1938

MCE

July 1, 1954

THE CUTANEOUS AND TOXICOLOGICAL APPRAISAL OF
PRODUCTS EVOLVED IN THE 2,4,5 TRICHLOROPHENOXYACETIC ACID SYNTHESIS
SUBMITTED BY THE MONSANTO CHEMICAL COMPANY, NITRO, W. VA.

DOW 751748
MN069799

I. General Objectives

A. To determine the potential acnegenicity and systemic effects of products evolved in the synthesis of 2,4,5 trichlorophenoxyacetic acid.

B. To further explore the pathogenesis of "occupational acne", and the syndrome which resulted from exposure to operations concerned with the production of 2,4,5-T, described in three (3) previous reports from this Laboratory.

II. Experimental Background

A survey of the literature revealed one paper which concerned itself with experimental work performed on rabbits. Adams et al.[1] discussed the reactions of rabbits to compounds reported to have caused acneform dermatitis in man. They concluded that their evidence "shows this acneform dermatitis to be the visible response of the skin to an irritant acting upon it from the exterior, and that this response took the form of epithelial hyperplasia, secondary inflammatory and degenerative changes, and finally, regenerative processes".[1]

The technique described by Adams and his co-workers, was employed in this Laboratory in connection with another evaluation in which known acnegens were applied in the prescribed manner and their results were not reproduced. This method was therefore regarded as useless for the present study.

16146

NOTICE: POOR COPY DUE TO
ILLEGIBLE ORIGINAL

DOW 751749

The observations of Parnell[2] on the postnatal development of the sebaceous glands of the rats appeared to provide a means of evaluating acnegens in this animal. Parnell described the following developmental changes in the skin of rats from birth to 31 days.

A.	Day 0 to day 17:	Sebaceous glands are added slowly and dermal fat increases.
B.	Day 18 to day 27:	Sebaceous cell enlarges and fills with sebum at the expense of dermal fat.
C.	Day 27 to day 28:	Sebum cells disappear in the secretory phase.
D.	Day 28 to day 31:	Stratum germinativum is activated, and sebaceous cells are replaced rapidly from the deeper layers of the cell wall.

Here then was a known pattern in sebaceous gland development whereby the application of a suspected acnegenic material, in the proper time sequence, might alter phase "C" of the cycle and perhaps lead to changes in the sebaceous glands and ducts comparable to those observed in acne.

Several other reasons supported the selection of the rat as an experimental animal. First, the hair cycle and concomitant epidermal changes in the rat were well known[3]. Secondly, the sebaceous glands of the rat are well defined and easily recognizable histologically. Thirdly, there were available data on the effect of applying various chemical substances to the epidermis of the rat[4]. 16147

In the initial group of experiments one of the suspected acnegens, 2,4,5 trichloroanisole and several control substances were applied for periods of varying duration and initiated during the first month of

NOTICE - POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 7517750

life (in consideration of Parnall's description of sebaceous gland development). When completely negative results were observed in these experiments, it was decided to apply the same substances in various phases of the hair cycle. It has been reported by one investigator that carcinogens are more effectively applied to mouse skin in anagen or the growth phase. Another investigator was of the opinion that methylcholanthrene 0.6% in benzene produced maximum cutaneous damage in mice when the hair was in the telogen phase or resting. Hence in the second group of experiments the rats were treated in various stages of the hair cycle.

III. Purpose of Present Experiments

A. To study the effects of the repeated application of the products submitted as well as known acnegens on the developing sebaceous gland and hair follicle of young rats.

B. To study the effects of the products submitted as well as known acnegens when applied during specific stages of the hair cycle, e.g., anagen, telogen.

C. To study the effects of the repeated application of the products submitted and known acnegens to the skin of human volunteers with particular reference to the pilosebaceous unit.

D. To obtain any information regarding the percutaneous toxicity of the substances submitted, when they are applied to rat or human skin over extended periods.

16148

NOTICE - POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 7517751

IV. Materials and Methods

A. Experimental Animals:

The rats were bred in this laboratory from an albino strain obtained from Carworth Farms, Rockland, New York. The resultant litters averaged 10 - 12 animals. Weaning was carried out at the end of three (3) weeks. Rats were not mated for at least one month following weaning. Only male rats were used throughout the experimental work.

B. Chemicals Employed:

Materials from the 2,4,5-T process:

1. 2,4,5 Trichloroanisole
2. Pentachloroanisole
3. 2,4,5 Trichlorophenoxyacetic acid
4. "Wringer Cake"

5. Acetone C.P., specific gravity 0.792.

Known acnegens employed as controls:

6. "Halowax 1001"
7. "Halowax 1014"

The test solutions used for application to the skin were prepared as 5% weight in acetone solution except in the case of "Wringer Cake" which was dissolved in water.

C. Techniques:

1. Animal Experiments

16149

A skin test site measuring approximately 1 cm in diameter, and located with its anterior edge at the shoulder level on the dorsum of the animal was used. Hair was removed from this site by means of an electric clipper, and this routine was repeated sufficiently often to keep the hair above the skin surface

TO DEFICIENT ORIGINAL

DOW 751752

less than 2 mm in length during the experiment.

Animals were painted daily with a fully wetted camel's hair brush (No. 1-3536 type camel's hair brush - W. Grumbacher, N.Y.). Originally a daily application consisted of three (3) separate paintings with a fully wetted brush. Later in the course of the experiments, five (5) fully wetted applications of the brush were made each day. The total dose per treatment for each substance applied was calculated and recorded. Daily painting of the animals was continued for an appropriately planned length of time. (See Tables for total dose applied in each experiment and duration of exposure.) Following this, the animals were anesthetized with Nembutal®, given intraperitoneally, and necropsied.

In the investigations with trichloroanisole and the Halowaxes treatment periods of varying duration were employed and initiated in different phases of the rat hair cycle.

The animal experimentation was controlled by treating a litter mate with the vehicle used as a solvent for the chemical being appraised. Other controls were used in different phases of the total experimental process, and were as follows:

16150

a. Untreated.

b. Rubbed: the skin of the animal was stroked with a 2" by 2" gauze pad for 20 times.

NOTICE: POOR COPY DUE TO ORIGINAL CONDITION

DOW 7517753

c. Brushed: the skin of the animal was stroked or brushed with a dry camel's hair brush for 15 times.

d. Petrolatum: the skin of the animal was rubbed with white petrolatum.

2. Human Experiments

A skin test site measuring approximately two (2) inches in diameter and located over the center of the scapula was used. The skin area was painted daily with one of the several materials under study for varying periods of time as indicated in Tables IV and V. The test site was observed at frequent intervals, and at the cessation of treatment, a biopsy of the skin was carried out, without anesthesia, using a rotary-driven electric punch. Excised tissues were fixed in 10% formalin, sectioned and stained with Hematoxylin and Eosin. Serial sections of each biopsy were studied microscopically.

D. Summary of Experimental Procedures:

The details of each experiment are recorded in Tables I - VI and may be summarized as follows:

1. Experiments #1 - #6 (Table I, Sections A and B).

2,4,5 trichloranisole and the control substances were applied repeatedly to the skin of young rats. These applications were initiated at various ages during the first months of life and maintained for periods of varying duration.

16151

NOTICE - POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751754

2. Experiments #7 - #8 (Table II).

2,4,5 trichloroanisole and the control substances were applied repeatedly to the skin of young rats during specific phases of the hair cycle. The first group of animals was treated during three (3) successive anagen phases and the second group during three (3) successive telogen phases of the rat hair cycle.

3. Experiment #9 (Table III).

2,4,5 trichlorophenoxyacetic acid, pentachloroanisole and "Ringer Cake" were applied repeatedly to rat skin for 60 daily applications in an attempt to determine the effect of prolonged exposure in animals prior to application of the same substances to human skin.

In experiments #1 - #9 inclusive the animals were carefully observed for evidence of systemic toxicity.

4. Experiment #10 (Table IV).

"Halowax 1014", a known acnegen, was applied repeatedly to the skin of four (4) human subjects with apparently normal skin for a period of 27 days.

5. Experiment #11 (Table V).

Pentachloroanisole and 2,4,5-T were applied repeatedly to the skin of two (2) groups of human subjects (two in each group) with apparently normal skin, for a period of 59 days. 2,4,5 trichloroanisole was applied to the normal skin of two (2) persons who had acne vulgaris for a period of 56 days.

16152

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751755

V. Summary of Findings

A. The repeated daily application to the skin of young, male rats of a 5% solution of trichloroanisole for varying periods up to 31 days produced neither gross or pathological changes in the skin nor evidence of systemic toxicity.

B. There were no differences in the results following the application of trichloroanisole when the exposures were initiated in either the anagen or telogen phase of the rat hair cycle and carried out in three (3) successive periods for a total application period of 31 to 39 days.

C. The repeated application to the skin of young, male rats of a 5% solution of pentachloroanisole, 2,4,5 trichlorophenoxyacetic acid and "Wringer Cake" for a period of 60 days failed to produce either clinical or pathological changes in the skin or other organs.

D. The application of 5% solutions of "Halowax 1001" and "Halowax 1014" to the skin of male rats for periods up to 31 days failed to produce clinical or pathological changes in the skin or other organ systems.

E. The application of a 5% solution of 2,4,5 trichloroanisole to human skin, extended over a three-month period, produced no clinical or pathological changes in the skin.

16153

F. The application of a 5% solution of pentachloroanisole and 2,4,5-T to human skin over a period of two (2) months produced no clinical or pathological changes in the skin.

NOTICE: POOR COPY DUE TO ORIGINAL

? send sample 10-50 g to Dr Seitz

C. The application of a 5% solution of "Halowax 1014" to human skin over a four-week period produced no clinical or pathological changes in the skin.

VI. Interpretations and Considerations for Future Investigation

It is fairly evident from the results of these experiments that neither the cutaneous changes nor the systemic effects noted in the clinical exposures have been reproduced with four (4) of the suspected materials or with the known acnegens such as "Halowax 1001" and "Halowax 1014" under the experimental conditions employed. In rats, as much as 372 mgms of trichlorocanisole and the Halowaxes were applied over a period of 36 days (31 applications). As much as 1.2 gms of 2,4,5-T and pentachlorocanisole were applied in 60 daily treatments without any noticeable effect, and 1.5 gms of "Stringer Cake" were applied during a similar period with no change. In humans, exposure to 0.46 gms of Halowax in 23 daily applications, 1.06 gms of pentachlorocanisole in 53 daily applications, 1.06 gms of 2,4,5-T in 53 daily applications, as well as 1.38 gms of 2,4,5 trichlorocanisole in a series of 98 daily applications, produced no clinical or pathological effects.

It is apparent that the circumstances which brought about the clinical problem in the plant exposure were not approached in either the animal or human experiments. Some pertinent questions to be raised in connection with our failure to induce clinical or pathological effects with either the four (4) specific materials from the 2,4,5-T process or with the known acnegens are these:

DOW 751756

16154

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL
NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751757

A. Is the rat an appropriate experimental animal for such a study?

B. Were the concentrations per dose applied to rat and man, adequate to stimulate cutaneous effects when applied epicutaneously?

C. Should not other routes of entry into the body be considered in the pathogenesis of "chloracne"?

By the answers to these questions we may be guided into the several experimental approaches to this problem which should be considered for the continuation of this investigative program:

1. Although the rat skin appears to have remained unaltered with the concentrations used and the manner of exposure employed, it may be possible to induce pilosebaceous changes in the rats with higher epicutaneous concentrations of acnegens and other types of exposure. Nevertheless, a variety of other animals should be tried for epicutaneous appraisal of known acnegens and the 2,4,5-T materials. It would seem logical to employ animals which spontaneously develop, or in which there may be induced, follicular inflammation such as in the dog or cat. It would be well to consider also specific sites in experimental animals in which there is an abundance of large sebaceous glands. Such a site is the external auditory canal of the rabbit (not the skin of the auricle which was employed by Adams and his co-workers). The usefulness of this area for investigation of sebaceous gland problems has been suggested by Montagna[5] and described recently by Hambrick and Blank[6].

16155

BEST AVAILABLE COPY DUE TO DEFICIENT ORIGINAL

DOW 7517758

2. Since the doses of materials were applied in uniform amounts throughout each experiment, the dose/weight relationship changed greatly in many instances with the rapid growth of the young rats, which growth was not inhibited by the exposures. For the three-day old rats the dose calculated in gm/kilo body weight was in some instances greater than 1.0, but with rapid increase in weight the dose in gm/kilo decreased to as little as 0.1. It is also possible that 5% concentrations were insufficient to produce pilosebaceous changes in animal or human skin. In future work it is

suggested that much higher concentrations be used over larger areas of skin in both experimental animals and human subjects.

3. In the clinical exposure at the Nitro plant there were opportunities not only for cutaneous contact with the chemical materials but for inhalation and possible ingestion of these materials as well. Since in so many cases the cutaneous involvement was very wide-spread, endogenous distribution of the toxic material to the affected organs including the skin, was a possibility. Respiratory absorption of the acnegens should be seriously considered in this connection. In future investigations exposure of animals to the suspected acnegens via inhalation and ingestion is recommended. It is

16156

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751759

likely that cutaneous absorption of acnegens under experimental conditions may be limited. To enhance such absorption the addition of surfactants to the applied solutions may be tried.

5. It is possible that some or all of the four (4) substances from the 2,4,5-T materials chosen for the initial study are not acnegenic. Nevertheless since the methods of appraisal produced negative results with known acnegens, these four (4) as well as the remainder of the materials submitted, should be investigated in future studies.

VII. Recommendations

The initial phase of the investigation which has been carried out with Monsanto materials and known acnegens should be regarded as exploratory. This initial experience indicates clearly that the entire problem required considerable probing at an experimental level and that investigative activity should be carried out simultaneously in several directions if progress is to be made.

That new cases arise currently in the 2,4,5-T synthesis, despite careful reorganization of the process and rigid hygienic precautions, indicates that acnegens are still being evolved and that the potential hazard is still present.

16157

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751760

It seems imperative, therefore, that a fundamental investigation of the pathogenesis of "chloracne" and a detailed appraisal of the materials which gave rise to the "Nitro Syndrome", be carried out. Methods of study must be developed before the major objectives can be approached. It will require the combined skill and experience of several scientific disciplines to make such methods of study and the appraisal of the suspected acnegens, possible. The study will require a long term program systematically organized, in which the problems may be assailed from several directions simultaneously. This study should be considered in terms of not less than a three- to five-year program.

The program for the next two (2) years should include the following general experiments:

A. Determine the cutaneous and systemic effects of known acnegens and the chemical materials from the 2,4,5-T process when applied in high concentrations and over large cutaneous areas to a variety of experimental animals including dogs, cats, rats, etc.

B. Determine the cutaneous and systemic effects of known acnegens and chemical materials from the 2,4,5-T process when administered repeatedly over an extended period by inhalation and by oral feeding. A variety of experimental animals should be used.

16158

C. Utilize the sebaceous-rich skin of the external auditory canal of rabbits and determine the effect of acnegens on such areas.

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 751761

D. Continue the human experiments in which known acnegens and chemical materials from the 2,4,5-T process are to be applied repeatedly in high concentrations to areas commonly affected, such as malar eminences, preauricular areas, ala nasi and to large areas of the back. Surface active agents may be used to increase percutaneous absorption of the acnegens and enhance pathological changes.

When a method of inducing pilosebaceous changes in animals comparable to man is developed, the cytologic and cytochemical appraisal of such changes which may be induced by individual acnegens should be carried out. This might include the consideration of lipids, phosphatases, ether esterases, glycogen, and keratin as determined histochemically.

From the Kettering Laboratory, in the Department of Preventive Medicine, College of Medicine, University of Cincinnati.

Experimental work and report by:

William R. Buckley, M.D.
Margaret Walker, B.S.
Raymond R. Suskind, M.D.

16159

Approved _____
Robert A. Kehoe, M.D., Director
The Kettering Laboratory

July 1, 1954

NOTICE: POOR COPY DUE TO DEFICIENT ORIGINAL

DOW 7517662

REFERENCES

1. Adams, E.M., Irish, D.D., Spencer, H.C., and Rowe, V.K.: The Response of Rabbit Skin to Compounds Reported to Have Caused Acneform Dermatitis. Industrial Medicine, 10 (Industrial Hygiene Section), 1, 1941.

2. Parnell, J.P.: Postnatal Development and Functional Histology of the Sebaceous Glands in the Rat. Am. J. Anat., 85:41, 1949.

3. Butcher, E.O.: The Hair Cycles in the Albino Rat. Anat. Record, 61:5, 1934.

4. Butcher, E.O.: The Effects of Applications of Various Substances on the Epidermis of the Rat. J. of Invest. Dermat., 16:85, 1951.

5. Montagna, W.: Personal communication.

6. Hendrick, O.W. and Blank, H.: Whole Mounts for the Study of Skin and Its Appendages, paper presented at meeting of Society for Investigative Dermatology, June 19, 1954.

16160

NOTICE - POOR COPY DUE TO ORIGINAL

DOW 751763

16161

TABLE I

The Effects of Repeated Application of 2,4,5 Trichloroanisole, "Halowax 1001", and Control Treatment to the Skin of Young Rats, Initiated During the First Month of Life

Summary of Experimental Conditions and Observations

(Section A) - Experiments #1 - #3a

Test Materials	Controls	No. of Animals (male rats)	Date of Birth	Age Start of Test (Days)	Age Completion of Test (Days)	Age at Necropsy (Days)	No. of Applications (Days)	Duration of Applications (Days)	Total Quantity Applied Grams	Clinical Findings	Microscopic Findings
	Untreated	1	8-11-53	-	-	32	-	-	-		
	Untreated	1	"	-	-	5	-	-	-		
	Untreated	1	"	-	-	9	-	-	-		
	Untreated	1	"	-	-	11	-	-	-		
	Acetone	1	"	3	10	11	7	8			
"Halowax 1001" (5% by wt. in acetone)		1	"	3	10	11	7	8	0.084	None	None
2,4,5 Trichloro-anisole (5% by wt. in acetone)		1	"	3	10	11	7	8	0.084	None	None
	Untreated	1	"	-	-	32	-	-	-		
	Acetone	1	"	3	31	32	24	29			
"Halowax 1001"		1	"	3	31	32	24	29	0.288	None	None
2,4,5 Trichloro-anisole		1	"	3	31	32	24	29	0.288	None	None
	Untreated	1	"	7	-	32		25	-		
	Acetone	1	"	7	31	32	21	25	-		
"Halowax 1001"		1	"	7	31	32	21	25	0.252	None	None
2,4,5 Trichloro-anisole		1	"	7	31	32	21	25	0.252	None	None

DOW 751764

16162

TABLE I

(Section A) - Experiments #1 - #3a (Con't.)

Test Materials	Controls	No. of Animals (male rats)	Date of Birth	Age at Start of Test (Days)	Age Completion of Test (Days)	Age at Necropsy (Days)	No. of Applications (Days)	Duration of Applications (Days)	Total Quantity Applied Grams	Clinical Signs
	Untreated	1	8-11-53	77	-	24	-	-	-	
	Acetone	1	"	77	23	24	15	17	-	
"Haloxax 1001"		1	"	77	23	24	15	17	0.18	
2,4,5 Trichloro-anisole		1	"	77	23	24	15	17	0.18	

(Section B) - Experiments #4 - #6

	Untreated	1	8-11-53	91	-	21	-	-	-	
	Untreated	1	"	91	31	32	-	-	-	
	Acetone	1	"	91	31	32	9	11	-	
"Haloxax 1001"		1	"	91	31	32	9	11	0.108	
2,4,5 Trichloro-anisole		1	"	91	31	32	9	11	0.108	
	Untreated	1	8-19-53	13	-	13	-	-	-	
	Untreated	1	"	13	-	57	-	-	-	
	Untreated	1	"	13	41	42	-	-	-	
	Acetone	1	"	13	41	42	23	28	-	
"Haloxax 1001"		1	"	13	41	42	23	28	0.276	
2,4,5 Trichloro-anisole		2	"	13	41	42	23	28	0.276	
	Untreated	1	8-21-53	85	-	85	-	-	-	
	Untreated	1	"	85	-	63	-	-	-	
	Untreated	1	"	85	-	63	-	-	-	
	Untreated	1	"	85	62	63	-	-	-	
	Acetone	1	"	85	62	63	31	36	-	
	Rubbed Control	1	"	85	62	63	31	36	-	
	Petrolatum Control	1	"	85	62	63	31	36	-	
"Haloxax 1001"		1	"	85	62	63	31	36	0.372	
"Haloxax 1001"		1	"	85	62	63	31	36	0.372	
2,4,5 Trichloro-anisole		1	"	85	62	63	31	36	0.372	

DOW 751765

16163

TABLE II

The Effects of Repeated Applications of 2,4,5 Trichloroanisole, "Halowax 1014" and Control Treatment, Administered During Three (3) Successive Anagen and Three (3) Successive Telogen Phases of the Rat Hair Cycle

Summary of Experimental Conditions and Observations

Experiments #7 - #8

Test Materials and Controls	No. of Animals	Date of Birth	Age Start of Test (Days)	No. of Applications (Days)	Age Completion of Test (Days)	Age Resumption of Test (Days)	No. of Applications (Days)	Age Completion of Test (Days)	Age Resumption of Test (Days)	No. of Applications (Days)	Age Completion of Test (Days)	Total Quantity Applied (grams)	Age Recovery (Days)	Incidence of Tumors Histologic Examination
Rubbed or Brushed Control	4	10-21-53	6	12	19	30	16	46	65	11	76	-	115	
Acetone	6	"	6	12	19	30	16	46	65	11	76	-	115	
"Halowax 1014"	3	"	6	12	19	30	16	46	65	11	76	0.78	115	None
2,4,5 Trichloroanisole	7	"	6	12	19	30	16	46	65	11	76	0.78	115	None
Acetone	1	11-18-53	21	10	31	49	10	60	81	11	92	-	138	None
2,4,5 Trichloroanisole	3	"	21	10	31	49	10	60	81	11	92	0.62	138	None

DOW 751766

16165

TABLE III

The Effects of Repeated Applications of 2,4,5 Trichlorophenoxyacetic Acid, Pentachloroanisole and Ringer Cake* to the Skin of Rats for 60 Days

Summary of Experimental Conditions and Observations

Experiment 99

Test Materials and Controls	No. of Animals	Date of Birth	Age Start of Test (Days)	No. of Applications (Days)	Age Completion of Test (Days)	Total Quantity Applied (grams)	Age Necropsy (Days)	Clinical and Microscopic Findings
Acetone	3	Jan. 1, 195	41	60	110		111	None
2,4,5 Trichlorophenoxyacetic acid	3	"	41	60	110	1.20	111	None
Pentachloroanisole	3	"	41	60	110	1.20	111	None
Ringer Cake* (5 gms dissolved in 100 gms distilled water)	3	"	41	60	110	1.50	111	None

DOW 751767

16166

TABLE IV

The Effect of Repeated Applications of "Halowax 1014" to the Normal Skin of Human Subjects

Summary of Experimental Conditions and Observations

Experiment #10

Subject Identification	Age (Years)	Present Status of Skin (Site of application)	Previous Skin Trouble	Test Material	Test Material	No. of Applications (Days)	Total Period of Application (Days)	Quantity Applied (grams)	Clinical Findings	Microscopic Findings
M.W. (female)	66	Normal	None	"Halowax 1014" (5 gms. in 100 gms. acetone)	"Halowax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
S.B. (female)	77	Normal	None	"Halowax 1014" (5 gms. in 100 gms. acetone)	"Halowax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
H.J. (female)	65	Normal	None	"Halowax 1014" (5 gms. in 100 gms. acetone)	"Halowax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
E.N. (female)	61	Normal	None	"Halowax 1014" (5 gms. in 100 gms. acetone)	"Halowax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal

DOW 751768

TABLE V

The Effects of Repeated Applications of Pentachloroanisole, 2,4,5 Trichlorophenoxyacetic Acid and 2,4,5 Trichlorophenol to the Skin of Human Subjects

Summary of Experimental Conditions and Observations

Experiment #11

Subject Identification	Age (Years)	Present Status of Skin (site of application)	Previous Skin Trouble	Test Material	No. of Applications (Days)	Total Period of Applications (Days)	Quantity Applied (grams)	Control	Clinical Findings	Microscopic Findings
G.F. (Female)	66	Normal	None	Pentachloroanisole (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	
G.C. (Female)	69	Normal	None	Pentachloroanisole (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	
V.R. (Female)	48	Normal	None	2,4,5 Trichlorophenoxyacetic Acid (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	
C.R. (Female)	60	Normal	None	2,4,5 Trichlorophenoxyacetic Acid (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	
F.P. (Male)	19	Normal	Severe Acne Vulgaris of face. Topical Therapy	2,4,5 Trichlorophenol (5 gms. in 100 gms. acetone)	69	96	1.38	Acetone	None	
E.D. (Female)	15	Normal	Acne Vulgaris, old. Face scarred. Recurrent Acne Vulgaris, face, shoulders. Topical Therapy	2,4,5 Trichloroanisole (5 gms. in 100 gms. acetone)	67	96	1.34	Acetone	None	

DOW 7517769

Summary of Preventional Data on Imported Guanogous Appliances of Known Toxicity

TABLE VI

Material	No. of Applications	Duration of Application	Clinical Microscopic Findings
Phosphorus	7	7 - 39 days	Rope burns
Phosphorus	11	11 - 39 days	Rope burns
Phosphorus	7	7 - 72 days	Rope burns
Phosphorus	36	36 - 72 days	Rope burns

* Animals were treated by oral administration only; one phase of burns (3rd degree) developed.

DOW 749666

"Pathogenesis of Chemically Induced Acne"

The objectives of this study are: (1), to compare quantitatively the responses of human skin with rabbit skin to chemical acneigens, and (2), to study in detail the structural pathophysiologic and bacteriologic changes which occur during the evolution of experimentally induced human disease.

a. The subjects will be healthy adult male prisoners -- inmates of the Philadelphia County Prison at Holmesburg, Philadelphia.

b. acneigens - 2 substances of different potency will be evaluated:

1. hexachlordiphenyl ether
2. tetrachlorodibenzol dioxane

c. procedures: - The initial studies will be carried out on the back applying .05 ml. of the aneigen in a suitable solvent to a 2" square of skin. The area will be covered with a square of gauze held in place by strips of adhesive. The applications will be made once daily. The principle variables will be concentration and total number of applications. The aim here will be to establish threshold concentrations for various numbers of exposure. This will require, of course, a considerable number of range finding experiments.

Both anergens will be applied to the skin of the same subject in the final tests. Moreover, tests of liver function will be included as follows: SGOT, BSP., Bilirubin, Alkaline Phosphatase, and Cephalin Flocculation.

d. pathogenesis - The skin will be examined for clinical signs of change. At appropriate times, punch biopsies will be removed and stained as follows:

H & E, Orcein-Giemsa, Hale, Mallory and Alkaline Phosphatase. These procedures will give detailed information of the effect on vasculature, the synthesis of mucopolysaccharides, collagen and elastin, and the general architecture. Enzyme content will be evaluated by histochemical procedures for ATP'ase and succinic dehydrogenase. As regards three dimensional views of the lesions, the epithelium will be separated by sodium bromide and mounted in tact as a sheet.

e. bacteriology - Comedones will be removed at various stages and the contents sampled qualitatively and quantitatively for bacterial contents. For comparison, a similar study will be conducted on comedones derived from patients with acne vulgaris. Lesions which become inflammed will be sampled for their bacterial content, either by biopsy or by aspirating fluid.

f. horny layer - The horny layer from treated sites will be separated as a sheet and studied structurally, physically and chemically. Values for normal horny layer have been obtained. We wish to know whether hyperkeratotic samples represent an increase in mitotic activity or whether the epidermis is pathologically altered at the same time. The rate of desquamation of the horny layer will be estimated by measuring the time required for the disappearance of a fluorescent dye which has been appropriately used to stain the above horny layer.

DOW 749667

CLOVER LABORATORIES, INC.
P. O. BOX 7686
PHILADELPHIA I, PENNSYLVANIA
EVERGREEN 6-3459

DOW 749668

December 21, 1964

To: Dr. V. K. Rowe
Biochemical Research Laboratory
1701 Building
The Dow Chemical Company
Midland, Michigan

From Albert M. Kligman, M. D.
Clover Laboratories, Inc.,
P. O. Box 7686
Philadelphia, Pa.

For: Study with Acnnigenic substances

Help and Overhead at Prison		$2,000.00
Subjects:		
Approx. 100, average cost @ $15.00 per subject =		1,500.00
Bacteriology		1,000.00
Histology and Histochemistry		1,000.00
Miscellaneous:		
Materials, Photos, Hormones, etc.		500.00
	Total	$6,000.00
University Overhead 20%		$1,200.00
	TOTAL	$7,200.00

Grant to be made out to Trustees of The University of Pennsylvania

16171

g. Prevention and Treatment - It is known that x-rays, estrogens, and keratolytic agents are helpful in acne vulgaris. The beneficial effects of these modalities will be evaluated.

h. miscellaneous studies - Limited attempts will be made to evaluate the effect of age, sex, and body area on the development of experimental acne.

Respectfully submitted,

Albert M. Kligman, M. D.

AMK/a

DOW 749669

16172

1987
J.H. Davidson
DOW 471026
MN001665

SPECTRUM

that have had implants. A spokesman for the Department of Agriculture told Environment that residues of DES are not ordinarily found in animals with implants because the levels of DES are below the sensitivity of the analytical techniques used, but that there were undoubtedly some residues. The spokesman also explained that the small farmer will be hurt by a partial ban on DES because whereas large farm operations can simply switch to implants, the small farmer does not have the equipment needed for implantation.

A SHARP INCREASE in the incidence of a particular type of liver cancer in North Vietnam may be related to herbicide spraying programs, according to Dr. Ton That Tung of the University of Hanoi. Dr. George Perera who visited Dr. Tung on behalf of the American Friends Service Committee told Environment that Dr. Tung had seen a greater than five-fold increase in liver hepatoma cases between the periods 1955-61 and 1962-66. Perera said that Dr. Tung suspects that dioxin, a contaminant of the herbicide 2,4,5-T which causes birth defects, can cause liver cancer. Human liver samples suspected of containing small amounts of dioxin have been sent to the U.S. for analysis, but techniques to test for low quantities of the chemical are still in the developmental stage. Although no herbicide spraying was conducted over North Vietnam, Dr. Tung claims that there is a great deal of movement between North and South, so that his patients in Hanoi may have been affected by 2,4,5-T in the South.

2,4,5-T is again in the courts. A federal district court judge in Arkansas issued a preliminary injunction June 23 against the Environmental Protection Agency (EPA) plan to hold public hearings concerning the fate of the registration of the herbicide for use on rice crops. In a case brought by Dow Chemical Company, a manufacturer of the herbicide, the judge ruled that William Ruckelshaus, administrator of EPA, must make a clearcut decision on whether or not to ban 2,4,5-T for the specified use before hearings are held. Last summer Ruckelshaus found that following the report of an advisory committee on 2,4,5-T, he had insufficient evidence to make a final decision and ordered a hearing. A spokesman for EPA explained to Environment that the administrator's order was sufficient, and said that a notice of appeal had been filed. When asked if Dow was using 2,4,5-T as a test case, a spokesman for the company told Environment that "We've got to stop this ridiculous mess somewhere; who else is going to stand up in a case like this?"

A CORRELATION may exist between air pollution and what is known as the sudden infant death syndrome, in which an apparently healthy infant is found dead in its crib. Preliminary analyses made by Dr. Bertram Carnow of the University of Illinois Medical School showed that when sulfur dioxide and particulate air levels were high, there was an associated rise in the incidence of sudden infant deaths. However, Dr. Carnow told Environment that further analyses have yet to back up the preliminary work. Dr. Carnow theorizes that the sudden deaths are actually due to a constriction of the throat opening caused by acute tracheobronchitis, which is aggravated by high air pollution levels.

A NEW PRICING regulation by the Federal Power Commission (FPC) may spur exploration and development of new natural gas resources in the U.S. The FPC, which now regulates gas prices, will allow gas producers to sell in the interstate market without being restricted to current area price ceilings at the wellhead, reports Coal News, August 4. However, the new ruling applies only to gas from reserves discovered after April 6 or gas diverted from intrastate markets. The Commission retained the right to ensure that gas prices will remain "in the public interest." Natural gas, one of the cleanest fuels, has been in short supply, a fact which Coal News says is attributable to "restrictive FPC price regulation of gas at the wellhead."

A NEW WAY of controlling mosquitoes is being studied by Clyde Umphlett of Clemson University. A report in BioScience, July, states that Umphlett is working with a microscopic fungus that attacks and kills mosquito larvae. If the fungus is found to be without unacceptable side effects, it could eliminate the need for spraying pesticides or oil on mosquito breeding grounds. The fungus is extremely effective on several species of

16173

THE DOW CHEMICAL COMPANY

MIDLAND
June 21, 1968

H. Ferguson

RECEIVED
JUN 21 1968
BIOPRODUCTS DEPT.
GHH 6/21/68

cc: M. G Wiltse
J. R. Ridner
C. A. Bryant
O. H. Hammer
A. J. Watson
W. J. McCoy

Your letter to Andy Watson has been passed to me for answering.

Enclosed are several pieces of literature dealing with the toxicological aspects of silvex.

A good summary of the effects of silvex ester and salts on fish has been assembled by Hammer.

The summary by Mullison lists several references that we could supply if you feel this is necessary.

The size of the market you mention in your letter was a bit surprising but perhaps this includes some other areas than New Zealand.

I have sent a copy of this letter to W. J. McCoy. He will advise you on his thoughts regarding the world market for silvex.

Let us know if we can be of further help.

John

John H. Davidson
Plant Science Research and Development
Agricultural Products Center

bk

encl

DOW 381612

2008

Oct 13, 1958

NATIONAL AGRICULTURAL CHEMICALS ASSOCIATION

CONFIDENTIAL

Inter-Office Correspondence

TO: Mr. Hitchner

FROM: Jack Dreessen

SUBJECT: July 28 Conference with USDA and FDA on the use of 2,4-D and 2,4,5-T on grazing lands.

DOW 3768842 MN053305

USDA arranged for a joint conference between FDA, NAC, and the USDA on July 28 in the office of Mr. R. S. Roe, Director, Bureau of Biological and Physical Sciences, FDA. Attending the meeting were:

H. L. Haller - Agricultural Research Service, USDA
W. B. Ennis, Jr. - Weed Sections, USDA
W. C. Shaw - Weed Sections, USDA
D. L. Klingman, Weed Sections USDA
J. T. Coyne - Pesticide Regulation Section, USDA
E. A. Walker - Pesticide Regulation Section, USDA
W. Ligon - Pesticide Regulation Section, USDA
J. Cummings - Pesticide Regulation Section, USDA
R. S. Roe - FDA
A. J. Lehman - FDA
F. Vorhes - FDA
F. J. McFarland - FDA
J. A. Noone - NAC
J. Dreessen - NAC

The writer had met with Dr. W. B. Ennis, Jr., in advance of the meeting and together we worked out a suggested agenda which in turn was forwarded to all participants by Dr. Haller who presided at the conference. The following is the suggested agenda as developed by Ennis and myself.

1. Purpose of Meeting - To determine the information needed to continue recommendations on the use of 2,4-D and 2,4,5-T on pastures and rangelands.

   H. L. Haller (5 min.)

2. Status of registration of 2,4-D and 2,4,5-T on grazing lands.

   J. T. Coyne, PPCD, ARS (5 min.)

3. What industry is doing to comply with Miller Amendment with reference to use of 2,4-D and 2,4,5-T on pastures and rangelands.

   Jack Dreessen, NACA (5 min.)

4. Necessity for continuing uses of 2,4-D.

   D. L. Klingman, ARS, (5 min.)

5. Analysis of existing data on residues of 2,4-D, 2,4,5-T and MCPA when used on grazing lands.

   J. T. Coyne, PPCD, ARS (5 min.)

1617

6. Analysis of existing data on the pharmacology of 2,4-D and 2,4,5-T.

A. J. Lehman, FDA (5 min.)

7. Discussion of questions raised concerning registration.

(a) On June 9, 1955, Mr. G. E. Lynn wrote to Mr. W. B. Rankin and the following excerpts are taken from that letter:

"I am writing to be sure that Mr. Sunderland and I have a clear understanding of the conclusions reached with respect to 2,4-D and the related compounds in our meeting with you on June 3.

"We explored at some length the problem of residues of 2,4-D and 2,4,5-T and MCP in meat. It was our opinion that the bulk of the spraying on range land and pastures was carried out in the spring or early summer. Stock grazing on such lands are not slaughtered for 4 to 6 months later. A high percentage of this stock is lot fed after they are removed from the range and before they are slaughtered. Thus it did not seem likely to us that early transient exposure to these chemicals would constitute a residue problem in stock slaughtered several months later. You took this matter up with your colleagues and gave us the opinion that it appeared there was no possibility of a residue problem under these circumstances and that you would not ask us to obtain data on this particular problem."

re exemption from meat residue data

DOW 376843

Mr. Rankin replied to Mr. Lynn's letter on June 15 as follows:

"This acknowledges receipt of your letter of June 9, 1955 which reflects accurately our understanding of the questions discussed at our conference on June 3.

"In reading the letter I realize that there is one additional comment which needs to be made about the problem of residues of herbicides on forage. If herbicide residues are present on forage, other than alfalfa, which moves in interstate commerce, on baled hay for example, tolerances or exemptions would need to be established for them."

The situation concerning 2,4-D, 2,4,5-T and MCPA applications where beef cattle may be grazing have not changed since 1955, i.e., the 2,4-D, 2,4,5-T and MCPA are normally applied early in the grazing season, application rates are relatively low and generally only one application per year is made. If the same conclusions be drawn today as in 1955 then there is no possibility of a residue problem under these circumstances and recommendations can be continued.

(b) How much and what kind of data are needed to determine whether there are residues in milk?

16176

(c) How sensitive a method is required in running residue studies on milk? Will data obtained by chemical, biological and radiological methods be evaluated on an equal basis?

In a letter dated April 12, 1955, from W. B. Rankin to Mr. L. S. Hitchner the following statements were made:

"The toxicity data on 2,4-D would not in our opinion justify the establishment of a tolerance higher than 5 parts per million for this herbicide.

"The method used to determine residues of 2,4-D should detect residues of 0.5 ppm and at a level of 5ppm should be accurate to plus or minus .0.5 ppm....."

(d) Can data accumulated on residues from one ester be applied to other esters? One salt to another?

(e) If residues are found, are there adequate pharmacological data to establish a tolerance for milk?

(f) If residues are found to occur in milk from animals grazing treated forage and it is found that these residues are no longer prevalent within x number of days after treatment will registration be continued by including on the label a statement to the effect "Do not allow milk animals to graze on pastures treated with 2,4-D within _____ days after treatment."

DOW 376844

8. Summarization

The various items on the agenda were discussed and the following are my impressions as to statements made by various persons attending the conference.

1. Dr. Haller merely expressed a desire to try to develop what is required for the Department of Agriculture to continue recommending the use of 2,4-D and 2,4,5-T on pastures and range lands.

2. Mr. Coyne stated that the status of registration of 2,4-D and 2,4,5-T on grazing lands has not changed as far as his section was concerned. He indicated that letters which have been sent to registrants in reference to clearing the use of 2,4-D on range and pasture lands had been mailed to put the Industry on notice that such uses had not been cleared in the Section's opinion but that registrations were not being cancelled. It was his opinion that the basic producers' action in removing the use of 2,4-D on range and pasture lands had been done at the Industry's own volition. He went on to state that the use of 2,4-D for range and pasture lands would continue to be registered until some other course of action is indicated by administrative superiors and stated that should the Industry members resubmit a label bearing these uses, it would be accepted. Attached as exhibit 1 is a copy of a letter to one of our member companies similar to those that have been sent to other registrants of 2,4-D in reference to this problem.

3. I commented that the Industry removed the use of 2,4-D on pasture and range lands from their labels based on letters the registrants had received from USDA and which were commented on by Mr. Coyne. I stated that it was the registrant's opinion that the cost of collecting data to clear the suggested uses under the Miller Amendment were not justified on the part of Industry based on the expected cost of collecting such data versus the expected profit from the continued sale of 2,4-D for such uses. These statements were made based on discussions at a meeting of the Herbicide Technical Subcommittee last fall and telephone conversations with members of the Subcommittee prior to the conference.

4. Dr. Klingman presented a very convincing case, pointing out the necessity for the continued use of 2-4,D and 2,4,5-T on grazing lands. A copy of Dr. Klingman's statement is attached as Exhibit 2. Dr. Klingman's statement brought to the attention of FDA the necessity for continuing the use of 2,4-D on pasture lands.

5. Mr. Coyne referred this item to Dr. Walker and others of his staff who reported that they had insufficient data to conclusively determine whether 2,4-D, 2,4,5-T and MCPA leave residues when used on grazing lands. They also stated that they did not have conclusive data as to whether any of these products were transmitted to meat and/or milk.

6. Dr. Lehman stated that if residues of 2,4-D and/or 2,4,5-T occur in meat or milk that further pharmacological studies would have to be conducted with these herbicides. It was pointed out by representatives of FDA that if residues of any chemical or its metabolites are present, that pharmacology of these and products would have to be determined before FDA could pass on their safety.

7. The various topics listed under item 7 were covered during the course of the conference and definite answers were not obtained in all cases.

(a) It was generally agreed that the situation concerning 2,4-D, 2,4,5-T and MCPA applications where beef cattle may be grazing have not changed since 1955 (refer to this item in the above agenda). However, based on previous statements, the Pesticide Regulation Section had continuously accepted these chemicals for this use and could see no reason why such registration should not be continued.

(b) This question was not adequately answered.

(c) No specific answer was given to the question raised in this item. FDA indicated that the nature of residues, pharmacology of residual material and many other factors would have to be considered before the sensitivity of a method could be passed upon.

(d) FDA indicated that they were of the opinion that the various esters and amine salts should be considered as different chemicals and that if data or pharmacology on residues is accumulated for one or more esters enough work should be done to indicate that this same data could be translated to other formulations. Indications are that FDA and the Pesticide Regulation Section would accept a limited

DOW376845

amount of data on some esters and amine salts if it can be shown that their properties are similar to other formulations on which more complete data has been accumulated.

(e) The answer to this question was No.

(f) It seemed to be the opinion of the group that label statements to the effect "Do not allow milk animals to graze on pastures treated with 2,4-D within _____ days after treatment" would be acceptable provided sufficient data had been accumulated to conclusively indicate the interval between application and grazing which would result in no residues occuring in milk.

The above represents some of the answers to specific questions raised in the suggested agenda. I would like to emphasize that FDA was quite positive in stating that they did not have enough pharmacological data concerning 2,4-D to establish tolerances should residues be found in meat or milk.

Jack Dreessen

Jack Dreessen

DOW 376846

KURON

TOXICOLOGICAL INFORMATION AND SUGGESTIONS REGARDING TREATMENT FOR PERSONS SWALLOWING KURON

DOW 323d09
MN022777

FIRST AID

Call a physician immediately. Keep the person warm. Do not attempt to induce vomiting.

TO THE PHYSICIAN

Kuron® contains 65.3% of 2-(2,4,5-trichlorophenoxy) propionic acid, propylene glycol (C_3H_6O to $C_9H_{18}O_3$) butyl ether esters and 35.5% of inert petroleum oils and emulsifying agents.

Kuron has a low acute oral toxicity. The LD_{50} values for various animal species are: rat, 1070 mg/kg; guinea pig, 850 mg/kg; rabbit, 850 mg/kg; mouse, 2140 mg/kg; and chicks, 2000 mg/kg.

The advisability of attempting to empty the stomach by emesis or gastric lavage is controversial since aspiration of a petroleum solvent is to be carefully avoided. Respiratory embarrassment caused by pulmonary edema and pneumonia is the primary danger from swallowing the petroleum solvent. In such a case, the administration of oxygen probably will be beneficial. Chemotherapy would have no effect on the so-called "chemical pneumonia" but it may have some beneficial effect in preventing or controlling possible subsequent bacterial invasion.

Depression of the central nervous system may also be a problem. Oil laxatives other than mineral oil should be avoided.

Liver damage may be minimized by promptly placing the patient on a diet low in fat and high in protein. The usefulness of lipotropic drugs has not been investigated. Kidney involvement is rarely sufficient to merit special treatment and for those rare cases where the kidneys are seriously involved, the treatment should be the same as for toxic nephritis of other etiology.

DOW 3234U

NOTICE

This information is given in good faith, but while it is believed to be correct, no warranty is made.

NOTE: In case of any questions, call The Dow Chemical Company at Midland, Michigan, on phone

MElrose 6-2376 (Toxicology Laboratory)

MElrose 6-2243 (Medical Department)

Biochemical Research Laboratory
The Dow Chemical Company
May 28, 1959

Biochemical Research Laboratory

The Dow Chemical Company

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON AGRICULTURAL CHEMICAL FORMULATION M-2121 CONTAINING 50% SILVEX EQUIVALENT

File T 2.30-237-1
K No. Sample 1
Chg. 1274-26
Ref. GH3-677-137
Sub.by G. Scoles
Rept.by K. J. Olson

Signed K. J. Olson, Jan. 17, 1962

Checked F. Oyen 1-17-62

DOW 23306
MN 02717

DISTRIBUTION

The Dow Chemical Co., Midland
R. H. Boundy
CRI
H. H. McIntyre
R. P. Perkins
A. Hart
N. Wright
G. E. Lynn (2)
G. D. Jones
K. Barrons (2)
G. Scoles

Midland Division
H. H. Gay
W. L. Tisdale
E. N. Luce
L. M. Greene
S. A. Shrader
W. J. Falkenstein

Louisiana Division
CRI

Texas Division
CRI
D. J. Kilian
R. W. Colby

Western Division
CRI
D. Elshere
J. F. Kagy

James River Division
CRI

T 2.30-237-1

THIS REPORT IS THE PROPERTY OF THE DOW CHEMICAL COMPANY

Biochemical Research Laboratory

The Dow Chemical Company

DOW 3233307

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON AGRICULTURAL CHEMICAL FORMULATION M-2121 CONTAINING 50% SILVEX EQUIVALENT

PROBLEM

A sample of M-2121 was submitted to the Biochemical Research Laboratory for toxicological testing and definition of industrial handling hazards. The material is a pelletized formulation containing Silvex Acid and Silvex Potassium Salt on Attaclay. The formulation is designed for use as a brush-killer in the undiluted dry granular form.

CONCLUSIONS

M-2121 possesses a low acute oral toxicity and is only slightly irritating to the eye as well as to intact and abraded skin. Excessive skin exposure to the moist material, particularly if confined on the surface, might result in some skin redness. Such exposure should be avoided. It is anticipated that no problems should arise from handling the subject material under conditions where reasonable care and cleanliness are practiced.

SUMMARY OF RANGE FINDING TOXICOLOGICAL DATA

Acute Oral Toxicity

Animal	Preparation Fed	Dose (g/kg)	No.Died No. Fed	Response-Remarks
Rat	5% in corn oil	0.50	1/2	Animal died four days after feeding, pathology ess. negative.
Rat	5% in corn oil	1.0	0/2	Pathology ess. negative.

Eye Contact - Rabbit

Material	Treatment	Response-Remarks
Undiluted	Washed and unwashed	Sl. conjunctivitis subsided in one week.

Skin Contact - Rabbit

Material	Condition of Skin	No.of Appl.	Site	Response-Remarks
Undiluted (Dry)	Intact	10	Belly	No irritation observed.
Undiluted (Dry)	Abraded	3	Belly	No irritation observed. Sl. scar in 21 days.

DOW3233308

Skin Contact - Rabbit (Cont'd.)

Material	Condition of Skin	No.of Appl.	Site	Response-Remarks
Undiluted (Wet)	Intact	10	Belly	Sl. to mod. hyperemia with sl. swelling and sl. exfol. Skin normal in 21 days.
Undiluted (Wet)	Abraded	3	Belly	Ess. the same as above with sl. burn following 3rd appl. Skin healed with sl. scar in 21 days.

NOTE: When applied wet to the rabbit skin under a bandage, the material adhered to the skin which probably resulted in the irritation.

Skin Absorption

There is no indication, from the skin irritation tests conducted, that this material is absorbed through the skin in toxic amounts.

FIRST AID MEASURES

EYE CONTACT	[X]	If the eyes are contaminated, they should be flushed immediately with copious amounts of flowing water for at least 15 minutes.
	[X]	Medical attention should be obtained if irritation persists or develops after washing of the eyes.
	[ ]	Medical attention should be obtained.
	[ ]	MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY.
SKIN CONTACT	[X]	Any injuries or irritations which may develop should receive medical attention.
	[X]	Contaminated clothing and shoes should be removed and not re-used until thoroughly cleaned.
	[X]	Wash contaminated skin with soap and plenty of water.
	[ ]	Contaminated clothing, including shoes, should be removed and the affected skin area should be washed thoroughly with soap and plenty of water.
	[ ]	Medical attention should then be obtained.
	[ ]	Contaminated clothing and shoes should not be re-used until thoroughly cleaned.
	[ ]	All contaminated clothing, including shoes, must be removed immediately and the affected skin area flushed thoroughly with water from a safety shower, or other suitable device and cleansed with soap and plenty of water.
	[ ]	MEDICAL ATTENTION MUST THEN BE OBTAINED AS RAPIDLY AS POSSIBLE.
	[ ]	Contaminated clothing including shoes, must not be re-used until thoroughly cleaned or must be discarded.
INHALATION	[ ]	If a person should experience any noticeable ill effects from breathing the vapor or fumes of this material, medical attention should be obtained promptly.
	[ ]	If a person should be overcome from breathing this material, he should be removed to fresh air at once, be made to rest, kept warm, and MEDICAL ATTENTION SHOULD BE OBTAINED IMMEDIATELY. If breathing stops, artificial respiration should be administered.
INGESTION	[ ]	If appreciable amounts of material are swallowed, vomiting should be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Medical attention should then be obtained.
	[ ]	If the material is swallowed, vomiting must be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY

COMMENTS:

nad

DOW3233309

16185

RESPONSE TO #5

MN420978

DOW 2773379

PART IV. RESPONSE OF HUMANS TO KURON AND OTHER FORMULATIONS OF SILVEX.

1. Wolf, M. A. 1959, Results of Human Skin Irritation and Human Skin Sensitization Studies on Kuron. Dow Internal Report No. BC T2.30-40-3.

2. Wolf, M. A. 1961, Results of Human Skin Irritation and Sensitization Tests on Kurosal SL (M-2033). Dow Internal Report No. T60.14-39291-2.

2074

DOW2712167

TUCSON DAILY CITIZEN, Thursday, November 27, 1969, page 3

Pinal People Plead Suit Over Herbicides

By NYLA CROCE
Citizen Staff Writer

GLOBE — Angry residents here plan to sue the federal government for several million dollars for damages to gardens, livestock and property they claim resulted from indiscriminate use of herbicides.

"In addition we expect to file a class action (for anyone affected) against the U.S. Forest Service for damage to the entire environment," says the spokesman of a group of 500 residents claiming damage.

She is Mrs. Willard Shoecraft, who has enlisted the help of scientists, her congressman and a national health organization to f[illegible] aerial spraying started in [illegible] by the U.S. Forest Service in cooperation with the Salt River Project.

The sprays — 2-4D, 2, 4, 5-T and Kuron, also called Silvex — are part of a chaparral management program to thin out native growth in the Pinal Mountain foothills, says Robert Courtney of Phoenix, Tonto National Forest supervisor.

The Salt irrigation project became involved because reduced brush cover increases water flow into reservoirs.

"The spray is definitely designed to kill shrubs and brush," Courtney said, "and probably would kill most broad-leafed plants."

Homeowners in nearby Russell Gulch and Kellner Canyon agree that the spray does, indeed, affect broad-leafed plants — not to mention fruit and shade trees, birds, animals, people and the water supply.

"I call it (Tonto National Forest) the crazy forest," says Mrs. Shoecraft. "The deadly sprays seem to stimulate the growth of the weed and kill the living." (All are chlorinated hydrocarbons, and do regulate growth.)

Dead plants really are not stimulated, of course. But Mrs. Shoecraft contends the herbicides bring about mutations. Vegetables flower out of season, for instance, and some flowering plants inexplicably are leafless.

The efforts of Mrs. Shoecraft have spawned a citizen's committee against the spraying. The committee has appealed to Rep. Sam Steiger (R, Ariz.) who visited the area last week to discuss the problem with several people."

Citizen sentiment concerning chemical spraying currently is being compiled by the Governor's Commission on Arizona Beauty. The commission also has consulted several University of Arizona scientists about the project.

"Nobody put down baselines before the treatment began," according to Dr. Paul S. Martin, UA ecologist. "The whole business is a matter of too much doing with too little know-how."

The same herbicides have been used in Vietnam (some have been used nowhere else on a widespread basis) to defoliate enemy areas. Martin, studying only slightly, says:

"At the rate they've been spraying, the Forest Service must have found a Viet Cong infiltration."

During the past several months he has been collecting in the Pinals abnormal plants apparently polluted by herbicides. The scientist also has taken UA students on field trips to the area.

Martin feels that the Forest Service has used a treatment whose total effects on the environment are unknown. "It may take us decades to find out," he criticizes.

Proof of pollution will come from tree-ring studies, Martin predicts. If trees are damaged, ring growth will not reflect annual moisture as it normally would.

Another concerned scientist is UA ornithologist Dr. Stephen M. Russell, who says that "the Forest Service has been lax in assuming that herbicides can't cause undue damage simply because they're not supposed to."

He referred to a report by Globe ornithologist Mrs. Robert R. McCusick on "abnormal occurrences during the last four years at Kellner Canyon." The following are excerpts from her study:

—"Between May 15 and 23, [illegible] 11 brown towhees were found in our yard's drinking water. They were unable to fly, had eyes discharging or swollen shut . . . unable to eat, drink or stand up. They were brought in and fed and watered, but all died within one to three days.

—"In 1967 a change in bird [illegible] was quite noticeable. Not only were the towhees absent, but there were no resident cardinals, jays, teasers, hardly any canyon wrens and no Bewick's wrens.

—"In April 1968 our hackberry trees which had shown top kill in 1967 shed all their tender leaves in one day and began to disintegrate with bark and branches falling off. Chicken, geese and goats left their grain stand and licked up all the leaves. The fowl became sick immediately.

—"Many young sparrows died during June and July, as many as two or three a day at our waterer."

Russell commented that "taken as a single instance, none of these would prove herbicide damage. But, viewed as a whole, they point to something pretty alarming."

Decayed, deformed and dead plants could be seen during a recent visit to the sprayed area:

A pine tree, verdant and normal on one side, had cones but no needles on the other. In a Globe garden, several rows of chiles were chile-green on one side. On the other, they were black and decomposed. And peaches rotted before they fruited fully.

Could natural diseases have caused all this? Dr. Stanley M. Alcorn, a UA plant pathologist, thinks not. Assays would have to be made to be sure, he says, but he believes it to be unlikely that any one virus would affect so many different plants in the same way.

To understate their mood, the Forest Service is somewhat inflamed by what one staff member termed "not too much rationale on the part of some people in Globe."

"Maybe folks are running a little scared," says A. B. Evanko of the Forest Service's division of range management at Washington.

He pointed out that "there's a tendency to throw a rope around pesticides. People make the same generalizations about pesticides, herbicides and DDT."

The federal government, nonetheless, last month restricted the use of 2, 4, 5-T after learning that it may be dangerous to animal life. Unless the Food and Drug Administration establishes a "safe legal tolerance in and on foods," the U.S. Department of Agriculture will ban the use of

16187

DOW271217

2, 4, 5-T on food crops effective Jan. 1, 1970.

In a prepared statement, Dr. ...ee A. DuBridge, presidential science adviser, pointed out that "the relationship of the effects of 2, 4, 5-T on laboratory animals to effects in man are not entirely clear at this time."

Not so, says the National Health Federation, a nonprofit foundation with headquarters in Monrovia, Calif. Since the federation began an investigation last July of the Globe spraying, it has "gathered enough material for a Senate investigation," says Vice President Howard C. Long.

Although the project in the Pinals was approved by the Federal Committee on Pesticide Control, the Forest Service has "delayed future herbicide treatment," admitting there was "some drift damage."

"We do feel that we've pretty thoroughly investigated all the knowledgable complaints," Evanko said last week.

— Photos Courtesy of UA Geochronology Dept.

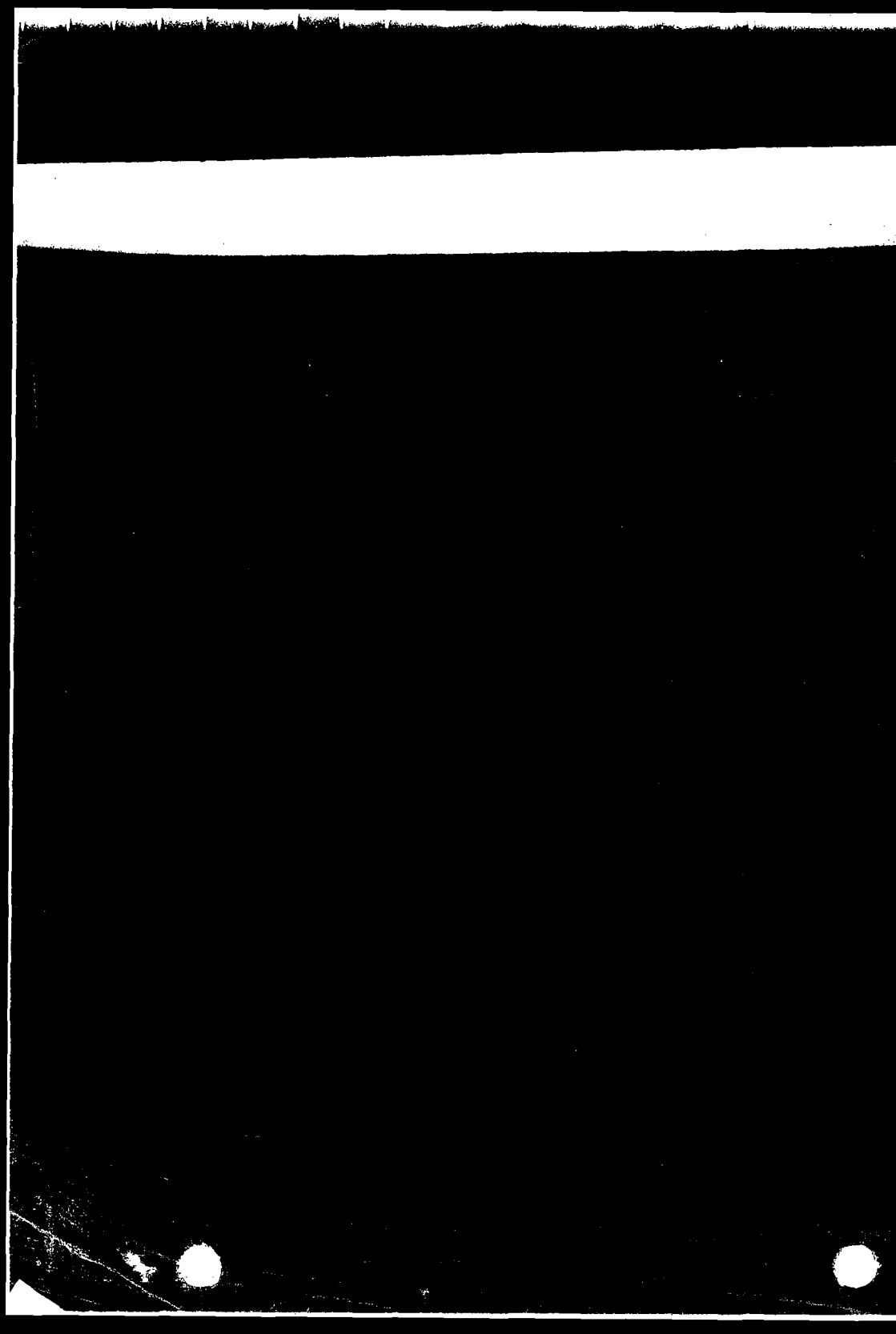
Withered Leaves

Chemical spray has burned this manzanita bush in the Pinal foothills, photographed by Dr. Paul S. Martin earlier this fall. The leaves should not then have turned brown, said the UA scientist.

Polluted Plant?

Mrs. Willard Shoecraft points out a spotted cotton plant in an area of the Pinal Mountains that has been sprayed with herbicides.

16188



16189

AGNEW V DOW
MOYER V DOW

2-4

AGNEW V DOW
MOYER V DOW

TESTIMONY OF A. T. TALCOTT

SHOECRAFT ET AL. VS. DOW

DOW 1469660

I am A. T. Talcott, administrator of Regulations and Labeling in the Quality Assurance Department of The Dow Chemical Company. I have a Bachelor of Science degree in chemistry from Alma College, Alma, Michigan, and have done graduate work at Michigan State University but have no advanced degree. I joined The Dow Chemical Company, Midland, Michigan, in 1957 as a chemist in their analytical laboratories and moved through a series of technical promotions in the analytical chemical field. In 1965 I moved to the Labeling/ Product Registration section of the Legal Department, becoming supervisor of that section in 1975. In 1976 I became manager of Product Safety Compliance in Dow's Quality Assurance Department, which included the functions of labeling, material safety data sheets, and product registration. In 1979 I assumed my present position where I am responsible for labeling policies on Dow products.

I am a member of the American Conference on Chemical Labeling, the Labeling Standard Revision Committee of the Chemical Manufacturers Association, a member or chairman of several committees dealing with regulations and labeling within the Chemical Specialties Manufacturers Association, a former member of the Labeling and Precautionary Information Committee of the Chemical Manufacturers Association, and the Labeling Committee of the National Paint and Coatings Association. I was also one of two industry representatives participating with EPA to put together their National Symposium on Pesticide Labeling in 1974.

I will testify on the history and procedures used in the development of labels by The Dow Chemical Company and to the registration requirements for labeling used by the Pesticide Registration Division of EPA which was part of the USDA in 1968 prior to the formation of EPA. I will also testify to

the relationship of the labeling policies used by Dow and independent standards as well as how the regulations promulgated by the Pesticide Registration Division which apply to the product KURON* weed and brush killer.

The procedures used at Dow allow us to get the input from our experts such as health professionals, technical specialists, and legal and regulatory counsel in areas pertinent to such a product.

The labeling used on KURON weed and brush killer in the time period 1967 through 1969 was in great part specified in government regulations for pesticides which were called economic poisons under the statute in effect at that time. These regulations required the product to bear a label containing the name of the product, the name and address of the manufacturer or registrant, the net contents, an ingredient statement which was prescribed in a special manner, a warning or caution statement as appropriate to regulations prescribing same, and directions for use--which if complied with, would be adequate for the protection of the public. These elements of a label were further prescribed in a certain manner by the regulations and interpretations promulgated by the Pesticide Registration Division and required a preclearance or acceptance by that Division before they could be used on the package of the pesticide. This labeling was also submitted to the state of Arizona and other states for review as part of the state registration of the product required prior to sale.

The product name KURON, the Company name, and certain other elements such as the registration number required to appear on the label are for the most part self-explanatory.

For precautionary labeling purposes, pesticides were placed in one of four different categories based on certain toxicity data that would be derived from animal tests provided to the Pesticide Registration Division by the manufacturer. These were identified as categories one, two, three, and four with category one being materials that were highly toxic and requiring the word "poison" and the skull and crossbones to be used on

*Trademark of The Dow Chemical Company

the label. The other categories moved in decreasing toxicity to category four which was for products determined not likely to cause injury under any reasonably foreseeable conditions of use. Each of the second and third categories were ten-fold decreases in toxicity from the lower-numbered category.

During this time precautionary statements used on pesticides were set forth in a document called "Interpretation 18, Revision 2" published in the Federal Register of March 9, 1962 as an interpretation of 7 CFR, Part 362. There were two ways in which this interpretation was used. One related to signal words and statements of hazard that were used on pesticides containing specifically-listed active ingredients and the other part set forth general procedures based on the toxicity categories previously mentioned which was applicable to KURON weed and brush killer.

Applying the principles stated in Interpretation 18, one comes up with the precautionary labeling which was accepted by the government as adequate to protect the public and used by Dow on its product KURON weed and brush killer.

Quoting from section 362.116(b)(2)(III), "Labels of products in the third category should carry the word "caution" in statements indicating the means of avoiding the principal hazards of use. Use of the skull and cross bones, the word "poison," and antidote statements are not necessary for these products."

Another part of the label which may need more explanation is the ingredient statement. Ingredient statements are required to be made on all pesticide products and must be stated in certain ways. The constituents of a product must be characterized as either active or inert ingredients. The active ingredients must be described by their chemical name.

The two ways in which ingredient statements can be listed were specified in section 362.7 of the regulations. These were to list each active ingredient and its percentage and the total of the inert ingredients or to list the total of the active ingredients and each individual active ingredient in descending order of percentage without the actual percentage of each ingredient plus the

DOW 1469662

total inert ingredients. Equal prominence is required to be given to all of the ingredient statements and these ingredient statements were required to be placed on the front panel of pesticide products. In other words, only those materials that were considered active ingredients from a pesticidal standpoint were allowed to be listed in the ingredient statement even though the product contained other materials that comprised the total.

It has been suggested that TCDD should have been listed on the label of this product. Such a listing would not have been acceptable from the government's standpoint since this is not an active pesticidal constituent; additionally, the initials TCDD do not represent a common chemical name acceptable to the federal government so such listing would again be prohibited. The other reason for not listing TCDD as an ingredient is that the limits of analytical detection at the time of production of the KURON in question were such that TCDD was not found in this product.

There are other sections of the label that are, of course, pertinent to the allegations made concerning the product KURON weed and brush killer. Among these are the instructions under a section titled "Warning" that "applications by airplane, ground rigs, and hand dispensers should be carried out only when there is no hazard from drift." Other instructions which are meaningful to a user of such products are the instructions that "coarse sprays are less likely to drift" as well as the instructions on application rates and times for specific applications of the product.

The procedures followed for labeling pesticidal products are an application of the principles that are included for labeling of chemicals set forth in the American National Standard for Precautionary Labeling ANSI Z129.1. This is a consensus standard which was not published until 1976, however, does have the benefit of review of such organizations as the American Society of Agricultural Engineers, the National Safety Council, the American Society of Safety Engineers, the Society of Toxicology, AFL-CIO, the American Conference of Governmental

Industrial Hygienists, and numerous other government, industry, and independent organizations with an interest in conveying precautionary information.

These principles prescribe a signal word, statement of hazard, precautionary measures, and as appropriate, instructions in case of contact or exposure as procedures for providing precautionary labeling. The labeling used by Dow on KURON weed and brush killer demonstrates these principles.

The procedures used within Dow to develop labeling in 1967 involved the initial labeling being developed from a use standpoint by a technical specialist from our Agricultural Products Department along with the individual responsible for submitting registration applications to the federal government, and precautionary information coming from a labeling specialist like myself. This draft copy then would be typed and sent for review by the numerous specialists within the Company with input on the health and safety aspects coming from toxicologists, industrial hygienists, medical doctors, individuals from our quality function in connection with our manufacturing people providing input on the composition of the product, trademark and patent comments coming from our attorneys, and the original three persons reviewing these inputs and those of our marketing personnel. Once these inputs had been composited, the labeling was sent to the federal government for their acceptance or suggestions for change if they did not accept it.

Upon return of a copy of the accepted labeling, the labeling specialist would initiate the actions responsible for getting the labels or the preprinted container copy to our production people for application on containers of the final product.

It is important to remember when developing or reviewing labeling for a product that the labeling must be based on the product as a whole; consequently, the labeling must be viewed in its entirety, not just in parts--since it is the whole product that is used, not a part.

I have compared the labeling used by other pesticide producers of similar products and find the labeling used, which also was required to be submitted to the Pesticide Registration Division before being used, to be essentially the same where applicable as that used on the product KURON weed and brush killer.

Based on my education, training, and experience, it is my opinion that the label used on the product KURON weed and brush killer in the time period 1967 through 1969 met or exceeded all regulations and standards of care pertaining to such products; and further the directions and precautions if followed were adequate to prevent accident or injury with the handling and application of the product.

DOW 1469965

Ex. 118

INFECTION AND IMMUNITY, Dec. 1975, p. 1319–1324
Copyright © 1975 American Society for Microbiology

Vol. 12, No. 6
Printed in U.S.A.

Increased Susceptibility to Bacterial Infection as a Sequela of Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

J. E. THIGPEN,* R. E. FAITH, E. E. McCONNELL, AND J. A. MOORE

National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Received for publication 12 August 1975

The effects of subclinical levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the response of mice to infection with either *Salmonella bern* or *Herpesvirus suis*, also known as pseudorabies virus, are reported. TCDD is a contaminant of certain commercially useful chemicals, such as chlorinated phenols or herbicides. It has been shown to cause thymic atrophy and to suppress cell-mediated immunity in laboratory animals. Sublethal levels of TCDD were used: 0.5, 1, 5, 10, or 20 μg/kg, given through a gastric tube once weekly for 4 weeks. A significant decrease in weight gain compared with control mice occurred at the 20-μg dosage. Dose schedules of 1 μg or more, followed by salmonella infection, resulted in significant increases in mortality and decreases in the time from infection to death. However, TCDD had no significant effect on mortality in the pseudorabies-infected mice. The most important finding in this study is that extremely low levels of TCDD, which do not produce clinical or pathological change, still have the capacity to affect host defense.

MN0697799

DOW 13691?1

Various environmental chemicals have been shown to affect immune responses or host resistance to infectious agents (Friend and Trainer [6], Gainer [8], Gainer and Pry [10], Hemphill et al. [11], Jones et al. [14], Koller and Thigpen [19], Koller and Kovacic [18], Koller [17], Verschuuren et al. [27], Vos and van Genderen [30], Vos et al. [29], Vos and Moore [28], Wassermann et al. [32]). These reports generally involve observed defects in antibody response (14, 17, 18, 19, 27, 30, 32) or on cell mediated immunity (28, 29, 30), although several (6, 8, 10, 11) also describe suppressed host resistance to infection.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) can occur as a highly toxic contaminant in the production of some chlorinated phenols or chemicals synthesized from chlorinated phenols such as the herbicide 2,4,5-trichlorophenoxyacetic acid and thus could be unknownly distributed in the environment. TCDD as an environmental contaminant can present health problems, both in man and other animals. It is associated with chloracne in man (16) and chick edema disease in chickens (5). More recently TCDD and 2,4,5-trichlorophenol were implicated as the probable cause of toxicity in a group of horses in eastern Missouri. In addition to the horses, insects, birds, and rodents were poisoned, and there were several cases of human illness attributed to TCDD in this incident (3).

TCDD has been observed to cause profound thymic atrophy and suppress cell-mediated immunity in rats, mice, and guina pigs (28, 29). It is not known whether TCDD-exposed animals are more susceptible to infectious agents. These studies were designed to determine the effects of subclinical levels of TCDD on the response of mice subsequently infected with either *Salmonella bern* or pseudorabies virus (PRV). The criteria used to measure these effects were clinical appearance, weight gains, mortality rates, time from infection to death, and pathological examination.

MATERIALS AND METHODS

Animal. Four-week-old male C57BL/6Jfh (J67) specific-pathogen-free mice were purchased from Jackson Laboratories, Bar Harbor, Me. The mice were caged individually under filter tops (Envirogard, Lab Products, Garfield, N.J.) following a rigid sanitary regimen and given food (Sterilizable Lab-Blox, Allied Mills, Inc., Chicago, Ill.) and water ad libitum.

TCDD. TCDD, greater than 99% purity (lot 351-144-II), was a gift from Dow Chemical Company, Midland, Mich. It was dissolved in reagent-grade acetone and subsequently diluted with at least 6 parts of corn oil. The exact volume of corn oil was adjusted to provide each mouse with a dose volume of approximately 0.2 ml.

Infectious agents. The bacterium used was a *S. bern* isolate from a naturally infected opossum. This organism was grown overnight at 35 to 37 C on Trypticase soy agar containing 5% sheep blood. Bacterial dilutions were prepared in sterile physiological saline, and the number of bacteria was quanti-

16136

tated turbidimetrically with a Coleman model 6/20 spectrophotometer at 540 μm. In addition, the number of total viable bacteria in all dilutions used for animal inoculation was confirmed by conventional total plate count procedures.

The viral agent used was *Herpesvirus suis* (PRV) (9). PRV was produced in 75-cm² Falcon flasks containing RK-13 monolayers. After a 4-plus cytopathogenic effect was observed, the medium from each flask was centrifuged at 800 × *g* to remove large cellular debris. The supernatants from each flask were pooled, thoroughly mixed, aliquoted in 2-ml vials, and stored at −79 C until used. At the time of inoculation the original suspension contained approximately 5.0×10^7 tissue culture infective doses/ml. The pooled viral suspension was culture negative for mycoplasma and bacteria.

Preliminary study. Lethal dose response curves were established for *S. bern* and PRV to determine challenge doses (0.2 ml intraperitoneally [i.p.]) that would produce 20% mortality in 8-week-old normal mice. All mice were observed for 14 days. Dead mice were examined by both microbiological and histopathological procedures to verify infection.

Experimental design. Two different experiments were performed. In each experiment 4-week-old mice were randomly divided into four groups. Each mouse was weighed and dosed by gavage once each week for 4 consecutive weeks. Mice were dosed with either TCDD in acetone-corn oil or with acetone-corn oil.

In the first experiment, each mouse received either 0, 5, 10, or 20 μg of TCDD/kg per week. Two days after the last dose of TCDD, when the mice were 8 weeks old, each group was randomly divided into three subgroups. Mice in one subgroup were injected i.p. with 10^6 viable *S. bern* organisms ($LD_{20/14}$ dose), the second subgroup received 10^4 TCID of PRV ($LD_{20/14}$ dose), and the third subgroup (control) was injected with saline only. ($LD_{20/14}$ is the number of bacterial or viral agents required to kill approximately 20% of the mice inoculated within a 14-day observation period.)

The second experiment was conducted in a manner identical to the first except that each mouse received either 0, 0.5, 1, or 5 μg of TCDD/kg per week and the PRV subgroup was eliminated.

Both experiments were performed as blind studies, and the order of inoculation was randomized. Neither the bacterium nor the virus appeared to lose or gain potency during the time of administration. The level of mortality seen in the final 25% of the animals inoculated was not statistically different from that observed in the first 25% or the middle 50%. Therefore, the order of inoculation was virtually irrelevant to subsequent survival.

After bacterial and viral challenge, the animals were observed at least twice daily for 14 days. Body weights were recorded at death or at termination of the study. Necropsies were performed on at least three mice from each subgroup, and selected tissues were obtained for histopathological and microbiological examination. Mice from subgroups with less than three deaths were sacrificed and necropsied on day 14.

The tissues selected for histopathological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and routinely stained with hematoxylin and eosin.

The criteria used to compare the effects of sublethal levels of TCDD in mice subsequently infected with *S. bern* or PRV were clinical appearance, weight gains, mortality rates, time from infection to death, and pathological examination.

Statistical analyses. For the mortality data, dose response trends were analyzed by the Bartholomew test (2), and pair-wise comparisons were made by the Fisher exact test for 2 × 2 tables (12). For the other variables, the significance of dose-response trends was determined by the Jonckheere test (13), and treatment-control differences were evaluated by the two-sided Mann-Whitney *μ* tests (12).

RESULTS

Effects of TCDD on weight gain, mortality rate, and time from infection to death. There was essentially no difference in mean group body weights prior to TCDD dosing. By week 4, the high-dose group (20 μg of TCDD per kg) gained significantly ($P < 0.01$) less weight than did the controls. The 10 μg of TCDD per kg dose group also gained less weight; all other TCDD group body weights were comparable to the group which received acetone-corn oil (Table 1). At a given level of TCDD there were no significant differences in the week 4 weights among the mice assigned to the bacteria, virus, or control groups. There was little association between body weight at the time of infection and subsequent survival.

Mortality rates in the bacteria and virus control groups that had not received TCDD were as anticipated based on the $LD_{20/14}$ preliminary study (Table 2). The groups of mice exposed to 1, 5, 10, or 20 μg of TCDD per kg and given *S. bern* showed a significant ($P < 0.05$ to 0.01) increase in mortality rates. The mortality rate

TABLE 1. *Effect of TCDD on weight gain of mice over a 3-week exposure period*

Weekly TCDD dose[a] (μg/kg of body wt)	No. of mice	Initial wt (g)[b]	Wt gain at challenge (g)[b]
Expt I			
20	80	18.68 ± 0.21	3.06 ± 0.20[c]
10	58	18.78 ± 0.22	5.21 ± 0.17
5	80	18.41 ± 0.22	5.77 ± 0.19
0	60	18.85 ± 0.19	5.68 ± 0.17
Expt II			
5	35	20.91 ± 0.21	4.56 ± 0.17
1	33	21.08 ± 0.22	4.50 ± 0.17
0.5	35	20.67 ± 0.24	4.06 ± 0.19
0	35	21.13 ± 0.24	4.29 ± 0.14

[a] TCDD in acetone-corn oil carrier via gastric intubation on an individual body weight basis.
[b] Mean ± standard error.
[c] $P < 0.01$.

DOW 1 369172

16137

in the 0.5 μg of TCDD per kg dose group was identical to the control group; therefore, the "no effect" level based on the parameters of this study would be somewhere between 0.5 and 1.0 μg of TCDD per kg (Table 2; Fig. 1).

The mortality rates in the groups that received virus were not seen to differ significantly with variations in the dose of TCDD.

There was a significant ($P < 0.01$) dose-related decrease in time from infection to death in the *S. bern*-challenged mice (Table 3; Fig. 1).

TABLE 2. *Comparison of 14-day mortality rates of mice exposed to sublethal levels of TCDD and challenged with either S. bern or PRV*

Weekly TCDD dose[a] (μg/kg of body wt)	Mortality rates (%)		
	Bacterial challenge[b]	Viral challenge[c]	Control[d]
Expt I			
20	95 (19/20)[e,f]	10 (2/20)	0 (0/20)
10	65 (13/20)[g]	0 (0/20)	0 (0/15)
5	65 (13/20)[g]	15 (3/20)	0 (0/20)
0	25 (5/20)	15 (3/20)	0 (0/20)
Expt II			
5	70 (14/20)[g]	ND[h]	0 (0/15)
1	65 (13/20)[g]	ND	8 (1/13)
0.5	25 (5/20)	ND	0 (0/15)
0	25 (5/20)	ND	0 (0/15)

[a] TCDD in acetone-corn oil carrier via gastric intubation on an individual body weight basis.
[b] Bacterial challenge (LD_{80-14}) of *S. bern* via i.p. injection on day 2 after the 4th weekly dose of TCDD.
[c] Viral challenge (LD_{80-14}) of PRV via i.p. injection on day 2 after the 4th weekly dose of TCDD.
[d] Control animals were injected i.p. with sterile saline on day 2 after the 4th weekly dose of TCDD.
[e] The numbers in parentheses indicate the number of mice dying per the total number of mice in the group.
[f] $P < 0.01$.
[g] $P < 0.05$.
[h] ND, Not done.



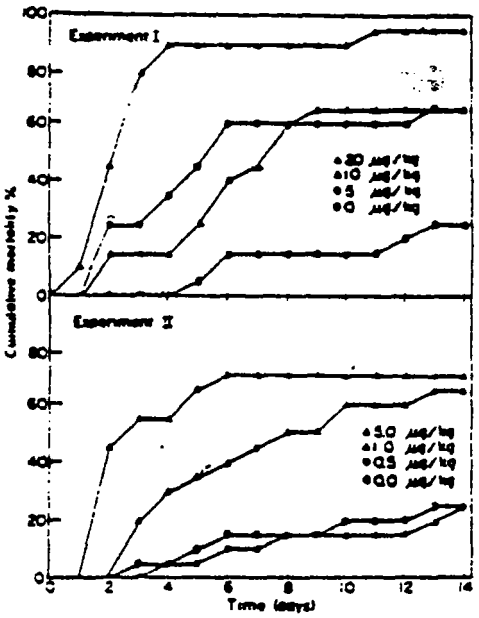


FIG. 1. *Cumulative mortality rates and time from infection to death in mice exposed to sublethal levels of TCDD and subsequently infected with S. bern.*

TABLE 3. *Average time from infection to death (days)*

Weekly TCDD dose[a] (μg/kg of body wt)	Bacterial challenge[b]		Viral challenge[c]	
	No. of nonsurvivors[d]	Time to death[e]	No. of nonsurvivors[d]	Time to death[e]
Expt I				
20	19	2.95 ± 0.49[f]	2	9.00 ± 5.00
10	13	5.69 ± 0.67	0	
5	13	4.54 ± 0.84[g]	3	6.33 ± 1.21
0	5	8.40 ± 1.69	3	4.67 ± 0.33
Expt II				
5.0	14	2.86 ± 0.38[f]		ND[h]
1.0	13	6.08 ± 0.92		ND
0.5	5	9.00 ± 1.95		ND
0	5	7.40 ± 1.81		ND

[a] TCDD in acetone-corn oil carrier via gastric intubation on an individual body weight basis.
[b] Bacterial challenge (LD_{80-14}) of *S. bern* via i.p. injection on day 2 after the 4th weekly dose of TCDD.
[c] Viral challenge (LD_{80-14}) of PRV via i.p. injection on day 2 after the 4th weekly dose of TCDD.
[d] The number of nonsurvivors was the number of animals dying within 14 days after bacterial or viral challenge.
[e] ± = standard error.
[f] $P < 0.01$.
[g] $P < 0.05$.
[h] ND, Not done.

DOW 1 369178

16198

Pathological observations. Postmortem examination of mice treated with TCDD only revealed dose-dependent lesions in the liver and thymus similar to those described by Vos et al. (31). The thymus from mice in the 20 μg/kg group was smaller than normal. Microscopic examination revealed cortical atrophy due to loss of small lymphocytes. Thymic atrophy was much less apparent and often equivocal in the 10 μg/kg group and was not observed at the lower doses. Interestingly, no lesions were found in the spleen.

The livers from the 20 μg/kg group were lighter in color than normal and were mottled. This was less evident in the 10 μg/kg animals, and no macroscopic lesions were visible in the livers from mice in the 5, 1, or 0.5 μg of TCDD per kg dose groups. Histopathological examination of livers from two high-dose groups revealed a nodular surface with variable degrees of hepatocellular cytomegaly and occasional foci of single cell necrosis. A few inflammatory cells (predominantly mononuclear) were found in the areas of necrosis. In addition, there were often intracellular accumulations of lipid in hepatocytes, especially in midzonal areas. Lesions were not observed at lower doses or in other organs.

The lesions described were still apparent in the mice from the two highest dose groups inoculated with virus or bacteria (both survivors and nonsurvivors). In addition, mice in the bacterial groups that died showed macro- and microscopic lesions typical of salmonellosis (1, 26). There was widespread infiltration of polymorphonuclear leukocytes, particularly in visceral organs. Focal necrotic hepatitis resembling microinfarction was attributed to adjacent areas of vasculitis and thrombosis. Lymphoid necrosis, thrombotic vasculitis, and suppuration were also observed in the spleen. Necrotic enteritis, purulent peritonitis, and occasionally suppurative meningitis were also noted.

In the virus portion of the experiment, lesions attributable to PRV were not observed in any of the mice examined. PRV in laboratory rodents is known to produce few lesions at the light microscopic level (21).

DISCUSSION

The data presented in this study clearly established that mice exposed to four weekly doses of 1, 5, 10, or 20 μg of TCDD per kg and subsequently infected with an LD_{50} dose of *S. bern* have a significantly higher rate of mortality with a shortened incubation period. Furthermore, the mice exposed to 1 or 5 μg of TCDD per kg appeared clinically normal, had a normal rate of weight gain, and showed no histopathological evidence of toxicity. This demonstrates the most important aspect of this study, i.e., that extremely low levels of TCDD which do not produce clinical or pathological change still have the capacity to affect host defense. These findings are significant in that humans may be exposed to environmental contaminants such as TCDD in minute quantities and therefore may be rendered more susceptible to certain types of infectious agents.

The fact that TCDD did not increase mortality in mice challenged with PRV suggests a difference in the pathogenesis of these two agents. The reason(s) for this difference is not understood.

There are several host defense factors which could account for the increased susceptibility to a facultative intracellular organism such as *S. bern*. These include: (i) a defect in cell-mediated immunity either through loss of mediating cells or by inhibition of cell function; (ii) a defect on the humoral system; (iii) a defect in one or more of the steps of phagocytosis; and (iv) a reduction or loss of phagocytic cells.

Cell-mediated immune reactions as evidenced by delayed hypersensitivity have been shown to play an important role in immunity and recovery from infections with salmonella and other facultative intracellular organisms (4, 15, 20, 22, 23, 25). Delayed hypersensitivity is thought to be mediated through production of lymphokines by specifically activated T cells in response to bacterial antigens (4, 20, 22, 23, 25). TCDD has been shown to suppress delayed hypersensitivity reactions and responses of T cells to activation by the mitogens concanavalin A and phytohemagglutinin (28, 29). These studies did not investigate the influence of TCDD on lymphokine production, but it would be surprising if TCDD did not also suppress their production since it has such a profound effect on other T-cell function.

Humoral factors do not appear to be dominant in impaired host resistance to salmonella infection in mice since full protection from challenge appears to depend upon the immune response that leads to delayed-type hypersensitivity, rather than to that which results in circulating antibody and arthus sensitivity (4). The effect of TCDD on humoral antibody production in mice is not known. Recent studies by one of the authors (R. E. Faith, unpublished data) indicates that TCDD has no effect on humoral antibody production to bovine gamma globulin in rats. In contrast, the guinea pig humoral response to tetanus toxoid was observed to be impaired (Vos et al. [29]).

DOW 1 369174

16199

Alterations in the functions of phagocytic cells, either polymorphonuclear or mononuclear, may be involved. This could result from necrosis of these cells and/or a decrease in production or blockage of functional abilities of these cells. A recent study by Weissberg and Zinkl (33) indicated no significant change in peripheral blood monocyte numbers in rats treated with TCDD. In addition, these authors report an elevation in peripheral blood neutrophil counts after TCDD treatment. This would suggest that the defect in host resistance is not the result of destruction of circulating phagocytes. However, this does not rule out the possibility of death or destruction of tissue-fixed macrophages. The number of circulating monocytes is small in relation to those that are tissue fixed, and the study of circulating monocytes does not necessarily reflect the state of production or function of mononuclear phagocytes. In this light, it is of interest to note the results of two studies (7, 24) which show that treatment with agents that interfere with monocyte production also suppress host resistance to bacterial infection.

Studies designed to investigate the possibilities of alteration in functional abilities of phagocytic cells and production of monocytes in relation to dioxin exposure are being undertaken in this laboratory. In addition, possible defect(s) in cooperation between T cells and phagocytic cells are being investigated. The results of these studies should lend insight into the mechanism(s) of suppressed host resistance to infection induced by exposure to TCDD.

ACKNOWLEDGMENTS

We wish to thank Mary Clements, Martha W. Harris, and Janet D. Allen for excellent technical assistance and J. K. Haseman for statistical analyses.

LITERATURE CITED

1. Bakken, K., and T. M. Vogelsang. 1974. The pathogenesis of *Salmonella typhimurium* infection in mice. Acta Pathol. Microbiol. Scand. 27:41–60.
2. Barthalomew, D. J. 1959. A test of homogeneity for ordered alternatives. Biometrika 46:36–48.
3. Carter, C. D., R. D. Kimbrough, J. A. Liddle, R. E. Cline, M. M. Zack, Jr., W. F. Barthel, R. E. Koshier, and P. E. Phillips. 1975. Tetrachlorodibenzodioxin: an accidental poisoning episode in horse arenas. Science 188:738–740.
4. Collins, F. M., and G. B. Mackaness. 1968. Delayed hypersensitivity and arthus reactivity in relation to host resistance in Salmonella-infected mice. J. Immunol. 101:830–845.
5. Firestone, D. 1973. Etiology of chick edema disease. Environ. Health Perspect. 5:59.
6. Friend, M., and D. O. Trainer. 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 170:1314–1316.
7. Gadsberg, O. V., J. M. Rhodes, and S. O. Larson. 1975. The effect of various immunosuppressive agents on mouse peritoneal macrophages and on the in vitro phagocytosis of Escherichia coli 04:K3:H5 and degradation of ^{125}I-labeled HSA-antibody complexes by these cells. Immunology 28:59–70.
8. Gainer, J. H. 1972. Increased mortality in encephalomyocarditis virus-infected mice consuming cobalt sulfate: tissue concentrations of cobalt. Am. J. Vet. Res. 33:2067–2072.
9. Gainer, J. H., J. Long, Jr., F. R. Hill, and W. L. Capps. 1971. Inactivation of the pseudorabies virus by dithiothreitol. Virology 46:91–100.
10. Gainer, J. H., and T. W. Pry. 1972. Effects of arsenicals on viral infections in mice. Am. J. Vet. Res. 33:2299–2307.
11. Hemphill, F. E., M. L. Kaeberle, and W. B. Buck. 1971. Lead suppression of mouse resistance to *Salmonella typhimurium*. Science 172:1031–1032.
12. Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, New York.
13. Jonckheere, A. R. 1954. A distribution-free k-sample test against ordered alternatives. Biometrika 41:133.
14. Jones, R. H., R. L. Williams, and A. M. Jones. 1971. Effects of heavy metal on the immune response. Preliminary findings for cadmium in rats. Proc. Soc. Exp. Biol. Med. 137:1231–1236.
15. Kaster, F. S. 1975. Infection, anergy and cell-mediated immunity. N. Engl. J. Med. 292:629–634.
16. Kimmig, J., and K. H. Schulz. 1957. Berufliche Akne (neg. chloracne) durch chlorierte aromatische zyklische ather. Dermatologica 115:540.
17. Keller, L. D. 1973. Immunosuppression produced by lead, cadmium and mercury. Am. J. Vet. Res. 34:1457–1464.
18. Keller, L. D., and S. Kovacic. 1974. Decreased antibody formation in mice exposed to lead. Nature (London) 250:148–150.
19. Keller, L. D., and J. E. Thigpen. 1973. Reduction of antibody to pseudorabies virus in polychlorinated biphenyl-exposed rabbits. Am. J. Vet. Res. 34:1605–1606.
20. Lane, F. C., and E. R. Unanue. 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. 135:1104–1112.
21. McFerran, J. B., and C. Dow. 1970. Experimental Aujeszky's disease (pseudorabies) in rats. Br. Vet. J. 126:173–179.
22. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. 129:973–992.
23. Mauel, J. 1974. Cell-mediated immune mechanisms in bacterial and protozoal infections. Prog. Immunol. II 4:109–116.
24. North, R. J. 1970. Suppression of cell-mediated immunity to infection by an antimitotic drug. J. Exp. Med. 132:535–545.
25. Ruskin, J., J. McIntosh and J. S. Remington. 1969. Studies on the mechanisms of resistance to phylogenetically diverse intracellular organisms. J. Immunol. 103:252–259.
26. Smith, A. W. 1973. The mouse. Aeromed. Rev. 19:11–15.
27. Verschuuren, H. G., E. J. Ruitenberg, F. Postema, P. W. Halleman, and G. J. van Esch. 1970. Influence of triphenyltin acetate on lymphatic tissue and immune responses in guinea pigs. Toxicol. Appl. Pharmacol. 16:400–410.
28. Vos, J. G., and J. A. Moore. 1974. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy 47:777–794.
29. Vos, J. G., J. A. Moore, and J. G. Zinkl. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune

DOW 1 369175

16200

system of laboratory animals. Environ. Health Perspect 5:149–162.
30. Vos, J. G., and H. van Genderen. 1973. Toxicological aspects of immunosuppression. p. 527–548. *In* Pesticides and the environment: a continuing controversy. Symposia Specialists, Miami.
31. Vos, J. G., J. A. Moore, and J. G. Zinkl. 1974. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57B1/6 mice. Toxicol. Appl. Pharmacol. 29:229–241.
32. Wassermann, M., D. Wassermann, Z. Gershon, and L. Zellermayer. 1969. Effects of organochlorine insecticides on body defense systems. Ann. N.Y. Acad. Sci. 160:383–401.
33. Weeneberg, J. S., and J. G. Zinkl. 1973. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon hemostasis and hematologic function in the rat. Environ. Health Perspect 5:119–123.

DOW 1 369176

16201

Shoecraft et al v. The Dow Chemical Company

LABORATORY TEST RESULTS*

OF

KAUKO SATAMA

* * *

BLOOD COUNT		
HGB	15.2	(3)
HCT	45.0	(3)
WBC	23 or 04.1	(3)
RBC	4.92	(3)
MCV	091	(3)
MCH	30.6	(3)
MCHC	33.7	(3)
PTT		
Control		
SR (Sed Rate)	7	(3)
PRO. TIME		
Control		
PL. CT.	255,000	
DIFF.		
SEGS	60	(3)
Lymphs	35	(3)
Mono	2	(3)
EOS	2	(3)
Baso.	1	(3)
Band		

TESTS ORDERED BY PLAINTIFFS' COUNSEL:	
IgD	
FSH/RIA	
IgE	
PLASMA ESTRADIOL	
TSH	

URINALYSIS:	
Color	yellow
Appearance	hazy
Specific Gravity	1.013
pH	5.0
Protein	--
Glucose	--
Ketones	--
Occult Blood	--
Bilirubin	--
Casts/LPF	--
WBC/HPF	1-2
RBC/HPF	--
Epithelial Cells	occasional
Bacteria	trace
Crystals	--
Mucus	1+

BLOOD CHEMISTRIES		
Albumin	4.0	(3)
Globulin	2.3	(3)
A/G Ratio	1.7	(3)
Total Proteins	6.3	(3)
Alkaline Phosphatase	76	(3)
Bilirubin	0.5	(3)
LDH	254	(3)
SGOT	41	(3)
SGPT	43	(3)
GGT (GGTP)	23	(3)
CO_2	32	(3)
Sodium (NA)	140	(3)
Potassium (K)	4.4	(3)
Chloride (CL)	101	(3)
Calcium	9.1	(3)
Triglycerides	75	(3)
Creatinine	1.1	(3)
Blood Urea Nitrogen	23	(3)
Uric Acid	4.6	(3)
T3	27.1	(3)
T4	7.6	(3)
Free Thyroxine Index	2.06	(3)
Cholesterol	201	(3)
HDL Cholesterol	57	(3)
Total Cholesterol/ HDL	3.53	(3)
LDL Cholesterol	129	(3)
LDL/HDL		
VDRL	NR	
VLDL		

OTHER/COMMENTS:	
GLUCOSE	95
PHOSPHORUS	3.5
DIRECT BILIRUBIN	0.1
INDIRECT BILIRUBIN	0.4

* (1) Gila County General Hospital; (2) Diagnostic Laboratory; (3) National Health Laboratories; (4) Metpath, and (5) Other

BIO-MEDICAL RESEARCH
2030 Dow Center
Midland

DOW

DOW 1 257785

Off

Let me know if I've
made any major errors

Cook

Their RR estimates are underestimates since matching was apparently not taken into account in the RR estimate. Jerry with potential bias and presumptions you have discussed. Minor point compared

RALPH R. COOK, M.D., M.P.H.
(517) 636-1383

MN07091

REVIEW OF TRANSLATED ARTICLE; HARDELL, L. AND SANDSTROM, A. CASE-CONTROL STUDY. MALIGNANT MESENCHYME SOFT TISSUE TUMOUR AND EXPOSE TO PHENOXY ACIDS OR CHLOROPHENOLS. LAKARTIDNINGEN, VOL. 75, NO. 40, 1978. (sic)

This was a confusing article to review: the Swedish to English translation was loose; references were not included; the data was incomplete; and, there were multiple inconsistencies or contradictions incorporated into the body of the article.

In this paper the authors addressed some interesting epidemiologic problems: confounding, recall, selection, consistency, and causation. I am not convinced they solved any of them satisfactorily. In addition, the methodology they used, case-control while rapid and flexible is prone to greater bias than the more rigorous cohort approach. Although phenoxy acids or chlorophenols may be associated with malignant mesenchyme soft tissue tumors, this study does not prove causation. It simply points out the need for "continued investigations."

Cases were drawn from patients hospitalized at the Oncologic Clinic in Umea, Sweden, 1970-1977. Males with any malignant mesenchymal soft tissue tumor (fibrosarcoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, angiosarcoma, osteosarcoma, etc.) were included. Each malignancy has a different age distribution of occurrence. Unless cases and controls were matched within fairly tight age ranges, the results were potentially confounded.

The selection factors leading to hospitalization at this clinic were not discussed. If foresters or pulp workers were more apt to be seen at this center, the implications are obvious, i.e., the relative risks were inflated.

MN0709Z1 DOW 1257786

Their matching criteria were not adequately presented. As a rule of thumb, the reader should be able to replicate the investigation using the article as a guide. I would be hard pressed to do that in this case. For example, I don't know what they mean by "otherwise matching" on page 2. In addition, they apparently used different matching criteria for live and dead cases. They also chose 8 controls for each live case and 10 controls for each dead case, and then limited their analysis to a 4-to-1 match. Why? They excluded suicides from the control group. Why? They made a point of selecting controls who had worked up to 2 years prior to death. Why?

One matches to control confounding. Having matched in the design, one has to match in the analysis. They did not.

The statistical methods they quote are Miettinen's. His methods do not have complete acceptance by biostatisticians and epidemiologists. What do they mean on page 3, under Statistical Methods, by "effect of s k"?

What percent participation did they have? They suggest 100% among cases and 99.23% among controls. This degree of success is difficult to believe. Unless other investigations by the same authors demonstrated a high degree of epidemiologic sophistication (which this analysis does not), I would suspect a fair amount of selection bias has been incorporated into this study.

Since they had such poor environmental measurements, they were really testing the risk of being a wood product worker. The chemicals they arbitrarily choose as labels were merely surrogates for the workers' total exposures. One has to ask, what were the workers other chemical exposures,

DOW 1257787

physical exposures and selection into or out of the jobs in question? Was there a dose response in any sense?

For me, the study did a better job of presenting questions than answers. What is a Swedish municipality? What are the problems associated with the register of population (RTB)? How did they use the register of dead persons? What was their definition of exposed? Was it greater than "totally 1 day's length"? Why were "all persons in the region with the studies tumor diseases ... not investigated at the clinic (sic)"? In 60% of the cases and controls, exposure histories were obtained from a member of the "bereaved family"; can your spouse list your exposures of twenty years ago?

R. R. Cook, M.D.
14 November 1978

DOW 1257783

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

November 10, 1976

W. B. Crummett - 574
B. B. Holder - 607
R. L. Johnson - 2040
M. J. Mintz - 1710
R. D. Olson - 607
E. S. Parsey - 834
B. A. Schwetz - 1803
W. W. Muelder - 9001
W. B. Seward - 9001

MN 117354

CHLORINATED DIOXIN ISSUE MANAGEMENT TEAM MEETING, November 17, 1976, 1:15 P.M., Planning Conference Room, Agricultural Products Department, 9008 Building.

AGENDA

M. J. Mintz

Progress in the process for minimizing the TCDD in 2,4,5-T and silvex products.

E. S. Parsey

Appropriate timing for announcing new TCDD product spec.

B. A. Schwetz

Review of NIEHS meeting.

B. B. Holder

Comments gathered from conversations with those concerned with TCDD problems - Italy, etc.

R. L. Johnson

Status of studies to determine how the chlorinated phenolic Dowicides are used and their fate in the environment.

LIFE IS FRAGILE · HANDLE WITH CARE ·

DOW 1515900

R. D. Olson

a) Current status of employer monitoring study.
b) Status of soil monitoring studies for TCDD around and in the Plant - including a protocol of the study.

W. B. Crummett

Current status of EPA Dioxin Implementation Plan.

W. W. Muelder

Status of investigations on occurrence of chlorinated dioxins and furans in Dow products.

W. B. Seward, J. H. Davidson

Status of legislation and P.R. effort to support phenoxy herbicides.

J. H. Davidson
Agricultural Products Department

nac

DOW 1515901

AGRICULTURAL EXPERIMENT STATIONS
UNIVERSITY OF FLORIDA
EVERGLADES EXPERIMENT STATION

2799

December 23, 1964

P. O. DRAWER A
BELLE GLADE, FLORIDA
33430

DOW

2893445

Dr. Lawrence Southwick
Agricultural Chemical Development
The Dow Chemical Company
Abbott Road Buildings
Midland, Michigan

Dear Larry:

The following comments are in response to Chuck Lichy's phone request concerning silvex (Kuron) for sugarcane in Florida.

Information on the performance of silvex under our conditions has been sent Dow on several occasions in the form of mimeo reports, data summaries and letters. Continued, long-time use of 2,4-D in parts of our cane area has permitted the development of broadleaf weed populations resistant to 2,4-D. Properly timed 2,4,5-T applications may control some, but not all, of these species effectively. These to which I refer are dogfennel, ground cherry, nightshade, purslane, ragweed and wild lettuce. Other low-growing, less competitive winter annuals which are less important in cane culture may not be controlled by 2,4-D and/or 2,4,5-T. The weeds which I listed are amenable to control with silvex.

I think that silvex is a needed and useful tool in cane culture which I would recommend if it were cleared. My use suggestions would be for postemergence application of 1 - 1½ lb./A ai. (and not over 2 lb./A in extreme cases) silvex to the species named after establishment of plant cane or ratooning of stubble cane up until the cane is closed-in. I would stress that the rate of application should be adjusted to weed maturity.

Please contact me if you wish further information.

Sincerely yours,

J. R. ORSENIGO
Associate Horticulturist

JRO:sdt

16209

2821

Reprinted from *BioScience*

The Public Health Implications of Widespread Use of the Phenoxy Herbicides and Picloram

Julius E. Johnson

DOW 712933

This paper is concerned primarily with 2,4-D, 2,4,5-T, silvex, and picloram—the phenoxies because of widespread use; picloram, 2,4,5-T, and 2,4-D more specifically because of their use as military defoliants. The purpose of this discussion is to explore possible public health consequences of widespread use. This presentation will be confined primarily to information as it may translate to the health and safety of man. The data presented will emphasize exposure, toxicity, and chemistry.

First, I will comment on exposure to food, water, and air. There is very little exposure of man to the phenoxy herbicides through food. The principal uses of these herbicides in growing food crops were originally registered on a no-residue basis. A change in policy now requires these registrations be supported by a negligible residue tolerance. An industry task force has formally requested FDA to establish these tolerances. (The request for 2,4,5-T has been temporarily withdrawn without prejudice to future filing.)

Table 1 shows a condensed version of tolerances proposed for 2,4-D, 2,4,5-T, MCPA, silvex, and picloram. Those marked with an asterisk are for tolerances at a permissible level for 2,4-D in rice and flax seed and for picloram in forage grasses and meat. The remainder are negligible residue tolerances.

Table 2 demonstrates the infrequency of residues of phenoxy herbicides in foods as confirmed by the FDA market basket survey of pesticides in composites of 12 categories of foodstuffs. Of the 1548 samples analyzed, only 36 positive identifications were made. All but one was less than 0.2 ppm. 2,4,5-T was found in three of the surveyed samples (Duggan et al., 1967; Martin and Duggan, 1968; Corneliussen, 1969).

Air and water are potential sources of exposure of man to these herbicides. Manigold and Schulze (1969) have reported on pesticide residues in selected streams in the West such as the Missouri, Yellowstone, Colorado, Arkansas, Rio Grande, Snake, and others. Phenoxy herbicides were detected as shown in Table 3. Of the 320 samples taken, fractional parts per billion concentrations were reported in 78 samples. The measurement of picloram residues in water has been reported following *direct spraying of a static* pond in Texas (Hoffman et al., 1969) and a *watershed* experiment was conducted in North Carolina using 2,4-D, 2,4,5-T, and picloram (Sheets and Lutz, 1969). The results of these studies are summarized in Table 4. It is noted that picloram persisted longer than 2,4-D or 2,4,5-T. Air samples collected in spring and summer from two wheat-growing areas in the State of Washington were analyzed for phenoxy herbicides by Bamesberger and Adams (1966). The results are summarized in Figure 1. Phenoxy herbicides are widely used for early postemergence control of weeds in these crops. There were detectable levels of 2,4-D and 2,4,5-T in the air up to 0.06 $\mu g/m^3$. Man will inhale about 30 m^3 of air per day. At a level of 0.06 $\mu g/m^3$, this would amount to an exposure of

TABLE 1. Tolerances proposed to FDA

	2,4-D	2,4,5-T	MCPA	Silvex	Picloram
Fruits	X	X		X	
Berries	X	X			
Grains	X	X	X	X	
Rice	X*	X	X	X	
Flax Seed	X*		X		
Potatoes	X				
Sugarcane	X	X		X	
Hay	X		X		
Forage Grasses					X*
Meat					X*
Milk					X

(All negligible residue tolerances except those marked*)

TABLE 2. FDA market basket survey pesticide residues 1965-68

	Number of Samples with Residues of 1548 Samples Analyzed			
	PPM			Total
	0.001 – <0.1	0.1 – <0.2	0.4	
2,4-D	23	4	0	27
MCPA	2	1	1	4
2,4,5-T	2	1	0	3
Silvex	2	0	0	2

The author is Vice-President and Director of Research, Dow Chemical Company.

Presented at the Symposium on Possible Public Health Implications of Widespread Use of Herbicides (Chairman: Dr. Arthur Galston) American Institute of Biological Sciences, Bloomington, Indiana, 26 August 1970.

Pesticide Monitoring Journal, 1967, 1968, 1969

Duggan, R. E., H. C. Barry, and L. Y. Johnson, 1967. *Pestic. Monit. J.* 1(1): 2-12.

Martin, R. J., and R. E. Duggan. 1968. *Pestic. Monit. J.* 1(4): 11-20.

Corneliussen, P. E. 1969. *Pestic. Monit. J.* 2(4): 140-152.

16210

DOW 712939

1.8 μg phenoxy herbicide per day per man. For a 70-kg man, this would be 0.025 μg per kilogram body weight per day of exposure.

A variety of pesticides were reported in dust trapped in rain collected in Cincinnati in 1965 (Weibel et al., 1966). The dust was created by high winds over the southwestern United States and moved in a cloud in a northeasterly direction to Ohio where the precipitation occurred. An amount of 0.04 ppm of 2,4,5-T was determined to be present based on the air dried weight of the dust in the rain. No value was given for the amount of dust in the water. If it were as much as 10%, the water could have contained a 4 ppb of 2,4,5-T. The significance of these findings are open to question, however, because of the (1) difficulties in analyzing for such small amounts, and (2) the alleged presence of a newer pesticide, ronnel, which is used in relatively small amounts as an additive to cattle feed and as a topical spray on cattle. The results are summarized in Table 5

Direct skin contact together with vapor and mist exposure are encountered in varying degrees by applicators and employees in manufacturing plants. The exposure of an infrequent user is much less than professional pest control operators or those engaged in manufacturing 5 days a week. A medical evaluation of the effect of 2,4-D and 2,4,5-T has been made of men engaged in their manufacture. The workmen were given extensive physical examinations including a battery of at least 20 laboratory tests. The results of these studies are given in Tables 6 and 7. No meaningful differences were noted when the clinical results on these workers were compared to those obtained on a control population of 4600 men not exposed to 2,4-D or 2,4,5-T. An important observation was that there were no chromosomal effects.[1]

In the fall of 1969, considerable apprehension was created in the minds of the public when an announcement was made of tests sponsored by the National Cancer Institute reporting that 2,4,5-T was teratogenic. Following that announcement, I made special efforts to trace the identity of the samples used and reported to representatives of the National Cancer Institute and the Mrak Commission that the test sample probacontained 2,4,7,8-tetrachlorodibenzo-p-dioxin in amounts which might have been a complicating factor. The sample employed in the NCI study was analyzed and found to contain about 27 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Teratology tests with 2,4,5-T and the impurity were conducted in Dow laboratories. Results of the first experiments were observed by representatives of the Department of Health, Education & Welfare.

TABLE 3. Residues in water from 20 monitoring sites in 15 western states 1965-68

	No. Positive per 320 Samples	Parts per Billion
2,4-D	36	0.01 – 0.35
2,4,5-T	28	0.01 – 0.07
Silvex	14	0.01 – 0.21

(*Pesticide Monitoring Journal*, Manigold and Schulze, 1969)

Following is a description of the series of experiments concerned with the teratology question.

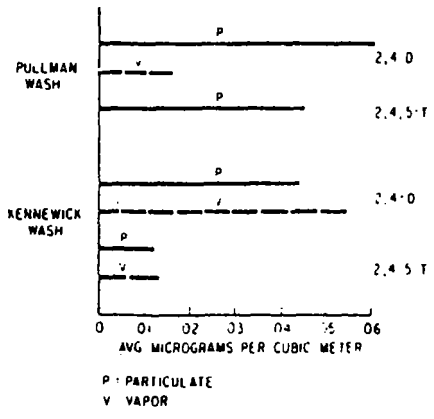


Fig. 1. Phenoxy herbicides in air (Bamesberger and Adams, 1966).

Commercial 2,4,5-T containing less than 1 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin was administered by gavage to groups of pregnant female rats on days 6 through 15 of the gestation period. The doses of 2,4,5-T administered were 0 (control), 1, 3, 6, 12, and 24 mg/kg/day. No clinical or gross pathological signs of adverse chemical effects were observed in treated dams during the period of treatment or gestation. Fetuses were harvested by cesarean section on day 20 of gestation. Visual and histopathological examination of the fetuses failed to reveal any teratogenic or embryotoxic effects (Emerson et al., 1970).

In another test, commercial production grade 2,4,5-T, given to rats at a dose of 50 mg/kg/day from days 6-15 of pregnancy, produced a minimal amount of fetotoxicity with one fetus in 203 having intestinal hemorrhage. When commercial grade 2,4,5-T was given at 100 mg/kg/day, a high incidence of maternal deaths occurred, and of the pregnant animals that survived, most had complete early resorptions. No fatal anomalies were observed as a result of the administration of the commercial production grade 2,4,5-T to rats. Likewise, no abnormalities were noted in litters of treated dams which were allowed to deliver and raise their young through lactation. Treatment had little effect on the survival of pups through this time period.[2]

Rabbits (New Zealand White) were used in another experiment to determine the embryotoxicity or teratogenicity of commercial 2,4,5-T containing less than 1 ppm of 2,3,7,8-tetrachlorodi-

TABLE 4. Residues in water

Type of Treatment	Herbicide	Lb/Acre	PPB in Water	Days After Spray
Direct spray on pond	Picloram	4	2400	<1
			700	1
			150	23
			6	180
Spray on 25% of watershed	2,4-D	4	1800	2
			40	21
	2,4,5-T	4	680	2
			90	21
	Picloram	4	4200	2
			340	21

[1] Unpublished data, 1970, Dow Chemical Co.

[2] Unpublished data, 1970, Dow Chemical Co.

16211

2,4,5-T	0.04

benzo-p-dioxin. Groups of pregnant female rabbits were given the compound by gavage from day 6 through 18 of gestation. The doses of 2,4,5-T administered were 0 (control), 10, 20, and 40 mg/kg/day. Fetuses were harvested by cesarean section performed on day 29. No clinical or gross pathological signs or adverse chemical effects were observed during the period of treatment or gestation. Examination of the fetuses showed no dose-related teratogenic or embryotoxic effects (Emerson et al., 1970).

In order to check the effect of the contaminant itself, a group of pregnant rats were administered 2,3,7,8-tetrachlorodibenzo-p-dioxin by gavage on days 6 through 15 of gestation. The dosages used were 0 (control), 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg/body weight/day. The fetuses were harvested on day 20 of gestation. No differences were observed in the fetuses taken from dams treated at the dosage of 0.03 μg/kg/day and those taken from dams that received the solvent vehicle only. At the 0.125 μg/kg/day dose level, there was evidence of intestinal hemorrhage and subcutaneous edema in some of the fetuses. At the 0.5 and 2.0 μg/kg/day doses, there were a substantial number of fetal deaths. Intestinal hemorrhage in the fetuses was frequent. The 8 μg/kg/day dosage was toxic to the dams. There were no viable fetuses in the dams which were examined on day 20 of gestation. All resorptions occurred early and no evidence of fetal tissue was found. Skeletal examinations revealed delayed ossification of some sternebra and skull bones. This occurred generally throughout the various groups, including controls, and was not considered to be of practical significance. The results of this experiment indicated a high level of maternal and fetal toxicity to be associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Its presence in 2,4,5-T at levels of 27±8 ppm could well have accounted for the observations reported and attributed to 2,4,5-T in the original NCI study conducted by the Bionetics Laboratories (Sparschu et al., 1970). 2,4,5-T, and (2) pure 2,4,5-T to which specific amounts of the 2,3,7,8-tetrachlorodibenzo-p-dioxin were added. The results of these studies show (1) that a dose of 50 mg/kg/day of 2,4,5-T from day 6 through day 15 of pregnancy (maximum tolerated dose for our strain of rats) did not cause teratogenic effects in the young, and (2) that when a toxic dose of the 2,3,7,8-tetrachlorodibenzo-p-dioxin was superimposed upon this dose of 2,4,5-T, cleft palates were observed in a few fetuses in addition to the typical toxic manifestations of the 2,3,7,8-tetrachlorodibenzo-p-dioxin when given alone. However, it is very important to note that when no effect levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 50 mg of pure 2,4,5-T were combined, no teratogenic and only minimal fetotoxic results were observed.[3] The results are summarized in Table 8.

[3]Unpublished data, 1970, Dow Chemical Co.

solvent. Although the *pure* 2,4,5-T was reported to have shown teratogenicity, dosages of 100 mg/kg were required to produce the cleft palates or kidney involvement.[4]

Steinfeld[5] also reported terata in hamsters injected with commercial 2,4,5-T containing <1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin at a dose of 100 mg/kg/day. Terata were also observed in hamsters injected with 20 mg of 2,4,5-T containing 45 ppm of the tetrachlorodibenzo-p-dioxin per kg/day.

Verrett has reported that 2,4-D 2,4,5-T and silvex produce terata and chick edema syndrome following injection into the yolk sac. The extreme

[4]Testimony before the U.S. Senate Committee on Commerce, Sub-Committee on Energy Water, Natural Resources and the Environment, 15 April 1970, Dr. Jesse Steinfeld pages 167-189, Report of the Committee Serial 91-60 (1970).

[5]Testimony before the U.S. Senate Committee on Commerce, Sub-Committee on Energy Water, Natural Resources, and the Environment, 18 June 1970, Dr. Jesse Steinfeld

TABLE 6. Industrial health experience—manufacture of 2,4-D the Dow Chemical Company

I.

No. Men Exposed	Work Experience	Range of Exposure	Results
220	0.5-22 years	30-40 mg/da	No differences when compared to contro population of 4600 me

II.

10 men exposed to 2,4-D were karyotyped.
Result. No effect on structural integrity or rearrangement of the genetic material of the lymphocyte chromosomes.

TABLE 7. Industrial health experience—manufacture of 2,4,5-T, the Dow Chemical Company

I.

Number of Days Exposed to 2-8 mg 2,4,5-T Daily	Number of Men Examined	Results
1 – 59	24	No differences when compared to control population of 4600 men
60 – 119	15	
120 – 239	6	
240 – 479	10	
480 – 959	6	
960 +	3	

II.

52 men exposed to 2,4,5-T were karyotyped.
Result: No effect on structural integrity or rearrangement of the genetic material of the lymphocyte chromosomes.

DOW 713940

16212

DOW 712941

sensitivity of this test procedure makes it difficult to interpret the meaning of the results from a public health point of view.[6]

Binns of Utah has tested the effects of 2,4,5-T exposures on the 14th to 36th day of gestation of sheep, the period in which they are most susceptible to teratogenic effects from the plant *Veratrum californicum*[7]. No anomalies were noted as a result of the exposure to 2,4,5-T (see Table 9).

TABLE 9. Teratology study of 2,4,5-T with sheep

Compound	Amount Fed	No. of Ewes	Number Pregnant	Results
None	None	20	15	15 ewes bore live, normal lambs. 1 twin. 1 ewe aborted 28 days, fetus normal.
2,4,5-T Dow Prod'n.	100 mg/kg (a)	11	10	10 ewes bore live, normal lambs.
PGBE ester of 2,4,5-T Dow Prod'n.	100 mg/kg (a)	11	9	7 ewes bore live, normal lambs, 1 twin. 1 ewe died 36 days, aspirated feed. Fetus = normal. 1 ewe died 35 days, profuse diarrhea from 7th day. Fetus normal.

W. Binns, Poisonous Plant Research Laboratory, USDA, Logan, Utah

[a]Compound given by stomach tube mixed with 0.5 lb. ground alfalfa. Dosed 14-36 days of gestation. *Veratrum californicum* fed this period induces cyclopia, short metatarsals and metacarpals, harelip and cleft palate.

A pilot study to determine the tolerance of pregnant female rats to silvex in preparation to conducting a conventional teratology study in rats has been completed. Silvex was fed to groups of pregnant rats (5 to 6 per group) by gavage on days 6 through 15 of the gestation period. Doses given were 100, 150, 200, and 300 mg/kg/day. There were no effects on either the dams or fetuses harvested on day 20 by cesarean section at the dose level of 100 mg/kg/day. At 150 mg and above there was toxicity noted in the dams with an increasing amount of maternal mortality with increasing dosage. No teratology was observed by gross examination in fetuses at these levels; however, there were some resorptions.[8]

Teratology experiments have also been conducted with 2,4-D. To date we have concluded studies on pregnant rats with 2,4-D acid and with 2,4-D propylene glycol butyl ether ester. In this study, involving about 320 rat litters, we administered corn oil as a control, the acid form of 2,4-D at dosage levels of 12.5, 25, 50, 75, and 87.5 mg/kg/day and the ester form at dosage levels of 20, 40, 81, 122, and 142 mg/kg/day. About one-half of the litters were taken by cesarean section on day 20 of gestation for examination for soft tissue and skeletal abnormalities and the other half were allowed to go to term. The offspring were observed for 21 days, after which they were sacrificed and examined. Fetal weights were reduced at a dose of 50 mg of 2,4-D acid/kg/day, and at a dose of the ester at 121 mg/kg/day. No teratogenic effects were noted. Among litters delivered naturally, treatment with 2,4-D during pregnancy had no effect on neonatal growth and survival.[9]

Groups of male and female rats were maintained on diets containing 0, 3000, 1000, or 300 ppm picloram through a three-generation reproduction study without evidence of adverse effect as judged by indexes of fertility, gestation, viability, lactation, by body weight, and by teratological examination of fetuses.[10]

A pilot study has been concluded to determine the tolerances of pregnant female rats to picloram in preparation for a conventional teratology study. Picloram was administered to groups of pregnant rats (5 to 6 per group) by gavage on days 6 through 15 of the gestation period. The doses given were 250, 500, 750, 1000, and 2000 mg/kg/day. There was no adverse effect on the dams, nor to fetuses taken at day 20 of gestation at the levels of 250, 500, and 750 mg/kg/day. At the 1000 mg/kg/day dosage, there was no adverse effect on the dams, but there was a small increase in the absorptions. The fetuses harvested showed no teratogenesis. At the 2000 mg/kg/day level, only one dam survived to the 20th day of gestation. Eleven fetuses were harvested from this dam all of which were viable and appeared normal by gross observation.[10]

Since October 1969, great interest has been created in 2,3,7,8-tetrachlorodibenzo-p-dioxin as a possible serious and continuing contaminant in the environment. It is important to emphasize that the tetrachlorodibenzo-p-dioxin is no longer a significant contaminant and probably never was in carefully prepared commercial 2,4,5-T. When it did occur—in 1964 and 1965—the contaminant carried over from the intermediate 2,4,5-trichloro-

TABLE 8. Teratology study in rats 2,3,7,8-tetrachlorodibenzo-p-dioxin added to 50 mg pure 2,4,5-T/kg body wt/day

Treatment	No. of Litters	No. of Fetuses	Litters with Cleft Palate	Litters with Intstnl. Hemorr.
Control (vehicle)	17	194	0	0
50 mg pure 2,4,5-T per kg	17	172	0	0
+0.01 μg TCDBD	15	150	0	0
+0.03 μg TCDBD	17	173	0	0
+0.06 μg TCDBD	17	155	1	8
+0.125 μg TCDBD	15	134	0	11
μg TCDBD	14	76	4	13.
+.0 μg TCDBD	15	46	5	8

[6]Testimony before the U.S. Senate Committee on Commerce, Sub-Committee on Energy, Water, Natural Resources, and the Environment. 18 June 1970, Dr. Jacqueline Verrett, pages 190-203, Report of the Committee, Serial 91-60 (1970).

[7]Binns, W. 1970. Poisonous Plant Research Laboratory, U.S.D.A., Logan, Utah. Unpublished data.

[8]Unpublished data, 1970. Dow Chemical Co.

[9]Unpublished data, 1970, Dow Chemical Co.

[10]Unpublished data, 1970. Dow Chemical Co.

16213

was established to ensure that no more than minimal amounts would occur in the product (the best method available had a sensitivity of 1 ppm). Improved assay methods now show the actual contaminant is less than 0.5 ppm.

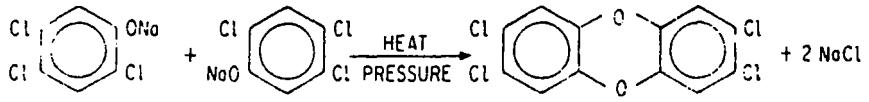


Fig. 2.

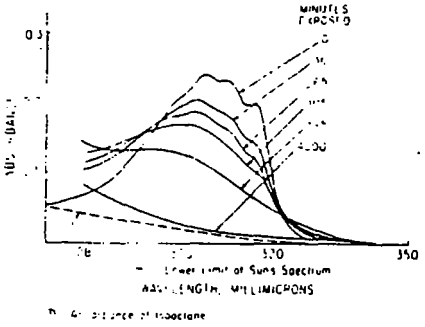


Fig. 3. Effect of radiation from G.E. sunlamp at distance of one meter on 12 ppm solution of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Incidentally, the 2,4,5-trichlorophenol submitted by the National Cancer Institute in 1964 to be tested by the Bionetics Laboratory *did not cause* a teratological response. This was a sample procured from Coleman Matheison Bell who got it from McKesson Robbins who got it from Dow.

The following information is known about the stability and properties of the 2,3,7,8-tetrachlorodibenzo-p-dioxin: (a) in solution it is rapidly degraded by ultraviolet light at wavelengths that appear in the spectrum of the sun (Fig. 3);(b) the tetra dioxin has one-fifth the solubility of DDT in water and one-sixteen hundredth the solubility of DDT in benzene (Table 10). Thus there is probably less tendency to concentrate in fat, but the question is really academic because the quantities of tetra dioxin in the environment are exceedingly small.

Concern has been expressed that the combustion of 2,4,5-T (even though the parent material contained no tetrachlorodibenzo-p-dioxin) would cause dioxin formation. A laboratory experiment was conducted wherein agent Orange (a defoliant containing equal quantities of the n-butyl esters of 2,4-D and 2,4,5-T) was applied to 18.5-cm filter paper at rate of 10.9 kg per 0.41 hectares. This is equal to approximately 4.5 kg of 2,4,5-T acid equivalent per 0.41 hectares. The paper was burned and combustion products plus air drawn through an absorber packed with glass beads cooled with liquid nitrogen. The combustion products plus the ash was solvent extracted and analyzed by gas chromatography (Fig. 4). The burning temperatures encountered were as follows:

	°F	°C
Paper surface	600 - 800	300 - 400
Body of flame	1000 - 1200	550 - 650
Front of flame	1300 - 1400	700 - 750

There was no 2,3,7,8-tetrachlorodibenzo-p-dioxin detected in the combustion products at a sensitivity of 5 ppm. A similar experiment was tried using wood as a substrate, but there were substances in the combustion products which interfered with the analysis. Speculative claims are widespread that 2,4,5-T residues on vegetation might be converted to 2,3,7,8-tetrachlorodibenzo-p-dioxin if the dead foliage is burned. All available evidence to date indicates this conversion does not occur. The alleged precursor is in dilute form on the substrate, the reaction is biomolecular—the molecules must be forced close together to react.

Concern has also been expressed that traces of preformed dioxin contaminating 2,4,5-T sprayed into the environment would accumulate. The evidence

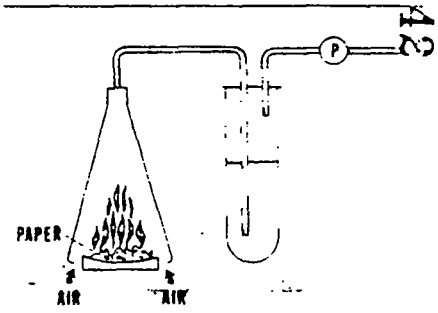
Benzene	0.047 g/100 ml	78 g/100 ml

24 C (75 F). This evidence of degradation is so slow it will require further observations to quantify.[11]

2,3,7,8-tetrachlorodibenzo-p-dioxin does not move in soil. It is strongly adsorbed on clay and organic matter and is practically insoluble in water (0.2 parts per *billion*). First studies on uptake by plants indicate none from water and very little, if any, from soil.[12]

TABLE 11. Solubilities (g/100 ml of solution at 25 C)

Solvent	Picloram	Picloram, Potassium Salt
Water	0.043	40.00
Benzene	0.02	–
Xylene	–	0.01



DOW 1970

Fig. 4.

Silvex is also made with 2,4,5-trichlorophenol as a starting material. A total of eight lots of silvex has been analyzed, obtained from 1967, 1968, and 1969 production. No detectable amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin have been found using a method having a sensitivity of 0.1 ppm. Before leaving the subject of contaminating chlorodibenzo-p-dioxins, it is noteworthy that no detectable dibenzo-p-dioxins (including the 2,3,7,8-tetrachloro- or the 2,7-dichloro-) have been found in 2,4-D samples analyzed. The sensitivity of the analytical method used was 1 ppm.[13]

[11] Unpublished data, 1970, Dow Chemical Co.

[12] Private communication from Dr. P. C. Kearney, 1970, USDA, Beltsville, Md.

[13] Unpublished data, 1970, Dow Chemical Co.

DOW
NI29423

16214

DOW 712943

TABLE 12. Acute oral toxicity, single oral dose

	LD_{50} as Milligrams per kg Body Weight	
	Rat	Guinea Pig
2,4-D	376.0 – 666.[a]	1000.0
2,4,5-T	500.0	380.0
Silvex	500.0	850.0
Picloram	8200.0	3000.0
DDT	150 – 800.0[a]	400.0
Parathion	1.7 – 30.0[a]	9.3-32.0[a]
2,3,7,8-tetrachlorodibenzo-p-dioxin	0.022 – 0.045[a]	0.0006

[a]Varies according to vehicle or sex.

The formation of 2,7-dichlorodibenzo-p-dioxin does not occur in the manufacture of 2,4-dichlorophenol since it is made by direct chlorination of phenol and not by alkaline hydrolysis of a trichlorobenzene. Reaction conditions do not favor biomolecular condensation to form a chlorodibenzodioxin. Moreover, 2,7-dichlorodibenzo-p-dioxin is more difficult to form than the 2,3,7,8-tetrachlorodibenzo-p-dioxin even when reaction conditions are favorable.

The persistence of pesticides in soil and water is an important factor in their potential to occur as residues in foods and water or to come in contact with nontarget organisms. Degradation of herbicides is accelerated by increased temperature, soil microflora, and organic matter. A comparison of degradation rates in soils has been published by Kearney et al. (see Fig. 5) (Gunther, 1969). It is true that picloram is more persistent. Unlike DDT, however, picloram forms salts that are water soluble. The solubilities are shown in Table 11. Picloram does not concentrate in fat. Moreover, the mammalian toxicity of picloram when compared with most other herbicides is low. The potential hazards from picloram will result primarily from effects on sensitive plant species. Up to the present time the bioassay is more sensitive than chemical techniques for determining the presence of picloram.

TABLE 13. No-effect levels (mg/kg/day)

Species	2,4-D	2,4,5-T	Silvex	Picloram
Reproduction and Fertility				
Rat	25	–	–	150
2-Year Dietary Feeding Toxicity				
Rat	62	–	5	150
Dog	12	–	5	150

TABLE 14. Grass production—Panama

	Tons of Vegetation/Acre 15 Months Deferred Grazing			
Treatment	Jaragua Grass	Other Grasses	Live Brush	Total
None	1.80	0.28	12.65	14.43
0.6 lb. picloram + 2.4 lb. 2,4-D Per acre	13.55	0.29	0.50	14.34

Swazey and Montano, 1968 (26)

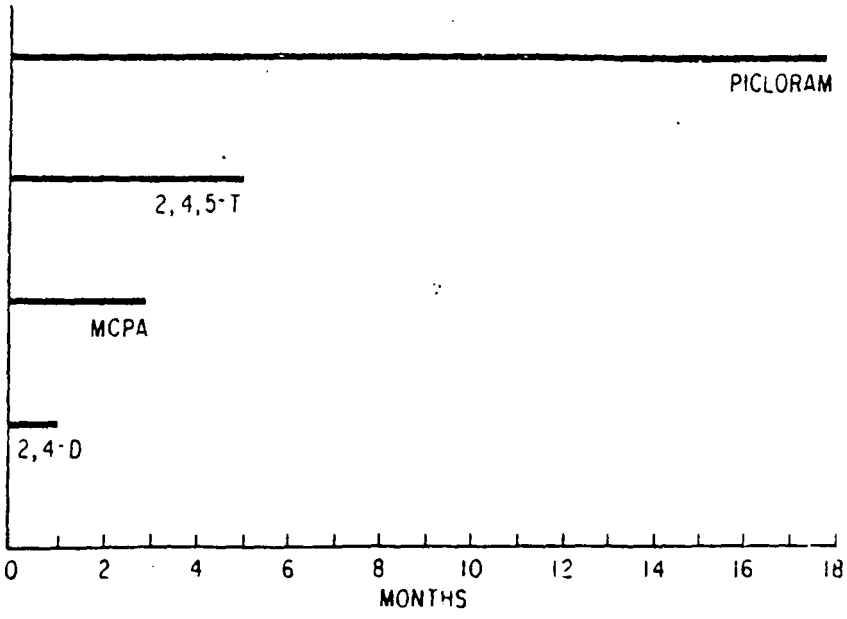


Fig. 5. Persistence of herbicides in soils. Length of bar represents time required for 75 to 100% loss of the compound (after Kearney, 1969).

The acute oral toxicities of 2,4-D, 2,4,5-T, silvex, and picloram are given in Table 12, in comparison with DDT, parathion, and the 2,3,7,8-tetrachlorodibenzo-p-dioxin. It is evident that this dioxin is highly toxic, in fact one of the most toxic substances known. This alone is a good and sufficient reason to keep this impurity at an absolute minimum. By contrast, the low order of acute toxicity of picloram is also of interest. The no-effect levels attained in long-term dietary feeding studies also indicate a low order of toxicity of picloram. Concentrations were not tested above 150 mg/kg/day (see Table 13). The reproduction and fertility tests were conducted through three generations of rats receiving continuous feeding of the test compounds. No birth anomalies or other adverse effects were observed with continuous intakes of 25 mg/kg/day of 2,4-D or with 150 mg/kg/day of picloram, respectively.

Some of the benefits of using herbicides are illustrated in Tables 14 and 15. The resulting increases in beef production are important to nutrition and

16215

TABLE 15. Grass production—Oklahoma

Application Per Acre	Weed	Months Deferred Grazing	Grass, Lb./Acre	
			Wet Years	Dry Years
None	Oaks	6	862	348
2 lb.-2,4,5-T	Oaks	6	2487	2200
3 lb.-2,4,5-T	Oaks	6	2266	2063
4 lb.-2,4,5-T	Oaks	6	2917	2083
4 lb.-2,4,5-T	Oaks	6	-	2750

Elwell, 1964 (27)

health of man (Gunther, 1969; Swezey and Montano, 1968).

In conclusion, I set forth the opinion that the widespread use of phenoxy herbicides has produced no demonstrable evidence of potential harm to man. The herbicides used most widely (2,4-D and 2,4,5-T) are degraded and do not bioconcentrate. Moreover, the comparative toxicity shows these materials to be well tolerated in a variety of test systems. Man is not exposed to harmful concentrations. Impurities can be an important factor—particularly the chlorodibenzo-p-dioxins—but these can be controlled by proper manufacturing techniques. As always, care in application is an important part of safe practice both from the standpoint of man and the ecosystem in which these tools are used.

References

Bamesberger, W. L., and D. R. Adams. 1966. Organic pesticides in the environment. *Advan. Chem Ser*

Corneliussen, P. E. 1969. *Pestic. Monit. J* 2(4): 140-152.

Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1967. *Pestic. Monit. J.*, 1(1): 2-12.

Elwell, H. M. 1964. *Agron. J.*, 56: 411-415.

Emerson, J. L., D. J. Thompson, R. J Strebing, C. G. Gerbig, and Robinson. 1970. Manuscript submitted for publication to *Food and Cosmetics Toxicology*.

Gunther, F. 1969. *Residue Reviews*, 29, Springer-Verlag, New York.

Hoffman, G. O., E. D. Robison, and M. G. Merkle. 1969 WSSA ABST. 75, Las Vegas, Feb.

Manigold, D. B., and J. A. Schulze. 1969 *Pestic. Monit. J.*, 3: 124-135.

Martin, R. J. and R. E. Duggan. 1968. *Pestic Monit. J.*, 1(4): 11-20.

Sheets, T. J., and V. F. Lutz. 1969. *Bull N. C. Exp. Sta.*

Sparschu, G. L., F. L. Dunn, and V. K. Rowe. 1970. Manuscript submitted for publication to *Food and Cosmetics Toxicology*.

Swezey, A. W., and A. Montano. 1968. *Down to Earth*, Summer, p. 6-9.

Weibel, S. R., R. B. Weidner, J. M. Cohen, and A. G. Christianson. 1966. *J. Amer Water Works Assoc.* 58: 1075-1084.

DOW 712344

16216

CRC Critical Reviews in Toxicology, 5: 67-101 (1977) Moore pp. 81-83

IMMUNE SUPPRESSION AS RELATED TO TOXICOLOGY

PARTIAL TEXT ONLY

Author: J. G. Vos
Laboratory of Pathology
National Institute of Public Health
Bilthoven, The Netherlands

Referee: John A. Moore
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

DOW0686016
MN072283

INTRODUCTION

Aberrant or defective immune responses are known to be the result of primary immunodeficiency diseases, e.g., in the DiGeorge syndrome (thymic hypoplasia) in man or in homozygous mice with the mutation "nude" (nu/nu), which are hairless and suffer from congenital thymic hypoplasia (Figure 1). Immune deficiency can also be secondary, e.g., a result of undernutrition, irradiation with γ- or X-rays, neonatal thymectomy, treatment with antilymphocyte serum, treatment with certain chemicals, and during infectious diseases. Regarding chemical compounds, relatively much information is available on the small number of immunosuppressive agents used clinically for immune suppression, e.g., some thiopurines and alkylating agents (reviewed by Bach).[1]

Surprisingly, only a limited number of chemicals have been shown in toxicity studies to have immunosuppressive properties, and particularly the cell-mediated immunity has been poorly studied. A likely reason for this fact is that current procedures for toxicity testing[2-6] underestimate the importance of the immune system: lymphoid organs and in general the immune system have been poorly examined. Therefore, in-depth investigations were performed with only a few chemicals as to their effect on the immune response. This raises the question of whether these chemicals represent only the tip of an iceberg, a question which can only be resolved by adequate toxicity testing that includes careful examination of the immune system both morphologically and functionally.

In this review, it will be stressed that knowlegde from the expansive growing immunology discipline should be incorporated into the field of toxicology. First, the immune system and the immunological response will be introduced. Next, attention will be given to the interdependence of the immune system and the macrophage system. Of special importance in toxicology is the discrimination between direct effects on the immune system and indirect effects such as nutritional deficiencies, pathogenic organisms, and changes in the endocrine balance. In this light, procedures are presented to detect immunosuppression in routine toxicity studies. Furthermore, function studies will be discussed, of the cell-mediated immune system, the humoral immunity, and the macrophage system; these function tests are necessary in order to gain insight into the mode of action of the chemical and to determine the functional significance of a

from Moore

81-85

lymphoid organs, that increase the susceptibility to infection, or that impair the immunological responsiveness. These parameters are considered important in the evaluation of the toxicity of these chemicals. Chemicals for which in depth information is available (including data on an indirect or a direct action of the chemical) will be discussed first. In addition, chemicals whose effects require further investigations will be reviewed.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

The compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (Table 3) is a highly toxic impurity that may be formed during the production of 2,4,5-trichlorophenol. Buu-Hoi et al.[93] were the first to describe, in addition to hepatotoxicity, severe thymus atrophy in rats exposed to TCDD (Figure 3). Since this first report, several studies with rats, mice, and guinea pigs have confirmed and extended this finding.[52,53,68,94–97] In contrast to the adult animal, rat and mouse pups that are exposed during the perinatal period by maternal treatment do not show major liver pathology, while severe effects are seen in the lymphoid organs, particularly in the thymus.[52] Liver lesions are mild in the adult guinea pig when compared with the severe atrophy of thymus and peripheral lymphoid organs.[95]

As shown by Moore and Faith[53] (Figure 7), rats exposed during the pre- and/or postnatal period by maternal treatment with TCDD had significantly lower body and thymus weights at weaning age. At an age of 39 days, pups exposed only postnatally gained more weight than animals exposed during the gestation and nursing period. After 145 days, the body and thymus weights of the former group did not differ significantly from

Xerox 81-85

TABLE 3

The Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Susceptibility to Infection and on Various Immunologic Parameters

Species	In vivo or in vitro treatment	Parameter	Effect[a]	Ref.
Rat	In vivo	Thymus atrophy	++[b]	52, 53, 68, 93–95, 97–99
Mouse	In vivo	Thymus atrophy	++	52, 68, 94–96
Guinea pig	In vivo	Thymus atrophy	++	68, 94, 95
Rat	In vivo	Serum α-foetoprotein	–	87
		Peripheral lymphocyte count	–	100
Mouse	In vivo	Peripheral lymphocyte count	+/–	96, 100
Guinea pig	In vivo	Peripheral lymphocyte count	+	100
Mouse	In vivo	Peripheral monocyte count	–	96
		Reduction serum α, β, and γ globulins	+	96
		Susceptibility *Salmonella bern* infection	+++	101
		Susceptibility pseudorabies infection	–	101
Rat	In vivo	Delayed-type hypersensitivity	–/+[c]	53, 68
Guinea pig	In vivo	Delayed-type hypersensitivity	+	68
Rat	In vivo	Graft vs. host activity	+	52
Mouse	In vivo	Graft vs. host activity	+/–[d]	52, 68
Rat	In vivo	Rejection of skin allografts	+	52
Mouse	In vivo	Rejection of skin allografts	+	52
Guinea pig	In vivo	Antibody response to tetanus toxoid	–/+[e]	68
Rat	In vivo	Antibody response to bovine gamma globulin	–/–[e]	53
		Lymphocyte transformation by PHA and Con A	++	52, 53
Mouse	In vivo	Lymphocyte transformation by PHA	+/–[d]	52
	In vitro	Lymphocyte transformation by PHA	–	52
Rat	In vitro	Lymphocyte transformation by PHA and Con A	–	52

[a]Denotes the increased susceptibility to infection and the suppressive effect on immunological parameters: +, slight; ++, moderate; +++, strong; –, no effect.

[b]Thymus atrophy also occurred in adrenalectomized and hypophysectomized rats and in mice injected with thymic "hormone" (thymosin).

[c]In young adult rats and in rats exposed during the perinatal period by maternal treatment, respectively.

[d]In young and adult animals, respectively.

[e]On the primary and secondary antibody response, respectively.

DOW0686017

1621 8

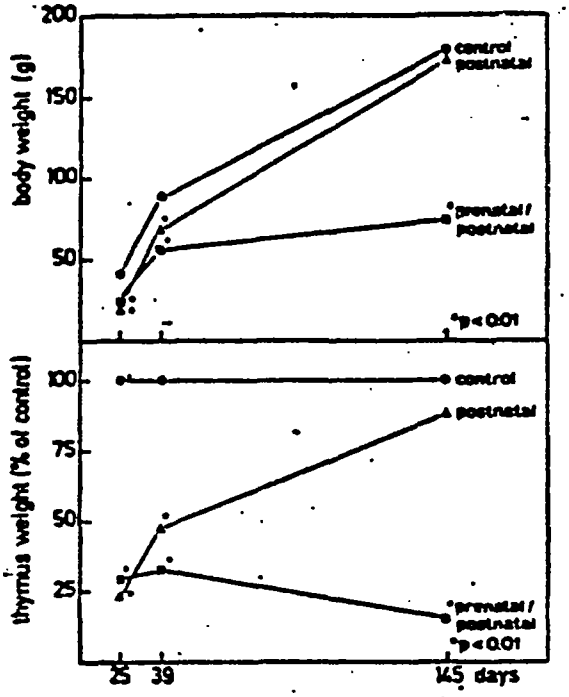


FIGURE 7. Body and thymus weights (as percentage of controls) of female Fisher rats that were exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin postnatally via maternal dosing (5 μg/kg body weight) on days 0, 7, and 14 and pre- and postnatally via maternal dosing on gestation day 18 and on days 0, 7, and 14. (Adapted from Moore and Faith).[53]

the control, whereas severe growth stunting and thymus atrophy were present in the latter group. Pre- and postnatal maternal treatment in mice can also produce a wasting syndrome in pups.[52] These findings clearly demonstrate the severe consequences of TCDD exposure during the developmental phase.

The effect on the thymus is not indirectly mediated by the pituitary or adrenals, since thymus atrophy occurred in TCDD-exposed rats that were adrenalectomized or hypophysectomized.[98] In addition, the influence of TCDD on the thymus is not caused by reduced food intake.[99] Also, recent results have shown that serum α-fetoprotein levels were not elevated in rats with TCDD-induced thymus atrophy.[87] Finally, preliminary results indicate that injections of thymic "hormone" (thymosin) did not protect TCDD-exposed mouse pups from developing thymus atrophy.[87] TCDD did reduce the number of peripheral lymphocytes in guinea pigs and mice, but not in rats.[100] In another study with mice,[96] no effect was seen on peripheral lymphocyte and monocyte counts, but serum concentrations of α, β, and γ globulins were reduced by TCDD exposure.

A very sensitive parameter of TCDD exposure is the resistance to *Salmonella bern* infection.[101] Intubation of mice, once weekly for 4 weeks with a dose as low as 1 μg TCDD per kilogram body weight, significantly increased the mortality rate and decreased the time from infection to death in animals infected with *Salmonella bern*. This dose of TCDD was one order of magnitude lower than the dose that caused atrophy of the thymus. In contrast, TCDD had no significant effect on mortality in mice infected with pseudorabies virus. The increased susceptibility of TCDD-treated mice to *Salmonella* infection is probably due to the endotoxin content of the bacteria, since recent results have shown that oral intubation, once weekly for 4 weeks with 50 μg TCDD per kilogram body weight (a dose that gave a moderate thymus atrophy), rendered mice 50 times more sensitive to endotoxin (LPS, *E. coli*).[87]

Of the different parameters of the cellular immunity, the delayed-type hypersensitivity to tuberculin was reduced in guinea pigs but not in young adult rats.[68] The delayed-type hypersensitivity to oxazolone was reduced when rats were exposed during the perinatal period by maternal treatment with TCDD; this reduction was even present in rats evaluated at 145 days of age.[53] The graft vs. host activity of spleen cells of rats exposed to TCDD during the postnatal period[52] and of young mice[68] was significantly reduced, but no reduced activity was seen when spleen cells of adult mice were used.[52] In both rats and mice, perinatal exposure to TCDD prolonged the rejection of skin transplants.[52]

Regarding the thymus-dependent antibody response, treatment of guinea pigs with TCDD reduced the secondary response to tetanus toxoid, but no effect was seen on the primary response.[68] In the rat, perinatal exposure to TCDD did not reduce the primary and secondary antibody response to bovine gamma globulin.[53]

The mitogenic response of lymphocytes from thymus and spleen of rats exposed during the perinatal period was suppressed on a cell-for-cell basis and in particular on an organ-for-organ basis.[52,53] The transformation of spleen cells by PHA was reduced in young mice, but not in adult animals.[52]

The responsiveness of mouse spleen cells and rat thymus cells to the mitogens PHA or Con A

CRC

By J. Vos

16219

DOW0686018

was not reduced when cultured in the presence of TCDD at concentrations up to 0.02 μg/ml medium.[52] Even the presence of 0.32 μg TCDD per milliliter medium has been reported not to be toxic for human lymphocytes.[102] However, in another study, TCDD at a concentration of 0.48 μg/ml medium was cytotoxic for several mammalian cell types,[103] whereas concentrations that are two orders of magnitude lower induce aryl hydrocarbon hydroxylase activity in human lymphocytes.[104] These results make it unlikely that TCDD needs metabolic activation, but indicate that the lymphocyte depletion of the thymus and the thymus-dependent lymphoid tissue is not caused by a direct cytotoxic action of TCDD on lymphocytes.

In conclusion, TCDD causes thymus atrophy in all mammalian species studied. Regarding the biological significance of this effect, it has been shown in several experiments that TCDD exposure results in functional impairment of the cellular immunity, but with quantitative different effects on subpopulations of T cells. In particular, T-helper cells, possibly with the exception of those in the guinea pig, seem less compromised. In addition, it seems that the suppression of the cell-mediated immunity is an age-related phenomenon, at least in the mouse and rat where TCDD exposure during ontogenesis of the immune system seems to be prerequisite; in this respect, TCDD mimics the effect of neonatal thymectomy. As discussed earlier,[52] it is tempting to speculate to what degree depressed cell-mediated immunity in young rats and mice and in adult guinea pigs contributed to their deaths, since severe effects were observed in the absence of major liver pathology found in adult mice and rats. Regarding the mode of action of the TCDD-induced atrophy of the thymus, it is probably not caused by a direct cytotoxic action on lymphocytes, neither indirectly by an effect on pituitary, adrenals, or production of thymic "hormone," nor via induction of α-fetoprotein or reduced food intake. However, as discussed by van Logten et al.[99] it is still possible that TCDD may impair the absorption and transportation of nutrients. An alternative hypothesis that needs investigation may be through an effect of TCDD on bone marrow stem cells as precursors of lymphocytes in the thymus. The increased susceptibility of mice to infection with *Salmonella bern* is remarkable. Host resistance to this type of infection primarily depends upon cell-mediated immunity;[105] however, since increased mortality occurred at dose levels of TCDD that do not produce thymus atrophy, another mode of action seems likely. This increased susceptibility is very probably due to the endotoxin content of the bacteria, which has also been demonstrated to be the cause of the greatly enhanced susceptibility to *E. coli* infection of rats exposed to lead.[106] Regarding this sensitizing effect of TCDD for endotoxin, it would be of interest to investigate whether TCDD impairs the endotoxin detoxifying properties of macrophages.

Di-n-octyltindichloride (DOTC) and Di-n-butyltindichloride (DBTC)

In-depth investigations were carried out on effects on the immune system of the chemicals di-n-octyltindichloride (DOTC) and di-n-butyltindichloride (DBTC) (Table 4). As shown by Seinen and Willems,[55] the main effect of DOTC in rats is on the thymus. Histologically, lymphocyte depletion was observed in thymus and thymus-dependent areas of spleen and lymph nodes. In cell suspensions of the thymus, a dose-related decrease was seen in the number and viability of thymocytes; the effect on spleen cell count and cell viability was less pronounced, whereas no effect was found on bone marrow count and viability and on peripheral lymphocyte and monocyte numbers.

As shown in Figure 8, in a series of dialkyltin compounds tested, both DOTC and DBTC produced a severe thymus atrophy (which was reversible upon discontinuation of exposure), whereas a less pronounced effect occurred with diethyltindichloride and di-n-propyltindichloride. In contrast, the other dialkyltin compounds (dimethyltindichloride, di-n-dodecyltindibromide, and di-n-octadecyltindibromide) as well as mono-n-octyltintrichloride, tri-n-octyltinchloride, and tetraoctyltin did not cause atrophy of the thymus.[56]

The selective effect of DOTC on thymus is not indirectly caused by stress-related release of glucocorticoids, since a similar effect on thymus was present in adrenalectomized rats.[55] A strong argument in favor of a direct action of DOTC and DBTC on rat thymus is their in vitro cytotoxicity for thymocytes of rats, but not of guinea pigs and mice; in these latter two species, thymus atrophy does not occur after exposure to these chemicals.[56]

In function studies of the thymus-dependent immunity, the delayed-type hypersensitivity to

DOW0E86019

16220

EXHIBIT E

DOW 289640
MN 022777

KURON Complaints:

1. E. L. Hand - Lake Jackson, Texas
2. John Norton - Glendale, Arizona

Final Report copied 8-10-71 by

Copies to: DSM
Product Sales Manager
Product Technical Specialist
Salesman

AGRICULTURAL DEPARTMENT

PRODUCT COMPLAINT QUESTIONNAIRE (PLANTS)

Information given here should be factual unless indicated otherwise.

DOW 28941

Written by: R. W. Swart Date: 8/4/71

Exact Name, Address and Telephone Number of Complainant: E. L. Hand, Highway 332, Lake Jackson, Texas 77566

Telephone No. 713-798-2590 County Brazoria State Texas

Detailed location of damaged and non-damaged area (locate on Geological Survey, highway, SCS or irrigation map): Buffalo Camp Bayou - north of Highway 332 - west of Lake Jackson, Texas

Name, address and telephone number of person receiving complaint: W. S. McGregor, Agricultural Department, Dow Chemical U.S.A., Lake Jackson, Texas

Who requested Dow assistance: Mr. Hand

Name and address of Dow and other personnel involved in investigation: D. M. Turner, Dow Texas Division Water Supply Dept., Freeport
R. W. Swart, Dow Agricultural Dept., Lake Jackson

Date of complaint: July 12, 1971

Product and Application:

Product: Kuron Date of Application June 21, 24, 25 1971 Date of purchase: Within 6 Mo.

Proof of puchase: Distributor or Dealer: Sprayco, direct from Dow

Condition of container immediately prior to use: Lot number 50-12-29

Amount of Product Used 35 Gal. 5 Gal. Plyac Emulsifier Area Treated- Rate/Acre Spot Spray Vol. Spray/Acre

Wind velocity and direction at time of application: None

Obtain original container with suspected contents: Spray water source:

Prior use of sprayer: None Other pesticides used in tank mix: None

Identification, exact name, telephone number and address of:

Contractor: Sprayco Systems, 1327 Judiway, Box 10025, Houston, Texas 77018
Mr. M. W. Held - 713-686-0598.

16222

DOW 2896422

Sub-contractor: _____

Contract applicator: _____

Weed commissioner: _____

Farmer applicator: _____

Other: _____

Equipment used– method of application (ground, air, boom or gun, nozzle, pressure, chisel, tarp, etc.): Pressure

– method of mixing (nurse tank or single batch): Spray from Boat

Is equipment used for application of other pesticides? _____

Application Site:

	Acres		Cropland		Acres
Utility ROW:	( ) _____		Planted: _____	Crop: _____	_____
Highway ROW:	( ) _____		Fallow: _____	Future Crop _____	_____
Railroad ROW:	( ) _____		Grassland _____	Grass Species _____	_____
Ind. Plant Site:	( ) _____				

Other: ( ) (Acres or size of structure): Buffalo Camp Bayou - spot spray for control of aquatic weeds

Organism Affected:

Human*: _____ Livestock: Cattle Wildlife: _____ Ornamental: _____

Crop: _____ Specific identification: _____

Number (plants, acres, animals, etc.): 3 Abortions Dollar value claimed: None

Proximity to treated area: Cattle drink from bayou

**Notify Dow Medical Director (Dr. H. L. Gordon): 2030 Dow Center, The Dow Chemical Company, Midland, Mich 48640- Phone (517)636-2105*

Suspected Mechanism of Movement:

Drift: _____ Volatility: _____ Water movement: 40,000 gal./minute Soil movement: _____

Crop residue: _____ Manure: _____ Faulty Appln. Equip.: _____ Other: _____

Field History (previous years):

Suspected pesticide: X Date Applied: _____

Other pesticides: X Date Applied: _____

Disease, insect or other pests: _____

Fertilizer (analysis): X Source: _____

Source of drinking water, if sick or dead animals involved Bayou where weeds were sprayed

Previous crops grown in field -

Soil type - Type of irrigation employed -

16223

Summary of Possible Contributing Factors Not Covered Above (including competence of applicator, other pesticides applied in area, and opinion of writer as to cause):

Mr. Hand is a retired Dow employee, living on 54 acres adjacent to Buffalo Camp Bayou. He has 16 cows and 1 bull that pasture on the area and drink from the bayou. The bayou carries water to the canal that supplies water to the Dow plant. It is a practice of the Water Supply Department of Dow to spot spray weeds in this bayou each summer.

Mr. Hand stated he has had 4 or 5 calves die soon after birth over a several year period, and had 3 abortions this year.

1970 - Bayou sprayed on June 29 and 30. There was 1 calf died soon after birth in the fall, several months after spraying.

1971 - Spraying was done on June 21, 24, and 25. Calves lost:

1	June 5	lived short while
1	June 27	about 6 mo.
1	July 9	" "

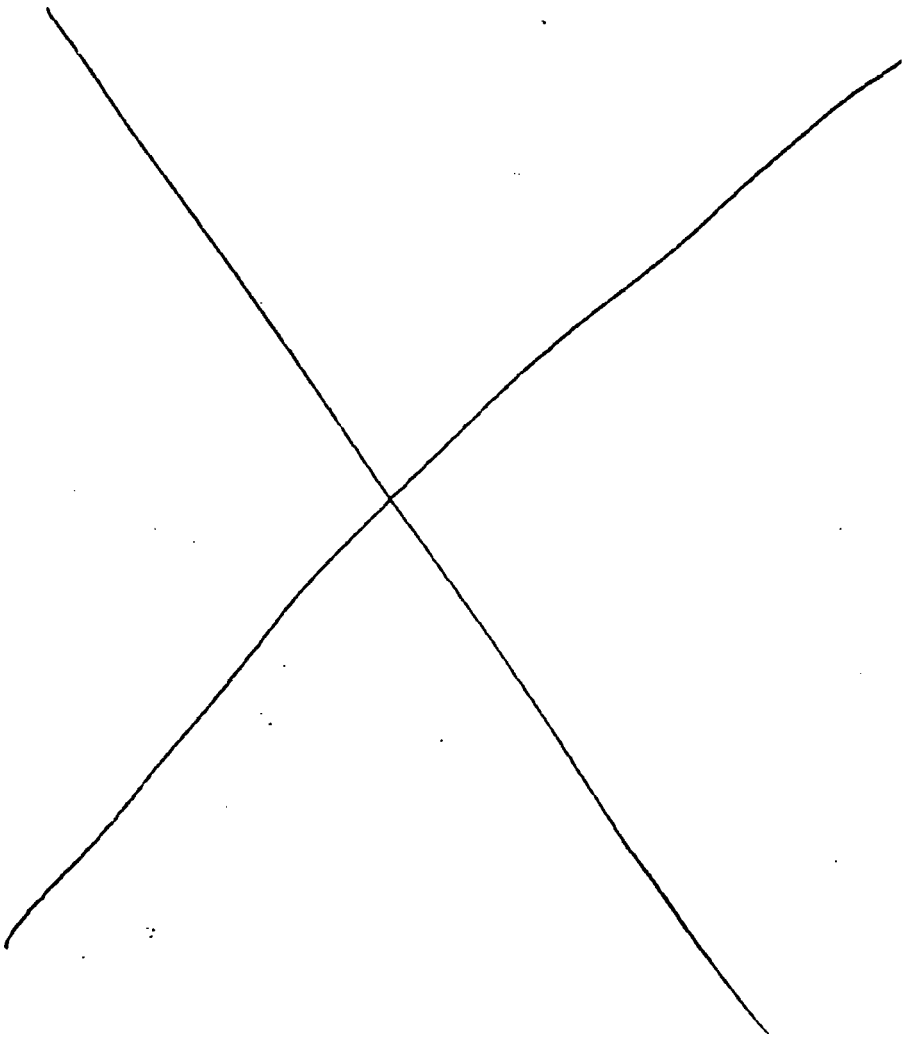
I discussed the happenings with Mr. Hand and thanked him for reporting to us. However, I do not think Kuron was the cause of his problem and Mr. Hand really doesn't either. Various causes of abortion were discussed, and it was suggested that he consult his veterinarian with the possibility of checking blood samples. He agreed to do this and thanked us for our interest, and will report their findings to us later.

DOW 289643

DOW 289641

SKETCH (to include the following in respect to area showing injury):

(1) Location of treated area showing damaged and non-damaged areas.
(2) Direction of surface water drainage.
(3) Location and flow of nearby streams and/or drainage ditches (with distances).
(4) Prevailing wind direction.
(5) Include location of other sensitive crops in the area and indicate whether or not affected.
(6) Have photographs taken, if appropriate, showing overall effect and closeup of alleged plant disorder.



Summary of Disposition of Claim or report:

Samples (samples to be taken only as a last resort). If independent laboratory, obtain:

Name _____ Address _____

Taken	Analized	Analytical results:
Soil	_____	_____
Water	_____	_____
Plant	_____	_____
Animal	_____	_____
Wildlife	_____	_____

Settled, closed: _____ Nature of settlement: None

Complaint active: _____ Recommended action: None

Mr. Hand, being a former Dow employee, said he thought we would be interested in checking any possible relation of our product to his problem. I assured him we were interested and again thanked him for calling us.

DOW 289643

16226

2878 GN 022777

Chloracne: the chemical disease

Chloracne is caused only by contact with chlorinated organic chemicals—it is truly a disease of modern civilisation. Although sometimes disfiguring, it is, in itself, not serious. But it could be a vit indication that sufferers have been exposed to chemicals with other, much more serious, effects





Dr Kenneth Crow is consultant dermatologist at Princess Margaret Hospital, Swindon and is a leading world authority on chloracne

One cannot usefully discuss chloracne without considering the chemistry, toxicology, and even the environmental impact of the chemicals which cause the disease. Chloracne is a cutaneous eruption which is generally symptomless but sometimes disfiguring. Its significance is as a marker, an indicator of the presence of a chemical which is likely to be toxic in other ways and may well have environmental effects. When chloracne has sounded its warning, our whole attention should be directed to these other, more serious, problems. They may not be present—indeed generally are not—but until there is conclusive proof to the contrary, chloracne should always be regarded as a sign of potential systemic poisoning and environmental hazard. Chloracne is produced by skin contact, ingestion or inhalation of certain chlorinated aromatic hydrocarbons. All known "chloracneigenic" chemicals can pass through the intact skin, but in doing so their toxic effect is lessened, so that systemic poisoning from skin contact alone is usually accompanied by very severe chloracne.

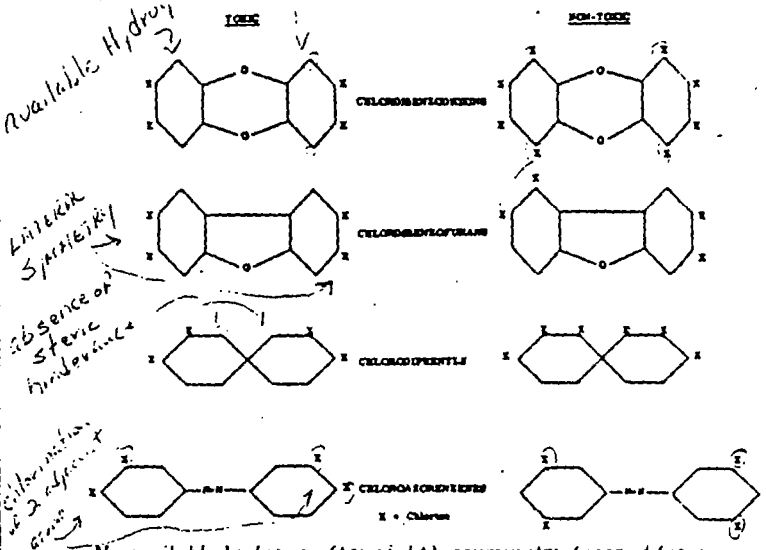
Clinically, chloracne is an eruption of blackheads, usually accompanied by small pale yellow cysts which, in all but the worst cases, are from pinhead to lentil size (above, left). In severe cases there may be inflammatory papules and even pustules (a papule is a spot, a pustule is a papule containing pus). The distribution of chloracne over the body is interesting: the disease has a predilection for the skin of the face, especially in a crescent outside and under the eyes (the malar crescent) and behind the ears. Thes areas are frequently affected when every other part of th skin is normal. The remainder of the face, neck, shoulder genitalia, chest and lower trunk can also be involved (i that order). The hands, feet and legs are rarely involve and then only in the worst cases. In cases of exception severity unusual findings may be encountered. Fo example, every single "hair" follicle in affected areas ma become a blackhead or acne cyst (above, right). In rare cases the entire body surface may be covered with sma horny spines like a nutmeg grater and in others generalise hyperpigmentation may occur to such an extent that th patient's racial origin may be in doubt.

There is every reason to believe that the response o the human skin to the same dose of causative chemical ma differ greatly in different areas of the body but this h never been tested experimentally. In mild cases, chloracn may closely resemble adolescent acne but the curiou concentration of blackheads and cysts round the eyes an on the ears is specific to chloracne. Its duration depen greatly on severity—the worst cases may still have activ skin lesions 15 years after contact with the chloracneigen chemical has ceased. Mild cases, however, clear with months; after three years all but a "hard core" of 20 p cent of cases are likely to have resolved themselves. Th only permanent skin effect is scarring, which may n occur at all in mild cases but in the worst is very sever

Apart from the human subject, chloracne (or somethi very like it) has been produced in only three experiment animals; the rhesus monkey (on the face, with loss of ha

16227

DOW281057



No available hydrogen (top right), asymmetry (second from top, right), masking of the reactive bridge by chlorine atoms (third from top, right), and unadjacent chlorine atoms (bottom right) all make the chemical relatively non-toxic

(causing steric hindrance) and the absence of adjacent chlorines all result in the relative non-toxicity of the compounds on the right of Figure 3.

Usually short- and medium-term toxic effects are only seen in heavily poisoned subjects with severe chloracne and, with few exceptions, have been produced by polychlorinated dibenzodioxins and polychlorinated dibenzofurans. The worst incident of poisoning by chloracneigenic chemicals was the Yusho incident in Japan in 1968, when over 1000 people were accidentally poisoned by consuming a rice-based cooking oil contaminated with a polychlorinated biphenyl (PCB) which contained an abnormally high amount of polychlorinated dibenzofurans. It is now believed that the dibenzofurans were largely responsible for the toxic effects. The symptoms of severe poisoning are known in detail only for TCDD and the PCDF/PCB mixture in the Yusho accident. The two chemicals differ little in their target organs. Chloracne, disturbances of the central and peripheral nervous systems, wasting, hepatic damage and hyperlipaemia (high blood lipid levels) have been most prominent. Recent experimental work revealing that TCDD, although known to cause birth defects, is in fact far more directly toxic to the fetus, is borne out by the history of pregnant Yusho victims: there was an excessive incidence of still-births in this group but no congenital malformations caused by exposure to the dioxin-like PCDFs. PCBs are known to be fetotoxic but not teratogenic.

An interesting toxic manifestation is porphyria—a metabolic abnormality in which the pigment porphyrin is retained in the tissues. For years porphyrin has been known to be associated with severe TCDD poisoning in man and can be produced in experimental animals by TCDD and PCBs—even pure (PCDF-free) PCB isomers, especially the 3,4,5,3',4',5' hexachlorobiphenyl. It now seems most likely that porphyria may be due to an effect on the enzyme delta-aminolaevulinic acid synthetase, the enzyme that limits the rate of porphyrin production. This enzyme is strongly induced *in vitro* by TCDD and it seems likely that the PCB isomers which strongly induce AHH also induce the synthetase enzyme.

Mild chloracne, such as that suffered after the much-publicised Seveso accident in Italy in July 1976, does not appear to be accompanied by short-term systemic effects and surveys of the Seveso population one year after the accident confirm this hypothesis. The absence of teratogenic effects at Seveso is as expected in view of the finding already quoted. The Seveso cases were most confusing because the initial damage was due to chemical the airborne dust consisting of particles of the released by the accident. This dust produced t and swelling seen in so many of the cases after sion. As the burns faded, so the chloracne ap almost every case it has been mild. Contact wit these cases appears to have been mainly exte firmed by the fact that (apart from the classic fa already described) only externally exposed are body were affected. All chloracneigens appe absorbed through the skin, although the extent tion varies greatly. Some systemic level of the invariably occur. But such systemic concentratio are unlikely to cause problems, except possibly i term, when much depends on rate of metabolism.

There is no possibility of consumer poisoning c mild chloracne from manufactured products, b the chemical industry the risk of chloracne although much diminished as awareness of the increases and as diagnosis (especially in mild c comes more accurate. From the environmental st the risk of low dose, long-term effects—in particul and damage to genetic material (mutagenicity)—, ably more important. Poorly metabolised materials a hazard in the long term even when the dose is sm

Experimental data on the long-term effects o acneigens on animals are limited to the rat and When fed to rodents in large doses throughout th time, PCBs produce liver tumours. The results of study in which varying amounts of TCDD were fed throughout their life revealed that tumours were only by a dose large enough to produce other evid chronic toxicity in various organ systems. Lower which did not appear to affect the health of the a produced no tumours. This suggests a dose-relatec Of further interest is a careful follow-up of 70 me severely poisoned by TCDD 24 years ago in an inc accident. So far there has been no evidence of ex or unusual malignant disease. In short, it seems p that TCDD and PCBs may be carcinogenic to man o high doses. There remains the possibility of addit synergistic effects between mild carcinogens, as w co-carcinogenesis, when a non-carcinogen can be act by interaction with other chemicals. As yet noth known about these phenomena among chloracneigen these are under intensive scrutiny following the recer covery of PCDFs in a wide variety of materials.

Controlling the risks

What can the chemical industry do to reduce the of chloracneigenic chemicals? One priority must be t lish and disseminate toxicological information v throughout the industry. Otherwise costly and le research would be wasted. Secondly, because acne potential in a chlorinated or brominated aromatic stance appears to imply various other toxicities whicl be serious and of environmental significance. I believ the simple, but extremely sensitive, rabbit ear test s become part of the standard battery of toxicological applied to every new substance (especially herbicide pesticides). If a contaminant is responsible for the a it may be possible to eliminate it altogether or reduc negligible levels (providing that long-term low dose have been adequately assessed). The headlong ru produce new chemicals after the Second World War that a great many new substances were inadeq examined toxicologically. Increasing awareness on th of the major manufacturers and government research tutions, especially in the US, makes such problems le less likely to recur.

* This article is based on a paper delivered last week to the annual meeting of the Occupational Hygiene Society.

16228

 DOW 281056

and disruption of glands in the eyelids—Meibomian glands—as in the human subject); the hairless mouse, and, most important of all, on the inner surface of the rabbit's ear. It is not possible to produce chloracne on any other part of the rabbit's skin. Indeed, even systematic absorption of chloracneigens by the rabbit produces chloracne only on the inner surfaces of the ears. Thus there is a clear parallel to the curious sensitivity of the malar crescents and ears in the human subject. The rabbit's ear appears to be the most sensitive biological surface known, reacting to a total dose of the most potent chloracneigenic chemicals as small as one microgramme. Thus the rabbit's ear affords a simple, easy and relatively cheap method of identifying chloracneigenic materials. The biological basis of chloracne, and in particular of its predilection for certain areas of the body, is quite unknown. Investigation of the disease is hampered by the lack of an analytical method accurate and sensitive enough to estimate quantitatively chloracneigenic chemicals which may be present in blackheads and cysts as opposed to other skin tissues.

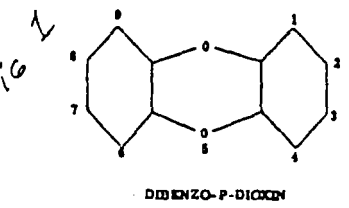
The chlorinated aromatic hydrocarbons which are known to have produced chloracne in humans are

- chlornaphthalenes
- commercial polychlorinated biphenyls (PCBs)
- polychlorinated dibenzofurans (PCDFs)
- polychlorinated dibenzodioxins (PCDDs)
- tetrachloroazobenzene (TCAB) and tetrachloroazoxybenzene (TCAOB).

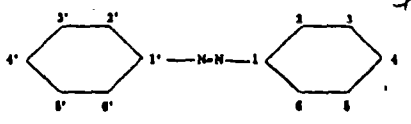
With the exception of the chlornaphthalenes and PCBs, all these compounds are contaminants formed accidentally during the manufacture of other chemicals. For example, although the PCBs have been shown to have a toxicity of their own, in their commercial form they are always contaminated by varying amounts of the much more toxic PCDFs. Again, the manufacture of 2,4,5 trichlorophenol produces as a contaminant the most toxic and chloracneigenic small molecule known, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) of Seveso fame. Crude pentachlorophenol production, as recent research has revealed, leads to the formation of a range of PCDF impurities. The latter have also recently been discovered in crude hexachlorbenzene. The contaminants TCAB and TCAOB occur during the chemical reduction of dinitrochlorbenzene to the corresponding diphenyl hydroxylamine and dichloroaniline, both industrially significant intermediates. TCAB and TCAOB are never found in the final products and exposure therefore occurs only in the chemical industry. Little is known of the detailed chemistry of the commercial chlornaphthalenes. Whether their toxicity is due to contaminants or the pure chlornaphthalenes is unknown.

The acneigenic potential of a chemical appears to be directly related to its overall toxicity. The importance of this fact is the implication that chloracne in an experimental animal can provide a very important screening test for systemic (not only skin) toxicity. This appears to hold true for all known chloracneigens with the exception of 3,4,3',4' tetrachloroazobenzene and possibly azoxybenzene. Recent work reveals a vast difference between the oral and cutaneous toxicity of these chemicals, presumably because of the well-known susceptibility of the azo bond to destruction in the mammalian gut.

Certain basic elements of their chemical structure determine the toxicity of chloracneigens. The crudest of all factors is the degree of chlorination. It appears that a



DIBENZO-P-DIOXIN



AZOBENZENE

How carbon atoms in aromatic hydrocarbons are numbered

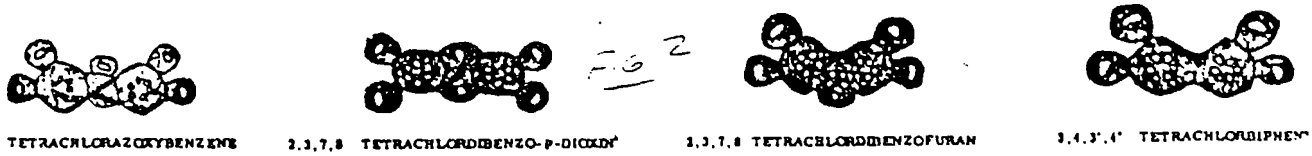
chlorine content somewhere between maximum and minimum produces the greatest toxicity. For example, the dioxins can have from one to eight chlorine atoms. Less than four, or more than six, chlorine atoms in a dioxin molecule greatly reduce toxicity: between four and six produce highly toxic compounds. However, the most important chemical property determining toxicity is isomerism, that is, not just the number of halogen atoms but their exact position in the molecule. By convention these positions are allotted a number (see Figure 1). Thus we may have 2,3,7,8 and 1,2,3,4 tetrachlorodibenzo-p-dioxins or, similarly, 3,4,3',4' and 3,5,3',5' tetrachloroazobenzene. The toxic potential of these isomer pairs varies so greatly that we must be able to distinguish them. The detailed study of as many isomers as possible is essential. Successful work in this field depends entirely on the chemist's ability to synthesise the various isomers in pure form. Once these are available as standards, modern instrumentation provides a means of relatively rapid, accurate and sensitive analysis, not only qualitative but also quantitative.

Simple test-tube test

Ironically, many toxic chemicals initiate their own destruction—they can activate (or "induce") cellular enzymes whose function is to metabolise and detoxify them. These enzymes can be separated from liver cells, a particularly rich source, and used for *in vitro* tests of toxic potential. Arylhydrocarbon hydroxylase (AHH) is the most useful for assessing acneigenic chemicals. The degree and duration of enzyme induction has proved to parallel closely the acneigenic and systemic toxicity of all chloracneigens so far studied, giving us an elegant and simple method for distinguishing toxic from less toxic isomers. On the other hand, *in vitro* tests will not reveal the sometimes great differences in susceptibility between species. Thus animal studies are still necessary but only for a very small number of selected compounds. As a result of recent intensive research with the enzyme induction technique we are now able to make some generalisations about the relationship between structure and toxicity.

Lateral symmetry in the molecule, the chlorination of two adjacent carbon atoms, the presence of available hydrogens and the absence of steric hindrance (where the spatial arrangement of a molecule "hides" reactive atoms, preventing certain reactions) all enhance toxicity. Recent work indicates that all chloracneigenic compounds are either exactly or approximately isosteric (that is, have the same distributions of electrons over the outside of the molecule—see Figure 2). The absence of free hydrogen, lateral asymmetry, chlorination next to the diphenyl bridge

Models show that the distribution of electrons (shading) is practically identical in all chloracneigens



3,4,3',4' TETRACHLORAZOXYBENZENE 2,3,7,8 TETRACHLORODIBENZO-P-DIOXIN 2,3,7,8 TETRACHLORODIBENZOFURAN 3,4,3',4' TETRACHLORODIPHENYL

16229

2885

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
PESTICIDES REGULATION DIVISION
WASHINGTON, D.C. 20250

FORM APPROVED
BUDGET BUREAU NO. 40-R1746

APPLICATION FOR AMENDED REGISTRATION OF ECONOMIC POISONS

(Under the Federal Insecticide, Fungicide, and Rodenticide Act)

1. DATE OF APPLICATION: January 10, 1973

IMPORTANT: READ INSTRUCTIONS ON REVERSE

2. NAME OF ECONOMIC POISON *(Must be same product name as on label—do not list active ingredients)*

KURON

3. NAME & MAILING ADDRESS OF REGISTRANT *(Include Zip Code)*

Dow Chemical U.S.A.
Ag-Organics Department
P.O. Box 1706
Midland, Michigan 48640
Attn: Dr. W. R. Mullison

4. REGISTRATION NO.: 464-162

5. PROPOSED EFFECTIVE DATE OF CHANGE: Immediately

DOW 28982

6. NATURE OF REVISION *(Check applicable item and give details in item 7, when required)*

- GENERAL REVISION OF LABELING [X]
- CHANGE IN FORMULATION [ ] *(Give description of exact change in item 7)*
- OTHER [ ] *(Specify in item 7)*
- ADDITIONAL USES ADDED TO LABELING [X] *(List new recommendations in item 7)*
- CHANGE IN PRODUCT NAME [ ] *(Give old name and new name in item 7)*

7. DETAILS REQUIRED BY REVISION CHECKED IN ITEM 6 *(Attach additional sheets if more space is needed)*

This application for amended registration involves a general label revision. The recommendations have been reorganized into four major headings: Industrial and Non-Cropland Areas, Aquatic Weed Control, Pasture and Rangeland, and Uses in Croplands. The precautionary statements have been expanded and strengthened.

No really new uses have been added but the label does have several recommendations which are practices that have been in common use but were not previously stated on the label. These are: forest conifer release, basal bark and stump, powered knapsack basal treatment, and modified basal treatment. These recommendations are all for Industrial and Non-Cropland areas.

The Aquatic section has been modified slightly to make it shorter and less complex.

[ ] CONTINUED ON ATTACHMENT

8. THE FOLLOWING MUST BE SUBMITTED WITH THIS APPLICATION

- Five (5) copies of revised labeling, including any printed or graphic matter which may accompany the sale of this product. Copies must be clearly legible and identical.
- If a change in formulation is involved, five (5) copies of a statement of revised formula showing the precise name and percentage of each active and each inert ingredient. *(This information is treated confidentially)*
- When appropriate, three (3) copies of Supporting Data.

IN ANY CORRESPONDENCE ON THIS PRODUCT REFER TO REGISTRATION NO. IN ITEM 4, ABOVE.

9. SIGNATURE OF AUTHORIZED FIRM REPRESENTATIVE

W. R. Mullison

10. TITLE: Registration Specialist

11. DATE SIGNED: 1/10/73

PR FORM 9-198
MAY 1969

EXISTING STOCK OF PR FORM 9-198 (AUG. 1968) WILL BE USED UNTIL EXHAUSTED

APPLICANT'S COPY

16230

ITEM

INSTRUCTIONS FOR PR FORM 9–198

1. Applications should be submitted as far in advance as possible prior to desired registration date. The time required to process applications may vary depending on the extent of review required. Applications which require consultation with other governmental agencies will take a longer time to process.

2. The name of the economic poison shown in the application must be the same product name as that shown on the labeling submitted. Do not list the active ingredients.

3. The name of the firm or person and address shown in your application is the person or firm to whom registration will be issued. If you are acting in behalf of another party, you must submit authorization from that party to act for them in registration matters. The address given in item three will be the mailing address permanently on record unless changed by the registrant.

4. The registration number assigned must appear on the label. The number must be the same as that appearing on the notice of registration and shall be preceded by the phrase "USDA Registration Number" or the phrase "USDA Reg. No." (Refer to Sec. 362.6(F) of the Regulations)

5. Changes in the labeling or changes in the formula must be submitted in advance of the proposed effective date.

6. Nature of revision — The registrant must describe the exact changes desired and upon request must submit a description of test results to justify such changes. Minor changes in formulation may be handled as revisions of existing registrations. This might include a change in the percentage of active ingredients or a change in inert ingredients. However, any basic change in formulation such as a change in the principal active ingredients would require a separate registration as a new product with a different product name.

7. Self Explanatory.

8. Labeling — All copies of labeling must be clearly legible and identical. Photo copy of lithograph labels should be submitted. Do not send containers. Your supplier should be able to furnish photo copies of the screen print. Submit reduced photo copies of labeling for dry agricultural fertilizer - insecticide, or herbicide bags. Any label smaller than half the size of a sheet of paper 8 inches by 10 inches should be stapled to a sheet of paper 8 inches by 10 inches before attaching to application.

Formulation — If a change in formulation is involved, five copies of a separate statement listing the correct name and percentage by weight of each active and each inert ingredient must be submitted with each application. This statement is treated confidentially. It cannot be used to support a customer's (formulator) application for subsequent registration without written authorization from the registrant. If necessary information on the formula is not available to the applicant (formulator or packer), he should: (a) Obtain the necessary information from the basic supplier and submit five copies of the statement with the application or (b) Request the basic supplier to furnish the necessary information to the Division along with a statement specifically authorizing the use of such information to support separate registration for the applicant. The statement from the basic supplier must clearly identify the applicant's product to which it applies.

ATTENTION: The submission of an application does not constitute registration. Comment or notice of registration will be sent after examination by the Pesticides Regulation Division of the information submitted.

DOW 289283

16231

2895
DOW018159
AG 03818

WILLARD AND BILLEE SHOECRAFT

VS.

THE DOW CHEMICAL COMPANY

EXHIBIT C

2,4,5-Trichlorophenol Analytical and Shipment Record.

2,4,5-TRICHLOROPHENOL ANALYTICAL AND SHIPMENT RECORD MICHIGAN DIVISION, DOW CHEMICAL U.S.A. FOR ALL LOTS PRODUCED DURING THE PERIOD MAY 27, 1967 AND MAY 2, 1968 THAT WERE USED IN THE MANUFACTURE OF KURON®.

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
05277	5/27/67	489	309		0.2	0.4	0.8	91.5	0.2	2.2	0.3	<1
06017	6/1/67	489	313		0.2	0.3	1.7	89.7	0.2	1.5	0.2	<1
06067	6/6/67	267	314	<0.5	0.2	0.3	2.5	89.7	<0.5	2.2	<0.5	
06077	6/7/67	489	313	<0.5	0.3	0.3	2.8	89.2	<0.5	2.4	<0.5	<1
06127	6/12/67	489	314	<0.5	<0.5	0.5	3.0	88.1	0.2	2.0	0.4	<1
06137	6/13/67	489	313									
06147	6/14/67	489	309	0.4	0.2	0.4	3.2	90.0	0.3	2.1	0.9	<1
06167	6/16/67	489	314	0.4	0.2	0.6	3.4	89.2	0.3	2.2	1.1	<1
06237-1	6/23/67	267	314	-	0.5	0.6	3.2	90.8		2.8	0.3	<1

16233

DOW 018160

SHIPMENTS

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol
06267	6/26/67	489	309	
06287	6/28/67	489	313	
06287-2	6/28/67	489	314	
06307	6/30/67	489	309	<0.5
07067	7/6/67	489	313	
07087	7/8/67	489	314	
07117	7/11/67	267	309	0.4
07137-1	7/13/67	489	313	0.4
07267-2	7/26/67	267	309	<0.5
08107-2	8/10/67	489	309C	0.3
08157	8/15/67	267	314	<0.5
08157-1	8/15/67	489	313	<0.5

OF TRICHLOROPHENOL

% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
0.4	0.5	3.1	91.0		2.6	0.4	<1
0.5	0.6	2.9	90.5		2.8	0.4	<1
0.4	0.6	3.3	90.0		2.8	0.4	<1
<0.3	0.4	3.0	92.7	<0.2	2.5	0.3	<1
							<1
							<1
0.1	0.4	3.1	90.3	<0.2	2.3	0.1	<1
0.1	0.4	2.8	89.9	0.2	2.2	0.1	<1
0.3	0.6	2.2	92.6	<0.2	1.8	0.4	
0.2	0.3	1.6	88.5	0.2	2.1	0.3	<1
<0.1	<0.5	1.7	87.9	<0.2	1.8	<1.0	<1
< .1	< .5	1.7	89.2	0.2	2.1	<1.0	<1

DOW 018161

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	(PPM) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
08167-2	8/16/67	489	309	<0.5	< .1	< .5	1.3	90.4	0.2	2.2	<1.0	<1
08247	8/24/67	489	313	<0.5	0.4	0.5	2.2	92.3	0.3	2.7	<1	<1
08217	8/21/67	489	314	<0.5	0.5	0.7	2.0	91.5	0.3	2.6	<1	Inter-ference
08287	8/28/67	489	314	<0.5	0.5	0.7	2.4	92.8	0.3	2.6	0.3	<1
08297	8/29/67	489	309	<0.5	1.2	0.5	3.8	89.0	0.2	3.0	0.3	<1
09087	9/8/67	267	309	0.4	0.1	0.4	2.6	89.0	-	2.2	<0.3	<1
09057	9/5/67	489	314	0.2	0.2	0.4	2.5	89.6	0.2	2.1	<0.3	<1
09017	9/1/67	489	313	0.3	0.2	0.4	2.6	89.0	0.2	2.0	<0.3	<1
09117-1 Day	9/11/67	267	294	-	0.2	0.3	2.9	93.5	0.3	2.0	-	<1
09147	9/14/67	489	313	-	0.4	0.3	2.3	93.2	0.2	2.2	-	<1
09167	9/16/67	489	314	-	0.6	0.5	1.8	91.0	0.1	2.6	<0.5	<1
09207-1	9/20/67	489	313	-	1.1	0.5	2.1	90.4	0.2	2.7	<0.3	<1

16235

DOW 018162

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
09207-2*	9/20/67	267	294		1.0	0.1	1.9	91.7	0.1	2.6	<0.3	<1
09227-1	9/22/67	267	294	<0.1	1.0	0.3	2.5	93.1	0.4	2.9	0.3	<1
09237	9/23/67	489	314	<0.1	1.0	0.5	2.8	92.5	0.3	2.5	0.3	<1
09267-1	9/26/67	489	313-C	<0.1	0.9	0.3	2.9	92.2	0.4	2.3	0.3	<1
09287-1	9/28/67	489	314-C	<0.1	0.7	0.4	2.7	93.2	0.3	1.7	0.2	<1
09297-1	9/29/67	489	313-C	<0.1	0.6	0.3	2.9	92.2	0.4	2.3	0.2	<1
10037-1	10/3/67	489	314-C	<0.1	0.7	0.8	2.8	90.0	0.1	3.1	<0.3	<1
10057-2	10/5/67	267	294-D	<0.1	0.5	0.8	2.7	91.0	0.1	2.7	<0.3	<1
10067-1	10/6/67	267	294-D	<0.1	0.8	0.3	3.1	89.9	0.3	2.4	<0.3	<1
10067-2	10/6/67	489	309-C	<0.1	0.3	0.5	3.3	90.0	0.2	2.3	<0.3	<1
10067-3	10/6/67	267	294-D	<0.1	0.6	0.4	3.3	90.1	0.2	2.0	<0.3	<1

162338

DOW 018163

SHIPMENTS OF

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol
10097-1	10/9/67	489	313-C	<0.1
10097-2	10/9/67	267	294-D	<0.1
10127-1	10/12/67	267	309-C	<0.1
10137-1	10/13/67	489	314-C	<0.1
10167-1	10/16/67	489	313-C	<0.1
10187-1	10/18/67	489	314-C	<0.1
10217-1	10/21/67	489	313-C	<0.1
10237-1	10/23/67	489	309-C	<0.1
10257-1	10/25/67	267	314-C	<0.1
10287-1	10/28/67	489	313-C	<0.1
10317-1D	10/31/67	489	309-C	<0.1

1328

TRICHLOROPHENOL

% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
0.5	0.5	2.7	89.6	0.2	2.0	<0.3	<1
0.5	0.3	2.9	92.1	0.2	1.9	<0.3	<1
0.6	0.3	2.4	90.0	0.1	1.8	<0.3	<1
0.7	0.4	2.5	91.2	0.2	2.2	0.3	<1
0.7	0.3	2.9	90.5	0.2	2.4	0.3	<1
0.6	0.3	2.7	90.5	0.2	2.1	0.3	<1
0.4	0.2	2.3	91.8	0.2	2.4	<0.3	
0.3	0.2	2.7	90.8	0.2	2.3	<0.3	<1
0.4	0.4	3.4	89.7	0.3	2.3	<0.3	<1
0.6	0.4	3.6	88.9	0.3	2.0		
0.6	0.3	4.0	88.3	0.3	2.4	<0.3	<1

DOW 018164-

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,4,6-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
11027-2D	11/2/67	489	313-C	<0.1	0.6	0.3	4.3	89.5	-	0.4	2.3		<1
11067-1D	11/6/67	489	309-C	<0.1	0.1	0.5	4.4	89.7	-	0.3	2.1	-	<1
11097-1F	11/9/67	267	313-C	<0.1	0.5	0.5	4.6	91.8	-	0.3	2.3	0.3	<1
11137-1F	11/13/67	489	309-C	<0.1	0.2	0.4	3.3	91.0	-	0.2	2.1	<0.5	<1
11247-1D	11/24/67	489	309-C	-	0.4	0.3	2.6	90.7	2.8	0.2	2.0	0.2	<1
11257-1D	11/25/67	267	313-C	-	0.6	0.3	2.4	89.8	3.5	0.2	2.2	0.3	<1
11277-1D	11/27/67	489	314-C	-	0.6	0.3	2.4	90.2	3.5	0.2	2.3	0.2	<1
12047-1D	12/4/67	489	314-C	-	0.6	0.3	2.7	90.5	-	0.3	2.2	0.3	
12087-1D	12/8/67	489	309-C	-	0.6	0.3	2.4	90.7	-	0.3	2.1	0.3	<1
12097-1D	12/9/67	489	313-C	-	0.6	0.3	2.5	90.5	-	0.2	2.3	0.3	<1
12107-1D	12/10/67	489	314-C	-	0.6	0.3	2.4	91.3	-	0.2	2.4	0.3	<1

16238

DOW0181657

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
12147-1D	12/14/67	489	314-C	<0.5	0.6	0.3	2.4	89.8	0.3	2.3	0.3	<1
01128-1D	1/12/68	489	313-C	-	0.6	0.7	2.7	90.2	0.2	2.4	0.3	<1
01138-1D	1/13/68	489	309-C	-	0.7	0.7	2.9	90.2	0.2	2.3	0.3	<1
01158-1D	1/15/68	489	314-C	-	0.7	0.7	3.0	89.3	0.2	2.3	0.2	<1
01188-1D	1/18/68	489	313-C	<0.5	0.8	0.6	3.3	90.5	0.2	2.3	0.3	<1
01218-1D	1/21/68	267	314-C	<0.5	0.7	0.7	2.8	91.2	0.2	2.2	0.3	<1
01278-1D	1/27/68	489	309-C	-	0.7	0.6	2.8	89.3	0.2	2.3	0.3	<1
01298-1D	1/29/68	489	313-C	-	0.7	0.7	2.8	90.7	0.2	2.3	0.4	<1
02038-1D	2/3/68	489	314-C		0.7	0.7	2.6	91.8	0.2	2.1	0.4	<1
02078-1	2/7/68	489	309-C	<0.1	0.6	0.5	2.8	91.7	0.2	2.2	<0.1	<1

16239

DOW018166

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
02108-1	2/10/68	489	314	<0.1	0.6	0.5	2.2	92.0	0.1	2.0	0.3	<1
02118-1	2/11/68	267	313	<0.1	0.6	0.7	2.2	91.0	0.1	1.9	0.3	<1
02168-1	2/16/68	489	314	<0.1	0.5	0.6	2.3	92.2	0.1	2.1	0.3	<1
02208-1	2/20/68	489	GATX 309-C	<0.1	0.6	0.6	2.4	91.9	0.2	2.2	0.3	<1
02288-1	2/28/68	489	314	<0.1	0.6	0.6	1.2	92.5	0.1	2.3	0.3	<1
03098-1F	3/9/68	267	309		0.6	0.1	2.5	91.6	0.2	2.0	0.3	<1
03208-1F	3/20/68	489	313		0.6	0.5	2.1	92.9	0.2	2.2	0.3	<1
03228-1F	3/22/68	489	314		0.6	0.5	2.3	92.4	0.2	2.2	0.4	<1
03268-1	3/26/68	489	309		0.6	0.5	2.3	92.2	0.3	2.0	0.3	<1
03288-1F	3/28/68	489	314									<1
03308-1	3/30/68	489	313		0.7	0.9	2.4	92.0	0.2	2.3	0.3	<1

DOW018167

162496

SHIPMENTS OF TRICHLOROPHENOL

LOT	DATE	BLDG	CAR	% 2,4-dichlorophenol	% 2,5-dichlorophenol	% 2,3,4-trichlorophenol	% 2,3,6-trichlorophenol	% 2,4,5-trichlorophenol	% 2,3,5,6-tetrachlorophenol	% 2,4-dichloro-5-methoxy phenol	% 4,5-dichloro-2-methoxy phenol	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (PPM)
04028-1	4/2/68	489	314		0.7	0.6	2.4	89.9	0.1	2.4	0.3	<1
04058-1F	4/5/68	489	309		0.6	0.6	2.2	91.7	0.1	2.3	0.3	<1
04098-1F	4/9/68	489	314		0.7	0.6	2.3	91.5	0.2	2.5	0.3	<1
04118-1	4/11/68	267	313		0.7	0.6	2.3	92.7	0.2	2.6	0.3	<1
04178-1F	4/17/68	489	GATX 70118		0.7	0.5	2.5	93.6	0.2	2.4	0.4	<1
04188-1F	4/18/68	267	314		0.7	0.6	2.5	91.7	0.2	2.5	0.2	<1
04228-1	4/22/68	489	313		0.5	0.6	2.1	93.2	0.2	2.3	0.3	<1
04268-1D	4/26/68	267	309		0.6	0.6	2.2	93.3	0.1	2.5	0.3	<1
04298-1D	4/29/68	489	314		0.6	0.6	2.2	90.6	0.1	2.2	0.3	<1
05018-1D	5/1/68	489	GATX 70116		0.6	0.6	2.2	92.1	0.1	2.2	0.4	<1
05028-1D	5/2/68	267	313		0.6	0.6	1.9	93.8	0.1	2.1	0.4	<1

DOW 0181681

DOW01816

...TA DICHLOROPHENOL SHIPMENTS
ANALYSIS RESULTS

LOT NO.	PLANT	DIP	2,4 Cl_2	2,5 Cl_2	2,3,+ Cl_3	2,3,6 Cl_3	2,4,5 Cl_3	2,3,5,6 Cl_4	A	B	D
03088-2	Check on 02298-1			0.7	0.2	2.4	91.8	0.1	2.0	0.3	
✓ 03098-1F	267	309		0.6	0.1	2.5	91.6	0.2	2.0	0.3	<
03128-1F	265	Pipe		0.6	0.5	2.2	91.8	0.1	2.2	0.4	<
03138-1	349	Pipe		0.6	0.5	1.5	92.7	0.1	2.1	0.3	
03138-2F	441	1212		0.6	0.5	2.1	92.5	0.1	2.1	0.4	<
03148-1F	441	1212		0.6	0.5	2.1	91.7	0.1	2.2	0.3	<
03158-1F	265	Pipe		0.6	0.5	2.0	92.4	0.1	2.2	0.3	<
03198-1F	265	Pipe		0.6	0.5	2.2	92.1	0.1	2.1	0.3	<
✓ 03208-1F	489	313 +		0.6	0.5	2.1	92.9	0.2	2.2	0.3	<
✓ 03228-1F	489	314		0.6	0.5	2.3	92.4	0.2	2.2	0.4	<
✓ 03268-1	489	309		0.6	0.5	2.3	92.2	0.3	2.0	0.3	<
03268-2	Check on 03088-1			0.6	0.5	1.9	92.6	0.1	2.5	0.4	-
03268-3	Check on 03208-1F	+		1.0	0.5	2.1	91.7	0.2	2.5	0.3	
03278-1F	265	Pipe		0.7	0.6	1.9	92.8	0.1	2.1	0.2	<
✓ 03288-1F	489	314									<
03298-1F	265	Pipe		0.7	0.6	2.6	93.0	0.2	2.6	0.3	<
✓ 03308-1	489	313		0.7	0.9	2.4	92.0	0.2	2.3	0.3	<
✓ 04028-1	489	314		0.7	0.6	2.4	89.9	0.1	2.4	0.3	<
04038-1F	Tiger Hall	GATX 70117		0.6	0.6	2.1	92.8	0.1	2.0	0.3	<
04048-1F	441	1212		0.7	0.6	2.3	91.0	0.2	2.5	0.3	<
✓ 04058-1F	489	309		0.6	0.6	2.2	91.7	0.1	2.3	0.3	<
04058-2F	441	1212		0.7	0.6	2.1	91.0	0.2	2.2	0.3	<
✓ 04098-1F	489	314		0.7	0.6	2.3	91.5	0.2	2.5	0.3	<
✓ 04118-1	267	313		0.7	0.6	2.3	92.7	0.2	2.4	0.3	<
✓ 04178-1F	489	GATX 70118		0.7	0.5	2.5	92.6	0.2	2.4	0.4	<
✓ 04188-1F	267	314		0.7	0.6	2.5	91.7	0.2	2.5	0.2	<
04195-1	Check on 04178-1F			0.6	0.5	2.3	92.2	0.1	2.6	0.3	-
04203-1F	Check on 04188-1F			0.7	0.6	2.4	92.3	0.2	2.5	0.2	-
✓ 04228-1	489	313		0.5	0.6	2.1	93.2	0.2	2.3	0.3	<
04233-1F	265	Pipe		0.5	0.5	2.1	93.2	0.1	2.6	0.2	<
04243-1	Tiger Hall	GATX 70110		0.6	0.6	2.2	93.3	0.2	2.5	0.3	<
✓ 04268-1D	267	309		0.6	0.6	2.2	93.3	0.1	2.5	0.3	<
✓ 04298-1D	489	314		0.6	0.6	2.2	90.6	0.1	2.2	0.3	<
04308-1F	265	Pipe		0.6	0.6	2.2	91.8	0.1	2.3	0.3	<
✓ 05018-1D	489	GATX 70118		0.6	0.6	2.2	92.1	0.1	2.2	0.3	<
✓ 05028-1D	267	309		0.6	0.6	1.9	92.8	0.1	2.1	0.3	<

16242

DOW018170

SHIPMENTS OF TRICHLOROPHENOL

LOT	BLD	CAR	2,4 Cl_2	2,5 Cl_2	2,3,4 Cl_3	2,3,6 Cl_3	2,4,6 Cl_3	2,3,5 Cl_3	A	B	DIOXIN	
05267	441	1212									<1	
05277	489	309		0.2	6.4	0.8	91.5	0.2	2.2	0.3	<1	
05277	265	-	—	<0.5	0.2	3.1	92.2	0.2	2.2	0.3	<1	
05307	265	-	-	0.2	0.2	1.6	90.5	0.2	1.9	0.2	<1	
06017	489	313		0.2	0.3	1.7	89.7	0.2	1.5	0.2	<1	
06057	338	297	<0.5	0.2	0.3	2.5	89.8	<0.5	2.2	<0.5	<1	
06067	267	314	"	0.2	0.3	2.5	89.7	"	2.2	"		
06067	338	297		0.2	0.3	2.7	89.6	<0.5	2.4	<0.5	<1	
06077	338	"										
06077	489	313	"	0.3	0.3	2.8	89.2	<0.5	2.4	<0.5	<1	
06087	338	297	"	0.2	0.4	2.8	88.7	<0.5	2.1	<0.5	<1	
06097	"	"	<0.5	0.6	0.5	2.9	93.0	0.2	2.6	0.4	<1	
06097	441	1212										
06107	338	297	<0.5	0.6	0.4	2.9	91.3	0.2	2.4	0.4	<1	
06117	"	"	<0.5	0.5	0.5	3.2	91.8	0.2	2.5	0.4	<1	
06127	489	314	<0.5	<0.5	0.5	3.0	88.1	0.2	2.0	0.4	<1	
06127	338	297					87.5				<1	
06137	489	313										
06147	489	309	0.4	0.2	0.4	3.2	~~90.0~~ 87.2	0.3	2.2	~~0.9~~ 1.1	<1	
06167	489	314	0.4	0.2	0.6	3.4	87.2	0.3	2.2	1.1	<1	
06187	338	297	<0.5	0.3	0.4	2.1	91.6	0.1	2.6	<0.5	<1	
06197-1	338	"	"	0.3	0.5	2.6	91.2	0.2	2.8	<0.5	<1	
06197-2	Thom Hatuwo	70116	<0.5	0.4	0.7	2.7	90.7	0.2	3.0	<0.5	<1	
06217	339	297	—	<0.2	0.4	3.0	90.2	—	2.2	0.3	<1	
06227	338	297	—	"	"	"	90.3	—	"	"	<1	
06237-1	267	314	—	0.5	0.6	3.2	96.8		2.8	0.3	<1	
06237-2	338	297		0.6	0.6	3.3	91.0		2.7	0.4	<1	
06257	338	297		0.6	0.6	2.6	90.6		2.6	0.4	<1	
06267	489	309		0.4	0.5	3.1	91.0		2.6	0.4	<1	
06287	489	313		0.5	0.6	2.9	90.5		2.8	0.4	<1	
06277	338	294		"	"	"	91.3		2.9	0.3	<1	
06287-1	338	311		"	"	3.3	91.7		2.7	0.3	<1	
06287-2	489	314		0.4	0.6	3.3	90.0		2.8	0.4	<1	
06297-2	338	297		0.4	0.6	3.2	88.6		2.7	0.4	<1	

DOW018171

TRICHLOROPHENOL SHIPMENTS

LOT	BLD	CAR	2,4 Cl_2	2,5 Cl_2	2,3,4 Cl_3	2,3,6 Cl_3	2,4,5 Cl_3	2,3,5,6 Cl_4	A	B
07017	338	297	~~0.05~~	0.2	6.1	2.9	93.0	<0.2	3.0	0.3
07027	338	297	0.5	6.3	0.6	2.6	93.0	<0.2	3.8	0.3
07037	THOM HAY	GATX 70117	<0.5	0.3	0.6	2.8	92.8	<0.2	3.4	0.3
07057	338	297	0.4	0.1	0.4	2.8	88.7	<0.2	3.0	0.3
✓ 07067	~~489~~	313								
06297-1	265	—		0.4	0.8	3.3	88.0	—	2.9	0.4
07067R2	338	297								
✓ 07087	489	314								
07107	338	297	0.4	<0.1	0.5	3.3	90.8	<0.2	2.3	0.1
✓ 07117	267	309	0.4	0.1	0.4	3.1	96.3	<0.2	2.3	0.1
07127	338	297	0.4	0.1	0.4	2.8	92.3	0.2	2.2	0.1
✓ 07137-1	~~489~~	313	0.4	0.1	0.4	2.8	89.9	0.2	2.2	0.1
07137-2	338	297	0.4	0.1	0.4	3.0	90.5	0.2	2.1	0.1
07167-1	THOM HAY	GATX 70117	<0.5	0.3	0.6	2.1	90.0	<0.2	2.6	0.3
07167-2	338	297								
07207	"	"	<0.5	0.3	0.8	2.2	88.8	0.3	2.2	0.3
07217	"	"	<0.5	0.3	0.7	2.4	90.3	~~2.1~~ 0.2	2.1	0.3
07127	265	—								
07267-1	THOM HAY HOUSTON	GATX 70118	<0.5	0.3	0.4	2.3	92.2	<0.2	2.2	0.4
✓ 07267-2	~~338~~ 267	~~297~~ 309	<0.5	0.3	0.6	2.2	92.6	<0.2	1.8	0.4
07267-3	~~338~~ 267	~~304~~ 297	<0.5	0.2	0.8	2.5	92.4	<0.2	2.3	0.4
07277-2	338	294	<0.5	0.2	0.6	1.4	93.8	<0.2	1.9	0.4
07317	338	294	<0.5	0.4	0.4	1.7	93.8	<0.2	2.7	0.3
08017-1	338	297	"	0.2	0.5	1.7	91.5	"	2.6	0.3
08027-1	338	297	"	0.4	0.5	2.0	93.7	"	2.7	0.3
07277-1	349	—	<0.5	0.3	0.5	1.2	92.8	<0.2	2.0	0.4
08037-2	338	297	"	0.4	0.5	2.1	92.3	"	2.7	0.3
08047	338	297	"	0.4	0.5	2.0	93.3	"	2.8	0.3
08107-1	338	297	<0.5	<1.0	<0.5	1.4	90.9	<0.2	2.1	<1.0
✓ 08107-2	~~465~~ 309C	309C	0.3	0.2	0.3	1.6	88.5	0.2	2.1	0.3
08077	THOM HAY	GATX 70113	<0.5	0.4	0.5	2.2	92.7	"	2.5	0.3
08037-1	265	—	<0.5	0.4	0.5	2.1	92.9	<0.2	2.7	0.3
08117	338	294	<0.5	0.1	<0.5	1.5	89.2	<0.2	2.2	<1
08127-1	"	297	<0.5	0.1	<0.5	1.5	90.4	0.2	2.2	1.6
~~08127-2~~	~~465~~	~~313~~								

DOW 018172

Trichlorophenol Shipments

LOT	BLD	CAR	2,4 Cl2	2,5 Cl2	2,3,1 Cl3	2,3,6 Cl3	2,4,5 Cl3	2,3,5,6 Cl4	A	B
✓ 08157	267	314	<0.5	<0.1	<0.5	1.7	87.9	<0.2	1.8	<1.0
✓ 08157-1	489	313	<0.5	<1	<.5	1.7	89.2	0.2	2.1	<1.0
08157-2	338	297	<0.5	<.1	<.5	1.4	90.1	0.2	2.0	<1.0
08167-1	"	291	<0.5	2.1	<0.5	1.8	89.1	0.2	2.0	<1.0
✓ 08167-2	489 THOM	309 GATX	<0.5	2.1	<.5	1.3	90.4	0.2	2.2	<1.0
08167	HAY	70116	0.3	0.4	0.4	2.4	90.3	0.1	2.0	<1.0
✓ 08247	489	313	<0.5	0.4	0.5	2.2	92.3	0.3	2.7	<1
08227	338	297	<0.5	0.5	0.7	2.3	91.2	0.3	2.8	0.3
✓ 08247	489	314	<0.5	0.5	0.7	2.0	91.5	0.3	2.6	<1
08257	THOM HAY	GATX 70111	<0.5	1.0	0.4	2.4	92.0	0.2	2.6	0.3
✓ 08287	489	314	<0.5	0.5	0.7	2.4	92.8	0.3	2.6	0.3
✓ 08291	489	309	<0.5	1.2	0.5	3.8	89.0	0.2	3.0	0.3
08307-2	265 THOM	— GATX								
08307	NAY	70118	<0.5	1.0	0.6	2.1	92.2	0.3	2.6	0.3
09057	349	—	"	0.8	0.4	3.0	91.5	0.2	3.0	0.3
09077	349	—	0.2	6.1	6.8	1.6	91.8	6.1	2.2	<0.3
✓ 09087	267	309	0.4	6.1	6.4	2.6	89.0	—	2.2	<0.3
✓ 09057	489	314	0.2	0.2	0.4	2.5	89.6	6.2	2.1	<0.3
✓ 09017	489	313	0.3	0.2	0.4	2.6	89.0	0.2	2.0	<0.3
09117-2	349	—	—	—	0.3	2.3	93.5	6.2	2.2	—
✓ 09117-1 DUP	267	294	—	6.2	0.3	2.9	93.5	0.3	2.0	—
09127-1	265	—	—	0.2	0.3	2.7	93.4	0.2	2.2	—
09127-2	349	—	—	0.2	0.2	2.3	95.1	0.2	2.2	—
✓ 09147	489	313	—	0.4	0.3	2.3	93.2	0.2	2.2	—
✓ 09167	489	314	—	6.6	6.5	1.8	91.0	0.1	2.6	<0.5
✓ 09207-1	489	313	—	1.1	6.5	2.1	90.4	0.2	2.7	<0.3
✓ 09207-2 *	267 THOM	294		1.0	0.1	1.9	91.7	0.1	2.6	<0.3
09217-1	NAVIGANT	70113		1.0	0.7	2.4	91.7	0.1	2.4	<0.3
✓ 09227-1	267	294	<0.1	1.0	0.3	2.5	93.1	0.4	2.9	0.3
✓ 09237	489	314	<0.1	1.0	0.5	2.8	92.5	0.3	2.5	0.3
✓ 09267-1	489	313-C	<0.1	0.9	0.3	2.9	92.2	0.4	2.3	0.3
✓ 09287-1	489	314-C	<0.1	0.7	0.4	2.7	93.2	0.3	1.7	0.2
✓ 09297-1	489	313-C	<0.1	0.6	0.3	2.9	92.2	0.4	2.3	0.2
✓ 10037-1	489	314-C	<0.1	0.7	0.8	2.8	90.0	0.1	3.1	<0.5
✓ 10057-2	267	294-N	<0.1	0.5	1.8	2.7	91.0			

TRICHLOROPHENOL SHIPMENTS

ANALYSIS RESULTS

SW01817.3

LOT NO.	BLDG	CAR	2,4 Cl_2	2,5 Cl_2	2,3,4 Cl_3	2,3,6 Cl_3	2,4,5 Cl_3	2,3,5,6 Cl_4	A	B	DIC
10057-1	Thomp Hay	GATX 70116	<0.1	0.5	0.8	2.7	91.0	0.1	2.7	<0.3	<
10067-1	267	294-D	<0.1	0.8	0.3	3.1	89.9	0.3	2.4	<0.3	<
10067-2	489	309-C	<0.1	0.3	0.5	3.3	90.0	0.2	2.3	<0.3	<
10067-3	267	294-D	<0.1	0.6	0.4	3.3	90.1	0.2	2.0	<0.3	<
10097-1	489	313-C	<0.1	0.5	0.5	2.7	89.6	0.2	2.0	<0.3	<
10097-2	267	294-D	<0.1	0.5	0.3	2.9	92.1	0.2	1.9	<0.3	<
10127-1	267	309-C	<0.1	0.6	0.3	2.4	90.0	0.1	1.8	<0.3	<
10137-1	489	314-C	<0.1	0.7	0.4	2.5	91.2	0.2	2.2	0.3	<
10167-1	489	313-C	<0.1	0.7	0.3	2.9	90.5	0.2	2.4	0.3	<
10187-1	489	314-C	<0.1	0.6	0.3	2.7	90.5	0.2	2.1	0.3	<
10217-1	489	313-C	<0.1	0.4	0.2	2.3	91.8	0.2	2.4	<0.3	
10237-1	489	309-C	<0.1	0.3	0.2	2.7	90.8	0.2	2.3	<0.3	<1
10107-1	265	Pipe	<0.1	0.5	0.4	2.8	90.5	0.2	1.9	0.3	
10257-1	267	314-C	<0.1	0.4	0.4	3.4	89.7	0.3	2.3	<0.3	<1
10287-1	489	313-C	<0.1	0.6	0.4	3.6	88.9	0.3	2.0		
10307-1	265	Pipe	<0.1	0.6	0.4	3.4	88.9	0.3	2.4	<0.3	
10317-1D	489	309-C	<0.1	0.6	0.3	4.0	88.3	0.3	2.4	<0.3	<1
11027-1D	338	294-D	<0.1	0.5	0.5	4.1	88.8	0.4	2.3	<0.3	<1
11027-2D	489	313-C	<0.1	0.6	0.3	4.3	89.5	0.4	2.3		<1
11027-3F	338	297-D	<0.1	0.2	0.6	4.1	88.0	0.3	2.2		<1
11037-1F	338	294-D	<0.1	0.1	0.5	4.9	88.9	0.3	2.3	—	<1
11067-1D	489	309-C	<0.1	0.1	0.5	4.4	89.7	0.3	2.1	—	<1
11077-1F	Thomp Hay	GATX 70111	<0.1	<0.5	0.5	4.6	90.5	0.3	2.7	—	<1
11077-2F	338	294-D	<0.1	0.4	0.3	4.1	91.8	0.3	2.1	0.2	<1
11097-1F	267	313-C	<0.1	0.5	0.5	4.6	91.8	0.3	2.3	0.3	<1
11097-2F	338	294-D	<0.5	0.1	0.4	3.9	90.2	0.3	2.4	0.2	
11137-1F	489	309-C	<0.1	0.2	0.4	3.3	91.0	0.2	2.1	<0.5	<1
11147-1F	338	294-D	<0.1	0.2	0.4	3.3	91.4	0.3	2.3	<0.5	<1
11147-2F	265	Pipe	<0.1	0.2	0.4	3.6	91.4	0.3	2.3	<0.5	
11157-1F	338	294-D	<0.1	0.2	0.3	3.3	91.7	0.3	2.3	<0.5	<1
11177-1F	338	294-D	<0.1	0.3	0.3	3.2	93.7	0.3	2.3	0.3	<1
11217-1F	338	294-D	<0.1	0.4	0.3	3.1	93.2	0.3	2.4	0.3	<1
11217-2F	265	Pipe	<0.1	0.3	0.4	3.0	93.4	0.3	2.4	0.3	<1
11227-1F	338	294-D	2,4,6 Cl_3 2.6	0.3	0.4	2.7	91.3	0.3	2.4	0.3	<1
11247-1D	489	309-C	2.8	0.4	0.3	2.6	90.7	0.2	2.0	0.2	<1
11257-1D	267	313-C	3.5	0.6	0.3	2.4	89.8	0.2	2.2	0.2	<1

BOM 018171

...TA CHLOROPHE... ...HIMENTS
ANALYSIS RESULTS

Lot No.	Plant	Bag	2,4,6 Cl_3	2,5 Cl_2	2,3 Cl_2	3,5 Cl_2	2,4,5 Cl_3	2,3,5,6 Cl_4	A	B	DI
11277-1D	489	314-C	3.5	0.6	0.3	2.4	90.2	0.2	2.3	0.2	<
11277-2F	Check on 11097-1F		3.5	0.2	0.2	3.6	89.5	0.3	2.4	0.3	—
11287-1F	338	294-D	—	0.5	0.4	3.2	88.8	0.2	2.4	0.1	<
11287-2F	265	Pipe	—	0.5	0.5	2.5	89.3	0.3	2.5	0.1	<
11307-1F	338	294-D	2.4 Cl_3 —	0.3	0.4	3.4	90.5	0.3	2.4	0.3	<
12017-1F	338	294-D									<
12017-2F	265	Pipe	—	0.5	0.3	2.8	90.8	0.3	2.3	0.3	<
12047-1D	489	314-C	—	0.6	0.3	2.7	90.5	0.3	2.2	0.3	
12057-1F	338	294-D	—	0.7	0.3	2.7	90.2	0.3	2.4	0.3	<
12077-1F	Thermo Hat	GATX 70113	<0.5	1.0	0.4	2.5	90.5	0.1	2.8	<0.5	
12087-1D	489	309-C	—	0.6	0.3	2.4	90.7	0.3	2.1	0.3	<
12087-2F	265	Pipe	—	0.6	0.3	2.5	91.2	0.2	2.3	0.3	<
12087-3F	338	294-D									<
12097-1D	489	312-C	—	0.6	0.3	2.5	90.5	0.2	2.3	0.3	<
12107-1D	489	314-C	—	0.6	0.3	2.4	91.3	0.2	2.4	0.3	<
12117-1F	338	294-D	—	0.6	0.3	2.4	90.4	0.2	2.4	0.3	<1
12117-2F	265	Pipe	—	0.5	0.3	2.5	91.5	0.2	2.3	0.3	<
12117-3F	Check on 12097-1F		—	0.6	0.3	2.5	92.3	0.2	2.3	0.3	—
12127-1F	338	294-D	—	0.5	0.3	2.4	91.3	0.2	2.5	0.3	<1
12137-1F	338	294-D	—	0.5	0.3	2.4	90.9	0.2	2.2	0.3	<1
12147-1D	489	314-C	<0.5	0.6	0.3	2.4	89.8	0.3	2.3	0.3	<1
12157-2F	338	294	<0.5	0.7	0.3	2.4	90.5	0.3	2.3	0.3	<1
12207-1F	338	294	"	0.5	0.3	2.4	92.1	0.2	2.3	0.3	<1
12217-1F	338	294	"	0.7	0.3	2.3	90.9	0.2	2.5	0.1	<1
12217-2F	265	Pipe	"	0.5	0.2	2.3	90.8	0.3	2.3	0.2	
12227-1F	338	294	"	0.6	0.3	2.2	91.6	0.2	2.1	0.2	<1
12287-1F	349	Pipe	"	0.3	0.1	1.7	93.3	0.2	2.4	0.3	<1
12287-2F	Therm Hat	GATX 70116	"	0.6	0.2	2.5	91.7	0.3	2.5	0.3	<1
12287-3F	338	294	"	0.3	0.1	1.7	92.0	0.2	2.6	0.3	<1
01028-1F	338	294	"	<0.1	<0.1	0.6	91.8	0.1	2.3	0.4	<1
01038-1F	338	294	"	0.5	0.5	1.7	92.1	0.1	2.4	0.4	<1
01038-2F	349	Pipe	"	0.6	0.6	2.0	92.2	0.1	2.3	0.3	<1
01058-1F	338	294	"	0.6	0.6	2.5	92.7	0.1	2.4	0.4	<1
01068-1F	349	Pipe	"	0.6	0.6	2.0	93.1	0.1	2.4	0.3	<1
01078-1F	265	Pipe	"	0.6	0.6	2.2	92.9	0.1	2.4	0.3	<1

16247

CHLOROPHENOLS ANALYSIS RESULTS

LOT NO.	SAMPLE	DATA	2,4,5 Cl3	2,5 Cl2	2,3,5 Cl3	2,3,4,6 Cl4	2,3,4,5 Cl4	2,3,5,6 Cl4	A	B	D
01088-1F	349	Pipe	<0.5	0.6	0.6	2.1	94.0	0.1	2.4	0.4	<
01098-1F	Thom Hay	GATX 70118	"	0.5	0.4	2.2	92.7	0.1	2.5	0.4	<
✓ 01128-1D	489	313-C	—	0.6	0.7	2.7	90.2	0.2	2.4	0.3	<
01128-2F	265	Pipe	—	0.6	0.6	2.4	90.2	0.2	2.5	0.4	<
✓ 01138-1D	489	309-C	—	0.7	0.7	2.9	90.2	0.2	2.3	0.3	<
✓ 01158-1D	489	314-C	—	0.7	0.7	3.0	89.3	0.2	2.3	0.2	<
01168-1F	265	Pipe	—	0.6	0.6	2.3	90.6	0.2	2.3	0.3	<
✓ 01188-1D	489	313-C	"	0.8	0.6	2.3	90.5	0.2	2.3	0.3	<
✓ 01218-1D	267	314-C	"	0.7	0.7	2.8	91.2	0.2	2.2	0.3	<
01238-1F	265	Pipe	"	0.6	0.6	2.7	92.4	0.1	2.7	0.3	<
01258-1F	Thom Hay	GATX 70117	"	0.6	0.5	2.9	91.0	0.1	2.6	0.3	<
01268-1F	265	Pipe	—	0.7	0.7	2.8	90.5	0.2	2.3	0.3	<
✓ 01278-1D	489	309-C	—	0.7	0.6	2.8	89.3	0.2	2.3	0.3	<
✓ 01298-1D	489	313-C	—	0.7	0.7	2.8	90.7	0.2	2.3	0.4	<
01298-2F	Sample Order		—	0.6	0.6	2.8	90.7	0.2	2.3	0.3	<
02028-1D	265	Pipe									<
02028-2F	Thom Hay	GATX 70113	—	0.7	0.7	2.7	92.3	0.2	2.1	0.4	<
✓ 02038-1D	489	314-C		0.7	0.7	2.6	91.8	0.2	2.1	0.4	<
02038-2D	Check on 01295-1D		—	0.7	0.6	2.6	92.6	0.2	2.0	0.4	—
✓ 02078-1	489	309-C	<0.1	0.6	0.5	2.8	91.7	0.2	2.2	<0.1	<
02088-1	265	Pipe	<0.1	0.6	0.5	2.7	91.8	0.1	2.1	<0.1	<
✓ 02108-1	489	314	"	0.6	0.5	2.2	92.0	0.1	2.0	0.3	<
✓ 02118-1	267	313	"	0.6	0.7	2.2	91.0	0.1	1.9	0.3	<
✓ 02168-1	489	314	"	0.5	0.6	2.3	92.2	0.1	2.1	0.3	<
02168-2	265	Pipe	"	0.5	0.6	2.3	92.2	0.1	2.1	0.3	
02178-1F	Thom Hay	GATX 70116	"	0.7	0.7	2.7	92.0	0.2	2.3	0.4	<
✓ 02208-1	489	GATX 309-C	"	0.6	0.6	2.4	91.9	0.2	2.2	0.3	<
02208-2	Check on 02108-1		"	0.6	0.6	2.4	91.9	0.1	2.3	0.3	—
02238-1	265	Pipe	"	0.6	0.6	2.4	91.9	0.2	2.4	0.4	<
02268-1	265	Pipe	"	0.6	0.3	1.8	92.0	0.1	2.1	0.3	<
02268-2F	Thom Hay	GATX 70116	"	0.2	0.6	1.5	93.3	0.1	2.0	0.3	<
02288-1	489	314	"	0.6	0.6	1.2	92.5	0.1	2.3	0.3	<
02278-1	?	GATX 70 [illegible]	"	0.6	0.3	1.3	92.5	0.1	2.1	0.3	<
03018-1	?	GATX 76069		0.6	1.3	2.2	91.7	0.1	2.1	0.3	
03078-1F	Sample Order										
03058-1	349	Pipe		0.5	0.6	2.1	93.2	0.1	1.9	0.4	—

2917

DOW 3511/43

Reprinted from *WEED SCIENCE*
Vol. 18, No. 6, November, 1970

16249

CCW 531746

Effects of Herbicides on Water and Its Inhabitants[1]

W. R. Mullison[2]

Abstract. There is little evidence that herbicides from agronomic or industrial usage are reaching or accumulating in our water supplies in amounts to cause a pollution problem. Fish tolerance to weed killers varies with their size and species as well as with differences in the aquatic site. There may be variation to different forms of the active ingredient. Other components in a formulation may be more toxic than the herbicide itself. Therefore, the LD_{50} of different formulations should be determined. In addition, the manner and other circumstances involving application of a herbicide may change its toxicity to water inhabitants. Our current knowledge of the effects of herbicides on fish, plankton, and other water inhabitants indicate that harmful effects with our present herbicides, when such exist, are only temporary. Available evidence suggests there is no biological magnification problem with herbicides.

Introduction

Today there is much professional and lay interest in our environment, particularly with respect to pollution problems. The unfavorable subtle effects of dichloro diphenyl trichloroethane (DDT) on certain organisms and its practically universal distribution in our environment have stimulated questions concerning the possibility of similar effects from other agricultural chemicals.

Herbicides are common today and their use is growing rapidly. Increasing concern will be directed toward the effects of weedkillers on non-target organisms as well as the ultimate fate of these chemicals in our environment. Our analytical methods are becoming increasingly sensitive and trace amounts of various herbicides are likely to be found in more and more places. However, interpreting the importance of such small quantities as parts per million and even parts per billion of the weed killers when found in our environment is the real problem. It appears timely, therefore, to survey the possible occurrence of herbicides in water and to summarize present knowledge concerning their effects on fish, plankton, and other water dwellers.

The residual effect of herbicides in the ecosystem that involves water supplies is of great importance. Drainage from watersheds and other areas treated with herbicides might result in secondary effects involving the health of domestic animals, wildlife, aquatic animals, and even man. It has been estimated (90) that more than 80% of the population will depend upon surface water for drinking by 1980. Thus, the prevention of water contamination is of the utmost direct importance to man. With the increasing use of herbicides on non-cropland as well as cropland, the significance of their occurrence in surface water must be evaluated. The accumulation of herbicides, if it occurs, by plankton and water animal could start the biological magnification process first s clearly demonstrated with the insecticide DDT (63 Thus, their effect on fish, plankton, and water crustacean is of considerable consequence.

There are several general references dealing with th toxicity of chemicals, particularly herbicides, to fish Applegate *et al.* (5) and Lawrence (73) both have exten sive fish toxicity data. Three reports from the Fish and Wildlife Service (135, 136, 137) are excellent summaries of investigations carried out by the U.S. Department of the Interior. The Herbicide Handbook (62) has fish toxicity data for many weedkillers.

There have been relatively few investigations on the effect of herbicides on plankton. Usually the work reported occurs as a brief note in conjunction with a herbicidal study of an aquatic weed problem. As the study of herbicides is becoming a more mature science and we have realized the possible existence of a biological magnification problem through herbicidal use, more emphasis and study will be placed on this aspect of the subject in developing new products.

This is not a review of herbicidal effectiveness on aquatic weed problems. Klingman (68), Fryer and Evans (43), and more recently the National Research Council (86) in their books have good sections on the application of various herbicides for aquatic weed control. A general review on this subject also is given by House *et al.* (57) and a good history of the development of aquatic weed control is given by Timmons (132). Instead, this publication is concerned with the ultimate fate of herbicides after use and also their effects on non-target organisms. In this paper, the organization has been according to the common names of herbicides taken up in alphabetical order. In certain instances, for convenience, more than one may be discussed under one heading.

Herbicides

Acrolein. Burdick *et al.* (17) reported that acrolein is very toxic to fish. The chemical has a 24-hr TL_m[3] of 46 ppb for fingerling brown trout (*Salmo trutta* L.) and 79 ppb for bluegills (*Lepomis macrochirus* R.).

Ferguson *et al.* (38) used acrolein to control aquatic weeds and snails (*Bulinus and Biomphalaria* spp.) in the Sudan. Phytotoxicity studies at 25 and 50 ppm showed minor or no injury to furrow-irrigated crops, but flood irrigation of vegetable seedlings at 15 ppm was toxic. Effective downstream carriage of acrolein was demonstrated for a distance of 1 mile. The snails were 98 to 99% killed and all submersed aquatic weeds were destroyed.

Unrau *et al.* (138), experimenting with acrolein in

[1]Received for publication October 24, 1969.

[2]Agricultural Department, The Dow Chemical Company, Midland, Michigan.

[3]Median tolerated limit.

16250

Egypt, also found it very effective against snails as well as the aquatic curlyleaf pondweed (*Potamogeton crispus* L.) in irrigation ditches. The 20 to 50 ppm concentration required for weed control had no adverse effect on local crops. Field tests demonstrated the importance of water control during the application period.

Arsenic. Mackenthun (77) reported on the long-term use of sodium arsenite. During the past 10 years, 218,000 lb of AS_2O_3 have been applied along a lake shoreline; uniform distribution of this quantity over the top inch of bottom mud or hydrosoil would amount to 380 ppm. Mud samples analyzed in the fall had 48 to 154 ppm and the following spring 10 to 89. Five species of fish 3 or 4 years old were analyzed for arsenic; highest concentration found in the fish flesh was 0.07 ppm in yellow perch (*Perca flavescens* M.). Highest concentration found in the organs was 0.27 ppm in skin and scales of bluegills. Water samples from the outlet were analyzed for 10 weeks starting in June and contained from 0.075 to 0.225 ppm of arsenic.

Ball (9) used ^{74}As-tagged sodium arsenite in a study of its effects on pond ecology. Treatment was at 8 ppm, the standard commercial rate. The sodium arsenite was taken up rapidly by the plants and recycled to the water and soil by decay and sedimentation of the plants within 5 days. One alga (*Spirogyra* sp.) was killed whereas another one (*Chara* sp.) absorbed the herbicide and appeared to be unaffected. The tagged material persisted in the water for the duration of the experiment at about one-half the concentration of the applied amount. The invertebrates (microcrustacea) showed great variation from very sensitive to very resistant in response to the herbicide. The arsenite moved into the soil and reached a depth of 3 inches in the hydrosoil during a 60-day period. General effect on the community metabolism of the pond as shown by the diurnal O_2 curve indicates that the herbicide had a drastic effect. It raises serious questions as to the advisability of using sodium arsenite in fish-producing ponds due to its deleterious effects on several levels of the fish food chain.

Gilderhus (45) studied the use of sublethal concentrations of sodium arsenite on bluegills and pond invertebrates. Applications that totaled 4 ppm reduced the survival and growth of the fish; immature fish were more affected than adults. Substantial quantities of arsenic were found in the water, hydrosoil, and the organs and flesh of bluegills at the end of the experiment. Bottom fauna and plankton populations were reduced or inhibited in several pools with the highest concentrations of herbicide.

Lawrence (71) and Surber and Meehean (125) both used 4 ppm arsenic as a herbicide for filamentous algae and found it to be toxic to desirable food organisms. Lawrence also noted that it decreased bluegill production.

Amitrole (3-amino-s-triazole). Marston *et al.* (81) made a study in Oregon on a stream draining a municipal watershed. An air application was made at 2 lb A to control the salmonberry (*Rubus spectabilis* Pursh). A maximum concentration of 155 ppb was found in water samples 30 min later, decreasing to 26 ppb at the end of 2 hr and none in 6 days. At 1.8 miles below the sprayed area, no amitrole was detected at any time. Water sampled during their study probably would have been safe for use in home irrigation systems. There was not sufficient amitrole present at any time to cause toxicity to warm-blooded animals.

In other tests with amitrole for salmonberry control, Tarrant and Norris (126) made aerial applications 2 successive years using 2 lb A in 10 gal of water. Level of amitrole in samples of stream water at the spray site was 400 ppb in samples taken 5 min after spraying; 10 hr later, it had decreased 100-fold to 4 ppb. Amitrole was not detected after 3 days. Norris *et al.* (93) also treated 260 acres with amitrole at 2 lb A. Afterwards, stream water contained 42 and 45 ppb of amitrole which totally disappeared in 48 hr.

Dalapon (2,2-dichloropropionic acid). It is generally recognized that dalapon has a very low degree of toxicity to fish. It caused zero mortality to goldfish (*Carassius auratus* L.) after 24 hr of exposure at 100 ppm (62). Thiegs (130) and Magee and Colmer (79) showed its rapid decomposition in soil.

Chancellor and Ripper (22) indicated that yellow perch, northern fathead minnow (*Pimephales promelas* R.), and pumpkinseed (*Lepomis gibbosus* L.) tolerated 300 ppm, although at 400 ppm 50% of the brown trout were killed. The sodium salt of dalapon apparently did not affect the Lake Emerald shiner (*Notropis atherinoides* R.) at 3,000 ppm. Dragon fly nymphs (*Odonata* sp.) were not killed by 2,000 ppm and several insects that normally are eaten by fish were unaffected by dosages between 400 to 800 ppm.

Jacorossi (65) in investigations on controlling weeds in canals reported no toxic effects on seven fish species exposed for 3 days to dalapon at concentrations far exceeding those likely to be obtained in field practice. Kochkin (69), in an organoleptic study of dalapon, concluded that 2 ppm is the maximum permissible concentration.

Dichlobenil (2,6-dichlorobenzonitrile). Frank *et al.* (41) investigated the effects of canal-bottom plots treated with dichlobenil and (2,3,6-trichlorophenyl)acetic acid (fenac) on irrigation water flowing over them. Concentrations of dichlobenil and fenac initially were 8.6 and 8.8 ppm, respectively. The bulk of the herbicides lost from the plots due to the water flow occurred quickly and safe levels were reached after 1 hr of water flow.

Van Valin (139) applied dichlobenil at 10, 20, and 40 ppm to ponds. Residues in both water and fish were highest within 3 days after treatment. Concentration in water 11 days after treatment was about 2% of that after 3 days. The residue in fish, whole body, dropped nearly as fast but could still be detected after 112 days.

Taylor (128, 129) reported that the LD_{50} of dichlobenil for most species of fish was 15 to 20 ppm; the usual recommended rate of 0.5 ppm did not adversely affect spawning. It had a low degree of toxicity to species such as shrimp (*Penaeus* sp.) and oysters (*Crassostrea virginica* G.); plankton tolerated 10 ppm. Median lethal concentration to the fresh water crustacean *Daphnia* sp., a common food chain organism, was approximately 10 ppm. Walker (142) reported that concentrations of 12 to

16251

22 ppm with a 96-hr exposure period produced 50% mortality in four warm water fish species.

Cope *et al.* (27) reported on pond studies in Oklahoma and Colorado. At the former site, only 3% of the dichlobenil applied at 10, 20, and 40 ppm remained in the water after 11 days; even with 40 ppm, only 1.5 ppm remained after 60 days. At that time, the same amount was left in the mud. But fish reproduction was affected adversely by the 40 ppm, and there were some pathologic effects in the fish organs. There was no immediate mortality, but survival after 16 weeks was 60% for the controls and 20, 5, and 2% in the ponds with the increasing dosages. Increase in weight was 67% for the controls but 169, 169, and 482%, respectively, for the other treatments mentioned above, presumably owing to a more abundant food supply.

In Colorado, 0.58 ppm of dichlobenil, resulted in maximum concentration of 0.32 and 0.43 ppm of the herbicide in the water after 3 weeks. After 63 days, it declined to 0.17 ppm when there was 1.98 ppm in the mud. No fish mortality occurred outside the cages although a lack of oxygen killed all the caged fish at the bottom.

Diquat 6,7-dihydrodipyrido 1,2-a:2',1'-c pyrazinediium ion and *1,1'-dimethyl-4,4'-bipyridinium ion (paraquat).* Allen *et al.* (3) found that the use of diquat at 2 ppm for 1 hr produced some prophylactic effect on adult and juvenile chinook salmon (*Oncorhynchus tshawytscha* W.) affected by the pernicious disease caused by the fungus *Dermocystidium* sp.

Beasley *et al* (10) found a maximum of 7.9 ppm of paraquat and 1.7 ppm of diquat in hydrosoils where the soils had been treated 4 years previously with 0.3 lb A. diquat and paraquat will persist in hydrosoils for long periods of time. Blackburn and Weldon (12) reported that diquat, originally applied at 2.5 ppm, persisted in water 8 and 11 days. No toxicity to fish was apparent. It appeared to have little effect on natural fauna and plankton.

Coats *et al.* (23) investigated the disappearance of diquat and paraquat when applied at 0.5 and 0.4 ppm in plastic pools and ponds. Paraquat in plastic ponds in water alone took 31 weeks to disappear and diquat 10 weeks. In contrast, neither was found in pond water after 15 days. This work shows the strong sorptive power of soil for these compounds. Daly *et al.* (31) found that water analysis revealed only a trace of paraquat and diquat 24 hr after they had been applied to control eurasian watermilfoil (*Myriophyllum spicatum* L.).

Frank *et al.*[4] in comparing the residual effect of paraquat, an ester of (2,4-dichlorophenoxy)acetic acid (2,4-D), dichlobenil, and fenac in pond water and soils, found paraquat was the least persistent in water; it was not detected 12 days after application. But the butoxyethanol ester of 2,4-D took 24 days to disappear. Dichlobenil and fenac persisted for 150 days and traces of fenac were still present in the water 1 year after application. After 85 days, the top inch of the hydrosoil contained 10 ppm paraquat and no 2,4-D. Fenac in the hydrosoil dropped from 12.4 ppm at 24 days to 0.26 ppm after 160 days; dichlobenil went from 10.6 ppm initially to 1.34 ppm after 120 days.

Later, Frank and Comes (42) studied herbicide residues in pond water and hydrosoil. They applied dichlobenil at 0.58 and 0.40 ppm, fenac at 1 and 1.56 ppm, 2,4-D at 1.33 ppm, 7-oxabicyclo (2.2.1)heptane-2,3-dicarboxylic acid (endothall) at 1 ppm, paraquat at 1.14 ppm, and diquat at 0.62 ppm. Fenac and dichlobenil residues persisted in soil and water for more than 160 days. Low amounts of 2,4-D were found in water for 24 days and in the hydrosoil for 55 days after which it disappeared. Endothall, paraquat, and diquat were less persistent and were not found in the water after 24, 8, and 4 days, respectively. Paraquat and diquat persisted at high concentrations in the hydrosoil for 85 and 160 days, respectively.

Gilderhus (46) exposed fingerling and adult bluegills to diquat at various concentrations and frequencies of treatment in outdoor pools for 24 weeks. Numbers among bottom fauna and plankton were unchanged. Treated fish did not show any changes that could be attributed to diquat. The herbicide was toxic to a freshwater crustacean, *Cladocera* sp.

Grzenda *et al.* (50) compared the persistence of diquat and paraquat, applied at 2.5 ppm in pond water, to fenac at 4.0 ppm and amitrole at 1.0 ppm. Both paraquat and diquat disappeared from the water in less than 1 month. Soil sediment in the water increased the rate of their disappearance; they did not appear to be desorbed from bottom clays. The paraquat and diquat treatments were repeated with essentially the same results. Fenac was reduced to 2.4 ppm by 202 days and amitrole to 0.13 ppm by 154 days after treatment. He concluded that fenac appears to have the least potential for use in sources of potable water because of its long persistence.

Rodriguez-Kabana *et al.* (117) studied the effect of paraquat on the growth of the pathogenic fungus, *Sclerotium rolfsii* Sacc., in liquid culture and in soil. In the former, growth was decreased at all concentrations. In soil experiments, evolution of CO_2 was lowered at all concentrations but greatly reduced at only the 0.5 ppm and 1 ppm rates. Yeo (150) studied the effects of diquat and paraquat in water on fish. In reservoirs, diquat applied at 1000 ppb dissipated to 9 ppb after 12 days. In fish-growing pools, diquat at 4000 ppb dissipated to 900 ppb or less. Paraquat was slower to disappear and exhibited unpredictable fluctuations.

Beasley *et al.* (10), using radioactive diquat, found that goldfish placed into water containing 8 ppm diquat for 72 hr and then into fresh water for 10 days had radioactivity in the flesh and various organs in a negligible amount except in the gastrointestinal tract. Gilderhus (46) investigated the effects of diquat on bluegills and their food organisms. He reported that diquat disappeared from the water at about the same rate during spring or summer. Residues were highest about 10 days after treatment and disappeared 6 to 12 weeks after treatment. Diquat at 1 and 3 ppm did not affect adult or fingerling bluegills. No hematological or histopathological effects were noted. It was quite toxic to the freshwater crustacean, *Cladocera* sp., but these returned

[4] Frank, P. A., R. D. Comes, and E. B. Hollingsworth. 1966. Persistence of herbicides in ponded water and soils. Weed Sci. Soc. Amer. Abstr. p. 88.

DOW 5317148

16252

to their normal numbers with the disappearance of diquat.

Holz (56) used paraquat for aquatic weed control in ditches. He reported that fish, frogs (*Rana* sp.), and aquatic insects appeared to be unharmed by paraquat. Cattle drinking the ditch water after treatment (how long not specified) showed no toxic effect. Riemer (116) used diquat at 0.31, 0.53, and 0.73 ppm to control bladderwort (*Utricularia* sp.). No apparent effects were noted on large mouth bass fry (*Micropterus salmoides* Lacepede), pickerel fry (*Esox* sp.), or young yellow perch; it did not affect the spawning of pumpkinseed sunfish. Bottom dwelling invertebrates, primarily chironomids, were unaffected. Plankton were present in an insufficient amount to make valid comparisons before and after treatment.

Blok (13), using paraquat and diquat at 0.4 to 0.8 ppm to control filamentous algae, noted no effect on fish or fish food organisms. Used at 0.8 ppm for control of filamentous algae, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) had no deleterious effects on fish; in shallow ditches, no fish were killed. He also noted that one species of rotifiers was present in large numbers and that one species of *Daphnia* increased in numbers. The phytoplankton were chiefly unicellular flagellates and *Volvox aureus* Ehr. Dichlobenil and 2,6-dichlorothiobenzamide (hereinafter referred to as chlorthiamid) at 1 ppm were equally well tolerated by the fauna. In another trial with them, Crustaceae, especially copepods and bottom fauna, increased in number, but the phytoplankton were restricted to flagellates and isolated diatoms. Cassie (21) used diquat at an estimated 0.5 ppm in New Zealand and noted a decreased number of diatoms and algae. The decrease in algae might have been associated with anaerobic conditions caused by the decomposing aquatic weeds.

Lawrence (74) gave a numerical and graphic presentation of aquatic weed data on nine different combinations of treatments over a 2-year period. He used paraquat, diquat, fenac, 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine), and 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) in various combinations. This reviewer interpreted the data as showing that plankton were unaffected by these treatments with the exception of the paraquat-linuron combination. This caused a 50% reduction in plankton the first year and a slight reduction the following year. Fish production appeared to have been adversely affected in all treatments with perhaps the exception of the fenac treatment at the end of the first year.

Newman (87) gave data on the use of diquat and paraquat as it affected population changes in the smaller arthropods. There was a two to three-fold increase in earthworms; a residual amount of the herbicide was found in them which was rapidly lost upon their transfer to untreated soil. Amounts found even following high application rates were negligible. In aquatic situations no drastic effects on any group of animals (insects, Crustaceae, mites, mollusca, worms, leeches, and flat worms) were found. He (89) also reported on the ecological effect of paraquat and diquat at 0.5 to 1.0 ppm at 10 different sites. No direct toxic effects to fish were noted and in one case an increase in Dipterous larvae, mainly Chiromedae, occurred which was associated with the breakdown of killed weeds.

Newman and Way (88) reported no direct effects of diquat and paraquat on aquatic invertebrates present in their experiments. But they noted severe oxygen depletion at one location following decay of the treated aquatic weeds. This resulted in some mortality among Hirudinea, Isopoda, Odonata, Coleoptera, Trichoptera, Gastropoda, Lambellebranchiata, and captive trout although free-living coarse fish and trout were generally unaffected. These herbicides were absorbed by the vegetation and the bottom mud which in one case contained 36% of the original dose in the top 2 inches 5½ months after treatment. They also reported on effects of paraquat and diquat at 0.5 to 1.0 ppm at 10 different sites. No toxic effects to fish were noted. An increase in dipterous larvae, mainly Chironomidae, associated with the breakdown of dead weeds occurred once.

Pierce *et al.* (109) used fenac, simazine, 2-chloro-4-(ethyl-amino)-6-(isopropylamino)-*s*-triazine (atrazine), 2,4-bis(isopropylamino)-6-methylmercapto-*s*-triazine (prometryne), diquat, and paraquat for aquatic weed control. The triazines were effective for control of filamentous algae. Microcystic blooms were removed and rapidly replaced with more desirable plankton algae when atrazine was used at 0.08 and 0.16 ppm. Diquat and paraquat used at 0.25 to 10 ppm had no adverse effect on fish, fish food organisms, or plankton.

Tatum and Blackburn (127) used diquat at 0.5 ppm in two sub-tropical ponds. Phytoplankton was temporarily affected immediately but recovered rapidly. Organic matter from decaying vegetation appeared to significantly increase certain benthic organisms.

Wile (148) studied the effect of diquat, fenac, and simazine on phytoplankton. Applications were at 1 and 2 lb/A. In two ponds where diatoms were dominant over the flagellates and in two where blue green algae were dominant, there was no reduction. There was a substantial reduction (80%) in one pond which had a distinct bloom at the time of spraying. In agreement with Hilsenhoff (55), no effects were found on bottom-dwelling organisms examined in dredge samples. No diquat was detected in the water 5 and 7 days later. Fenac was used at 1 to 3 ppm. In three ponds, there was no effect on the phytoplankton. In three others, the numbers dropped, but total recovery was observed in 6 to 10 weeks. In one pond, the population was reduced 75% and remained there for the rest of the season. No effect was noted on bottom-dwelling organisms. Simazine was applied at 0.5 to 3.0 ppm to control aquatic weeds, but phytoplankton studies were not made. It has potential in control of filamentous algae.

Endothall. Lindaberry (75) reported that although the normal dosage of endothall is 1 to 2 ppm, 5 ppm endothall completely disappears in 4 days. He also reported no residue in fish which were placed in water containing 6 and 12 ppm. Bluegills tolerated, with no mortality, 100 ppm for 21 days. There was no mortality in three species of minnows (*Cyprinidae* sp.) at 40 ppm. Salmon (*Salmo* sp.), rainbow trout (*Salmo gaird-*

UOW 531749

16253

nerii R.), and bass (*Micropterus* sp.) suffered no mortality at 10 ppm.

Simes (120) applied endothall at 5 ppm in ponds or trout-rearing raceways in a Pennsylvania fish hatchery and noted no indication of distress by the fish. Steucke (123, 124) reported that 2 ppm of endothall in fish hatchery ponds did not affect the fish population—large mouth bass, bluegills, goldfish, or fish food organisms. Walker (141) reported that it took 25 days for disappearance of residue from fish flesh exposed to 1 to 3 ppm of endothall; some residues were found in fish food organisms 3 weeks later.

Fenac. Pierce (104, 105, 106) applied fenac to ponds at 1 to 3 ppm. There was no effect on plankton, small invertebrates, frogs, turtles (*Chelona* sp.), and fish. Young fish were present.

Paraquat. See section on diquat.

Phenoxyaliphatic acids in water. A study of the effect of 2,4-D in irrigation water on red Mexican beans (*Phaseolus vulgaris* L.) has been reported by Bruns (15). When the herbicide at 2 lb/A in the water was applied to beans in the seedling stage, root systems were severely injured; recovery began within 16 days. When the dosage was increased to 6 lb/A, yield was reduced 40%. The 2 lb-rate, when applied in the bloom stage, caused no significant reduction in yield; but the loss at 6 lb/A was 29%.

Aldhous (2) used the nonyl ester of 2,4-D at 4 lb/ae 12 gal water to treat a forest of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) for control of scotch heather (*Calluna vulgaris* Salisb.). Water samples, from a ditch that drained about 3 acres, were taken before spraying and up to 4 weeks. Analysis showed 0.0 ppm after 28 days. Aly and Faust (4) found that 2,4-D disappeared in 35 days from lake bottom soil that had been previously treated; on untreated hydrosoil, it took 65 days.

Averitt (6) in one field test applied an amine formulation of 2,4-D to water hyacinth (*Eichhornia crassipes* (Mart.) Solms). At 4 lb 2,4-D/A, 10 ppb were found at 3 weeks and 0.6 ppb at 20 weeks. In another field test, when 4 lb 2,4-D A were injected into the water at two different locations, he found 689 and 967 ppb 1 day after application; 31 days later, he found 11 and 19 ppb. In other tests, when the original applications were 4 and 5 lb/A, he found 4 and 15 ppb after 29 days. Averitt[5] also applied the methyl amine salt of 2,4-D at 4 lb/A. He found 802 ppb the second day, 446 the third, 74 the fourth, and then a gradual decline until 402 days when no 2,4-D was detected. Similar results were produced by 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) and a polypropylene glycol butyl ether ester of 2,4-D.

Bailey *et al.*[6] applied to ponds the polypropylene glycol butyl ether ester of silvex at 8 lb/A. Half of the ester was hydrolyzed to the acid in 5 to 7 hr and 99% in 33 to 49 hr. There was absorption of both acid and ester upon the bottom sediments with complete dissipation of both after 5 weeks. Cochrane *et al.* (24) applied 8 lb/A of the same ester of silvex to three plastic ponds, each with a different soil type at the bottom. In 2 weeks the ester hydrolyzed almost totally to the acid which gradually dissipated over 19 weeks. Absorption of both ester and acid apparently occurred on the hydrosoil, followed by gradual diminution. There is a possibility that silvex acid and/or a degradation product may be desorbed and re-enter the water. Therefore, they concluded that it should not be applied directly to water supplies for human consumption.

There have been few reports dealing with the effects of herbicides applied to watersheds and reservoirs where the water is destined for human consumption. One U.S. Government publication (134) lists 0.1 ppm for 2,4-D, 2,4,5-T, and silvex as permissible criteria for surface water for public water supplies. No such criteria are given for other herbicides. The same reference has a very useful table giving the likely concentration of herbicides in irrigation water resulting after a typical application rate and also the crop injury threshold in ppm.

Faust *et al.* (35, 37) investigated drinking water quality as affected by 2,4-D and 2,4-dichlorophenol (hereinafter referred to as 2,4-DCP). The latter is a most potent offender in water as it causes an objectionable medicinal taste at 8 ppb and has an odor even at 2 ppb. In laboratory carboy tests using 2,4-D, 9.5 and 16.7 ppb of 2,4-DCP were released in 7 days. Maximum concentrations of 20.7 ppb developed in 218 days. When the carboys were aerated and in the presence of small amounts of organic matter, this phenol disappeared rapidly. Under acid conditions and water unfavorable for biological oxidation, about half of the 2,4-DCP persisted.

Faust *et al.* (36) in later field tests applied the isooctyl ester of 2,4-D which contained 2,4-DCP as an impurity. It was applied at 20 lb/A to control eurasian watermilfoil. This gave a theoretical concentration in the lake of 1310 ppb 2,4-D and 4.36 ppb of 2,4-DCP. This did not cause an odor. There were 13 ppb of 2,4-DCP although it did not appear to accumulate as the result of biological degradation of 2,4-D. Maximum level of 2,4-D was 19.5 ppb, dropping to 13 ppb after 132 days.

Frank and Comes (42) introduced into a pond 1.33 ppm of 2,4-D from a 20% granular formulation. Initial concentration was 0.024 ppm. The maximum, 0.067 ppm, occurred after 18 days and fell to 0.019 ppm after 24 days. This formulation persisted longer in the hydrosoil but could not be detected there after 55 days. Gribanov (48) reported on the contamination of water in a reservoir with the butyl ester of 2,4-D which rendered it unfit for use by organoleptic criteria. No cases of poisoning were reported.

Grzenda (49), in a public health report on weed control in potable water supplies, gave data concerning six common herbicides and their persistence in water after varying periods of time. Application was a maximum of 4 ppm. After some days, no more than 1 ppm was found. For the dimethyl amine salts of 2,4-D, 62 days after treatment, the amount had dropped to 0.001 ppm.

Gratekowski (47) and Tarrant and Norris (126) did aerial forest spraying with a commercial 50-50 formu-

[5]Averitt, W. K. 1968. A summary of a study of the persistency and residues of some herbicides in surface waters. Weed Sci. Soc. Amer. Abstr. p. 65.

[6]Bailey, G. W., J. D. Pope, Jr., and D. R. Cochrane. 1968. The degradation kinetics and persistence of silvex under impounded conditions. Weed Sci. Soc. of Amer. Abstr. p. 45.

DOW 031750

16254

lation of low volatile esters of 2,4-D and 2,4,5-T diluted with diesel oil and applied at 2 lb A. This gave residues of 0.2 to 70 ppb in the water. Usually the level fell in a few days to 0.2 ppb. Maximum time for total disappearance of 2,4-D was 2 days at one test station and 17 days at another.

Krammes and Wellets (70) sprayed a brush-covered area on a steep slope to encourage conversion to grass. Application rate was 1½ lb 2,4-D and 1½ lb A 2,4,5-T as low volatile esters in a 20-gal mixture of water plus 1 gal of diesel oil. A follow-up hand treatment of brush was made with 1½ gal of 2 lb each 2,4-D and 2,4,5-T 100 gal of water that contained 1 gal of diesel oil. No herbicide was found in surface water. In soil samples, small amounts of herbicide were detected 8 days later. None was found after 11½ months.

Noll and Burdick (92) applied to a lake an ester of silvex at 2 ppm. When heated before treatment, the water had an earthy, musty odor. After treatment, a kerosene-like odor developed. This odor lessened considerably in 1 week although it was still detectable 3 weeks after treatment. The lake was treated in two sections; a number of large and small fish were killed when the second half was treated although this was not noted for the first half. A second lake was treated at a concentration of 1 ppm and no fish were killed.

Norris *et al.* (93) applied by helicopter a phenoxy brush killer formulation to 476 A at a rate of 1 lb of 2,4-D and 1 lb of 2,4,5-T 10 gal of oil A. Another area was spot-treated in 25 locations resulting in 407 treated acres. A stream drained each area. Samples taken from both streams were analyzed. Measurable quantities of herbicide were not found in any samples although a total of 3½ to 5¼ inches of rain had fallen over the treated area. Norris (94) cites work by Linden and Muller (76) who used diesel oil at rates in excess of 500 gpa, which was followed by leaching with 4 in of rain water. A maximum of 2 ppm of diesel oil was found in a sandy loam soil at a depth below 21½ inches. It was concluded that application of diesel oil at rates of 50 gpa to soil surfaces presented no threat to ground water quality.

Reigner *et al.* (114) discuss treatment of vegetation along two stream banks with the usual amounts of acid and ester formulations of 2,4,5-T. Water samples were taken for analysis periodically for 3 weeks. Odor tests were conducted. They concluded that 2,4,5-T, if applied with normal precautions, could be used on municipal watersheds without creating any water contamination.

Reinhart (115) applied 1325 gal (740 gal of diesel oil plus 52 gal of an ester of 2,4,5-T containing 6 lb gal) on 30 A of a timber watershed. This was a basal spray applied to all trees 1 inch dbh or larger. This treatment is heavier than would be used in a riparian area and diesel oil normally would not be used. An odor panel found no contamination in numerous water samples taken from the stream draining the treated area. Schwartz (119) reported a laboratory study of aqueous solutions of 2,4-D under conditions favorable for microbiological activity. At least 60% of the 2,4-D persisted 3 to 6 months.

Smith and Isom (121) applied a 20% formulation of the butoxy ethanol ester of 2,4-D to control eurasian watermilfoil. Application of 888 tons of the 2,4-D was made to 8000 infested acres in seven TVA reservoirs at rates of 40 to 100 lb A. Analyses showed little uptake of 2,4-D by fish but some by mussels (*Mytilus* sp.); all mud samples showed some 2,4-D. Eight of nine water treatment plants showed less than 1 ppb of 2,4-D in the water. The ninth had its water intake directly under a 2,4-D application plot and its highest concentration was 1 and 2 ppb. They also reported that mosquito larvae (*Anopheles quadrimaculatus* L.) in the laboratory survived 2,4-D treatments at 100 ppm; adults from these larvae were carried to the F_2. No difference in hardiness or reproductive ability could be detected. They concluded that high rates of 2,4-D applied in TVA reservoirs have not produced adverse effects upon aquatic fauna or water quality.

Wershaw *et al.* (147) reported on a literature review of an ionization constant study of 2,4-D. Various authors showed 2,4-D could persist for periods up to 6 months in natural waters. Winston and Ritty (149) discussed the fate of phenoxy herbicides when applied to a watershed. At least eight species of soil bacteria use phenoxyaliphatic herbicides as an energy source. They concluded that the phenoxyaliphatic herbicides are decomposed into carbon dioxide, inorganic chloride ions, and water, and when properly applied to watershed areas will not create a water pollution problem.

Phenoxyaliphatic acids and fish. Beaven et al. (11) had caged oysters, blue crabs (*Callinectus sapidus* Ratabun), clams (*Mya arenaria* L.), and fish near the center and outside of 1-A tidewater plots set up to control eurasian watermilfoil. The butoxyethanol ester of 2,4-D was used at 30, 60, and 150 lb A. The two latter treatments were replicated three times. When normal aerobic conditions were maintained, treatments at 30 lb A resulted in no mortality to the native microfauna and the valuable native plants.

Butler (20) exposed oysters continuously for 7 days to flowing sea water containing 0.1 ppm of the butoxy ethanol ester of 2,4-D. No mortality occurred. The oysters had accumulated 18 ppm 2,4-D acid. Half the treated oysters then were flushed with normal sea water for 7 days at which time they had no 2,4-D residue. In other experiments no effects of 2,4-D were noted on pigtoe mussels (*Pleurobema cordatum* Green). Data are given for effects of 37 different herbicides on oysters, shrimp, fish, and phytoplankton. He concluded that herbicides in general are less toxic than most other pesticides.

Hughes (60) and Hughes and Davis (61) reported on variations of different formulations of 2,4-D, 2,4,5-T, silvex, and 2-(2,4-dichlorophenoxy)propionic acid (dichloroprop) to bluegills. The number of different formulations varied from three with dichlorprop to 21 for 2,4-D. Differences in toxicity of various formulations of the same herbicide were great and may be greater than the difference in toxicity between different herbicides. Amine salts were generally less toxic than esters. The actual formulation must be evaluated to determine the toxicity.

Lawrence *et al.* (72) ran experiments for aquatic weed control with 2,4-D acid and silvex both at 5 ppm.

UCW 5317751

168255

diquat at 2 ppm, diquat at 1 ppm plus 3 ppm (4-chloro-*o*-tolyl) oxy) acetic acid (MCPA), endothall at 2 ppm, and amitrole at 1.5 ppm – 3 ppm MCPA. They placed fathead minnows and redear sunfish (*Lepomis microlophus* G.) in their plastic pools. No deaths up to 9 months after treatment occurred with either fish; in several instances they had offspring. Mackenthun (78) noted in using 2,4-D granules to control aquatic weeds in fish hatchery ponds that applications of 25 to 30 lb 2,4-D/A had no harmful effects. Rao and Murty (112) found that using the sodium salt of 2,4-D at 8.8 lb/A in 100 gal of water to control *Ipomea reptans* L. caused no fish mortality.

Rawls (113) worked with blue crabs, eastern oysters, soft shell clams, and various fish species in conjunction with herbicidal experiments. The following herbicides were applied at these rates as lb A: 2,4-D acetamide—20; polypropylene glycol butyl ether esters of 2,4-D—20; silvex—40; butoxy ethanol ester of 2,4-D —20 to 120; and isooctyl ester of 2,4-D—20 to 60. At the end of 1 month, he concluded that only the 2,4-D acetamide appeared dangerously toxic.

Thomas and Duffy (131) investigated both in laboratory and field the effect of the butoxyethanol ester of 2,4-D for control of eelgrass (*Zostera marina* L.). They quote Mount and Stephen (84) who stated that continuous exposure to 0.3 ppm 2,4-D had no effect on reproduction of fathead minnows. Rawls (113) also is quoted as saying that 6 ppm 2,4-D had little effect on oyster egg development; there is evidence that 2,4-D residues in shell fish are eliminated in 10 months. Butler (18, 19) also indicates that continuous exposure to 3.75 ppm 2,4-D reduced oyster shell growth by 50%; 1 ppm for a 4-hr exposure period reduced phytoplankton productivity by 16%. In their own laboratory experiments, Thomas and Duffy found that with application of 40 lb/A, the highest concentration of 2,4-D detected in the water was 0.1 ppm; the last detectable amount occurred 20 days after treatment. In the field experiments at 30 lb/A and in water samples taken for 3 consecutive weeks, no 2,4-D was detected after 21 days. Highest field concentration was 1.4 ppm. In the laboratory experiment, highest 2,4-D residue detected in oysters was 3.07 ppm; no 2,4-D residue was detected 17 days after its disappearance from the water. Benthos samples from the field showed generally increasing fauna biomass during 4 months following treatment; total number of animals also rose. No mortality of natural fauna or flora was observed and relative percentage of species remained unchanged.

Tschirley (133) discussed the effects in Vietnam of using both phenoxyaliphatic weed killers and 4-amino-3,5,6-trichloropicolinic acid (picloram) applied over more extensive areas and at higher dosages than normally used on crops. Statistics on the fish caught over a 3-year period, including fresh and salt water fish and also cuttlefish (*Sepia* sp.), mollusks, shrimp, and crabs, indicated that the aquatic food chain had not been seriously disturbed.

Estes (31) applied to polypropylene glycol butyl ether esters of silvex at 8 lb A to control aquatic weeds. Within 30 min after spraying where the average depth of water was 3 ft, some fish seemed distressed and some pickerel (*Esox* sp.) died. In a second area, no distressed fish were observed. Analyses of fish after the treatments showed less than 0.01 ppm silvex; there was no change in the benthic population. Gaylor and Houser (41) reported on 3 years' work with silvex used for aquatic weed control. Results are given for eight species of aquatic weeds with good results for three of the most troublesome. No dead fish were observed, although the fish hunted new habitats when their old plant cover was destroyed.

Houser and Gaylor (58) used endothall and silvex for control of water weeds. These herbicides caused no fish mortality, nor were there reports of off-flavor in the fish caught by many anglers in that area. Houser (59) reports that three species of fish in two ponds treated with silvex experienced a weight loss in the 2 years following application. It is not known whether this was directly due to the silvex or indirectly through the destruction of the weed beds. Mathews (83) reported that in New Zealand, trout (*Salmo* sp.) were unaffected by 50 ppm silvex.

Mullison (85) reviewed the silvex literature and gave toxicological data for rats, guinea pigs (*Cavia porcellus* L.), rabbits, mice, chickens (*Gallus gallus* L.), dogs (*Canis familiaris* L.), and two species of fish. Silvex apparently does not constitute a hazard to game birds such as bobwhite quail (*Colinus virginianus* L.), mallard ducks (*Anas platyrhyncho* L.), and ring-neck pheasants (*Phasianus colchicus* L.). There seemed to be fish species variation in regard to tolerance of ester formulations of silvex, but in most situations where a silvex ester was used, fish populations were not seriously affected. Nonetheless, the potassium salt of silvex was less toxic to fish than the ester of silvex. Effects of the ester of silvex on plankton were variable; some injury was observed in fresh water, but this was temporary and populations soon were back to normal.

Phenoxyaliphatic acids and plankton. Butler (20) reported physiological data on decrease in carbon fixation of a natural salt water phytoplankton community, chiefly dinoflagellates and diatoms. It was given a 4-hr exposure to 1 ppm of various herbicidal formulations. There was a wide variation in response even among members of the same group of herbicides such as different esters of 2,4-D. The same paper contains much EC_{50}[2] data on effects of herbicides on oysters, shrimp, and fish.

Cowell (28) used silvex at concentrations of 2 ppm in a study on aquatic vegetation and plankton in farm ponds. It gave excellent control of *Potamogeton* sp. and *Lemna* sp. but not satisfactory control of the alga, *Chara* sp. There were no adverse effects on the phytoplankton and zooplankton; no fish mortality was observed. Cowell (29) reported that neither silvex nor sodium arsenite, at the dosages used, had any effect on phytoplankton; 2 ppm of silvex were not too toxic to zooplankton. But sodium arsenite at 4 ppm drastically reduced zooplankton and heavily decreased rotifiers, copepods, and cladocerans. Silvex treatments had no such effects.

Crosby and Tucker (30) studied the effect of various herbicides on *Daphnia magna* L. after a 26-hr exposure. They reported 2,4-D and MCPA were innocuous, but

[2] An emulsifiable concentrate which causes response in 50% of the individuals.

16256

2,3-dichloro-1,4-naphthaquinone (hereinafter referred to as dichlone), *S*-ethyl hexahydro-1*H*-azepine-1-carbothioate (molinate), 3′,4′-dichloropropionanilide (propanil), and dichlobenil could present a danger to *Daphnia* under field conditions if used at excessive rates.

De Oliveira (33) reported that a Brazilian lake sprayed with an amine of 2,4-D temporarily increased in plankton. This was attributed to a better food supply caused by the dying weeds. Harp and Campbell (54) studied the effects of the potassium salt of silvex on benthos organisms of a farm pond. It was applied at 2.8 to 4.6 ppm and the pond was sampled at 28-day intervals for 3 months. The benthos population doubled. There was an increase in tendipedids, aligochaetes, and chaoborus correlated with an increased food supply from the decaying plants. Chaoborus and libellulids increased greatly within 1 year after treatment. Snails, leeches (*Hirudo* sp.), and coenagrionids appeared unaffected. Lacewing flies (*Chrysops* sp.) decreased quickly but reappeared in smaller numbers during the last 2 months.

Petruk (97) applied 2,4-D and trichloroacetic acid (TCA) on saprophytic microorganisms in ponds. The second or third day after the sodium salt of 2,4-D was administered, the population of microorganisms in the pond increased about 50%, but it gradually diminished after 12 to 14 days and approached its original value by 25 days. Similar results occurred with TCA. Variations of the microorganism population, particularly with 2,4-D, strongly suggested that the herbicides were being used as food.

Pierce (98, 99, 100) has studied in depth the polypropylene glycol butyl ether esters of silvex. Plankton is represented by about 50 species in the following classes. Annelida, Chlorophyceae, Crustacea, Desmidaceae, Dictomaceae, Flagellata, Myxophyceae, Nematoda, Rotifera, and other protozoa. Benthic organisms were represented by the following groups: Amphipoda, Annelida, Diptera, Ephemerida, Gastropoda, Pelecypoda, and Odonata. Also present were large aquatic vertebrates, such as fish, frogs, and turtles. Silvex at 1.2 ppm temporarily decreased the plankton population, but it was back to normal within 2 weeks. The benthic organisms, fish and other larger aquatic vertebrates were unaffected. Pierce (103) compared two formulations of the potassium salt of silvex at 2.2 ppm and observed no effects on plankton or benthic organisms. Fish of all ages, frogs, and turtles were present throughout the summer and were unaffected. Pierce (101, 102) made another series of investigations using granular 2,4-D at rates of 1.5 to 6.2 ppm. No effect on the benthic or plankton population was noted, although in one trial there might have been a temporary reduction in plankton which returned to normal in 3 weeks.

Pierce (107) determined the effects of a number of herbicides on plankton and other pond organisms. Dichlobenil, fenac, 2-methoxy-3,6-dichlorobenzoic acid (dicamba), endothall, the potassium salt of silvex, and 2,4-D acid in a granular formulation were used at dosages of 1 to 2.5 ppm. There were no significant changes in the number of individuals or species in the plankton. Neither the benthic organisms, consisting chiefly of chironomid larvae and gammarids, nor any other of the pond dwellers showed any injury. Pierce (108) continued her ecological investigations using a combination of 2,4-D (7.2%) and endothall (3.6%) to give a concentration of 2 ppm. This had no deleterious effects on plankton, large and small fish, tadpoles, frogs, turtles, and water snakes (*Natrix* sp.).

Walker (110) reported that bottom-dwelling organisms in fish ponds were significantly reduced in number by 2,4-D, 1-butyl-3-(3,4-dichlorophenyl)-1-methylurea (neburon), sodium arsenite, and copper sulfate during the year following treatment but were not affected by endothall and simazine. Dosages were not stated except as "reasonable concentrations". He discussed the possibility of sublethal dosages changing the species composition and how that would affect pond ecology.

Whitney[3] studied the effects on aquatic fauna of the polypropylene glycol butyl ether ester of silvex applied at 8 lb/A. Fish deaths were observed in half the silvex applications: gizzard shad (*Dorosoma* sp.), bluegills, and largemouth bass were the principal species affected. Highest value found was 19 ppm 24 hr after treatment in a forage fish. This decreased to a negligible amount 1 to 3 weeks later. Benthic organisms increased significantly in number after treatment. The normal phytoplankton consisting of numerous species was destroyed and replaced by *Euglena* sp. and dinoflagellates. It did not return in 3 weeks. Rotifers and crustacea also decreased following application but regained their normal numbers within 1 to 3 weeks.

Picloram (4-amino-3,5,6-trichloropicolinic acid). Davis *et al.* (32), after using picloram at an unusually high rate (9.3 lb/A in a pellet formulation) on a watershed area, found detectable amounts of picloram in the stream after rains. Highest concentration found in the stream was 0.37 ppm. After 16 months, during which a total of 40 inches of rain fell, no picloram was detected in the stream.

Butler (20) using a formulation of picloram and 2,4-D at 1 ppm, reported no effect on oysters, shrimp, fish, and phytoplankton. Kenaga (67) studied and reviewed the effect of picloram on 15 fish species and three game birds. This work indicated that it presents an extremely low potential hazard, if any, to such wildlife.

Hardy (53) showed that picloram at 1 ppm did not retard the growth of algae or *Daphnia*. Guppies were grown in water with 1 ppm picloram and fed *Daphnia* reared in such treated water. The guppies were normal in appearance, behavior, and reproduction. The *Daphnia* also grew and reproduced normally with no evidence of biological magnification of the picloram in them.

Sodium PCP (sodium pentachlorophenate). Ishizawa and Matsuguchi (64) reported that the sodium salt of PCP mixed with soil at a rate of 1200 ppm stimulated bacterial growth, *Pseudomonas* sp. becoming predominant. Ammonification and iron reduction were depressed at concentrations greater than 120 ppm.

The Okubos (95) reported that an extensive number of fish and clams were killed in 1961 and 1962 on the coast of the Ariake Sea, Kyushu. A sodium PCP application had been followed at once by a heavy rain. They concluded that the resistance of a native clam (*Venerupis*

[3] Whitney, E. W. 1968. The toxicity of silvex to aquatic fauna. Weed Soc. of Am., Abstr. p. 65.

531753

16257

sp.) to PCP decreases to one-tenth its normal value under stress of the diluted sea water.

Saito *et al.* (118) used the sodium salt of PCP as a 25% granular material applied at 6.6 lb A on rice (*Oryza sativa* L.) paddy fields. Samples of river water were collected at 24 different spots near the treated fields. Maximum quantity of PCP detected was 30 ppb; they concluded there was no danger from this use of the herbicide.

s-Triazines and *α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine (trifluralin).* Axe *et al.* (8) studied the residual effect in farmers' fields of atrazine, 2-chloro-4,6-bis(isopropylamino)-s-triazine (propazine), and trifluralin. Only 14% of the trifluralin was found in the soil after 3 months, 20% propazine after 5 weeks, and 33% of the atrazine after 5 days. Highest concentrations of herbicide in the runoff water were 0.04, 0.05, and 0.04 ppm for atrazine, propazine, and trifluralin, respectively. It was assumed that much of the herbicides must have been on or in the plants.

Avraman (7) studied the effect of prometryne on the taste of water and its toxicity to mammals. The herbicide, within its solubility limits, did not affect the color or odor of the water. The taste threshold was 1.2 ppm. The short-term LD_{50} value for mice (*Mus musculus* L.) was 9.3 to 20.6 gr lb[-1] and 19.2 to 24.6 gr lb[-1] for rats (*Rattus norvegicus* L.). Daily doses of 4.3×10^{-4} gr lb[-1] in long-term studies had no significant effect on rabbits (*Oryctolagus cuniculus* L.) and rats. He concluded that a level of 1 ppm would be the maximum permissible concentration in water.

Martynyuk and Gzhegotskii (82) investigated simazine, atrazine, propazine, 5-amino-4-chloro-2-phenyl-3 (2*H*)-pyridazinone (pyrazon), sodium TCA, and diuron in water. Water containing 0.1 to 5.0 ppm of these herbicides did not differ from control samples in its organoleptic properties even after heating to 158 F. TCA excepted, increasing the concentration of these herbicides to 50 to 200 ppm markedly changed the organoleptic properties of the drinking water, particularly for pyrazon. Diuron excepted, ground water containing 600 to 1000 ppm of these herbicides stored in bottles for 8 to 20 months at temperatures of 43 to 71 F showed no drop in concentration. Ground water containing 50 to 100 ppm of these herbicides was treated with 300×10^{-3} gr/qt of calcium hypochlorite; this completely decontaminated the water.

Weldon and Blackburn (145) reported that 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (ametryne) persisted in plastic-lined pools nearly unchanged for 32 days after application. In the field, however, no residues were found under treated water hyacinth 1 day after application.

Snow (122) used simazine at 2 ppm in two ponds. In one pond, adult redear sunfish were killed although no small fish died; none died in the other. Another pond was treated at 3 ppm and no fish died. The phytoplankton developed slowly after 4 to 6 weeks. Fish production was low to average, but the evidence was inconclusive as to the cause.

Walker (143) experimented with simazine and other *s*-triazines in fish habitats. Simazine was less toxic than propazine, 2,4-bis(isopropylamino)-6-methoxy-*s*-triazine (prometone), or atrazine to fish. Laboratory tests on bottom organisms showed simazine had an LD_{50} of 28 ppm; however, field observations did not demonstrate any serious reproduction problems. Atrazine is more toxic than simazine to bottom organisms, but they recovered in 4 to 6 months. Prometone and propazine were not critically examined, but no fish mortality was observed.

Flanagan (39) reported simazine as non-toxic to fish at 3 ppm. Guse (51), in a summary of reports on aquatic weed control, mentioned that ametryne at 4 and 5 ppm caused irritation or death to some fish. Prowse (111) noted that TCA at 100 ppm caused no distress to fish after several days' contact. He also noted that simazine at 120 ppm caused 70% mortality in 4 hr; after 12 hr the tanks were no longer toxic. Wellborn (146) investigated the fish toxicity of diquat, diuron, and simazine and found simazine to be the most toxic to bass (*Roccus* sp.) with an LC_{50} of 0.25 ppm.

Trifluralin. Cope (25) stated the LC_{50} concentration of trifluralin for a 24-hr exposure of 2 to 4-inch rainbow trout was 14 to 210 ppb and for smaller bluegills it was 23 to 120 ppb. Increasing the temperature increased the toxic response; e.g., trifluralin once was 130 times as toxic at 85 as at 45 F. In some cases, there was greater toxicity in soft than hard water and in other cases there was no difference. Cope (26) later reported the EC_{50} values of trifluralin to be 11 to 19 ppb for rainbow trout and bluegills.

Lawrence[10] incorporated trifluralin in soil 7 days before putting it in water-filled plastic ponds. The ponds then were planted with several species of water weeds and stocked with fathead minnows. There was no mortality to the fish during 4 weeks after flooding; they reproduced satisfactorily.

Parka and Worth (96) found that trifluralin had an LC_{50} of 58.2 ppb to bluegills; when put on soil and this soil added to water, 48 to 227 times more were required to obtain an LC_{50}. In a simulated field test using swimming pools, trifluralin was sprayed at the rate of 1 lb A on soil and tilled into it. It then was given 10 inches of simulated rainfall; water draining from the treated area was not toxic to bluegills. Analysis of the sediment showed 55 ppb trifluralin. This chemical has a water solubility of 50 ppb, is strongly absorbed by soil, and leaches very little even on sandy soils.

Substituted ureas. Walker (144) found monuron, diuron, fenuron, neburon, and mixtures of them with TCA effectively controlled certain aquatic weeds. Monuron and fenuron were less toxic than diuron and neburon to fish. These urea herbicides mixed with TCA were somewhat more toxic than the urea herbicides alone. Species differences were manifested. Fingerlings were more sensitive than adults. Fish food organisms were noticeably reduced in the treated areas.

Frank (40) studied the disappearance of monuron and neburon in an aquatic environment. Significant loss did not occur until after 8 weeks for monuron and 16 weeks for neburon. The rate of loss then accelerated

[9]Avoirdupois units.

[10]Lawrence, J. M. 1966. Effects of soil applied trifluralin on fish production in ponds. Weed Soc. of Amer. Abstr. pp. 89–90.

16258

until about 50% of the herbicides disappeared, after which it continued at a decreasing rate. After 128 weeks approximately 15% of the monuron and 39% of the neburon remained.

Bungenberg de Jong (16) used diuron at concentrations up to 0.4 ppm in fish spawning ponds; it did not inhibit the growth of pike. First and second year carp suffered some growth inhibition attributed to the retarded development of fish food organisms. Hambric (52) used diuron at 2 lb A or 0.2 lb A foot of water on two lakes in Texas and no fish were killed. Maloney (80) used monuron at concentrations of 0.004 to 32.0 ppm to control algae. Toxicity data are given for 32 species of algae with exposure periods of 7 and 28 days. He also noted that ponds treated at 1 and 1.6 ppm were not toxic to fish. Pierce (110) used diuron at 0.25, 0.33, and 0.5 ppm to control common duckweed (*Lemna minor* L.) and noted light to moderate fish mortality at all concentrations. He attributed this to a low oxygen supply and not to the herbicide. No plankton bloom developed in these ponds within 6 weeks.

Alabaster (1) in a study involving 164 different chemicals, many of them herbicides, pointed out that the toxicity of formulated pesticides can not be predicted from knowing the toxicity and percentage of the active ingredient. It is essential to test the actual formulation under consideration because many additives are markedly toxic, some more so than the active pesticidal ingredient. Nishiuchi and Hashimoto (91) studied three kinds of fresh water fish and two kinds of fresh water *Daphnia* and their susceptibility to 76 different pesticides. Although some herbicides were toxic to the species tested, they concluded most herbicides were non-toxic to both fish and *Daphnia*.

Discussion

This literature survey presents a very considerable mass of evidence concerning the fate in our aquatic environment of various herbicides. Evidence at hand shows few signs of either a contamination problem or an accumulation in our waters if our presently available weed killers are used according to label directions.

The toxicity of herbicides to fish is a complex problem. There is the usual biological variation in size, age, and species as well as physical and chemical differences at the aquatic site. There also may be variation with different forms of the active ingredient: for example, the esters of phenoxyaliphatic herbicides are more toxic than sodium or amine salts. Additives to a formulation may be markedly toxic, some more so than the active pesticide ingredient. Therefore, the specific formulation should be evaluated to determine its LD_{50}. In addition, the LD_{50} may not indicate the actual hazard to fish because of the circumstances involving the herbicidal application. For example, trifluralin when applied to the soil is strongly adsorbed and moves little. This creates no real hazard to fish except for accidental contamination, even though the compound *per se* is extremely toxic to them.

Plankton studies indicate that our present herbicides, including those used in aquatic sites, have at most only temporary harmful effect.

There is no evidence of a biological magnification problem with herbicides in the food chain organisms. Theoretically, the intrinsic fact that herbicides kill plants which then decay and are not generally eaten should prevent biological contamination at one beginning part of the food chain, the plankton.

Johnson *et al.* (66) discussed the studies on pesticide residues being carried out cooperatively by several government departments. He found that of the many pesticidal chemicals now in use the most important to fish, wildlife, and estuaries are DDT, its metabolites, and other chlorinated hydrocarbon insecticides. Not a single herbicide is on his list. Brown and Nishioka (14) discussed the results of their monitoring program. Eleven streams in the western part of the United States were checked monthly for nine insecticides and 2,4-D, 2,4,5-T, and silvex. No herbicide residues were found.

Most herbicides now in common use are low in toxicity to man and animals, and they break down in soil and disappear from water. In addition they are applied in such a way that they normally do not accumulate to create a hazard in our environment. Nonetheless, complacency is not justified. Continued careful study of each new herbicide is essential with the express purpose of preventing environmental contamination in the future. Man's tools frequently interfere with nature, and herbicides are a powerful tool. It is the wisdom with which this extremely useful agricultural tool is used that will determine whether it has a beneficial or harmful ecological effect.

Literature Cited

1. Alabaster, J. S. 1969. Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. Int. Pest Contr. 11:29–35.
2. Aldhous, J. R. 1967. 2,4-D residues in water following aerial spraying in a Scottish forest. Weed Res. 7:239–241.
3. Allen, R. L., T. K. Meekin, G. B. Pauley, and M. Paul Fujihara. 1965. Mortality among chinook salmon associated with the fungus *Dermocystidium*. J. Fish Res. Board Can. 25:2467–2475.
4. Ay, O. M. and S. D. Faust. 1964. Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J. Agr. Food Chem. 12:541–546.
5. Applegate, V. C., J. W. Howell, A. E. Hall, and M. A. Smith. 1957. Toxicity of 4,346 chemicals to larvae, lampreys and fishes. Fish and Wildlife Serv., U.S. Dep. of Int. Washington, D.C. Spec. Sci. Rep. Fisheries 207. p. 157.
6. Arbiter, W. K. 1967. An evaluation of the persistence of 2,4-D amine in surface waters in the state of Louisiana. Proc. So. Weed Conf. 20:342–347.
7. Arkhangel'skaya, E. V. 1968. Maximum permissible concentration of 'propanil' in bodies of water. Gigiena i Sanit. Hyg. Sanit. 33:103–107.
8. Arle, J. A., V. C. Mathers and A. F. Wiese. 1969. Disappearance of atrazine, propazine, and trifluralin from soil and water. Proc. So. Weed Conf. 22:367. (Abstr.)
9. Ball, R. C. 1966. Use of ^{76}As-tagged sodium arsenite in a study of effects of a herbicide on pond ecology. Proc. of Symp., Oct. 25–29, 1965. Vienna, Austria. pp. 149–163.
10. Beasley, P. G., J. M. Lawrence, and H. H. Funderburk, Jr. 1965. The absorption and distribution of C^{14}-labeled diquat in the goldfish (*Carassius auratus* L.). Proc. So. Weed Conf. 18:551. (Abstr.)
11. Beaven, G. F., C. K. Rawls, and G. E. Beckett. 1962. Field observations upon estuarine animals exposed to 2,4-D. Proc. No. East. Weed Contr. Conf. 16:419–428.
12. Blackburn, R. D. and L. W. Weldon. 1963. Results of three years' testing diquat as an aquatic herbicide in Florida. Proc. So. Weed Conf. 16:365. (Abstr.)

UOW 531755

16259

13. Blok, E. 1969. Experiments with herbicides in fishing waters 1964–1967. Weed Abstr. 18:117. No. 746.
14. Brown, E. and Y. A. Nishioka. 1967. Pesticides in selected western streams—A contribution to the national program. Pest. Moni. J. 1:38–41.
15. Bruns, V. F. 1951. The response of certain crops to 2,4-dichlorophenoxyacetic acid in irrigation water. Weeds 1:359–376.
16. Bungenberg de Jong, C. M. 1969. The use of diuron in pond pisciculture. Weed Abstr. 18:118. No. 753.
17. Burdick, G. E., H. J. Dean, and E. J. Harris. 1964. Toxicity of aqualin to fingerling brown trout and bluegills. N.Y. Fish Game J. 11:106–111.
18. Butler, P. A. 1963. Commercial fisheries investigations. U.S. Fish and Wildlife Serv. Circ. 167:11–25.
19. Butler, P. A. 1964. Commercial fisheries investigations. U.S. Fish and Wildlife Serv. Circ. 199:5–28.
20. Butler, P. A. 1965. Effects of herbicides on estuarine fauna. Proc. So. Weed Conf. 18:576–580.
21. Cassie, V. Effects of spraying on phytoplankton in Lake Rotorua. Proc. Rotorua Seminar on Water Weeds, sponsored by U. Ext. Serv. Univ. Auckland, New Zealand. Oct. 15, 1966. 31–40.
22. Chancellor, R. J. and W. E. Ripper. 1960. Control of reeds and other emergent water weeds in drainage ditches, ponds and water courses. The effects of dalapon on fish. Weed Abstr. 9:No. 696.
23. Coats, G. E., H. H. Funderburk, Jr., J. M. Lawrence, and D. E. Davis. 1964. Persistence of diquat and paraquat in pools and ponds. Proc. So. Weed Conf. 17:308–320.
24. Cochrane, D. R., J. D. Pope, Jr., H. P. Nicholson, and G. W. Bailey. 1967. The persistence of silvex in water and hydrosoil. Water Resour. Res. 3:517–523.
25. Cope, O. B. 1965. Some responses of fresh-water fish to herbicides. Proc. So. Weed Conf. 18:439–445.
26. Cope, O. B. 1966. Contamination of the fresh-water ecosystem by pesticides. J. Appl. Ecol. 3(Supplement):33–44.
27. Cope, O. B., J. P. McCraren, and L. Eller. 1969. Effects of dichlobenil on two fish pond environments. Weed Sci. 17:158–165.
28. Cowell, B. C. 1965. The effects of silvex on aquatic vegetation and plankton in central New York farm ponds. Down to Earth 21(1 & 2):19–20.
29. Cowell, B. C. 1965. The effects of sodium arsenite and silvex on the plankton populations in farm ponds. Trans. Am. Fish. Soc. 94:371–377.
30. Crosby, D. G. and R. K. Tucker. 1966. Toxicity of aquatic herbicides to *Daphnia magna*. Science 154:289–291.
31. Daly, R. W., H. H. Funderburk, Jr., and J. M. Lawrence. 1968. Persistence of diquat, paraquat, and 2,4-DBEE when used for eurasian watermilfoil (*Myriophyllum spicatum* L.) control. Proc. So. Weed Conf. 21:299.
32. Davis, E. A., P. A. Ingebo, and C. P. Pase. 1968. Effect of a watershed treatment with picloram on water quality. U.S. Forest Res. Note RM-100. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 4 p.
33. DeOliveira E. Silva, S. L. 1961. Influence of 2,4-D amine on plankton of marshy areas (preliminary note). Weed Abstr. 10:No. 1080. Not paged.
34. Estes, R. D. 1966. Some preliminary observations and findings on effects of silvex on the aquatic environment. Proc. So. Weed Conf. 19:416–419.
35. Faust, S. D., R. J. Tucker, and O. M. Aly. 1961. A preliminary report on the effect of some aquatic herbicides on water quality. Proc. No. East Weed Contr. Conf. 15: 546–548.
36. Faust, S. D., R. R. Younger, J. T. Tucker, and O. M. Aly. 1962. Observations on the occurrence and persistence of 2,4-D and 2,4-dichlorophenol in lake water. Proc. No. East Weed Contr. Conf. 16:459–465.
37. Faust, S. D. and O. M. Aly. 1963. Some effects of 2,4-D and 2,4-DCP on drinking water quality. Proc. No. East Weed Contr. Conf. 17:160–170.
38. Ferguson, F. F., E. K. Dawood, and R. Blondeau. 1965. Preliminary field trials of acrolein in the Sudan. World Health Org. Bull. 32:243–248.
39. Flanagan, J. H. 1969. A review of simazine for aquatic weed control. Proc. No. East Weed Contr. Conf. 14:502–505.
40. Frank, P. A. 1966. Persistence and distribution of monuron and neburon in an aquatic environment. Weeds 14:219–222.
41. Frank, P. A., R. H. Hodgson, R. D. Comes. 1967. Residues of two herbicides in water irrigation canals. Weeds 15:353–355.
42. Frank, P. A. and R. D. Comes. 1967. Herbicidal residues in pond water and hydrosoil. Weeds 15:210–213.
43. Fryer, J. D. and S. A. Evans. 1968. Weed Control Handbook, Vol. I, Principles. Blackwell Scientific Publications, Oxford and Edinburgh. 494 p.
44. Gaylor, J. and A. Holser. 1962. Three years' results with silvex for aquatic plant control in Oklahoma. Down to Earth 18(3):2–4.
45. Gilderhus, P. A. 1966. Some effects of sublethal concentrations of sodium arsenite on bluegills and the aquatic environment. Trans. Am. Fish. Soc. 95:289–296.
46. Gilderhus, P. A. 1967. Effects of diquat on bluegills and their food organisms. Prog. Fish Cult. 29:67–74.
47. Gratkowski, H. 1965. Project 3: Undesirable woody plants. Res. Comm. Prog. Rep. West. Weed Contr. Conf. 21:35–50.
48. Gribanov, O. I. 1967. Contamination of a surface water supply source of the Shortandy Station with herbicide 2,4-D and butyl ester. Gig. Sanit. 32:97–98.
49. Grzenda, A. R. 1963. Public health aspects of weed control in potable water supplies. Proc. So. Weed Conf. 16: 420–424.
50. Grzenda, A. R., H. P. Nicholson, and W. S. Cox. 1965. The persistence of four herbicides in pond water. Proc. So. Weed Conf. 18:521–525.
51. Guse, L. R. 1961. Aquatic weed control research in Indiana. Res. Rep. No. Cent. Weed Contr. Conf. 18:102.
52. Haveric, R. N. 1969. Testing diuron as an economical herbicide in recreation lakes. Proc. So. Weed Conf. 22: 321–323.
53. Hardy, J. L. 1966. Effect of Tordon herbicides on aquatic chain organisms. Down to Earth 22(2):11–13.
54. Harp, G. L. and R. S. Campbell. 1964. Effects of the herbicide silvex on benthos of a farm pond. J. Wildlife Manage. 28:309–317.
55. Hilsenhoff, W. 1966. Effects of diquat on aquatic insects and related animals. J. Econ. Entomol. 59:1520–1521.
56. Holz, W. 1963. Chemical weed control in ditches: trials on the control of submerged plants. Weed Abstr. 12:100. No. 507. Not paged.
57. House, W. B., L. H. Goodson, H. M. Gaberry, and K. W. Dockter. 1967. Assessment of ecological effects of extensive or repeated use of herbicides: final report. Midwest Res. Inst., Kansas City, Missouri. 369 p.
58. Holser, A. and J. Y. Gaylor. 1962. Results of endothall and two formulations of silvex for the control of aquatic plants in Oklahoma. Proc. So. Weed Conf. 15:244–255.
59. Holser, A. 1963. Loss in weight of sunfish following aquatic vegetation control using the herbicide silvex. Proc. Okla. Acad. Sci. 43:232–237.
60. Hughes, J. S. 1962. The toxicity of fish of different formulations of 2,4,5-T, 2-(2,4-DP) and silvex. Proc. So. Weed Conf. 15:265–266.
61. Hughes, J. S. and J. T. Davis. 1963. Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds 11: 50–53.
62. Hull, H. M. (Chm.). 1967. Herbicide Handbook. Weed Soc. of Amer. 293 p.
63. Hunt, E. G. 1966. Biological Magnification of Pesticides Scientific Aspects of Pest Control 251–262. Nat. Acad. Sci., Washington, D.C. 470 p.
64. Ishizawa, S. and T. Matsuguchi. 1966. Effects of pesticides and herbicides upon microorganisms in soil and water under waterlogged conditions. Bull. Nat. Inst. Agr. Sci. (Japan), Ser. B. 16:1–90.
65. Jacorossi, F. 1965. The toxicological problems of controlling weeds in canals. Weed Abstr. 14:267–268. No. 1506.
66. Johnson, R. E., T. C. Carver, and E. H. Dustman. 1967. Indicator species near top of food chain chosen for assessment of pesticide base levels in fish and wildlife—clams, oyster, and sediment in estuarine environment. Pest. Monit. J. 1:7–13.
67. Kenaga, E. E. 1969. Tordon herbicides—Evaluation of safety to fish and birds. Down to Earth 25(1):5–9.

16260

68. KLINGMAN, G. C. 1965. Weed Control: As a Science. John Wiley & Sons, Inc., New York. 421 p.
69. KOCHKIN, V. P. 1967. Experimental data for the hygienic standardization of the dalapon level in bodies of water. Gig. Sanit. 32:7–11.
70. KRAMMES, J. S. and D. B. WELLETS. 1964. Effects of 2,4-D and 2,4,5-T on water quality after spraying treatment. U.S. Forest Serv. Res. Note PSW 32:1–4.
71. LAWRENCE, J. M. 1957. Recent investigations on the use of sodium arsenite as an algacide and its effects on fish production in ponds. Southeast. Assoc. Game and Fish Commissioners 11:281–286.
72. LAWRENCE, J. M., R. D. BLACKBURN, and P. G. BEASLEY. 1961. Aquatic herbicide evaluation tests using plastic pools. Proc. So. Weed Conf. 14:309–316.
73. LAWRENCE, J. M. 1962. Aquatic herbicide data. Agr. Handbook No. 231. Agr. Res. Serv., U.S. Dep. of Agr. 471 p.
74. LAWRENCE, J. M. 1965. Graphic presentation of aquatic herbicide data. Proc. So. Weed Conf. 18:568–573.
75. LINDABERRY, H. L. 1961. Considerations regarding the use of aquathol in potable watersheds. Proc. No. East Weed Contr. Conf. 15:481–484.
76. LINDEN, G. and A. MULLER. 1963. Further investigations on the effect of diesel oil on the soil. Allg. Forstzeitschr 18: 791–793.
77. MACKENTHUN, K. M. 1960. Some limnological investigations on the long-term use of sodium arsenite as an aquatic herbicide. Proc. No. Centr. Weed Contr. Conf. 17:30–31.
78. MACKENTHUN, K. M. 1961. Observations on the use of 2,4-D granules in fish hatchery ponds. Res. Rep. No. Centr. Weed Contr. Conf. 18:105.
79. MAGEE, L. A. and A. R. COLMER. 1959. Decomposition of 2,2-dichloropropionic acid by soil bacteria. Can. J. Microbiol. 5:255–260.
80. MALONEY, T. E. 1958. Control of Algae with chlorophenyl dimethyl urea. J. Amer. Water Works Assoc. 50:417–422.
81. MARSTON, R. B., D. W. SCHULTS, T. SHIROYAMA, and L. V. SNYDER. 1968. Amitrole concentrations in creek waters downstream from an aerially sprayed watershed sub-basin. Pest. Monit. J. 2:123–128.
82. MARTYNIUK, V. Z. and M. I. GZHEGOTSKII. 1967. Toxic chemical level in water and soil treated with chloroganic herbicides. Gig. Sanit. 32:96–98.
83. MATHEWS, L. J. 1960. Aquatic weed control. Proc. New Zealand Weed Contr. Conf. 13:58–61.
84. MOUNT, D. I. and C. E. STEPHEN. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Amer. Fish Soc. 96:185–193.
85. MULLISON, W. R. 1966. Some toxicological aspects of silvex. Proc. So. Weed Conf. 19:426–435.
86. NATIONAL ACADEMY OF SCIENCES—NATIONAL RES. COUNCIL. 1966. Principles of Plant and Animal Pest Control. Vol. 2. Weed Control. Nat. Acad. Sci., Washington, D.C. 471 p.
87. NEWMAN, J. F. 1965. Methods of assessing wildlife hazards. Proc. Brit. Invest. and Fungic. Conf. 3:342–357.
88. NEWMAN, J. F. and J. M. WAY. 1966. Some ecological observations in the use of paraquat and diquat as aquatic herbicide. Proc. Brit. Weed Contr. Conf. 582–585.
89. NEWMAN, J. F. 1968. The ecological effects of paraquat and diquat when used to control aquatic weed. Weed Abstr. 17:361. No. 2211.
90. NICHOLSON, H. P. and J. R. THOMAN. 1965. Pesticide persistence in public water, their detection and removal. pp. 181–189. *In* Research in Pesticides. Academic Press, Inc., New York.
91. NISHIUCHI, Y. and Y. HASHIMOTO. 1967. Toxicity of pesticide ingredients to some fresh water organisms. Bull. Inst. Insect Contr. 32:5–11.
92. NOLL, D. C. and R. BURDRICK. 1961. Interim report on aquatic weed control of recreational waters above potable water. Proc. No. East Weed Contr. Conf. 15:470–475.
93. NORRIS, L. A., M. NEWTON, and J. ZAVITKOVSKI. 1967. Stream contamination with amitrole from forest spray operations. Res. Prog. Rep., West. Weed Contr. Conf. pp. 33–35.
94. NORRIS, L. A. 1968. Stream contamination by herbicides after fall rains in forest land. Res. Prog. Rep., West. Soc. of Weed Sci. pp. 33–34.
OKUZO, K. and T. OKUBO. 1965. Influence of diluted sea water on the physiological activity of baby-neck clam, *Venerupis japonica*, and the toxic effect of an herbicide PCP, pentachlorophenate. Bull. Tokai. Reg. Fish Res. Lab. 44:31–40.
96. PARKA, S. J. and H. M. WORTH. 1965. The effects of trifluralin on fish. Proc. So. Weed Conf. 18:469–473.
97. PETRUK, G. F. 1965. Effect of herbicides on heterotrophic microorganisms of ponds. Weed Abstr. 14:330. No. 1837.
98. PIERCE, M. E. 1958. The effect of the weedicide Kuron upon the flora and fauna of two experimental areas of Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 12:338–343.
99. PIERCE, M. E. 1959. Further study of the effect of the weedicide Kuron upon the flora and fauna of Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 13:310–314.
100. PIERCE, M. E. 1960. Progress report of the effect of Kuron upon the biota of Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 14:472–475.
101. PIERCE, M. E. 1960. A study of the effect of the weedkiller, 2,4-D Granular, on three experimental plots of Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 14:483–487.
102. PIERCE, M. E. 1961. A study of the effect of the weedkiller 2,4-D Aqua Granular on six experimental plots of Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 15:539–544.
103. PIERCE, M. E. 1962. A comparative study of the application of three weedicides, Kurosal G, Kurosal SL and 2,4-D ester to three areas in Long Pond, Dutchess County. Proc. No. East Weed Contr. Conf. 16:435–441.
104. PIERCE, M. E. 1963. Progress report of the application of fenac granular to six small plots in Long Pond, Dutchess County, New York. Proc. No. East Weed Contr. Conf. 17:451–455.
105. PIERCE, M. E. 1966. Application of fenac to four small ponds on Vassar Campus. Proc. No. East Weed Contr. Conf. 20:470–475.
106. PIERCE, M. E. 1967. Study of a second year application of fenac to two small ponds on Vassar Campus. Proc. No. East Weed Contr. Conf. 21:530–533.
107. PIERCE, M. E. 1968. The effect of several herbicides on eight test areas in Nobska Pond, Woods Hole, Massachusetts. Proc. No. East Weed Contr. Conf. 22:195–203.
108. PIERCE, M. E. 1969. The effect of a combined formulation of 2,4-D and endothall upon a small pond. Proc. No. East Weed Contr. Conf. 23:376–379.
109. PIERCE, P. C., J. E. FREY, and H. M. YAWN. 1965. Field evaluations of newer aquatic herbicides. Proc. So. Weed Conf. 18:497–506.
110. PIERCE, P. C. 1966. Results of field testing newer aquatic herbicides with emphasis on spot treatment applications. Proc. So. Weed Conf. 19:374–375.
111. PROWSE, G. A. 1960. Notes on the toxicity to fish of some common agricultural insecticides and weedicides. Weed Abstr. 9:No. 1665. Not paged.
112. RAO, K. N. and M. S. MURTY. 1968. Control of *Ipomoea reptans* Poir with herbicides in Andhra Pradesh. Weed Abstr. 17:364. No. 2233.
113. RAWLS, C. K. 1965. Field tests of herbicide toxicity to certain estuarine animals. Chesapeake Sci. 6:150–161.
114. REIGNER, I. C., W. F. SUPPER, and R. R. JOHNSON. 1968. Will the use of 2,4,5-T to control streamside vegetation contaminate public water supplies? J. Forest. 66:914–918.
115. REINHART, K. G. 1965. Herbicidal treatment of watersheds to increase water yield. Proc. No. East Weed Contr. Conf. 19:546–551.
116. RIEMER, D. N. 1964. Observations on some acid water lakes treated with diquat. Proc. No. East Weed Contr. Conf. 18:489–493.
117. RODRIGUEZ-KABANA, R., E. A. CURL, and H. H. FUNDERBURK, JR. 1967. Effect of paraquat on growth of *Sclerotium rolfsii* in liquid culture and soil. Phytopathology 57:911–915.
118. SATO, M., M. KITAYAMA, and T. KITSUTAKA. 1968. Studies on the prevention of poisoning by agricultural chemicals:12. Influence of herbicide sodium pentachlorophenol spread on paddyfield regions with river water. Rep. Hokkaido Inst. Pub. Health 17:103–107.
119. SCHWARTZ, H. G., JR. 1967. Microbial degradation of pesti-

DOW 5311757
16261

cides in aqueous solution. J. Water Poll. Contr. Fed. 39: 1701–14.
120. Simes, J. C. 1961. Control of the pondweed, *Potamogeton crispus* in both flowing and static situations with endothall. Proc. No. East Weed Contr. Conf. 15:556–559.
121. Smith, G. E. and B. C. Isom. 1967. Investigation of effects of large-scale applications of 2,4-D upon aquatic fauna and water quality. Pest. Monit. J. 1:16–21.
122. Snow, J. R. 1963. A preliminary report on the control of Pithophora with simazine. Proc. So. Weed Conf. 16:329–335.
123. Steucke, E. W. 1961. Observations on the use of disodium endothall in fish hatchery ponds. Res. Rep. No. Centr. Weed Contr. Conf. 18:104.
124. Steucke, E. W. 1963. Observations on the use of disodium endothall in fish hatchery ponds. Weed Abstr. 12:197. No. 1036.
125. Surber, E. W. and O. L. Meehean. 1931. Lethal concentrations of arsenic for certain aquatic organisms. Trans. of Amer. Fisheries Soc. 61:225–239.
126. Tarrant, R. F. and L. A. Norris. 1967. Residues of herbicides and diesel oil carriers in forest water. Proc. Symp. Herb. in Veg. Manage. pp. 81–88.
127. Tatum, W. M. and R. D. Blackburn. 1965. Preliminary study of the effects of diquat on the natural bottom fauna and plankton in two subtropical ponds. Weed Abstr. 14:26. No. 144.
128. Taylor, J. L. 1966. Casoron a new aquatic herbicide. Hyacinth Contr. J. 5:20–21.
129. Taylor, J. L. 1969. Dichlobenil aquatic herbicide for use in non-flowing water. Proc. So. Weed Conf. 19:376–377.
130. Thiegs, B. J. 1955. The stability of dalapon in soils. Down to Earth 11(2):1–4.
131. Thomas, M. L. H. and J. R. Duffy. 1968. Butoxyethanol ester of 2,4-D in the control of Eelgrass (*Zostera marina* L.) and its effects on oysters (*Crassostrea virginica* Gmelin) and other benthos. Proc. No. East Weed Contr. Conf. 22:186–193.
132. Timmons, F. L. 1962. Present and future of aquatic weed control. Proc. No. East Weed Contr. Conf. 16:1–8.
133. Tschirley, F. H. 1969. Defoliation in Vietnam. The ecological consequences of the defoliation program in Vietnam are assessed. Science 163:779–786.
134. U.S. Dep. of Int. 1968. Report of the Committee on Water Quality Criteria. Fed. Water Poll. Contr. Admin., Washington, D.C. 234 p.
135. U.S. Dep. of Int. 1963. Fish and Wildlife Service. Pesticide-Wildlife Studies. Review of Fish and Wildlife Service investigation during 1961 and 1962. Circ. 167. 109 p.
136. U.S. Dep. of Int. 1964. Fish and Wildlife Service. Pesticide-Wildlife Studies. Review of Fish and Wildlife Service investigations during calendar year 1963. Circ. 199. 130 p.
137. U.S. Dep. of Int. 1965. Fish and Wildlife Service. Pesticide-Wildlife Studies. Circ. 226. 77 p.
138. Unrau, G. O., M. Farooq, K. Dawood, L. Miguel, and B. Dazo. 1965. Field trials in Egypt with acrolein herbicide-molluscicide. World Health Org. Bull. 32:249–260.
139. Van Valin, C. C. 1966. Persistence of 2,6-dichlorobenzonitrile in Aquatic Environments in Organic Pesticides in the Environment. A Sym. Amer. Chem. Soc. Advances in Chemistry Series 60:271–279.
140. Walker, C. R. 1961. Toxicological effects of several herbicides to bottom dwelling fish-food organisms in Missouri ponds. Res. Rep. No. Centr. Weed Contr. Conf. 18:104–105.
141. Walker, C. R. 1963. Endothall derivatives an aquatic herbicide in fishery habitats. Weeds 11:226–232.
142. Walker, C. R. 1964. Dichlobenil as a herbicide in fish habitats. Weeds 12:267–269.
143. Walker, C. R. 1964. Simazine and other *s*-triazine compounds as aquatic herbicides in fish habitats. Weeds 12: 134–139.
144. Walker, C. R. 1965. Diuron, fenuron, monuron, neburon, and TCA mixtures as aquatic herbicides in fish habitats. Weeds 13:297–301.
145. Weldon, L. W. and R. D. Blackburn. 1967. The control of floating aquatic weeds with ametryne. Proc. So. Weed Conf. 20:312–318.
146. Wellborn, T. L., Jr. 1969. The toxicity of nine therapeutic and herbicidal compounds to striped bass. Prog. Fish-Cult. 31:27–32.
147. Wershaw, R. L., M. C. Goldberg, and D. J. Pinckney 1967. The determination of the ionization constant of 2,4-D in water. Water Resour. Res. 3:511–516.
148. Wile, I. 1968. Aquatic plant control with diquat, fenac, and simazine in Ontario farm ponds. Proc. No. East Weed Contr. Conf. 22:173–185.
149. Winston, A. W., Jr. and P. M. Reily. 1951. What happens to phenoxy herbicides when applied to a watershed area? Proc. No. East Weed Contr. Conf. 15:396–401.
150. Yeo, R. R. 1967. Dissipation of diquat and paraquat, and effects on aquatic weeds and fish. Weeds 15:42–46.

16262

2918

PROGRESS REPORT ON THE USE OF KURON, 2,4-D and 2,4,5 TP GRANULES AS AQUATIC HERBICIDES

by

Roy R. Younger 1/
Assistant Fisheries Biologist
N. J. Division of Fish and Game

MN057107 DOW 14285521

The purpose of this report is to summarize the results of three year's experimentation using Kuron, and one year's progress in using 2,4-D and 2,4-5 TP granules as aquatic herbicides.

Kuron

The first experimental work with Kuron was conducted at Ramapo Lake, Passaic County in 1956. 2/ The following year thirteen lakes and ponds were treated. The results of these experiments have been presented in a previous paper. 3/

At Ramapo Lake, the herbicide was applied at a concentration of 2.5 p.p.m. to control water milfoil, Myriophyllum heterophyllum; fanwort, Cabomba caroliniana; and white water lily, Numphaea odorata. No further treatment was necessary in 1957, however, in 1958 the treated areas were completely reinfested with the same noxious weeds.

Good control of the most predominant species - water milfoil, Myriophyllum sp., yellow water lily, Nuphar sp., and white water lily, Nymphaea sp., was obtained in five ponds receiving total applications of Kuron in 1957 at concentrations ranging from 0.5 to 2.5 p.p.m. In 1958 these ponds needed no further treatment because the water milfoil had not reappeared and the water lily stands were greatly reduced. Considering the size and extent of the lily root systems it is not difficult to understand why total control in one application is not possible.

Plant reinfestation seems most likely to occur, at least in New Jersey, from seeds and plant fragments entering from untreated lakes upstream, rather than from the germination of seeds after the application of Kuron. There is some evidence

---

1/ The author is now employed as a technical representative of Halco Chemical Company, Kenilworth, N. J.

2/ Huckins, Robert K. 1956. Job completion report, Project F-1-R, Aquatic Weed Control, unpublished.

3/ Younger, Roy R. 1957. Preliminary studies using Kuron as an aquatic Weed Control Conference, New York, January, 1958. All this work has been conducted under federal aid in Fish & Wildlife Restoration Acts, New Jersey project numbers F-10-R & F-14-D.

16263

reduced prior to treatment greater plant destruction was achieved. Therefore, it is recommended that lakes and ponds receive total treatment and that the lake volume be reduced to insure sufficient contact time.

The concentration recommended to obtain adequate control with Kuron is 2.0 to 2.5 p.p.m. The Dow Chemical Toxicological Laboratory has determined the L.D. 50 for the emerald shiner, Notropis atherinoides, is 7.0 p.p.m. The recommended concentration is far below the toxic level and fishkills should not occur, providing other chemicals are not used before or after treatment. Furthermore, damage to shoreline vegetation should be negligible at this concentration. 5/

3. Bottom application

Spraying the exposed bottom with Kuron at the rate of 4 gallons per acre has proven successful in one instance. It might be worthwhile to more fully explore this technique, thereby reducing the cost and making it possible to spray at an earlier period in the spring.

Conclusions

The results of three years of experimentation would seem to indicate that longer periods of control are obtained with Kuron than with other herbicides.

2,4-D and 2,4,5 TP granules

As a result of Dr. Grigsby's 6/ work in Michigan, the New Jersey Division of Fish and Game became interested in further field testing of 2,4-D and 2,4,5 TP granules. Thirty-six plots containing 2,500 square feet (.057 acres) were laid out to evaluate the effectiveness of 2,4-D and 2,4,5 TP granules of either 15/30 or 8/15 mesh size. The "TP" granules were tested in three plots and the "D" in thirty-three plots.

5/ There have been some fishkills in the past. In one instance the fishkill was caused by the addition of copper sulfate for algae control prior to treatment with Kuron. Another fishkill in a farm pond was attributed to the rinsing of spray equipment previously used to apply Malathion. A third fishkill has never been explained due to so many extraneous factors which occurred either prior to or immediately following application.

6/ Grigsby, B. H. and R. H. Hamilton, 1958. New techniques in the application of herbicides for control of aquatic plants. Presented at the 1958 meeting of the Weed Society of America, Memphis, Tenn. January, 1958.

DOW 14285 23

16264

DOW 1428525

Conclusions:

The advantage of using granules is ease of distribution since no cumbersome spray equipment is required. This makes it possible for lake front property owners to treat their portion of the lake.

If the mechanics of the granules can be improved, and if the rates of application can be determined, they will definitely have a place in aquatic weed control.

16265

DOW 1428527

16266

Table I Contd.

SUMMARY OF 2,4-D and 2,4,5 TP EXPERIMENTS IN NEW JERSEY FOR 1958

Area treated	Type of vegetation	Control
Applegate's Pond Monmouth County	Fanwort, Cabomba sp. Spatterdock, Nuphar sp.	None
Medford Lakes Camden County	Water milfoil, Myriophyllum sp. White water lily Nymphaea sp.	None
Musconetcong Lake Morris County	Water milfoil, Myriophyllum sp.	2,4-D control only with 12 and 18 lbs/acre. 2,4,5 TP control with 9 lbs/acre 1/
Hopatcong Sussex County	Water milfoil, Myriophyllum sp. Spatterdock, Nuphar sp. Pond weed Potmegeton sp.	Good control at all rates 2/
Grovers Mills Mercer County	Fanwort, Cabomba sp.	None 3/
Estelville Lake Atlantic County	Bladderwort Utricularia sp.	None 3/
Saxtons Falls Warren County	Water milvoil Myriophyllum sp.	None 3/

1/ At time of application no Vallisneria was observed. After treatment and destruction of Myriophyllum, the Vallisneria took over the treated area.

2/ Regardless of rates of application, good control was had for a short period of time since weed fragments from untreated areas settled to the bottom, putting out advantitious roots, thus reinfesting the treated area.

3/ In these plots the 2,4-D was sprayed on soft attaclay which broke down immediately upon contact with water.

Reprinted from the Proceedings of the Thirteenth Annual Meeting of the Northeastern Weed Control Conference January 7, 8 and 9, 1959

ACD File No. HA-8

DOW 288954

REPRINT OF PAPER PRESENTED AT THE

TWELFTH ANNUAL MEETING

OF THE

NORTHEASTERN WEED CONTROL CONFERENCE

Hotel New Yorker

New York City

January 8, 9, 10, 1958

16867

PRELIMINARY STUDIES USING KURON AS AN AQUATIC HERBICIDE[1/]

by
Roy R. Younger
Assistant Fisheries Biologist
New Jersey Division of Fish and Game

DOW 288955

In the summer of 1956 the New Jersey Division of Fish and Game, under Dingell-Johnson Project No. F-1-R, Aquatic Weed Control, experimented with Kuron on one-acre plots in two northern New Jersey lakes. The chemical called Kuron - 2(2,4,5-Trichlorophenoxy Propionic Acid - is a hormone-type growth regulating compound which is absorbed by the leaf and translocated to other parts of the plant. The above mentioned plots were heavily infested with water milfoil, Myriophyllum heterophyllum; fanwort, Cabomba caroliniana, and white water lily, Nymphaea odorata. One week after application good control of the submerged species was evident; the emergents were not materially affected. (Huckins, 1956, ms.) It was felt that no further treatment was necessary after examination of the plots in the spring of 1957.

The results did seem to indicate that further evaluation was desirable and a testing program was initiated in the summer of 1957[2/].

Methods and procedures

Lakes and ponds, in order to be included as part of this testing program, had to meet certain requirements:

1. As a group they had to represent a cross-section of existing lakes and ponds throughout the state with regard to weed species, both emergent and submerged, alkalinity and hydrogen-ion concentration.

2. They had to have a woody shoreline so that the effect of Kuron on woody plants could be evaluated.

In treating a pond the procedure was to calculate the active ingredient in Kuron required to produce the predetermined concentration in parts per million.

To determine the effectiveness of Kuron at various concentrations the herbicide was tested at 0.5 p.p.m., 1.0 p.p.m., 2.0 p.p.m., and 2.5 p.p.m. One reclaimed lake in southern New Jersey was treated at 3.5 p.p.m.

The herbicide was applied by a Bean Estate Sprayer, skid mounted, with split intake lines and the by-pass was used for circulation and agitation. A spray boom was devised and a thirty-gallon drum served as a spray tank. The entire rig was mounted on a 11.5 foot aluminum boat and was operated by two men. The herbicide was applied in rows with a slight overlap between them.

1/ Misc. Report #21, New Jersey Fisheries Laboratory, Division of Fish and Game, Milltown, New Jersey.

2/ This program would not have been possible without the assistance of Dr. E. Evaul and Neal Munch of the Soil Conservation Service who obtained ponds for testing; Dr. Mark Wiltse of the Dow Chemical Company supplied the Kuron.

16268

After the ponds had been treated water samples were taken and forwarded to the Dow Chemical Company for Kuron analyses. The water samples were taken from two to twenty-four days after application. (Table I)

Ponds were checked at seven day intervals for the first month with monthly checks thereafter.

Findings

During the course of the summer eight lakes received total applications and five lakes received plot treatments of a half-acre in area. A summary of these applications is presented in Table II.

The concentration of 0.5 p.p.m. was effective on white water lily, Nymphaea. The submerged species were only slightly affected. At 1 p.p.m. good control of water weed, Anacharis sp., yellow water lily, Nuphar, and mud plaintain, Heteranthea sp., was obtained. Complete control of water milfoil, Myriophyllum, and bladderwort, Utricularia, was achieved with concentrations of 2.0 p.p.m. and 2.5 p.p.m. From these data there is every indication that adequate control of most species can be obtained at 2 p.p.m. however, further testing of the effects of Kuron on pondweed, Potamogeton, is necessary since none of the concentrations employed controlled this species. Duckweed, Lemna minor, turned brown forty-eight hours after application of Kuron and then recovered fully.

DOW 288956

As to the effects of Kuron on trees, either in the water or surrounding the lake, no damage was evident on such species as silver maple, black gum, pitch pine, willow and white cedar.

Since this herbicide contains phenol the possibility of its tainting fish flesh was considered. Fish were collected from a treated area and taste tests revealed that Kuron did not taint in any manner.

As mentioned earlier, measurements of pH, alkalinity and temperature were recorded. None of these water characteristics appeared to affect the herbicidal effects of Kuron.

Observations suggest that Kuron is slow to act. Seven days after treatment submerged plants appeared wilted and emergents were curled. Fourteen days after treatment open areas were visible; submerged vegetation had started to rot, while the emergents continued to curl. It was not until twenty-one days after treatment that the submergent weeds were completely disintegrated and leaf and stem systems of the emergent vegetation had broken free from the roots. One month after application root systems of both types of weeds were observed in windrows along the shore.

Since the affected plants did seem to break down quite slowly, there was some suggestion that no serious oxygen lag occurred. However, this cannot be substantiated since an insufficient number of D. O. determinations was taken. Observations also suggested the absence of algae blooms, sometimes characteristic of certain herbicidal applications, caused by the release of essential nutrients from the rapid breakdown of plant tissues. However, this lack of algae blooms could also indicate possible algacidal effects of Kuron.

Further evidence of the slow breakdown of Kuron was indicated by the water samples which were forwarded to the Dow Chemical Company for Kuron analyses. In the total applications the concentration of Kuron remained fairly constant while in plot applications the concentrations were affected by dilution, or a build-up in stream application. Residual effects cannot be determined until next spring when the viable seeds, if any, will germinate.

Plot applications were not as effective as total applications since reinfestation was possible when shoots and seeds from untreated areas floated into treated areas. The possibility also exists that once the vegetation has been destroyed other aquatic species, either desirable or undesirable, may over-run the treated area. Total applications were most effective since reinfestation was less likely to occur.

Summary and conclusions

The experiments conducted to date have more or less been designed to determine the herbicidal effects of Kuron on aquatic vegetation. Success has been obtained on the most predominant weed species in the thirteen lakes and ponds so treated, using a concentration of 2.0 p.p.m. The period necessary for total plant destruction is normally twenty-one days. The usual algal blooms were not observed following treatment with Kuron; preliminary data suggest no serious oxygen lag. This could be attributed to the slow breakdown of the plant tissues but the apparent scarcity of phytoplankton also suggests the possibility of algacidal properties.

None of the concentrations employed have affected the trees either in the water or along the shoreline. Neither pondweed, Potamogeton, nor duckweed, Lemna minor, has been effectively controlled by any concentrations of Kuron.

There are many important questions still to be answered regarding the possible use of Kuron as an aquatic herbicide. These questions include: At what time of year will application achieve the most effective control of weeds? Does this herbicide have any undesirable side effects on certain micro fauna and flora important to game and pan fish species? Will it control filamentous algae? Does it have a residual effect? Will its cost affect its use?

The results to date have been promising and the proposed questions are in the process of being investigated. In the meantime Kuron can be considered as one of the most important aquatic herbicides on the market since the development of sodium arsenite.

DOW 288957

16270

Table I. AMOUNT OF KURON DETECTED FROM FIVE TREATED PONDS 2 TO 24 DAYS AFTER APPLICATION
(Analyses conducted by Dow Chemical Company)

Name of Pond	Kuron applied p.p.m.	No. days sample taken after application	Kuron detected p.p.m.
Rte. No. 1	2.0	2 days	2.0
Golomb's Pond	2.5	24 days	1.5
Keswick Pond	2.0	14 days	1.8
New Waywayanda	2.0	3 days	2.1
Panther Pond	2.0	8 days	Less than 0.5

DOW 288958

16271

Table II SUMMARY OF KURON APPLICATIONS - 1957

Name of lake or pond	Kuron applied p.p.m.	pH	Tot.alk. p.p.m.	Temp.	Dominant weed species	Degree of Control
Taylor Pond	1.0	7.2	13.0	86°	Heteranthera dubia	Complete
Mohawk Lake	1.0	8.4	82.0	78°	Anacharis canadensis Potamogeton crispus Typha latifolia Scirpus americanus Sparganium sp. Lemna minor Nuphar variegatum	Excellent control of submergents, no control of emergents except those individual plants which came in contact with spray
Bell Lake	3.5	8.3	105.0	68°	Anacharis canadensis Nuphar variegatum	Excellent control of both species
Ludlum Lake	2.5 (plot)	6.4	14.0	81°	Eriocaulon septangulare Myriophyllum heterophyllum Vallisneria americana	Excellent control Reinfestation
Cape May Park Pond	3.0	6.6	12.0	79°	Nymphaea odorata Potamogeton sp. Anacharis canadensis Utricularia purpurea	Excellent control
Barker Pond	0.5	7.3	6.0	78°	Myriophyllum heterophyllum	In doubt. Pond was previously treated with sodium arsenite
Applegate Pond	0.5	7.0	27.0	80°	Nymphaea odorata Potamogeton sp. Vallisneria americana Cabomba caroliniana Myriophyllum heterophyllum	Only species affected was water lily

336.

Pond 288959

2797

Table II Cont.

Calomb Pond	2.0	6.7	12.0	81°	Myriophyllum heterophyllum Nuphar variegatum Scirpus sp.	Excellent control
Foo Lake	0.5-plot #1 0.1-plot #2 2.0-plot #3	5.4	53.0	80°	Nuphar variegatum Cabomba caroliniana Myriophyllum Lemna minor Anacharis canadensis	Plot #1 only Nuphar. Plot #2 and Plot #3 good control No control of Lemna
Keswick Colony Lake	2.5	5.6	2.0	78°	Utricularia purpurea Nymphaea odorata Potamogeton sp.	Excellent control except for Potamogeton
Panther Lake	2.5	8.8	103.0	74°	Myriophyllum heterophyllum	Excellent control
New Waywayanda Lake	2.0	8.8	107.0	76°	Nymphaea odorata Myriophyllum heterophyllum	Excellent control
MacDowell Pond	2.0	6.8	76.0	80°	Scirpus americanus Potamogeton sp.	Only Scirpus was controlled

16273

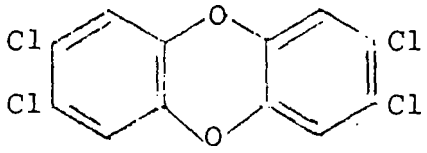
DOW0288960

SUMMARY OF TOXICOLOGICAL AND ANALYTICAL INFORMATION ON THE CHLORACNEGEN IMPURITY FOUND IN SOME SAMPLES OF 2,4,5-T

OCCURRENCE AND IDENTITY

Several impurities can occur in 2,4,5-T arising from their presence in 2,4,5-trichlorophenol. They can be minimized by proper operating procedures. The most important impurity which causes the chloracne problem is:

symmetrical tetrachlorodibenzo-p-dioxin



Dow 1413086

TOXICOLOGY

Note. All doses given as MICROGRAMS (10^{-6} grams)

Skin Contact

Human

1. Known to cause severe chloracne by skin contact. Very small amounts can cause the lesions.

Rabbit

1. Inner surface of rabbit ear treated with various levels of the chloracnegen in 100 microliters (ul) of a test solution in acetone benzene or chloroform on an area of 1 to 2 sq. in. (5 animals per dose):

Total Dose	Applications	
0.2 ug	repeated	= no effect
0.5 ug	repeated	= marginal effect*
1-2 ug	sometimes from one application	= usually causes an effect
4-8 ug		= almost always causes an effect, usually severe, but not lethal

2. Shaved belly of rabbits treated with 10% solution in acetone, 5 animals per dose. LD_{50} by skin absorption = 275 (142-531)** ug/kg body wt. Mortality generally occurred 12-22 days after dosing.

For parathion, $NO_2-C_6H_4-O-P(=S)-(OC_2H_5)_2$, the LD_{50} by skin absorption has been reported to be:

rabbits = 40,000 ug/kg body wt.
rats(M) = 21,000 ug/kg
rats(F) = 6,800 ug/kg

Intraperitoneal

When administered as a 10 mg % suspension in corn oil to rabbits (5 animals per dose level):

I.P. LD_{50} = 283 (114-703)** ug/kg body wt.

*Follicular prominence and/or epithelial thickening begins 1 to 3 weeks after exposure.

**95% confidence limits.

FDA 1413087

[For parathion, the intraperitoneal LD_{50} has been reported[1] to be:

rat = 5,500 ug/kg
guinea pig = 12,000 ug/kg
dog = 12,000-20,000 ug/kg]

EPA 1413088

Acute Oral (single dose)

Rabbit

When administered as a 10 mg % suspension in corn oil (5 animals per dose level): Acute oral LD_{50} = 100 (29-389)** ug/kg. Two rabbits died at 32 ug/kg, 1 died at 16 ug/kg. Typical clinical picture was severe liver and kidney injury (histopathologic examination) at doses approaching LD_{50}. Have seen histopathology at single dose of 3 ug/kg. Dose of 500-1000 ug/kg was always fatal; sometimes took 10-20 days for death to occur.

[For parathion, the acute oral LD_{50} in the rabbit = 10,000 ug/kg]

Rat

5 mg % in 1:1 corn oil/acetone (5 animals per dose):

Acute LD_{50}, female rat = 45 (36-65)** ug/kg
male rat = 22 ug/kg

[For parathion, the acute oral LD_{50} is:

rat(M) = 5,000-30,000 ug/kg
rat(F) = 1,750-5,000 ug/kg]

---

(1) Data for parathion taken from "Evaluation of The Toxicity of Pesticide Residues in Food". FAO/WHO Joint Expert Committee on Pesticide Residues. FAO Mtg. Rept. No. PL/1965/10/1.

** 95% confidence limits.

Guinea Pig

50 mg % in corn oil (5 animals per dose)

Acute oral LD_{50}, female guinea pigs = 0.6 (0.4-0.9)** ug/kg

[For parathion, the acute oral LD_{50} is 9,300-32,000 ug/kg for guinea pigs]

D0W 1413089

ANALYTICAL METHODS

Vapor Phase Chromatography can detect 1 ppm. Need 100 g. for assay. (Method attached.)

Liquid Chromatography can quantitate 1 ppm in a 2 g. sample.

Bioassay (see under TOXICOLOGY, Skin Contact, Rabbit). Can detect the chloracnegen in smaller samples and should be used to verify absence or presence of "dioxin" in 2,4,5-T used in toxicity tests.

DOSE RELATIONSHIP OF 2,4,5-T/"DIOXIN" WHERE "DIOXIN" PRESENT AT 10 PPM IN 2,4,5-T

2,4,5-T mg/kg	2,4,5-T ug/kg	10 ppm "Dioxin" ug/kg
1	1,000	0.01
10	10,000	0.1
50	50,000	0.5*
100	100,000	1.0

*Equal to the acute oral LD_{50} for the "dioxin" to female guinea pigs and 1/6 of the single oral dose which causes histopathology in liver of rabbits.

**95% confidence limits.

DEFICIENT ORIGINAL

MN0012332

Present:

H. Gay	C. Kramer	V. Rowe
H. Gordon	R. Stewart	H. Doyle
L. Mathews	R. Holmes	A. Wilson
B. Holder	A. Leuke	L. Silverstein
L. Bollinger	E. Coad	K. Olson

The meeting was called to order at approximately 12:30 by Dr. Gordon.

Larry Mathews presented a literature review on chloracne. He indicated that the treatment of chloracne is the greatest of the puzzles to be solved. Basically, as with any disease of this type, treatment consists of removing the patient from the area. However after removal the lesions may continue to appear for up to two years. Many methods have been and are being used on the lesions themselves including radiation, antibiotics steroid, expression of comedones and keratolytic agents. Presently, he said, we are treating our patients with expression of the comedones and frequent brisk washing of the exposed area with soap and a rough wash cloth. Steroid therapy is being tried at some institutions but no reports of success or failure has as yet been reported. Despite all attempts, the duration of the chloracne appears to be unchanged. Until more suitable measures of treatment are found, the prevention of exposure is the only satisfactory way of eliminating the problem.

L. Silverstein present a chronological chart of the process changes, etc.; relating such changes to the chloracne problem.

Silverstein indicated that the oil draw-off operation in the process is the most vulnerable spot for exposure to acneform producers.

A flow-sheet chart of the process was presented and discussed by Ray Holmes. Ray emphasized that he would like to have an analytical method which could be correlated with the acneform materials in order to hasten the clean-up work.

V. K. Rowe reviewed the work done by the Germans. He put the structural formula on the board for tetrachloro benzodioxane which has been incriminated as being at least one of the most potent of the materials which produces the acneform dermatitis and perhaps the ... etc. It is our understanding that Ken Bradley is in the process of ... this material which will be sent to Bloomington for study.

The following recommendations were agreed upon by the group:
1. Substantial cleanup of operating area, tools and equipment.

Ray Holmes has contacted Dowell personnel for advice on the cleanup procedure. He indicated a desire to start on Saturday, August 29. The men will wear rubber suits for the job.

2. Review personal hygiene practices and clothing policies with men in 199 building.

4. Continue surveillance of potentially exposed personnel.

The Medical personnel stated that there is no indication at the present time of any systemic injury. Kramer said that all men should have periodic physicals.

Dr. Stewart reviewed the benzene exposure problem in the plant. He presented the formula for determining MCV values the normal of which is reported as being 87 ± u^3. He further indicated that there seems to be some discrepancy in this value which may be a function of our present methodology conducted in Medical. This methodology should be resolved. In order to do this Biochem. has offered the use of the Coulter Counter.

The matter of accumulating normal values for our area was discussed extensively.

Koyle listed a number of materials that might effect the benzene exposures. He also indicated that benzene exposure is at a safe level in the plant and that the MCV values conducted on many of these people may represent a normal value insofar as our area personnel are concerned.

Respectfully submitted

T. L. Gibson

T17.4-12-13

UCM 0100

MATERIALS TESTED

(Data in Table I)

Sample No.	Process Stage	Materials	Bldg. No.
1	Coil Reactor	A composite of all materials in reactor.	199
2	Decanter	Oil material decanted and disposed of in burner.	199
3	Steaming-out	Condensate from top of column. Contains chloroanisols, chloroether, and methyl alcohol.	199
5	Drying column	Tops stripped from the product from drying column (contains chlorophenol concentrate).	349
6		Tops consisting essentially of 2,4-dichlorophenol.	349
7	Finishing column	Residue from distillation of 2,4,5-trichlorophenol. Contains chlorinated diphenylether, chlorinated resorcinols, methoxy dichlorophenols, etc. Goes to waste disposal.	349

8-20-64
RDH

TABLE IX

T17.4-12-13

SKIN CONTACT IRRITATION - RABBIT Mar 1 ?

8-20-64

2,4,5-T Oil (T17.4-12-13, K-2777-55, Mar. 23, 1964)

Material	Animal No.	Condition of Skin	No. of Applications	Site	Response-Remarks
5% DPM	115 F.	Intact	1	R. ear	Moderate erythema, edema and burn. Animal died in one week.
1% DPM	115 F.	Intact	4	L. ear	Slight erythema after 3rd day; follicles prominent. (same animal as above)
1% DPM	164 M.	Intact	10	ear	Slight erythema only. Follicles prominent. Animal died after 10th application.
0.25% DPM	555 F.	Intact	12	ear	Follicles prominent. Animal died after the 12th application.
0.25% DPM	555 F.	Intact	12	belly	Slight exfoliation, no hyperplasia. Same animal as above.
0.10% DPM	938 M.	Intact	14 (17 days)	ear only	Moderate folliculitis.
0.01% DPM	940 M.	Intact	14 (17 days)	ear only	Very slight folliculitis.

16281

TABLE III

T17.4-12-14

SKIN CONTACT IRRITATION - RABBIT

2,4,5-T Oil (T17.4-12-14, K-2777-56, April 5, 1954)

Temp 21°C
Time ~45 min

Material	Animal No.	Condition of Skin	No. of Applications	Site	Response-Remarks
5% in DPM	375 M.	Intact	8	ear	Essentially no irritation. Follicles prominent.
5% in DPM	375 M.	Intact	5	belly	Extensive hyperemia and swelling after last application. Animals sacrificed.
5% in DPM	375 M.	Abraded	3	belly	Essentially the same as above
1% in DPM	557 F.	Intact	5	ear	No irritation, no folliculitis Animal died after last application.
1% in DPM	557 F.	Intact	5	belly	Same as above, no hyperplasia
0.25% in DPM	608 M.	Intact	6	ear	No irritation, follicles prominent after last application. Animal died.
0.25% in DPM	608 M.	Intact	6	belly	Same as above; no hyperplasia
0.1% in DPM	932 M.	Intact	14 (17 days)	ear only	Moderate folliculitis.
0.01% in DPM	934 M.	Intact	14 (17 days)	ear only	Slight folliculitis.

DOW 979336

16282

TABLE I

T17.A

REACTION IN THE RABBIT EAR TO PROCESS SAMPLES

Sample No.	Material Tested	1957	1964 (May 26)
1 199 Bldg Crude Reactor Prod	10% in water	Very slight folliculitis (no thickening of ear)	Severe folliculitis (5 X thickening of ear) *
2 199 Bldg Oil from Decanter	10% in water	Severe folliculitis (3 X thickening of ear) *	Severe folliculitis (4 X thickening of ear) *
3 199 Bldg Stripper Phenate	10% in water	No folliculitis (no thickening of ear)	Essentially no folliculitis (no thickening of ear)
4	Undiluted	No folliculitis (no thickening of ear)	No Sample
5 349 Bldg Drying Column	10% in DPM	No folliculitis (no thickening of ear)	Moderate to severe folliculitis (3 X thickening of ear) *
6 349 Bldg Tops Neutrex Distillate	10% in water	No folliculitis (no thickening of ear)	Very slight folliculitis (2 X thickening of ear)
7 349 Bldg Still Residue	10% in DPM	Very slight folliculitis (2 X thickening of ear)	Very slight folliculitis (slight thickening of ear died after 8th application

* Potent acneform producers.

Rec. 8-26-64
RDH

16283

DOW 979338

+ slight
++ more it

TEST V. E17.4-12-13

Wipe Tests from 199 Building 7/27/54

Chloracne Potential	Sample Number	Description
++	1	Laboratory hood - table top and sash.
+	2	Laboratory bench tops.
-	3	Laboratory - Phenate sample bottles.
-	4	Lunch Room - coffee pot, hot plate and table tops.
-	5	Locker Room - benches.
++++	6	Stripper and Methanol Pump Area - valve handle
+++	7	Oil draw-off - valves and chains.
+++[1]	8	Oil Dumpster and pipe-in oil spills.
+++	9	Taylor's shoes - 6-8 months old.
++	10	Tool boxes and Taylor's tools.
+++	11	Operator's desk and chair at panel.
+++	12	Caustic - methanol pumps - valves.
+++	13	Roof - railing around stripper columns (former vent).
++++	14	Roof - railing around methanol condenser (where Phoenix men hooked up pipes).
++++	15	Roof - railing and surface where process operator walks and stands. (See H/o on back-end)
++	16	Caustic - methanol screen area - valves.
++	17	Caustic screens - valves.
+++	18	Taylor's gloves (rubber) after he had drawn off oil on 7/27/54.
-	19	Control gloves.
+++[2]	20	1954 Sample 2 from Process caustic insoluble oi
+++[3]	21	K-2777-55 (Caustic insoluble oil) (Mar. 10, 196
+++[4]	22	K-2777-56 (Caustic insoluble oil)(April 30, 196

Collected by L. G. Silverstein and D. Garland, M.D. with A. F. Lucek on 7/27/54.

1. Animal sacrificed after 8th application. Bleeding from both ears.
2. Animal prostrate and autopsied after 5 applications and 15 days.
3. Animal prostrate and autopsied after 3 applications and 15 days.
4. Animal died on 10th day with 3 applications.

NOTE: White gloves were contaminated by wiping the fingertips on the areas described above. The contaminated areas were cut from the gloves and extracted with benzene and adjusted to 10 cc vol. 0.2 cc of the extract was applied to the rabbit ear for 7 applications; 0.1 cc 1 day thereafter.

16284

1205

HERCULES INCORPORATED

SYNTHETICS DEPARTMENT • WILMINGTON, DELAWARE 19899 • TELEPHONE: 302-656-9811

November 27, 1967

Dr. Harry Hays, Director
Pesticide Regulation Division
U. S. D. A.
Washington, D. C.

extension granted 12/27/67
see PR-Bulletin 68-1

Dear Mr. Hays:

I am writing to request that the effective date for possible cancellation of the registrations for use of phenoxy herbicide viz., 2,4-D, 2,4,5-T. MCPA, and Silvex, on range and pasture grass be extended for at least one year beyond the December 31, 1967 date established for cancellation of no-residue registrations.

The NACA Industry Task Force on Phenoxy Herbicide Tolerances has assembled petitions requesting tolerances to cover uses previously registered on a "no-residue" basis. The Department of Agriculture has cooperated with this group in several ways. Dr. L. L. Danielson was assigned the function of Department liaison with our group, and has provided information and data from USDA sources. Dr. Danielson and other members of the Department have also consulted with us at various stages in the preparation of our petition.

On November 17, representatives of the Task Force met with you and other USDA officials in the office of Dr. Robert J. Anderson to inform the group of progress in the preparation of petitions. The status of range grass and pasture grass as raw agricultural commodities and tolerances which might be necessary for them were also discussed. Pursuant to the results of that discussion, we are deleting from our petitions requests for the 20 ppm tolerances for grasses that had been considered, and requesting that an extension be granted so that data necessary for tolerances at a level higher than 20 ppm may be developed.

Continuation of the registrations for use of phenoxy herbicides on grass is in the best interests of both the agricultural industry and the public. The usefulness of these materials in recovering and improving of grass lands and pasture for livestock production was emphasized by USDA representatives at our meeting on November 17. There is no hazard to animals, as evidenced by the extensive use of these materials for more than a decade,

DOW 852478

16285

nor is the presence of residues in meat or milk a regulatory problem. Evidence from feeding studies concerning residues in meat and milk is included in the petitions being submitted in support of negligible tolerances for no-residue uses.

We have requested a cooperative program with USDA to resolve the matter of residue tolerances for phenoxy herbicides used on grass, and where necessary, for meat products. We intend to pursue this by a series of conferences to establish a program that will meet the needs and desires of the various agencies concerned with the agricultural use of phenoxy herbicides, as well as those of the regulatory agencies. A meeting with Pesticide Regulation Division to further discuss such a program will be scheduled in the near future.

Petitions seeking negligible tolerances for uses of 2,4-D, 2,4,5-T, MCPA, and Silvex presently registered on a no-residue basis (except for range and pasture grasses) are being submitted by Hazleton Laboratories, Inc., in behalf of the Industry Task Force. These should be in your hands within the next week.

Very truly yours,

C. L. Dunn

C. L. Dunn, Chairman
NACA Industry Task Force on
Phenoxy Herbicide Tolerances

CCD:alc

cc: Dr. George W. Irving, Administrator
Agricultural Research Service
U.S.D.A.
Washington, D. C.

Dr. R. J. Anderson, Deputy Administrator
Regulatory and Control Programs
Agricultural Research Service
U.S.D.A.
Washington, D. C.

Dr. L. L. Danielson
Agricultural Research Service
Beltsville, Maryland

DOW 8524479

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

1206

Pesticide Petition No. 8F0675

April 16, 1968

Mr. C. L. Dunn, Chairman
NACA Industry Task Force on
Phenoxy Herbicide Tolerances
1155 Fifteenth Street, N.W.
Washington, D.C. 20005

Dear Mr. Dunn:

This refers to Pesticide Petition No. 8F0675 requesting tolerances for negligible residues of the herbicide silvex at 0.2 ppm on apples, pears, prunes, rice, and sugarcane. We have completed our review of the petition and find that we are unable to establish the tolerances as requested for the reasons listed below.

Our conclusions with regard to the residue data are as follows:

1. The metabolism and degradation of silvex and its ester and salt formulations are not adequately delineated. Data are also required to identify and quantitate the major toxic metabolites which may be present in plants at normal harvest; of particular concern are conjugates of silvex such as those reported by Meagher, W.R., J. Agr. Food Chem. 14, 374-6 (1966). If such metabolites are found to constitute a significant residue at harvest, an analytical method for their determination will be required.

2. The methods of analysis outlined in the Pesticide Analytical Manual, Vol. I, 6.11, 6.21, 6.31(A), and 6.33(A), would not be expected to determine the metabolites mentioned above.

3. The present residue data are not sufficient to conclude whether the proposed tolerances are adequate to cover all toxic residues, including the metabolites mentioned above. In addition, residue data (including toxic metabolites if found present) are required (a) for apples reflecting the concentrated spray application; (b) for dried prunes; (c) for rice bran and straw; (d) for sugarcane bagasse and sugarcane products (syrup, molasses, sugar) derived from cane bearing residues of 0.2 ppm. (The present study of residues in refined products does not indicate whether residues were found on the cane from which the products were derived). The application rate and PHI used in the present residue data for prunes should be furnished. The additional residue data should represent application of the most persistent esters or salts of silvex. If residues are

DOW852471

16287

concentrated in any of the above processed foods, appropriate food additive tolerances should be proposed.

4. Depending on the level of residues found in livestock feed items (rice bran, rice straw, bagasse, and molasses), we may need additional tissue storage data as a basis for concluding whether or not residues would transfer to meat and milk.

We are unable to draw final conclusions with regard to the adequacy of the toxicity data until the necessary information is received. You may amend the petition or you may withdraw it and refile when the necessary information is available. Otherwise it may be necessary to establish a tolerance of zero for residues of silvex on the crops in the petition. Please let us have your reply within 30 days.

Sincerely yours,

William Stokes

William Stokes
Petitions Control Branch
Bureau of Science

cc Pesticides Regulation Division
ARS - USDA

DOW 852172

Copied to:
M. L. Leng
ITFPHT File

NATIONAL AGRICULTURAL CHEMICALS ASSOCIATION

THE MADISON BUILDING
1155 FIFTEENTH STREET, N.W.
WASHINGTON, D.C., 20005

Telephone: 296-1585
Area Code 202

12,07

July 11, 1968

DOW 852456
PN 700814

Mr. Drew M. Baker, Jr.
Petitions Control Branch
Bureau of Science
Department of Health, Education, and Welfare
Food and Drug Administration
Washington, D. C. 20204

Dear Mr. Baker:

I am writing in regard to Pesticide Petition Nos. 8F0669 (2,4,5-T); 8F0670 (2,4-D); 8F0675 (Silvex); and 8F0676 (MCPA). Letters detailing objections, primarily concerned with questions concerning metabolic products, were sent to us in April, 1968. On April 25 representatives of our Task Force met with you, Mr. Cummings, and Mr. Ramsey to discuss these objections.

At the meeting we stated that we would prepare an additional section for the petition reviewing the scientific literature with respect to metabolism of phenoxy herbicides and emphasizing the application to residues. Work on that project was started shortly after our meeting, and I wish to report on its status at this time.

Publications dealing with the metabolism of phenoxy herbicides in plants and animals as well as recent residue studies have been screened. From this material, about 60 references and 4 review articles have been selected as being pertinent to this review. Documents have been obtained, examined in detail, and information tabulated. In some cases authors have been written or called for additional information or clarification of their research findings. The writing of various portions is currently in progress, and it is estimated that the draft will be completed in about two weeks. Revision and reproduction of this somewhat bulky document will require an additional several weeks, and we are aiming at submission of the document by about August 15.

RECEIVED
JUL 15 1968
BIOPRODUCTS DEPT.

16289

An outline of the section that is being prepared is as follows:

Table of Contents

Summary Statement

Glossary of Terms

I. Metabolism in Plants
   A. Introduction
   B. Tabulation of Conditions Used in Metabolic Studies in Plants
   C. Metabolism of 2,4-D
      1. Hydrolysis of Esters and Salts
      2. Decarboxylation
      3. Conjugation
         (a) Formation of Water Soluble Unknown 1
         (b) Formation of Water Soluble Unknown 3
      4. Transformation to a Different Ether Soluble Compound
   D. Metabolism of 2,4,5-T and 2,4,5-TB
   E. Metabolism of MCPA and MCPB
   F. Metabolism of Silvex (2,4,5-TP)

II. Metabolism in Animals

III. Recent Residue Studies by Newer Methods

IV. Significance of Metabolites as Residues in Foods and Feed

Please let me know if there are further questions about our activity in the execution of our commitment to prepare this additional information discussed at our April 25 meeting.

Very truly yours,

C. L. Dunn

C. L. Dunn, Chairman
NACA Industry Task Force on
Phenoxy Herbicide Tolerances

CLD:mea

cc: USDA Pesticides Regulation Division

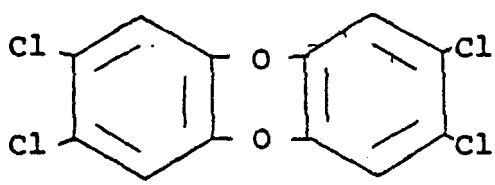
DOW 852457

CHLORACNE

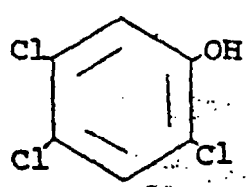
DOW EXPERIENCE

MN0067862

DOW 1 767165



2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN -- TCBD



2,4,5-TRICHLOROPHENOL -- TCP

CHLORACNE IN HUMANS

1. LIKE TEENAGE ACNE, BUT MORE BLACKHEADS.

2. APPEARS FIRST ON FACE, MAY SPREAD TO THE BACK AND OTHER PORTIONS OF THE BODY.

3. LONG-TERM -- 2 TO 5 YEARS TO RECOVER, WITH OR WITHOUT TREATMENT.

4. USUALLY NOT DISABLING BUT MAY BE FATAL.

5. LIVER INJURY -- DOCUMENTED IN EUROPEAN EXPERIENCE AND RABBIT TESTS, NOT DETECTED IN DOW CASES BY LIVER FUNCTION TESTS, ONE BIOPSY ON A SEVERE CASE.

6. MANY OTHER SYMPTOMS CLAIMED -- LISTLESSNESS, TIRE MORE EASILY, ETC.

DOW 1 767166

DOW EXPERIENCE -- 1964 TO PRESENT

1. About 100 cases.

2. About 60 in TCP incident described below.

3. Most of remainder in Dowicides production.

4. Some cases in laboratory research.

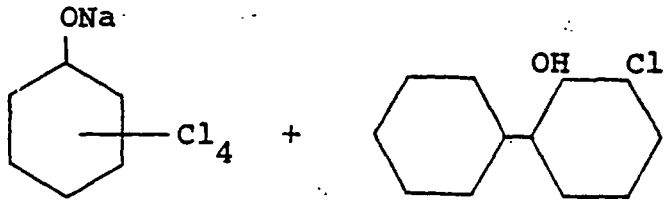
5. Three extremely severe cases, about fifteen severe cases, about three "acute" cases, about four people lost ~~life~~ Time.

6. Most cases are mild, many were found in health inventory activity by "sensitized" M.D.'s.

DOW 1767167

SIGNIFICANT CASES -- DOW

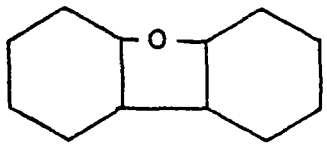
1934-1936 DOWICIDE P PRODUCTION -- SEVERE OUTBREAKS AMONG EMPLOYEES.



SODIUM TETRA-CHLOROPHENATE

O-CHLOROPHENYLPHENOL

1930's LABORATORY CASE FROM "CHLORINATED DIPHENYLENE OXIDE"



Cl_{3-6} Active

DIPHENYLENE OXIDE, ALSO DIBENZOFURAN

DOW 7671168

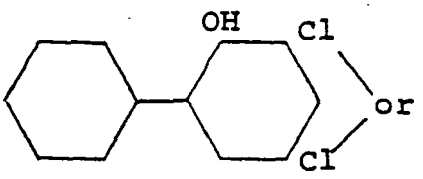
16294

1941

D. D. IRISH AND E. M. ADAMS PUBLISHED PAPER ON RABBIT EAR TEST FOR PREDICTING ACNEGENS: STILL THE MOST SENSITIVE TEST AVAILABLE.

1940's

DOW CUSTOMERS CONTRACTED CHLORACNE FROM THEN DOWICIDE 3 (NOW DOWICIDE 31 & 32, HIGH PURITY THAN DOWICIDE 3).



MIXTURE OF 4- AND 6-CHLORO-2-PHENYL PHENOL

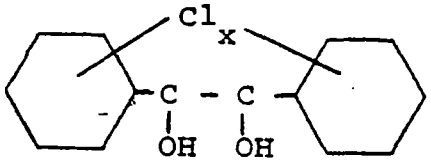
1966

PRODUCT AND PROCESS WERE CLEAN EXCEPT FOR TAR.

USDC 7671169

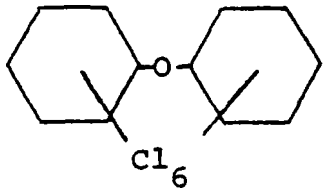
1942

BENZENE RESEARCH LAB -- ONE SEVERE CASE FROM RECYCLING TCP RESIDUE IN GLYCOL SOLVENT. AN EXOTIC MOLECULE WAS IDENTIFIED BY IR AND HYPOTHESIZED AS CULPRIT, BUT NOT CONVICTED BY THE EVIDENCE.



1950's TO 1960's

DOW 6X PRODUCTION -- AT LEAST ONE SEVERE CASE, SOME MILD CASES. DOW TERMINATED PRODUCTION BECAUSE IT WAS LOW-VOLUME, LOW-PROFIT ITEM, NOT WORTH THE COST OF RE-ENGINEERING TO PREVENT FURTHER INJURY.



Cl_{4-6} Active

Cl_8 Inactive

DOW 1 767140

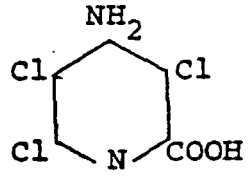
16296

1963-1964

CHEMICAL-PHYSICS LAB -- SEVERAL CASES, AT LEAST ONE SEVERE, FROM RECYCLING CAUSTIC INSOLUBLE OIL IN ALCOHOLIC HYDROLYSIS OF TETRACHLOROBENZENE. AT LEAST ONE EXPLOSION OCCURRED.

1965

WESTERN DIVISION TORDON PROCESS -- FOUR MILD CASES. RABBIT TESTS FAILED, SUGGESTING NEW FAMILY OF ACNEGENS IN CHLORINATED PYRIDINE CHEMISTRY.



4-AMINO-3,5,6-TRICHLOROPICOLINIC ACID

DOW 1 7671741

1966

DOW CORNING - RESEARCH LAB CHEMIST HEATED TCP FOR RESEARCH PURPOSE UNRELATED TO DOW PROCESSES, ISOLATED TCBD AS AN UNKNOWN BUT INTERESTING BY-PRODUCT, BECAME ONE OF OUR BETTER SOURCES OF TCBD.

CASUAL DISPOSAL OF LAB WASTES RESULTED IN ONE MILD CASE -- THE JANITOR!

DOW 1767172

1966

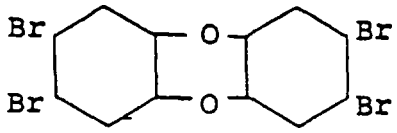
FORT SASKATCHEWAN, DOW CANADA -- ABOUT TEN CASES RELATED TO DOWICIDE PRODUCTION, PROBABLY DOWICIDE G DRYER AND HIGH TEMPERATURES (WITNESS HAD SEEN AT LEAST THREE FIRES IN THE DRYER).

PLANT WAS CLEAN BY THE TIME WE GOT THERE TO WIPE TEST.

DOW 767173

1966

UNIVERSITY OF IOWA -- 2,3,7,8-TETRABROMODIBENZO-P-DIOXIN APPEARS TO BE AS POTENT AS TCBD. AT LEAST ONE SEVERE CASE; DID NOT RELATE IT TO HIS RESEARCH UNTIL ASKED FOR A SAMPLE AND DESCRIBED THE PROBLEM.



2,3,7,8-TETRABROMODIBENZO-P-DIOXIN

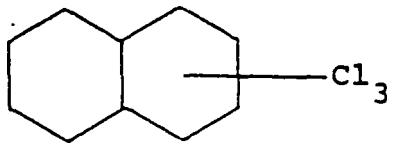
1968

ONE MILD CASE RESULTED FROM DICHLOROPHENOL TARS FROM 349 BUILDING. THE TAR WAS USED IN A PUMP TESTING SET UP AT 562 BUILDING. POSSIBLE HAZARD HAD BEEN MENTIONED BUT NOT EMPHASIZED TO USERS. VICTIM DISMANTLED CONTAMINATED PUMPS WITH NO PRECAUTIONS.

OTHER EXPERIENCE

1930's

MANY SEVERE CASES INCLUDING LIVER INJURY RESULTED FROM CHLORINATED NAPHTHALENES, THREE CHLORINE EQUIVALENTS OR MORE.



1950's

FRENCH -- MANY SEVERE CASES. REVISED PROCESS. WERE VERY SECRETIVE. USED MOUSE TEST IN PLACE OF RABBIT, APPEARED TO BE IGNORANT OF MANY IMPORTANT ASPECTS OF PROBLEMS.

DOW 1767175

1955 GERMAN -- SEVERE CASES INCLUDING SOME SUSPECTED FATALITIES. SHUT DOWN AND RESEARCHED NEW PROCESS. SHOWED AGAIN THAT RABBIT EAR AND LIVER ARE MOST SENSITIVE TESTS.

1957 SENT A REMARKABLE LETTER TO ALL KNOWN PRODUCERS OF TCP DESCRIBING PROCESS PARAMETERS NECESSARY TO AVOID CHLORACNE HAZARD -- LETTER WAS FILED AND FORGOTTEN!

1964 GERMANS WERE QUITE KNOWLEDGEABLE AND VERY COOPERATIVE. DOW BOUGHT PROCESS INFORMATION.

THE ANILINE MOUNTAIN STORY -- 199 BUILDING

1946	BATCH PROCESS START UP, RAN UNTIL 1952.
1952	BEGAN CONTINUOUS COIL REACTOR PROCESS.
1957	C. I. OIL AT 10% ——→ SEVERE FOLLICULITIS.
1962	EXPERIMENTED WITH 73% AND 24% CAUSTIC TO REPLACE FLAKE.
	C. I. OIL AT 10% ——→ FOLLICULITIS.
	NO DIFFERENCE IN SEVERITY BETWEEN FLAKE PRODUCED AND 24% CAUSTIC PRODUCED OILS.

DOW

17671177

1963	JULY	NEW EQUIPMENT START UP, PLUGGED LINES PROBABLY PRODUCED MORE EXPOSURE.
	NOVEMBER	PUSHED PROCESS TO INCREASE PRODUCTION, RAISED TEMPERATURE FROM 195° C TO 205-225° C. MADE MORE OIL, BEGAN SAMPLING IT.

DOW 1671178

1964	FEBRUARY	SUPERVISORS, LAB MAN AND OPERATORS WITH CHLORACNE.
		PHOENIX SPRINKLER MEN MOVED IN.
		REDUCED EXPOSURE BY EQUIPMENT AND PROCEDURE CHANGES.
	APRIL 1	C. I. OIL AT 0.25% KILLED RABBITS
	APRIL 15	PHOENIX MEN MOVED OUT.
	APRIL 20	C. I. OIL AT 0.25% KILLED RABBITS.
	MAY	C. I. OIL AT 10% ——→ SEVERE FOLLICULITIS.
	JULY	PHOENIX MEN WITH CHLORACNE.

DOW 1 7671179

16305

MID-1964

MANAGEMENT ASKED TWO QUESTIONS OF MEDICAL, INDUSTRIAL HYGIENE AND SAFETY:

(1) CAN PLANT BE OPERATED SAFELY AT ALL?

(2) WHAT IS NEEDED TO ENSURE SAFETY?

DOW 1 7671180

ANSWER TO (1) WAS YES.

ANSWER TO (2) COST MANY $100,000's AND MUCH MANPOWER.

PROCESS CONTINUED 1964-1966 WITH NO NEW CASES OR ADVERSE EFFECTS ON OLD ONES.

TWO NEW CASES IN 1966 -- DID NOT FOLLOW RULES.

DOW 17671181

DISPOSITION OF 199 BUILDING

PRIZE WINNING SUGGESTION --

"BURY IT AND MAKE A SKI RESORT,
CALL IT ANILINE MOUNTAIN."

DISMANTLED IN 1968 -- NO INJURIES.

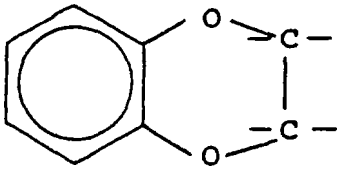
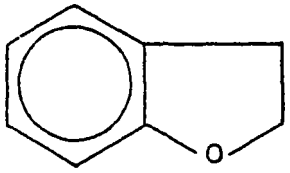
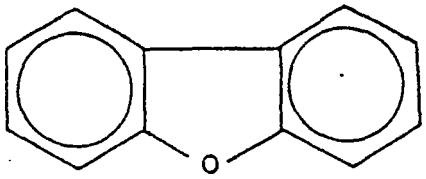
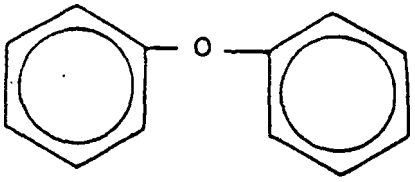
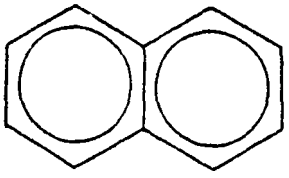
DOW 1 767182

NEW PLANT -- 804 BUILDING

C. I. OIL	30-50 PPM TCBD — 4-7 PPM TCDD (PROCESS CHANGE 3/74)
PRODUCT	ALWAYS LESS THAN ~~1 PPM~~ < .02 PPM
WIPE TESTS	CLEAN

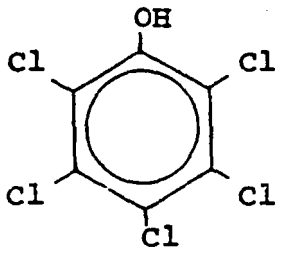
DOW 1 767183

KNOWN ACNEGENS

COMPOUNDS	ACTIVITY
	"VERY ACTIVE" DATA NOT CONCLUSIVE
	Cl_{1-x} UNKNOWN ACTIVITY
	Cl_{1-2} UNKNOWN ACTIVITY Cl_{3-6} SOME VERY ACTIVE. Cl_8 NOT ACTIVE
	Cl_{4-6} SOME ACTIVITY Cl_8 NOT ACTIVE
	Cl_3 + SOME ACTIVITY

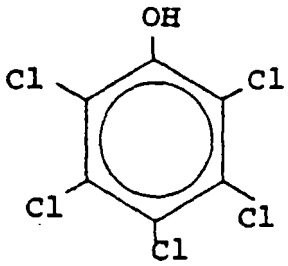
DOW 1767184

MATERIALS POSSESSING UNEXPLAINED CHLORACNE ACTIVITY



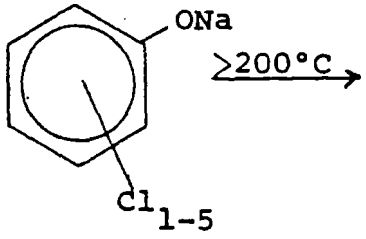
SOME MIDLAND BATCHES ARE MILDLY ACTIVE

MANY CANADIAN BATCHES ARE MILDLY REACTIVE

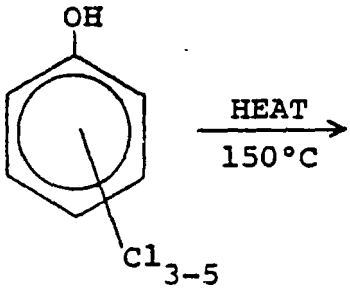


DOWICIDE G

ALL SLUDGES ARE ACTIVE



ALL DECOMPOSITIONS ARE ACTIVE



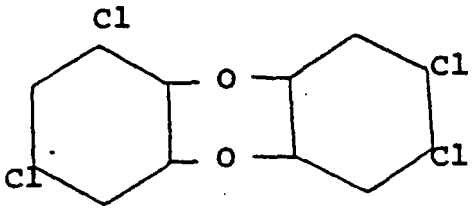
SOME HAVE ACTIVITY

MOD

7671185

16311

COMPOUNDS KNOWN TO BE INACTIVE

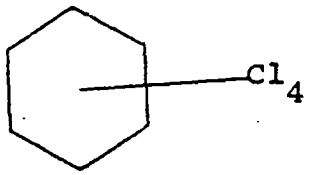


1,3,7,8-TCBD

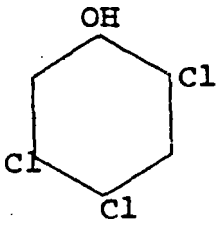
AT LEAST 10^{-3} LESS ACTIVE THAN 2,3,7,8-TCBD

2,7-DICHLOROBENZODIOXIN

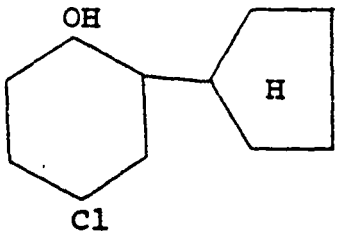
OCTACHLOROBENZODIOXIN



DOW TETRACHLOROBENZENE



DOW 2,4,5-TCP & T ACIDS



DOWICIDE 9

4-CHLORO-2-CYCLOPENTYL PHENOL

CARBON TETRACHLORIDE

ACCUSED IN LITERATURE!

DOW J 767186

16312

SUMMARY

1. PLATITUDES

2. AVOID TEMPERATURES GREATER THAN 150° C.

3. BEWARE OF TARS AND OILS

4. LABORATORY SCALE IS HAZARDOUS

5. USE RABBIT TEST EARLY

6. COMMUNICATE -- INDUSTRIAL HYGIENE, MEDICAL

DOW 1967187

DOW 746820

THE DOW CHEMICAL COMPANY

MIDLAND, Michigan
August 16, 1966

C. A. Highhill
2,4-D Plant
489 Building

cc: E. C. Staehling, Org. Chem. Prodn., 258 Building
F. C. Amstutz, Herbicide Section, 441 Building
F. I. Chase, 2,4-D Products, 489 Building
B. B. Holder, M.D., Medical Department, 607 Building
A. W. Wilson, Safety Department, 401 Building
V. K. Rowe, Biochemical Research Laboratory

I would like samples from your first run with 2,4,5-trichlorophenol in the direct ester process. The animal test will detect the presence of chloracne-producing compounds.

Please send me a sample of the final product and any intermediates that you think should be tested. If activity is detected, the intermediate may tell us where the trouble starts.

Because of the concern about chloracne, I think we should run animal tests on 2,4,5-T esters for a time to prove the lack of hazard, even if the first tests are negative.

L. G. Silverstein
Biochemical Research Laboratory
1701 Building

LGS:sjl

C
1329473

Dow herbicide history traced

Continued from page 1

dioxin, according to the EPA. CAST estimates 2,4,5-T used to contain as much as 80 ppm TCDD before the hazards of dioxin were firmly established.

When Dow began making 2,4,5-T in 1948, no chemical analysis method existed to detect TCDD. But Dow did know that if the then-unknown agent exceeded a certain limit, workers in the 2,4,5-T area could exhibit chloracne, the first sign of dioxin exposure in humans.

Chloracne was first diagnosed by a German doctor in 1888. It was then termed an occupational skin disease due to worker exposure to chlorine ions in the production of caustic potash.

In 1939, Dow developed a rabbit ear test, in which the animal's very sensitive ears were exposed to chemicals suspected of containing the still-unidentified chloracne agent. The test was sensitive to about 1 ppm TCDD, and if a rabbit developed chloracne on its ears, it was assumed the production sample was exceeding that amount.

It was not until 1964 that Dow developed an analytical test for TCDD that rivaled the rabbit ear test for sensitivity. Until that time, Dow monitored its 2,4,5-T productin via the rabbit ear test, according to John Davidson, a technical advisor in Dow's Agricultural Products Department.

1964 also was the year 80 of Dow's Michigan Division employees became contaminated with dioxin during production of trichlorophenol. The dioxin was present in an oily waste stream which at the time was allowed to run openly in troughs at the plant.

Many of the 80 men exhibited chloracne, which first appears near the eyes and ears as a eruption of blackheads usually accompanied by small, pale-yellow cysts.

Mild cases clear within months, while severe cases may have active skin lesions for up to 15 years after contact. Some of the Michigan Division workers also exhibited liver toxicity and neurological problems, according to Dow.

When the extremely toxic effects of TCDD began to be recognized, production methods were changed and carefully controlled by manufacturers.

As Dow's analytical methodology improved, the level of TCDD in its 2,4,5-T was lowered, first to .5 ppm and then to .1 ppm after 1971, Davidson said.

Dow's laboratories once prepared a special, purified sample of 2,4,5-T for a Dow scientist that contained only .0003 ppm TCDD.

But the purification process took eight months, and doing the same for commercial 2,4,5-T would not be economically feasible, Dow contends.

"Aside from the cost, we don't feel it represents an unreasonable risk," said Robert Charlton, public relations manager for Dow's Agricultural Products Department, of the .1 ppm TCDD in 2,4,5-T.

Chemical terms defined

Defoliant — A harsher form of plant killer generally containing several herbicides. Intended to remove all vegetation from the target area.

Dioxin — The generic name for the family of compounds called polychlorinated dibenzo-p-dioxins. Although there are 75 dioxins, the term usually refers to the most toxic form, 2,3,7,8-tetrachlorodibenzo-p-dioxin or 2,3,7,8-TCDD.

Herbicide — A substance capable of killing plants, particularly weeds. Some herbicides are selective in what they kill, while others such as 2,4,5-T are broad-spectrum plant killers.

Mono — A prefix to a chemical name which in this case refers to the number of chlorine atoms attached to the dioxin structure. Mono means one, so the dioxin with only one chlorine atom attached is called monochlorodibenzo-p-dioxin. Dioxins occur with prefixes di- (two), tri- (three), tetra- (four), hepta- (five), hexa- (six), septa- (seven), up to octa, or eight chlorine atoms.

Oncogenicity — The ability of a substance to cause tumors (neoplasms) in humans or animals. If the tumors are malignant neoplasms or cancers, the substance causing them is called a carcinogen.

Part per million (ppm), billion (ppb) or trillion (ppt) — Toxic chemicals such as TCDD often are measured in these tiny amounts. One ppm is equal to one cent out of $100,000, while one ppb would be one penny out of $10 million and one ppt equals one cent for every $10 billion. One ppb also equals 1 square foot in 36 square miles, while one ppt is the same as the width of a hair compared to a trip around the world.

Teratogenicity — The ability of a substance to cause birth defects or malformations. These substances are called teratogens.

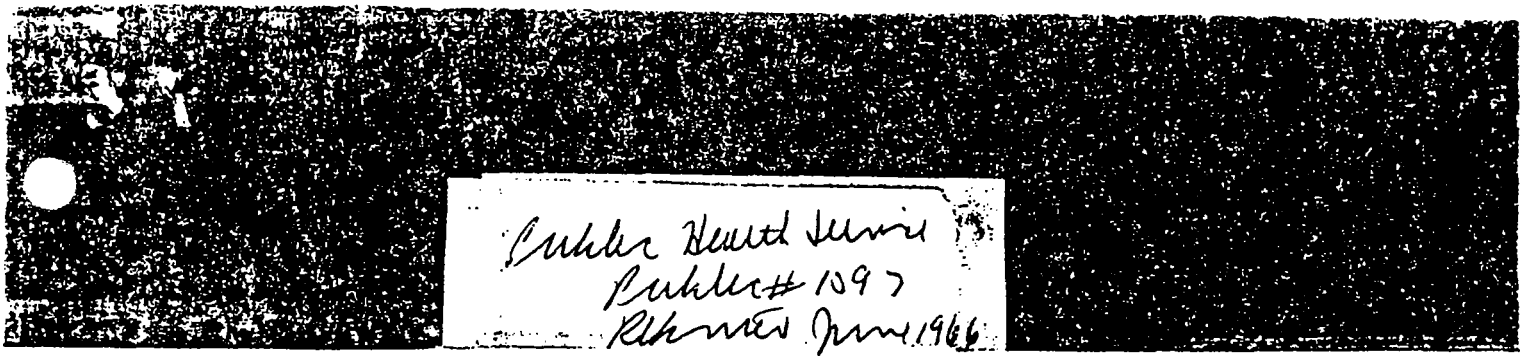
Toxic — The harmful effect to the human body caused by exposure to a poisonous substance either by physical contact, ingestion or inhalation.

Isomer — One of two or more chemical substances having the same composition of elements and molecular weight but differing in structure, and therefore in properties. For instance, there are 22 isomers of dioxin with four chlorine atoms attached (tetrachlorodibenzo-p-dioxins). All have the same chemical building blocks and weigh the same, but the most toxic isomer by far is that with the four chlorine atoms attached at the two, three, seven and eight positions — 2,3,7,8-TCDD.

Phenoxy herbicides — This group of herbicides is made from phenoxyacetic acid, a light tan powder soluble in a variety of liquids. Phenoxy herbicides are weak acids that are combined with other ingredients such as solvents, emulsifiers, thickeners and wetting agents to make commercial herbicides for specific uses. Three common phenoxy herbicides are 2,4,5-T, silvex and 2,4-D.

16315

1235



Public Health Service
Public # 1097
Revised June 1966
U.S. Govt. Printing Office.

OCCUPATIONAL DISEASES

Lectures on 1949

A Guide To Their Recognition

DOAM 1 123543
MN0719 56

In 1944 Donald Bernstein was Chief, Dermatology Section

Rewritten and Enlarged Edition of
Occupation Hazards and Diagnostic Signs

W. M. GAFAFER, D. Sc., Editor
Division of Occupational Health

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

16316

may be damaged by maceration, mechanical or chemical trauma, and certain internal diseases. Lipid-soluble and nonionizable (organic) substances are able to bypass the barrier layer and enter by way of the transfollicular pathway. Gases, except for carbon monoxide, are readily exchanged through the epidermis.

Protection against actinic stimuli is afforded by increased melanin production and a concomitant thickening of the keratin layer. An antimicrobial action is attributed to certain fatty acids in the surface lipid film, and the subcorneal barrier layer also offers some protection against microorganisms.

Primary Irritants

Primary irritation causes most of the cases of contact dermatitis in industry. A primary irritant is an agent which will cause dermatitis by direct action on the normal skin at the site of contact if it is permitted to act in sufficient intensity or quantity for a sufficient length of time. Thus, the normal skin will almost always react to a primary irritant if the necessary conditions are present.

Irritants can act in several ways to injure the skin. Several examples have already been given, such as removal of lipid film, denaturation of keratin, and interference with the subcorneal barrier layer. Other examples of primary irritation include dehydration by inorganic acids, anhydrides, and alkalis; protein precipitation by heavy metal salts and tanning agents; and oxidation by bleaches, chlorine compounds, and per- salts.

Sensitizers

Ordinarily, no more than 20 percent of contact dermatitis in industry is caused by allergens. However, certain of the strong sensitizers may affect many workers. Almost any chemical can be a sensitizer, but the mode of action usually differs from that exhibited by primary irritants. The cutaneous sensitizer, therefore, does not necessarily cause demonstrable cutaneous change on first contact, but may effect such specific changes in the skin so that after 5 to 7 days or more, further contact on the same or other parts of the body may cause dermatitis.

The difference between the irritant and the sensitizer, therefore, is generally a matter of time as well as mode of action. The irritant will usually act within a matter of minutes to a few hours, whereas the sensitizer requires at least 5 to 7 days because sensitization must build up during the period of incubation. Low-grade irritants such as soap may require prolonged or repeated contact before a dermatitis appears, and this prolonged reaction time may be confused with allergic incubation.

Secondary Effects

After the cutaneous defenses have been broken down and a contact dermatitis is present, the oozing or fissured surface offers ideal conditions for the entrance and growth of bacteria. Thus, it is not unusual for a contact

dermatitis to be secondarily infected. Another secondary effect commonly seen in contact dermatitis is over-treatment by irritating or sensitizing medication.

Diagnosis

Diagnosing an occupational dermatosis is generally contingent upon satisfying certain criteria. The disease should look like a contact dermatitis or one of the other clinical types of occupational dermatoses. It should be located on sites of exposure, and the time of appearance of the eruption as well as periods of remisson and exacerbation should correlate with the history of work exposures. When the patient does not get well following complete withdrawal from the suspected contact agent, stimuli of nonoccupational origin should be reinvestigated. Multiple factors not associated with the occupation can perpetuate a chronic dermatosis.

Patch tests are helpful in differentiating between a primary irritation dermatitis and an allergic contact dermatitis. A nonirritating concentration of the suspected allergen is applied to the normal skin of a patient for 24 to 48 hours in an attempt to reproduce an eczematous dermatitis beneath the covering patch.

Classification of Lesions

The clinical lesions seen among occupational exposures are multiple, varying from the mildest erythema to lesions of neoplastic nature. However, occupational skin disease can be classified as follows:

(1) Acute contact eczematous dermatitis characterized by erythema, edema, papules, vesicles, or bullae, crusts and finally desquamation. These effects are generally the result of contact with either a primary irritant or a sensitizer, or with both.

(2) Chronic contact eczematous dermatitis characterized by erythema, lichenification, and fissuring of the skin, usually resulting from contact with dehydrators, fat solvents, soaps, and detergents.

(3) Folliculitis and acneform types, including chloracne, characterized by plugged sebaceous follicles and suppurative lesions. Chloracne also shows numerous straw-colored cystic lesions. These forms of occupational dermatoses are usually caused by contact with oils, tars, waxes, and certain chlorinated hydrocarbons.

(4) Neoplastic (benign and malignant) types, characterized by keratoses, papillomata and epitheliomata of the exposed areas, usually caused by petroleum products, coal tar and certain derivatives, sunlight, and ionizing radiation.

(5) Pigmentary disturbances characterized by increase or decrease of pigment in the epidermis. Increased pigmentation can result from contact with

DOW 1 1235544

o-Dichlorobenzene workers
Dry cleaners
Dye makers
Fumigant workers
Greasemakers
Gum makers
Heat transfer workers
Hide processors
Insecticide workers
Lacquerers
Lacquer workers
Metal degreasers
Metal polish makers
Organic chemical synthesizers
Paint remover workers
Paint workers
Polishing compound makers
Resin makers
Rubber makers
Solvent workers
Stainers
Stain makers
Sulfur processors
Tannery workers
Tar makers
Tar remover workers
Termite exterminator workers
Varnish makers
Varnish remover workers
Wax makers
Wood preservative workers
Wool processors

p-Dichlorobenzene
Deodorant makers
p-Dichlorobenzene workers
Disinfectant workers
Drug makers
Dye makers
Insecticide workers
Moth ball makers
Soil fumigators

Trichlorobenzenes
Dye makers
Electric equipment makers
Heat transfer workers
Insecticide workers
Lubricant makers
Trichlorobenzene workers

Hexachlorobenzene
Fungicide workers
Hexachlorobenzene workers
Organic chemical synthesizers
Seed disinfectors

DOW 1 123545

References

HOLLINGSWORTH, R. L.; ROWE, V. K.; OYEN, F.; HOYLE, H. R., AND SPENCER, H. C.: Toxicity of paradichlorobenzene; determinations on experimental animals and human subjects. *A.M.A. Arch. Indust. Health* 14: 138, 1956.

HOLLINGSWORTH, R. L.; ROWE, V. K.; OYEN, F.; TORKELSON, T. R., AND ADAMS, E. M.: Toxicity of o-dichlorobenzene; studies on animals and industrial experience. *A.M.A. Arch. Indust. Health* 17: 180, 1958.

(48) Chlorinated Diphenyls and Naphthalenes

Harmful Effects

Local Prolonged contact with fume or the cold wax leads on exposed skin to comedones, sebaceous cysts and pustules, known as chloracne.

Routes of Entry Inhalation of fume or vapor; percutaneous absorption of liquid.

Systemic Acute or chronic exposure can produce varying degrees of liver damage depending on amount of chlorine in compound and preexisting

16318

state of liver. Symptoms include jaundice, anorexia, nausea, indigestion, abdominal pains, and edema. Death from acute yellow atrophy of liver has occurred.

Special Diagnostic Test

None.

Recommended Threshold Limit

Chlorodiphenyl (42 percent chlorine), 1 milligram per cubic meter of air. Should be reduced when also absorbed percutaneously.

Chlorodiphenyl (54 percent chlorine), 0.5 milligram per cubic meter of air. Should be reduced when also absorbed percutaneously.

Chlorinated diphenyl oxide, 0.5 milligram per cubic meter of air.

Pentachloronaphthalene, 0.5 milligram per cubic meter of air. Should be reduced when also absorbed percutaneously.

Trichloronaphthalene, 5 milligrams per cubic meter of air. Should be reduced when also absorbed percutaneously.

Potential Occupational Exposures

Aniline dye makers
Cable coaters
Carbon removers
Chlorinated diphenyl workers
Condenser impregnators
Crankcase oil additive makers
Dye makers
Electric equipment makers
Electricians
Electroplaters
Flameproofers
Gum processors
Heat transfer workers
Herbicide workers
Ink makers
Insecticide workers
Insect proofers
Lacquerers
Lacquer makers
Light fixture makers
Machinists
Metal degreasers
Mineral oil processors
Moisture proofers
Paint makers
Paper treaters
Petroleum refinery workers
Plasticizer makers
Plastic makers
Rayon makers
Resin makers
Rubber workers
Solvent workers
Stainers
Stain makers
Textile flameproofers
Transformer workers
Upper cylinder oil makers
Varnish makers
Vegetable oil processors
Wax makers
Wire coaters
Wood preservers

Reference

MEIGS, J. W.; ALBOM, J. J.; AND KARTIN, B. L.: Chloracne from an unusual exposure to arochlor. *J. Am. Med. Assoc.* 154: 1417, 1954

(.

Harmful Effects

Local Extreme irritation of sion of teeth.

Route of Entry Inhalation of

Systemic Acute respiratory pain, dyspnea, and cyanosis. nia, and pulmonary edema ma

Special Diagnostic Test

None.

Recommended Threshold

1 part per million parts of a of air.

Potential Occupational Ex

Aerosol propellant makers
Alkali salt makers
Aluminum purifiers
Benzene hexachloride maker
Bleachers
Bleaching powder makers
Bromine makers
Broom makers
Carpet makers
Chemical synthesizers
Chloride of lime makers
Chlorinated solvent makers
Chlorine workers
Color makers
DDT makers
Disinfectant makers
Dye makers
Ethylene glycol makers
Ethylene oxide makers
Flour bleachers
Freon makers
Gasoline additive workers
Gold extractors
Ink makers
Iodine makers
Iron detinners
Iron dezinkers

UNIT 1 1235546

Herbicides, or weed killers, may be classified as pesticide chemicals. They can kill plants on contact, or they can be translocated; that is, absorbed by one part of the plant and carried to other parts where they exert their primary toxic effect. Most of the commonly used herbicides—ammonium sulfamate, dalapon, phenoxyacetic acid derivatives, carbamate derivatives, petroleum oils, sodium borate, Crag® herbicide—have a low toxicity and have caused little difficulty among users.

Some herbicides pose more serious problems; for example, the central nervous system effects of maleic hydrazide or the methemoglobinemia and central nervous system depression of sodium chlorate. Pentachlorophenol, a metabolic stimulant, has been responsible for several deaths because of hyperthermia. Amino triazol has produced cancer in experimental animals, but there have been no untoward effects reported in man.

Herbicides with cutaneous effects include trichloroacetic acid, a corrosive irritant of the skin and mucous membranes; maleic hydrazide, a producer of allergic contact dermatitis; pentachlorophenol, a producer of a primary irritant type of contact dermatitis; and creosote, a primary irritant and photosensitizer.

Reference may be made to chemicals in the section on Chemical Hazards for the toxicity of the following herbicides: Arsenic trioxide and sodium arsenate (see Arsenic), copper sulfate (see Copper and Compounds), creosote compounds (see Cresol and Phenol), dinitrophenols (see Dinitrophenol), kerosine, and phenylmercuric acetate (see Mercury and Compounds).

Threshold limit values, in milligrams per cubic meter of air, have been recommended for the following herbicides as shown:

Ammonium sulfamate (Ammate®)	15 mg. per cu. m.
Crag® herbicide	15 mg. per cu. m.
2,4-D (2,4-dichlorophenoxyacetic acid)	10 mg. per cu. m.
Pentachlorophenol (PCP)	0.5 mg. per cu. m.*
Phenylmercuric acetate (PMA)	0.01 mg. per cu. m.* (for organic mercury)
2,4,5-T (2,4,5-trichlorophenoxyacetic acid).	10 mg. per cu. m. (tentative)

*Should be reduced when also absorbed percutaneously.

Fumigants

Fumigants are pesticides which may be applied in the solid, liquid, or gaseous state. A combination of high volatility with high pest toxicity is generally desired; however, compounds with low volatility may be preferred for soil fumigation. The possibility of excessive exposures exists wherever

houses, barns, ships, mills, freight cars, and greenhouses.

Each of the following compounds has found use as a fumigant. Because they have other industrial applications as well, they are discussed individually in the section on Chemical Hazards.

Acrylonitrile
Carbon Disulfide
Carbon Tetrachloride
p-Dichlorobenzene (see Chlorinated Benzenes)
Dioxane
Ethylene Dibromide
Ethylene Dichloride
Ethylene Oxide
Hydrogen Cyanide
Methyl Bromide (see Bromine and Compounds)
Methylene Chloride
Methyl Formate
Naphthalene
Perchloroethylene
Propylene Dichloride
Sulfur Dioxide
Tetrachloroethane
Trichloroethylene

References

ARTERBERRY, J. D.; DURHAM, W. F.; ELLIOTT, J. W., AND WOLFE, H. R.: Exposure to parathion. Measurement by blood cholinesterase level and urinary p-nitrophenol excretion. *Arch. Environ. Health* 3: 476, 1961.

ASSOCIATION OF AMERICAN PESTICIDE CONTROL OFFICIALS: *Pesticide Chemicals Official Compendium*. A. B. Heagy, University Post Office, College Park, Md., 1962. Loose-leaf service.

COMMITTEE ON PESTICIDES: Outlines of information on pesticides. Part 1. Agricultural fungicides. *J. Am. Med. Assoc.* 157: 237, 1955.

COMMITTEE ON TOXICOLOGY: Occupational dieldrin poisoning. *J. Am. Med. Assoc.* 172: 2077, 1960.

COMMUNICABLE DISEASE CENTER, PUBLIC HEALTH SERVICE: Clinical memoranda on economic poisons. Pub. Health Service Pub. No. 476. U.S. Government Printing Office, Washington, D.C., 1956.

DIVISION OF BIOLOGY AND AGRICULTURE, NATIONAL RESEARCH COUNCIL-NATIONAL ACADEMY OF SCIENCES: *Handbook of Toxicology*. Vol. 3, Insecticides. Vol. 5, Fungicides. W. B. Saunders Co., Philadelphia, 1959.

DURHAM, W. F.; GAINES, T. B., AND HAYES, W. J. JR.: Paralytic and related effects of certain organic phosphorus compounds. *A.M.A. Arch. Indust. Health* 13: 326, 1956.

DURHAM, W. F. AND HAYES., W. J., JR.: Organic phosphorus poisoning and its therapy; with special reference to modes of action and compounds that reactivate inhibited cholinesterase. *Arch. Environ. Health* 5: 21, 1962.

FREAR, D. E. H. (EDITOR): *Pesticide Index*. College Science Publishers, State College, Pa., 1961.

GAINES, T. B.: The acute toxicity of pesticides to rats. *Toxicol. Appl. Pharmacol.* 2: 88, 1960.

GOLZ, H. H. AND SHAFFER, C. B.: *Toxicological Information on Cyanamide Insecticides*. American Cyanamid Co., New York, 1960.

HAYES, W. J., JR.: The toxicity of dieldrin to man; report on a survey. *Bull. WHO* 20: 891, 1959.

HAYES, W. J., JR.: Pesticides in relation to public health. *Ann. Rev. Entomol.* 5: 379, 1960.

218-695 O-66-17

DOW 1 123547

DOW 537307

Petition for the Establishment of
Tolerances for the Pesticide Chemical,
2,4,5-Trichlorophenoxyacetic Acid
on Raw Agricultural Commodities

SECTION C

Full Reports of Investigations Made with
Respect to the Safety of the Pesticide
Chemical, 2,4,5-Trichlorphenoxyacetic Acid

December 8, 1967

DOW 537308

Acute Studies

Oral Administration - Rats, Mice, Guinea Pigs, and Chicks

The acute oral LD_{50} of 2,4,5-trichlorophenoxyacetic acid for young adult male and female albino rats, mice, and guinea pigs and for young chicks, as reported by Rowe, et al. (1), was as follows:

Species	LD_{50} (mg/kg)	Confidence Limits	Slope
Rats	500	391-640	1.49
Mice	389	245-619	1.80
Guinea Pigs	381	307-472	1.21
Chicks	310	211-456	1.40

The material was administered as a 3.0 or 10% suspension in olive oil. Gross toxic signs consisted of ataxia and myotonia, particularly at the higher doses.

Oral Administration - Dogs

According to Drill and Hiratzka (2), the acute oral LD_{50} of 2,4,5-trichlorophenoxyacetic acid for adult mongrel dogs is probably in the range of, but slightly higher than, 100 mg/kg of body weight. Only 25 % mortality occurred at this level. Gross toxic signs consisted of anorexia, body weight loss, slight or moderate stiffness in the hind legs, and ataxia. Histologic changes included some necrosis and inflammation of the intestinal mucosa, moderate diffuse hepatic necrosis, and a mild degree of renal tubular degeneration.

DOW537309

Acute Oral Administration - Rats, Mice, Guinea Pigs, Rabbits, Chicks, and Steers

Rowe and Hymas (3) reported the acute toxicity of various formulations of 2,4,5-trichlorophenoxyacetic acid and mixtures of 2,4-dichlorophenoxyacetic acid and 2,4,5-T. The reported LD_{50} values and/or range of toxicity are as follows:

Esteron 245 (old formulation containing 33.3% isopropyl ester of 2,4,5-T and 12.1% mixed amyl esters of 2,4,5-T)

Adult female albino rats - Estimated LD_{50} of 1000 mg/kg of body weight (range 300-2000 mg/kg).

Esteron 245 (new formulation containing 65.3% mono-, di-, tripropylene glycol butyl ether esters of 2,4,5-T)

Adult male albino rats - Estimated LD_{50} 800 mg/kg of body weight (range 600-1000 mg/kg).

Isopropyl ester of 2,4,5-T

Male and female albino rats - LD_{50} 495 mg/kg of body weight (confidence limits 420-584 mg/kg).

Male albino mice - LD_{50} 551 mg/kg of body weight (confidence limits 380-799 mg/kg)

Male guinea pigs - LD_{50} 449 mg/kg of body weight (confidence limits 362-557 mg/kg).

Mixed Butyl esters of 2,4,5-T

Male rats - LD_{50} 481 mg/kg of body weight (confidence limit 313-739 mg/kg).

DOW 537310

Male rabbits - Estimated LD_{50} 712 mg/kg of body weight (range 500-1000 mg/kg).

Male mice - LD_{50} 940 mg/kg of body weight (confidence limits 674-1312 mg/kg).

Guinea pigs - Estimated LD_{50} 750 mg/kg of body weight (range 500-1000 mg/kg).

Mixed Amyl ester of 2,4,5-T

Female rats - Estimated LD_{50} 750 mg/kg of body weight (range 500-1000 mg/kg).

Brush Killer T (52.2% butyl esters of 2,4,5-T)

Female rats - LD_{50} 1200 mg/kg of body weight (confidence limits 783-1850 mg/kg).

Female guinea pigs - LD_{50} 1410 mg/kg of body weight (confidence limits 875-2290 mg/kg).

Female mice - LD_{50} 1230 mg/kg of body weight (confidence limits 938-1620 mg/kg).

Male rabbits - LD_{50} 849 mg/kg of body weight (confidence limits 604-1190 mg/kg).

Chicks - LD_{50} 2000 mg/kg of body weight (confidence limits 1350-2960 mg/kg).

Brush Killer 50-50 (formulation containing 27.2% butyl esters of 2,4-D and 26.5% butyl esters of 2,4,5-T).

Female rats - LD_{50} 1070 mg/kg of body weight (confidence limits 700-1650 mg/kg).

DOW 537311

Female guinea pigs - LD_{50} 1100 mg/kg of body weight (confidence limits 820-1630 mg/kg).

Female mice - LD_{50} 800 mg/kg of body weight (confidence limits 624-1070 mg/kg).

Female rabbits - Estimated LD_{50} 1420 mg/kg of body weight (range 500-2000 mg/kg).

Chicks - LD_{50} 4000 mg/kg of body weight (confidence limits 2700-5900 mg/kg).

Esteron Brush Killer (old formulation containing 25.6% isopropyl ester of 2,4-D and 24.4% isopropyl esters of 2,4,5-T)

Male rats - Estimated LD_{50} 1000 mg/kg of body weight (range 300-3000 mg/kg).

Esteron Brush Killer (new formulation containing mono-, di-, and tripropylene glycol butyl ether esters of 2,4-D 34.8% and 2,4,5-T 33.0%)

Male and female rats - LD_{50} 860 mg/kg of body weight (confidence limits 800-930 mg/kg).

Male guinea pigs - LD_{50} 1220 mg/kg of body weight (confidence limits 1040-1430 mg/kg).

Female guinea pigs - LD_{50} 1600 mg/kg of body weight (confidence limits 1390-1840 mg/kg).

Male and female rabbits - LD_{50} 960 mg/kg of body weight (confidence limits 790-1160 mg/kg).

Chicks - Estimated LD_{50} 2000 mg/kg of body weight (range 1000-3000 mg/kg).

Steers - Acute oral LD_{50} greater than 1000 mg/kg of body weight.

DOW 537312

Eye Application - Rabbits

According to a report by the Dow Chemical Company (4), a 10% solution of 2,4,5-T in propylene glycol produced severe conjunctivitis and moderate corneal damage which persisted for longer than five days.

Acute Oral Administration - Rats; Acute Dermal and Eye Application-Rabbits

DOW537313

The results of acute studies on Nu Amine D/T, a formulation containing 1,3-propylene diamine salts of 2,4-D (11.95%) and 2,4,5-T (11.85%) was reported to Diamond Alkali Company February 23, 1962 by Hazleton Laboratories, Inc. (5).

The acute oral LD_{50} of Nu Amine D/T for adult male albino rats is 708μl/kg of body weight with confidence limits from 447-1120μl/kg. Deaths occurred within 1 to 14 days, and toxic signs were mainly characterized by depression, weight loss, labored respiration, ataxia, abnormal gait, circling movements, depressed or absent reflexes, and hematuria.

A single application of 0.05 ml. of Nu Amine D/T to the eyes of albino rabbits produced marked irritation, including corneal damage, which did not completely subside within seven days. However, no evidence of systemic toxicity from mucous membrane absorption of the test material was observed.

The estimated acute dermal LD_{50} of undiluted Nu Amine D/T for albino rabbits is 1.2 ml/kg of body weight. A corrosive-type skin reaction was produced at dosage levels of 0.1, 0.5, and 2.0 ml/kg of body weight. At 2.0 ml/kg, toxic signs included depression, labored respiration, depressed righting and placement reflexes, incoordination of hindquarters, abnormal gait, prostration, sluggish pupillary response to light, absent

pain response in hind limbs, running movement of hind limbs, and coma. Necropsies revealed definite liver and kidney changes at the 2.0 ml/kg level and possible liver and/or kidney damage at the two lower levels.

Subacute Studies

Oral Administration - Dogs

According to Drill and Hiratzka (2) oral ingestion of 2,4,5-T by adult male and female mongrel dogs for 13 weeks at levels of 2.0, 5.0, and 10 mg/kg of body weight produced no adverse effects. At a dosage level of 20 mg/kg, total mortality occurred between days 11 and 75, and gross toxic signs included weight loss, weakness, slight hind leg stiffness, difficulty in swallowing food, and bleeding from the gums.

Dietary Feeding - Rats

The results of a 90-day feeding study involving Dowanol 97B (ester of 2,4,5-T) was reported by Biochemical Research Laboratory of the Dow Chemical Company (9).

Ingestion of Dowanol 97B by young male and female albino rats for 90 days produced no adverse effects at dietary levels of 100 and 300 ppm. At 1000 ppm, increased kidney weights were noted in the male animals and histologic alterations were noted in the livers of both sexes and in the kidneys of the females. At 3000 ppm, growth suppression and increased liver and kidney weights were noted only in the male rats, while gross and microscopic changes in the livers and kidneys and increased terminal serum alkaline-phosphatase values were noted in both male and female animals.

DOW 537314

Oral Administration - Steers

Repeated oral administration of Esteron Brush Killer (formulation of ester of 2,4-D and 2,4,5-T) to steers, one per level, was also reported by Rowe and Hymas (3). Results of these studies were as follows:

1. Fifteen (15) consecutive daily doses of Esteron Brush Killer at a level of 100 mg/kg/day produced no gross toxic effects. However, necropsy performed 48 hours after the last dose revealed some intestinal irritation and histologic alterations were noted in the liver and kidneys.

2. Two (2) consecutive daily doses at 500 mg/kg/day produced slight toxic signs 24 hours after the last dose but apparent recovery within an additional 24 hours.

3. Three (3) consecutive daily doses at 500 mg/kg/day produced no gross toxic signs.

4. Three (3) consecutive daily doses at 1000 mg/kg/day produced death (three days after the last dose).

Consequently, assuming field usage at two quarts/acre, a steer grazing on 744 sq. ft. of treated forage would consume a maximum of 100 mg/kg of the formulation which, as shown above, was not lethal after 15 daily doses.

Oral Administration - Sheep and Cattle

Palmer and Radeleff (7) reported the following results of repeated oral studies in sheep and cattle (one animal per level).

DOW 537315

The propylene glycol butyl ether ester of 2,4,5-T was lethal to sheep following 369 consecutive doses at a daily dosage level of 100 mg/kg of body weight and to sheep and cattle following seven consecutive doses at a daily dosage level of 250 mg/kg. The triethylamine salt of 2,4,5-T produced no toxic effects in one sheep after 481 daily doses at a dosage level of 100 mg/kg of body weight.

DOW 537316

Repeated Dermal Applications - Rabbits

The results of repeated dermal applications of Nu Amine D/T was also contained in the report to Diamond Alkali Company (5), and a supplemental report (5a) dated December 16, 1965 presented findings from microscopic examination of bone marrow from three animals.

Aqueous emulsions of Nu Amine - D/T at field strength (4 oz/gal. or 3.1%) and at three (12 oz/gal. or 9.4%) and five (20 oz/gal. or 15.6%) times field strength were applied at a constant daily dose of 1.0 ml/kg/animal five days per week for 20 applications to intact abdominal skin. Moderate dermal irritation and evidence of systemic toxicity from percutanious absorption of the test material were produced at all concentrations. Toxicity was characterized by depression, labored respiration, sprawling of the limbs, weakness, and body weight loss; prostration, incoordination or partial loss of use of hind limbs, depressed reflexes, clonic convulsions, and death was also noted in one animal at each concentration.

Depressed hemoglobin values, increased percentage of segmented neutrophils, and increased urinary bilirubin values were noted in each test group. Histologically, evidence of compound effect was noted in

liver, kidney, and skin at field strength and at five times field strength (middle level tissues not examined). No distinctive bone marrow effect was noted at three and five field strength.

DOW 537317

Fish and Marine Life Toxicity

Toxicity studies on 2,4,5-trichlorophenoxyacetic acid and its various derivatives in fish and other marine species have been performed by The Bureau of Commercial Fisheries and reported in Fish and Wildlife Service Circular No. 167, No. 199, and No. 226. Data pertaining to 2,4,5-T has been excerpted and is summarized below.

Oysters -

0.14 ppm of the polyglycol butyl ether ester of 2,4,5-T caused 50% decrease in oyster shell growth (96- hour EC_{50}) with recovery within one week (8); 2.0 ppm of the acid of 2,4,5-T produced no toxic effect (9).

Brown Shrimp -

1.0 ppm of the polyglycol butyl ether ester of 2,4,5-T produced 10% mortality after 24-hours exposure and 20% mortality after 48-hours exposure, and 1.0 ppm of the acid of 2,4,5-T produced no effect after 48-hours exposure (10).

Fish -

50.0 ppm of the acid of 2,4,5-T produced no effect in Longnose Killifish after 48-hours exposure (9); 1.0 ppm of the polyglycol butyl ether ester of 2,4,5-T produced 0.32% mortality in Spots after 24- and 48-hour exposure (10).

The potency ratio of 2,4,5-T derivatives for Bluegill relative to that of DDT was quoted in Table 2 (11) as 0.10.

Although no specific studies or mortality data on only 2,4,5-T for wildlife were found in the above Fish and Wildlife Service Circulars, the potency ratio of 2,4,5-T derivatives for Bobwhite Quail, Ring-Necked Pheasants, and Mallard Ducks relative to that of DDT was quoted in Table 2 (11) as approximately 0.50.

DOW 537318

Conclusion

Although considerably more toxicity data, particularly regarding subacute toxicity is available on 2,4-D than on 2,4,5-T, the available toxicity data indicates that the two materials are comparable. The acute oral toxicity of 2,4-D and 2,4,5-T and their various derivatives for laboratory animals is in the same range, 300-1000 mg/kg of body weight. Short-term (90-day) dietary feeding of esters of 2,4,5-T to rats at 100 and 300 ppm level, and short-term (13-weeks) oral administration of the acid of 2,4,5-T to dogs at level of 2.0, 5.0, and 10 mg/kg of body weight produced no adverse effects. Sheep tolerated 481 doses of the salts of both 2,4-D and 2,4,5-T at a dosage level of 100 mg/kg of body weight, while the ester of 2,4,5-T was lethal to both sheep and cattle at levels of 2,4-D which were only slightly toxic or non-toxic. However, 369 doses of the ester of 2,4,5-T at a level of 100 mg/kg of body weight were administered to one sheep before death occurred. The toxicity of 2,4-D and 2,4,5-T for fish and wildlife appears to be comparable. Particularly, since the long history of usefulness has not produced evidence of unusual toxicity.

DOW 537319

BIBLIOGRAPHY

1. Rowe, V. K., McCollister, D. D., and Spencer, H. C., The Acute Oral Toxicity of 2,4,5-Trichlorophenoxyacetic Acid to Rats, Mice, Guinea Pigs, and Chicks; Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan, 1950.

2. Drill, V. A. and Hiratzka, T., Toxicity of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid, A Report on Their Acute and Chronic Toxicity in Dogs; Industrial Hygiene and Occupational Medicine, 7:61-67, 1953.

3. Rowe, V. K. and Hymas, T. A., Summary of Toxicological Information on 2,4-D and 2,4,5-T Type Herbicides and an Evaluation of the Hazards to Livestock Associated with Their Use; American Journal Vet. Res. 15: 622-629, 1954.

4. Results of Toxicological Tests on 2,4,5-Trichlorophenoxyacetic Acid, Reported by Biochemical Research Department, The Dow Chemical Company, Midland, Michigan, August, 1950.

5. Toxicity Tests on Nu Amine D/T (1,3-propylene diamine esters of 2,4-D and 2,4,5-T; Report to Diamond Alkali Company dated February, 1962 by Hazleton Laboratories, Inc.

5a. Microscopic examination of bone marrow sections from the above repeated dermal study, Report to Diamond Alkali Company, dated December 16, 1965 from Hazleton Laboratories, Inc.

6. Results of 90-day Dietary Feeding Studies of Dowanol 97B (Ester of 2,4,5-T) in Rats, Report by Biochemical Research Laboratory, The Dow Chemical Company, dated November 27, 1961.

7. Palmer, J. S. and Radeleff, R. D., The Toxicologic Effects of Certain Fungicides and Herbicides on Sheep and Cattle, Annals of N. Y. Acad. of Sc. Bol. III, (2); 729-732, 1964.

8. Butler, P. A.; Commercial Fishery Investigations, Acute and Chronic Toxicity Studies, U. S. Department of the Interior, Fish and Wildlife Service Circular 199, 5-8 and 25, 1964.

9. Butler, P. A.; Commercial Fishery Investigations, Laboratory Studies and Toxicology, U. S. Department of the Interior, Fish and Wildlife Service Circular 167, 11-15, 22 and 24, 1963.

DOW 537320

10. Butler, P. A.; Commercial Fishery Investigations, Acute Toxicity (Biological Laboratory, Gulf Breeze, Florida), U. S. Department of the Interior, Fish and Wildlife Service Circular 226, 69-73, 1965.

11. George, J. L., Recommendations for Minimizing Dangers of Pest Control and Pesticides to Fish and Wildlife, U. S. Department of the Interior, Fish and Wildlife Circular 167, 101-105, 1963.

DOW 537321

1248

ROBERT E. JENKINS, M. D.
3311 N. MERIDIAN ST.
INDIANAPOLIS 8, INDIANA
WINTHROP 2500

June 7, 1954

DOW-1 278634

Dr. Harold H. Gay
Medical Director,
The Dow Chemical Co.
Midland, Mich.

Dear Dr. Gay:

Mr. James R. Mayfield has recently brought in several letters from you and other interested parties regarding an alleged contact dermatitis he may have sustained in August 1953 from the use of 2,4,5 T.-or something.

I have seen Mr. Mayfield on several different occasions since his first visit here on August the 14th 1953. At that time he presented a severely excoriated dermatitis of the perianal area. From the history at that time he was stated to have had pruritis ani for ten years. There was some dermatitis of the elbow extensor then but I was not clinically impressed with the dermatitis venenata picture.

He has been treated with various topical medicaments and with some success but still has some dermatitis. I also had him seen by Dr. R. Kierland at the Mayo Clinic and they felt much as I do, namely that there might have been an element of contact early but that would not explain the pruritis ani of long duration except from the "over-treatment" standpoint.

Sincerely,

R. E. Jenkins

Robert E. Jenkins, MD

16336

THE DOW CHEMICAL COMPANY
MIDLAND MICHIGAN

April 12, 1954

DOW 1 278633

Dr. J. S. Vandemark
Assistant Professor of Horticulture
Purdue University Agricultural Extension Service
Department of Horticulture
Lafayette, Indiana

Dear Dr. Vandemark:

Your letter to our Mr. V. K. Rowe is now in my hands for comment.

As Mr. Rowe has told you, this is certainly not a reaction typical of 2,4,5-T contacts. I am assuming that Mr. Mayfield has had no subsequent contacts with 2,4,5-T since the original back in August, and if this is true then it follows apriori that the dermatosis which he now has, is not the result of contact with 2,4,5-T because contact dermatoses by definition are cleared certainly within two months after discontinuance of the contact.

If contact has continued however since that time, then the first thing that must be done is to remove him from any further contacts, and it must be remembered that contact does not necessarily mean direct contact with the material. It may be contact through re-wearing of clothing which are impregnated with the substance.

I hesitate to offer any suggestions for therapy without knowing more than the fact that "this area turned red in the field and now remains rather red and irritated." I think the most effective way for me to offer any help would be to talk to some physician who has recently seen the skin condition. If you could give me the name of the physician who last saw him, I should be glad to call and talk with him.

Very sincerely,

Harold H. Gay, M.D.
Medical Director

HHG:EW

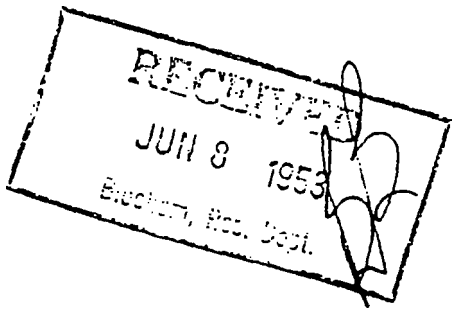
CC: V. K. Rowe, Biochem. Res Dept., 280 Bldg.

THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

DOW 1278635

June 5, 1953.



V. K. Rowe
BIO-CHEMICAL LAB.
2-280 Bldg.

cc:
H. L. Smith

SUBJECT: REPORT OF CALL BY CLOFT, BOSTON OFFICE ON LUCAS TREE EXPERT COMPANY

Attached you will find a copy of a report of call from Harry Cloft wherein he requests some information describing the effect of a 4% solution of 2,4,5-T in kerosene on eyes.

Perhaps some action has already been taken on this matter; however, Hillard Smith will be in town on Monday, June 8. Could you call Hillard Monday to decide what action, if any, should be taken at this time?

Yours very truly,

Clyde A. Bryant

Clyde A. Bryant
Agricultural Chemical Sales
Ext 8804

rms

-COPY-

PURDUE UNIVERSITY
Agricultural Extension Service
Lafayette, Indiana

Department of Horticulture

March 24, 1954

DOW 1 278632

Mr. V. K. Rowe
Dow Chemical Research Department
Dow Chemical Company
Midland, Michigan

Dear Mr. Rowe:

I am inquiring as to whether you or anyone in your organization might know of a treatment for an apparent allergy developed by 245 T.

One of our growers in this state received severe toxicological reaction appearing to be a burn over a portion of his body while spraying fence rows with 245 T. This occured last August. This area turned red in the field and now remains rather red and irritated. Mr. Mayfield was quite concerned over this and local physicians were not able to give him any satisfaction and he went up to Mayo Clinic last January. They didn't seem to know any treatment for this type of skin injury. Could you possibly suggest what might be done, if anything, or refer us to anyone who is more familiar with this type of a toxicological problem.

Very truly yours,

/s/ Joe

J. S. Vandemark
Assistant Professor in
Horticulture

JSV/pf

COPY

EUGENE A. HAND, M.D.
Diseases of the Skin
211-12 Bearinger Bldg.
Saginaw, Michigan

DOW 1278626

June 2, 1954

John T. Woodruff
Consumers Power Company
Bay City, Michigan

RE: Richard Guillet
vs. Consumers Power Company

Dear Sir:

This man returned to my office 5-24-54 for the patch tests as you requested.

The results are as follows:

	24 hrs	48 hrs
Crude oil	4 plus	4 plus
Estron 245	negative	negative
Timber Tar	4 plus	negative
Poison Ivy	4 plus	4 plus

These tests would indicate the cause of the recent diffuse dermatitis was the crude oil used as a vehicle for the Estron 245 weed killer.

I think it is only fair to give this man another trial at this same job before transferring him. He was instructed on how to avoid the irritant.

Hoping this information will be of service to you, I remain,

Sincerely yours,

Eugene A. Hand (Signed)

Eugene A. Hand M. D.

EAH:mes:cd

16340

CONFIDENTIAL

THE DOW CHEMICAL COMPANY
MIDLAND MICHIGAN

July 12, 1955

DOW 1278615

Dr. Andrew G. Goesl
Daniel-Harrell Clinic
Medical Arts Building
317 State Line Avenue
Texarkana, Arkansas-Texas

Dear Dr. Goesl:

Mr. R. A. Crandall of our Agricultural Chemical Division at St. Louis has asked me to reply to your letter of June 25th addressed to that office.

I am very sorry to hear that you have a patient who is suffering from mild hepatitis following use of our Esteron 245. Our studies of the toxicology of this material have been conducted on laboratory animals and livestock, and while mild hepatic symptoms have occurred, the dosages in all cases have been rather massive and by the oral route. Furthermore, effects upon the hepatic or renal systems seemed to appear only after rather large doses were given and after other more drastic effects such as myotonia, anorexia, and gastritis were experienced. Thus, if the patient had swallowed the material in substantial amounts, it would not be unreasonable to expect some transient hepatic and perhaps kidney injury to follow other primary symptoms.

It is recognized, of course, that such experimental work is suggestive but not conclusive as far as human subjects are concerned.

As you are probably aware, we have manufactured and sold Esteron 245 and related materials for several years. Millions of gallons have been used and many people have been exposed. To our knowledge, there has never been a case of systemic toxicity attributed to these materials except perhaps where it has been swallowed accidentally or with suicidal intent. An occasional case of dermatitis has been reported, but in most of these cases, the diluent oil (fuel oil or kerosene) has been shown to be the causative agent.

The significance of this plant and field experience is not appreciated until one realizes that there are many custom spraying firms in this country and abroad whose employees do little

DOW 1 278618

else but apply these materials. It is not unusual for these men to have their clothing wet with the material for hours a day, day after day. We, of course, do not recommend such practice and do our best to discourage it, but nevertheless, it does occur.

In view of our laboratory findings and the experience we have had, we doubt very much if the application of Esteron 245 by your patient could have been in any way related to his hepatitis.

I am sending a copy of your letter together with a copy of this reply to Dr. H. H. Gay, Director of our Medical Department, and thereby will ask him to contact you directly with his opinions in the matter.

If we can be of any further assistance, please do not hesitate to contact us.

Sincerely yours,

VKR

V. K. Rowe
Biochemical Research Department
2-280 Building

VKR/bb

enc.

P.S. I am enclosing a reprint entitled, "Summary of Toxicological Information on 2,4-D and 2,4,5-T Type Herbicides and Evaluation of Their Hazards to Livestock Associated with Their Use." Recognizing that this information does not apply directly to human beings, we believe it is indicative of the type of thing which can be expected.

V.K.R.

cc: Dr. H. H. Gay ✓
R. A. Crandall
H. L. Smith
G. J. Williams

RESULTS OF TOXICOLOGICAL TESTS ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

DOW 688872

PROBLEM

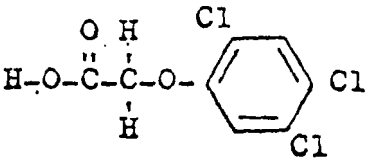
The widespread handling of 2,4,5-trichlorophenoxyacetic acid made it desirable for us to have a significant amount of toxicological information. Data on the oral toxicity was necessary for presentation to the F.D.A. Hearing on residue tolerances.

MATERIAL

Name: 2,4,5-Trichlorophenoxyacetic acid

Formula:

Structural:



Empirical: $C_8H_5Cl_3O_3$

EXPERIMENTAL RESULTS

The acute oral toxicity of 2,4,5-trichlorophenoxyacetic acid for rats, mice, guinea pigs and chicks is given in the attached summary which was presented in evidence at the F.D.A. Hearing. In concluding our testimony on this material we stated,

"Because of the similarity in chemical structure and in toxicity and physiological effect between 2,4-D and 2,4,5-T, it seems that a residue tolerance for the latter on fresh fruits and vegetables should be the same as that established for 2,4-D; it would seem that a residue of 20 p.p.m. would provide a wide margin of safety".

DOW 668873

Eye Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in propylene glycol was introduced into the eye of a rabbit, it produced marked pain and slight conjunctival irritation immediately. Five hours later, severe conjunctivitis and moderate corneal damage was apparent. This picture remained about the same for two days before healing began. Five days after the exposure mild conjunctivitis and corneal damage was still apparent.

Skin Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in butyl carbitol acetate was applied repeatedly to the rabbit ear and bandaged repeatedly to the shaven abdomen, a slight simple irritation developed in both instances.

SUMMARY

The toxicological properties of 2,4,5-trichlorophenoxyacetic acid may be summarized as follows:

1. The material is moderate in acute oral toxicity for the four species, rat, mouse, cavie, and chick. Of these species the rat seems to be the more resistant and the chick the most susceptible.
2. The material is markedly irritating to the eyes.
3. The material is slightly to moderately irritating to the skin upon prolonged exposure.

23 HANDLING HAZARDS AND PRECAUTIONS FOR SAFE HANDLING

Swallowing

It does not seem likely that 2,4,5-trichlorophenoxyacetic acid can be swallowed accidentally in amounts dangerous to life, although this might be possible if concentrated solutions of the material were available to children. If substantial quantities of the material were to be swallowed by anyone, serious ill effects could be anticipated.

If the material should be swallowed, induce vomiting by giving a common emmetic such as 2 tablespoonfuls of table salt in a glass of warm water. Call a doctor.

es

The 2,4,5-trichlorophenoxyacetic acid is capable of causing serious damage to the eyes. Precautions should be taken when handling this material to prevent possible eye contacts. It is ommended when handling strong solutions of 2,4,5-trichlorophenoxy-cetic acid that goggles, face shield, or other similar device be orn to prevent accidental splashes in the eye. It is believed hat the handling of the solid does not present a hazard simply ecause of its insolubility in aqueous medium.

If the material in any form should get into the eyes, they puld be promptly washed with flowing water for at least 15 inutes and medical attention obtained.

kin

Prolonged and repeated contact with the skin may result in ritation. Occasional contacts for short periods are not expected cause irritation. Strong solutions are no doubt more likely to use difficulty than the dry solid.

In case of contact wash the exposed area with soap and water. not wear clothing or shoes which have been contaminated with solu-ons of the material.

DOW 688874

Inhalation

No studies of toxicity upon inhalation of 2,4,5-trichlorophenoxyacetic acid dust have been made. We believe, however, that in view of the similarity with this material to that of 2,4-dichlorophenoxyacetic acid, that it is unlikely that toxic concentrations of the dust would be inhaled, primarily because of the irritation it will produce upon the upper respiratory passages.

DOW 668875

Ind. Hygiene and Occupational Medicine,
7:61, 1953

C-T-L

DOW6883857

TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID

A Report on Their Acute and Chronic Toxicity in Dogs

VICTOR A. DRILL, Ph.D., M.D.
AND
TOMIHARU HIRATZKA, M.D.
DETROIT

BOTH 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may act as plant hormones and herbicides [1] and are now being fairly widely used for the control of certain types of weeds on farm lands. They are being sprayed from airplanes on many acres of specialized farms and grazing land and are present in trademark preparations sold at retail for home gardeners. With the widespread use of these herbicides, possible toxic effects are important, as accidental ingestion may occur or residues may be inadvertently encountered in food. In an earlier report, 2,4-D was mentioned as being nontoxic to animals and man when administered orally.[1a,d] However, the injection of large doses of 2,4-D in animals produced symptoms similar to those seen in clinical myotonia.[2] More detailed observations were reported by Hill and Carlisle who studied the acute and subacute effects of 2,4-D in various species of animals.[3] There have been no reports concerning the toxic properties of 2,4,5-T. The present study concerns the acute oral toxicity and chronic oral toxicity of 2,4-D and 2,4,5-T in dogs.

EXPERIMENTAL PROCEDURES

Adult mongrel dogs of both sexes were used. Most of the animals were housed in the laboratory for a period of two to three months before the study was started. During this control period they were immunized against distemper with the Green vaccine. The dogs were fed a standard stock diet (Friskies) ad libitum. Both 2,4-D and 2,4,5-T were commercial materials, with a purity of 98.5 and 98.9% and freezing points of 132.8 and 150.6 C., respectively.

In the acute studies, the calculated dose of 2,4-D or 2,4,5-T was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days, at which time the sur-

This study was supported by a grant from the Dow Chemical Company

From the Department of Physiology and Pharmacology and the Department of Pathology, Wayne University College of Medicine.

1. (*c*) Hildebrand, E. M.: War on Weeds, Science **103**:465-468, 1946. (*b*) van Overbeek, J., and Vélez, I.: Use of 2,4-Dichlorophenoxyacetic Acid as a Selective Herbicide in the Tropics, Science **103**:472-473, 1946. (*c*) Smith, F. G.; Hamner, C. L., and Carlson, R. F.: Control of Ragweed Pollen Production with 2,4-Dichlorophenoxyacetic Acid, Science **103**:473-474, 1946. (*d*) Marth, P. C., and Mitchell, J. W.: 2,4-Dichlorophenoxyacetic Acid as a Differential Herbicide, Bot. Gaz. **106**:224-232, 1944.

2. Bucher, N. L. R.: Effects of 2,4-Dichlorophenoxyacetic Acid on Experimental Animals, Proc. Soc. Exper. Biol. & Med. **63**:204-205, 1946.

3. Hill, E. V., and Carlisle, H.: Toxicity of 2,4-Dichlorophenoxyacetic Acid for Experimental Animals, J. Indust. Hyg. & Toxicol. **29**:85-95, 1947.

16347

vivors were autopsied. In the chronic studies, the 2,4-D or 2,4,5-T was administered orally in capsules five days a week over a 13 week period. Each capsule was imbedded in a 4 to 5 gm. piece of commercial canned dog food, which the animals consumed readily, thus avoiding the continued trauma of the stomach tube. During the study the animals were weighed twice a week. Control blood counts were taken before the administration of the drug, on the 30th day, and on the 90th day at the completion of the study. Hemoglobin was determined with a photoelectric colorimeter.[4] Upon death or at the completion of the study, the animals were autopsied. Tissues were taken from the lung, heart, liver, kidney, adrenals, spleen, thyroid, and ovary or testes, fixed in 10% formalin, and stained with hematoxylin and eosin.

TABLE 1.—*Effect of Single Oral Doses of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid in Dogs*

Dog No.*	Dose, Mg./Kg.	Weight Loss, Kg.	Symptoms†	Death Day‡	Mortality
		2,4-D			
1 F	400	−0.1	+	2	2/2
2 M	400	−0.6	++	3	
3 F	250	−1.8	+++	8	3/3
4 M	250	−2.0	++	7	
5 M	250	−0.4	++	4	
6 F	100	−2.5	+	8	2/3
7 F	100	−2.0	0	9	
8 F	100	−1.7	+	9	
9 F	100	−2.1	0	8	
10 F	25	0.0	0	8	0/3
11 F	25	+0.2	0	8	
12 M	25	+0.2	0	8	
		2,4,5-T			
13 M	400	−1.1	+	2	1/1
14 M	250	−1.4	+	3	1/1
15 F	100	−0.5	0	8	1/4
16 F	100	−1.0	0	7	
17 F	100	−1.0	0	8	
18 F	100	−0.4	0	8	
19 M	50	+0.3	0	S	0/4
20 M	50	−2.3	0	S	
21 F	50	+0.1	0	8	
22 M	50	−0.6	0	S	

* F in this column means female; M, male.
† Column indicates general severity of symptoms as discussed in text. 0 means no change from normal
‡ S in this column means survived 14 day test period.

RESULTS

1. Acute Oral Toxicity.—(*a*) Mortality: The effect of single oral doses of 2,4-D or 2,4,5-T on survival for 14 days is shown in Table 1. The deaths were delayed and occurred two to nine days after the compounds were administered. The oral $L.D._{50}$ of 2,4-D was approximately 100 mg. per kilogram of body weight; for 2,4,5-T it was in the range of 100 mg./kg. or higher.

(*b*) Body Weight: The large oral doses of 2,4-D or 2,4,5-T produced a decrease in body weight (Table 1). Such animals developed various degrees of anorexia, and those that died refused even canned dog food toward the end of their survival.

4. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter with Light Filters, J. Biol. Chem. **115**:63-75, 1936.

DOW 6633558

16348

(*c*) Symptoms: In dogs that died the effect produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myotonia. Initial signs were often noted six hours after the oral administration of 2,4-D. At this time the animals were more quiet than normal, and a slight ataxia was occasionally present. The hind legs were always affected first; the forelegs later or not at all. There was usually a progressive increase in spasm in the hind legs associated with increasing ataxia, and some dogs spontaneously extended the hind limbs in a spastic movement lasting a few seconds. The knee jerk was either normal or hyperactive.

In the early stages of acute poisoning with 2,4-D, the animals were usually quiet. If the animal was lifted and made to walk, the spasm and ataxia were clearly noticed, but after it had walked for a while, the spasm and ataxia tended to decrease. In the later stages of intoxication, the animal, when placed on its feet, was unable to stand and would then sit or lie in an awkward position with spastic limbs marked particularly by spasm of the hind legs. Other dogs stumbled and rolled on the floor in attempts to right themselves. An occasional dog showed evidence of irritation or pain when the skin over the back of the neck was grasped. Some dogs, when held up by the shoulders, would show extension and crossing of the hind limbs. As the toxic effects of the drug progressed, the dogs eventually refused the various types of food offered. Occasionally, sneezing, rubbing of the eyes, and diarrhea were observed, but not vomiting.

Such signs were also seen in one dog that survived a lower dose of 2,4-D for 14 days (Table 1). In this instance the changes were relatively mild and were limited to spasm of the hind legs and a slight ataxia.

Marked effects were not observed in the dogs receiving 2,4,5-T. The changes were limited to a slight or moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses. No symptoms were evident in the one dog that died after a single dose of 100 mg./kg. (Table 1).

(*d*) Pathology: Changes observed were limited to the gastrointestinal tract and lungs. Four dogs (1, 2, 4, and 6) receiving 2,4-D and two dogs (13 and 17) fed 2,4,5-T showed mild to diffuse redness of the mucosa in the small intestine. In some dogs pneumonia was present. Histologically, the above dogs showed some necrosis and inflammation of the intestinal mucosa. One dog (13) had a moderate, diffuse hepatic necrosis. Two dogs (13 and 16) had a mild degree of renal tubular degeneration. In most dogs that died the findings were limited to nonspecific changes, such as hepatic congestion. In many cases death was apparently due to pneumonia, which followed the development of anorexia, weight loss, and myotonia.

II. Chronic Oral Toxicity.—(*a*) Mortality: All dogs receiving 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight survived the 90-day test period. The dogs receiving 20 mg./kg. of these compounds died during the study. The administration of 20 mg./kg. in divided doses twice a day produced death in both dogs receiving 2,4,5-T and in one dog receiving 2,4-D (Table 2).

(*b*) Body Weight: The only significant change in body weight occurred in the animals that failed to survive the study (Table 2). Loss of weight in these dogs began 7 to 12 days before the death of the dog.

(*c*) General Symptoms: Dogs that survived the oral administration of 2,4-D or 2,4,5-T for 90 days were free of any symptoms.

DOW 668859

16349

The three dogs that died while receiving the highest dose of 2,4-D showed signs differing somewhat from those observed in the acute studies. The dogs were quiet, weaker, and less responsive than normal. Muscle tonus was higher in the hind legs, particularly on passive extension. The hind legs were held more stiffly than usual when walking, and a slight ataxia was present. During the last two to three days of their survival two of the dogs showed difficulty in chewing or swallowing and eventually even refused a small bolus of canned dog food (usually readily consumed). There was also some oozing of blood from the gums and buccal mucosa.

TABLE 2.—*Body Weight and Survival of Dogs Fed 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid for 90 days*

Dog No.*	Dose, Mg./Kg.	Weight			Death Day‡
		Initial, Kg.	Final, Kg.	Diff., Kg.	
		2,4-D			
23 F	None	14.9	16.4	+1.7	S
24 F	None	9.3	10.9	+1.6	S
25 M	2	6.8	9.5	+2.7	S
26 F	2	15.2	13.8	−1.4	S
27 M	5	9.5	9.5	0.0	S
28 F	5	12.0	12.5	+0.5	S
29 M	10	14.7	14.8	+0.1	S
30 M	10	11.6	11.9	+0.3	S
31 M	10	17.2	17.8	+0.6	S
32 M	20†	13.0	13.7	+0.7	S
33 F	20†	9.8	5.9	−3.9	49
34 M	20	12.2	10.1	−2.1	18
35 M	20	13.4	9.7	−3.7	25
		2,4,5-T			
36 F	None	7.3	7.7	+0.4	S
37 M	None	12.1	14.5	+2.4	S
38 F	2	11.3	12.7	+1.4	S
39 M	2	8.9	10.0	+1.1	S
40 F	5	8.6	8.4	−0.2	S
41 M	5	12.3	12.5	+0.2	S
42 F	10	12.3	11.5	−0.8	S
43 M	10	13.0	12.2	−0.8	S
44 F	10	9.8	9.2	−0.6	S
45 M	20†	11.8	9.2	−2.6	49
46 F	20†	10.5	7.9	−2.6	75
47 F	20	14.9	12.7	−2.2	11
48 M	20	10.9	8.2	−2.7	59

* F in this column means female; M, male.
† Daily dose divided and administered one half in a. m. and one-half in p. m.
‡ S in this column means survived 90 day test period.

In dogs that died while receiving 2,4,5-T, the prominent effects were weakness, slight stiffness in the hind legs, difficulty in swallowing food, and, in one dog, bleeding from the gums.

(*d*) Blood Count: The administration of 2,4-D or 2,4,5-T did not have any significant effect on the hemoglobin, red cell count, or total white cell count of animals that survived or died during the study. The differential blood count remained normal in the surviving animals. In three animals that died a terminal fall in the percentage of lymphocytes was observed (Table 3).

(*e*) Organ Weights: There was no significant change in the weight of the thyroid gland, adrenal gland, heart, liver, or kidney in animals that survived the 90-day period of study. In two of the three dogs that died during the administration

DOW 668860

16356

DOW 668861

of 2,4-D, there was a slight increase in heart and kidney weight. A similar change was noted in two of the four dogs that died as a result of 2,4,5-T.

(*f*) Gross Pathology: Two dogs receiving 2,4-D (26 and 34) showed areas of redness in the duodenum. With the administration of 2,4,5-T there developed in dog 42 a diffusely reddened duodenum and jejunum, while in dog 48, autopsied on the 59th day, a generalized icterus was demonstrated.

TABLE 3.—*Effect of 2,4-D and 2,4,5-T on Hemoglobin, Total Blood, and Differential Counts*

Dog No.*	Dose, Mg./Kg.	Day	Hb, Gm. %	RBC, Mil.	WBC	Differential Count: Neut., %	Lymph., %	Mon., %	Bas., %	Eosin., %	[illegible]
					Control Dogs						
23 F.....	None	0	13.20	5.72	17,800	56	38	0	0	6	
		30	13.82	5.93	15,600	64	29	0	0	7	
		90	13.70	6.09	13,850	71	27	2	0	0	
24 F.....	None	0	12.80	6.01	10,900	86	11	3	0	0	
		30	14.72	6.66	13,150	79	12	7	0	2	
		90	14.85	6.16	7,100	79	21	0	0	0	
26 F.....	None	0	9.60	5.08	16,100	67	26	1	0	6	
		30	10.75	5.63	13,500	56	16	2	0	3	
		90	12.74	5.87	14,700	81	18	1	0	0	
37 M.....	None	0	12.29	6.53	14,500	55	39	2	0	4	
		30	13.31	5.78	14,900	63	26	4	0	7	
		90	13.95	6.47	6,400	48	51	1	0	0	
					2,4-D						
32 M.....	20†	0	10.43	5.02	13,800	64	27	5	0	4	
		30	12.44	6.16	16,400	66	26	4	0	4	
		90	11.52	6.35	12,100	70	28	6	0	0	
33 F.....	20†	0	13.18	5.56	14,850	54	41	2	0	3	
		30	14.44	5.88	9,700	47	46	4	0	3	
		49	14.36	5.67	16,600	79	14	7	0	0	
34 M.....	20	0	13.20	5.72	15,200	59	44	4	0	3	
35 M.....	20	0	14.96	6.47	13,150	76	11	1	0	12	
		25	14.59	6.34	9,100	68	27	4	0	1	
					2,4,5-T						
45 M.....	20†	0	12.54	5.35	16,400	72	12	6	0	10	
		30	8.64	3.98	16,458	77	13	3	0	7	
		49	3.94	3.07	6,900	94	4	2	0	0	
46 F.....	20†	0	12.18	7.10	16,050	63	29	2	0	6	
		30	14.66	6.82	11,200	71	28	0	0	1	
47 F.....	20	0	12.44	5.73	13,800	63	32	4	0	1	
48 M.....	20	0	12.48	6.03	16,800	51	44	2	0	3	
		30	12.93	6.81	14,650	78	18	4	0	0	
		59	11.97	6.14	7,175	92	9	1	0	0	

* F in this column means female; M, male.
† Daily dose divided and administered one half in a. m. and one half in p. m.
To conserve space, only data on control dogs and the highest doses are given.

(*g*) Microscopic Changes: The heart, lungs, thyroid, adrenal, ovary, or testes did not demonstrate any significant changes. Three of the dogs receiving 2,4,5-T and two receiving 2,4-D showed an occasional area of focal necrosis in the liver. This finding, however, was not related to either the dose of the compounds or the death of any animal (Table 4) and was judged to be of no significance. The duodenum of dogs 26, 34, 42, and 48 showed varying degrees of hyperemia and some early infiltration of cells in the mucosa. The submucosa and serosa were normal.

16351

DOW 668862

A slight increase in the number of casts in kidney sections was noted in some dogs (Table 4), but again this was unrelated to dosage and was of doubtful significance.

COMMENT

In the acute studies, single large oral doses of 2,4-D administered to dogs may produce signs characteristic of clinical myotonia. This effect of 2,4-D was similar to that observed after various experimental species had received injections.[5] In addition, some dogs showed signs of irritation and pain when the skin of the back of the neck was grasped, which may indicate meningeal irritation. The extension and crossing of the hind limbs, when lifted by the shoulders, may point to a spinal or

TABLE 4.—*Histological Changes in Liver and Kidney of Dogs Fed 2,4-D or 2,4,5-T for 90 Days*

Dog No.	Dose, Mg./Kg.	Death Day	Liver		Kidney	
			Congestion	Focal Necrosis	Casts	Tub. Injury
			2,4-D			
23	None	..	0	0	0	0
24	None	..	0	0	0	0
25	2	..	0	0	0	0
26	2	..	0	0	1+	0
27	5	..	1+	1+	0	0
28	5	..	2+	0	1+	1+
29	10	..	0	0	0	0
30	10	..	0	0	0	0
31	10	..	1+	0	0	0
32	20	..	0	0	0	0
33	20	49	1+	1+	0	0
35	20	25	2+	0	0	0
			2,4,5-T			
36	None	..	0	0	0	0
37	None	..	0	0	0	0
38	2	..	0	0	1+	0
39	2	..	0	0	0	0
40	5	..	0	0	0	0
41	5	..	0	1+*	2+	1+
42	10	..	2+	0	1+	0
43	10	..	0	0	0	0
44	10	..	1+	0	0	0
45	20	49	1+	1+	0	0
46	20	75	1+	1+	1+	0
48	20	59	2+	0	0	0

* Also a 1+ fibrosis.
Tissues graded on basis of 0, 1+, 2+, 3+, and 4+.

central lesion. Other effects occasionally observed were sneezing, rubbing of the eyes, and diarrhea. The effects of 2,4,5-T, previously studied, were not as severe, being limited to stiffness in the hind legs and ataxia. The higher doses of each compound produced anorexia and weight loss. Although initial effects may be seen six hours after the compounds are given, the syndrome developed slowly in the dogs, and death was delayed, occurring several days after the administration of the compounds.

In the chronic studies, the dogs survived doses of 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight without apparent ill effects. However, the daily oral administration of 20 mg./kg. of 2,4-D produced death in three of four dogs within 18 to 49 days. This delayed death may indicate a cumulative effect of 2,4-D. The signs observed in animals dying from the 20 mg./kg. dose of 2,4-D

16352

DOW668863

differed somewhat from those seen in the acute studies. With the chronic administration, the animals exhibited chiefly a stiffness of the hind legs and ataxia, weakness, difficulty in chewing or swallowing, and occasionally bleeding from the gums. All four dogs which received 20 mg./kg. of 2,4,5-T succumbed between the 11th and the 75th day of the study. Toxic effects in these animals consistently included slight muscle spasm and difficulty in swallowing food. Thus, the chronic oral toxic dose for 2,4,5-T in dogs seems to be in the same range as that obtained with 2,4-D. With both drugs, there was a terminal loss in body weight.

Dogs surviving the chronic doses of 2,4-D or 2,4,5-T did not show any significant change in hemoglobin, total blood counts, or differential blood count. In three of the dogs (64, 57, and 105) which died while receiving 20 mg./kg. of 2,4-D or 2,4,5-T, there was a definite decrease in the percentage of lymphocytes in the peripheral blood. Hill and Carlisle [3] have observed a fall in the polymorphonuclear leucocyte count in one dog and a decrease in the per cent of lymphocytes in two dogs to which 50 mg./kg. of 2,4-D was administered intravenously each day for six days. In mice which received chronic injections there was no alteration in the peripheral blood picture.[2]

Large doses of 2,4-D administered intravenously or, in the present study, orally occasionally produced some necrosis in the liver. However, the administration of chronic oral doses produced only a slight and inconsistent focal necrosis of doubtful significance (Table 4). Deaths during the chronic oral administration of 2,4-D or 2,4,5-T were not correlated with significant lesions in the liver, kidney, or other organs examined.

SUMMARY

The acute oral $L.D._{50}$ of 2,4-dichlorophenoxyacetic acid (2,4-D) in dogs was approximately 100 mg. per kilogram of body weight. Doses in this range or higher produced a definite myotonia accompanied by anorexia and weight loss. The acute oral $L.D._{50}$ for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was in the range of 100 mg. per kilogram or higher. Such toxic doses produced only signs of a mild spasticity.

All dogs survived the oral feeding of 2, 5, or 10 mg. per kilogram of 2,4-D or 2,4,5-T 5 days a week for a period of 90 days. These doses did not produce any symptoms or changes in body weight, organ weights, or blood count.

Three of the four dogs receiving repeated doses of 20 mg. per kilogram of 2,4-D died, and all four dogs receiving 20 mg. per kilogram of 2,4,5-T died during the study. Toxicity to 2,4-D at the dosage level was accompanied by bleeding from the gums, necrotic changes in the buccal mucosa, and some difficulty in chewing and swallowing, but there was only little evidence of clinical myotonia. A terminal fall in the percentage of lymphocytes was observed in three of the animals.

Death during the repeated administration of 2,4-D or 2,4,5-T was not related to pathological changes in the liver, kidney, or other organs examined.

16353

THE ACUTE ORAL TOXICITY OF 2,4,5-TRICHLOROPHENOXYACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS

DOW668852

V. K. Rowe
D. D. McCollister
and
H. C. Spencer

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

1950

Single oral doses of 2,4,5-trichlorophenoxyacetic acid as a 10 percent suspension in olive oil were administered by stomach tube to mixed sex groups of rats, mice and guinea pigs. Aliquots of a 3 percent solution in olive oil were administered to chicks. The white rats and the guinea pigs were selected from the stock colonies of this laboratory. White mice were obtained from the Carworth Farms and New Hampshire Red chicks were purchased from a commercial hatchery. Mature, young adult animals were used in each instance except for the chicks which were approximately three weeks of age.

DOW668853

Table I summarizes the concentrations and volumes of olive oil solutions containing 2,4,5-trichlorophenoxyacetic acid administered to each species.

TABLE I

SINGLE ORAL ADMINISTRATION OF
2,4,5-TRICHLOROPHENOXYACETIC ACID

Species	Percent Material in olive oil	Volume Given by Stomach Tube	
		Minimum (cc.)	Maximum (cc.)
Rats	10	0.7	2.9
Mice	10	0.03	0.3
Guinea Pigs	10	1.0	2.9
Chicks	3	0.3	5.8

Mortalities resulting from the various single oral dosages of 2,4,5-trichlorophenoxyacetic acid to the experimental animals are given in Table II. All surviving animals were observed until it was certain that they had fully recovered (usually about two weeks). Symptoms of poisoning, particularly with the higher doses, were ataxia and myotonia.

16355

3

TABLE II

MORTALITIES RESULTING FROM THE ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4,5-TRICHLOROPHENOXYACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS

Dosage (gm./kgm.)	Rats		Mice		Guinea Pigs		Chicks	
	No.fed	No.died	No.fed	No.died	No.fed	No.died	No.fed	No.died
0.1			3	0			6	0
0.2							6	0
0.3	10	0	6	2	5	0	6	4
0.4					5	4		
0.5	10	5	6	4	5	4	6	5
.7	10	8	6	6	5	5	6	6
1.0	10	10	6	6			3	3

DOW 668854

The acute oral LD_{50} values with their 19/20 confidence limits were determined for each species by use of the method described by Litchfield and Wilcoxon[1]. The results of the statistical treatment of the dosage-response data are presented in Table III.

TABLE III

ACUTE ORAL LD_{50} VALUES FOR 2,4,5-TRICHLOROPHENOXYACETIC ACID WHEN ADMINISTERED TO RATS, MICE, GUINEA PIGS AND CHICKS

Species	LD_{50} (19/20 confidence limits) gm./kgm.	Slope Function*
Rats	0.500 (0.391 - 0.640)	1.49
Mice	0.389 (0.245 - 0.619)	1.80
Guinea Pigs	0.381 (0.307 - 0.472)	1.21
Chicks	0.310 (0.211 - 0.456)	1.40

* the fold change in dosage required to produce one unit standard deviation in response along the line.

16356

1. Litchfield, J. T., Jr., and Wilcoxon, F.: J. Pharm. and Expt. Ther. 96: 99, 1949.

The Litchfield and Wilcoxon tests for parallelism of two lines and estimate of relative potency revealed that the 2,4,5-trichlorophenoxyacetic acid was significantly (19/20 probability) more toxic to the chicks than to the rats. There were no significant differences in either parallelism or relative potency as a result of the following comparisons: rats versus mice, rats versus guinea pigs, mice versus guinea pigs, mice versus chicks and guinea pigs versus chicks.

DOW 668655

12 44 R I File Copy

DOW 1 453181 MN0719 56

Biochemical Research Department

The Dow Chemical Company

RESULTS OF SKIN TESTS ON:

1. CONCENTRATED IMPURITIES IN DOW'S 2,4,5-TRICHLOROPHENOL (K 2777-40)
2. CONCENTRATED IMPURITIES IN 2,4,5-TRICHLOROPHENOL MADE FROM HOOKER'S 1,2,3,4-TETRACHLOROBENZENE (K 2777-41)
3. CONCENTRATED IMPURITIES FROM 2,4,5-TRICHLOROPHENOL FROM DOW'S 1,2,3,4-TETRACHLOROBENZENE (K 2777-42)
4. CONCENTRATED IMPURITIES FROM HOOKER'S 2,4,5-TRICHLOROPHENOL (K 2777-43)
5. IMPURITIES FROM DOW'S TETRACHLOROBENZENE (K 2696-5)
6. IMPURITIES FROM HOOKER'S TETRACHLORO-BENZENE (K 2696-6)

File T16.4-60-4
~~K No.~~ T17.4-12-9
K No. 2777-40
2777-41
2777-42
2777-43
Chg. 1219-2
Rept. By Mark A. Wolf

Be T 16.4-60-4

THIS REPORT IS THE PROPERTY OF THE DOW CHEMICAL COMPANY

COPIED IN MIDLAND

16358

THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

July 12, 1956

DOW 14531182

Mr. R. Reese
Bisphenol Plant
349 Building

SUBJECT: SKIN IRRITATION OF SOME IMPURITIES IN 2,4,5-TRICHLOROPHENOL AND TETRACHLOROBENZENE FROM VARIOUS SOURCES

The skin tests on the impurities in 2,4,5-trichlorophenol made from various sources have been completed. These tests were undertaken to help explain, if possible, the differences in skin responses produced by various samples of 2,4,5-trichlorophenol. Previous tests have shown that only the impurities found in Hooker's 2,4,5-trichlorophenol were capable of causing skin responses which were unusual. Other possible impurities were shown to be similar in their effects on the skin to 2,4,5-trichlorophenol.

The materials submitted for testing were as follows:

1. Concentrated impurities from Dow's 2,4,5-trichlorophenol (K 2777-40).

2. Concentrated impurities from 2,4,5-trichlorophenol made from Hooker's 1,2,3,4-tetrachlorobenzene (K 2777-41).

3. Concentrated impurities from 2,4,5-trichlorophenol from Dow's 1,2,3,4-tetrachlorobenzene (K2777-42).

16359

DOW 1453183

4. Concentrated impurities from Hooker's 2,4,5-trichlorophenol (K 2777-43).

All of these materials were applied to the intact rabbit's skin as a 10% solution in Dowanol 50B. When tested in this manner, all of these materials gave essentially the same response in that they were slightly irritating to the rabbit's skin. This response was characterized by slight reddening of the skin and slight exfoliation or scaliness. This is considered typical of the response caused by 2,4,5-trichlorophenol at this concentration.

These results show that the impurities, as obtained in these samples, should not contribute significantly to the irritating properties of 2,4,5-trichlorophenol regardless of source of the raw material. This finding is not in accord with those reported earlier in which it was suggested that an impurity of Hooker's 2,4,5-trichlorophenol may cause acneform dermatitis in humans (See letter of January 27, 1956).

Two possible explanations for this difference in response were suggested. The first was that the "acneform" effect was weak in nature and may have been overlooked in this series of tests although they were repeated on several rabbits with no significant change in response.

The second possible explanation was that there may have been differences in the original samples used even

16360

DOW_1453184

though the impurities were concentrated in a similar manner. Consequently, the samples were analyzed by infrared scanning with the following results.

ANALYSES OF CONCENTRATED IMPURITIES

Dow's 2,4,5-trichloro-phenol	2,4,5-trichlorophenol made from Hooker's tetrachloro-benzene	2,4,5-trichlorophenol made from Dow's tetra-chlorobenzene	Hooker's 2,4,5-tri-chlorophenol	Identification Of Constituents
59 ± 3	75 ± 3	69 ± 3	91 ± 2	% 2,4,5-trichloro-phenol
12 ± 2	1.3 ± .5	7 ± 1	1.9 ± .5	% 2,3,6-trichloro-phenol
<0.5	<0.5	<0.5	<0.5	% 2,4-dichlorophenol
0.6 ± 0.3	1.7 ± .5	5 ± 1	3 ± 1	% 2,5-dichlorophenol
12 ± 2	13 ± 2	10 ± 2	<1	% 2,4-dichloro-5-methoxyphenol (impurity A)
8 ± 2	6 ± 2	6 ± 2	<1	% 4,5-dichloro-2-methoxyphenol (impurity B)

The remaining materials in the above samples are not identified but are similar in structure to the methoxy dichloro phenols and dimethoxy dichloro benzenes. This would indicate the possibility of dimethoxy chloro phenols and dichloro catechol and dichloro resorcinol.

Unfortunately a similar analysis was not performed on the previously reported "concentrated impurity" from Hooker's 2,4,5-trichlorophenol. It is apparent, however, that the sample reported on here must have been concentrated

DOW 1453185

from a material of a high order of purity, being 91 ± 2 % of 2,4,5-trichlorophenol. It is reasonable to assume that any impurities that were in the submitted sample were present only in minor amount in the original product thus reducing the possibility of its producing the skin effects observed in the first sample.

The skin irritation tests on the two samples of impurities from tetrachlorobenzene have been completed also. The samples submitted were:

K 2696-5 Residue from Dow 1,2,4,5 tetrachlorobenzene.

K 2696-6 Residue from Hooker 1,2,4,5-tetrachlorobenzene.

When applied repeatedly to the rabbit's skin, both of these materials, as a 5% solution in Dowanol 50B, caused only very slight irritation and slight to moderate exfoliation. A similar response was obtained when the Hooker 1,2,4,5-tetrachlorobenzene residue was applied as a 1% solution. This response is typical of 2,4,5-trichlorophenol at this level. Hence the impurities, as represented in these two samples, should not affect materially the toxicological response of 2,4,5-trichlorophenol.

I hope this information proves useful to you. If I can be of any further help, please let me know.

-Mark A. Wolf-

Mark A. Wolf
Biochemical Research Department
12-634 Building

MAW/mc

16362

DOW 1453189

UNIT INDEX

Effects of skin tests of various impurities from 2,4,5-trichlorophenol and 1,2,4,5-tetrabenzene from various sources are given.

INDEX HEADINGS

C.R.I. Name: Phenol; 2,4,5-trichloro; impurities from, ~~toxicity~~

Benzene; 1,2,4,5-tetrachloro-, impurities from, ~~toxicity~~

Impurities from 2,4,5-trichlorophenol

Impurities from 1,2,4,5-tetrachlorobenzene

DISTRIBUTION

Director of Research - R. Boundy
Central Research Index
Western Central Research File
Texas Central Research Index
Bisphenol Plant - R. Reese
Spectorscopy Laboratory - K. Bradley

16363

File - Authorization S-399

1267

CABLE ADDRESS DOWCHEMCO
BRANCH SALES OFFICES
NEW YORK CITY
SAN FRANCISCO
PHILADELPHIA
LOS ANGELES
WASHINGTON
SAINT LOUIS
CLEVELAND
HOUSTON
CHICAGO
SEATTLE
DETROIT
BOSTON

THE DOW CHEMICAL COMPANY

MIDLAND • MICHIGAN

December 9, 1949

TRADE DOW MARK REG. U. S. PAT. OFF.

DOW 14645058

MN058810

Dr. W. R. Veazey
Main Office
47 Building

Subject: Request for authorization to finance acute and sub-acute toxicity tests on dogs with 2,4-D and 2,4,5-T.

(1) In preparing toxicological information suitable for presentation at the FDA hearings on agricultural chemicals, we believe it would be desirable to have information on the acute and sub-acute toxicity of 2,4-D and 2,4,5-T on dogs. This work will be in addition to similar work which we have conducted on smaller laboratory animals and in addition to that which appears in the literature. The advisability of conducting this work has been discussed with representatives of the Agricultural Sales Department and the Legal Department.

(2) We have discussed this problem with Dr. V. A. Drill, Head of the Department of Pharmacology, School of Medicine, Wayne University, Detroit, and have received word from him that he is willing to undertake this work. It is our feeling that a fair price for the work we have in mind would be about three thousand dollars ($3,000.00).

(3) We therefore request that an authorization for three thousand dollars be set up to cover the acute and sub-acute toxicity studies on dogs with 2,4-D and 2,4,5-T.

(4) If you have any questions regarding this work we will be happy to discuss them with you.

Sincerely yours,

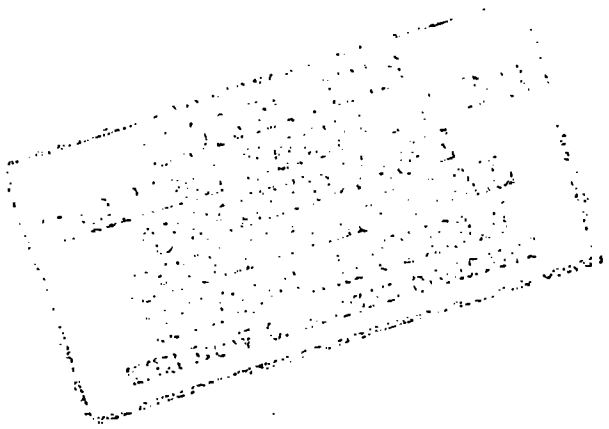
V. K. Rowe
Biochemical [redacted] Department

VKR/mg

cc: W. W. Allen
M. E. Putnam

16364

1268



DOW 7977669

4. Acute and Semi-Chronic Toxicity of Silvex to Laboratory Rats

Biochemical Research Department
The Dow Chemical Company

16365

ACUTE AND SEMI-CHRONIC TOXICITY OF "SILVEX" TO LABORATORY RATS

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

DDMAC 10/28/54

MATERIAL

"Silvex" is a trade name that has been coined for the material known chemically as 2(2,4,5-trichlorophenoxy) propionic acid. The test substance used in these studies was a sodium salt preparation in the form of a brown, rosin-like, hygroscopic solid. This material assayed 90% acid equivalent and was shown to contain one molecule of water of hydration.

SINGLE ORAL ADMINISTRATION

Procedure

The toxicity of Silvex was determined when administered in single oral doses to young adult white rats of both sexes from the stock colonies of this laboratory. Aliquots of a 10% solution of the acid (as the sodium salt) in water were fed by intubation. Eight animals (4 of each sex) were used at each of 4 dosage levels differing in quantity by a geometric ratio of 2. All surviving animals were observed for about 2 weeks or until they had fully recovered from any loss of weight and were gaining normally.

Results

The dosage-response data were treated statistically according to the method of "moving-averages" described by Thompson. The LD_{50} for rats was found to be 0.50 g./kg. with 19/20 confidence limits of 0.39 to 0.65 g./kg.

SEMI-CHRONIC DIETARY FEEDING

Procedure

All of the rats used in this work were progeny of stock obtained by this laboratory from the Wistar Institute in 1938. These animals were maintained on the stock diet from the time of weaning until they were 45 days of age, when they were divided according to body weights into well-matched groups of 10 rats of each sex and started on the control or experimental diets.

The control and basic ration used in this investigation was the usual stock diet employed in this laboratory. The following ingredients are used in the preparation of this stock diet:

	Percent by Weight
Whole wheat, freshly ground	55.0
Dried whole milk	26.5
Dried extracted liver	12.0
Dried brewers' yeast	5.0
Iodized table salt	0.5
Calcium carbonate	1.0

The experimental diets were prepared by thoroughly mixing the finely ground test material with the stock diet in amounts calculated to give diets containing the equivalent of 1.0, 0.3, 0.1, 0.03, or 0.01 percent 2(2,4,5-trichlorophenoxy) propionic acid.

Five rats of the same sex were caged together in wire bottom cages and were allowed free access to food and water at

MOW 797671

all times. All of the rats were weighed twice a week for the first 30 days, and weekly thereafter throughout the remainder of a 90-day experimental feeding period. Records were kept of body weight, general appearance, and average daily food consumption per animal. Whenever possible, failing animals were sacrificed when moribund and examined in an effort to ascertain the cause of impending death.

At the end of the 90-day experimental period, representative groups of female rats were selected for hematological examination. All of the surviving rats were then fasted overnight, weighed, killed by decapitation, and examined. The lungs, heart, liver, kidneys, and spleen from all the rats and the testes from the male rats were weighed. Hematoxylin-and-eosin sections of these organs, as well as from the pancreas and adrenals, were prepared for histologic examination.

DOW 797672

Results

1.0 Percent

The diet containing the equivalent of 1% Silvex was not acceptable to the groups of male and female rats that received it. Food consumption for each sex averaged but 5.3 grams per rat per day compared to 16.0 g. for the control male rats and 12.3 g. for the control females. The animals became rough and unkempt in appearance. The average body weight records (see charts) showed a severe depression. Within 32 days six of the ten male rats and two of the ten female rats had died. At this time, the surviving

animals were sacrificed and examined. Histopathologic findings were as follows:

(a) Male and Female Rats.

Heart - Cloudy swelling of muscle fibers.

Liver - Generalized cloudy swelling and granular degeneration of parenchymal cells together with foci of necrotic cells especially portally.

Kidney - Cloudy swelling of tubular epithelium and vacuolation of epithelium cells of the convoluted (proximal and distal) tubules.

(b) Male Rats.

Testes - Degeneration of the tubules with necrosis of germinal epithelium.

Histopathological examination of tissues from the control animals revealed no abnormalities.

0.3 Percent

The groups of rats that received the diet containing 0.3% Silvex for 90 days exhibited a marked retardation of growth. Food consumption records indicated that the animals were accepting the ration but eating slightly less (12.1 g./rat/day for the males; 10.9 g. for the females) than the animals receiving the control diet.

Four of the ten male rats and three of the ten female rats failed to survive the 90-day experimental period. These deaths were believed to be due to spontaneous infections of the lungs or middle ear. The incidence of this type of illness was similar in the control and experimental groups.

DOW 797673

Terminal hematological values were normal (Table 1). Organ weight studies (Table 2) revealed a large increase in the average weight of the livers and kidneys for both sexes. Histopathologic examination revealed:

(a) Male and Female Rats.

Heart - Slight to moderate fatty degeneration of the muscle fiber.

Liver - Marked generalized cloudy swelling of the parenchymal cells. Foci of necrosis in the portal areas involving 3 to 12 cells.

Kidney - Degeneration of numerous uriniferous tubules. Vacuolation and cloudy swelling of the epithelium cells of many remaining convoluted and collecting tubules with accumulation of debris in their lumen but without cast formation. Degeneration of several glomeruli.

(b) Male Rats.

Testes - Numerous degenerated tubules and degenerated germinal epithelium cells in other tubules but there was still evidence of spermatogenesis.

DOW 797674

0.1 Percent

The groups of male and female rats maintained for 90 days on the diet containing 0.1% Silvex seemed normal as far as gross appearance, behavior, hematological values (Table 1), and food consumption records were concerned. However, the male rats exhibited a moderate depression of growth (see chart). A suggestive, but not statistically significant, depression of growth was observed for the female rats. Liver and kidney weights were increased significantly. Histopathologic examination revealed:

NOW 79-7673

(a) Male and Female Rats.

Heart - Very slight cloudy swelling and fatty degeneration in some of the animals.

Liver - Marked generalized cloudy swelling of the parenchymal cells. Foci of necrosis in the portal areas involving 3 to 12 cells.

Kidney - Degeneration of numerous uriniferous tubules. Vacuolation and cloudy swelling of the epithelium cells of many remaining convoluted and collecting tubules with accumulation of debris in their lumen but without cast formation. Degeneration of several glomeruli.

0.03 Percent

The group of male rats that received the diet containing 0.03% Silvex exhibited a slight depression of growth, but the female rats grew essentially as well as the control animals (see charts). Liver and kidney weights each were increased significantly in the males, but liver weight only was affected in the female rats. Microscopic examination of the tissues revealed:

(a) Male and Female Rats.

Liver - Marked generalized cloudy swelling of the parenchymal cells. Foci of necrosis in the portal areas involving 3 to 12 cells.

Kidney - Degeneration of numerous uriniferous tubules. Vacuolation and cloudy swelling of the epithelium cells of many remaining convoluted and collecting tubules with accumulation of debris in their lumen but without cast formation. Degeneration of several glomeruli.

0.01 Percent

The groups of male and female rats that received the diet containing 0.01% Silvex for 90 days showed no evidence of adverse

(a) Male and Female Rats.

Heart - Very slight cloudy swelling and fatty degeneration in some of the animals.

Liver - Marked generalized cloudy swelling of the parenchymal cells. Foci of necrosis in the portal areas involving 3 to 12 cells.

Kidney - Degeneration of numerous uriniferous tubules. Vacuolation and cloudy swelling of the epithelium cells of many remaining convoluted and collecting tubules with accumulation of debris in their lumen but without cast formation. Degeneration of several glomeruli.

NOW 497676

0.03 Percent

The group of male rats that received the diet containing 0.03% Silvex exhibited a slight depression of growth, but the female rats grew essentially as well as the control animals (see charts). Liver and kidney weights each were increased significantly in the males, but liver weight only was affected in the female rats. Microscopic examination of the tissues revealed:

(a) Male and Female Rats.

Liver - Marked generalized cloudy swelling of the parenchymal cells. Foci of necrosis in the portal areas involving 3 to 12 cells.

Kidney - Degeneration of numerous uriniferous tubules. Vacuolation and cloudy swelling of the epithelium cells of many remaining convoluted and collecting tubules with accumulation of debris in their lumen but without cast formation. Degeneration of several glomeruli.

0.01 Percent

The groups of male and female rats that received the diet containing 0.01% Silvex for 90 days showed no evidence of adverse

effects as judged by growth (see charts), gross appearance and behavior, mortality, or food consumption records. Organ weights (Table 2) were normal except for slight increases in the average weight of the liver from the female rats and of the kidneys from the male rats. Microscopic examination revealed:

(a) Male and Female Rats.

Liver - Very slight cloudy swelling of some of the parenchymal cells in the periphery of the lobe, together with foci of necrosis of cells in a few portal areas.

Kidney (Male Rats) - Very slight cloudy swelling and vacuolation of epithelium cells of numerous convoluted tubules together with accumulation of debris in the lumens of their tubules but without case formation.

DOW 7976771

SUMMARY

Male and female rats (10 of each sex per group) were maintained for 90 days on diets containing the equivalent of 0.0 (control), 0.3, 0.1, 0.03, and 0.01 percent 2(2,4,5-trichlorophenoxy) propionic acid (Silvex). The actual test material was in the form of the monohydrate sodium salt. A similar group of rats that were offered a diet containing 1% Silvex did not accept the ration satisfactorily and this portion of the experiment was terminated after 32 days.

Growth was retarded for the male rats at all levels except 0.01%, but for the females retardation occurred only at the 0.3% level.

Terminal hematogical values were determined on groups of 5 female rats each from the 0.3 and 0.1% levels and normal results were obtained.

Organ weight studies showed significant increases in the average weights of the livers and kidneys from the male rats that received dietary concentrations of 0.3, 0.1, or 0.03%. Kidney weights were increased also for the 0.01% male animals. For the female rats, the average weights of the liver were increased for all groups, including the 0.01% level, but kidney weights were elevated only for the female rats on the 0.3 or 0.1% diets.

Histopathologic examination revealed evidence of adverse effects, appearing predominantly in the liver and kidneys, from rats of both sexes and at all levels of dietary concentration, except that the kidneys from the female rats fed the lowest dietary concentration (0.01%) were not affected.

DOW79678

TABLE 1

AVERAGE HEMATOLOGICAL VALUES FROM FEMALE RATS THAT RECEIVED DIETS CONTAINING SILVEX FOR A PERIOD OF 90 DAYS

Determination	Average Value Per Group of 5 Rats		
	Control	0.3%	0.1%
Total Erythrocytes ($X10^6$)	8.93	8.99	8.57
Total Leucocytes ($X10^3$)	18.8	17.1	19.1
Hemoglobin (g./100 cc.)	13.2	13.1	13.0
Differential Count			
% Neutrophiles	21.8	13.8	27.4
% Lymphocytes	74.4	84.8	70.0
% Monocytes	1.2	0.8	0.8
% Eosinophiles	2.6	0.6	1.8

MOW 797679

TABLE 1

AVERAGE HEMATOLOGICAL VALUES FROM FEMALE RATS THAT RECEIVED DIETS CONTAINING SILVEX FOR A PERIOD OF 90 DAYS

Determination	Average Value Per Group of 5 Rats		
	Control	0.3%	0.1%
Total Erythrocytes ($X10^6$)	8.93	8.99	8.57
Total Leucocytes ($X10^3$)	18.8	17.1	19.1
Hemoglobin (g./100 cc.)	13.2	13.1	13.0
Differential Count			
% Neutrophiles	21.8	13.8	27.4
% Lymphocytes	74.4	84.8	70.0
% Monocytes	1.2	0.8	0.8
% Eosinophiles	2.6	0.6	1.8

MOW 497680

TABLE 2

AVERAGE FINAL BODY AND ORGAN WEIGHTS FROM MALE AND FEMALE RATS THAT RECEIVED DIETS CONTAINING SILVEX FOR A PERIOD OF 90 DAYS

Percent in Diet	No. of Rats	Sex	Body Wt. (g.)	Organ Weights (g./100 g. body wt.)					
				Lung	Heart	Liver	Kidney	Spleen	Testes
Control	6	M	359	0.50	0.31	2.45	0.62	0.24	0.88
0.3	6	M	201	0.69	0.38	5.98	0.89	0.32	1.32
0.1	9	M	263	0.61	0.37	5.38	0.78	0.22	1.02
0.03	9	M	310*	0.58	0.31	3.15	0.75	0.31	0.91
0.01	9	M	342	0.51	0.30	2.46	0.72**	0.26	0.87
Control	9	F	208	0.66	0.36	2.46	0.69	0.30	
0.3	7	F	152	0.78	0.43	5.72	0.81	0.30	
0.1	8	F	192	0.62	0.36	4.78	0.76*	0.28	
0.03	10	F	196	0.70	0.35	3.19	0.72	0.29	
0.01	10	F	209	0.71	0.36	2.70*	0.72	0.27	

* $p = 0.01$ to 0.05

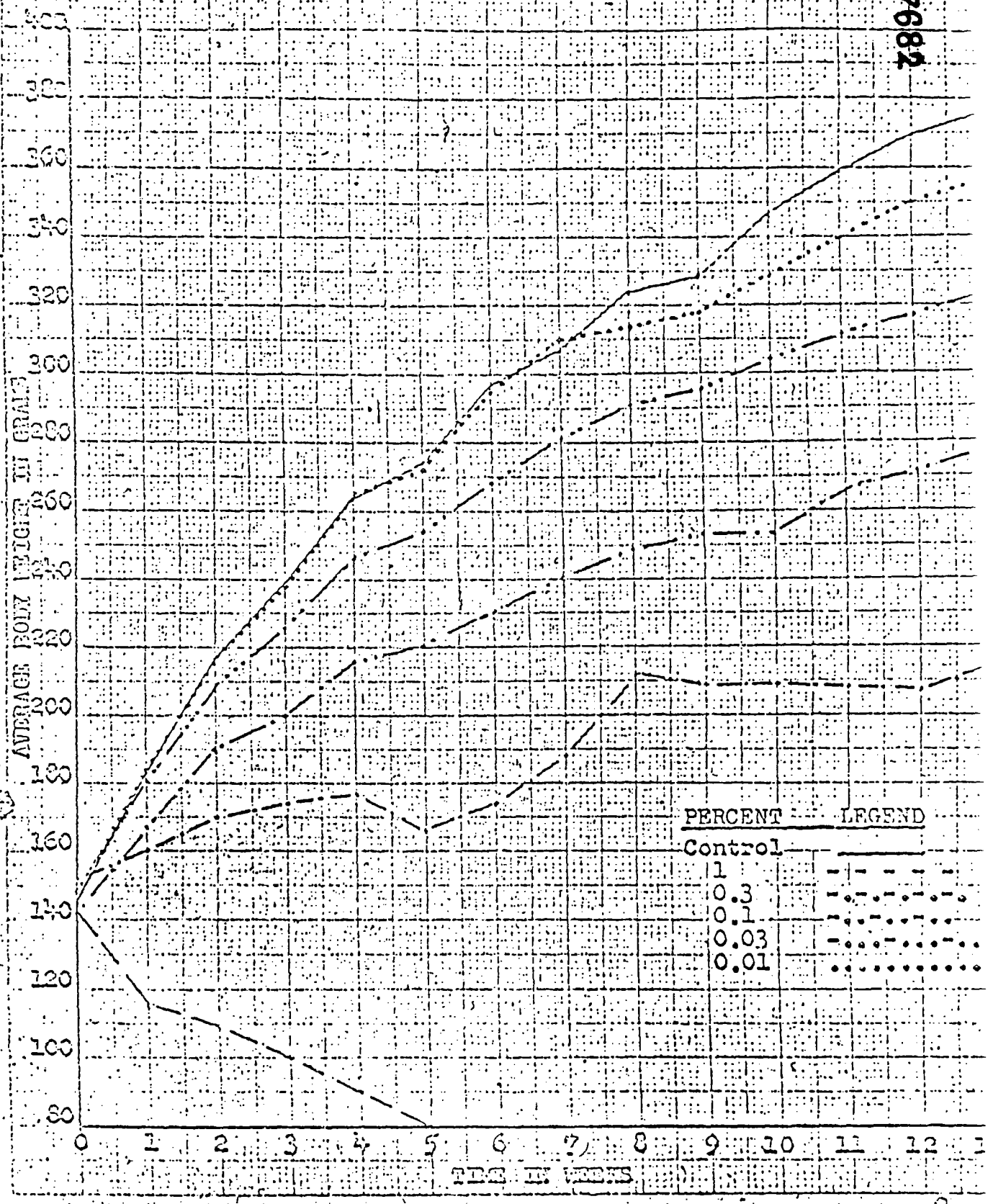
** $p = < 0.01$, as calculated by "t" test.

DOW 979681

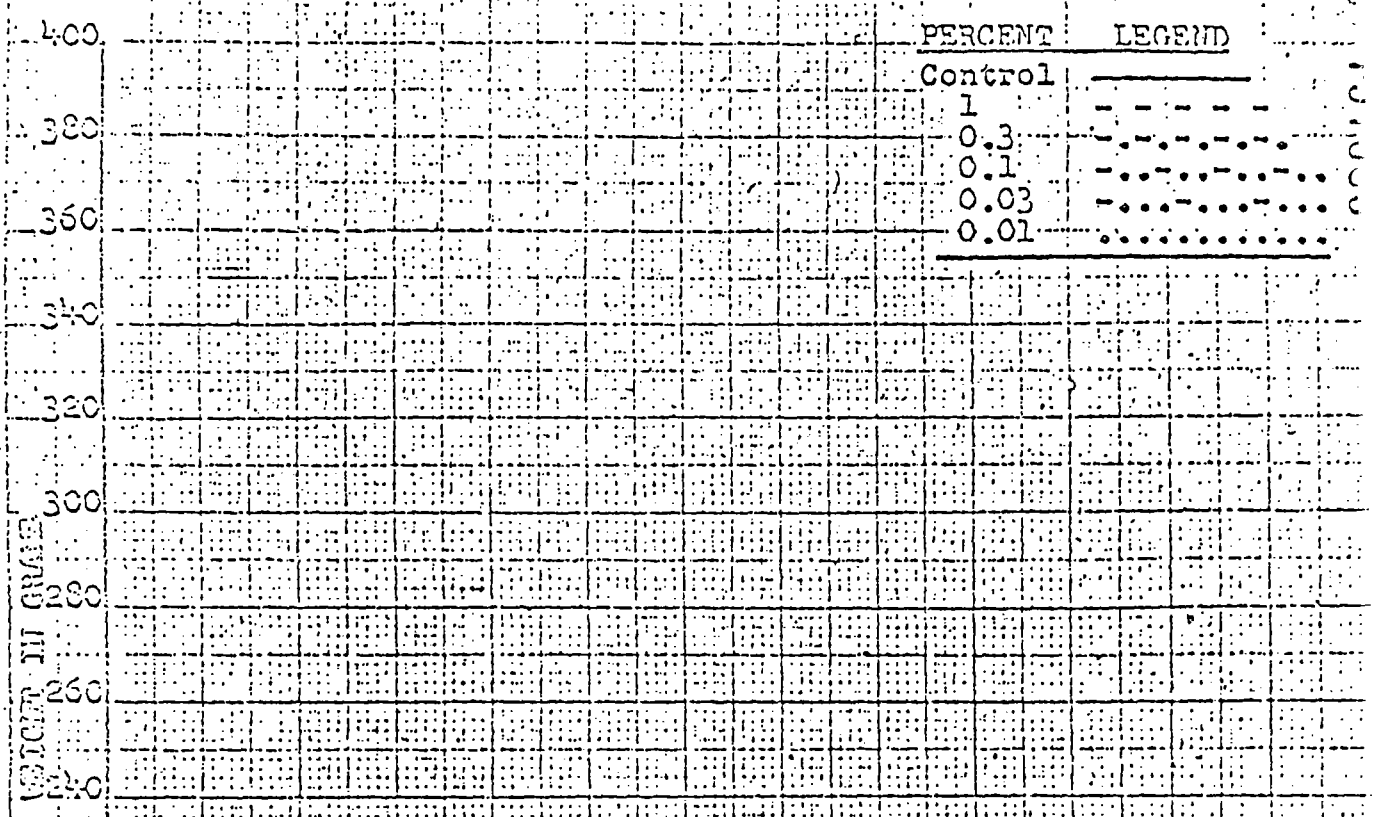
16377

DOW 797682

AVERAGE BODY WEIGHT CURVES FOR GROUPS OF MALE RATS THAT WERE MAINTAINED FOR 90 DAYS ON DIETS CONTAINING SILVEX, SODIUM SALT



AVERAGE BODY WEIGHT CURVES FOR GROUPS OF FEMALE RATS THAT WERE MAINTAINED FOR 90 DAYS ON DIETS CONTAINING SILVEX, SODIUM SALT



16379

DOW 1977684

2. Results of 90-Day Dietary Feeding of Propylene Glycol Isobutyl Ether Ester of Silvex (Dowco 171) to Rats. Biochemical Research Laboratory. The Dow Chemical Company, Midland, Michigan. June 19, 1962

2436

PBW

Toxicology, 9 (1978) 75—86
© Elsevier/North-Holland Scientific Publishers Ltd.

STUDIES ON 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-INDUCED IMMUNE SUPPRESSION AND DECREASED RESISTANCE TO INFECTION: ENDOTOXIN HYPERSENSITIVITY, SERUM ZINC CONCENTRATIONS AND EFFECT OF THYMOSIN TREATMENT

J.G. VOS, J.G. KREEFTENBERG, H.W.B. ENGEL, A. MINDERHOUD and L.M. VAN NOORLE JANSEN

National Institute of Public Health, P.O. Box 1, Bilthoven (The Netherlands)

(Received August 23rd, 1977)
(Accepted October 3rd, 1977)

DOW0686133
MN072283

SUMMARY

Sublethal doses of the highly toxic chemical 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) cause thymus atrophy in several species and induce suppression of cell-mediated immunity as measured by different parameters. The selective effect on thymus is not likely caused by a cytotoxic effect on lymphocytes or by an effect on pituitary or adrenals. In the present study, mice received daily injections with thymosin in order to study whether reduced production of thymic hormones could be involved in the atrophy. Also, serum zinc concentrations were measured. As thymosin injections did not increase thymus weight and mitogenic responsiveness of thymocytes, and zinc levels were not depressed, the mode of action of TCDD-induced thymus atrophy remains unknown. It has been reported that TCDD markedly decreased resistance of mice to infection with *Salmonella bern.* In this study, data are presented that indicate that the increased susceptibility is likely due to the endotoxin content of the bacteria: pre-treatment of mice with single or repeated doses of TCDD markedly enhanced their susceptibility to endotoxin even at dose levels that did not produce thymus atrophy. Finally, possible effects of TCDD on macrophage functions were studied. As treatment with TCDD did neither impair non-specific killing and phagocytosis of *Listeria monocytogenes*, nor macrophage reduction of nitro-blue tetrazolium, it seems likely that the immunosuppression is only due to a, to date unknown, effect on T-lymphocytes, and not due to a combined effect on both T-cells and macrophages, and that the endotoxin hypersensitivity is not the result of alteration in phagocytic function of macrophages.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a chemical that can be

16381

formed in small, but significant, amounts in the production of some chlorinated phenols or chemicals synthetized from chlorophenols, such as the herbicide 2,4,5,-trichlorophenoxyacetic acid. It is one of the most toxic chemicals known [1], it is a teratogen [2,35], it is associated with chick edema disease in chickens [4] and with several industrial incidents resulting in cases of chloracne in man [5,6]. TCDD exposure has been shown to induce thymus atrophy in rats, mice, guinea pigs [7—12] and monkeys [McConnell and Moore, in press]. Thymus atrophy appeared to be one of the most consistently sensitive parameters of TCDD exposure. Since lymphocytes derived from the thymus (T-cells) play a central role in cell-mediated immunity, several funciton studies of the thymus-dependent immunity have been carried out in mice, rats and guinea pigs. TCDD exposure did result in functional impairment of diffferent parameters of cellular immunity (delayed type hypersensitivity, allograft rejection, graft-versus-host activity and responsiveness of lymphocytes to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) [11,13, Moore and Faith, in press] but the suppression of the cell-mediated immunity seems to be an age-related phenomenon, at least in the mouse and the rat where TCDD exposure during the ontogenesis of the immune system seems to be pre-requisite in order to obtain immune suppression. TCDD mimics in this respect the effect of neonatal thymectomy.

Since the TCDD-induced atrophy of the thymus can be explained in many ways and no clear-cut mode of action has been described, we were interested to examine whether a reduced production of thymic hormones by thymus-epithelial cells could be involved. Therefore, in the present study, the effect of daily administration of thymosin on TCDD-induced thymus atrophy was studied. As shown by Thigpen et al. (14), TCDD exposure markedly decreased the resistance of mice to infection with *Salmonella bern*, whereas TCDD has no significant effect on mortality in pseudorabies virus-infected mice. In the present study, data are presented that indicate that the increased susceptibility to *Salmonella* infection is very likely due to the endotoxin content of the bacteria. Since on the one hand zinc deficiency can result in severe atrophy of lymphoid organs [15,16] and on the other hand parenteral administration of zinc increases the resistance of mice to endotoxin [17], serum zinc concentrations were also measured in TCDD-exposed mice. Finally as macrophages play an important role in the immune response and in the detoxification of endotoxin, function studies of macrophages (non-specific phagocytosis and killing of *Listeria monocytogenes* and reduction of nitro-blue tetrazolium by macrophages) were also included.

MATERIALS AND METHODS

TCDD

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (purity 98.6%, Lot No. 851:144-II,

DOW0686134

16382

kindly provided by Dow Chemical Company, Midland, Mich., U.S.A.) was dissolved in reagent grade acetone at a concentration of 50 μg/ml. For the animal experiments, this stock solution was diluted with at least 9 parts of arachis oil to provide the different concentrations. Mice were intubated orally with 0, 1.5, 5, 15, 50 or 100 μg TCDD/kg body weight.

Animals

Specific pathogen-free outbred Swiss mice were reared at the Institute. They received food and water ad libitum. Because of the high toxicity of TCDD, all animal experiments were carried out under non-sterile conditions in plastic Trexler isolators, and all contaminated material was incinerated at temperatures over 800° C (N.V. Roteb, Rotterdam, The Netherlands).

Zinc analysis

For the determination of zinc in serum, an atomic absorption spectrometer with acetylene-air slot burner and zinc hollow-cathode lamp was used. Fifty μl of serum was diluted with 500 μl of distilled water. After mixing thoroughly 100 μl of the sample was aspirated into the flame and the resulting atomic absorption signal was recorded. Peak heights were compared with results obtained from the aspiration of aqueous standard solutions under the same conditions and zinc concentrations were calculated.

Experiment with thymosin

The effect of thymosin injections on TCDD-induced thymus atrophy was studied in mouse pups that were exposed to TCDD by postnatal maternal treatment. At birth the number of neonates was standardized to 6. Groups of 5 nursing mice received 0 or 10 μg TCDD/kg body weight (in a volume of 0.1 ml/30 g), postnatally on days 1, 4, 8, 11, 15 and 18. Calf thymosin (fraction 5, Lot No. BPM 390, kindly provided by Dr. A.L. Goldstein, Galveston, Tex., U.S.A.) was dissolved in sterile saline at a stock concentration of 15 mg/ml, passed via Millipore bacteria filter and aliquots stored at −20° C. Just prior to injection, a dilution (1 mg/ml) was made in sterile phosphate-buffered saline (PBS). In the first week, groups of 2 pups per litter received daily subcutaneous injections of a high (50 μg) or low (15 μg) dose of thymosin. In addition, 3 pups per control litter received PBS. In the second and third week, the doses were doubled and tripled respectively. The endotoxin content of the thymosin (1 mg/ml) solution was estimated with the Limulus assay [18] and contained between 12.5 and 62.5 ng endotoxin/ml. After the 22-day experimental period, the animals were killed. Zinc concentration was measured in serum.

The thymus of half of the number of mice was removed under sterile conditions. Cell suspensions were made by using a glass homogenizer, the viability was determined with a dye exclusion test and the final suspension

DOW0686135

16383

was adjusted to a concentration of 3×10^6 viable nucleated cells/ml in minimum essential medium (MEM, Gibco F14) buffered with Tris (0.025 M) and supplemented with 20% inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 IU/ml). A total volume of 1 ml was cultured in polystyrene tubes (Nunc, Denmark) and 12.5 μl reconstituted phytohemagglutinin (PHA, Wellcome Labs., Beckenham, Kent, U.K.), 6 μg concanavalin A (Con A, Pharmacia, Uppsala, Sweden) or 50 μl reconstituted pokeweed (PWM, Gibco) were added. After 24 h cultivation, 10 μCi of [6-^{3}H]thymidine (specific activity 600 mCi/mmole) was added. Twenty-four hours later the cells were harvested on glass fiber filters using a multiple cell culture harvester (Skatron, Lierbyen, Norway). After drying, the filters were placed into scintillation vials and 2 ml of scintillation fluid were added. Counting was done in a Packard liquid scintillation counter. Results (median value of triplicate cultures) were expressed as the difference between stimulated and non-stimulated cultures (in counts/min (cpm) per 3×10^6 cells).

Experiments with endotoxin

In 3 experiments, the effect of TCDD on the susceptibility of mice to endotoxin was studied. Since there is no major difference in the biological activity of endotoxins of *Salmonella* and *E. coli* species, *E. coli* endotoxin was used in the present study. Endotoxin (lipopolysaccharide, *E. coli*, O 127: B 8, Difco Labs, Detroit, Mich.) was dissolved in sterile saline. In two studies, 3—4 week-old male Swiss mice were intubated orally, once a week for 4 weeks, with 0, 1.5, 5, 15 or 50 μg TCDD/kg (0.1 ml/10 g body weight). Two days after administration of the final dose of TCDD, the mice were injected intravenously with 10, 20, 100, 250 or 500 μg endotoxin in 0.1 ml. The mortality due to endotoxin shock was scored after 48 h. Surviving animals were killed and examined macroscopically.

In the third experiment, 3—4 week-old female mice received a single oral dose of 0 or 100 μg TCDD/kg body weight (0.2 ml/10 g). Animals were challenged with endotoxin 5 days after intubation. In the second and third experiment, additional animals were intubated and killed to determine the effect of TCDD on body weight and on the weight of thymus and spleen. Also, serum zinc concentrations were measured in the third experiment.

Clearance of Listeria monocytogenes

The resistance of *Listeria monocytogenes* is a combination of non-specific phagocytosis and cell-mediated immunity; non-specific phagocytosis and killing can be measured shortly (within 2 days) after the intravenous inoculation of *Listeria monocytogenes* [19]. Male Swiss mice (3—4 weeks old) were intubated orally, once a week for 4 weeks, with 0 or 50 μg TCDD/kg body weight. Four days after the last intubation the animals were inoculated intravenously with 2.4×10^3 *Listeria monocytogenes* organisms (Strain L 242/73 type 4b). One or two days after the injection, the spleens were weighed,

D0W0686136

16384

homogenized and serial dilutions were plated to determine the viable counts of *Listeria* [20]. The number of bacteria was expressed as ^{10}log/spleen.

Reduction of nitro-blue tetrazolium

Lymphokine activated macrophages show increased glucose oxidation via the hexose-monophosphate pathway; an increase that can be quantitated by the reduction of soluble nitro-blue tetrazolium (NBT) to insoluble formazan. Krueger et al. [21] demonstrated a correlation between delayed type hypersensitivity skin tests and NBT reduction by lymphokine-activated macrophages. Similarly Engel and Sekhuis (unpublished data) showed an increase in NBT reduction in macrophages of mice that were injected with *Corynebacterium parvum*, without the addition of lymphokines to the culture chamber. In the present experiment, the effect of TCDD on NBT reduction of macrophages was determined in order to study an effect on the baseline activity of non-activated macrophages. For that purpose, 3—4-week-old male Swiss mice were intubated orally, once a week for 4 or 5 weeks with 0 or 50 μg TCDD/kg body weight. Five days after the last intubation, peritoneal macrophages were harvested in 2 washings with 2.5 ml Eagle's Basal Medium (Gibco, G 13) supplemented with 20% inactivated calf serum, 40 mM L-glutamine and 5 U heparin/ml. Of this suspension 0.5 ml was added to an 8-chambered tissue culture slide and incubated at 37° C in 10% CO_2 in air for 2 h. At this time chambers were washed 3 times to remove non-adherent cells. Of each mouse, one chamber was used for the identification of macrophages (Giemsa stain); the other was used for the NBT assay according the technique described by Krueger et al. [21]. One hundred cells were individually scored at 1000 × on the quantity of formazan precipitate as a measure of NBT reduction (no precipitate = 0; slight precipitate = 1; moderate precipitate = 2; strong precipitate = 3). Results were expressed as the sum of the scores per 100 cells.

Statistical analysis

Student's *t* test was used to make one-sided treatment—control comparisons.

RESULTS

The effect of daily thymosin injections on body and thymus weights of 22-day-old mice is given in Table I. Body and thymus weights of the 3 TCDD groups were significantly lower when compared with the control group. Treatment of TCDD-exposed pups with thymosin significantly reduced body weight, but did not alter thymus weight. Serum zinc concentrations were the same in control and TCDD-exposed pups. The responsiveness of thymus cells to the mitogens PHA, Con A and PWM is presented in Table II. In general, the response of the TCDD groups to these mitogens was approximately half

DOW0686137

16385

TABLE I

BODY AND THYMUS WEIGHTS[a] AND SERUM ZINC CONCENTRATIONS OF 22-DAY-OLD TCDD-TREATED MICE THAT RECEIVED DAILY INJECTIONS WITH THYMOSIN[a]

Dose of TCDD (μg/kg)	Thymosin treatment	Body weight (g)	Thymus weight (mg)	Zinc (mg/l)
0	0	13.1 ± 0.9	85 ± 5	1.1 ± 0.2
10	0	12.4 ± 0.8[b]	60 ± 10[c]	1.2 ± 0.6
10	low doses[e]	11.2 ± 1.0[c,d]	60 ± 10[c]	
10	high doses[e]	11.2 ± 1.2[c,d]	58 ± 7[c]	

[a] Mean values ± S.D. (body weight 8—10 animals, thymus weight and zinc analyses of 4—5 animals). Mice were exposed to 0 or 10 μg TCDD/kg by maternal treatment postnatally on days 1, 4, 8, 11, 15 and 18.
[b] Significantly ($P < 0.05$) different from control group (no TCDD and no thymosin).
[c] Significantly ($P < 0.001$) different from control group (no TCDD and no thymosin).
[d] Significantly ($P < 0.025$) different from thymosin control group (10 μg TCDD and no thymosin).
[e] For schedule see text.

DOW0686138

of the response seen in the control group. Since the results are expressed on a cell-for-cell basis and thymus weight was reduced by TCDD treatment, the total response per thymus is likely even more decreased. Thymosin treatment of TCDD-exposed mice did not result in a significant increase in the mitogenic responsiveness of the thymus cells.

The results of the first two experiments with endotoxin are given in Table III. Pretreatment of mice with TCDD markedly enhanced their susceptibility

TABLE II

MITOGENIC RESPONSE OF THYMUS CELLS FROM 22-DAY-OLD TCDD-TREATED MICE THAT RECEIVED DAILY INJECTION WITH THYMOSIN[a]

Dose of TCDD (μg/kg)	Thymosin treatment	Δ PHA (cpm)	Δ Con A (cpm)	Δ PWM (cpm)
0	0	19,001 ± 10,774	20,895 ± 6,517	6,494 ± 2,647
10	0	8,097 ± 2,731[b]	10,779 ± 6,345[b]	2,108 ± 1,879[b]
10	low doses[c]	9,985 ± 8,248	14,616 ± 4,989	3,714 ± 1,485
10	high doses[c]	11,878 ± 4,677	11,244 ± 2,595[b]	2,542 ± 1,135[b]

[a] Mean values ± S.D., 4—5 animals per group. Mice were exposed to 0 or 10 μg TCDD/kg by maternal treatment postnatally on days 1, 4, 8, 11, 15 and 18. Results are expressed as the difference between stimulated and non-stimulated cultures (in cpm/3 × 10^4 cells).
[b] Significantly ($P < 0.05$) different from control group (no TCDD and no thymosin).
[c] For schedule see text.

16388

TABLE III

EFFECT OF 4 WEEKLY DOSES OF TCDD ON THE SUSCEPTIBILITY OF MICE TO ENDOTOXIN

Dose of TCDD (μg/kg)[a]	Incidence of mortality[b]		
	10 μg endotoxin	250 μg endotoxin	
0	0/4	0/5	
1.5	0/4	1/5	
5	0/4	6/6	
15	1/4	6/6	
50	2/4	ND[c]	
	10 μg endotoxin	100 μg endotoxin	500 μg endotoxin
0	0/6	0/6	2/6
1.5	0/6	1/6	3/6
50	2/5	5/6	6/6

[a] In two experiments, male mice (3—4 weeks of age) were intubated orally, once a week for 4 weeks, with TCDD in acetone-arachis oil carrier.
[b] Intravenous injection with endotoxin (E. coli. LPS) two days after last intubation. Mortality scored after 48 h.
[c] ND = not done.

to endotoxin. After the injection of 10 μg endotoxin, 2 out of 4 animals died in the 50 μg TCDD group and 1 in the 15 μg TCDD group. Injection of 250 μg endotoxin was lethal for all mice in the 5 and 15 μg TCDD groups and caused one death in the 1.5 μg TCDD group, whereas all controls surviv-

TABLE IV

EFFECT OF 4 WEEKLY DOSES OF TCDD ON BODY AND ORGAN WEIGHTS OF MICE[a]

Dose of TCDD (μg/kg)	Body weight (g)	Thymus weight (mg)	Spleen weight (mg)
0	30.7 ± 2.2	66 ± 20	106 ± 13
1.5	28.7 ± 2.5	65 ± 12	97 ± 23
50	26.0 ± 2.3[c]	34 ± 10[c]	89 ± 8[b]

[a] Mean values ± S.D., 6 animals per group. Male mice, 3—4 weeks of age, were intubated orally, once a week for 4 weeks, with TCDD in acetone-arachis oil carrier.
[b] $P < 0.025$.
[c] $P < 0.005$.

DOW0686139

16387

TABLE V

EFFECT OF A SINGLE ORAL DOSE OF TCDD ON BODY AND ORGAN WEIGHTS AND ON SERUM ZINC CONCENTRATION OF MICE[a], AND ON THE SUSCEPTIBILITY TO ENDOTOXIN

Dose of TCDD (μg/kg)	Body weight[a] (g)	Thymus weight[a] (mg)	Spleen weight[a] (mg)	Zinc[a] (mg/l)
0	19.3 ± 1.8	70 ± 15	120 ± 23	1.0 ± 0.2
100	18.7 ± 2.4	51 ± 14[b]	97 ± 19[b]	1.4 ± 0.5
	Incidence of mortality[c]			
	20 μg endotoxin	100 μg endotoxin	500 μg endotoxin	
0	ND[d]	0/5	5/5	
100	5/5	5/5	ND	

[a] Mean value ± S.D., 7 animals per group. Female mice, 3—4 weeks of age, were intubated once orally with TCDD in acetone-arachis oil carrier and killed after 5 days.
[b] $P < 0.05$.
[c] Intravenous injection with endotoxin (E. coli, LPS) 5 days after intubation. Mortality scored after 48 h.
[d] ND = not done.

ed. In the second experiment, animals were given 0, 1.5 or 50 μg TCDD/kg body weight and were challenged with 10, 100 or 500 μg endotoxin. The 10 μg dose of endotoxin caused 2 deaths out of 5 animals in the 50 μg TCDD group, and a similar mortality (2 out of 6) was scored in the controls injected with 500 μg endotoxin. Thus, the susceptibility to endotoxin was approximately 50-fold enhanced in the 50 μg TCDD group when compared with the controls. Even at the 1.5 μg TCDD level there was a slightly increased mortality after injection of endotoxin. This latter dose of TCDD being more than one order of magnitude lower than the dose that caused thymus atrophy at gross inspection. As shown in Table IV, body weights and weights of thymus and spleen of mice of the 50 μg TCDD group were significantly decreased, whereas these parameters in the group intubated with 1.5 μg TCDD/kg body weight did not differ from the controls. A single oral dose of 100 μg TCDD/kg body weight significantly reduced thymus and spleen weight of female mice, killed after 5 days; but body weights and serum zinc levels were not altered (Table V). Also, this treatment enhanced the susceptibility to endotoxin: a 100% mortality occurred in the TCDD-exposed animals after injection with 20 μg LPS, whereas a similar mortality was seen in controls receiving 500 μg LPS.

The results of the study of the effect of TCDD on non-specific phagocytosis and killing of *L. monocytogenes* are given in Table VI. The number of viable *Listeria* organisms, determined one or two days after inoculation of this

DOW0686140

16388

TABLE VI

EFFECT OF 4 WEEKLY DOSES OF TCDD IN MICE ON BODY AND SPLEEN WEIGHTS AND ON DAY 1 AND DAY 2 SPLEEN COUNTS OF LISTERIA MONOCYTOGENES[a]

Dose of TCDD (μg/kg)	Body weight (g)	Spleen weight (mg)	Listeria count[b] (^{10}log/spleen)
Day 1 p.i.			
0	23.4 ± 1.4	140 ± 10	4.89 ± 0.40
50	21.7 ± 0.6[c]	96 ± 18[d]	4.58 ± 0.24
Day 2 p.i.			
0	21.7 ± 1.5	178 ± 23	5.13 ± 0.31
50	21.2 ± 2.4	124 ± 29[d]	5.08 ± 0.27

[a] Mean values ± S.D., 5 animals per group. Male mice,3—4 weeks of age, were intubated orally, once a week for 4 weeks with TCDD in acetone-arachis oil carrier.

[b] Animals were inoculated intravenously with 2.4×10^3 Listeria monocytogenes organisms 4 days after the last intubation; the number of viable Listeria organisms per spleen was determined after 1 and 2 days.

[c] $P < 0.05$.

[d] $P < 0.01$.

organism, was the same in spleens from treated and control mice. Spleen weights were significantly lower in TCDD-exposed animals. In addition, body weights of mice killed one day after inoculation were significantly reduced in the TCDD group. Finally, as shown in Table VII, in two experiments TCDD did not alter the number of peritoneal macrophages, nor did it cause a significant effect on the macrophage reduction of NBT.

TABLE VII

EFFECT OF 4 OR 5 WEEKLY DOSES OF TCDD IN MICE ON NUMBER OF PERITONEAL MACROPHAGES AND ON MACROPHAGE REDUCTION OF NBT[a]

Dose of TCDD (μg/kg)	No. of peritoneal macrophages (× 10^4)	Reduction of NBT[b]
Four doses		
0	1.94 ± 0.68	32.2 ± 22.2
50	2.23 ± 0.35	37.0 ± 8.9
Five doses		
0	1.81 ± 0.54	23.8 ± 17.1
50	2.08 ± 0.31	57.0 ± 14.0

[a] Mean values ± S.D., 4—5 animals per group. Male mice, 3—4 weeks of age, were intubated orally, once a week for 4 or 5 weeks with TCDD in acetone oil carrier.

[b] For details see text.

DOW0686141

16389

DISCUSSION

From the present results it can be concluded that thymosin treatment of mice at the employed doses does neither protect the animals from thymus atrophy nor from a reduced mitogenic responsiveness of thymocytes. The presence of a minor quantity of endotoxin in the thymosin solution could not conceal a possible increase, since treatment of mice with *E. coli* LPS has been shown to increase the PHA and Con A responsiveness of thymocytes [22]. Thus, it seems unlikely that the depletion of lymphocytes in the thymus, as induced by TCDD, is caused by a reduced production of thymic hormones, since calf thymosin has been shown in several studies to potentiate various parameters of thymus-dependent immunity [23]. Neither seems zinc deficiency to be a cause for the thymus atrophy. A direct cytotoxic action of TCDD on lymphocytes is also unlikely, since the mitogen responsiveness of mouse or rat lymphocytes was not reduced when cultured in the presence of TCDD at concentrations up to 0.02 μg/ml medium [13]. Even the presence of 0.32 μg TCDD/ml medium has been reported to be not toxic for human lymphocytes [24], whereas concentrations that are two orders of magnitude lower do induce aryl hydrocarbon hydroxylase activity in human lymphocytes [25]. In another study [26] a cytotoxic action of TCDD at a concentration of 0.48 μg/ml was reported for several mammalian cell types. These results make it unlikely that TCDD does need metabolic activation, but they do indicate that the lymphocyte depletion of the thymus and the thymus-dependent lymphoid tissue are not caused by a direct effect on lymphocytes, but rather by an indirect way. There are several conditions that can cause thymus atrophy [27]. Regarding the selective effect of TCDD on thymus, it is not likely caused by an effect on pituitary, on adrenals or by reduced food intake, since thymus atrophy did occur in TCDD-exposed rats that were hypophysectomized or adrenalectomized and in rats that were placed on a restricted diet [Van Logten, Gupta and Moore, in preparation] though it is still possible that TCDD may impair the absorption and transport of certain nutrients. In addition, serum α-fetoprotein levels are not elevated in TCDD-exposed rats [Vos and Boekestein, unpublished data], thus the observed immunosuppression is not due to a α-fetoprotein induction. In conclusion, the mode of action of the TCDD-induced thymus atrophy remains unknown.

The results of the present experiments with endotoxin show a marked increase in susceptibility to endotoxin of mice treated with a single or with repeated doses of TCDD. This sensitizing effect of TCDD for endotoxin offers a logical explanation for the decreased resistance of TCDD-exposed mice to infection with (endotoxin-containing) *Salmonella* bacteria as observed by Thigpen et al. [14]. Another explanation for the increased susceptibility to this type of infection, which is primarily dependent upon cell-mediated immunity [28] is by general suppression of the thymus-dependent immunity. This explanation is, however, less likely since increased mortality by *Salmonella* occurred at dose levels of TCDD that did not produce thymus

16390

atrophy. The endotoxin hypersensitivity, which has to date only been studied in mice, is a phenomenon of particular interest when considered in connection with: (1) terminal hemorrhages that were seen in TCDD-exposed rats and guinea pigs [8,29] and mice [12,30], (2) thrombocytopenia, observed in rats and guinea pigs [10,31], and (3) presence of thrombi in blood vessels of heart and lung in the rat after a lethal dose of TCDD [7,8]. These lesions are also seen in endotoxin shock and since TCDD, at least in the mouse, also induces endotoxin hypersensitivity, one has to consider endotoxin shock as a possible cause of death. Regarding the mechanism of endotoxin hypersensitivity, several possibilities exist (see review of Cook et al. [32]). With regard to the endotoxin hypersensitivity produced by TCDD it would be worthwhile to investigate the role of the enzyme glutathione-S-transferase B, a cytosolic glutathione-conjugating enzyme, since sulfhydryl containing enzymes are necessary for endotoxin inactivation. TCDD has been reported to increase the activity of both hepatic and renal glutathione-S-transferase B [33].

Finally, as treatment of mice with TCDD did neither impair the non-specific killing and phagocytosis of *L. monocytogenes* nor the macrophage reduction of NBT, it seems likely that the TCDD-induced suppression of the cell-mediated immunity is only due to a, to date unknown, effect on T-lymphocytes and not due to a combined effect on both T-cells and macrophages. Similarly, the results of the experiment with *L. monocytogenes* indicate that the endotoxin hypersensitivity is not the result of alteration in phagocytic function of macrophages but, as discussed in the previous paragraph, perhaps due to impaired endotoxin detoxifying properties of macrophages.

DOW0686143

REFERENCES

1 B.A. Schwetz, J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson and C.G. Gerbig, Environ. Hlth Perspect., 5 (1973) 87.
2 K.D. Courtney and J.A. Moore, Toxicol. Appl. Pharmacol., 20 (1971) 396.
3 D. Neubert, P. Zens, A. Rothenwallner and H.J. Merker, Environ. Hlth Perspect., 5 (1973) 87.
4 D. Firestone, Environ. Hlth Perspect., 5 (1973) 59.
5 K.D. Crow, Trans. St. John's Dermatol. Soc., 56 (1970) 79.
6 J. Kimmig and K.H. Schulz, Dermatologica, 115 (1957) 540.
7 N.P. Buu-Hoi, P.H. Chanh, G. Sesqué, M.C. Azum-Gelade and G. Saint-Ruf, Naturwiss., 4 (1972) 174.
8 B.N. Gupta, J.G. Vos, J.A. Moore. JG. Zinkl and B V. Bullock, Environ. Hlth Perspect., 5 (1973) 125.
9 M.W. Harris, J.A. Moore, J.G. Vos and B.N. Gupta, Environ. Hlth Perspect., 5 (1973) 101.
10 R.J. Kociba, P.A. Keeler, C.N. Park and P.J. Gehring, Toxicol. Appl. Pharmacol., 35 (1976) 553.
11 J.G. Vos, J.A. Moore and J.G. Zinkl, Environ. Hlth Perspect., 5 (1973) 149.
12 J.G. Vos, J.A. Moore and J.G. Zinkl, Toxicol. Appl. Pharmacol., 29 (1975) 229.
13 J.G. Vos and J.A. Moore, Int. Arch. Allergy Appl. Immunol., 47 (1974) 777.

16391

14 J.E. Thigpen, R.E. Faith, E.E. McConnell and J.A. Moore, Infect. Immun., 12 (1975) 1319.
15 W. Groth, Zbl. Vet. Med. A, 23 (1976) 31.
16 B.L. O'Dell, P.M. Newberne and J.E. Savage, J. Nutr., 65 (1958) 505.
17 S.L. Snyder and R.I. Walker, Infect. Immun., 13 (1976) 998.
18 J.G. Kreeftenberg, H.G. Loggen, J.D. van Ramshorst and E.C. Beuvery, Develop. Biol. Stand., 34 (1977) 15.
19 S.P. Tripathy and G.B. Mackaness, J. Exp. Med., 130 (1969) 1.
20 E.J. Ruitenberg and L.M. van Noorle Jansen, Zbl. Bakt. Hyg., I. Abt. Orig. A, 231 (1975) 197.
21 G.G. Krueger, B.E. Ogden and W.L. Weston, Clin. Exp. Immunol., 23 (1976) 517.
22 L. Adorini, L. Ruco, S. Uccini, G. Soravito de Franceschi, C.D. Baroni and G. Doria, Immunology, 31 (1975) 225.
23 A. White, Thymus hormones: a new field in endocrinology and its significance for host immunological competence. In: D.W. van Bekkum (Ed.), Activity of Thymic Hormones, Kooyker Scientific Publications Rotterdam, 1975, p. 17.
24 P.W. Beatty, K.J. Lembach, M.A. Holscher and R.A. Neal, Toxicol. Appl. Pharmacol., 31 (1975) 309.
25 R.E. Kouri, H. Ratrie, S.A. Atlas, A. Niwa and D.W. Nebert, Life Sci., 15 (1374) 1585.
26 A. Niwa, K. Kumaki and D.W. Nebert, Mol. Pharmacol., 11 (1975) 399.
27 J.G. Vos, Crit. Rev. Toxicol., 5 (1977) 67.
28 F.M. Collins and G.B. Mackaness, J. Immunol., 101 (1968) 830.
29 K.S. Khera and J.A. Ruddick, Adv. Chem. Ser., 120 (1973) 540.
30 G. Jones and J.B. Greig, Experientia, 31 (1975) 1315.
31 J.G. Zinkl, J.G. Vos, J.A. Moore and B.N. Gupta, Environ. Hlth Perspect., 5 (1973) 111.
32 J.A. Cook, N.R. DiLuzio and E.O. Hoffman, Crit. Rev. Toxicol., 3 (1975) 201.
33 R. Kirsch, G. Fleischner, K. Kamisaka and I.M. Arias, J. Clin. Invest., 55 (1975) 1009.

D0W0686144

16392

2433

Lennart Hardell*: Malignant mesenchymal tumors and exposure to phenoxy acids - A clinical observation [Maligna mesenkymala tumorer och exposition for fenoxisyror - en klinisk observation].
Lakartidningen, 74, (33), pp. 2753-2754, 1977.

MN053968

DOW063154S

*Dept. of Oncology, Regional Hospital, Umeå.

DOW AGRICULTURAL PRODUCTS DEPARTMENT

INDEX
M. J. Traynor - 2030
B. B. Holder - 2030
H. C. Scharnweber - 2030
R. J. Kociba - 1803
B. A. Schwetz - 1803
P. J. Gehring - 1803
V. K. Rowe - 1803
J. G. Gleeson - 2030
V. B. Robinson - IN
H. C. Spencer - AZ
M. L. Leng
D. D. McCollister

FROM: John H. Davidson 11/23/77

Translated from Swedish by the Ralph McElroy Co., Custom Division, 2102 Rio Grande, Austin, Texas 78705 USA

16393

Code: 101-8715

MN0539968

D0W0631546

MALIGNANT MESENCHYMAL TUMORS AND EXPOSURE TO PHENOXY ACIDS - A CLINICAL OBSERVATION

Seven cases of malignant mesenchymal tumours observed at the dept of oncology in Umeå during the years 1970-76 and with exposure to phenoxy acids over a period of 10-20 years are described. The exposure was direct and comparatively massive in five of the cases. The latent period is in agreement with that assumed for chemical carcinogenesis. The distribution between the sexes in this material, of 87 patients in all, deviates from the national average, with a dominance of males. No definite conclusions concerning a possible causal connection can be drawn from these cases; this would call for more extensive investigations.

The possibility that phenoxy acids (for example, hormoslyr) and the contaminants that occur in them may have harmful effects in humans and animals has been the object of an intensive debate. No cases of carcenogenesis in man have been reported so far.

In this article we shall describe seven patients suffering from malignant mesenchymal tumors observed at the oncological clinic in Umeå during the years 1970-76. In these patients the exposure to phenoxy acids had occurred 10-20 years before the diagnosis of the tumors. These cases do not, naturally, prove any possible connection between such an exposure and the malignant tumors; to prove or disprove such a connection extensive epidemiological studies are required. However, we believe that adequate evidence is available to report the observations in a casual form.

During the years 1970-76, the oncological clinic in Umeå received (and checked) 87 patients who had malignant mesenchymal tumors. 32 of these were women (37%) and 55 were men (63%). 43 of the men have been employed; occupation is missing, however, in one case. Nine were forestry workers (20.9%), four worked in farming and forestry (9.3%) and six in saw mills or the pulp industry (14.0%).

16394

Cases

Case 1. A 62 year old man, forest worker until retirement in 1971; presenting symptoms, diarrhea and pains on the left side of the stomach. Smoker. Treated for tuberculosis in 1959. Otherwise, previously basically healthy. In this work he had sprayed the phenoxy acid 2,4,5-T roughly one week in 1963 and 1964, two weeks in 1965, one month in 1966, one month in 1967, and two weeks in 1968. Since August 1976 increased pain in the right fossa iliaca and also swelling in the right leg. Laparotomy revealed a tumor in the region of the small of the back, attached to the wall of the hip bone. DIAGNOSIS: Rather highly differentiated leiomyosarcoma.

Case 2. A 57 year old forest worker, nonsmoker. High blood pressure since 1960. Treated for postinfectious arthritis in 1974. In the 1950's and 1960's, for three or four summers each, and for each summer for three weeks he had worked using a mixture of 2,4-D and 2,4,5-T for spotting and to a lesser extent for back spraying. Since August 1976 ventral tumor growth, on the proximal portion of the right thigh. DIAGNOSIS: Mesenchymal tumor, probably relatively highly differentiated fibroid liposarcoma.

Case 3. A 49 year old man, workshop owner. For many years gastric symptoms, but otherwise healthy. On his workshop premises, from 1961 to 1972, he had stored thousands of liters of phenoxy acids for a forestry company during the nonspraying season, about 11 months per year. The acid was stored partially in open containers. The patient had come in contact with the substance, which emitted a constant odor in the shop. In August 1976 melena and stomach pains. In November again acute stomach symptoms which led to laparotomy. This revealed a tumor in the region of the small of the back and involvement of the rectum, urinary bladder and overgrowth to the small intestines. DIAGNOSIS: Mesenchymal tumor, more specifically, neurosarcofibroma.

DOW0631547

Case 4. A 60 year old forest worker, moderate smoker. In 1938 appendectomy, but otherwise healthy. During the years 1961-66 for 20 workdays per year he had sprayed with phenoxy acids. In August 1971 saw a doctor for defecation trouble. Palpation of the rectum and sphincter muscle revealed submucous tumor growth. DIAGNOSIS: Sarcoma recti (leiomyosarcoma).

Case 5. A 44 year old man, who had previously been in perfect health. During the years 1945-46 for 3 weeks each year he had been exposed to herbicides while doing farm work in Denmark. Employed in the oil-industry in 1954-68. During the summers of 1960-68 for about two weeks each year he had "rather carelessly" handled phenoxy acids both with a sprayer and for spotting. Visited a doctor in 1974 with complaint of increasing peripheral resistance of palm and right underarm. DIAGNOSIS: Rhabdomyosarcoma.

Case 6. A 76 year old man, who had been healthy until 1972, when he was operated on for a perforated ulcus ventriculi. Reported some exposure to phenoxy acids for 4-5 summers in the 50's, at which time he had cut and collected grass along roadsides, which had been sprayed by the highway department. The patient also reported that he had worked in forests which had been sprayed with phenoxy acids. Visited a doctor for trouble with numbness and pain in right arm. The palpation showed a 1 decimeter long tumor on the inside of the right upper arm. DIAGNOSIS: Myxofibrosarcoma with suspected lipoblastic differentiation.

Case 7. A 67 year old forest worker with three earlier cases of pneumonia and gastric anamnesis. The patient had worked in the forests until 1970. According to his own account, had done cutting and clearing from 1956 to the beginning of the 1960's within a phenoxy-sprayed area. Indicated work in spraying for a few years after this time. Since June 1969 he had noticed a peripheral

DOW0631548

resistance in the left underarm. Visited a doctor in March 1970. DIAGNOSIS: Polymorphocellular sarcoma, possibly a rhabdomyosarcoma.

In addition to these seven patients, among whom the exposure to the phenoxy acids had been direct, at least in the first five cases, there are also a couple of patients - both forestry workers - whose places of work in the 1950's and 1960's have been occasionally located in proximity to sprayed areas. In one case, harvesting berries after spraying was also reported. DIAGNOSIS: Rhabdomyosarcoms and neurofibrosarcoma, respectively.

DOW0631546

DISCUSSION

The latent period for chemical induction of malignant, solid tumors in man is generally assumed to be very long, on the average ranging from 15 to 30 years. The latent period is possibly dependent on dose (Heuper, Konway 1964). In the reported cases the exposure occurred 10-20 years earlier. The contact had been through the skin or through the respiratory system.

Of course we cannot draw any conclusions about the possible causal connection between exposure to the phenoxy acids and contaminants and the occurrence of malignant tumors, solely on the basis of the reported cases. Exposure to the phenoxy acids should be relatively common within the area of the three forest provinces, from which the Umeå clinic receives patients for treatment. These cases very well could have been chance occurrences.

However, what is remarkable about these cases and should give rise to continued investigations are the facts that these tumors are of a rare type, that the exposure in all cases has been rather massive, that the latent period agrees well with that assumed for chemical carcinogens, and also that the distribution by sex for malignant mesenchymal tumor at the Umeå clinic strongly deviates from the national average. In a follow-up investigation of possible causal connections, extensive studies are needed, for example, of epidemiology and industrial-medicine.

Missing from H. Warwick 10/13/77

Maligna mesenkymala tumörer och exposition för fenoxisyror – en klinisk observation

MN0539968

Sju fall med malign mesenkymal tumör iakttagna vid onkologiska kliniken i Umeå åren 1970–76 och med exposition för fenoxisyror 10–20 år tillbaka beskrivs. Expositionen har varit direkt och relativt massiv för fem av fallen. Latenstiden stämmer med den för kemisk karcinogenes antagna. Könsfördelningen i detta material om totalt 87 patienter avviker från den genomsnittliga för riket med manlig dominans. Några säkra slutsatser om ett eventuellt kausalsamband kan givetvis ej dras av dessa fall, utan för detta krävs mer omfattande undersökningar.

Frågan om fenoxisyror (t ex hormoslyr) eller i dessa ingående föroreningar kan utöva skadeeffekter på människa och djur har varit föremål för en omfattande debatt. Någon cancerogenicitet hos människa har hittills ej rapporterats.

I denna artikel beskrivs sju patienter med malign mesenkymal tumör iakttagna vid onkologiska kliniken i Umeå åren 1970–76 och hos vilka exposition för fenoxisyror förelegat 10–20 år före diagnosen av tumörsjukdomen. Dessa fall bevisar naturligtvis inget om ett eventuellt samband mellan sådan exposition och malign tumör – för att bevisa eller utesluta ett sådant samband krävs ingående epidemiologiska undersökningar – men det har ändå ansetts motiverat att rapportera iakttagelserna i kasuistisk form.

Åren 1970–76 har vid onkologiska kliniken i Umeå mottagits sammanlagt 87 patienter med malign mesenkymal tumör. Av dessa var 32 kvinnor (37 proc) och 55 män (63 proc). Av männen har 43 varit yrkesverksamma; yrkesbeteckning saknas dock i ett fall. Nio var skogsarbetare (20,9 proc), fyra arbetade inom jord- och skogsbruk (9,3 proc) och sex vid sågverk eller massaindustri (14,0 proc).

LENNART HARDELL är underläkare vid onkologiska kliniken, regionsjukhuset, Umeå.

Kasuistik

☐ *Fall 1.* 62-årig man, skogsarbetare fram till pensionering 1971 på grund av diarré och smärtor till vänster i buken. Rökare. Vårdad för tbc 1959. För övrigt väsentligen frisk tidigare. I sitt arbete hade han besprutat med fenoxisyran 2,4,5-T knappt en vecka 1963 och 1964, två veckor 1965, en månad 1966, en månad 1967 samt två veckor 1968. Sedan augusti 1976 ökande värk i höger fossa iliaca samt svullnad av höger ben. Vid laparotomi påvisades en tumör i lilla bäckenet adherent till bäckenväggen. PAD: Medelhögt differentierat leiomyosarkom.

☐ *Fall 2.* 57-årig skogsarbetare, icke rökare. Hypertoni sedan 1960. Behandlad för postinfektiös artrit 1974. På 1950- och 60-talen under vardera tre eller fyra somrar och varje sommar under tre veckor hade han använt en blandning av 2,4-D och 2,4,5-T för fickning och mindre utkning för ryggbesprutning. Sedan augusti 1976 tillväxande tumör ventralt, proximalt på höger lårben. PAD: Mesenkymal tumör, troligen medelhögt differentierat fibroserande liposarkom.

☐ *Fall 3.* 49-årig man, verkstadsägare. Mångåriga gastritsymtom men för övrigt frisk. I sin verkstadslokal hade han åren 1961–72 under icke besprutningssäsong, ca 11 månader årligen, förvarat tusentals liter av fenoxisyror för skogsbolags räkning. Förvaringen hade delvis skett i öppna hinkar. Patienten hade även handskats med medlet. Uppgav ständig lukt på arbetsplatsen. Augusti 1976 melena och buksmärtor. I november ånyo akuta buksymtom som föranledde laparotomi. Härvid påvisades tumör i lilla bäckenet med engagemang av rektum, urinblåsa samt överväxt till tunntarm. PAD: Mesenkymal tumör, snarast neurofibrosarkom.

☐ *Fall 4.* 60-årig skogsarbetare, måttlighetsrökare. År 1938 appendektomi men för övrigt frisk. Åren 1961–66 hade han ca 20 arbetsdagar per år besprutat med fenoxisyror. Sökte i augusti 1971 för defekationsbesvär. Palpatoriskt i rektum innanför sfinktern submuköst växande tumör. PAD: Sarcoma recti (leiomyosarkom).

☐ *Fall 5.* 44-årig man som tidigare varit helt frisk. Åren 1945–46 under vardera tre veckors tid hade han exponerat sig för herbicider under jordbruksarbete i Danmark. Anställd i oljeindustri åren 1954–68. Sommartid åren 1960–68 hade han två veckor årligen handskats »ganska ovarsamt» med fenoxisyror både med spruta och i form av fickning. Sökte 1974 för tilltagande resistens volart höger underarm. PAD: Rabdomyosarkom.

☐ *Fall 6.* 76-årig man som varit frisk till 1972 då han opererades för perforerat ulcus ventriculi. Angav viss exponering för fenoxisyror under 4–5 somrar på 50-talet, varvid han slagit och tillvaratagit gräs längs vägrenar som vägverket besprutat. Patienten uppgav dessutom att han arbetat och vistats i skog som besprutats med fenoxisyror. Sökte 1972 för domningar och värk i höger arm. Palpatoriskt påvisades en decimeterlång tumör på insidan av höger överarm. PAD: Myxofibrosarkom med misstänkt lipoblastisk differentiering.

☐ *Fall 7.* 67-årig skogsarbetare med tre tidigare pneumonier samt gastritanamnes. Patienten hade arbetat i skogen till 1970. Hyggesrensat enligt egen uppgift från 1956 till början av 60-talet inom fenoxisyrabesprutade områden. Angav arbete i anslutning till besprutning till ett par år senare. Sedan juni 1969 hade han noterat en resistens på vänster underarm. Sökte för denna i mars 1970. PAD: Polymorfcelligt sarkom, möjligen ett rabdomyosarkom.

Utöver dessa sju patienter, hos vilka expositionen för fenoxisyror har varit direkt åtminstone i de fem första fallen, finns ytterligare ett par patienter – bägge skogsarbetare – som haft sina arbetsplatser på 1950–60-talen tidvis förlagda i anslutning till besprutade områden. I det ena fallet har dessutom bärplockning efter besprutning förekommit. PAD: Rabdomyosarkom respektive neurofibrosarkom.

Diskussion

Latenstiden för kemisk induktion av maligna, solida tumörer hos människa anses i allmänhet vara mycket lång och i genomsnitt av storleksordningen 15–30 år. Latenstiden är möjligen dosberoende (Hueper, Conway 1964). I de relaterade fallen ligger exponeringen 10–20 år bakåt i tiden. Kontakten har skett via hud och inandningsorgan.

Några slutsatser om ett eventuellt kausalsamband mellan exposition för fenoxisyror och däri ingående föroreningar samt maligna mesenkymala tumörer kan självfallet ej dras från de relaterade fallen. Expositionen för fenoxisyror torde vara relativt vanlig inom de tre skogslän som utgör Umeå-klinikens upptagningsområde och det kan mycket väl ha varit fråga om ett slumpmässigt sammanträffande.

Vad som dock är anmärkningsvärt och kan motivera fortsatta undersökningar är att det är fråga om en sällsynt tumörtyp, att expositionen i samtliga fall varit ganska massiv, att latenstiden stämmer med den för kemisk karcinogenes antagna samt att könsfördelningen för maligna mesenkymala tumörer vid Umeå-kliniken starkt avviker från den genomsnittliga i

2753
16398

Ammoniumnitratdamm och nitrit

Företagsläkare har å ena sidan svårt att själva genomföra stora epidemiologiska undersökningar inom sitt ansvarsområde men å andra sidan svårt att engagera arbetsbelastade universitetsinstitutioner för sina problem. En liten expositionsstudie som den här redovisade har väckt debatt i press och radio. Sådana studier borde, liksom debatten inom lantbruket och dess framställare av kvavehandelsgödsel och liksom debatten om vårt av övergödsling kväveförorenade dricksvatten, kunna förmå de stora institutionerna att satsa än mera på epidemiologiska frågor om nitrat och nitrit.

I LKAB:s gruvor i Malmberget använder man sedan 1965 ett sprängmedel bestående av 95 proc nitritfritt ammoniumnitrat blandat med 5 proc dieselolja. Det kallas här AN, på andra ställen ANFO.

Som ett lätt luftblandat pulver sprutas AN vid laddning med tryckluft genom slang in i förut drivna borrhål. Hela tiden men särskilt när dessa börjar bli färdigfyllda dammar det kraftigt på personalen. Tekniskt förebyggande åtgärder mot dammet har hittills ej varit framgångsrika och oro över tänkbar explosionsrisk har därför utbrett sig.

Nitrat har länge förekommit inom industrin, särskilt vid framställning av kvävehandelsgödsel och dessas senare hantering i lantbruket. Man har hittills ansett hälsorisken av ammoniumnitrat som ringa. I våra dagar har dock tillkommit oro för cancerrisk av nitrosaminer, bildade av vissa aminer och nitrit. Sådan risk förutsätter förutom närvaron av nitrat–nitrit även förekomst av sekundära aminer. Det är för närvarande oklart om aminer förekommer i gruvmiljö mer än i varje annan miljö och vad typ de har. Christoffer Rappe, organokemist i Umeå och nitrosaminspecialist, studerar för närvarande denna fråga.

AN-sprängmedlet innehåller som tillsats fettsyreaminer som anses minska dammbildningen. Av dessa är emellertid endast 5 proc sekundära och dessutom långkedjiga, dvs innehåller 14–18 C-atomer. Risken för att det bildas karcinogena nitrosaminer anses upphöra när aminradikalen innehåller 10 C-atomer eller fler.

Här redovisade studie är inriktad på nitritsidan av problemet.

Arbetet utgår från LKAB, Malmberget, där SEIJA ABRAHAMSSON är företagssköterska, LEIF KÅGSTRÖM skyddsingenjör, KARL GÖRAN S:T CLAIR RENARD företagsläkare och KURT STENLUND skyddsingenjör.

I artikeln redovisade analyser av methemoglobin är utförda vid kliniskt kemiska laboratoriet, Centrallasarettet, Boden.

Nitrat-omvandlingen

Nitrat kan omvandlas i vatten till nitrit. Omvandlingen går långsamt men gynnas av alkalisk miljö, t ex i munhålan. Den sker ej alls i magsäckens sura miljö men oresorberat nitrat bör längre ned i tarmen åter kunna ombildas till nitrit genom bakterieinverkan.

Otänkbart om än ej särskilt sannolikt är det ej heller att nitrat på luftvägarnas slemhinnor skulle kunna omvandlas till nitrit och upptagas av kroppen.

Nitrit binds i blodet till hemoglobinet och bildar methemoglobin. Bindningen är på samma sätt som motsvarande med kolmonoxid en »direkt» reversibel bindning. Detta till skillnad från bindningen med andra kväveföreningar som anilin, som binds »indirekt» och icke reversibelt och under samtidig förstöring av hemoglobinmolekylen. Den indirekta bindningen torde ej vara aktuell i gruvmiljön. Båda bindningarna för till methemoglobin.

Methemoglobin används som parameter för nitrat–nitrit-expositionen.

Metod för bestämning av *små* mängder methemoglobin är sedan 1976 införd vid kliniskt kemiska laboratoriet, Centrallasarettet, Boden. Övre normalgräns för methemoglobin är 2 proc av det totala hemoglobinet.

Hygieniskt gränsvärde för dricksvatten är för:

nitrit 0,02 mg/liter,

nitrat 30 mg/liter för barn och 50 mg/liter för vuxna.

Vid 100 mg nitrat per liter anses vatten otjänligt som livsmedel.

Expositionsmätning

Stiftelsen Svensk Detonikforskning har på uppdrag av Stiftelsen Bergteknisk forskning tagit upp riskproblemen vid AN-(ANFO-)laddning. Preliminära NH_4NO_3-mätningar har gjorts på damm, dels på filter och dels på nitrat i vattenfas med impinger. NH_4NO_3 är starkt hygroskopiskt. Kalkyler av expositioner har gjorts (Skarped 1975).

Emellertid har man hittills ej gjort några mätningar av den verkliga belastningen på människa i samband med laddning. Med anledning därav utförde vi i Malmberget natten till den 12 maj 1976 en länge diskuterad expositionsmätning på tio laddare under jord (observerad grupp) och nio borrare under jord (referensgrupp). Som parameter valde vi methemoglobin i blod.

Under det aktuella kvällsskiftet gjordes kontinuerliga filtermätningar av totaldamm på laddningsarbetsplatserna. Samtidigt bestämdes även halt av NO_2, $NO + NO_2$ och NH_3. Serie 1–20.

En likartad undersökning utfördes natten till den 19 oktober 1976 på elva laddare och elva borrare. Serie 21–42.

Storleken av grupperna begränsades av resurserna på Bodens centrallaboratorium och våra egna mätningstekniska tillgångar.

Samtliga laddare och borrare hade samlats för instruktion dagen före mätningarna. Samarbete med fackföreningen upprättades. Man kom överens om att andningsskydd inte skulle användas av laddare vid undersökningsskiftet (används för övrigt sällan på grund av att ammoniumnitrat kittar ihop filtret) och att grönsaker, särskilt då spenat, ej skulle ingå i skiftmålet. (Nitrat, se nedan!)

Efter skiftslutet togs kl 20.35–23.05 prover i gruvmatsalen 500 m under jord. Proverna, 10 ml venblod i vakuum-heparinrör placerades omedelbart i kylskåp och snabbehandlades sedan.

I serie 21–42 blev analysen försenad ett dygn genom ett tekniskt missöde inom kliniskt-kemiska laboratoriet i Boden.

Samtidigt togs prover på nattpersonal och patienter på Gällivare och Boden lasarett.

riket. För en fortsatt utredning av ett eventuellt kausalsamband krävs ingående undersökningar av t ex epidemiologisk och arbetsmedicinsk karaktär.

Litteratur

HUEPER W C, CONWAY W D: Chemical Carcinogenesis and Cancers. Springfield, Ill: Thomas 1964

Malignant Mesenchymal Tumours and Exposure to Phenoxy Acids–A Clinical Observation

LENNART HARDELL, MD. Dept of Oncology, Regional Hospital, S-901 85 Umeå

LÄKARTIDNINGEN 74: 2753–2754 1977

Seven cases of malignant mesenchymal tumours observed at the dept of oncology in Umeå during the years 1970–76 and with exposure to phenoxy acids over a period of 10-20 years are described. The exposure was direct and comparatively massive in five of the cases. The latent period is in agreement with that assumed for chemical carcinogenesis. The distribution between the sexes in this material, of 87 patients in all, deviates from the national average, with a dominance of males. No definite conclusions concerning a possible causal connection can be drawn from these cases; this would call for more extensive investigations.

16339

2446

ECOLOGICAL BULLETINS No 27

MN001665 DOW0442043

Chlorinated Phenoxy Acids and Their Dioxins

Mode of Action, Health Risks and Environmental Effects

C. Ramel (ed.)

© 1978. Ecological Bulletins, Swedish Natural Science Research Council.

ISBN 91-546-0234-3
Production and distribution: NFR Editorial Service
LF/ALLF 209 77 002
Printed in Sweden by Berlings, Lund 1978

Copies of the bulletins can be ordered from the Editorial Service of NFR, Box 23136, S-104 35 Stockholm, Sweden. The Editorial Service, which provides financial and practical assistance for the publishing of scientific works, is responsible for the distribution of all NFR publication.

Report from a conference arranged by the
Royal Swedish Academy of Sciences
Stockholm, Sweden, 7–9 February 1977

16400

DOW0442044

LIST OF CONTENTS

Editor's preface *C. Ramel*	9	cc'd to
CONCLUSIONS AND RECOMMENDATIONS (attached)	11	all
CHEMISTRY	17	
Summary	19	
Chemical background of the phenoxy acids and dioxins *C. Rappe*	28	
Analysis of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin in the Seveso area *P. Adamoli, E. Angeli, G. Bandi, A. Bertolotti, E. Bianchi, L. Boniforti, I. Camoni, F. Cattabeni, G. Colli, M. Colombo, C. Corradi, L. De Angelis, G. De Felice, A. Di Domenico, A. Di Muccio, G. Elli, R. Fanelli, M. Fittipaldi, A. Frigero, G. Galli, P. Grassi, R. Gualdi, G. Invernizzi, A. Jemma, L. Luciano, L. Manaro, A. Marinella, F. Merli, S. Nicosia, F. Rizzello, C. Rossi, G. Rossi, G. Salvatore, A. Sampolo, G. P. Schmidt, F. Taggi, G. Tebaldi, E. Zaino & G. Zapponi.*	31	
The 2,3,7,8-tetrachlorodibenzo-*para*-dioxin problem: a review *D. Firestone*	39	
✓ **Analysis of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin in environmental samples** *J. D. McKinney* NIEHS	53	WBC
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin decontamination *F. Pocchiari* Seveso	67	
✓ **Confirmatory tests for pesticide residue analysis** *G. Widmark*	71	
✓ **Photochemical reactions of phenoxy acids and dioxins** *B. Åkermark*	75	MEG

16401

PLANT PHYSIOLOGY 83

Summary 85

The herbicidal effects of phenoxy compounds 86
B. Åberg & L. Eliasson

TOXICOLOGY 101

Summary 103

The phenoxy acid problem in Sweden 108
J. Bäckström

Phenoxy acids: effects and fate in mammals 122
P. J. Gehring & J. E. Betso

Toxicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin 134
J. A. Moore

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin and enzyme induction 145
A. Poland & E. Glover

Phenoxyacetic acids: sublethal effects 149
P.-O. Sjödén & U. Söderberg

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin: effects and mechanisms 165
J. G. Vos

GENETICS

Summary 179

Genetic effects of phenoxyacetic acids in animals 182
C. Ramel

Genetic effects of chlorophenoxyalkanoic acids on plants 186
L. Ehrenberg

Mutagenicity tests of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid in genetically stable and unstable strains of *Drosophila melanogaster* 190
B. Rasmuson & H. Svahlin

Genetic effects of phenoxy acids on microorganisms 193
G. Zetterberg

16402

ECOLOGY AND ECONOMY 205

Summary 207

Chemical weed control in the tropics 210
G. E. Blackman & J. D. Fryer

The use of phenoxy acid herbicides in Swedish forestry: amounts, types and modes of application 219
U. S. M. Bärring

The use of phenoxyacetic acid herbicides in Swedish agriculture 231
B. Granström

Phenoxyacetic acid herbicides as a tool in forest management: silvicultural considerations 235
B. Häggström

Effects of the silvicultural use of phenoxy acid herbicides on forest vegetation in Sweden 240
T. Ingelög

Bioaccumulation of 2,3,7,8-tetrachloradibenzo-*para*-dioxin 255
A. R. Isensee

Effects of phenoxyacetic acid herbicides on soil organisms 263
L. Torstensson

Ecological considerations regarding massive environmental contamination with 2,3,7,8-tetrachlorodibenzo-*para*-dioxin 285
A. H. Westing

List of participants 295
List of observers 297
List of additional authors 299

cc'd to MEG DAL etc.

Nonsense

DOW042049

16403

Ramel, C. (ed.) 1978.
Chlorinated Phenoxy Acids and Their Dioxins
Ecol. Bull. (Stockholm) 27:13–16.

DOW04420047

CHLORINATED PHENOXY ACIDS AND THEIR DIOXINS: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The phenoxy acid herbicides are not formed naturally in the environment. There is no evidence that dioxins are formed from phenoxy acid herbicides in the environment. The only dioxin of environmental concern with respect to the phenoxy acid herbicides is 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), the dioxin found in the herbicides, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP). TCDD is found neither in 2,4-dichlorophenoxyacetic acid (2,4-D) nor in 4-chloro-2-methylphenoxyacetic acid (MCPA).

Levels commonly encountered in soil after normal applications of phenoxy acid herbicides are 1–2 parts per million (ppm); due to heterogeneity of soils and irregularity of application, these levels could be somewhat higher or lower locally within sprayed areas. The half-life of the phenoxy acid herbicides is of the order of 3–4 weeks; that of 2,4,5-T is up to 10 weeks. The rate of degradation generally increases with increasing microbiological activity and decreases at lower pH, especially below pH 4. Repeated applications of 2,4-D and MCPA result in somewhat faster microbiological degradation.

The level of phenoxy acid herbicides in the green parts of target vegetation shortly after normal spraying is 200–500 ppm. After 10 weeks, this level decreases to 20–50 ppm. During the cold winter period this value seems in some cases to be nearly constant, although in other cases it decreases. Injected phenoxy acid herbicides may persist for 4–6 years within the woody parts of vegetation, however, the ecological consequences of such persistence in wood are probably not very great.

Photolysis is the primary mode of degradation of TCDD on environmental surfaces; microbiological degradation occurs slowly in soil. Its halflife in soil has been estimated in the United States to be 1–1.5 years (on the basis of studies in which high levels of dioxin-contaminated phenoxy acid herbicides were used).

Wind-drift of sprayed phenoxy acid herbicides may reach non-target areas. To avoid such risks, strict regulations with regard to weather conditions during spraying and with regard to spraying techniques are required.

There appears to be virtually no downward movement of TCDD in the soil: it can be estimated that no more than 1% is transported from the site of application annually. Movement in the air would occur largely *via* airborne particles. Vegetation takes up very little or no TCDD from soil.

16404

There is no evidence of bioaccumulation of phenoxy acids; bioaccumulation of TCDD resulting from normal use of 2,4,5-T is insignificant.

The fate of phenoxy acids in plant tissues, changes in plant composition induced by these compounds and their mechanism of toxic action are understood only imperfectly. It can be concluded that further studies in this field would be of importance for understanding the response of crop plants to phenoxy acids and for the development of more selective and efficient methods of herbicide usage. Such studies would also provide important information about aspects of herbicide toxicity at the cellular level that may be relevant to their toxicity in animals.

Possible levels of exposure to phenoxy acids and/or dioxins vary among different populations, such as: employees in factories where phenoxy acid herbicides and/or trichlorophenol are made; persons who prepare and apply the herbicides, and members of the general public who enter sprayed areas and/or consume treated vegetation. People representing potential risk groups, pregnant women and individuals with chronic liver disease, should be excluded from activities involving high exposures, such as manufacturing and occupational spraying.

Only very low levels of phenoxy acid herbicides are normally found in foodstuffs. Levels in Swedish wildlife, fish and waters are also low. The residue levels in woodland berries from recently sprayed areas might be higher, of the order of 10 ppm. A daily intake of as much as 1 kg of such berries would correspond to a dose of 0.2 mg kg^{-1} day^{-1} of phenoxy acids for a person weighing 50 kg. This level probably represents the same degree of exposure as that realized by herbicide sprayers. Calculations based on extreme conditions (high exposure from food and lowest dose of 2,4,5-T that caused teratogenic effects in mice) still provide a safety factor of at least 100.

If the levels of TCDD in phenoxy acid herbicides remain below 0.1 ppm, considerations of health risks associated with these products should be based primarily on the potential toxicity of the phenoxy acids *per se* and not on that of its contaminants.

In evaluating the risk associated with the presence of TCDD in phenoxy acid herbicides, potential exposure to compounds that have similar toxic effects, i.e., other halogenated dibenzodioxins, dibenzofurans, naphthalenes and azoxybenzenes, should also be considered.

Studies to assess the tumorigenicity of phenoxy acids and TCDD provide no defintive indication that these agents are or are not carcinogenic. Additional, more extensive evaluations are underway.

The only statistically significant demonstration that phenoxy acids induce mutations transmissible to the offspring was obtained in the recessive lethal test in *Drosophila melanogaster*. Work on microorganisms sustains the conclusion that the phenoxy acids are mutagenic under the test conditions used. Two investigations on 2,4,5-T and one on 2,4-D, both using pure acids, showed an increased number of recessive lethals in *Drosophila*. From a tentative comparison of the radiation dose that would give a similar effect, the mutagenic effects of 2,4,5-T and 2,4-D were concluded to be weak.

Somatic mutations have been observed in *Drosophila*, and chromosomal aberrations have been shown to be induced in plants and mammals. The relevance of these latter findings to a risk of mutation from phenoxy acids (associated or not

16405

DOW044204B

with heritable damage) requires further studies. Observation in one study of a possible role of the emulsifier and/or solvent show the necessity of including these constituents of phenoxy acid formulations in future studies.

In view of positive mutation tests in bacteria and findings of chromosomal aberrations in one mammal and in one plant species with TCDD, it must be concluded that this compound is mutagenic. The fact that other tests of a similar kind were negative may have many explanations, such as problems in keeping the compound in solution. Such difficulties make it impossible at present to quantify genetic risks from TCDD.

With regard to behavioral effects of the phenoxy acids in mammals, it was concluded that further studies of dose-response relationships are necessary before it would be possible to make an evaluation of similar effects, if any, of the doses of phenoxy acids to which humans could be expected to be exposed in connection with normal herbicide usage.

No counterpart to the physiological mechanism involved in the major herbicidal effects of phenoxy acids in plants is known to exist in animals. In plants, high concentrations of phenoxy acid compounds cause less specific effects, e.g., the uncoupling of oxidative phosphorylation; this effect appears to be weak in mammalian organisms.

Recommendations

1. The stability and bioaccumulation of TCDD should be studied in realistic situations, under normal field conditions, in various types of climates and soils.
2. Risks associated with exposure to TCDD could be greatly reduced if manufacturers would utilize processes, currently available, that minimize the formation of TCDD from trichlorophenol. Manufacturers should also use techniques that minimize potential contamination of the surrounding environment by TCDD in the event of industrial accidents. In addition, strict precautions should be taken to ensure safe disposal of TCDD-containing residues.
3. Failure of manufacturers to utilize techniques that minimize TCDD formation or contamination should lead to a re-evaluation of the feasibility of continued use of trichlorophenol or of products derived from it, such as the phenoxy acid herbicides, 2,4,5-T and 2,4,5-TP.
4. The use of other synthetic processes that eliminate formation of TCDD as a contaminant should be investigated.
5. At present, the mechanism for the toxicity of TCDD is unknown. A further understanding of how TCDD and related compounds act is necessary.
6. Further sutdies of the mutagenic activity of the phenoxy acids are important and should comprise all constituents of formulations and their impurities. In order to make quantitative estimates of risk to man, it is essential that the mechanisms for the genetic effects observed be clarified.
7. Studies of mutagenicity should also aim at safe-guarding plant genotypes.
8. It is recommended that epidemiological investigations be made of human groups exposed to phenoxy acid herbicides and dioxins and that these comprise all effects that have a possible genetic mechanism.
9. In forest use of herbicides, it is recommended that consideration be taken

16406

D0M04204 9

of non-target plants within target areas, especially those of recreational and scientific interest. Information about the effects of herbicides on non-target flora is insufficient, and further research within this field is recommended.

10. It is recommended that special consideration be given to the ecological importance of field borders and other ecotones in any spraying program.
11. In choosing herbicides, their selectivity for intended target species and other species within target areas should always be considered.
12. The ecological importance of habitat, including both food and cover, to vertebrate and other fauna was recognized; it is recommended that this factor be taken into consideration in the use of herbicides and in other manipulations of habitat. Further research on the possible significance of alterations to wildlife habitats is recommended. Both vertebrates and invertebrates should be considered.
13. The conference stressed the importance of establishing and maintaining reference areas representative of major ecological types. With respect to herbicide use, it is recommended that areas are set up in which herbicides would not be employed and in which other management operations are strictly controlled.

BOW04205

16407

DOW CHEMICAL U.S.A.

9008 Building
MIDLAND, MICHIGAN 48640

May 21, 1974

CONFIDENTIAL - SUBJECT TO INJUNCTION
D.C., E.D. MI. 4-4-78; DOW/EPA AGREEMENT 9-79

DOW048285

J. H. Davidson, Manager
2,4,5-T Project
9008 Building

cc: E. H. Blair
M. C. Feigelson
G. E. Lynn
D. D. McCollister
J. M. Theis, 834
M. J. Traynor
M. R. Wessel
C. S. Williams

SILVEX AND ERBON - REGISTRATION AND TOXICOLOGY

On May 16, EPA published a notice of intent to broaden the 2,4,5-T hearing to include all registered uses of herbicides derived from 2,4,5-trichlorophenol, including silvex and erbon (Federal Register 39, No. 96, p. 17466). Dow is the principal manufacturer of silvex and the only manufacturer of erbon. Current Dow registrations for silvex are:

KURON® Low Volatile Brush and Weed Herbicide (EPA Reg. No. 464-162)
Silvex Acid for Manufacturing Use Only (EPA Reg. No. 464-455)
Silvex Butoxypropyl Esters for Manufacturing Use Only (464-461)
Silvex Isooctyl Esters for Manufacturing Use Only (464-436)

Registration for other Dow silvex products was cancelled in October 1966 when Dow indicated they would not renew their registrations as required every five years.

GARLON® Grass and Weed Killer (464-217)
KUROSAL® SL Aquatic Herbicide (464-277)
KUROSAL® G Aquatic Herbicide (464-276)
COLORSET® for control of preharvest drop in apples (464-267).

Other products similar to KURON® include:

SILVI-RHAP® LV-4TP (formerly Hercules, now Transvaal)
WEEDONE® 245-TP (Amchem)
Silvex 4TP (Diamond-Shamrock)
Silvex 4L (Chipman Division, Rhodia, Inc.)
DED-WEED® Silvex LV (Thompson-Hayward).

Salt formulations used for aquatic purposes are registered by Pennwalt, as mixtures with endothall (e.g. AQUATHAL®). Dilute formulations of amine salts are used as growth regulators in fruit crops (e.g. Arcadian STA-SET 400, EPA Reg. No. 218-492, Allied Chemical Corporation). A large portion of the estimated

LIFE IS FRAGILE HANDLE WITH CARE

1.5 million pounds of silvex produced annually is used in the home and garden market (e.g. WEED-B-GONE®, Chevron).

With regard to erbon, we have three registered products:

ERBON 4 Non Selective Herbicide (formerly BARON®, 464-172)
NOVEGE® Weed and Grass Killer (464-255)
ERBON R for Manufacturing Use Only (464-335).

The latter contains 42% erbon and 15% related chemicals which are not designated but are claimed as active ingredients. It is sold to many formulators for registration under their labels for home and garden use. Examples include:

Greenfield Contact Killer (1471-33, Elanco or Eli Lilly)
Best Liquid Edger (7001-RIA, Occidental)
Best Ded n' Gon or All-Out Weed Killer (7001-88-AA, Occidental)
Four Seasons Edge or Trim Grass and Weed Killer (7393-UE, Wonder)
Ferti-Lome New Improved Perma Trim (Voluntary Purchasing Groups)
Weed and Grass Killer No. 1 and No. 2 (407-xx, Imperial)
Green Light Liquid Lawn Edger (869-61)
Sears Liquid Edger (539-254)

Uses, rates of application, toxicology, residues, and status of registration are discussed individually below for silvex and erbon. Additional details can be provided as necessary.

Silvex is the common name for 2-(2,4,5-trichlorophenoxy)propionic acid. (Note that it is a mixture of D and L forms of the chemical due to the presence of an asymmetric carbon on the side chain.) According to the current EPA Summary of Registered Agricultural Pesticide Chemical Uses, it is registered for use as an herbicide at rates up to 4 lb/A in pasture and rangeland, 1.5 lb/A in rice, 5 lb/A in sugarcane (Florida, Louisiana and Hawaii), and 40 lb/A in lakes and ponds. It is also registered for use as a growth regulator applied postharvest at 7.5 ppm on pear trees and preharvest at 20 ppm in apples and prunes, with a maximum rate equivalent to 60 grams per acre. Uses for control of brush in industrial and other non-cropland areas include rates up to 16 lb/A as approved by EPA in October 1973 for KURON®. KURON® contains 69.2% propylene glycol butyl ether esters of silvex, or 45.8% silvex acid equivalent, which amounts to 4 lb a.e. per gallon.

We have considerable toxicology data for silvex, including reports on subacute 90-day studies with the sodium salt and the PGBE ester in rats and with the potassium salt in dogs. We also have chronic 2-year studies with the potassium salt in rats and dogs, and recent teratogenic studies with the acid and the PGBE esters in rats. The results of these are summarized in the attached excerpts from Section C of Pesticide Petition No. 8F0675. EPA has indicated that they are satisfied with the studies but that a multigeneration reproduction study would be needed for tolerances greater than 0.1 ppm (negligible residue) in food crops.

DOW0485286

All registered uses for silvex in food crops are covered by PP 8F0675 first submitted in December 1967 by the Industry Task Force for Phenoxy Herbicide Tolerances (formally disbanded in April 1974). Amendments to the petition were submitted by Dow in September 1968, December 1970, October 1971 and January 1973, for a total of nine volumes to date. The petition is currently being held in abeyance until 11/1/74 pending completion of a study on persistence of silvex in soil and of another feeding study in cattle.

The residue pattern for silvex in milk and meat resembles that for other phenoxy herbicides in some cases but not in others. For example, residues were not detected in milk of cows fed silvex, 2,4-D or MCPA until the level in total diet was raised to 1000 ppm, but residues of 2,4,5-T and/or 2,4,5-trichlorophenol occurred in milk when the cows were given as little as 100 ppm 2,4,5-T in the diet. Residues in meat tissues, particularly liver and kidney, were much higher for silvex than for 2,4-D and MCPA and were substantially higher than for 2,4,5-T at levels of 300 to 2000 ppm in the diet of cattle and sheep. However, residues of the 2,4,5-trichlorophenol metabolite were low or non-detectable in milk and meat of animals fed silvex in contrast to relatively high levels of the phenol in milk, liver and kidney of animals fed 2,4,5-T. Additional feeding studies are currently being conducted at reasonable levels of 300 ppm in the diet, to determine the preslaughter intervals required to reduce residues of silvex (or of 2,4,5-T plus trichlorophenol) to below the 0.1 ppm negligible residue level in liver and kidney. We have proposed limiting the rate of use to 2 lb/A in pasture and rangeland, with no cutting of hay during the season of application of silvex or 2,4,5-T.

Interim tolerances were established in late 1972 for many pesticides formerly registered on a no-residue basis in food crops. Included was silvex at 0.1 ppm in or on apples, plums (prunes), rice and sugarcane. (Federal Register 37, No. 169, p. 1775, August 30, 1972). However, a subsequent order proposing additional interim tolerances, including 2,4-D and MCPA in grass, stated that interim tolerances could not be established for 2,4,5-T in any crop, nor for silvex in or on grasses (pasture and rangeland), grass hay, and in water. The reason given was because the data in petitions and otherwise available data were judged inadequate to rule out the likelihood of injury to consumers from residues from these uses (Federal Register 37, No. 178, p. 18565-18566, September 13, 1972). Dow appealed this for silvex in grass but the appeal was denied. In February 1974, we renewed the request for an interim tolerance for silvex in grass but EPA replied on April 5, 1974 that data from the additional feeding study were needed before they could finalize the usage on grass, i.e. application rates, withdrawal times, etc.; hence they could not establish interim or permanent tolerances for grass at this time.

Use of silvex as a growth regulator in pears was cancelled by EPA in March 1972 when they denied our request for exemption from the need for tolerances. We had hoped they would consider it a non-food use since the 7.5 ppm spray is applied to the trees after harvest of the pears,

i.e. a year before harvest of the next crop. However, EPA ruled that this use would reasonably be expected to result in small residues in or on harvested food or feed. Subsequently, we provided all available data on silvex in pears for submission by Inter-Regional Project No. 4 on behalf of the State of Washington. Their Pesticide Petition No. 3E1339 was deemed adequate by EPA and a permanent tolerance of 0.05 ppm was established for silvex in pears (Federal Register 38, No. 165, p. 22894, August 27, 1973, §180.340 of the Code of Federal Regulations).

Uses of silvex as an aquatic herbicide are covered by Pesticide Petition No. 1E1012 submitted by the U.S. Corps of Engineers in conjunction with the Bureau of Reclamation, USDI. However, EPA has repeatedly found their petition inadequate. We have supplied them with information for incorporation in their petition and have authorized EPA to consult our petition 8F0675 on their behalf. EPA has raised questions about residues of metabolites in fish, and the possibility that TCDD might be formed by cooking of fish containing residues of trichlorophenol. Their last amendment was submitted in November 1973 and their cover letter included the statement:

> "We understand that a hearing will be held by the Environmental Protection Agency in April 1974. If this hearing includes the aquatic use of silvex, we would like to defend our position on the basis of Technical Report No. 5 - Aquatic Use Patterns for Silvex."

This report dated October 1973 is 1/2 inch thick. A copy was sent to M. Leng by Dr. E. O. Gangstad of the Corps of Engineers as part of the inter-communication between Dow and the petitioners for tolerances in water and fish. The first sentence of the Foreword states that an announcement by Dr. Lee A. DuBridge in October 1969 listed "actions being taken by governmental agencies to restrict the use of the herbicide 2,4,5-trichlorophenoxyacetic acid (silvex)". Apparently they don't know the difference between the various compounds since they also reported finding 2,4,5-T as a metabolite of silvex in birds. (Dick Otten of Amchem is a member of the Registration Work Group of the Interagency ad hoc Committee on Use of Herbicides in Aquatic Sites.)

Erbon is the common name for the herbicide chemical 2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate. It is the alcohol form of 2,4,5-T combined as an ester with Dalapon. It degrades slowly in soil, presumably to 2,4,5-T and Dalapon which kill broadleaf weeds and grasses, respectively. When used at high rates, it eradicates all vegetation.

Dow's original product was called BARON® Non Selective Herbicide, which was reregistered in 1971 as ERBON 4 Non-Selective Herbicide under the same registration number (464-172) after we sold the trademark BARON® for other purposes. It contains 30.5% erbon and 10.5% related compounds for a total of 4 lb/gal. It is recommended for use in industrial areas such as storage and parking areas, tank farms, rail sidings, substations, outdoor theaters, drainage ditch banks, fence lines, pole yards, lumber yards, elevators, equipment storage areas, around farm sheds, and in non-crop agricultural land. It is usually applied in late May or early June in northern areas or in early winter or mid-winter in California. Recommended rates are 3 to 4 quarts per thousand square feet or 30 to 40 gallons per acre. This amounts to a maximum of 160 lb/A including 40 lb/A "related products".

DOW 0482289

Dow also has a private label product called NOVEGE® Weed and Grass Killer (EPA Reg. No. 465-255). This contains 9% erbon and 3% related compounds for a total of 1 lb/A. It is recommended for use in driveways, walkways, parking areas, under fences, around garages and other buildings, and in similar areas where vegetation is not wanted, as well as for edging various areas. The rates are described as 1 qt in 2 gal of water applied to 250 sq ft, or to 100 sq ft when extended control is desired. This amounts to a maximum of 1/4 pound per 100 sq ft or about 110 lb/A including about 30 lb/A "related products". Presumably, other products formulated from ERBON R (42% erbon, 15% related products) are recommended for use at similar rates.

Acute toxicity data on erbon and ERBON 4 indicate an acute oral LD_{50} of 1 to 2 g/kg. Precautionary labeling indicates it may cause skin irritation and eye irritation. Toxicity studies have been conducted in fish (2/28/63) and in birds (submitted to EPA on 9/26/73). According to Warren Crummett, analyses for TCDD are complicated by the high content of other materials in our technical erbon.

→ No - Formulated [illegible]

Marguerite

Marguerite L. Leng
Government Registration
Health & Environmental Research

enc:3

s

P. S. See attached article on ERBON 4 plus KURON® in the latest issue of the Dow publication "Industrial Vegetation Management" (Vol. 6, No. 1, 1974) by Juell Johnson of the South Dakota Department of Highways. Newly approved uses of KURON® are listed in an excerpt from the same magazine as an addendum to an Update on the Status of 2,4,5-T by L. Southwick.

Toxicology

A summary of known information on the toxicology of silvex was included in the December 1967 submission, and was repeated as Sections C.0.1 and C.0.2 in the amendment submitted in December 1970. Additional reports on toxicity studies in cattle and sheep were included in Section C.1 in December 1970.

Data presented in these submissions show that the oral LD_{50} of silvex and its various formulations is in the range of 500 to 1000 mg/kg of body weight for various species of animals. A no-effect level of 7 mg silvex per kilogram per day was found in 90-day feeding studies with the sodium salt and the propylene glycol butyl ether ester in rats, and with the potassium salt in dogs. The no-effect level in 2-year feeding studies was essentially 2.6 mg/kg/day in both species.

No significant adverse effect was produced in livestock given salts and esters of silvex orally at levels of 25 or 50 mg/kg/da However, 100 mg/kg/day was lethal to cattle and sheep after 29 and 11 daily doses, respectively, and 250 mg/kg/day produced death in cattle after five doses. Chickens showed reduced weight gain after ten doses of 100 or 250 mg/kg/day.

The lethal dose of the butoxyethanol ester of silvex was >9000 mg/kg of body weight for pheasants and young Bobwhite quail. Mortality was produced in 50% of young Bobwhite quail given the ester at 5000 ppm in the diet for less than 10 days, in young pheasants at 5000 ppm for less than 100 days, and in adult Mallard ducks at 2500 ppm for less than 100 days.

Summary of Toxicological Data on Silvex in Rats and Dogs

Report and date	Formulation used	Test animal	Duration of test	No-effect level given in report	mg/kg/day silvex equiv.
Bio-Test Labs 6/19/62	Dowco 171* (PGBE ester)	Rats	90 days	10 mg/kg/day	6.8
Dow Chemical 10/26/54	Sodium salt	Rats	90 days	0.01% in diet	5-10 (calculated)
Dow Chemical 10/30/63	Kurosal SL (K salt)	Dogs	89 days	0.03% in diet	7 (based on daily food intake)
Dow Chemical 3/17/65	Kurosal SL (K salt)	Rats	2 years	100 ppm in diet	2.6
Dow Chemical 4/8/65	Kurosal SL (K salt)	Dogs	2 years	190 ppm in diet	2.6**

*Propylene glycol butyl ether esters of silvex (67.5% silvex acid equivalent). KURON® weed and brush killer contains approximately 6.8% PGBE ester of silvex (45% silvex acid equivalent).

**A slight histopathological effect was noted in male dogs but not in female dogs at 2.6 mg/kg/day silvex equivalent after two years of continuous feeding.

Prepared by Marguerite Leng, Agricultural Department, The Dow Chemical Company, December 29, 1966
Revised September 10, 1970

15412

Section C.2.0 Summary of Teratology Studies with Silvex in Rats, January 1973.

OOW048292

Numerous teratology studies were conducted with phenoxy herbicides by various government and industrial groups following disclosure on October 29, 1969, by Dr. Lee A. DuBridge, Science Adviser to the President, that studies conducted in 1965-1968 by Bionetics Laboratory in Falls Church, Virginia, indicated teratogenic responses were produced in mice and rats given high doses of 2,4,5-T. The significance of these and other studies conducted at exaggerated levels by non-representative routes of administration is still under discussion by experts in the field of teratology.

As part of this program, studies were conducted by The Dow Chemical Company on silvex and its propylene glycol butyl ether (PGBE) esters by oral administration in rats. The two studies were reported in Sections D.2.1 and D.2.2 of this amendment to PP 8F0675 for silvex. Groups of Sprague Dawley rats were given silvex at doses of 0, 50, 75 or 100 mg/kg/day on days 6 through 15 of gestation, or from day 6 of gestation through lactation. The test compound was administered orally once daily as a corn oil suspension. Additional groups were given silvex at 25 to 150 mg/kg/day or its PGBE ester at 35 to 200 mg/kg/day, on days 6-15, 7-9 or 9-11 of gestation as described in the reports. Certain groups were utilized for teratologic studies while others were used for postnatal studies.

The following parameters, where applicable, were examined in these studies with silvex and its PGBE esters: clinical observations; body weights; food consumption; gross visceral

examination of dams; position of fetuses in utero; numbers of live fetuses, resorptions and corpora lutea; individual pup weights (days 0, 5, 21) and sex; external skeletal and visceral examinations on teratology litters; histologic examination of selected tissues; examination of weanling rats to determine degree of skeletal ossification.

048293

Conclusion

No overt clinical signs of toxicity, major visceral or skeletal anomalies or undesirable effects on other reproductive parameters resulted from treatment with silvex at 25 mg/kg/day or silvex PGBE esters at 35 mg/kg/day (equivalent to 23 mg of silvex per kilogram of body weight per day) on days 6 through 15 of gestation . Doses of 50 mg/kg/day resulted only in fetal toxicity as reflected by decreased fetal weight and minor skeletal variations, but no major abnormalities were observed at this dosage level. Maternal and fetal toxicity and fetal anatomical alterations were observed at levels of 75 mg/kg/day or more for silvex and at 100 mg/kg/day for silvex PGBE esters. Maternal death occurred when silvex was administered at 100 mg/kg/day for 10 days during gestation.

2435
CONFIDENTIAL - SUBJECT TO PROTECTIVE ORDER
D.C., E.D. Mi. 4-4-73; DOW/EPA AGREEMENT

DOW CHEMICAL U.S.A.

DOW037195

MIDLAND, MICHIGAN 48640

9008 Building
August 10, 1976

M. W. Sauerhoff
Health & Environmental Research
1803 Building

cc: E. H. Blair, 2020
G. E. Blau, 1707
M. B. Chenoweth, 607
J. H. Davidson, 9001
P. J. Gehring, 1803
H. L. Gordon, 2030
J. E. LeBeau, 1803
D. D. McCollister/Reg. Section
B. A. Schwetz, 1803

REPORT ON SILVEX STUDY IN HUMANS

Thank you for sending me a copy of your report on Fate of Silvex Following Oral Administration to Humans, dated May 21, 1976. It represents a tremendous amount of work and should be very helpful in resolving some of our problems with this herbicide. However, as we discussed by telephone, a somewhat different treatment of the data could provide even more useful information on rate of excretion of silvex by humans, for comparison with 2,4-D and 2,4,5-T.

First of all, I believe the half-life should be based on total excretion of silvex plus silvex conjugate in urine and feces rather than on only free silvex in urine. This would take care of variations in proportion of silvex in free and conjugated form in urine of the various subjects, ranging from a ratio of 5.19 for subject 1 to 0.66 for subject 4 (page 12). Would it be possible to have Gary Blau run the computer analysis of data for silvex plus silvex conjugate for successive 24 hr (or 12 hr) intervals in urine? It would provide better comparison with rates of excretion of 2,4-D and 2,4,5-T in humans where only small amounts, if any, were in conjugated form.

Secondly, fecal samples should be analyzed for longer than the first two days after dosing to see whether fecal excretion was delayed, at least for those subjects where total recovery in urine was less than 75% (subjects 5 and 6). It is possible that silvex taken up by the second compartment in the body would end up in feces. However, the first two fecal samples may have been taken as early as 0.5 and 24.5 hr after dosing which doesn't allow much time for uptake, possible conjugation in the liver or elsewhere, and delayed elimination as conjugate(s) in feces.

Thirdly, Figure 2 for excretion in urine should be based on silvex plus silvex conjugate to represent total rate of elimination of the chemical from the body. Differentiation should be made between "metabolism" in

LIFE IS FRAGILE HANDLE WITH CARE

which the parent chemical can be regenerated unchanged from the conjugate, and true metabolism in which the parent molecule is irreversibly altered (such as by hydroxylation of the ring, cleavage of the ether link to release the trichlorophenol, etc.). Similarly, hydrolysis of silvex ester(s) to give silvex and silvex conjugate(s) is not comparable to true metabolism/degradation of the herbicide molecule.

It will be interesting to see whether reanalysis of the data for silvex plus silvex conjugate will take care of the biphasic pattern of elimination developed by the computer. This pattern is not readily apparent from the plot of silvex excretion by the eight subjects (Figure 2). Perhaps total excretion will fit a one compartment model as found for 2,4-D and 2,4,5-T.

Were any studies done on ease of dissociation of the conjugate(s)? As far as I can see, the samples were simply adjusted to pH 1 or 13 and refluxed to hydrolyze any and all conjugates. On the other hand, a slight change in pH might have been adequate to release the silvex. If the conjugates are very labile, this could account for the variability in proportion of free vs conjugated silvex in the urine among the various subjects.

The apparent difference between silvex and 2,4-D and 2,4,5-T may also be due to the fact that the silvex administered was a racemic mixture of optically active isomers. Perhaps one isomer forms soluble salts like 2,4-D and 2,4,5-T whereas the other isomer may preferentially form a conjugate with some optically active amino acid. Only one isomer of silvex has herbicidal properties according to Loos in a book issued recently (Kearney et al. 1976).

As we discussed, I would greatly appreciate having recalculated half-live(s) based on total excretion of silvex in urine as soon as possible. I am currently finalizing a paper on comparative metabolism of phenoxy herbicides in animals for presentation at the ACS meeting in San Francisco on September 2. One slide deals with the three studies conducted by Dow in humans, comparing the excretion pattern and rates of elimination for 2,4-D, 2,4,5-T and silvex following single oral doses of 5, 5, and 1 mg/kg, respectively. It would help if the half-lives were all based on total excretion.

Joe LeBeau has suggested that we meet soon to discuss the data generated in this study. Milt Getzendaner is commenting separately on the need for more actual analytical data, such as on analyses of individual samples. We are also concerned about inclusion of a paragraph on page 16 which postulates that "the fraction of silvex not recovered in some subjects may represent metabolism of silvex and excretion in urine or bile of a heretofore unidentified metabolite(s)." The report also suggests that studies without a ^{14}C tag are of limited value. Such statements should not be made in reports which may be submitted to government agencies or used in court actions.

Marguerite

Marguerite L. Leng
Government Registration
Health and Environmental Research

abc

2503
DOW028637

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

November 8, 1978

Irving J. Selikoff, M.D.
Environmental Science Laboratory
Mt. Sinai School of Medicine
100th Street and 5th Ave.
New York, N.Y. 10029

Dear Dr. Selikoff,

It was good to talk to you yesterday. This letter is to confirm with you that Dow scientists are pleased to accept your invitation to come to Mt. Sinai and present a seminar on phenoxy herbicides on Wednesday, December 20, 1978.

It is my (our) understanding that you wish us to present the scientific data we have on 2,4-D, 2,4,5-T and related substances. The purpose is to acquaint you and your staff with the data so that you will be better able to plan and execute the proposed epidemiologic study in the Pacific Northwest.

The people we have selected to present the seminar are:

John Davidson, Technical Advisor to the Director of Research and Development, Agricultural Chemicals Department.

Bernard Schwetz, Director of Dow's Toxicology Research Laboratory in Health and Environmental Research.

Richard Kociba, Head of Pathology in the Toxicology Research Laboratory.

Benjamin Holder, Medical Director for Dow USA and Director of Biomedical Research in Health and Environmental Research

Warren A. Crummett, Technical Manager of Analytical Laboratories.

V. K. Rowe, Director of Toxicological Affairs, Health and Environmental Sciences (I may come along as moderator or whatever).

You asked if we had any objection to your inviting a few scientific colleagues from such places as NIEHS and NYU. Certainly not.

DOW 028638

I mentioned to you that Walter Melvin, Jr. M.D., M.P.H. and Sc.D. of the Institute of Rural Environmental Health, Colorado State University at Fort Collins might well be willing to contribute. Dr. Melvin is well informed and has had a wealth of human experience in the phenoxy herbicide area. He served with the Air Force for many years and has been directly involved in Viet Nam, with the disposal of the Air Forces' controversial inventory of Agent Orange, with the Seveso incident and presently is engaged in an environmental or epidemiological study of some sort in the Oregon-Washington area.

You asked what papers your people could benefit from reading before the seminar. I suggest the following:

1. Toxicology of Phenoxy Herbicides and Hazard Assessment of their Use in Reforestation by Frank N. Dost, Ph.D.
2. Long-term Hazards of Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans. WHO - IARC Technical Report No. 78/001, a report of the Joint NIEHS/IARC Working Group.
3. Chlorinated Phenoxy Acids and Their Dioxins. Ecology Bulletin No. 27 from Royal Swedish Academy of Sciences, C. Ramel (ed).
4. Embryo Problems Posed by the Seveso Accident. H. Tuchmann-Duplessis, M.D., LeConcours Medical No. 44, Nov. 26, 1977.

I have included copies of the Titlepage of the first three of these so as to help in identification. There are, of course, hundreds of papers but these reviews will give a good overall perspective and references to specifics. I am enclosing a copy of a translation of number 4 because I did not expect that you had seen it or might not be able to get it in time and I do think it is important.

I hope I have answered all the questions you asked. If you have specific items you would like to have discussed, let me know and I will do our best to include them.

With best personal regards,

VKR

V. K. Rowe, Sc.D.
Director, Toxicological Affairs
Health and Environmental Science
1803 Building
(517) 636-2376

Enclosure

krr

bcc: E. Blair
W. Crummett
J. Davidson
J. Donalds
P. Gehring
B. Holder
R. Kociba
B. Schwetz

P.S. As of now we are planning to take the Dow plane to New York and back on December 20. I will get you more details later.

16420

2510

DOW 0011 285

TOXICOLOGY OF PHENOXY HERBICIDES AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

Frank N. Dost, D.V.M.

Department of Agricultural Chemistry and
Environmental Health Sciences Center
Oregon State University
Corvallis, Oregon 97331

February 1978

16421

TOXICOLOGY OF PHENOXY HERBICIDES AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

Introduction 1

Toxicology of TCDD 2

- History of Human Exposure 2
- General Toxicity in Laboratory Animals 4
- Pathologic Morphology Resulting From TCDD Intoxication . . . 7
- Effects of TCDD Upon Reproductive Functions 9
- Carcinogenic and Mutagenic Potential of TCDD 11
- Absorption, Distribution, Metabolism, and Excretion of TCDD . 12
- Behavior of TCDD in the Physical Environment and in Submammalian Species 14
- Induction of Microsomal Enzymes 18

2,4,5-T 22

- General Toxicity in Laboratory Animals 22
- Effects of 2,4,5-T on Reproduction 25
- Carcinogenic and Mutagenic Potential of 2,4,5-T 28
- Absorption, Distribution, Metabolism, and Excretion of 2,4,5-T 29
- Behavior of 2,4,5-T in the Environment and Effects on Submammalian Species 32

2,4-D 35

- Toxicity to Humans 35
- General Toxicity to Laboratory Animals 36
- Effect of 2,4-D on Reproductive Function 38
- Carcinogenic and Mutagenic Potential of 2,4-D 39
- Absorption, Metabolism, Tissue Distribution, and Excretion of 2,4-D 40
- Behavior of 2,4-D in the Environment and Effects on Submammalian Species 42

Silvex 44

- Biological Effects of Silvex and Its Derivatives 44
- Effect of Silvex on Aquatic and Invertebrate Species 46
- Metabolic Fate of Silvex in Mammals 48

References 51

16422

TOXICOLOGY OF PHENOXY HERBICIDES AND 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

Frank N. Dost, D.V.M.

Introduction

The phenoxy herbicides 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxypropionic acid (silvex) are important agents for reforestation site preparation and for release of conifer plantings. To be used, the compounds must be inserted into a segment of the environment that may be occupied by humans or may have a potential for migration to points of human contact or consumption. This document is a review of the pertinent literature describing biological effects and environmental behavior of the three herbicides, and of 2,3,7,8-tetrachlorodibenzo-p-dioxin, an extremely toxic contaminant found in small quantities in 2,4,5-T and silvex.

For various reasons, the greatest public attention has been focused on 2,4,5-T and its unique contaminant, TCDD. Since 1969 public and scientific attention to TCDD has overshadowed the specific concerns about 2,4-D, which has no TCDD, and about 2,4,5-T and silvex, which are contaminated.

The unusual nature of the TCDD contaminant has made a special case of the herbicide 2,4,5-T, and has made it the subject of an unusually vehement public, political, and scientific controversy. An enormous scientific effort has evolved in an attempt to learn about the unique character of the compound, and to provide a rational basis for regulatory decisions. Hopefully, this effort to bring the existing literature into focus will aid in the decision-making process.

As matters presently stand, there is a division in the collective public and scientific minds on the safety and benefits of use of the phenoxy herbicides. As in any controversy, both views include unreasonable people, but the bulk of the argument is among sincere citizens and competent scientists on either side. It is my hope that the reviews and assessments in this document are dispassionate and without bias; otherwise it will be of limited utility.

In preparing this review, emphasis has been placed on information about the toxic properties of the respective agents. An assessment of hazard necessarily considers along with biological effects, factors governing the probability of exposure, and the probability that the chemical once contacted will enter the organism.

With the exception of the section on TCDD, I have chosen to touch only lightly upon the environmental behavior of the compounds. This information about phenoxy herbicides is well established and is much less complex than the behavior of TCDD. A more thorough treatment of the environmental behavior of TCDD is necessary here because: 1) this contaminant is clearly the most important of the four compounds under

16423

DOW 0014

study; 2) the high toxicity demands thorough understanding of the compound as it exists outside the target of toxic effect; 3) the environmental chemistry of TCDD is incompletely studied and subject to some controversy, whereas that of the herbicides themselves is well established.

The organization of this review and assessment includes sections reviewing the scientific literature on TCDD, 2,4,5-T, 2,4-D, and 2,4,5-TP (silvex), an assessment of the hazard potential of their distribution in the environment, recommendations on some aspects of methods of use of the herbicides and recommendations of needed research. A shortened version of the review and assessment is also attached. Organization within the review differs slightly among the chemical sections because of differences in research emphasis or the character of the agent itself. In certain instances, for example in considering the enzyme induction capacity of TCDD, an area may be dealt with that is not entirely germane to the issue of hazard but of considerable importance in understanding the biology of the compound.

A number of useful reviews of interaction of phenoxy herbicides or TCDD with animal systems have been or are being published. The most recent one by Leng (1977), discussing metabolic fate of phenoxy acids in domestic animals, Mercier (1976) in response to the Seveso accident which spread large amounts of TCDD through several Italian communities, and McQueen et al. (1977) refuting alleged association between herbicide use and fetal abnormalities in humans. McKenzie et al. (1975), Norris et al. (1972), Buhler (1977), and the EPA position paper arising from the Dioxin Working Group (presently in draft) are also all of considerable value.

TOXICOLOGY OF TCDD

History of Human Exposure

Probably the first clinical description of human TCDD exposure arose from the study by Kimmig and Schulz (1957) of workers in chlorophenol factories in Germany. The principal symptom was a persistent skin lesion (chloracne; so-called because it is characteristic of a number of related compounds and was at one time thought to be caused by free chlorine.

The active agent was established as TCDD, and animal studies showed that the same lesions appeared on rabbit ears after application of as little as 0.002% TCDD (Kimmig and Schulz, 1957). High topical doses and oral doses of 50 µg/kg or greater caused liver necrosis. A similar industrial intoxication in France was reported by Dugois and Colomb (1957). Pathology of the skin lesions is well described by Kimbrough (1974).

A study of 29 phenoxy herbicide workers by Bleiberg et al. (1964) disclosed chloracne and a high frequency of uroporphyrinuria, and tissue deposition of porphyrins related with hyperpigmentation and skin fragility. This assemblage of symptoms is commonly called porphyria cutanea tarda. The excessive production and excretion of porphyrins apparently results from a hyperactivity of the enzyme δ-amino levulinic acid (ALA) synthetase, the first and rate limiting step in porphyrin formation. Porphyrins are

16424

DOW 0011 789

eventual components of heme proteins, such as hemoglobin and cytochromes. Effects on ALA synthetase and other enzyme systems will be discussed in another section. Poland et al. (1971) examined the same factories later, after somewhat more satisfactory industrial hygiene measures were established. Among the 73 male workers observed, no clinical porphyria was apparent even though some degree of chloracne still persisted in most of the subjects. There was also apparently a significant psychological effect as measured by the Minnesota Multiphasic Personality Inventory.

The absence of porphyria and persistence of chloracne in the later study has led Poland et al. (1971) to conclude that while both may be caused by TCDD, the mechanisms are probably dissimilar.

Several severe accidental exposures to TCDD have occurred. In several cases, runaway chlorophenol plant reactions caused widespread exposures within the factory or in the surrounding community. An accident in Germany in 1953 exposed a large number of workers, said to have suffered liver, kidney, splenic, eye, cardiovascular, and nervous symptoms. A worker who apparently was in extensive contact with a contaminated segment of the plant five years after the accident became ill and later died. An explosion in a Dutch plant in 1963 released 30-200 g of TCDD into the main gallery of the factory exposing 50 people. In the subsequent two years, four died, but no clear association with TCDD intoxication could be shown (Hay, 1976). An explosion in a British plant in 1968 resulted in 79 cases of chloracne (May, 1973). In Czechoslovakia a pentachlorophenate production stream overheated under pressure, producing large amounts of TCDD (Jirazek et al., 1974). (It should be noted in this and some earlier German work a different numbering convention was used. 2,3,6,7-TCDD and 2,3,7,8-TCDD are the same compound.) Of 80 exposed workers, 76 developed chloracne, porphyria cutanea tarda, disorders of plasma lipids, hepatic damage, neural lesions, and neurasthenia.

An explosive reaction in a plant at Seveso, Italy, in 1976 scattered TCDD, apparently in kilogram amounts, over a wide area that included several communities. Details are still not widely disseminated but great numbers of animals were killed and many people were made ill. A review of the incident was prepared by Mercier (1976) a few months after the incident but the slow onset and persistent effects did not permit a detailed assessment of the damage. Since that time the Seveso disaster has been given unprecented publicity and as might be expected this tragedy has been sensationalized in press coverage, in part because there may have been an effort to minimize or cover up the extent of the problem. There were apparently defects in the administration of the plant that may have resulted in the accident itself, and management of public warning and information early after the accident was questionable. Further contributing to the extensive public attention and sensationalizing of the incident is a virtual absence of objective reporting in the scientific literature. As far as I am aware, there has been no scientific evaluation of the aftermath in publication since the review by Mercier (1976), shortly after the accident.

In the U.S., TCDD extracted from hexachlorophene production was stored in oil, then mistaken for waste lubricating oil and sprayed on several horse arenas and farms in Missouri, for dust control. The

16425

incidents occurred in spring and summer of 1971. On one horse breeding farm, 62 of 85 horses exercized in the sprayed area became ill and 48 died, the last in January, 1974, two and a half years later. Hundreds of birds and rodents died, along with a number of dogs and cats. Several children were exposed through play in the arena soil. One developed a severe hemorrhagic cystitis and others showed clear evidence of chloracne. All recovered. The TCDD concentration in the soil was later established at more than 30 ppm, a truly massive amount. (This concentration amounts to 120 lb per acre per foot of depth, assuming 4×10^6 lb soil/acre-foot.) The details of the latter event are described in detail by Carter et al. (1975) and Kimbrough et al. (1977).

All of these exposures were to a mixture of TCDD with other agents. Possibly the only published account of exposure to pure TCDD is the description by Oliver (1975) of laboratory contact by three individuals associated with synthesis experiments. The individuals all had utilized routine protective procedures but sufficient contact developed nonetheless. The exposures were not sufficient to cause porphyrinuria or liver damage but serum cholesterol was raised. In two cases personality changes and neural disorders developed two years after exposure. The amount of TCDD contacted is not known.

General Toxicity in Laboratory Animals

This section is a review of data on effects of TCDD other than reproduction and teratology, carcinogenesis, and its enzyme inducing properties upon mammals. These will all be considered independently, as will studies of tissue distribution and metabolism.

Schwetz et al. (1973) have determined lethalities for several species. The guinea pig is exceedingly sensitive, with an LD_{50} of only 0.6 µg/kg; rats are less sensitive; LD_{50} for males is about 20 µg/kg and for females the LD_{50} is in excess of 40 µg/kg. Rabbits are still less sensitive at more than 100 µg/kg. Dogs were found to require even higher effective doses. In these studies, responses of mice were highly erratic and were not included. McConnell et al. (1977a) have determined a single 30 day LD_{50} for mice of 284 µg/kg, and 2 µg/kg for guinea pigs.

An important characteristic of TCDD is a very long delay before lethality, often 2-3 weeks and occasionally 7-8 weeks.

The pathology associated with TCDD toxicity may include thymic atrophy, liver necrosis, and degeneration in the kidney and thyroid. The guinea pig is unique in that it suffers limited hepatic damage, even at doses that cause substantial thymus depletion (Gupta et al., 1973). The degree of thymic atrophy is generally dose dependent; the studies of Gupta et al. (1973) did not establish a zero effect dose, but the lowest dose in the rat of 1 µg/kg/day for 31 days caused moderate thymus and liver damage, and kidney and thyroid degeneration. Guinea pigs treated with 0.2 µg/kg weekly for eight weeks had limited thymus damage and no other pathology. Studies of guinea pigs at lower doses suggest a no observed effect level at 0.008 µg/kg/weekly for eight weeks, a total of 0.064 µg/kg, although there was a slight decrease in lymphocytes at that dose (Vos et al.,

DOW 0011 791

1973). Animals given tetanus toxoid at the fourth week to measure humoral immunity were found to have somewhat lower thymus weights than controls, although the difference was not significant.

According to the data of Kociba et al. (1976) the no observed effect level in rats given TCDD in the diet 5 days a week for 13 weeks is 0.001 µg/kg/ day, totaling 0.065 µg/kg, almost identical to the value found in guinea pigs. Again, thymus weight appeared to be the most sensitive parameter. The Kociba study dealt with a very broad range of clinical, chemical, and hematological measurements. At a dose of 1.0 µg/kg/day, 2 of 10 rats died and weight gain decreased sharply, erythrocyte numbers and volume decreased, reticulocytes increased in males. In females thrombocytes decreased and total leukocytes increased. Differential counts were not altered. Urinary porphyrins, creatine and δ amino levulinic acid (ALA) all increased. Serum bilirubin, BUN and alkaline phosphatase also were elevated. At an intake of 0.1 µg/kg/day red cell numbers and volume were still slightly decreased in males and porphyrin, ALA, bilirubin, and alkaline phosphatase was elevated in females. No changes in blood parameters occurred below these levels.

In a similar study Zinkl et al. (1973) found no changes in SGPT, bilirubin, or erythrocytes after 31 days of treatment at 1.0 µg/kg/day and no change in SGOT, cholesterol, or serum protein at 0.1 µg/kg/day. There was a modest depression in blood glucose at the lower dose rate.

Mice treated with 1.0 µg/kg/week for six weeks were found to have depleted thymus weight (Vos et al., 1974) but no changes were evident at the lowest dose, 0.2 µg/week. Hematological changes occurred only at a dose of 25 µg/kg/week, for six weeks. Some changes in serum proteins did occur at an intake of 0.2 µg/kg/weekly. Unfortunately, there is no data suggesting the magnitude of the no-effect single dose in any species.

Studies in primates have been limited. McNulty administered TCDD at a rate of 20 parts per billion in the diet to a single monkey and caused its death in a few days. A 10-fold lower concentration permitted survival for about two months. Total intake was estimated as about 6 µg/kg (McNulty, 1977). A study of eight female Rhesus monkeys fed 500 parts TCDD per trillion has recently been completed in the laboratory of J.R. Allen. The animals showed dermatitis and menstrual irregularities within six months after initiating treatment. One monkey died prior to the seventh month and by nine months of exposure, all survivors experienced a severe anemia and leukopenia. Food intake was normal but all of the animals lost weight. After 11 months, five of the eight monkeys died, all with severe anemia and destruction of blood forming tissues. The total dose was estimated at about three µg/kg (Allen et al., 1977a, 1977b) or about .3 µg/month.

A number of questions arise from these data. The monkey studies are limited, but they do suggest the possibility that the lethal dose is similar regardless of the time over which it is administered. The implications are quite interesting. If it is true that TCDD dosage is time independent in the monkey, then the effect is truly cumulative. No chemical is known to have such properties, and if TCDD behaves in this fashion,

16427

DOW 0011792

any estimate of acceptable dose must assume additive effects over long periods. TCDD does not remain in the body of monkeys (Van Miller et al., 1976) or other species (Van Miller et al., 1976; Fries and Marrow, 1975; Rose et al., 1976). A cumulative effect, if it exists, must then entail irreversible effects remaining after the molecule leaves active sites. TCDD is apparently not metabolized to a significant extent (Rose et al., 1976; Vinopal and Casida, 1973; Van Miller et al., 1976) so it is conceivable as well that a single molecule could interact several times before leaving the body. A possible mechanism may be intercalation of the planar TCDD molecule into DNA (Hussain, 1973), production of misinformation and departure of the agent to interact at another DNA site.

Data in other species, especially the guinea pig do not support the idea of extensive cumulative effect. A dose rate of 0.2 µg/kg/weekly for eight weeks provided a total dose almost three times the LD_{50}, but no deaths occurred and organ changes, while measurable, were not severe (Vos et al., 1973). Rats also accepted about three times the LD_{50} of 20 µg/kg over 13 weeks, without lethality. Some mice, on the other hand, died at a total dose less than the acute LD_{50}, distributed over a four week period (Vos et al., 1974).

There are a few other biological responses to TCDD that appear after high doses of from 10-25 µg/kg. One of the more interesting is a depressed capacity for biliary excretion. TCDD sharply decreases removal of PCBs from the liver via the bile (Yang et al., 1977) and decreases plasma clearance of ouabain into bile (Hamada and Peterson, 1977). In the latter work, steroidal inducers of microsomal enzymes reversed the TCDD effect. Whether this effect has potential significance in the response to secondary toxicity can only be speculated upon. In any case the doses required for effect are quite high and probably not attainable under realistic environmental conditions.

Because TCDD causes increased microsomal foreign compound metabolizing activity in the kidney cortex (Fowler et al., 1975, 1977), Pegg et al. (1976) evaluated proximal tubular function in intoxicated rats. They found little change at any but large doses (25 and 50 µg/kg).

There have been few observations of metabolic changes caused by TCDD. Incorporation of ^{3}H-sodium acetate into liver lipid fractions seems to be impaired at TCDD doses above 0.1 µg/kg (Cunningham and Williams, 1972), but the significance of this effect has apparently not been explored further.

Evidence of thymic atrophy after TCDD treatment has stimulated more direct study of effects upon the immune responses. Vos and Moore (1974) and Vos et al. (1973) found lymphocytic depletion in the cortex of the thymus and depressed cellular immunity in rats and mice exposed during gestation and the post-natal period. Guinea pigs were similarly affected (Vos et al., 1973). Thigpen et al. (1975) attempted to translate the effect into terms of infectivity and found that doses of 1 µg/kg or more increased mortality and time to death of mice infected with Salmonella bern. Course and severity of disease due to pseudorabies virus was not altered by TCDD. With all the study of TCDD effects, no pattern of

16428

DOW 0011 793

pathology, tissue distribution, and biochemical or physiological change has emerged that is consistent enough to suggest a mechanism of lethal effect. McConnell et al. (1977a) quote papers in preparation by Van Logten et al. which show no association of death in TCDD intoxication with adrenal or pituitary hormones or malnutrition.

Pathologic Morphology Resulting From TCDD Intoxication

Much of the more pronounced tissue change resulting from TCDD has been observed following high acute doses of the compound. There is some question whether the acute damage seen represents that observed in a low level longer term experiment or in a field exposure.

Before TCDD as such became an issue Norback and Allen (1969) studied the membrane changes in liver following ingestion of hexachlorohexahydrophenanthrene, a constituent of so-called "toxic fat." The lesions found under electron and light microscopy anticipated the damage later to be found after TCDD. Most striking was a great increase in the smooth endoplasmic reticulum (SER), with reorganization into a concentric arrangement, and the rough ER became folded and rolled over a period of days to form dense arrays of concentric separate, not spiraling, paired membranes. The authors speculate that SER proliferation in response to intoxication is related to acceptance of enzymes synthesized by the RER. The increased membrane mass may also be a means of sequestering chlorinated hydrocarbon in the lipid phase of the membrane in close proximity to foreign compound metabolizing enzymes. (This paper contains very useful schematic representations of the membrane development sequence.) The same authors later reported similar lesions in rats following administration of 1 µg TCDD/kg/day for 21 days (Norback and Allen, 1973). They found hypoplasia of lymph tissue and bone marrow with progressive anemia and leukopenia. The resultant reduced resistance caused substantial losses due to infection. Monkeys and chickens in the same study were much more sensitive in the latter parameters than rats. They also developed extensive fluid accumulation in all body cavities, probably due to decreased osmotic activity of blood due in turn to serum protein decreases. (Relative sensitivity of chickens has also been noted by Greig et al. (1973).) Monkeys were subject to extensive skin lesions, apparently corresponding to the chloracne of humans, but rats and chickens did not suffer skin damage. Monkeys and chickens also exhibited degeneration of the testes and decreased activity of seminiferous tubules (see also Allen et al., 1975).

Large doses of TCDD (single doses of 50 or 100 µg/kg; 16 to 31 daily doses of 10 µg/kg) to the rat caused severe liver and thymus damage, with icterus and disseminated hemorrhages, especially in the myocardium. The liver cells increase in size and cellular regenerative attempts occur. Renal tubule cells became vacuolated. No pathological change occurred at doses of 0.1 µg/kg/day over 31 days, at 1.0 µg/kg weekly for 6 weeks or 5 µg/kg in a single dose (Gupta et al., 1973). Fowler et al. (1973) studied the progressive changes in rats over 28 days following a single dose of 5 or 25 µg TCDD/kg. Increased SER, roughly dose responsive, was evident by day three, especially in cells adjacent to biliary drainage. Both RER and SER were greatly increased by day 9, SER was almost at normal levels by day

16429

DOW 0011 794

16, and both were indistinguishable from those of control animals by day 28. Jones and Butler (1974) carried out a similar experiment with rats given a single 200 µg/kg treatment. (A massive dose, considering the minimum effective dose.) Most change was found in centrilobular cells, with degeneration and necrosis of parenchymal cells evident within a week after treatment. They suggest that the multinucleate cells seen in increasing numbers through two months after treatment are formed through membrane disturbance and coalescence of hepatocytes. By 10 weeks some recovery was evident but fibrosis of central veins and sinus dilation still prevailed. No mention of lethality was made, although the dose was on the order of five (female) to ten (male) times the LD_{50}. Longer term treatment of rats (0.001-1.0 µg/kg/day, 5 days weekly x 13) caused almost complete thymus involution at a dose rate of 1.0 µg/kg/day, somewhat decreased cortical thymocyte and lymphoid cell numbers after 0.1 µg/kg/day. Splenic morphology was essentially unchanged at 1.0 µg/kg/day. The latter dose also caused some gross edema, and one of five males had decreased spermatogenic activity. Females at the highest dose tended toward formation of cuboidal epithelium in the uterus and decreased size and numbers of corpora lutea. Ovarian tissue was also abnormal. At all lower doses no effects were observed (Kociba et al., 1976). Two year continuous feeding studies at TCDD intakes of 0.001 to 0.1 µg/kg/day have just been terminated and histopathologic evaluation is not complete. Gross examination showed that in some rats given 0.1 or 0.01 µg TCDD/kg/day there were increased numbers of nodules in the liver (R.J. Kociba, Dow Chemical Company, preliminary status report; personal communication).

Lesions generally similar to those seen in rats were found in mice given single oral doses up to 200 µg/kg and weekly doses up to 25 µg (Vos et al., 1974). At a dose rate .1 µg/kg/week for 6 weeks decreased thymus weight occurred. At 25 µg/kg/week, serum globulins were decreased, and porphyria and bile duct epithelial proliferation occurred. Similar changes were also reported following treatment of mice with an LD_{50} (30 day) TCDD (McConnell et al., 1977a).

McConnell et al. (1977b) treated rhesus monkeys with heavy single doses of TCDD (in excess of 70 µg/kg) which were fatal in every case. Integumental changes were pronounced, anemia and lymphopenia and an increased neutrophil count followed treatment. Liver, adrenal and kidney appeared to increase in weight relative to body weight, but this effect may have been due to general wasting. With the skin hyperplasia, similar changes occurred in the epithelium of some hollow organs. The major difference from effects in similarly treated rats was the absence of significant liver lesions.

Chronic low level intoxication (500 ppt in diets, about 0.01 µg/kg/day) of monkeys until death at 7-12 months caused anemia and pancytopenia, with extensive hemorrhage. Bone marrow and lymphatic tissues were hypoplastic, and epithelial structures were hyperplastic (Allen et al., 1977).

The pathological changes resulting from absorption of TCDD by horses and cats during the Missouri horse arena episode have been recently described in detail by Kimbrough et al. (1977). The most pronounced and

DOW 0011 295

consistent findings were the generalized hepatic fibrosis and necrosis and gastric ulceration. There was an appreciable incidence of tubular degeneration and abscesses in the kidney and capsular thickening of the spleen. Lesions in cats were generally similar.

Effects of TCDD Upon Reproductive Functions

Of the many toxic responses to TCDD, the potential for teratogenicity has received perhaps the greatest notoriety. The early findings of 2,4,5-T teratogenicity raised a considerable public consciousness and the finding that the herbicide was contaminated by high levels of TCDD, also found to cause fetal malformation, focused additional attention on the contaminant. In evaluating this aspect of TCDD hazard it should be noted that the teratogenic potential of a toxicant can be considered in two ways. The absolute teratogenic dose of TCDD to the pregnant animal on a body weight basis is very low, as is true of all TCDD effects. A more realistic way of relating the teratogenic potential, however, is by comparison with the dose required to cause general toxic effects in the mother. For example, in the guinea pig, TCDD teratogenesis has not been studied because the teratogenic dose is apparently higher than the lethal dose.

This section is limited to studies of relatively pure TCDD upon reproductive function in experimental animals. There is reference elsewhere to reproductive studies of 2,4,5-T and silvex, which contain at least some TCDD.

Teratogenic defect resulting from TCDD are rather specific in experimental animals; the principal effects are increased frequency of cleft palate (reported only in mice) and an abnormality in which the central collection region of the kidney becomes enlarged, with an associated fluid accumulation (Neubert et al., 1973). Limited limb deformation may be seen in a few animals (Sparschu et al., 1971). Intestinal hemorrhage is also seen; this effect is not teratogenic but rather is direct toxic consequence of fetal exposure to TCDD. Fatty degeneration of fetal livers is in the same category (Becker, 1974) as is subcutaneous edema and delayed ossification.

In teratogenicity studies of rodents the usual procedure is to administer intoxicant at some point during, or throughout days 6-15 of pregnancy, which covers the organ forming period. Sparschu et al. (1971) found that the lowest effective dose of 1.25 µg/kg/day caused some fetal mortality, resorptions, and intestinal bleeding. Skeletal abnormalities were limited to delayed ossification. Renal defects tended to occur at about the same frequency regardless of dose. These changes are all a result of direct toxicity to the fetus and are not developmental. Intestinal hemorrhage has been reported in rat fetuses at maternal dose rates of 0.25 µg/kg/day (Khera and Ruddick, 1973). At higher doses up to 8 µg/kg/day the same kind of effects occurred with greater frequency; 0.5 µg/kg/day caused decreased maternal weight gain and higher doses resulted in severe toxicity.

Courtney and Moore (1971) examined rats and three mouse strains and found that a dose of more than 1 µg/kg/day was required to produce kidney

16431

DOW 0011 796

defects in rats, and the cleft palate and kidney changes in mice appeared at about 3 µg/kg/day, over days 6-15 of gestation. The C57B1 mouse strain was much more sensitive than the others tested. A somewhat larger group of NMRI mice showed a cleft palate frequency of about 3% (Neubert and Dillman, 1972), similar to the low frequency groups of Courtney and Moore. The period of maximum sensitivity for TCDD is at the eleventh day of gestation, as distinguished from dexamethasone, for example, which is most effective at day 13 (Neubert et al., 1973).

Continuous administration of 0.001 µg/kg/day to male and female rats for 90 days prior to mating and through weaning of offspring produced no effect on fertility (Murray et al., 1977). The study continued through three generations, with each preceeding generation sacrificed at weaning of its offspring; the third generation was maintained until two years from the beginning of treatment of the f_0 generation. Toxicity and poor litter survival dictated termination of feeding at 0.1 µg/kg/day; 0.01 µg/kg/day caused decreased fertility in generation f_1 and f_2 but not f_0. f_2 and f_3 litters were smaller and growth and survival were decreased after treatment at 0.01 µg/kg/day, but no pathological change was observed in liver, kidney, or thymus.

More recently, Smith et al. (1976) have treated CF-1 mice at doses ranging from 0.001 µg to 3.0 µg/kg/day through days 6-15 of gestation. There was no maternal toxicity at any dose. Cleft palate appeared at 1 µg, renal dilatation appeared at 3 µg/kg/day. No significant effects occurred at 0.1 µg.

A recent report by Courtney (1976) described results of treating CD-1 mice with high doses of TCDD (25-400 µg/kg/day) on days 7-16 of gestation. These very high doses caused no maternal deaths during the gestation period, but resulted in high fetal mortality. The nature of observed terata were typical of TCDD intoxication, but the enormous doses render the study useless for hazard evaluation. However, the substantially greater incidence of terata after subcutaneous administration, compared with that following oral TCDD suggests that hepatic metabolism may affect TCDD more than has been believed. A dose rate of 25 µg/kg/day orally caused 3% incidence cleft palate; the same dose subcutaneously caused 82% incidence of cleft palate.

There has been little research on effects of TCDD on primate reproduction. Experimental animals are in limited supply and the numbers of offspring are so low that a satisfactory study is almost impossible.

Allen et al. (1977) fed TCDD to eight female rhesus monkeys at a concentration of 500 ppt in the diet, which provided an intake of about 0.3 µg/kg/month. Skin lesions, endocrine, and menstrual changes occurred in all of the subjects by 3 months. Six females of the eight were bred after 7 months of treatment; one monkey died before mating and another was excluded from the reproductive study. Three of the animals conceived; two aborted, and one completed a normal pregnancy. Two of the three barren animals were bred four times, the other was bred twice. This dose rate is on the order of 0.01 µg/kg/day and comparison with the rat study of Murray et al. (1977) suggests that reproductive toxicity in monkeys may be somewhat greater than in rats, but is by no means proportional to the lethal dose.

16432

DOW 00117797

Carcinogenic and Mutagenic Potential of TCDD

Van Miller and Allen (1977) have evaluated pathological changes in rats fed TCDD in dietary concentrations ranging from 1 ppt to 1000 ppb, beginning at animal weights of about 60 g. Estimated weekly intakes of TCDD ranged from 0.0003 µg/kg at a dietary level of 1 ppt to 2 µg/kg at 5 ppb. Actual intake of TCDD at dietary concentrations of 50-1000 ppb was not clear. At 65 weeks the abdominal viscera of surviving animals were examined surgically and any observed tumors were biopsied. The diet with TCDD was then continued through the 78th week and all surviving animals sacrificed at 95 weeks.

There were 10 animals at each dose range; all those at 50-1000 ppb TCDD died by the fourth week. In the group given 1 ppt there were no tumors, but of those fed 5, 50, 500, 1000, and 5000 ppt 23 of 50 had developed tumors of several types. No control animals for this or a parallel experiment developed neoplasms. The great variety of tumors suggested to the authors the possibility that TCDD is a promoter rather than inducer of neoplastic activity, in view of the usual narrow spectrum of tumors caused by many chemical carcinogens. General pathologic changes have been described in a previous section.

Two studies of carcinogenesis in rats have utilized continuous feeding of TCDD over a two-year period. One program has been completed but the analysis of histopathology and gross tumor incidence have not been completed. The other study, by Dow Chemical Company, has been completed and a preliminary report filed with EPA. Dietary levels were 21, 210, and 2200 ppt TCDD. The maximum dose caused increased tumor incidence in some organs and decreased incidence in others. There was substantial mortality resulting from general toxic effects. The intermediate dose caused moderate systemic toxicity but no tumors and the lowest dose was without effect other than slight liver changes such as induction of increased drug metabolizing capability. These latter changes were seen only in females (J. Davidson, personal communication). The reasons for differences between the Dow and Van Miller studies is not apparent; it is paradoxical that the Dow work had a higher incidence of tumors in control animals, while showing a lower incidence of effect in treated animals.

Cytogenetic studies of TCDD by Green and Moreland (1975) suggested that the dioxin does not have potential for producing chromosomal abnormalities in bone marrow. The dose schedule provided up to 15 µg/kg/daily for 5 days, with sacrifice on day 5. A single injection experiment with sacrifice at four weeks was also negative. Khera and Ruddick (1973) were also unable to find mutagenic activity of 2,4,5-T.

Since it is not possible to obtain an absolutely TCDD-free preparation of 2,4,5-T, the studies of the herbicide may also be of value in considering TCDD effects. Effects of 2,4,5-T and TCDD on dividing African blood lily endosperm cells are not explicitly pertinent to animal studies, but the relation shown between herbicide and contaminant is of interest. Highly purified 2,4,5-T at 10^{-4} M was not effective, but 0.2

DOW 06-1 798

µg TCDD/L with or without 2,4,5-T, and commercial 2,4,5-T all caused dramatic mitotic inhibition and chromosomal aberations (Jackson, 1972) (0.2 µg/L = 200 ppt).

Hussain (1972) found that TCDD was somewhat mutagenic using the Ames procedure at doses that were intrinsically very toxic to the organisms. The authors suggested that the limited mutagenic activity was by intercalation of TCDD into DNA. Seiler (1973) has also obtained data suggesting that TCDD is mutagenic in the S. typhimurium TA 1532 strain. There was no indication of the concentrations necessary to produce effects.

Absorption, Distribution, Metabolism, and Excretion of TCDD

The biological action of a foreign compound is highly dependent on its logistics. A chemical must obviously be absorbed from the environment in order to interact, and it must be carried in blood to cells and move to or across cell membranes to sites of reaction. The agent in its original form may interact with sensitive systems, or it may be converted to an active derivative after entering an organism. Such conversion usually takes place in the liver. A given organ or cell type may have a particular affinity or sensitivity for a toxicant and thereby be singled out for attack. The chemical nature of the agent or its product will also dictate the extent and rate of excretion in urine or bile or respiratory gases. Even with an active excretion mechanism such factors as sensitivity of bladder epithelium or an active enterohepatic circulation may result in toxic effects after elimination seems to have been accomplished.

In spite of the enormous capacity for induction of mixed function oxidases (discussed below) TCDD is metabolized to a very limited extent. Rats given a single dose of 50 µg/TCDD-U-^{14}C/kg by stomach tube excreted about 30% in feces during the two days following treatment, and then 1-2% daily over the 19 days following, to a total of 53%. Urinary-^{14}C was about 13% and respiratory excretion totalled 3.2%. After the initial 2 days, total clearance half-time (T/2) was about 17 days. Residual TCDD at 3, 7, and 21 days was greatest in liver and fat (Piper et al., 1973). The long residence time and limited respiratory and urinary excretion suggested that TCDD was essentially unchanged but this was not verified. As a general rule lipophilic compounds are either sequestered in fat, eliminated as conjugates in bile, or converted to a water soluble form and excreted by kidneys. Absence of urinary excretion therefore implies limited conversion. In a later study of rats given a lower single dose of 1 µg/kg, TCDD was identified in feces but not urine, and again, liver and fat contained the highest concentrations. T/2 was 31 days (Rose et al., 1976). In the same investigation, repeated doses 5 days a week for 7 weeks resulted in some urinary loss, but most TCDD was excreted into feces. In this program of essentially continuous intake, T/2 was calculated as 24 days. The residual radioactivity in the liver was identified as unchanged TCDD. The report of Vinopal and Casida (1973) is suggestive that TCDD does not metabolize only because after giving a dose of 130 µg TCDD-^{3}H/kg to mice, no mention was made of tritium in the urine. Allen et al. (1975) found that over a 25 day period about 4.5% of a dose of 50 µg TCDD/kg appeared in urine. The daily fraction of total intake excreted gradually increased, which seems indicative of an unusually stable molecule, because at least a modest exchange of tritium with body water should be expected.

16434

DOW 0011799

The variety of indirect evidence indicating that TCDD is not metabolically altered must be considered in the perspective of other indirect evidence that metabolic change may occur. Beatty and Neal (1975) have shown that pretreatment with phenobarbital decreases and castration increases TCDD toxicity. The former treatment increases and the latter diminishes drug metabolizing capacity. The teratology study of Courtney (1976) also is suggestive that liver metabolism of TCDD occurs. An oral dose of 25 µg/kg/day through the organogenesis period caused only 3% cleft palate among the experimental fetuses, but subcutaneous administration of that dose resulted in 82% cleft palates. In view of the apparent affinity of TCDD for the increased endoplasmic reticulum following induction (Van Miller et al., 1976; Poland et al., 1976), it is possible that induction merely increases the capacity for sequestration of TCDD in a temporarily non-active system, but the remarkable difference seen by Courtney is difficult to ascribe to distribution differences only.

The selective distribution of TCDD into liver and fat has also been shown by Fries and Marrow (1975), Allen et al. (1975), and Van Miller et al. (1976) in rats and by Van Miller et al. (1976) in primates. The liver TCDD appeared to be sequestered on smooth endoplasmic reticulum (SER), principally that induced by presence of the toxicant (Allen et al., 1975). While liver storage of TCDD in monkeys was much lower than in rats, the difference appeared to be due to the characteristic lesser proliferation of SER in the monkey that has been described by Van Miller et al. (1976). Considering the difference between species in total induced ER membrane, TCDD appeared to bind to endoplasmic reticulum to about the same extent in both species (Van Miller et al., 1976).

Unlike the behavior of other chlorinated hydrocarbons in fasted or underfed animals, TCDD is not lost from adipose tissue with fat mobilization (Allen et al., 1975). In the case of PCB, for example, the fat soluble chlorinated hydrocarbon is liberated with mobilized fat and eventually may reaccumulate in the liver or other target organs. The reason for the difference is not clear, but it seems possible that TCDD may nonspecifically partition into fat, then selectively bind to adipose cell membrane protein. Liver TCDD residues do decrease during fasting, possibly because increased gluconeogenesis makes demands on available labile protein including the endoplasmic reticulum to which the TCDD may be bound. Hepatic subcellular distribution has been further defined by Poland and his associates (Poland and Glover, 1974, 1975; Poland et al., 1976; Nebert et al., 1975); and by Chhabra et al. (1974) in studies in which the genetically determined susceptibility for TCDD induced benzpyrene hydroxylase induction was found to correlate directly with the extent of TCDD binding in liver.

The T/2 for TCDD residence in the body has ranged from about 16-20 days for single dose experiments (Piper et al., 1973; Allen et al., 1975), although Rose et al. (1976) arrived at a figure of 31 days. When TCDD was administered in the diet over 12 days at dose rates of about 0.5 and 1.5 µg/kg/day, T/2 was found to be 12 days for males and 15 for females (Fries and Marrow, 1975). Rose et al. (1976) calculated a half-time of 24 days after a seven week feeding at dose rates of 1-0.01 µg/kg/day.

16435

DOW 00011 800

The concentration of TCDD reaches a maximum at a given dose rate. Fries and Marrow (1975) found that after 6 weeks of administration retention approached a steady state which would be 10.5 times the daily intake. Rose et al. (1976) found similarly in 7 weeks that a steady state concentration of a little more than 10 times the daily intake could be predicted, agreeing remarkably well with the Fries and Marrow estimate.

The difference in distribution between primates and rats is of interest. Seven days after equivalent doses of TCDD-^{3}H, livers of adult monkeys contained 0.09% of total dose/gm tissue, rats contained 4.54% (Van Miller et al., 1976). In adipose tissue the relationship was monkey, 0.16, rat 3.46, suggesting that storage or retention is not strictly related to binding on inducible membranes. In terms of total body distribution, the rat liver contained 40% of the dose and that of the monkey, 10%. This is a much closer ratio (4/1) than that of liver concentrations (4.54/.09). Skin as a whole stored a much higher percentage of administered TCDD, because of the much higher amount of adipose tissue, in spite of a concentration ratio (3.5/0.16) that was also quite unfavorable.

Whether these differences bear any relation to differences in sensitivity to toxic effects is not known. The difference in liver is at least associated in amount and time with the much greater capacity for induction in the rat. There is no data available to indicate whether the thymus of species like the guinea pig, which is subject to more extensive TCDD induced thymus damage, has a selective affinity for the toxicant as does the liver in the rat. The thymus of infant monkeys is expected to be highly active, but appeared less retentive compared with other tissues, while the adult rat thymus accumulated more TCDD/unit weight than most organs (Van Miller et al., 1976), further confusing this issue because liver damage is thought to be the primary lesion in the rat.

Each of the single administration experiments utilized heavy doses of TCDD, taking advantage of the long delay in onset of symptoms and death to obtain improvement in radiochemical detection capability. Studies in which 400 µg/kg (about 20 x LD_{50}) were administered (Van Miller et al., 1976) produced liver concentrations of about 3.6 ppm. Kociba et al. (1976) caused discernable but minor hepatic lesions at a total dose of 0.65 µg/kg given over seven weeks. The expected concentration of TCDD in liver at this intake is on the order of 20 ppb (Rose et al., 1976). A ten-fold lower dose caused no histopathologic damage. It is not surprising therefore that the beach mice studied by Young et al. (1974) which accumulated 300-500 ppt TCDD in their livers, did not show liver morphologic lesions.

Behavior of TCDD in the Physical Environment and in Submammalian Species

The extremely high toxicity of TCDD and the very small amounts of the agent present in 2,4,5-T and in the environment present a problem that is different less in philosophy than in scale. There is a tendency on the one hand to dismiss TCDD as a pollutant because the amount available at any one point or even sector is minute, and on the other to assume that any amount in excess of true zero residue is catastrophic, because of the enormous toxicity of the material. The amount of TCDD distributed on vegetation after a typical treatment is so small as to defy direct analysis and the quest for residues in biological materials has forced analytical

16436

sensitivity closer to zero than has been the case with any other pollutant. In spite of these unique characteristics, TCDD must be considered in the same way as any other chemical: How much is present in the human environment? How persistent is it? Can it move to unintended targets? If so, will the amount assimilated by humans or other organisms reach harmful proportions?

Present manufacture of 2,4,5-T contains about 0.02 parts TCDD per million parts 2,4,5-T, although manufacturers claim only about 0.05 ppm. Some lots contain only about 0.01 ppm. A two pound per acre application of 2,4,5-T will, therefore, distribute a little less than 20 µg TCDD/acre. It is necessary to predict what may be expected to happen to that material by examining existing laboratory data.

The environmental persistence of TCDD is highly dependent on the conditions to which it is exposed. When mixed into soil, degradation was found to be very slow, with a half time of one year (Kearney, 1972). Because the material was introduced as an acetone solution there has been some question about possible crystallization and limited access of TCDD to soil organisms. However, TCDD in 2,4,5-T deposited on leaves and exposed to direct sunlight was found to degrade with a half-time of a little more than an hour (Crosby et al., 1977). The latter study shed a good deal of light on earlier studies in which pure TCDD was carefully coated on clean glass slides, water, or dry sterile soil for exposure to sunlight, and found to be almost insensitive to photodecomposition (Plimmer et al., 1973; Kearney et al., 1972). Crosby et al. noted that reduction would proceed in methanol, and that the reaction slowed significantly in highly purified reagent. The finding that benzene accelerated the degradation of TCDD in aqueous systems (Plimmer et al., 1973) suggested that a good hydrogen donor was essential to photodecomposition. This conclusion has been verified in more recent work (Crosby et al., 1977). In that study TCDD with 2,4,5-T was also applied on soil, and a half-time of 50-55 hours could be inferred from the data. This finding provides some suggestion about the reaction time in indirect light, because the rough surface will provide shaded areas where reaction must be slower. As yet, studies of the effect of various parts of the spectrum emerging from light transmitted through leaves, or reflected from various surfaces has not been done. The absorption maximum for TCDD is known (Crosby et al., 1973) but the relative sensitivity to degradation at that wave length apparently is not.

Accumulation of TCDD in the biota has been studied both in the laboratory and in the field, but there are still many gaps in the needed data.

In spite of the limited solubility of TCDD (0.2 ppb), it can accumulate in aquatic organisms to the extent it is available on sediments or other reservoir (Matsumura and Benezet, 1973). In their experiments, TCDD was deposited on sand, in solvent which was evaporated to leave the dioxin as a film on sand particles. The sand was placed in a small confined static aqueous system and various aquatic organisms added. Brine shrimp reached a concentration of 157 ppb, mosquito larvae concentrated TCDD to 4150 ppb and silverside fish accumulated virtually no TCDD. It may be assumed that the concentration of TCDD in water was maintained throughout the experiment by the excess dioxin residue on sand.

16437

DOW 001, 802

When sufficient TCDD to represent 162 ppb in the aquarium was ingested by algae which were then placed in an aquarium with Daphnia and with Ostracods, the latter species accumulated TCDD to concentrations of 879 and 279 ppb, respectively. They also showed that mosquite fish concentrate TCDD to a lesser extent than do the bottom feeding larvae on which they feed. It is clear that accumulation does occur, but depends on maintaining saturation of the ambient water.

Matsumura and Benezet (1975) also examined movement of TCDD which had been bound to sand, to a sandy loam soil (inorganic to organic soil). Very little TCDD was translocated by a slow leaching with water. Subsequently, a more complex food chain system of soil, water, algae, duckweed, snails, daphnids, gambusia, and catfish was assembled (Isensee and Jones, 1975). ^{14}C-TCDD bound to soil in concentrations varying from 0.0001 to 7.45 ppm was placed in aquaria. The residues found in the various species after 33 days exposure tended to peak at the Daphnia stage. Gambusia were added at the end of the exposure period with access to water and other organisms for 3 days. Catfish fingerlings were exposed for 6 days, beginning after all other organisms were removed. The catfish accumulated TCDD to roughly the concentration found in the first biological stage, the algae. Nonetheless, at a water concentration of 7 ppt TCDD a terminal accumulation ratio of more than 10,000 was found in catfish, representing TCDD concentrations of about 100 ppb (Isensee and Jones, 1975). The work also shows, however, that as soil or water concentrations decrease, so also do concentrations in the organisms of the system. Toxicity was not studied although the higher tissue accumulations exceeded mammalian toxic doses, assuming uniform distribution of dosage. The authors point out that with the long latency of TCDD toxicity, the fish may not have had time to develop lesions and die.

Ward (1976) examined the persistence of TCDD in lake water and sediments. As expected, TCDD is almost entirely bound to lake sediments and is subject to very limited but nonetheless real metabolic degradation when microorganisms were present. The slow microbial activity is enhanced by presence of nutrients. Ward suggested that water-mediated evaporation of TCDD occurred to a limited extent.

Toxicity of TCDD to coho salmon has been assayed by Miller et al. (1973). TCDD was administered in the diet and dosage expressed as ng TCDD per gram organism mass. At 13.1 ng/g only about 30% of the experimental group survived for 80 days past exposure, and at 5.4 ng/g almost half the fish did not survive. Deaths often did not occur until 10 days after the end of exposure, and some lethally affected fish survived 60 days or more. In studies of rainbow trout there were no apparent effects at intakes of 6.3 ng TCDD/g or greater. Mosquito larvae pupated at a normal rate in water containing 200 ppt TCDD; snails reproduced normally and oligochaete worms suffered some reproductive deficit at the same concentration. Beatty et al. (1976) found Rana catesbiana tadpoles to be remarkably resistant, with no apparent lethal effect by intraperitoneal doses of 1000 µg TCDD/kg. Doses up to 500 µg/kg did not affect adult frogs, and no histopathological lesions appeared.

16438

DOW 0011 803

Field studies of fish and mammals have provided evidence both supporting and contradicting claims of TCDD accumulation in higher animals. An evaluation of biota in the area used by USAF for training bomber crews in application of military defoliants showed little accumulation of TCDD in fish living in the streams of the area. The area was literally saturated with 2,4,5-T, receiving on the order of 1000 lb/acre during the peak year, containing up to 2-10 ppm TCDD. Mice in the area accumulated tissue burdens of several hundred ppt as measured at the end of the spray program, when the sandy soil had accumulated residues up to 700 ppt. These animals demonstrated no pathological changes (Young et al., 1974), which is not surprising when extrapolating the data of Rose et al. (1976) and Kociba et al. (1976) to relate tissue levels and effective dose rates in rats. Given the extended period of exposure, it seems possible that the mouse population could adapt through survival of non-responding strains over the estimated 30 generations of exposures. It may be also that fish strains have evolved similarly as non-accumulators of TCDD, although such adaptation seems unlikely.

Fish have also been analyzed in drainages from areas in Texas and Arkansas which had been extensively treated with 2,4,5-T (Shadoff et al., 1977). The Texas samples were from a pond which collected run-off from a watershed on which the herbicide was used for brush control. The Arkansas site was a pond into which adjacent rice fields were drained and from which irrigation water was obtained, in essence recycling any contaminants. Treatment was 1.25 lb 2,4,5-T/acre, 4-8 weeks prior to flooding. The cycle had been in use for 18 years. Fish from the Texas site did not contain TCDD, at detection limits of 5-7 ppt. Mud at a detection limit of 3 ppt and water at 0.1 ppt were also negative. Six human milk samples obtained from the San Angelo area near the Texas site were also negative at a detection limit of 3 ppt. No TCDD was found in the Arkansas fish samples. Meselson and O'Keefe (correspondence to Representative James Weaver, 1977) have presented preliminary findings of about 1-2 ppt TCDD in human milk from the San Angelo area, and from an area in western Oregon.

Cows milk samples from 2,4,5-T-treated areas of Oklahoma, Arkansas, and Missouri were found to be negative for TCDD by Mahle et al. (1977) with detection limits on the order of 1 ppt. TCDD in beef fat was undetectable in two series of animals which had grazed on 2,4,5-T treated pastures in Oklahoma, Texas, and Missouri. In a third group of seven animals confined in an entirely sprayed pasture, three samples were positive at the detection limit of 3-4 ppt (Kocher et al., 1977). TCDD has been found, however, in several beef cattle maintained in a field experiment on fields which had received 1, 2, 3, or 4 lbs 2,4,5-T/acre. Average TCDD in fat was 20.3 ppt in the 4 lb/acre group, but there were only three samples, one of which was extremely high. At 3 lb/acre the average was 12.8 ppt, again derived from some high values and many negatives. Interpretation of this data by EPA is still in process, and is subject to some argument.

A number of wild animal samples were obtained in treated forest areas of western Oregon, several of which contained unusually high concentrations of TCDD. The unique character of the data raises some questions, since

16439

DOW 0011 804

there seems to be few or no samples containing TCDD concentrations intermediate between the low background level and the 100-200 ppt values in a few animals.

An issue has been raised about formation of TCDD during combustion of 2,4,5-T, and subsequent distribution in air. Stehl and Lamparski (1977) ignited grass treated with 2,4,5-T in a system where combustion was self supported and found less than 0.0002% converted to TCDD. Combustion of 2,4,5-T and trichlorophenol on filter paper led to production of even less TCDD. If the conversion of a field application were at this rate, about 2 parts of available 2,4,5-T per million would be converted, which is considerably more than the residual contaminant level. It should be remembered, however, that burning would not be done until foliage dries, and with a 1-2 week half time it is doubtful whether much herbicide would be available for conversion. Even this maximum level of conversion is probably not of great concern, since the product would be diluted with combustion gases and air in millions of cubic meters of atmosphere. The effect of exposure to light on decomposition while in the atmosphere is unknown. Masking or wavelength shifts may have an inhibitory influence on photodegradation, but the duration of atmospheric suspension and light exposure should enhance the reaction.

Induction of Microsomal Enzymes

TCDD has been found to be a remarkably potent inducer of many enzymes which act on foreign chemicals. The effect is apparently dependent upon synthesis of additional enzyme, rather than activation of partially synthesized or otherwise inactive complete protein. TCDD also increases activity of δ-aminolevulinic acid synthetase in some species, resulting in an accumulation of porphyrins in tissues and excretory routes. The potency of TCDD for inducing some enzymes is between four and five orders of magnitude greater than that of such classical inducing agents as 3-methyl cholanthrene or phenobarbital. According to Hook et al. (1975a) TCDD induces cytochrome P-450 at doses of fewer molecules of TCDD than there are of P-450 in the liver. This property is important because the induced enzymes may increase conversion of other organic intoxicants to non-toxic products, or conversely may increase conversion of non-toxic materials to highly reactive intermediates which exert profound toxic effects. Many carcinogens are activated in this way. The changes in "drug-metabolizing" enzymes also have been used to describe profound genetic differences among animal strains, and their respective response to intoxication.

Enzyme induction may also serve as a predictor of general toxicity, since the induction potency seems well correlated with lethal, acnegenic, and teratologic potential (Schwetz et al., 1973; Poland and Glover, 1973a).

The enzymes which oxidize foreign chemicals have evolved in response to reactive groupings on molecules found in nature. While natural and synthetic chemicals may represent an almost infinite variety of structures, the building blocks are the same, and they exist in relatively limited numbers. Consequently, organisms have not had to develop an infinite number of enzymes to survive, but rather have developed enzymes specific for reactive groups.

16440

DOW 0011805

The effects on these systems are among the more sensitive changes found to be caused by TCDD. To simplify presentation the following table identifies specific enzymes and the TCDD doses at which responses have been observed. Unfortunately, few explorations of the entire dose response curve have been made; the "no observed effect" intake has usually not been determined, nor has there been an adequate study of the impact of low level chronic studies on the microsomal enzymes. Most of the data in the table refers to rats. Relatively few studies of mice have been made, and in the guinea pig the effective doses approach the lethal dose, and observed changes are minor. Hook et al. (1975b) administered 0.175 µg/kg to guinea pigs and found a slight increase in liver biphenyl-4-hydroxylase and biphenyl-2-hydroxylase, and no change in aryl hydrocarbon hydroxylase or UDP glucuronyl transferase.

Several generalizations appear applicable to the enzyme inductive properties of TCDD. While most microsomal enzymes are increased after TCDD, the enzymes identified as demethylating aminopyrine, benzphetamine and morphine are decreased (Lucier et al., 1973).

Females seem to be more sensitive to the inducing effects of TCDD (Lucier et al., 1975b). While effects on UDP glucuronyl transferases are substantial, steroid glucuronyl transferases are not changed. The bulk of induction occurs in liver but a similar but usually lesser increase can occur in the kidney and some other tissues (see table). In kidney, activity seems concentrated in the "outer stripe" of the medulla and in the cortex. This region contains the terminal straight segments of the proximal tubules, which appear to be the only significantly inducible cells in the kidney (Fowler et al., 1977).

The induction of aryl hydrocarbon hydroxylase (AHH) by TCDD has been very useful in explaining genetic variability in receptor and effector mechanisms. TCDD is about 3×10^4 times more potent than 3-methylcholanthrene, producing half maximal stimulation in the rat liver at 0.85 nmoles/kg and in the C3H/HeN mouse (female) at a dose of 0.42 nmoles TCDD per kg (Poland and Glover, 1974). While the susceptibility to AHH induction is about the same in the species tested (ED_5 = 0.4-1.2 nmole/kg), and the LD_{50}s differ by about two orders of magnitude, this difference does not detract from the value of the system in studying the genetics of drug response and the great problems of non-homogeneity of exposed populations.

There is known to be a profound difference in the responses of various strains of mice to AHH induction by 3-methylcholanthrene (3-MC), leading to a designation of "responsive" and "non-responsive" strains. Even at high doses, 3-MC fails to cause induction in the non-responsive animals. TCDD, however, will elicit an amplified enzyme activity in such mice (Poland and Glover, 1975; Chhabra et al., 1974).

Poland and Glover (1975) and Neubert et al. (1975) have shown that the genetically non-responsive mice have the genetic apparatus necessary for expression of the inducible activities and suggest that the difference arises from a mutant form of inducer-binding receptor with a diminished affinity for aromatic hydrocarbons. The induction process is apparently an increase in de novo synthesis of enzyme, rather than activation of preexisting protein or decreased degradation of protein (Haugen et al.,

16441

Induction of Foreign Compound Metabolizing Enzymes by TCDD

Enzyme	TCDD Dose (µg/kg)	Extent of Initial Induction (x normal)
p-Nitrophenol-UDP glucuronyl transferase	3	14
	3	7
	25	6
	5	5
	0.2	2.5
Benzpyrene (aryl hydrocarbon) hydroxylase	3	14
	3	2
	2.5	14
	2.5	100
	0.2	7
	0.2	8
	1.0	1.5
	25	3
	25	>70
	10	180
	10	10
	10	100
	.08	3
NADPH diaphorase (dehydrogenase)	90	13
Aniline hydroxylase	5	2
	0.2	1.2
Cytochrome P-450	1.0	1.6
Cytochrome B_5	1.0	1.4
Biphenyl-2-hydroxylase	25	13
	25	18

LSA43

(1)

Duration of Increased Activity	References	Remarks (liver unless other tissue specified)
>34d	Lucier et al., 1975a	pregnant rats (2)
>34d	Lucier et al., 1975a	neonate, (3)
73d	Lucier et al., 1975b	male rat
>28d	Hook et al., 1975b	
--	Lucier et al., 1973	
>21d	Lucier et al., 1975a	pregnant rats (2)
	Lucier et al., 1975a	neonate, (4)
--	Berry et al., 1976	pregnant rat, (5)
--	Berry et al., 1976	fetal liver
--	Lucier, 1973	male rats (6)
--	Hook et al., 1975b	female rats (7)
--	Hook et al., 1975b	male (7)
--	Hook et al., 1975b	male rat liver
--	Hook et al., 1975b	male rat kidney (8)
--	Poland and Glover, 1974	rat kidney
--	Poland and Glover, 1974	rat lung
--	Poland and Glover, 1974	rat intestine
--	Poland and Glover, 1974	C3H/HeN mouse
>15d	Beatty and Neal, 1976	male rat liver (9)
>15d	Lucier et al., 1973	
--	Lucier et al., 1973	
--	Lucier et al., 1973	male, 3 day
--	Lucier, 1977	male, 3 day
--	Hook et al., 1975b	rat liver
	Hook et al., 1975b	rat kidney (11)

Induction of Foreign Compound Metabolizing Enzymes by TCDD (cont)

Enzyme	TCDD Dose (µg/kg)	Extent of Initial Induction (x normal)	Duration of Increased Activity	References	Remarks (liver unless other tissue specified)
Biphenyl-4-hydroxylase	25	2		Hook et al., 1975b	rat liver
	25	40		Hook et al., 1975b	rat kidney
Amino levulinic acid synthetase	25	none	--	Woods, 1973	
	25	2		Goldstein, 1973	mouse (11)
	1.5 ng/egg	2		Poland and Glover, 1973c	chick embryo

[1]This table is selective and is intended to show the variety of work done, the dose ranges studied, and duration of effects. In most cases the lowest dose producing effect has been shown and data for higher doses omitted. The references noted should be consulted for greater detail.

[2]Pregnant rats, treated on day 10 of 23 day gestation, sacrificed 21 days post partum.

[3]Mother treated at day 5 of gestation; activity measured at day 21 postnatal. No increase in activity before parturition.

[4]Same as 2 except measured at day 8 postnatal.

[5]TCDD at day 17 of gestation, sacrificed at day 20.

[6]Male rats, sacrificed 3 days after TCDD.

[7]Male rats did not respond at this dose; there was a small but significant ($P < 0.05$) increase in amino pyrine demethylase and cytochrome P-450 in females at 0.2 µg TCDD/kg.

[8]Resting activity below detection limit.

[9]Maximal activity in cytosol at 7 days, still rising in microsomes at 15 days.

[10]5 µg/kg produced no effect.

[11]Resting activity very low in these tissues; factor of increase approximate.

DOW 00111 807

DOW 0011 8u8

1976). Whether the effect of TCDD on inducible enzymes has implications on other aspects of protein synthesis can only be speculated upon. Poland and Glover (1975) showed about a 10-fold difference in the dose necessary to cause induction. Through the finding that heterozygous offspring of C57Bl/6J (responsive) and DBA/2J (non-responsive) strains are intermediate in sensitivity, they support the contention of Poland and Glover (1975) that the difference is a receptor site mutation. Later studies have strengthened that premise in finding that hepatic accumulation of ^{14}C-TCDD administered intraperitoneally was greater in responsive than non-responsive strains (Poland et al., 1976). In the same study, in vitro examination of binding in the soluble fraction of liver cells showed that specific binding sites exist, and that their affinity correlates with the genetic capability for induction. Furthermore, tests of an extended series of halogenated dioxins and dibenzofurans showed that cytosolic binding corresponded closely with the induction potency of the chemical.

Niwa et al. (1975) have measured AHH induction by TCDD in a variety of cell cultures. They used 10 established cell lines, human lymphocytes, and primary fetal cultures from chick, rat, rabbit, hamster, and four strains of mice. Generally, kinetics of TCDD induction in each cell type is similar to that of 3-MC, and the induction was inhibited by actinomycin-D and cyclohexamide, which are inhibitors of protein synthesis. They found no relation between inducibility and cytotoxicity of TCDD, which is further evidence that TCDD itself is not metabolized. The sensitivity of some cell lines is such that the authors suggest use of H-4-II-E cells (derived from Reuber hepatoma H-35 in rats) as a bioassay, with a suggested sensitivity below 1 pMole ($\sim$ 0.0003 µg) in 3 ml of culture medium.

The same kind of genetic differences have been studied in human lymphocyte cultures, which represent the genetic background of the cell donor and are therefore useful in making relatively non-invasive studies of potential human response to intoxication. The sensitivity to TCDD induction of AHH is about 50-fold greater than to 3-MC induction, considerably less than the 30,000-fold difference in responsive mice (Kouri et al., 1974). Atlas et al. (1976) have carried these studies forward with lymphocytes from human tissues, but have as yet dealt only with 3-MC, not TCDD. Part of the stimulus for examining human cells lies in the suggestion that extent of induction at contact sites (i.e., skin, lung) may relate to the probability of cancer initiation by activated carcinogens (Kellerman et al., 1973). Although TCDD is as yet not established as a carcinogen such research may provide suggestions about the extent of expected variations in other TCDD responses in humans.

2,4,5-T

General Toxicity in Laboratory Animals

Apparently the first published account of 2,4,5-T acute toxicity was that of Drill and Hiratzka (1953). The LD_{50} for dogs was about 100 mg/kg; the principal symptom was a mild incoordination. When fed five days weekly for 90 days, doses up to 10 mg/kg/daily were without evident

16445

DOW 0011 809

effect, but 20 mg/kg/day was lethal to all 4 treated animals between 11 and 75 days after the first dose. Symptoms were limited to muscle twitching and impaired swallowing. A field study by Grigsby and Farwell (1950) [as quoted by Rowe and Hymas (1954)] exposed a variety of stock on pasture immediately after spraying 2,4,5-T at 2-4 times usual levels, with no effect.

Rowe and Hymas (1954) summarized the available data at that time and estimated the acute oral median lethal dose for male rats to be about 500 mg/kg, male mice 389 mg/kg, and guinea pigs 381 mg/kg. LD_{50}s for various esters were higher than for the acid. A sheep fed the propyl glycol butyl ester of 2,4,5-T died after 369 daily doses of 100 mg/kg and another sheep and a cow died after seven daily doses of 250 mg/kg (Palmer and Radeleff, 1964). The triethylamine salt of 2,4,5-T at 100 mg/kg caused no observable effect after 481 days of treatment at 100 mg daily. As single animal observations these can be considered rough estimates only, but they convey the generally high doses necessary to cause harm in ruminants. 2,4,5-T has been shown to cause decreased volatile fatty acid production in vitro at concentrations of 500 µg/ml or greater (Kutches et al., 1970); this concentration is probably greater than can be maintained by a survivable daily dose of 2,4,5-T.

Subchronic (90 day) feeding of male and female rats caused no effects below 30 mg/kg/day, but body weight and food intake were depressed after treatment at 100 mg/kg/day. Alkaline phosphatase and SGPT were elevated and erythrocyte count and hemoglobin was decreased. The histopathologic findings were limited and inconsistent (McCollister and Kociba, 1970). Rats were able to tolerate 186 mg/kg/day treatment with mixed mono-, di-, and tripropylene glycol butyl ether esters of 2,4,5-T over 90 days but developed some indications of toxicity. At a dose of 18.6 mg/kg no evidence of toxicity was observed (Dow Chemical Company, 1961).

A two year study of rats given 3 to 30 mg 2,4,5-T/kg/day has very recently been completed by Dow Chemical Company. Much of the analysis has yet to be completed, including morphologic pathology. Animals that received 30 mg 2,4,5-T/kg/day were found to have increased urinary porphyrin excretion after 4 months of treatment, and this change continued through the entire 2 year period. No changes were found in any other hematologic, urinary, or clinical chemistry measurement at that rate of intake, and the increased porphyrin excretion did not occur at 10 or 3 mg/kg/day (R.J. Kociba, Dow Chemical Company, preliminary status report; personal communication).

A feeding study of reindeer was prompted by allegations that a high incidence of death and abortion occurred in 1970 in a herd after use of phenoxy herbicides the previous year. Fifteen of thirty pregnant reindeer were fed birch leaves which had been sprayed with a 2,4-D/2,4,5-T mixture, through a 1.5 month period late in gestation. The daily dose of phenoxy acid was about 1 mg/kg/day. No clinical chemical changes could be detected, nor did any changes appear at autopsy either in the adult animal or the full term fetus (Erne, 1976).

16446

DOW 0011 810

The toxic effects of 2,4,5-T in rodents have been studied in much more detail than the gross toxicity studies already described. Highman et al. (1976a, 1976b) established that lethal doses in pregnant or nonpregnant mice caused myocardial lesions, bone marrow aplasia, and lymphocytic depletion in thumus, spleen, and lymph nodes, and that animals which remained apparently healthy despite similar doses did not suffer the same severity of lesions. A mild hemolytic anemia did occur in such animals. Pregnancy did not augment the toxic effect. It was also clear that major differences exist in the response of specific strains. Many NCTR mice were seriously affected at doses below 60 mg 2,4,5-T/kg/day (6-9 days of treatment while most CRBL mice remained unaffected at doses as high as 90-120 mg/kg/day at the same time. The histopathology, hematology, and blood chemistry changes in treated mice were also studied. In many monitored animals, some myocardial fibers were found to be pale and swollen, with longitudinal striations. The condition was rarely seen in treated apparently non-intoxicated animals. Necrotic changes were also often seen in the outer myocardium. High doses caused thymic atrophy, with almost no lymphocytes in the cortex and increased numbers in the medulla (this effect was enhanced in late pregnancy by the normal tendency to cortical involution in pregnant mice). Spleens were often atrophied, and thyroid follicles were enlarged with enlarged epithelial cell. In the liver, glycogen was often depleted. Blood chemistry changes appeared not to be marked.

2,4,5-T has been found to cause increased liver weight in rats at doses of 167-334 mg/kg over a two-day period, apparently through stimulation of protein and RNA synthesis, but not as newly formed 2,4,5-T metabolizing enzymes. The change reverses after withdrawal (Chang et al., 1974; Rip and Cherry, 1976). Liver nuclei isolated from treated animals were more active in RNA synthesis than those from controls. 2,4,5-T at a concentration of 4 mmolar is capable of sharply inhibiting in vitro incorporation of mevalonate-^{14}C into non-saponifiable lipids by rat liver (Olson et al., 1974). If data obtained in mice by Nony et al. (1976) can be applied, this concentration is approachable at single doses of 50 to 100 mg/kg, which are survivable by most species.

Koschier and Berndt (1976a, 1976b, 1976c) have described the effect of 2,4,5-T upon renal physiology. Large doses of 2,4,5-T appear to impair secretion of itself by decreasing the activity of the organic acid transport system in the proximal tubule. Organic base transport was also depressed. Small doses of 2,4,5-T were excreted very rapidly, but large daily doses led to renal depression and retention of the compound. The authors consider the data to support the conclusion that transport of phenoxy herbicides is an active process.

The psychopharmacology of 2,4,5-T does not seem to be a popular field of study. In the one study found, single doses of up to 100 mg/kg were given on day 7, 8, or 9 of pregnancy. The male offspring of females given the highest dose exhibited more exploratory open field behavior, but no difference was found in females. The highest doses caused decreased litter size but no increase in malformations (Sjödén and Söderberg, 1972).

DOW 0011 811

Poultry seem relatively insensitive to 2,4,5-T. Whitehead and Pettigrew (1972) found that a single oral dose of 900 mg/kg to 4 week old chicks caused 40% lethality. However, feeding of 1000 mg 2,4,5-T per kg diet/day for three weeks to chicks, beginning at one day of age caused only some slowing of growth; 5000 mg/kg diet was lethal. At levels not causing gross toxicity, plasma calcium and magnesium were not affected. Given a choice, the birds rejected the treated diet in favor of non-contaminated food. Turkeys fed 2,4,5-T at a rate equivalent to 62 mg 2,4,5-T acid daily for 11 days were unaffected (Roberts and Rogers, 1957). Bjorklund and Erne (1971) introduced various 2,4,5-T derivatives into water and feed of chickens, quail, pheasants, and ducks. In water the LC_{100} of the triethanolamine salt for chickens over a 29 week period was 1000 ppm acid equiv. (about 200 mg/kg). In the diet of other species over a 7-day period, the LC_{50} was in excess of 2000 ppm. Kenaga (1975) has extensively reviewed avian toxicity and safety of birds in areas treated with herbicides and concludes that the no observed effect levels are substantially above amounts that might be contacted in a field application.

Effects of 2,4,5-T on Reproduction

The teratogenic potential of 2,4,5-T has received wide publicity since 1969 when allegations were made that its use as a military defoliant had caused fetal malformations in the Vietnamese population. A succession of studies over the next three years confirmed the teratogenicity of 2,4,5-T, but the implication of the herbicide in any increase in birth defects has not been supported. The initial report of 2,4,5-T teratogenic effect (Courtney et al., 1970; Bionetics Research Laboratories, 1970) described cleft palate and cystic kidneys at doses of 46 and 113 mg/kg/day on days 6 through 14 of gestation. Of the two lesions, cystic kidney appeared to be a more sensitive indicator. The 2,4,5-T used in the study was found to contain 30 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the respective toxicities of the two agents were still unclear at that time. The data was also criticized for an unusually high and variable incidence of embryotoxicity in control animals (Neubert and Dillman, 1972).

Emerson et al. (1971) evaluated a commercial 2,4,5-T with less than 0.5 ppm TCDD and found that 24 and 40 mg/kg/day through days 6-15 of gestation caused no teratogenesis in rats. Sparschu et al. (1971) then found that 50 mg was also nonteratogenic, although there was a slight increase in incidence of delayed skull ossification. (Such alteration in development is not considered teratologic, because the abnormality disappears with age. One criterion of teratogenic effect is irreversibility.) Treatment at 100 mg/day for days 6-10 caused generalized maternal toxicity, with only 4 survivors of 25 treated animals. Intestinal hemorrhage, considered a common fetotoxic but not teratologic lesion, was found in only one pup in this study. Khera and McKinley (1972) found a similar dose response.

In mice the effective dose is similar to that in rats. At 50 mg 2,4,5-T/kg/day, through days 6-15 of gestation, the frequency of cleft palates was increased from 4.7% to 20% and resorption frequency increased. The incidence was raised to 73% by a dose of 110 mg/kg/day with a decrease

1644

DOW 0011812

in fetal weight (Bäge et al., 1973). The high dose also caused a two-fold increase in rib and vertebral malformation, but only marginal increase in dilated renal pelvis. Neubert and Dillman (1972) found increased cleft palate in NMRI mice at doses over 200 mg/kg/day through days 6-15, increased embryo lethality at doses over 45 mg/kg/day reduction in fetal weight above 15 mg/kg/day. Strain differences in mice are significant. The dose causing at least one cleft palate in 50% of litters was 25 mg/kg/day in the most sensitive of 5 strains tested and 105 mg/kg/day in the least sensitive (Gaines et al., 1975). Roll (1971) found detectable teratogenesis at doses above 35 mg/kg/day through days 6-15 of pregnancy. The no-effect level with respect to teratogenesis was established as 20 mg/kg/day.

Hamsters are less sensitive to cleft palate, but do have a somewhat higher incidence of delayed head ossification which is not generally defined as a teratologic manifestation (Collins and Williams, 1971). The doses ranged from 40-100 mg/kg and there was a considerable difference among samples of 2,4,5-T of different sources and levels of TCDD contamination.

Fetal rabbits are apparently unaffected by maternal doses up to 40 mg 2,4,5-T/kg/day through days 6-18.

Ruminants are apparently also quite resistant to teratogenic effects of 2,4,5-T. Binns and Balls (1971) fed 100 mg 2,4,5-T/kg to 11 ewes from the 14th to 36th day of gestation, and fed 100 mg 2,4,5-T(PGBE)kg to another group during the same period. No evidence of deformity appeared in any of the lambs. The TCDD content was 1 ppm.

The no apparent effect levels in rodents reported in the preceeding studies contrast sharply with data in abstracts of reports by Konstantinova (1974a, 1974b), who is said to have found that 4.2 mg/kg/day of the butyl ester of 2,4,5-T through the entire gestation period caused embryotoxicity, nervous and hematologic change and histopathology in the mother. A dose rate of 0.42 mg/kg affected growth and development, nervous, liver and kidney functions and lowered fertility. The succeeding generation was found to have minor changes in organ weights. (Direct translations of these papers are not available; data quoted is obtained from Chemical Abstracts.)

There has been limited study of the teratogenic effect of 2,4,5-T in primates. Dougherty et al. (1975) administered 0.5, 1.0, and 10 mg/kg/day to groups of 10 pregnant rhesus monkeys from day 22 through 38 of gestation. The high dose was established after finding that 12 mg or more 2,4,5-T/kg daily for 18 days caused vomiting and weight loss in monkeys of both sexes. No teratogenesis occurred, although there were 1 or 2 abortions, premature births, or neonatal deaths in all groups, including the control.

The importance of TCDD in the teratogenicity of 2,4,5-T was examined soon after the contamination was recognized. Courtney and Moore (1971) tested 2,4,5-T with 0.5 and 0.05 ppm TCDD, 2,4,5-T and TCDD together and

16449

DOW 0011813

TCDD alone in mice. It was evident that the concentrations used or addition of 1 µg TCDD/kg/day through days 6-15 did not alter the apparent teratogenicity of 2,4,5-T. Neubert and Dillman (1972) reached a similar conclusion, suggesting that to potentiate 2,4,5-T effect on pregnant mice at least 1.5 ppm TCDD is necessary. Collins and Williams (1971) observed additive effects of 2.9 and 45 ppm TCDD contamination of 2,4,5-T administered to hamsters, but many of the changes appeared characteristic of primary TCDD intoxication.

Nutritional status seems to have little influence on the effect of 2,4,5-T on reproductive functions. Hall (1972) fed 250 and 1000 ppm 2,4,5-T with diets containing 20% and 60% casein. Resorption and stillbirth incidence and litter size were similar in all groups, although fetal organ and placenta weights were decreased in the high 2,4,5-T, high protein group. Effects of low protein intake were augmented by the high concentration of herbicide.

Incubating eggs have been considered to be particularly vulnerable to herbicides because they remain in one place, and because the eggshell is porous. A number of studies of 2,4-D effect on eggs have appeared since 1967, with conflicting results (see section on 2,4-D), but 2,4,5-T has not received much attention. Somers et al. (1973) sprayed hen eggs with a formulation containing 2,4,5-T and 2,4-D in a treatment equivalent to 11.2 kg/hectare (10 times normal field application rates) prior to incubation without effect on hatchability or early survivability of chicks. They then treated eggs of the pheasant, as a genetically more heterogeneous species, in the same way, without adverse effect. Entry of herbicide into the egg was verified analytically. The treatment did, however, increase weight gain in male chicks during the first four weeks of life (Somers et al., 1974). The LD_{50} of 2,4,5-T in DMSO, injected directly into the air space of chicken eggs at doses up to 125 mg/kg has been calculated to be 62 mg/kg, and in acetone carrier, 133 mg/kg (Strange et al., 1976). No teratogenic effect was evident. The solvent toxicity was shown to be substantial, but it seems clear that acquisition of an effective dose of 2,4,5-T by an egg in the field is highly unlikely.

In fact, immersion of hen eggs in 1% 2,4,5-T was ineffective, and a 5% solution was only moderately effective. The eggs were immersed for 10 seconds, then returned to incubation (Gyrd-Hansen and Dalgaard-Mikkelsen, 1974).

Insects may be more sensitive; Dävring and Sunner (1971) and Dävring (1975) have shown that while Drosophila are highly resistant to lethal effects of 2,4,5-T ester (LD_{50} = 4700 ppm in the diet), 1 ppm caused disturbed egg follicle development and chromosomal defects in developed oocytes.

A study on a species of killifish has shown that concentrations of 20 ppm have substantial teratogenic effect. The embryos developed several cardiovascular anomalies, and occasional eye and splenic defects. The 20 ppm treatment reduced hatchability to less than 50%, and 25 ppm allowed less than 5% to hatch. No anomalies were found after treatment with 14 ppm 2,4,5-T, and the hatch was reduced only 4% (Schreiweis and Murray, 1976).

16450

DOW 0011 814

Carcinogenic and Mutagenic Potential of 2,4,5-T

Probably the most important issue about any chemical introduced into the environment by human activity is the possibility that it may increase the incidence of cancer in the human population. The probability of mutagenic activity is of almost equivalent concern, both because of the possibility of genetic alteration and because mutagenesis may be a useful predictor of carcinogenic activity. The relationship is by no means constant. However, McCann et al. (1975) have assembled data from a number of laboratories using microbial systems ("Ames test") and find that 85% of known carcinogens are positive, 10% of non-carcinogens are active. This is not to say that almost every agent identified as a mutagen will be carcinogenic, but the test should become an aid in deciding priorities for direct screening.

Only a limited number of experimental studies of 2,4,5-T carcinogenicity have been made. A screening program for 120 pesticides and industrial chemicals was reported by Innes et al. (1969). Eleven of the agents caused an elevated incidence of tumors; none of the phenoxy herbicides, including 2,4,5-T, caused increased tumor formation at a dose previously determined to be the maximum tolerable daily dose over 20 days, without lethality (21.5 mg/kg/day for 2,4,5-T). Muranyi-Kovacs et al. (1976) treated two strains of mice with 80 ppm 2,4,5-T (TCDD content 0.05 ppm) in the diet for more than 500 days. Daily dosage was about 12-15 mg/kg. In one strain survival time was significantly decreased in males, in the other significantly increased in females. An apparently significant small increase in tumor incidence occurred with the increase in life span. The authors are concerned that altered life span may confound the analysis, but consider that 2,4,5-T can not yet confidently be considered non-carcinogenic, and requires further analysis.

A two year study of rats given up to 30 mg 2,4,5-T/kg/day has just been terminated by Dow Chemical Company, but the pathological evaluation had not been completed at the time of this preparation.

One epidemiological study has been made of tumor incidence in a group of Swedish railroad employees. It was possible to isolate groups who had handled phenoxy acids exclusively, and a cohort of 207 persons with at least 45 days of exposure, totaling 1747 person-years. No increase in tumor incidence could be detected in these workers although increased incidence appeared in groups exposed to other herbicides (Axelson and Sundell, 1974).

Mutagenic assessments have been carried out on a number of systems. Buselmaier et al. (1973) were not able to show 2,4,5-T induced mutations in Salmonella typhimurium G46 his-, and Serratio marcescens a21 Leu- and a31 his- in a host mediated assay in mice. Serum from animals treated orally with 2,4,5-T also did not induce mutants in S. typhimurium his- (Styles, 1973). Anderson et al. (1972) were unable to demonstrate any mutagenic properties of 2,4,5-T or other phenoxy herbicides when tested against eight histidine requiring mutants of S. typhimirium.

16451

DOW 0011 815

Sex linked lethal tests of adult male Drosophila showed 2,4,5-T to be negative, although fertility was decreased (Vogel and Chandler, 1974). Majumdar and Golia (1974) were able to show an increase in sex linked recessive lethal mutations after 15 day feeding on 1000 ppm 2,4,5-T. A non-significant increase was apparent after feeding 250 ppm.

In gerbils six-day feeding of up to 150 mg 2,4,5-T/kg produced no increase in chlorosomal abnormalities, but higher doses did cause some increase (Majumdar and Hall, 1973). Jenssen and Renberg (1976) tested 2,4,5-T for mutagenicity by seeking micronuclei in erythrocytes of mouse bone marrow, which is a high resolution detection system. The system was cytogenetically negative, although some mitotic depression was detected. It was noted that only about 5% of circulating 2,4,5-T found its way into the cells, which was suggested as indicating a limited hazard due to lack of access.

A limited survey of pesticide applicators who had been in contact with a variety of agents showed some increase in chromatid gaps and breaks during the spray season (Yoder et al., 1975). Recovery in the off season reduced frequency below that of controls, suggesting an augmented repair system. 2,4,5-T was the least frequently used agent, and it is not likely that any observed effect can be attributed to it.

Dow Chemical Company has maintained a program of continuing health monitoring of 2,4,5-T production workers. Cytogenetic evaluations of 52 men in early 1970 were reported to be negative (Johnson, 1971), and a second analysis in 1974 also found no evidence of abnormality (Kilian, 1975). An interesting finding, however, was that the fraction of each exposed group found to have no abnormal cells was above 80%, while of the control group only 74% had no abnormal cells.

Absorption, Distribution, Metabolism, and Excretion of 2,4,5-T

2,4,5-T has a rather short residence time in all species. A cow given 450 mg of 2,4,5-T acid in four daily doses excreted the entire amount in urine in six days (St. John et al., 1964). Erne (1966a, 1966b) administered 100 mg of the amine salt/kg to rats and swine and found plasma half time for the rat to be three hours, and 10 hours for pigs. Kidney, liver, lungs, and spleen concentrations of 2,4,5-T could occasionally be forced above plasma levels, but apparently none of the material entered the brain or adipose tissue. Tissue half times ranged from 5-30 hours. When the agent was administered repeatedly plasma levels tended to lessen, with increased excretion. Of the 2,4,5-T in blood, about 20% was in erythrocytes. 2,4,5-T is excreted more slowly by mice than by rats, at a rate of 1-4% of the original dose per hour (Zielinski and Fishbein, 1967).

By overloading sheep (four 250 mg doses) it is possible to produce residues in tissues. Maximum accumulations were about 100 ppm in fat and muscle. In each case residues were found in the acid form regardless of the form in which it was fed (Clark et al., 1970). The latter finding is at some variance with Clark et al. (1971), in which the PGBE ester of 2,4,5-T was reported to remain as the ester in urine and tissues. Cattle fed 0.15

16452

DOW 0011 816

and 0.75 mg 2,4,5-T propylene glycol or butyl ether esters/kg/day for 32 weeks, produced no increase over background residue. Clark et al. (1971) later reported feeding sheep 2000 ppm 2,4,5-T in the diet and finding 1.0 ppm 2,4,5-T in muscle. No residue could be detected 7 days later. Deer browsing on land treated for reforestation were found not to contain significant tissue residues; measurable 2,4,5-T was found in stomach contents, urine, and feces up to 43 days after herbicide application (Newton and Norris, 1968). The implication, again, is that sustained field intake will not cause detectable tissue residues.

Grunow et al. (1971) found about 50-70% of administered 2,4,5-T in urine, and found that a substantial fraction emerged as derivatives, one of which was identified as N(2,4,5-trichlorophenoxy acetyl) glycine. The taurine conjugate and 2,4,5-trichlorophenol have also been identified (Grunow and Böhme, 1974). Nony et al. (1976) have measured 2,4,5-T, its glycine amide and alkaline hydrolyzable conjugates in blood, urine, and feces. The proportion of metabolites is quite low in blood, and increases substantially in urine from 24-72 hours after administration of the herbicide. In the feces the glycine amide is found in only limited amounts, but other conjugates may account for as much as 25% of the total recovery.

Research with labeled 2,4,5-T in rats showed that the half-time (T/2) of plasma clearance was about 4.5 hours and essentially dose independent below doses of 50 mg/kg but increased as dosage increased to 19.4 hrs at 100 mg/kg and 25.2 hr at 200 mg/kg. The T/2 of a 5 mg/kg dose in dogs was much longer, 77 hours. About 90% of the 2,4,5-T in blood was reversibly bound to plasma protein, over a wide range of concentrations. It was found also that as the dose was elevated, a small amount of fecal excretion took place. Detectable, minute amounts of ^{14}C emerged in the respiratory gases (Piper et al., 1973). Fang et al. (1973) also showed a non-dose dependent T/2 at lower doses. Nony et al. (1976) found in excess of 10% of the total excreted material in feces. The extent of protein binding has been further defined by Kolberg et al. (1973); bovine serum albumin binds 13-14 moles 2,4,5-T/mol BSA.

As suggested by the slower T/2, the percentage of dose excreted/day decreased sharply at the higher doses, indicating a limit in excretory capacity by the kidney. Rat kidney tissue slices can concentrate 2,4,5-T about 15 times, slices from dogs can concentrate about 9 times. Berndt and Koschier (1973) found an energy dependent uptake of phenoxy herbicides by renal cortical tissue. Metabolic inhibitors depress the process. Acetate and lactate as substrates enhance the accumulation. Increased 2,4,5-T in the medium slows the concentrating capacity of the kidney.

The above work was later carried forward by resorting to intravenous injection of 2,4,5-T in order to take advantage of useful pharmacokinetic models (Sauerhoff et al., 1976). Doses of 5 mg/kg and 100 mg/kg were used, and samples were taken through 36 hours (5 mg) and 72 hours (100 mg). After 5 mg/kg rats excreted about 50% of the body burden every 12 hours, in spite of a 4.3 hour plasma T/2, further illustrating the concentrating capacity of the kidney. At 100 mg/kg the plasma T/2 was 23.1 hours for the first 36 hours, then was comparable to the animals given a lower dose.

16453

In humans (Gehring et al. (1973) found that a 5 mg/kg dose was excreted with a T/2 of 23 hours for both plasma and whole body clearance. Essentially all of the administered material emerged in urine unchanged.

The phenoxy acids are relatively strong organic acids, which is one of the reasons they are excreted in large part as the parent molecule. The possibility of hydrolysis by rumen flora or by tissue enzymatic processes was first considered by Wright et al. (1970) who found that after feeding of 2(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate to sheep, 2,4,5-trichlorophenol residues could be detected in tissues. Clark et al. (1975) measured phenoxy acid and phenol residues in muscle, fat, liver, and kidney of sheep fed 2000 ppm 2,4,5-T for 28 days, and found 1.00, 0.27, 2.29, and 27.2 ppm 2,4,5-T and 0.13, <0.05, 6.1, and 0.9 ppm 2,4,5-trichlorophenol, respectively. Seven days after withdrawal, 2,4,5-T residues were well below 0.1 ppm or below the detection limit of 0.05 ppm. The phenol remained at significant levels in both liver (4.4 ppm) and kidney (0.81 ppm).

According to Koschier and Berndt (1976c) the rate of excretion rises almost immediately to about 80% of input in animals treated daily, then continues to rise for 7-9 days until excretion approximately equals input, where it remains through the duration of treatment. The rate of administration was 20 mg/kg/day. As dose rate increased the initial percentage excreted was less, but 7-9 days was still required to reach a rate equal to input. An apparent over-shoot then took place for 5-6 days.

The markedly different rates of 2,4,5-T excretion by rats and dogs was studied in vitro by Hook et al. (1974). They found that kidney of both species actively accumulated 2,4,5-T by a saturable, oxygen dependent process which was depressed by other organic anions. Rat kidney had a greater dependence on potassium, and dog kidney increased accumulation of 2,4,5-T in the presence of acetate. The capacity of kidney to transport the standard test anion, para-amino hippuric acid (PAH), can be competitively inhibited by 2,4,5-T, indicating that both are transported by the same mechanism. The authors concluded that the greater capacity for PAH transport by rat tissues accounts for the difference in retention between rats and dogs. Apparently lower renal excretion is also responsible for the extended half-time of 2,4,5-T in new-born rats observed by Fang et al. (1973); Hook et al. (1974) have found that kidneys of 10 day old rats are much less efficient than adults. Subsequent work by the same group (Hook et al., 1976) indicated that plasma binding in dogs was more tenacious than in rats.

The high specificity of 2,4,5-T in causing cleft palate has been investigated by Dencker (1976) as a distribution phenomenon. The sensitive period has been shown to be late in fetal development, during days 12-13 (Neubert and Dillman, 1972). Palatal closure occurs very late in organogenesis and is complete at about day 15.

Dencker (1976) points out that no unusual uptake of 2,4,5-T in these structures occurs, but overall uptake in the fetus is greatly increased between days 11 and 18. It may well be that this increase, at a time when formation of most other structures have progressed far enough that they are not sensitive, is responsible for cleft palate.

DOW

16454

DOW 0011 818

It is possible for 2,4,5-T to reach milk if a sufficient level is included in the diet. At 30 ppm in the diet for two weeks, followed by one week on untreated feed, no 2,4,5-T or trichlorophenol was found at a detection level of 0.05 ppm; at 100 ppm trichlorophenol was barely detectable. At 1000 ppm 0.31-0.54 ppm 2,4,5-T and 0.16-0.27 ppm trichlorophenol was found in milk, and somewhat less was found in cream (Bjerke et al., 1972). These figures can be put in the perspective of forage consumption. Residues on grass are on the order of 100-150 ppm/lb/acre (Morton et al. 1967) immediately after application. These amounts drop sharply to about 20% of initial levels during the initial two weeks post-application. This factor, with the rapid excretion characteristic of 2,4,5-T make even limited human exposure through dairy products or meat highly unlikely unless products are obtained immediately after heavy spraying.

Behavior of 2,4,5-T in the Environment and Effects on Submammalian Species

One factor in the hazard potential of an herbicide is determined by its persistence after application and its movement and reactions in soil, water, or in plants.

Appearance of 2,4,5-T in forest streams has been shown to result only if the herbicide was introduced directly to the stream, and diminished from about 0.1 ppm to less than 0.01 ppm within one day after treatment (Norris, 1967). Norris (1968) also found that heavy rains six months after treatment did not move detectable 2,4,5-T into streams.

The herbicide is usually degraded on the forest floor quite rapidly. Norris (1969) found that nearly 90% disappeared in two months, although cold weather, sterile soil, and lack of moisture may extend the period of degradation over as much as 9 months. The trichlorophenol hydrolysis product of 2,4,5-T degrades faster than the parent compound (Alexander and Aleem, 1961). Evaluation of several soils from South Vietnam indicates that concentrations on the order of those expected after a typical forest application should be virtually gone in seven weeks (Byast and Hance, 1975). More than 20% of the radioactive carbon of labeled 2,4,5-T had become incorporated in the soil, but actual residues of herbicide were usually about 1%. 2,4,5-T has also been found short lived in various Texas soil by Bovey and Baur (1972). Altom and Stritzke (1973) studied 2,4,5-T in three Oklahoma soils and found half-times of 24, 14, and 21 days. The shorter time was on grass lands, the others were forests.

Leaching tests show that 2,4,5-T bound in soil remained in the upper 6 inches of test columns even after application of 4.5 inches of water (Wiese and Davis, 1964).

The various esters of 2,4,5-T are often intrinsically more toxic than the parent acid, probably because they are more fat soluble. In an aqueous system, however, they hydrolyze rapidly to the acid, and the rate is enhanced by soil and microorganisms (Teasley and Williams, 1970, as quoted by Kenaga, 1974). It seems reasonable to expect a fairly rapid hydrolysis in any system with some water present.

16455

DOW 0011819

In essence, 2,4,5-T disappears relatively rapidly after application, probably within two months in a forest environment. The evidence also seems firm that 2,4,5-T deposited on the ground will not move into a water course, and will not leach into deeper layers.

It is difficult to separate physical behavior after application from effects on lower organisms, because there is a very close interface between an agent bound to soil or in water, and insects or fish. Lower organisms are of direct importance in assessing environmental impact of any introduced chemical. Food chain effects may predict repercussions at higher levels, and specifically sensitive organisms may be useful indicators of pollution. Ecological displacement of any kind must be accepted only after careful examination, whether secondary to the intended impact of the chemical on plant species or as a result of direct toxic effect of the applied chemical.

The various derivatives of 2,4,5-T differ in toxicity to fish. The parent herbicide, the amine salts and isooctyl esters are relatively limited in toxicity, but the propylene glycol butyl ether and butoxy ethanol esters are quite toxic to some species. There is a large number of gross effect studies on various aquatic species; these generally use an end point of death or immobilization. Shellfish studies measure shell growth as an index of effect.

Standardization of methods has not received as much attention as seems justified. Study periods vary from 24-96 hours, water temperatures sometimes appear outside the normal acceptable range for the species, and most evaluations seem to be carried on under static conditions. Nonetheless, the relative gross effects can be useful in reference to a given application in the field. As a very rough approximation, a 2 kg/hectare application over a water course would produce a concentration of about 2 mg/L or 2 ppm, if an assumption of dilution in the upper 10 cm of water is accepted. Flowing water will diminish the concentration below detectable limits in a few hundred yards (Evans and Duseja, 1973), and application over deeper water will dilute accordingly.

Concentrations of 2,4,5-T or its derivatives found to have no effect are listed below as acid equivalents:

	Species	Exposure Period	No Effect Acid Equivalent Concentration ppm	Reference
2,4,5-T	killifish	48 h	50	Butler, 1963
	mullet	48 h	50	Butler, 1963
	sea lamprey	72 h	2	Applegate et al., 1957
2,4,5-T Na salt	bluegill	12 d	46	Hiltibran, 1967

16456

DOW 0011 820

2,4,5-T butyl ester	trout	24 h	4.1	Applegate et al., 1957
	bluegill	24 hr	4.1	Applegate et al., 1957
	lamprey	24 h	4.1	Applegate et al., 1957
2,4,5-T isooctyl ester	bluegill	8 d	7	Hiltibran, 1967
	green sunfish	8 d	7	Hiltibran, 1967
	bluegill	12 d	0.7	Hiltibran, 1967

Median lethal concentrations of 2,4,5-T derivatives are similarly tabulated:

	Species	Exposure Period (hours)	LC_{50} Acid Equivalent Concentration ppm	Reference
2,4,5-T DMA salt	bluegill	48	144	Hughes and Davis (1963)
2,4,5-T TEA salt	bluegill	24	54	Davis and Hughes (1963)
2,4,5-T Oleic-1,3-propylene diamine salt	bluegill	48	2.9	Davis and Hughes (1963)
2,4,5-T isopropyl ester	bluegill	48	1.7	Davis and Hughes (1963)
2,4,5-T isooctyl ester	bluegill	48	31	Hughes and Davis (1963)
2,4,5-T butoxyethanol ester	bluegill	48	1.4	Hughes and Davis 1963
2,4,5-T PGBE esters	bluegill	48	17	Hughes and Davis, 1963
	spot	24	0.21	Cope, 1965
	harlequin	48	1.0	Alabaster, 1969

While the more complex esters of 2,4,5-T tend to greater toxicity in water at pH 6.5, they hydrolyze to the parent acid in about a day (Teasley and Williams, 1970, as quoted by Kenaga, 1974).

1645

Effects of 2,4,5-T on other aquatic organisms have also been measured. In flowing salt water 1 ppm 2,4,5-T caused no mortality in brown shrimp and 2 ppm was without effect on oyster shell growth (Butler, 1963). The PGBE ester of 2,4,5-T at 0.14 ppm (0.09 ppm acid equivalent) caused a 50% decrease in oyster shell growth after 96 hour exposure (Butler, 1963).

Studies of terrestrial non-mammals seem to have been limited to honey bees. Honey bees are an important component of the crop cycle and in many areas are systematically managed for pollination. Phenoxy herbicides, including 2,4,5-T, fed in 60% sucrose-water solution at 10 ppm did not affect hatching, but reduced brood development when fed at 100 ppm (Morton and Moffett, 1972). When sprayed in a water carrier at field application rates, 2,4,5-T was non-toxic. (Petroleum solvents alone caused high mortality during the first day after spraying.)

Aerial spraying did not result in herbicide accumulation in honey sacs or colonies (Moffett and Morton, 1972). The point was made that the primary damage would be failure of flowers and loss of nectar, rather than direct toxicity. When fed directly to newly emerged bees, the phenoxy herbicides were essentially non-toxic at concentrations up to 1000 ppm in the diet (Morton et al., 1972).

2,4-D

Toxicity to Humans

For some reason there have been reported several incidents of 2,4-D poisoning in humans, but virtually no reports of human intoxication by 2,4-T. Probably the best known was a suicide by a single dose of 2,4-D DMA salt of which more than 90 mg/kg was estimated to have been retained by the victim, after extensive vomiting (Nielsen et al., 1965). An accidental poisoning with a formulation containing 49% S-ethyldipropylthiocarbamate, 9% kerosene, and 36.5% 2,4-D isooctyl ester caused symptoms and laboratory findings attributable to 2,4-D intoxication. Among these were twitching and paralysis of intercostal muscles, hemoglobinuria, and myoglobinuria. No evidence of peripheral neurological damage was evident during 36 months of follow-up observations (Berwick, 1970). Goldstein et al. (1959) reported on three cases in which 2,4-D esters were absorbed through the skin, resulting in peripheral neurological changes. One patient spilled 60 ml of a 10% solution of 2,4-D ester on the forearms and did not wash. Unusual fatigue developed in a few hours, and over 10 days after exposure there was extended nausea and vomiting, with considerable weight loss. A second exposure caused similar symptoms, then pain and numbness in all digits, loss of skin from the palms, and within six weeks there was substantial general neural damage. Another patient eventually developed metacarpal pain and swelling in both hands, and later became partially paralyzed. The third experienced gastrointestinal disturbance and vertigo, then paresthesia in the arms and legs and persistent general muscle fasciculations. In two of the cases, there were second exposures which appeared to cause greater impact than the initial event. Similar cases have been described by Todd (1962), Berkeley and Magee (1963), and Wilson (1956).

16458

DOW 0011822

Seabury (1963) attempted use of 2,4-D in treatment of a terminal case of disseminated coccidoidomycosis, on the rationale that the 2,4-D as a synthetic plant hormone might alter the course of the fungus infection. At the time of the treatment (1949) there was no therapeutic agent for the disease. In 24 treatments over a period of more than a month the dosage was raised to a final treatment of 3600 mg. No prior doses, up to 2000 mg caused any response, but the final administration caused extreme quiescence, and fibrillation of muscles in the face and hands, followed by deep stupor and reflex failure. The patient recovered from the 2,4-D within 48 hours and died of the fungus disease about two weeks later.

General Toxicity to Laboratory Animals

One of the earliest published studies of 2,4-D toxicity was by Bucher in 1948. She found an acute LD_{50} of 280 mg/kg in mice, and found that single doses of 150-200 mg/kg would produce a myotonia persisting several hours. The animals remained awake and alert, but when moved exhibited gross incoordination. They were capable of working out of the syndrome with continued exercise, but if allowed to remain quiet the myotonia would recur. The chemical also caused diarrhea in mice at the higher doses, and gastrointestinal and upper respiratory irritation in dogs. Mice were able to tolerate daily doses of 1/2 LD_{50} for three months. Hill and Carlisle (1947) established LD_{50} values for several species: mice, 375 mg/kg; rats, 666 mg/kg; rabbits 800 mg/kg; and guinea pigs, 1000 mg/kg. They also treated monkeys and were able to give up to 214 mg/kg without severe residual effect. Twice that dose caused vomiting, incoordination, and loss of muscle tone. Rowe and Hymas (1954) summarized lethality data on 2,4-D and five salts or esters; in general the lethal doses were very high, with the exception of a few dogs given 2,4-D itself, for which the LD_{50} was estimated at 100 mg/kg. The latter data was apparently derived from the observations of Drill and Hiratzka (1953). Hansen et al. (1971) conducted a two-year feeding study on rats, at dosages of 5 to 1250 ppm 2,4-D in the diet, without appreciable change in growth, hematologic values, or organ weight. In the same study dogs were maintained for two years with little effect at doses up to 500 ppm 2,4-D. Drill and Hiratzka (1953) found that 20 mg/kg daily was fatal in 3 of 4 dogs within 49 days of a 90-day study.

Grazing species of animals might be expected to suffer exposures in the field from eating treated vegetation. Palmer and Radeleff (1964) evaluated a series of herbicides at high doses in limited numbers of ruminants. One sheep tolerated 481 daily doses of 100 mg 2,4-D alkanolamine salt, another was unaffected by the same treatment with 2,4-D propylene glycol butyl ethyl ester. The first compound was lethal after 7-500 mg/kg doses; the second was lethal after 9-250 mg doses. A bovine tolerated 112 doses of 50 mg of the alkanolamine salt, but another developed digestive problems after 80 doses of 100 mg/kg. Palmer (1963) administered daily doses of 50-250 mg 2,4-D/kg as the alkanolamine salt to a group of steers. The steer given 100 mg/kg developed ruminal atony after 86 days, presumably as a result of the herbicide. Animals given 200 and 250 mg/kg became intoxicated in 34 and 15 days, respectively, with dessicated mucous membranes and tendency to nosebleed when restrained.

16459

DOW 0011 823

Ruminants on sprayed pasture or cows given 5.5 gm daily did not show clinical evidence of toxicity, nor did production decline. 2,4-D appeared in serum of a cow fed 5.5 gm/day for 106 days, but none was passed in milk (Mitchell et al., 1946).

Chickens and other fowl also appear relatively insensitive. Bjorn and Northen (1948) found no impairment in weight gain at doses up to 28 mg 2,4-D alkanolamine/kg three times weekly for four weeks; at 280 mg/kg there was a marked decrease in gain. Single doses of 765 mg/kg were lethal, 380 mg/kg was not. 2,4-D did not cause decrease in growth rate at dose rates up to 1000 ppm in the diet, but at 7500 ppm growth essentially stopped (Whitehead and Pettigew, 1972). The acute LD_{50} was estimated at 900 mg/kg. Whitehead (1973) tested dietary 2,4-D at concentrations up to 100 mg/kg diet, and found that at dietary levels of 10 mg/kg diet or greater, growth rate was depressed. Because food conversion efficiency was not affected, it was suggested that palatability was affected resulting in decreased consumption. Chickens will discriminate against 2,4-D contaminated food (Whitehead and Pettigrew, 1972). The U.S. Fish and Wildlife service tested a number of chemicals by feeding to various game bird species in the early 1960s. 2,4-D acetamide given to young quail at 2500 ppm caused 72% mortality in 12 days, the butoxyethanol ester at 5000 ppm caused 28% mortality in 135 days, and 2500 ppm dimethylamine salt caused 12% death in 138 days. In older birds 2500 ppm was almost without effect after 50 days, and after 111 days of 1000 ppm feeding. Tests on Coturnix indicated a similar tolerance (Stickel, 1964). More recently, Hill et al. (1975) have shown the LC_{50} for 2,4-D acetamide in the diet of bob white, coturnix, pheasant, and mallard to be in excess of 5000 ppm. The butoxy ethanol ester and DMA salt have a similar low toxicity.

A number of other biological effects of 2,4-D have been found experimentally, usually at very high doses. Dybing and Kolberg (1967) suggested that 2,4-D was actively reabsorbed, and if so would competitively decrease the clearance of p-aminohippurate without interfering with creatinine clearance. Studies with rabbits given 100 mg priming doses, then 2 mg 2,4-D/min produced decreased PAH clearance at doses of 62-115 mg/kg. Chang et al. (1974) studied a number of effects on rat liver following treatment with 2-5 gm/kg 2,4-D over 4-7 weeks. Glycogen content was 50-100% higher, and liver nuclei synthesized RNA more actively in vitro than did controls. DNA content per liver was decreased. In this work, 2,4-D was compared with 2,4,5-T; the latter compound caused increased liver weight and protein accumulation, while 2,4-D decreased liver weight and lessened RNA content, with no protein accumulation. Speculation is justified that TCDD in 2,4,5-T was responsible for the difference. A dietary supplement of 60 ppm 2,4-D to lambs had no effect on rumen liquor or sheep or serum proteins. Weight gain was slightly decreased over a 12 week feeding period (Abou-Akkada et al., 1975). The same authors (1973) had previously found that 2,4-D did not alter ruminal microbial function. At fairly high doses (80 mg/kg/day, for seven days) 2,4-D increases ^{131}I intake by the thyroid. The effect only occurs in a normally functioning thyroid gland; it was not seen after hypophysectomy or iodine depletion (Florsheim and Velcoff, 1962). The effect is apparently due to lowered thyroxine binding to serum protein (Florsheim et al., 1963) but whether this is secondary to 2,4-D binding or is a specific pharmacologic effect is not known.

16460

DOW 0011 824

There has been limited in vitro study of 2,4-D. Weiss and Beckert found stimulated mitotic activity and increased chromatin after cultured monkey kidney cells, Girardi heart cells, and trout gonad cells were exposed to 10 or 50 ppm 2,4-D for 72 hours. 2,4-D inhibits mevalonate incorporation into non-saponifiable lipids of liver, but only at concentrations in excess of 1 mM (Olson et al., 1974). Oxidative phosphorylation in rat mitochondria appears to be sensitive to concentrations as low as 10^{-4} M; at 10^{-3} M respiration was normal but P/O decreased to 20% of normal (Brody, 1952). In L929 cells in monolayer culture, 2,4-D causes triglyceride accumulation when present at a concentration of 500 µg/ml (Kolberg et al., 1972) and inhibits all growth at 50 µg/ml (Kolberg et al., 1971).

2,4-D at high doses is used also as a chemical inducer of experimental myotonia, as a model of the congenital disease (Iyer et al., 1977; Eyzaguirre et al., 1948). The action is apparently due to an increase in membrane resistance and reduced chloride transport. Electrical activity of the brain was found to be reversibly inhibited by doses of 200 mg 2,4-D/kg, in rats (Desi et al., 1962). The chemical also produces a primary myopathy that is useful in study of the family of diseases which includes white muscle disease in lambs (Heene, 1969). These effects are not of toxicologic significance except in cases of massive acute intake resulting in myoneural symptoms.

Effect of 2,4-D on Reproductive Function

As with 2,4,5-T, the possibility that 2,4-D has teratogenic potential was first studied by the Bionetics Research Laboratories study (Bionetics Research Laboratories, Inc., 1970). The data were suggestive that incidence of failed lower jaw formation was somewhat greater than that resulting from the DMSO carrier. Schwetz et al. (1971) measured teratogenic and fetotoxic effects of 2,4-D and two esters on rats. The higher daily doses (75 mg 2,4-D/kg; 75 mg propylene glycol butyl ester of 2,4-D/kg; 87.5 mg isooctyl ester of 2,4-D/kg, each just below the maternal toxic dose) caused decreased fetal weight, subcutaneous edema, delayed bone ossification and wavy ribs. Most of these changes are fetotoxic rather than teratogenic. The last two are developmental effects but have no effect on survivability. No teratogenic responses were found at any dose.

There is some difference in definition of teratogenic response among authors in the field. A variety of skeletal defects that do not interfere with postnatal survival were found by Khera and McKinley (1972); most effects observed were wavy ribs or fused sternum. The increased incidence of these changes was evident at doses as low as 25 mg/kg/day. 2,4-D teratogenesis was studied by Båge et al. (1973) but only in presence of 2,4,5-T. The mixture caused some teratogenesis, but was less effective than 2,4,5-T alone, so the impact of 2,4-D in the system was difficult to evaluate.

Hamsters are subject to a teratogenic effect of high doses of 2,4-D. Collins and Williams (1971) found that 2,4-D from three different sources

16461

DOW 0011 825

caused a low incidence of anomalies, usually fused ribs, at doses of 100 mg/kg/day through days 6-10 of gestation. There was no satisfactory dose response relationship. Dietary 2,4-D at 500 and 1000 ppm did not alter reproductive function in a three-generation, 6 litter study with rats. Percentage of pups surviving to weaning and weanling weight was decreased at 1500 ppm, however (Hansen et al., 1971).

Sheep are apparently not subject to 2,4-D induced teratogenesis. Binns and Johnson (1970) administered 2 grams daily for 30, 60, and 90 days following breeding and caused no malformation. There were presumably 6 sheep per group but the number in this specific experiment was not stated.

Eggs are peculiarly vulnerable to exposure to herbicides and several studies have been directed toward defining the hazard of 2,4-D to chicken or game bird eggs. An injected dose of 10 mg 2,4-D/egg caused 50% mortality, 5 mg/egg resulted in 30% loss, and at 0.5 mg/egg 90% of the eggs hatched. No deformities occurred at any dose (Dunachie and Fletcher, 1967). A later study by Dunachie and Fletcher (1970) confirmed these findings for 2,4-D and 2,4-DB (2,4-dichlorophenoxybutyric acid).

In contrast, Lutz-Ostertag and Lutz (1970) sprayed pheasant and grouse eggs with 2,4-D at rates commonly used in the field and caused embryonic mortality and terata. Somers et al. (1973, 1974) were not able to support these findings after spraying mixtures of 2,4-D and picloram at usual field rates (2.8 kg/ha) and 2,4-D and 2,4,5-T on hen eggs (1973) and pheasant eggs (1974) at 10 times field concentrations (11.2 kg/ha). They found no "adverse effect on hatching success, incidence of malformed embryo or subsequent chick mortality". Kopischke (1972) also found no effect on pheasant eggs sprayed at field concentrations, but found that diesel fuel as a carrier blocked hatching completely. Dipping hen eggs in 1% 2,4-D for 10 seconds, then continuing incubation was similarly ineffective (Gyrd-Hansen and Dalgaard-Mikkelsen, 1974).

An interesting observation of 2,4-D distribution in mouse fetuses has been made by Lindquist and Ullberg (1971). Labeled 2,4-D given late in gestation accumulated early in the yolk sac, passed on to the fetus and was almost completely eliminated by 24 hours after administration. Distribution among tissues was non-selective, and concentrations tended to parallel those of the dam, perhaps explaining in part the lack of teratogenic effect.

Carcinogenic and Mutagenic Potential of 2,4-D

Innes et al. (1969) screened 120 compounds for tumorigenic properties in mice. 2,4-D and several of its esters were included; none caused increased tumor incidence. Hansen et al. (1971) carried out a carcinogenesis study in rats and concluded that although tumors were found, the observed incidence did not support a finding that 2,4-D is carcinogenic. There seemed to be some inconsistencies in interpretation that may have confused the issue. Apparently no other evaluations of cancer potential of 2,4-D have been made. A number of mutagenic screens have included 2,4-D, however. Jenssen and Renberg (1976) found that 2,4-D

16462

would not induce increased micronuclei in mouse bone marrow erythrocyte, but the compound did slightly depress mitotic activity. Sex-linked lethality assay of 2,4-D in male Drosophila was also negative for mutagenic activity (Vogel and Chandler, 1974). Styles (1973) treated rats with 2,4-D, then used serum from the animals in a host mediated assay with histidine-requiring S. Typhimurium mutants. No effect of 2,4-D was evident. In a screen of 110 compounds with the "Ames test," using eight histidine-requiring mutant strains, Anderson et al. (1972) was unable to detect mutagenic activity by 2,4-D.

Absorption, Metabolism, Tissue Distribution, and Excretion of 2,4-D

2,4-D was absorbed rapidly from the lung of rats in which the herbicide was injected intratracheally as 0.1 ml of 0.01-10 mM solution. The animals were sacrificed from one half to 120 minutes later. The half-time for 2,4-D absorption was 1.4 minutes, and concentration had little influence on the rate (Burton et al., 1974). When ingested, 2,4-D is absorbed primarily by the portal circulation; the lymphatic drainage accounts for very little 2,4-D absorption, as might be expected from the limited fat solubility of the herbicide (Sieber, 1976). Blood concentration of ^{14}C from radiolabeled 2,4-D administered orally to sheep has been shown to rise very rapidly (Clark et al., 1964). The rate of absorption of 2,4-D from the rumen is not known; the immediate elevation in blood ^{14}C suggests that at least some material was removed directly from the rumen. However, Gutenmann et al. (1963) fed 5 ppm 2,4-D to cattle and found that the concentration in the rumen contents decreased from 3.5 to 0.5 ppm over a 24 hour period, which does not suggest rapid absorption, but rather dilution with passage of rumen content. The chemical did not disappear in an artificial rumen.

Kohli et al. (1974) gave 5 mg orally to six human volunteers and found peak plasma concentrations by seven hours; the average plasma concentration one week later was about 10% of the peak concentration. Half time for excretion was calculated at 33 hours.

Clinical observations of human patients who had extensive skin contact with 2,4-D showed systemic toxic responses within a few hours (Goldstein et al., 1959; Todd, 1962), indicating ready absorption through the skin.

Application of 2,4-D as the DMA salt, isooctyl ester and butyl ester to the body surface of rabbits was relatively ineffective (Kay et al., 1965). Treatment was with 15 ml of aqueous or oil solution applied on a 4 x 3 gauze patch, covered tightly with plastic film. Contact was seven hours daily, five days a week for three weeks; concentrations of 2,4-D were 0.626 and 3.13% acid equivalent. Some animals were treated on abraded skin. A few animals died during the experiment but the pathology and symptoms were not typical of 2,4-D poisoning; most of the fatalities were among the animals with skin damage. No neurological lesions were found, nor were any other observed parameters changed. The only lesions observed were at the site of application.

DOW 970

16463

DOW 0011 827

There seems to be general agreement, other than the report by Kohli et al. (1974), that significant amounts of 2,4-D do not remain in an animal for much more than one or two days. Zeilinski and Fishbein (1967) compared whole body residence times of two esters of 2,4-D and the 2,4-D acid after subcutaneous injection of 100 mg/kg to mice. Of the butyl ester, 95% was gone after 6 hours, and in animals that had been pretreated with five similar daily doses, the rate of disappearance was further enhanced. The isooctyl ester was considerably slower to disappear, with a half time of somewhat less than four hours, and the acid itself was retained slightly longer. The authors did not specifically note whether hydrolysis of esters to 2,4-D acid was identified as part of the metabolic process, but the method appeared to account for the disappearance of all forms of the compound. As a comparison, the disappearance half-time of 2,4,5-T acid was on the order of 20 hours.

Khanna and Fang (1966) administered 2,4-D-^{14}C to male and female rats at very high (80 mg/rat) and relatively low (1 mg/rat) doses and found tissue residues to be greatest at about 6 hours, and almost undetectable by 30 hours after treatment. Extremely high concentrations were found in the stomach but there was no information about separation of stomach content from the tissue proper. No label was found in respiratory gases. A very small fraction of urinary label was found to be an unidentified metabolite, but the data indicate that almost all 2,4-D is excreted without change by rats.

Although 2,4-D binds reversibly to bovine serum albumin (Kolberg et al., 1973) and therefore probably to other plasma proteins, there seem to be no specific tissue binding sites as are found in plants. Morre (1974) has examined fish muscle and rat liver after exposure to 2,4-D, seeking specific binding sites and has concluded that none exist.

2,4-D apparently does not move into milk of lactating animals in significant amounts, whether treated by direct administration of the herbicide or by grazing on treated land (Bjerke et al., 1972; Gutenmann et al., 1963; Bache et al., 1964; St. John et al., 1964; Klingman et al., 1966). Under forcing conditions, however, (1000 ppm 2,4-D in diet), the level of 2,4-D residue in cows milk was forced up to 0.06 ppm, and were still barely detectable seven days later.

A substantial capacity for conjugation of 2,4,5-T has been described elsewhere in this report (Nony et al., 1976). Grunow and Böhme (1974) have found that the ability to conjugate 2,4-D with glycine and taurine exists but is considerably less than that for 2,4,5-T. In dogfish and flounder, however, a major fraction of urinary 2,4-D is excreted as the taurine conjugate (James and Bend, 1976), and in three of the four dogfish studied 10-20% of the label was found in bile after 48 hours.

In spite of the rapid loss of 2,4-D by fish, persistence of the herbicide or its products may be prolonged. Treatment of pond weeds with up to 9 kg/ha usually left no detectable residues in fish by 28 days post-application, even at the highest rates (Schultz and Harman, 1974). Schultz (1973) has found, however, that some unidentified products may be present in fish 2-3 months after treatment.

16464

DOW 0011 828

The rapid appearance of intact 2,4-D in urine is apparently mediated by a presumably active and saturable tubular excretion mechanism (Erne and Sperber, 1974). The extensive excretion of 2,4-D in urine has been noted in steers (Lisk et al., 1963), sheep (Clark et al., 1964), rats (Sieber, 1976), and humans (Kohli et al., 1974), and apparently is rapid enough to remove all but massive exposures before significant damage can occur.

Behavior of 2,4-D in the Environment and Effects on Submammalian Species

This report is primarily concerned with effects of herbicides on non-plant species, but a cursory qualitative survey of literature on field persistence is useful in judging how long a given amount of herbicide may remain available.

Water concentrations are a major concern, in relation both to aquatic species and to water supplies for the human population. 2,4-D applied in a watershed area appears in streams to a very limited extent (White et al., 1976; Krammes and Willetts, 1964). Movement into subsurface water flows also appears negligible (White et al., 1976). In aerated lake water 2,4-D persists up to 120 days, but in lake mud 2,4-D hydrolyzes very rapidly, because of microbial activity (Aly and Faust, 1964). Half-time of 2,4-D disappearance seems overall to be much less than two weeks in aqueous systems.

For some applications on water, relatively massive applications of up to 40 lbs. per acre are necessary. Two reservoirs on the Tennessee River were treated at 20 and 40 lbs/acre depending on the nature of the water milfoil infestation. The treatments did not affect fish or other fauna, and had little adverse effect on most other plants. Plankton did retain significant amounts of herbicide over extended periods, and 2,4-D was detectable on occasion in finished domestic water from the reservoirs (Wojtalik et al., 1971).

In soils, 2,4-D has been found to have a half-time of 4-5 days by Altom and Stritzke (1973). White et al. (1976) found that 95% of 2,4-D in the top 0.5 cm of soil would disappear in 7 days. However, an *in vitro* study by Alexander and Aleem (1961) indicated that 2,4-D would be detectable for as much as 94 days in one type of soil and only 23 days in another. The preparation included 100 ml of nutrient medium and 4 mls of soil as an inoculum; whether such a system represents actual breakdown conditions in soil is possibly questionable. Sufficient material to cause detectable phytotoxicity persists for about a month, according to Mullison (1972). In areas where 2,4-D is used annually, the breakdown may be more rapid in the later years than following the first treatment (Hurle and Rademacher, 1970). Wiersma et al. (1972) reported a total of 28 which had been treated with 2,4-D were found to have from 0.01 to 0.03 ppm 2,4-D present. It seems unlikely that 2,4-D would persist in either water or soil for more than a month.

2,4-D has been used extensively in control of aquatic weeds, particularly water hyacinth and water milfoil, and has probably been subjected to more study of aquatic toxicity than any other herbicide. A

16465

DOW 00111829

number of short term screens have been conducted with fish. Alabaster (1969) reported on 164 compounds, including 2,4-D sodium salt, which was found to have a 24 hour LC_{50} of 1160 ppm for harlequin fish, and the butoxyethylester of 2,4-D which was much more toxic with a 24 hour LD_{50} of 1 ppm, for the same species.

The 24-96 hr LC_{50} of PGBE esters of 2,4-D for rainbow trout were slightly above 1 ppm (Cope, 1964). For mullet and killifish the LC_{50} (24 hr) was 5 ppm for both the PGBE and butoxyethanol esters. The parent acid was ineffective at 50 ppm (Butler, 1963).

Oyster shell growth was 50% inhibited by 3.75 ppm butoxyethanol ester, but the acid and dimethylamine salt were not effective at 2 ppm (Butler, 1963). Exposure to the DMA salt for 24 hrs at 2 ppm caused no mortality in shrimp; 48 hour exposure caused 10% lethality. The butoxyethanol and PGBE esters caused no mortality to shrimp at 1 ppm x 48 hour. The ethyl hexyl ester of 2,4-D caused 38% decrease in oyster shell growth at 5 ppm over 96 hrs (Butler, 1965). The considerable differences in 2,4-D effect among various species were illustrated by Sanders (1970a) 2,4-D acid has a 48 hour median response concentration (TL_{50}) of 100 mg/L (100 ppm) for Daphnia and 3.2 mg/L for scud. (Crosby and Tucker (1966) found the same value for Daphnia.) The PGBE ester TL_{50} varies from 0.1 for Daphnia to 2.6 for scud to more than 100 ppm for crayfish. The DMA salt TL_{50} is more than 100 ppm in bluegill but TL_{50} for the butyl ether ester in bluegill is 1.1 ppm, and 100 ppm for crayfish.

Stonefly naiads are less sensitive to 2,4-D itself (LC_{50} x 96 hr = 15 ppm) than the butoxy ethanol ester (LC_{50} x 96 hr = 1.6 ppm) (Sanders and Cope, 1968). Sanders (1970b) has also reported a 96 hr TL_{50} for 2,4-D amine of 100 ppm for tadpoles.

The lethal effect on tadpoles is apparently limited, but Buslovich and Borushko (1976) found that 2,4-D sodium salt would inhibit metamorphosis of Rana temporaria tadpoles, and blocked thyroidin stimulation of the process. The DMA salt was effective at 2 ppm. The authors speculate that 2,4-D antagonizes thyroid hormone.

Few studies of truly chronic exposure of fish to 2,4-D have been made. Mount and Stephan (1967) compared the 96 hour LC_{50} for 2,4-D butoxyethanol ester (5.6 ppm) with various 10 month exposures, and found that 1/19th of the LC_{50}, or 0.3 ppm could be tolerated by fathead minnows without effect on growth or reproduction. Eggs were much more sensitive than adults over 48 hour exposures. Schultz (1973) placed bluegill, channel catfish, and largemouth bass in solution of 2,4-D DMA salt, labeled with ^{14}C at concentrations of 0.5, 1.0, or 2.0 mg/L (ppm). Fish and water samples were removed at intervals up to 84 days for analysis of ^{14}C and 2,4-D content.

Considerable radioactivity remained in fish tissues but apparently none was associated with 2,4-D, suggesting extensive metabolism. The first tissue in which radioactivity appeared was the gall bladder of catfish and bluegills, and eventually ^{14}C appeared in every tissue analyzed.

16466

DOW 0011830

In both catfish and bluegill, the amount of radioactivity in muscle tended to increase through the collection period. No toxicity was evident in any of the exposed fish.

To relate concentrations of one ppm to field condition, an application of 2 kg/hectare (10000 M^2) to water will give a concentration of 2 mg/L or 2 ppm if the water is 10 cm deep. Deeper water will result in further dilution. If applied to flowing water, water movement will dilute the herbicide quite rapidly, and that material distributed on soil and vegetation will tend to remain in place. Obviously a spill or other accident presents different problems.

Among insects, the effects of 2,4-D on honey bees has received the most attention. Palmer-Jones (1964) has listed a number of investigators who concluded that 2,4-D is safe for bees, as well as others who have shown toxic effects, some of which may depend on the species of plant on which the herbicide is deposited. In a field investigation of a three lb/acre application Palmer-Jones found that a 22% mortality occurred in 48 hours after dusting. However, when bees at the hive were heavily dusted directly, there was no mortality, leading to the conclusion that the bees in the field were acquiring intoxicant by some mechanism other than surface contact. Moffett et al. (1972) sprayed caged bees directly with various phenoxy herbicides at a one pound/acre rate with very little effect. When included in the diet at concentrations up to 100 ppm, 2,4-D did not decrease lifetime of bees and the ester used was ineffective at 1000 ppm (Morton et al., 1972). The herbicide does cause decreased brood development when fed at 100 ppm but has no reproductive effect at 10 ppm (Morton and Moffett, 1972).

Treatment of Coccinellid beetle larvae with 2,4-D at a rate equal to 8 oz. acid equivalent/acre caused greatly increased mortality, regardless of the age of the larvae through day 12 at time of spraying (Adams, 1960).

SILVEX

There appears to be a much more limited accumulation of data on biological effects of silvex and related compounds than is available for other phenoxy herbicides. The descriptions of biological effects of silvex will be categorized in two broader sections rather than the more detailed entries prepared for the other agents.

Biological Effects of Silvex and Its Derivatives

The acute median lethal dose of silvex and its esters is quite high, and falls in a relatively narrow range among species and among the derivatives. Rowe and Hymas (1954) summarized data which had been established at that time. Silvex and its mono-, di-, and tri-propylene glycol ether esters were of almost identical LD_{50} in guinea pig of 1200 and 1350 mg/kg. The lethalities to rats were also similar at about 650 mg/kg. The rat appears to be most sensitive, and chicks and mice require doses similar to the guinea pig.

16467

DOW 0011831

Subchronic (90 day) studies of rats fed 10-600 mg/kg/day of the PGBE ester resulted in more than 50% lethality at the highest dose. The time of death ranged from 15-85 days. Growth was depressed in rats fed 300 mg/kg. There is somewhat of a paradox in the findings, because pathology in the dead animals indicated malnutrition rather than herbicide toxicity. A paired feeding study at 300 and 600 mg/kg/day indicates that depressed growth was not entirely due to inadequate food consumption. Dose rates of 30 and 100 mg/kg/day caused an increase in liver weight but 10 mg/kg/day caused no change. (Mullison, 1966). The study was followed by a 90 day feeding of up to 10000 ppm silvex (sodium salt) in the diet. 10000 ppm is 1% of the diet, and if the animals consumed 10 gm daily and weigh 300 gm, the dose rate would be on the order of 300 mg/kg/day. That dose rate was highly lethal and was abandoned, but did produce swelling and granular degeneration of hepatocytes and ultimate cellular necrosis. Renal tubule cells were swelled and vacuolated, and seminiferous tubules were degenerated. At levels above 10 ppm (about 3 mg/kg/day) some growth depression occurred. Mullison (1966) also fed Kurosol (potassium salt of silvex, containing 53.3% silvex acid) for two years at concentrations up to 300 ppm. The highest dose caused a slightly increased kidney/body weight ratio in males, but 100 ppm and lower dose rates caused no change in food consumption, growth, gross and microscopic morphology hematology or bone marrow. 100 ppm was stated by the author to be equivalent to 2.6 mg/kg/day of silvex.

Kurosol was without effect on beagles at a feeding rate of 56 ppm (about 19 mg/kg/day) over 2 years. Females fed 190 ppm suffered some hepatic degeneration and necrosis after a year of feeding, but at the end of two years no damage could be found. At the 56 ppm intake, there were no changes over a very broad spectrum of hematologic, clinical chemistry and morphologic analyses.

The liver damage at higher levels included hepatocyte necrosis, bile duct proliferation, and bile pigment deposition throughout the liver and in the epithelium of kidney tubules.

In a subchronic study in cattle by Palmer et al. (1964), one yearling Brahma-cross fed 100 mg silvex/kg/daily died after 29 days. Two other animals given 25 and 50 mg/kg for 73 days showed no evidence of toxicity. A 90 day experiment with 50 mg/kg/day, either by drench or by injection into a rumen fistula resulted in death of one of the latter group of three animals. Two of those treated orally developed severe inflammations in the parotid area, apparently due to local irritation.

The survey by Innes et al. (1961) screened silvex against two strains of mice for 18 months. The concentration of silvex was 121 ppm, which was the maximum tolerable dose rate, and there was no increase in tumors in either strain. The mutagenic screen by Anderson et al. (1972) did not detect point mutations resulting from silvex treatment in either T_4 phage or S. typhimurium (histidine requiring).

Silvex is teratogenic at high doses. Courtney (1975) found that 398 mg/kg/day, on days 12-15 of gestation, caused 3% cleft palate in the

16468

DOW 0011 332

group given silvex in DMSO subcutaneously, and 7% where given orally in corn oil. Fetal mortality increased to 25% in the group treated subcutaneously.

The Dow Chemical Co. has also conducted a series of teratology studies with silvex. Dose rates of 75, 100 and 150 mg/kg/day from day 6 to day 15 caused several cardiovascular anomalies; 50 mg/kg/day caused retarded ossification in the sternum and skull (Dow Chemical Co., 1972). The no adverse effect level was considered to be 25 mg/kg/day. The PGBE ester caused skeletal changes at an intake of 50 mg/kg/daily but no changes were found after 35 mg/kg/day (23 mg/kg silvex acid equivalent).

Birds seem peculiarly resistant to the herbicides. For example, DeWitt et al. (1963) fed 5000 ppm BEE ester of silvex to young bob white quail, pheasant and mallard ducks. The average lethal intakes were 9350, 9240 and 21,000 mg/kg, and time of death varied from 10 to 100 days. Mallards were able to consume 100 ppm for 100 days with no lethality, but reproduction was impaired.

Silvex acid at 5000 ppm in the diet was tolerated for 5 days with no mortality by Coturnix. The 5 day LD_{50} for pheasants was 4500 ppm of silvex BEE ester, for bobwhite quail the LD_{50} was 30 ppm over 5 days, for Coturnix it was in excess of 5000 ppm, for pheasants LD_{50} was less than 3000, and no mallards died after 5 days on 5000 ppm. All birds were two weeks old at the initiation of the study (Heath et al., 1972).

Stickel (1964) reported US Fish and Wildlife studies in which bobwhite were fed 1000 ppm of an unspecified silvex ester; 50% were dead by day 34 with average total dose of 17000 mg/kg. Of a group fed 5000 ppm, the average daily dose was 2300 mg/kg. Half of the birds survived more than 4 days, 40% survived more than 10 days, and an additional 4% died during the remaining 14 days. The average dose to birds that survived 24 days was 54,500 mg/kg.

These findings indicate that wild fowl should not be adversely affected by any field application of silvex, or for that matter even a gross over-application.

Effect of Silvex on Aquatic and Invertebrate Species

Silvex has become an herbicide of choice for control of surface and underwater water needs. As a consequence, there has perhaps been more attention paid to its impact on aquatic species than any of the other phenoxy herbicides. There is an unfortunate diversity of experimental methods and conventions for expressing concentration and other factors which sometimes makes comparison of data difficult.

Table 1 has been constructed to simplify consideration of the various reports of toxicity to aquatic species.

Among factors that influence toxicity to fish are the chemical form of the agent, whether the formulation is granular or liquid, and water hardness. Generally the esters are more toxic, and liquid formulations

16489

Table 1. Gross Toxicity of Silvex and Its Derivatives to Fish

Species	Form of Silvex	Concentration (Acid Equiv.) ppm	Effect	Period	Reference
Bluegill	BEE ester	0.36	LC_{50}	48 h	Cope, 1964
	BEE ester	1.7	LC_{50}	48 h	Hughes and Davis, 1966
	Isooctyl ester	1.4	LC_{50}	48 h	
(young)	K salt liquid	83	LC_{50}	48 h	Hughes and Davis, 1965
	K salt granular	100	LC_{50}	48 h	Hughes and Davis, 1965
(fry)	PGBE ester	0.3	tolerated	96 h	Jones, 1962
	K salt, liquid	100	tolerated	96 h	Jones, 1962
	K salt, granular	150	tolerated	96 h	Jones, 1962
(eggs)	PGBE ester	10	no effect on hatch		Wilber and Whitney, 1973
(fry)	PGBE ester	5	100% lethal	36 h	Wilber and Whitney, 1973
	PGBE ester	1	no effect	80 days	Cope, 1964
	PGBE ester	3	liver degen.	2 weeks	Cope, 1964
	K salt	10	no effect	2 1/2 mo	Cope, 1964
	K salt	75	35% lethal	2 1/2 mo	Cope, 1964
Stoneroller	K salt	25	95% hatch	72 h	Hiltibran, 1967
(eggs)	PGBE ester	5	45% hatch	72 h	Hiltibran, 1967
Chorus frog	BEE	20	LC_{50}	24 h	Sanders, 1970b
(tadpole)	BEE	10	LC_{50}	96 h	Sanders, 1970b
Fowler's toad	BEE	22	LC_{50}	24 h	Sanders, 1970b
(tadpole)					

16470

DOW0011 833

DOW 0011834

are more toxic. Data on water hardness is contradictory; but the tendency to greater toxicity in soft water seems to be associated with the salt rather than the esters (Surber and Pickering, 1962).

Distribution rates for silvex treatment of aquatic weeds may be as high as 40 lbs/acre. An assumption of dilution throughout an 8 foot depth is apparently conventional, which would give a concentration of 1.8-1.9 ppm. If this concentration of an ester formulation were to hold in a given area, it would clearly be lethal to many fish. The salt should have up to a 100 fold safety factor for fish, depending on mineral content of the water. Such application is not a consideration in forest operation, but accidental overwater dilution could occur. Terrestrial application is at rates at least 10 fold less than 40 lb/acre but many water courses are much shallower than 8 feet, thereby leaving a similar hazard. If flowing, dilution will be rapid and periods of exposure should be limited. In static water the agent will diffuse away from the application site with time except in small shallow ponds, where some damage might possibly occur.

Lower aquatic organisms have also been well studied because of silvex use on water weeds. Representative though not comprehensive data is compiled in Table 2. Again, it appears that the salts of silvex are much less toxic than the more complex esters. The most vivid contrast is in the effect on Daphnia magna, which is 50% immobilized by 100 ppm silver potassium salt, and by only 0.18 ppm silvex PGBE ester. Some species, however, are particularly resistant to even the PGBE ester of silvex. The crayfish LC_{50} is in excess of 100 ppm; for a number of other organisms this factor is less than 1 ppm.

It is not necessary to catalogue the entire literature on silvex effects on either fish or other aquatic species. The range of effective concentrations is well illustrated as well as the extent, if not details, of species differences.

Effects of silvex on terrestrial insect life have been evaluated in honey bees by USDA workers at Tucson. All of the phenoxy herbicides including silvex were found to be relatively non-toxic to bees when applied in water at field concentration (Morton et al., 1972; Moffett et al., 1972). When fed at 100 or 1000 ppm, silvex reduced brood production, but at 10 ppm, no adverse effect was found. In each case, when the toxicant was removed, the colonies regained their original reproductive efficiency (Morton and Moffett, 1972).

Metabolic Fate of Silvex in Mammals

There have been few studies of the disposition of silvex after absorption by mammals. Bjerke et al. (1972) fed silvex at 1000 ppm in feed and found 0.12 ppm 2,4,5-trichlorophenol (TCP) in milk and 0.16 ppm in cream. In one week off contaminated feed the concentrations decreased below 0.05 ppm. Leng (1972) in a review, reported work in which 2,4,5-TCP was not detected; however, residues of silvex were substantial in the liver. After feeding 300 ppm silvex 28 days, 4 ppm were found in liver, 18 in kidney, 0.6 in muscle and 0.9 in fat. After 2000 ppm in the diet, 12, 30, 2 and 4 ppm were found in the respective tissues. In a

16471

Table 2. Gross Toxicity of Silvex and Its Derivatives

Species	Form of Silvex	Concentration (Acid Equiv.) ppm
Daphnia magna	PGBE ester	0.18
	BEE ester	2.1
	K salt	100
Scud	PGBE ester	1.8
	PGBE ester	0.8
	BEE ester	1.2
Seed Shrimp	PGBE ester	0.2
	BEE ester	4.9
Glass Shrimp	PGBE ester	3.2
	BEE ester	8.0
Sowbug	PGBE ester	0.5
	BEE ester	40
Crayfish adult	PGBE ester	100
Daphnia pulex	PGBE ester	210
Simocephalus Serrulatus	PGBE ester	2.4
Stonefly naiad	acid	5.2
		.34
	PGBE ester	5.6
		.34
Brown shrimp adult	PGBE ester	0.28

LC_{50} median lethal concentration

IC_{50} median concentration to cause immobilization

*all first in star unless otherwise noted

16472

to Invertebrate Aquatic Species*

Effect	Period	Reference
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
IC_{50}	26 h	Crosby and Tucker, 1966
LC_{50}	24 h	Sanders, 1970a
LC_{50}	96 h	Sanders, 1970a
LC_{50}	24 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
LC_{50}	48 h	Sanders, 1970a
IC_{50}	48 h	Sanders and Cope, 1966
IC_{50}	48 h	Sanders and Cope, 1966
IC_{50}	24 h	Sanders and Cope, 1968
IC_{50}	96 h	Sanders and Cope, 1968
IC_{50}	24 h	Cope, 1965
LC_{50}	96 h	Cope, 1965
LC_{50}	24 h	Butler, 1965

DOW 00111835

DOW 0011836

similar experiment Clark et al. (1975) found roughly similar values, with traces of 2,4,5-TCP in each tissue. In a single cow experiment, 5 ppm kuron was fed for four days at 5 ppm (St. John et al., 1964). The kuron hydrolyzed to silvex and was excreted as a salt in the urine. Urinary concentrations peaked at 4.4 ppm on day 4 and declined to 0.32 on day 6. About 67% of the total fed was found in urine; feces were not analyzed. No detectable residues of Kuron or silvex were found in milk and no kuron as such entered the urine.

The pharmaco-kinetics of silvex have been studied in rats given 5 or 50 mg/silvex/kg. The compound was ring-labeled with ^{14}C. The higher dose appeared to be saturating because plasma clearance was not linear, as was the case at 5 mg/kg. Half-time for plasma clearance was 9-10 hours at both doses. At the lower dose 76% of the labeled material emerged in bile in 72 hours and 90% of the high dose. Almost all of the biliary excretion was in the first 24 hours. Most of the material was subject to entero-hepatic recirculation and eventually left the body in urine. Total silvex excreted in 192 hours in urine was 78%, and 16% appeared in feces (Sauerhoff et al., 1977a).

Sauerhoff et al. (1977b) have also examined silvex disposition by humans. One mg/kg was given orally to seven men and one woman, and plasma urine and feces were analyzed for 168 hours. Clearance from plasma and appearance in urine were bi-phasic, each stage following apparent first-order kinetics, maximum plasma concentrations occurred in 2-4 hours, 65% of the dose was excreted in urine in 24 hours. Half times for the two phases in plasma were 4 and 16.5 hours and in urine were 5 and 26 hours. The largest amount recovered in feces was 3.2%.

16473

DOW 0011 837

REFERENCES

Abou Akkada, A.R., M.A. Hassan, and M.A. Naga (1973). Effect of some pesticides and herbicides on the rumen micro-organisms of sheep. J. Agr. Sci. 81:231-236.

Abou Akkada, A.R., M.A. Hassan, and M. Naga (1975). A note on the effect of herbicides on the metabolism and performance of lambs. Anim. Prod. 20:311-313.

Adams, J.B. (1960). Effects of spraying 2,4-D amine on coccinellid larvae. Can. Jour. Zool. 38:285-288.

Alabaster, J.S. (1969). Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents, and miscellaneous substances. Internat. Pest. Control 11:29-35.

Alexander, M., and M.I.H. Aleem (1961). Effect of chemical structure on microbial decomposition of aromatic herbicides. J. Agr. Food Chem. 9:44-47.

Allen, J.R., J.P. Van Miller, and D.H. Norback (1975). Tissue distribution, excretion and biological effects of [^{14}C]tetrachlorodibenzo-p-dioxin in rats. Fd. Cosmet. Toxicol. 13:501-505.

Allen, J.R., D.A. Barsotti, and J.P. Van Miller (1977a). Reproductive dysfunction in non-human primates exposed to dioxins. Presented at 16th Annual Meeting, Soc. Toxicol., Toronto, 1977. Abstract 111.

Allen, J.R., D.A. Barsott, J.P. Van Miller, L.J. Abrahamson, and J.J. Lalich (1977b). Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food and Cosmetics Toxicol. (in press).

Altom, J.D., and J.F. Stritzke (1973). Degradation of dicamba, picloram, and four phenoxy herbicides in soils. Weed Science 21:556-560.

Aly, O.M., and S.D. Faust (1964). Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J. Agr. Food Chem. 12:541-545.

Anderson, K.J., E.G. Leighty, and M.T. Takahashi (1972). Evaluation of herbicides for possible mutagenic properties. J. Agr. Food Chem. 20:649-656.

Applegate, V.C., J.H. Howell, and A.E. Hall (1957). Toxicity of 4,346 chemicals to larval lampreys and fishes. U.S. Fish and Wildlife Service Spec. Scientific Report--Fish. 207.

Atlas, S.A., E.S. Vesell, and D.W. Nebert (1976). Genetic control of interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. Cancer Res. 36:4619-4630.

1647

DOW 0011 838

Axelson, O., and L. Sundell (1974). Herbicide exposure, mortality and tumor incidence. An epidemiological investigation on Swedish railroad workers. Work-Environ. Health 11:21-28.

Bache, C.A., D.D. Hardee, F.R. Holland, and D.J. Lisk (1964). Absence of phenoxyacid herbicide residues in the milk of dairy cows at high feeding levels. J. Dairy Sci. 47:298-299.

Båge, G., E. Cekanova, and K.S. Larsson (1973). Teratogenic and embryotoxic effects of the herbicides di- and trichlorophenoxyacetic acids (2,4-D and 2,4,5-T). Acta Pharmacol. et Toxicol. 32:408-416.

Beatty, P.W., and R.A. Neal (1975). Effect of alteration of hepatic mixed function oxidase activity on the toxicity of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) to rats. Toxicol. Appl. Pharmacol. 33: 151.

Beatty, P.W., and R.A. Neal (1976). Induction of DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Biophys. Res. Comm. 68:197-204.

Beatty, P.W., M.A. Holscher, and R.A. Neal (1976). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in larval and adult forms of rana catesbeiana. Bull. Environ. Contam. Toxicol. 16:578-581.

Becker, B.A. (1975). Teratogens. In: Toxicology. The Basic Science of Poisons. L.J. Casarett and J. Doull (eds). MacMillan, New York. pp. 331-332.

Berkeley, M.C., and K.R. Magee (1963). Neuropathy following exposure to a dimethylamine salt of 2,4-D. Arch. Int. Med. 111:351-352.

Berndt, W.O., and F. Koschier (1973). In vitro uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by renal cortical tissue of rabbits and rats. Toxicol. Appl. Pharmacol. 26:559-570.

Berry, D.L., P.K. Zachariah, M.J. Namkung, and M.R. Juchau (1976). Transplacental induction of carcinogen-hydroxylating systems with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 36:569-584.

Berwick, P. (1970). 2,4-Dichlorophenoxyacetic acid poisoning in man. J. Am. Med. Assoc. 214:1114-1117.

Binns, W., and A.E. Johnson (1970). Chronic and teratogenic effects of 2,4-D (2,4-dichlorophenoxyacetic acid) and atrazine (2-chloro-4-ethylamino-6-isopropyl amino-s-triazine) to sheep. North Central Weed Control Conference Proc. 1970:100.

Binns, W., and L. Balls (1971). Nonteratogenic effects of 2,4,5-trichlorophenoxyacetic acid and 2,4,5-T propylene glycol butyl esters herbicides in sheep. Teratology 4:245.

16475

DOW 000111839

Bionetics Research Laboratories, Inc. (1970). Evaluation of the carcinogenic, teratogenic, and mutagenic activity of selected pesticides and industrial chemicals. Vol. III. Evaluation of the teratogenic activity of selected pesticides and industrial chemicals in mice and rats. (Report to the National Cancer Institute on contracts PH 43-64-57 and PH 43-67-735).

Bjerke, E.L., J.L. Herman, P.W. Miller, and J.H. Wetters (1972). Residue study of phenoxy herbicides in milk and cream. J. Agr. Food Chem. 20:963-967.

Björklund, N-E., and K. Erne (1971). Phenoxy acid induced renal changes in the chicken. Acta Vet. Scand. 12:243-256.

Bleiberg, J., M. Wallen, R. Brodkin, and I.L. Applebaum (1964). Industrially acquired porphyria. Arch. Dermatol. 89:793-797.

Bovey, R.W., and J.R. Baur (1972). Persistence of 2,4,5-T in grasslands of Texas. Bull. Environ. Contam. Toxicol. 8:229-233.

Brody, T.M. (1952). Effects of certain plant growth substances on oxidative phosphorylation in rat liver mitochondria. Proc. Soc. Exp. Biol. Med. 80:533-536.

Bucher, N.L.R. (1946). Effects of 2,4-dichlorophenoxyacetic acid on experimental animals. Proc. Soc. Exp. Biol. Med. 63:204-205.

Burton, J.A., T.H. Gardiner, and L.S. Schanker (1974). Absorption of herbicides from the rat lung. Arch. Environ. Health 29:31-33.

Buselmaier, W., G. Röhrborn, and P. Propping (1973). Comparative investigations on the mutagenicity of pesticides in mammalian test systems. Mutation Res. 21:25-26.

Buslovich, S., and N. Boruschko (1976). Chlorine derivatives of phenoxyacetic acid as antagonists of thyroid hormones. Farmakol. Toksikol. 39:481-483.

Butler, P.A. (1963). Pesticide-wildlife effects. U.S. Fish and Wildlife Service Circular 167. pp. 11-26.

Butler, P.A. (1965). Effects of pesticides on fish and wildlife. U.S. Fish and Wildlife Circular 226. pp. 65-77.

Byast, T.H., and R.J. Hance (1975). Degradation of 2,4,5-T by South Vietnamese soils incubated in the laboratory. Bull. Environ. Contam. Toxicol. 14:71-76.

Carter, C.D., R.D. Kimbrough, J.A. Liddle, R.E. Cline, M.M. Zack, Jr., W.R. Barthel, R.E. Koehler, and P.E. Phillips (1975). Tetrachlorodibenzodioxin: An accidental poisoning episode in horse arenas. Science 118:738-740.

Chang, H-C., J.W. Rip, and J.H. Cherry (1974). Effects of phenoxyacetic acids on rat liver tissues. J. Agr. Food Chem. 22:62-65.

18476

DOW 0011.810

Chhabra, R.S., J.M. Tredger, R.M. Philpot, and J.R. Fouts (1974). Selection of inducers: An important factor in characterizing genetic differences to induction of aryl hydrocarbon hydroxylase in strains of mice. Life Sci. 15:123-130.

Clark, D.E., J.E. Young, R.L. Younger, L.M. Hunt, and J.K. McLaran (1964). The fate of 2,4-dichlorophenoxyacetic acid in sheep. J. Agr. Food Chem. 12:43-45.

Clark, D.E., J.S. Palmer, and C.H. Ayala (1970). Residual and toxicological aspects of 2,4,5-T and an ester in sheep and cattle. Presented at meeting of Am. Chem. Soc. Pesticide Division, and Chemical Inst. of Canada. Toronto, 1970.

Clark, D.E., and J.S. Palmer (1971). Residual aspects of 2,4,5-T and an ester in sheep and cattle with observations on concomitant toxicological effects. J. Agr. Food Chem. 19:761-764.

Clark, D.E., J.S. Palmer, R.D. Radeleff, H.R. Crookshank, and F.M. Farr (1975). Residues of chlorophenoxy acid herbicides and their phenolic metabolites in tissues of sheep and cattle. J. Agr. Food Chem. 23: 573-578.

Collins, T.F.X., and C.H. Williams (1971). Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. Bull. Environ. Contam. Toxicol. 6:559-567.

Cope, O.B. (1964). Pesticide-wildlife studies. U.S. Fish and Wildlife Service Circular 199. pp. 29-43.

Cope, O.B. (1965). Effects of pesticides on fish and wildlife. U.S. Fish and Wildlife Service Circular 226. pp. 51-77.

Courtney, K.D., and J.A. Moore (1971). Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 20:396-403.

Courtney, K.D., D.W. Gaylor, M.D. Hogan, H.L. Falk, R.R. Bates, and I. Mitchell (1970). Teratogenic evaluation of 2,4,5-T. Science 168: 864-866.

Courtney, K.D. (1976). Mouse teratology studies with chlorodibenzo-p-dioxins. Bull. Environ. Contam. Toxicol. 16:674-681.

Crosby, D.G., and R.K. Tucker (1966). Toxicity of aquatic herbicides to Daphnia magna. Science 154:289-290.

Crosby, D.G., and A.S. Wong (1977). Environmental degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Science 195:1337-1338.

Crosby, D.G., K.W. Moilanen, and A.S. Wong (1973). Environmental generation and degradation of dibenzodioxins and dibenzofurans. Env. Health. Persp. 5:259-266.

16477

DOW 0011 841

Cunningham, H.M., and D.T. Williams (1972). Effect of tetrachlorodibenzo-p-dioxin on growth rate and the synthesis of lipids and proteins in rats. Bull. Environ. Contam. Toxicol. 4:45-51.

Davis, J.T., and J.S. Hughes (1963). Further observations on the toxicity of commercial herbicides to bluegill sunfish. Proc. Southern Weed Conference 16:337-340.

Dävring, L. (1975). Effects of a 2,4,5-T ester on early oogenesis, fertility and development in Drosophila melanogaster. Hereditas 80:255-262.

Dävring, L., and M. Sunner (1971). Cytogenetic effects of 2,4,5-trichlorophenoxyacetic acid on oogenesis and early embryogenesis in Drosophila melanogaster. Hereditas 68:115-122.

Dencker, L. (1976). The herbicide 2,4,5-T: Early placental barrier and accelerated fetal uptake with advancing gestation. In: Tissue Localization of Some Teratogens at Early and Late Gestation Related to Fetal Effects. Acta Pharm. Toxicol. [suppl.] 39:59-72.

Desi, I., J. Sos, J. Olasz, F. Bule, and V. Markus (1962). Nervous system effects of a chemical herbicide. Arch. Environ. Health 4:95-102.

DeWitt, J.B., W.H. Stickel, and P.F. Springer (1963). Pesticide-wildlife studies. U.S. Fish and Wildlife Service Circular 167. pp. 74-105.

Dougherty, W.J., M. Herbst, and F. Coulston (1975). The non-teratogenicity of 2,4,5-trichlorophenoxyacetic acid in the rhesus monkey (Macaca mulatta). Bull. Environ. Contam. Toxicol. 13:477-482.

Dow Chemical Company, Internal Communication (1961). Results of 90-day dietary feeding studies on Dowanol 9713 ester 2,4,5-T in rats.

Drill, V.A., and T. Hiratzka (1953). Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Ind. Hyg. Occup. Med. 7:61-67.

Dugois, P., and L. Columb (1957). Chloric acne: Case reports arising from the preparation of 2,4,5-trichlorophenol. J. Med. Lyon 38: 899-903.

Dunachie, J.F., and W.W. Fletcher (1967). Effect of some herbicides on the hatching rate of hen's eggs. Nature 215:1406-1407.

Dunachie, J.F., and W.W. Fletcher (1970). The toxicity of certain herbicides to hen's eggs assessed by the egg-injection technique. Ann. Appl. Biol. 66:515-520.

Dybing, F., and A. Kolberg (1967). Inhibition of the renal tubular transport of p-aminohippurate-(Tm-PAH) in the rabbit caused by subtoxic doses of dichlorophenoxyacetate (2,4-D). Acta Pharm. et Toxicol. 25: Suppl. 4:51.

16478

DOW 0011 842

Emerson, J.L., D.J. Thompson, R.J. Strebing, C.G. Gerbig, and V.B. Robinson (1971). Teratogenic studies on 2,4,5-trichlorophenoxyacetic acid in the rat and rabbit. Fd. Cosmet. Toxicol. 9:395-404.

Erne, K. (1966a). Distribution and elimination of chlorinated phenoxyacetic acids in animals. Acta Vet. Scand. 7:264-271.

Erne, K. (1966b). Studies on the animal metabolism of phenoxyacetic herbicides. Acta Vet. Scand. 7:264-271.

Erne, K. (1975). Phenoxy herbicide residues in Swedish fish and wildlife. Environ. Quality & Safety (Suppl.) 3:192-195.

Erne, K., and I. Sperber (1974). Renal tubular transfer of phenoxyacetic acids in the chicken. Acta Pharmacol. Toxicol. 35:233-241.

Evans, J.O., and O.R. Duseja (1973). Herbicide contamination of surface runoff waters. USEPA Report 2-73-266.

Eyzaguirre, C., B.P. Folk, K.L. Zierler, and J.L. Lilienthal, Jr. (1948). Experimental myotonia and repetitive phenomena: The veratrinic effects of 2,4-D in the rat. Am. Jour. Physiol. 155:69-77.

Fang, S.C., E. Fallin, M.L. Montgomery, and V.H. Freed (1973). The metabolism and distribution of 2,4,5-trichlorophenoxyacetic acid in female rats. Toxicol. Appl. Pharmacol. 24:555-563.

Florsheim, W.H., and S.M. Velcoff (1962). Some effects of 2,4-dichlorophenoxy acetic acid on thyroid function in the rat: Effects of iodine accumulation. Endo. 71:1-6.

Florsheim, W.H., S.M. Velcoff, and A.D. Williams (1963). Some effects of 2,4-dichlorophenoxy acetic acid on thyroid function in the rat: Effects on peripheral thyroxine. Endo. 72:327-333.

Fowler, B.A., G.W. Lucier, H.W. Brown, and O.S. McDaniel (1973). Ultrastructural changes in rat liver cells following a single oral dose of TCDD. Environ. Health Perspec. 5:141-148.

Fowler, B.A., G.E.R. Hook, and G.W. Lucier (1977). Tetrachlorodibenzo-p-dioxin induction of renal microsomal enzyme systems: ultrastructural effects on pars recta (S_3) proximal tubule cells. Jour. Pharmacol. Exp. Therap. (in press).

Fries, G.F., and G.S. Marrow (1975). Retention and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rats. J. Agr. Food Chem. 23:265-269.

Gaines, T.B., J.F. Holson, Jr., C.J. Nelson, and H.J. Schumacher (1975). Analysis of strain differences in sensitivity and reproducibility of results in assessing 2,4,5-T teratogenicity in mice. Toxicol. Appl. Pharmacol. 33:174-175.

16479

DOW 0011 843

Gehring, P.J., C.G. Kramer, B.A. Schwetz, J.Q. Rose, and V.K. Rowe (1973). The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to man. Toxicol Appl. Pharmacol. 26:352-361.

Goldstein, N.P., P.H. Jones, and J.R. Brown (1959). Peripheral neuropathy after exposure to an ester of dichlorophenoxyacetic acid. J. Am. Med. Assoc. 171:1306-1309.

Goldstein, J.A., P. Hickman, H. Bergman, and J.G. Vos (1973). Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. Res. Comm. Chem. Pathol. Pharmacol. 6:919-928.

Greig, J.B., G. Jones, W.H. Butler, and J.M. Barnes (1973). Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fd. Cosmet. Toxicol. 11:585-595.

Grunow, W., C. Böhme, and B. Budczies (1971). Renale ausscheidung von 2,4,5-T bei ratten. Fd. Cosmet. Toxicol. 9:667-670.

Grunow, W., and C. Böhme (1974). Über den stoffwechsel von 2,4,5-T und 2,4-D bei ratten und mäusen. Arch. Toxicol. 23:217-225.

Gupta, B.N., J.G. Vos, J.A. Moore, J.G. Zinkl, and B.C. Bullock (1973). Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5:125-140.

Gutenmann, W.H., D.D. Hardee, R.F. Hardee, and D.J. Lisk (1963). Residue studies with 2,4-dichlorophenoxy acetic acid herbicide in the dairy cow and in a natural and artificial rumen. J. Dairy Sci. 46:1287-1288.

Gyrd-Hansen, N., and S. Dalgaard-Mikkelsen (1974). The effect of phenoxy-herbicides on the hatchability of eggs and the viability of the chicks. Acta Pharmacol. Toxicol. 35:300-308.

Hall, S.M. (1972). Effects on pregnant rats and their progeny of adequate or low protein diets containing 2,4,5-Trichlorophenoxy acetic acid (2,4,5-T) or p,p'-DDT. Fed. Proc. 31:726.

Hamada, N., and R.E. Peterson (1977). Reversal of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced depression in biliary excretion of ouabain by pregnenolone-16α-carbonitrile and spironolactone in rats. Int. Cong. Toxicol., Toronto. Abstracts p. 25.

Hansen, W.H., M.L. Quaife, R.T. Haberman, and O.G. Fitzhugh (1971). Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. Toxicol. Appl. Pharmacol. 20:122-129.

Haugen, D.A., M.J. Coon, and D.W. Nebert (1976). Induction of multiple forms of mouse liver cytochrome P-450. Evidence for genetically controlled protein synthesis in response to treatment with β naphthaflavone or phenobarbital. Jour. Biol. Chem. 251:1817-1827.

16480

DOW 0011 844

Hay, A. (1976). Toxic cloud over Seveso (Editorial, no references). Nature 262:636-638.

Heene, R. (1969). Redeutung experimentell-histochemischer Befunde für die pathogenese von muskeldystrophien. Klin. Wschr. 47:389-390.

Heath, R.G., J.W. Spann, E.F. Hill, and J.F. Kreitzer (1972). Comparative dietary toxicities of pesticides to birds. Bur. Spt. Fisheries and Wildlife, Spec. Scientific Report (Wildlife) 152.

Highman, B., T.B. Gaines, and H.J. Schumacher (1976a). Sequential histopathologic, hematologic, and blood chemistry changed induced in mice by a technical and a purified preparation of 2,4,5-trichlorophenoxyacetic acid. J. Toxicol. Environ. Health 1:469-484.

Highman, B., T.B. Gaines, H.J. Schumacher, and T.J. Haley (1976b). Strain differences in histopathologic, hematologic, and blood chemistry changes induced in mice by a technical and a purified preparation of 2,4,5-trichlorophenoxyacetic acid. J. Toxicol. Environ. Health 1:1041-1054.

Hill, E.V., and H. Carlisle (1947). Toxicity of 2,4-dichlorophenoxyacetic acid for experimental animals. J. Indust. Hyg. Toxicol. 29:85-95.

Hill, E.F., R.G. Heath, J.W. Spann, and J.D. Williams (1975). Lethal dietary toxicities of environmental pollutants to birds. U.S. Fish and Wildlife Service Spec. Scientific Report (Wildlife) 191. 61 pp.

Hiltibran, R.D. (1967). Effects of some herbicides on fertilized fish eggs and fry. Trans. Am. Fish. Soc. 96:414-416.

Hook, G.E.R., T.C. Orton, J.A. Moore, and G.W. Lucier (1975a). 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced changes in the hydroxylation of biphenyl by rat liver microsomes. Biochem. Pharmacol. 24:335-340.

Hook, G.E.R., J.K. Haseman, and G.W. Lucier (1975b). Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing enzyme systems by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chem.-Biol. Interactions 10:199-214.

Hook, J.B., M.D. Bailie, and J.T. Johnson (1974). In vitro analysis of transport of 2,4,5-trichlorophenoxyacetic acid by rat and dog kidney. Fd. Cosmet. Toxicol. 12:209-218.

Hook, J.B., R. Cardona, J.L. Osborn, and M.D. Bailie (1976). The renal handling of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the dog. Fd. Cosmet. Toxicol. 14:19-23.

Hughes, J.S., and J.T. Davis (1963). Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds 11:50-53.

16481

DOW 0011845

Hughes, J.S., and J.T. Davis (1965 (1962 conference)). Comparative toxicity to bluegill sunfish of granular and liquid herbicides. Proc. Conf. Southeast Assoc. of Game and Fish Comm. 16:319-322.

Hughes, J.S., and J.T. Davis (1966). Toxicity of pesticides to bluegill sunfish tested during 1961-1966. Louisiana Wildlife and Fisheries Comm. 8 pp.

Hurle, K., and B. Rademacher (1970). Studies on the effect of long-term application of DMOC and 2,4-D on their breakdown in the soil. Weed Research 10:159-164.

Hussain, S., L. Ehrenberg, G. Löfroth, and T. Gejvall (1972). Mutagenic effects of TCDD on bacterial systems. Ambio 1:32-33.

Innes, J.R.M., B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta, R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell, and J. Peters (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Nat. Cancer Inst. 42:1101-1114.

Isensee, A.R., and G.E. Jones (1975). Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic model ecosystem. Environ. Sci. Technol. 9:668-672.

Iyer, V., M. Whiting, and G. Fenichel (1977). Neural influence on experimental myotonia. Neurology 27:73-76.

Jackson, W.T. (1972). Regulation of mitosis. III. Cytological effects of 2,4,5-trichlorophenoxy acetic acid and of dioxin contaminants in 2,4,5-T formulations. Jour. Cell Sci. 10:15-25.

James, M.O., and J.R. Bend (1976). Taurine conjugation of 2,4-dichlorophenoxyacetic acid and phenylacetic acid in two marine species. Xenobiotica 6:393-398.

Jenssen, D., and L. Renberg (1976). Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. Chem.-Biol. Interact. 14:291-299.

Jirazek, L., J. Kalensky, K. Kubek, J. Pazderova, and E. Lukas (1974). Acne chlorina, porphyria cutanea, and other manifestations of general intoxication during manufacture of herbicides. Czech. Dermatol. 49:145-157.

Johnson, J.E. (1971). The public health implications of widespread use of the phenoxy herbicides and picloram. Bioscience 21:899-905.

Jones, G., and W.H. Butler (1974). A morphological study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. J. Path. 112:93-97.

16482

DOW 0011 846

Jones, R.O. (1965 (1962 conference)). Tolerance of the fry of common warm-water fishes to some chemicals employed in fish culture. Proc. Conf. Southeast Assoc. Game and Fish. Comm. 16:436-445.

Kay, J.H., R.J. Palazzolo, and J.C. Calandra (1965). Subacute dermal toxicity of 2,4-D. Arch. Environ. Health 11:648-651.

Kearney, P.C., E.A. Woolson, and C.P. Ellington, Jr. (1972). Persistence and metabolism of chlorodioxins in soils. Environ. Science Technol. 6:1017-1019.

Kellermann, G., M. Luyten-Kellermann, and C.R. Shaw (1973). Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. Amer. Jour. Human Genet. 25:327-331.

Kenaga, E.E. (1974). 2,4,5-T and derivatives: Toxicity and stability in the aquatic environment. Down to Earth 30(3):19-25.

Kenaga, E.E. (1975). The evaluation of the safety of 2,4,5-T to birds in areas treated for vegetation control. Residue Rev. 99:1-19.

Khanna, S., and S.C. Fang (1966). Metabolism of C^{14}-labeled 2,4-dichlorophenoxyacetic acid in rats. J. Agr. Food Chem. 14:500-503.

Khera, K.S., and J.A. Ruddick (1973). Poly(chloro)dibenzo-p-dioxins. Perinatal effects and the dominant lethal test in Wistar rats. ACS Adv. Chem. Series 120:70-84.

Khera, K.S., and W.P. McKinley (1972). Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats. Toxicol. Appl. Pharmacol. 22:14-28.

Kilian, D.J., M.C. Benge, R.V. Johnston, and E.B. Whorton, Jr. (1977). (Personal communication, unpublished manuscript). Cytogenetic studies of personnel who manufacture 2,4,5-T.

Kimbrough, R.D. (1974). The toxicity of polychlorinated polycyclic compounds and related chemicals. CRC Crit. Rev. in Toxicol. 2:445-498.

Kimbrough, R.D., C.D. Carter, J.A. Liddle, R.E. Cline, and P.E. Phillips (1977). Epidemiology and pathology of a tetrachlorodibenzo-dioxin poisoning episode. Arch. Environ. Health 32:77-85.

Kimmig, J., and K.H. Schulz (1957). Berufliche Akne (sogenannten Chlorakne) durch chlorierte aromatische zyclische Äther. Dermatologica 115: 540-546.

Klingman, D.L., C.H. Gordon, G. Yip, and H.P. Burchfield (1966). Residues in the forage and in milk from cows grazing forage treated with esters of 2,4-D. Weeds 14:164-167.

Kocher, C.W., N.H. Mahle, R.A. Hummel, and L.A. Shadoff (1977). A search for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in beef fat. Bull. Environ. Contam. Toxicol. (in press).

16483

DOW 00111817

Kociba, R.J., P.A. Keeler, C.N. Park, and P.J. Gehring (1976). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35:553-574.

Kohli, J.D., R.N. Khanna, and B.N. Gupta (1974). Absorption and excretion of 2,4-dichlorophenoxyacetic acid in man. Arch. Int. Pharmacodyn. Therap. 210:250-255.

Kolberg, J., K. Helgeland, J. Jonsen, and O. Tjelveit (1971). The herbicide 2,4-dichlorophenoxyacetic acid. I. Effects on L cells. Acta Pharmacol. et Toxicol. 29:81-86.

Kolberg, J., K. Helgeland, and J. Jonsen (1972). The herbicide 2,4-dichlorophenoxyacetic acid. II. Triglyceride accumulation in L cells. Acta Pharmacol. 31:481-487.

Kolberg, J., K. Helgeland, and J. Jonsen (1973). Binding of 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acid to bovine serum albumin. Acta Pharmacol. Toxicol. 33:470-475.

Konstantinova, T.K. (1974a). Effect of a herbicide, the butyl ester of 2,4,5-T on the progeny of experimental animals. Gig. Tr. Prof. Zabol. 9:29-32 (Chem. Abstr. 82:26816p).

Konstantinova, T.K. (1974b). Effect of the herbicide 2,4,5-T butyl ester on the maternal body and on the fetus under experimental conditions. Gig. Sanit. 8:101-102 (Chem. Abst. 82:26851w).

Kopischke, E.D. (1972). The effect of 2,4-D and diesel fuel on egg hatchability. J. Wildlife Management 36:1353-1355.

Koschier, F.J., and W.O. Berndt (1976a). Studies on the mechanism of renal transport depression caused by pretreatment with 2,4,5-trichlorophenoxyacetate. J. Toxicol. Environ. Health 2:323-334.

Koschier, F.J., and W.O. Berndt (1976b). Specificity of 2,4,5-trichlorophenoxyacetate on tetraethylammonium transport. Toxicol. Appl. Pharmacol. 38:297-306.

Koschier, F.J., and W.O. Berndt (1976c). In vitro uptake of organic ions by renal cortical tissue of rats treated acutely with 2,4,5-trichlorophenoxyacetic acid. Toxicol. Appl. Pharmacol. 35:355-364.

Kouri, R.E., H. Ratrie III, S.A. Atlas, A. Niwa, and D.W. Nebert (1974). Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sciences 15:1585-1595.

Krammes, J.W., and D.B. Willets (1964). Effect of 2,4-D and 2,4,5-T on water quality after a spraying treatment. U.S. Forest Service Res. Note PSW-52, Pacific SW Forest and Range Experiment Station, Berkeley. 4 p.

16484

DOW 0011 848

Kutches, A.J., D.C. Church, and F.L. Duryee (1970). Toxicological effects of pesticides on rumen function in vitro. J. Agr. Food Chem. 18: 430-433.

Leng, M.L. (1972). Residues in milk and meat and safety to livestock from the use of phenoxy herbicides in pasture and rangeland. Down to Earth 28(1):12-20.

Leng, M.L. (1977). Comparative metabolism of phenoxy herbicides in animals. In: Fate of Pesticides in Large Animals. G.W. Ivie and H.W. Dorough (eds.). Academic Press, New York.

Lindquist, N.G., and S. Ullberg (1971). Distribution of the herbicides 2,4,5-T and 2,4-D in pregnant mice. Accumulation in the yolk sac epithelium. Experientia 27:1439-1441.

Lisk, D.J., W.H. Gutenmann, C.A. Bache, R.G. Warner, and D.G. Wagner (1963). Elimination of 2,4-D in the urine of steers fed 4-(2,4-DB) or 2,4-D. J. Dairy Sci. 46:1435-1437.

Lucier, G.W., O.S. McDaniel, G.E.R. Hook, B.A. Fowler, B.R. Sonawane, and E. Faeder (1973). TCDD-induced changes in rat liver microsomal enzymes. Environ. Health Persp. 5:199-209.

Lucier, G.W., B.R. Sonawane, O.S. McDaniel, and G.E.R. Hook (1975a). Postnatal stimulation of hepatic microsomal enzymes following administration of TCDD to pregnant rats. Chem. Biol. Interactions 11:13-20.

Lucier, G.W., O.S. McDaniel, and G.E.R. Hook (1975b). Nature of the enhancement of hepatic uridine diphosphate glucuronyltransferase activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Biochem. Pharmacol. 24:325-334.

Lutz-Ostertag et M.H. Lutz (1970). TOXICOLOGIE--Action néfaste de l'herbicide, 2,4-D sur le developpement embryonnaire et la fécondité du gibier à plumes. C.R. Acad. Sc. Paris Series D 271:2418-2421.

MacKenzie, C.J.G., W.K. Oldham, and W.D. Powrie (1975). Relating to the control of the use of pesticides and herbicides and use of animal and plant pest-control mechanisms. Final Report of the Commissioners, Royal Commission of Inquiry into the Use of Pesticides and Herbicides.

Mahle, N.H., H.S. Higgins, and M.E. Getzendaner (1977). Search for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in bovine milk. Bull. Environ. Contam. Toxicol. (in press).

Majumdar, S.K., and R.C. Hall (1973). Cytogenetic effects of 2,4,5-T on in vitro bone marrow cells of Mongolian gerbils. The Journal of Heredity 64:213-216.

16485

DOW 0011 849

Majumdar, S.K., and J.K. Golia (1974). Mutation test of 2,4,5-trichlorophenoxyacetic acid on Drosophila melanogaster. Can. J. Genet. Cytol. 16:465-456.

Matsumura, F., and H.J. Benezet (1973). Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Persp. 5:253-258.

May, G. (1973). Chloracne from the accidental production of tetrachlorodibenzodioxin. Brit. Jour. Indust. Med. 30:276-283.

McCann, J., E. Choi, E. Yamasaki, and B.N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. 72:5135-5139.

McCollister, S.B., and F.J. Kociba (1970). Results of 90-day dietary feeding study on 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in rats. Dow Chemical Co. Internal Report.

McConnell, E.E., J.A. Moore, J.K. Haseman, and M.W. Harris (1977a). The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. (in press).

McConnell, E.E., J.A. Moore, and D.W. Dalgard (1977b). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rhesus monkeys (macaca mulatta) following a single oral dose. Toxicol. Appl. Pharmacol. (in press).

McNulty, W.D. (1977). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin for rhesus monkeys: brief report. Bull. Environ. Contam. Toxicol. 18:18-19.

McQueen, E.G., A.M.O. Veale, W.S. Alexander, and M.N. Bates (1977). 2,4,5-T and human birth defects. Report of the Division of Public Health, New Zealand Department of Health.

Mercier, M.J. (1976). 2,3,7,8-tetrachlorodibenzo-p-dioxin: An overview. Proceedings of the expert meeting on the problems raised by TCDD pollution. Commission of the European Communities.

Miller, R.A., L.A. Norris, and C.L. Hawkes (1973). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic organisms. Environ. Health Persp. 5:177-189.

Mitchell, J.W., R.E. Hodgson, and C.F. Gaetjens (1946). Tolerance of farm animals to feed containing 2,4-dichlorophenoxyacetic acid. Jour. Animal Sci. 5:226-232.

Moffett, J.O., H.L. Morton, and R.H. MacDonald (1972). Toxicity of some herbicidal sprays to honey bees. Jour. Ec. Entom. 65:32-36.

Morre, D.J. (1974). Brush control along agricultural drainage ditches. Environmental safety and efficacy of herbicide formulations. Tech. Report, Purdue University Water Resource Research Center.

16488

DOW 0011 850

Morton, H.L., E.D. Robison, and R.E. Meyer (1967). Persistence of 2,4-D, 2,4,5-T, and dicamba in range forage grasses. Weeds 15:768-771.

Morton, H.L., and J.O. Moffett (1972). Ovicidal and larvicidal effects of certain herbicides on honey bees. Environ. Entom. 1:611-614.

Morton, H.L., J.O. Moffett, and R.H. MacDonald (1972). Toxicity of herbicides to newly emerged honey bees. Environ. Entom. 1:102-104.

Mount, D.I., and C.E. Stephan (1967). A method for establishing acceptable toxicant limits for fish--malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fisheries Soc. 96:185-193.

Mullison, W.R. (1972). Ecological effects of herbicides. Down to Earth 28(2):21-24.

Mullison, W.R. (1966). Some toxicological aspects of silvex. Presented at the Southern Weed Conference, Jacksonville, Florida, 18 Jan 1966.

Muranyi-Kovacs, I., G. Rudali, and J. Imbert (1976). Bioassay of 2,4,5-trichlorophenoxyacetic acid for carcinogenicity in mice. Brit. Jour. Cancer 33:626-633.

Murray, F.J., F.A. Smith, K.D. Nitschke, C.G. Humiston, R.J. Kociba, and B.A. Schwetz (1977). Three-generation reproduction study of rats ingesting 2,3,7,8-tetrachlorodibenzo-p-dioxin. Presented at 16th Annual Meeting, Soc. of Toxicol., Toronto. Abstract 166.

National Academy of Sciences and National Research Council (1977). Drinking water and health. (In press).

Nebert, D.W., J.R. Robinson, A. Niwa, K. Kumaki, and A.P. Poland (1975). Genetic expression of aryl hydrocarbon hydroxylase activity in the mouse. J. Cell Physiology 85:393-414.

Neubert, D., and I. Dillmann (1972). Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Naunyn-Schmiedeberg's Arch. Pharmacol. 272:243-264.

Neubert, D., P. Zens, A. Rothenwallner, and H-J. Merker (1973). A survey of the embryotoxic effects of TCDD in mammalian species. Environ. Health Persp. 5:67-79.

Newton, M., and L.A. Norris (1968). Herbicide residues in blacktail deer from forests treated with 2,4,5-T and atrazine. Proc. West. Soc. Weed Sci. 32-34.

Nielsen, K., B. Kaempe, and J. Jensen-Holm (1965). Fatal poisoning in man by 2,4-dichlorophenoxyacetic acid (2,4-D): Determination of the agent in forensic materials. Acta Pharmacol. et Toxicol. 22: 224-234.

16487

DOW0011 851

Niwa, A., K. Kumaki, and D.W. Nebert (1975). Induction of aryl hydrocarbon hydroxylase activity in various cell cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Molec. Pharmacol. 11:399-408.

Nony, R., M.C. Bowman, C.L. Holder, J.F. Young, and W.L. Oller (1976). Trace analysis of 2,4,5-trichlorophenoxyacetic acid, its glycineamide, and their alkaline hydrolyzable conjugates in mouse blood, urine, and feces. Jour. Pharm. Sci. 1810-1816.

Norback, D.H., and J.R. Allen (1969). Morphogenesis of toxic fat-induced concentric membrane arrays in rat hepatocytes. Lab. Invest. 20(4):338-346.

Norback, D.H., and J.R. Allen (1973). Biological responses of the non-human primate, chicken, and rat to chlorinated dibenzo-p-dioxin ingestion. Environ. Health Persp. 5:233-240.

Norris, L.A. (1967). Chemical brush control and herbicide residues in the forest environment. In: Herbicides and Vegetation Management Symposium, Oregon State University, pp. 103-123.

Norris, L.A. (1968). Stream contamination by herbicides after fall rains on forest lands. Western Society for Weed Science Res. Prog. Rept., pp. 33-34.

Norris, L.A. (1969). Degradation of several herbicides in red alder forest floor material. West. Soc. for Weed Sci. Res., Prog. Rept., pp. 21-22.

Norris, L.A., H. Gratkowski, C. Graham, and W.E. Currier (1972). Report on background information for the phenoxy herbicides 2,4-D, 2,4,5-T, and 2,4,5-TP. Internal Report, U.S. Forest Service.

Oliver, R.M. (1975). Toxic effects of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin in laboratory workers. Brit. Jour. of Industr. Med. 32:49-53.

Olson, R.J., T.E. Trumble, and W. Gamble (1974). Alterations in cholesterol and fatty acid biosynthesis in rat liver homogenates by aryloxy acids. Biochem. Jour. 142:445-448.

Palmer, J.S. (1963). Chronic toxicity of 2,4-D alkanolamine salts to cattle. Jour. Am. Vet. Med. Assoc. 143:398-399.

Palmer, J.S., and R.D. Radeleff (1964). The toxicologic effects of certain fungicides and herbicides on sheep and cattle. Ann. N.Y. Acad. Sci. 111:729-736.

Palmer, J.S., D.E. Clark, and L.M. Hunt (1964). Toxicologic effects of silvex on yearling cattle. J. Amer. Vet. Med. Assoc. 144:750-755.

Palmer-Jones, T. (1964). Effect on honey bees of 2,4-D. N.Z. Agric. Res. 7:339-342.

16488

DOW 0011 852

Pegg, D.G., W.R. Hewitt, K.M. McCormack, and J.B. Hook (1976). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on renal function in the rat. Toxicol. Environ. Health 2:55-65.

Piper, W.N., J.Q. Rose, and P.J. Gehring (1973). Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Environ. Health Persp. 5:241-244.

Piper, W.N., J.Q. Rose, M.L. Leng, and P.J. Gehring (1973). The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to rats and dogs. Toxicol. Appl. Pharmacol. 26:339-351.

Plimmer, J.R., V.I. Klingebiel, D.G. Crosby, and A.S. Wong (1973). Photochemistry of dibenzo-p-dioxins. ACS Advances in Chemistry Series 120:44-54.

Poland, A.P., D. Smith, G. Metter, and P. Possick (1971). A health survey of workers in a 2,4-D and 2,4,5-T plant. Arch. Environ. Health 22:316-327.

Poland, A., and E. Glover (1973a). Studies on the mechanism of toxicity of the chlorinated dibenzo-p-dioxins. Environ. Health Persp. 5:245-251.

Poland, A., and E. Glover (1973b). Chlorinated dibenzo-p-dioxins: Potent inducers of δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase. II. A study of the structure-activity relationships. Molec. Pharmacol. 9:736-747.

Poland, A., and E. Glover (1973c). 2,3,7,8-tetrachlorodibenzo-p-dioxin: A potent inducer of δ-aminolevulinic acid synthetase. Science 179: 476-477.

Poland, A.P., E. Glover, J.R. Robinson, and D.W. Nebert (1974). Genetic expression of aryl hydrocarbon hydroxylase activity. Jour. Biol. Chem. 249(17):5599-5606.

Poland, A., and E. Glover (1974). Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. Molec. Pharmacol. 10:349-359.

Poland, A., and E. Glover (1975). Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-p-dioxin: Evidence for a receptor mutation in genetically non-responsive mice. Molec. Pharmacol. 11:389-398.

Poland, A., E. Glover, and A. Kende (1976). Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Jour. Biol. Chem. 251:4936-4946.

Rip, J.W., and J.H. Cherry (1976). Liver enlargement induced by the herbicide 2,4,5-trichlorophenoxyacetic acid. Jour. Agr. Food Chem. 24:245-250.

16489

DOW 0011853

Roberts, R.E., and B.J. Rogers (1957). The effect of 2,4,5-T brush spray on turkeys. Poultry Sci. 36:703-705.

Roll, R. (1971). Untersuchungen über die teratogene. Wirkung von 2,4,5-T bei Mäusen. Food Cosmetic Toxicol. 9:671-676.

Rose, J.Q., J.C. Ramsey, T.H. Wentzler, R.A. Hummel, and P.J. Gehring (1976). The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36:209-226.

Rowe, V.K., and T.A. Hymas (1954). Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. Amer. Jour. Vet. Res. 15: 622-629.

Sanders, H.O. (1970a). Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Pollut. Cont. Fed. 42:1544-1550.

Sanders, H.O. (1970b). Pesticide toxicities to tadpoles of the western chorus frog Pseudacris triseriata and Fowler's toad Bufo woodhousii fowleri. Copeia 1970:246-251.

Sanders, H.O., and O.B. Cope (1966). Toxicities of several pesticides to two species of Cladocerans. Trans. Amer. Fisheries Soc. 95:165-169.

Sanders, H.O., and O.B. Cope (1968). The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13:112-117.

Sauerhoff, M.W., W.H. Braun, G.E. Blau, and P.J. Gehring (1976). The dose-dependent pharmacokinetic profile of 2,4,5-trichlorophenoxy acetic acid following intravenous administration to rats. Toxicol. Appl. Pharmacol. 36:491-501.

Sauerhoff, M.W., W.H. Braun, and J.E. LeBeau (1977a). Dose-dependent pharmacokinetic profile of silvex following intravenous administration in rats. Jour. Toxicol. Environ. Health 2:605-618.

Sauerhoff, M.W., M.B. Chenoweth, H.L. Gordon, W.H. Braun, G.E. Blau, and P.J. Gehring (1977b). The fate of silvex following oral administration to humans. Presented at 16th Annual Meeting of the Society of Toxicology, Toronto. Abstract 75.

Schreiweis, D.O., and G.J. Murray (1976). Cardiovascular malformations in Oryzias latipes embryos treated with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Teratology 14:287-290.

Schultz, D.P., and P.D. Harman (1974). Residues of 2,4-D in pond waters, mud, and fish, 1971. Pestic. Monit. Jour. 8:173-179.

Schultz, D.P. (1973). Dynamics of a salt of (2,4-dichlorophenoxy) acetic acid in fish, water, and hydrosol. J. Agr. Food Chem. 21:186-192.

16490

DOW 0011 854

Schwetz, B.A., G.L. Sparschu, and P.J. Gehring (1971). The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. Fd. Cosmet. Toxicol. 9:801-817.

Schwetz, B.A., J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson, and C.G. Gerbig (1973). Toxicology of chlorinated dibenzo-p-dioxins. Environ. Health Persp. 5:87-99.

Seabury, J.H. (1963). Toxicity of 2,4-dichlorophenoxyacetic acid for man and dog. Archives of Environ. Health 7:86-93.

Shadoff, L.A., R.A. Hummel, L. Lamparski, and J.H. Davidson (1977). A search for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an environment exposed annually to 2,4,5-trichlorophenoxyacetic acid ester (2,4,5-T) herbicides. Bull. Environ. Contam. Toxicol. (in press).

Seiler, J.P. (1973). A survey of the mutagenicity of various pesticides. Experientia 29:622-623.

Sieber, S.M. (1976). The lymphatic absorption of p,p'-DDT and some structurally-related compounds in the rat. Pharmacology 14:443-454.

Sjödén, P-O., and U. Söderberg (1972). Sex dependent effects of prenatal 2,4,5-trichlorophenoxy acetic acid on rats open-field behavior. Physiology and Behavior 9:357-360.

Smith, F.A., B.A. Schwetz, and K.D. Nitschke (1976). Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol. Appl. Pharmacol. 38:517-523.

Somers, J., E.T. Moran, Jr., B.S. Reinhart, and G.R. Stephenson (1973). Effect of external application of pesticides to the fertile egg on hatching success and early chick performance. 1. Pre-incubation spraying with DDT and commercial mixtures of 2,4-D: Picloram and 2,4-D:2,4,5-T. Bull. Environ. Contam. Toxicol. 11:33-38.

Somers, J., E.T. Moran, Jr., and B.S. Reinhart (1974). Effect of external application of pesticides to the fertile egg on hatching success and early chick performance. 2. Commercial-herbicide mixtures of 2,4-D with picloram or 2,4,5-T using the pheasant. Bull. Environ. Contam. Toxicol. 11:339-342.

Sparschu, G.L., F.L. Dunn, R.W. Lisowe, and V.K. Rowe (1971). Study of the effects of high levels of 2,4,5-trichlorophenoxyacetic acid on fetal development in the rat. Food Cosmet. Toxicol. 9:527-530.

Sparschu, G.L., F.L. Dunn, and V.K. Rowe (1970). Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Food Cosmet. Toxicol. 9:405-412.

16491

DOW0011 855

Stehl, R.H., and L.L. Lamparski (1977). Combustion of several 2,4,5-trichlorophenoxy compounds: Formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Science 197:1008-1009.

St. John, L.E., Jr., D.G. Wagner, and D.J. Lisk (1964). Fate of atrazine, kuron, silvex, and 2,4,5-T in the dairy cow. J. Dairy Sci. 47:1267-1270.

Stickel, L. (1964). Pesticide-wildlife studies. U.S. Fish and Wildlife Service Circular 199. pp. 77-115.

Strange, J.R., P.M. Allred, and W.E. Kerr (1976). Teratogenic and toxicological effects of 2,4,5-trichlorophenoxyacetic acid in developing chick embryos. Bull. Environ. Contam. Toxicol. 15:682-688.

Styles, J.A. (1973). Cytotoxic effects of various pesticides in vivo and in vitro. Mutation Res. 21:50-51.

Surber, E.W., and Q.H. Pickering (1962). Acute toxicity of endothal, diquat, hyamine, dalapon, and silvex to fish. Progressive Fish Culture 24:164-171.

Thigpen, J.E., R.E. Faith, E.E. McConnell, and J.A. Moore (1975). Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Infection and Immunity 12:1319-1324.

Todd, R.L. (1962). A case of 2,4-D intoxication. Jour. Iowa Med. Soc. 52:663-664.

Van Miller, J.P., R.J. Marlar, and J.R. Allen (1976). Tissue distribution and excretion of tritiated tetrachlorodibenzo-p-dioxin in non-human primates and rats. Fd. Cosmet. Toxicol. 14:31-34.

Van Miller, J.P., and J.R. Allen (1977). Chronic toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Fed. Proc. 36:396.

Vinopal, J.H., and J.E. Casida (1973). Metabolic stability of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian liver microsomal systems and in living mice. Arch. Environ. Contam. Toxicol. 1:122-132.

Vogel, E., and J.L.R. Chandler (1974). Mutagenicity testing of cyclamate and some pesticides in Drosophila melanogaster. Experientia 30:621-623.

Vos, G.J., J.A. Moore, and J.G. Zinkl (1973). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health Persp. 5:149-162.

Vos, G.J., J.A. Moore, and J.G. Zinkl (1974). Toxicity of 2,3,7,8-tetrachloridibenzo-p-dioxin (TCDD) in C57B1/6 mice. Toxicol. Appl. Pharmacol. 29:229-241.

1649

DOW 0011 856

Vos, J.G., and J.A. Moore (1974). Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy Appl. Immunol. 47:777-794.

Ward, C.T. (1976). Studies on the degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in lake water and sediments. M.S. Thesis, University of Wisconsin, Madison, Wisconsin.

Weiss, S.V., and W.H. Beckert (1975). Herbicide effects on cultured animal cells. Jour. Cell. Biol. 67:451a.

White, A.W., Jr., L.E. Asmussen, E.W. Hauser, and J.W. Turnbull (1976). Loss of 2,4-D in runoff from plots receiving simulated rainfall and from a small agricultural watershed. J. Environ. Qual. 5:487-490.

Whitehead, C.C., and R.J. Pettigrew (1972). The subacute toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid to chicks. Toxicol. Appl. Pharmacol. 21:348-354.

Whitehead, C.C. (1973). Growth depression of broilers fed on low levels of 2,4-dichlorophenoxyacetic acid. Br. Poult. Sci. 14:425-427.

Wiersma, G.B., H. Tai, and P.F. Sand (1972). Pesticide residue levels in sails, FY 1969. National soils monitoring program. Pesticide Monit. Jour. 6:194-228.

Wiese, A.F., and R.G. Davis (1964). Herbicides movement in soil with various amounts of water. Weeds 12:101-103.

Wilbur, R.L., and E.W. Whitney (1973). Toxicity of the herbicide kuron (silvex) to bluegill eggs and fry. Trans. Amer. Fish Soc. 3:631-633.

Wilson, W.E. (1956). Toxicity of 2,4-dichlorophenoxy acetic acid (weed spray). Jour. Am. Med. Assoc. 162:1269.

Wojtalik, T.A., T.F. Hall, and L.O. Hill (1971). Monitoring ecological conditions associated with wide-scale applications of DMA 2,4-D to aquatic environments. Pesticides Monit. Jour. 4:184-203.

Woods, James S. (1973). Studies of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on mammalian hepatic δ-aminolevulinic acid synthetase. Environ. Health Persp. 5:221-225.

Wright, F.C., J.C. Riner, J.S. Palmer, and J.C. Schlinke (1970). Metabolic and residue studies with 2(2,4,5-trichlorophenoxy)ethyl 2-dichloropropronate (Erbon) in sheep. J. Agr. Food Chem. 18:845-847.

Yang, K.H., P.D. Guiney, J.L. Seymour, and R.E. Peterson (1977). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced depression in biliary excretion of polychlorinated biphenyls (PCBs) in rats. Presented at 16th Annual Meeting. Soc. of Toxicol., Toronto 1977. Abstract 103.

16493

Yoder, J., M. Watson, and W. Benson (1973). Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mut. Res. 21:335-340.

Young, A.L. (1974). Ecological studies on a herbicide-equipment test area. Technical Report AFATL-TR-74-12 USAF Systems Command, Eglin AFB, Florida.

Zielinski, W.L., Jr., and L. Fishbein (1967). Gas chromatographic measurement of disappearance rates of 2,4-D and 2,4,5-T acids and 2,4-D esters in mice. J. Agr. Food Chem. 15:841-844.

Zinkl, J.G., J.G. Vos, J.A. Moore, and B.N. Gupta (1973). Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Persp. 5:111-118.

16494

DOW CHEMICAL U.S.A.

2511

MIDLAND, MICHIGAN 48640

June 3, 1974

George E. Lynn
10622 El Capitan Circle
Sun City, Arizona 85351

cc: J. Davidson, 9008
M. J. Traynor, 2030
M. R. Wessel, Washington,D.C.
E. H. Blair, 2020
W. B. Crummett, 574
P. J. Gehring, 1803

TOXICOLOGY OF POSSIBLE CONTAMINANTS IN 2,4,5-T

On May 6, Lefty asked that the toxicity of possible contaminants of 2,4,5-T be made available should EPA or EDF people ask for such information. I have searched our files for our information with the following results. Note that the possible contaminant list was supplied by Andy Watson.

I found no tox. data on the following:

Amounts Likely in wt. percent	
0.4	bis-(2,4,5-trichlorophenocy)acetic acid
<0.02	2,3,6-trichlorophenoxyacetic acid
3.2	4,5-dichloro-2-methocyphenoxyacetic acid
4.1	2,5-dichloro-4-methocyphenoxyacetic acid
	2,4-dichloro-5-methoxyphenoxyacetic acid
<0.05	2,3,6-trichlorophenol
	2,5-dichloroquinone monomethyl ether
	4,6-dichlororesorcinal monomethyl ether
0.03	4,5-dichlorocatechol monomethyl ether
0.02	2,6-dichlorophenocyacetic acid
0.26	2,5-dichlorophenoxyacetic acid
0.03	2,4,6-trichlorophenoxyacetic acid

LIFE IS FRAGILE · HANDLE WITH CARE

The following contaminants had some data:

0.07 Dichlorophenol

2,3-dichlorophenol

Acute oral guinea pig in range of 0.3 to 2.6 g/kg
Acnegenic activity - none

2,4-dichlorophenol

Acute oral guinea pig in range of 0.3 to 2.0 g/kg
Skin - old sample - caused skin burn in 15 sec.
- new sample - non-corrosive by DOT test
Acnegenic activity - negative
Odor Threshold
Dow 0.02 ppm
Taste Threshold
Dow - Fish 0.005 ppm
Lit. - water - 0.002 mg/l
Fish Tox.
LC100 5 to 100 ppm in water
Lit. values
Acute oral Rat LD50 o- 4.5 g/kg
o> 3.67 g/kg
Mouse LD50 1.6 g/kg
Chronic oral
6 mo. - mice
No effect 100 mg/kg/day
Adverse effect 200 mg/kg/day
Liver wt. - slight depression
SGPT - slight depression

2,5-dichlorophenol

Acute oral guinea pig in range of 0.1 to 2.8 g/kg

2,6-dichlorophenol

Acute oral guinea pig in range of 0.8 to 2.0 g/kg
Acute oral rat >2.0 g/kg
Eye - moderate superficial burn which should heal in a week or so.
Skin - a burn upon 24 hr. contact
- a superficial burn in 1½ hr. heals in 24-48 hrs.
Lit. Ref. says 2,6-dichlorophenol may be a sex hormone in a tick species.

<0.02 2-Chlorophenoxyacetic acid

Acute oral - Rat in range of 1 to 3 g/kg
Skin - minor irritation
Skin absorption - no indication in skin tests

<0.09 4-Chlorophenoxyacetic acid

Acute oral - Rat ♂ LD50 1.54 g/kg
Acute oral - Chicks LD50 1.60 g/kg
Skin - dry powder - no irritation

0.06 2,4-Dichlorophenoxyacetic acid

Acute oral LD50

Rat	375 mg/kg	
Mouse	368 mg/kg	
G. Pig	469 mg/kg	
Dog	100 mg/kg	Lit. Ref.
Chicks	541 mg/kg	

Chronic oral

Rats fed 114 day in diet
0.03% - no effect level
0.1% - adverse effects - minor liver and kidney path. and growth depression

Skin - Rabbit

Powder (100%) dry - no irritation
- slight irritation

Skin - Human

1 application for 4 to 5 days, then rest 3 wks. followed by challenge 24 hr. exposure

"not a sensitizer"
no to slight irritation depending on sample used

Chloracne - negative

DO

Teratology and Fetal Toxicity

87.5 mg/kg/day to dam during 6-15 day
fetal toxicity - positive
teratology - negative

Odor Threshold

60 ppm ±

Metabolism

72 hrs. - Sheep - 96% excreted - urine unchanged
1.4% excreted - feces unchanged

<0.02 3,4-Dichlorophenoxyacetic acid

Acute oral - Rat 250-500 mg/kg
Eye - a transient burn lasting 2-3 days, completely healed in t days.
Skin - 100% dry - no irritation
- 10% sol. - slight irritation
Skin absorption - no indication in skin tests run.

0.22 2,4,5-Trichlorophenol

Acute oral - Rat - male - LD50-2830 mg/kg body wt.
Lethality - Rat - female - LD50-2460 mg/kg body wt.

Chronic oral - Rat Purified 2,4,5-trichlorophenol was fed in food at 100, 300, 1000, 3000 and 10,000 ppm for 98 days. No adverse effects were detected at levels of 1000 ppm or less; 3000 and 10,000 ppm caused mild diuresis and slight pathological changes in the liver and kidney.

Eye Irritation - Rabbit - The product caused conjunctival membrane burns, severe conjunctival chemosis, slight to moderate chemosis, and slight to moderate corneal injury.

Skin irritation - Rabbit - A single short exposure resulted in slight erythema (minutes), slight edema, and a slight to moderate burn (1-1½ hr.).

Rabbit - Twenty daily applications of a 10% chloroform solution of the product to the ears of rabbits on a bioassay test for chloracnegenic activity resulted in no activity.

<0.02 2,4,6-Trichlorophenol

Acute oral - Rat - male - LD50-3250 mg/kg body wt.
Lethality - Rat - female - LD50-2830 mg/kg body wt.

Eye Irritation - Rabbit - The product caused moderate to severe conjunctival redness and swelling, slight to moderate iritis, and slight to moderate corneal injury.

Skin irritation - Rabbit - A single short exposure of the product on intact skin resulted in slight erythema (1 hr), and moderate erythema, slight edema and slight necrosis (4-5 hr).

Prolonged exposure (24 hr) of the product on intact and abraded skin resulted in moderate to severe erythema, very slight to slight edema, and a moderate necrosis (random abraded skin areas).

Rabbit - Twenty daily applications of a 10% chloroform solution of the product to the ears of rabbits on a bioassay test for chloracnegenic activity resulted in no activity.

Inhalation - Human - Experience has shown that the dust is irritating to the nose and throat.

If any of you know of further data, please supply me with copies of it so I can update this.

The data given is a quick summary. Should more detail be needed, please let me know..

Mark A. Wolf

Mark A Wolf
Information Services
Toxicology Research Laboratory
1803 Building

2500

bcc: S. B. Sachs
G. E. Lynn--Action File--
KURON

December 30, 1964

DOW 289344

Mr. George M. Downard, Head (2)
Registration Section
Pesticides Regulation Division
Agricultural Research Service
U. S. Department of Agriculture
Washington, D. C. 20250

Dear Mr. Downard:

Subject: Proposed use of KURON® on Sugar Cane in Florida
(KURON - USDA Registration No. 464-162)

By copy of the attached communication from J. R. Orsenigo, Associate Horticulturist, University of Florida, Everglades Experiment Station, dated December 23, 1964, it will be noted that certain weeds of sugar cane plantings in Florida are not now controlled with 2,4-D and 2,4,5-T herbicides. In some cases the long continued use of 2,4-D has apparently resulted in development of resistance of the weeds and in others the weeds have never been susceptible to the use of 2,4-D and/or 2,4,5-T.

Several of these weeds, such as the nightshades, fennels, ground cherry, purslane and wild lettuce do respond to treatment with silvex, the name of the active ingredient in KURON®.

Since KURON is registered for use in sugar cane plantings in Louisiana and Hawaii, and since it is effective against the weeds in question in Florida when used at the same rates and under similar application conditions, it seems appropriate to apply for use registration in Florida, as indicated by the attached proposed addition to the KURON label.

We therefore respectfully apply for registration as indicated and will appreciate the earliest practicable action by the Pesticides Regulation Division pertaining thereto.

Very truly yours,

O H Hammer

O. H. Hammer
Registration Section
Bioproducts Department

cc: W. W. Sunderland, Dow Washington Office

abc

16500

ACD FILE NO. 44-26

2546

DOW 331106

AQUATIC VEGETATION CONTROL IN RECREATIONAL AREAS

by

Mark G Wiltse[1]

Introduction

Aquatic weed growth has become an increasing problem in recreational areas of the eastern half of the United States. Nature does abhor a vacuum in the plant world, and in aquatic areas of shallow water this is especially true. A succession of biological organisms and algal growth followed by the invasion of aquatic weeds, emergent aquatic growth, and higher forms of emerged aquatic vegetation and terrestrial plants, is nature's way of converting aquatic areas to dry land.

Aquatic vegetation has restricted utilization of water for such purposes as hunting, fishing, bathing, water skiing, boating, and in some instances has destroyed the normal beauty of aquatic areas. Fish production can be limited by excessive growth of water weeds. Aquatic vegetation is often a problem in water areas with limited water movement. Man has been trying to interrupt this evolution of aquatic areas for some time, in order to maintain the aquatic or water areas so they can be of maximum use to man.

Aquatic vegetation control measures have been used for many years and haveconsisted primarily of mechanical techniques. These contrivances have ranged from underwater cutting bars to hand rakes. Although these mechanical methods are often effective for a short time, regrowth from roots or rooting of stem fragments often increases the problem. The control of water levels, or draining and reflooding water areas, has also been used to some degree. These methods have generally been laborious and provide only temporary relief. In recent years, chemical control methods have offered a new opportunity in aquatic vegetation control. In discussions on aquatic vegetation, it is desirable to consider the various aquatic weeds, depending upon their habitat and type of growth, since the control measures for the various groups differ considerably. For this discussion, let us consider control measures for the following broad classification of aquatic vegetation--submerged, floating, and emergent aquatic weeds.

Control of Submerged Aquatic Weeds

Submerged weeds are probably the major weed problem in recreational waters or at least causes the greatest concern where the water is used for bathing, boating, fishing, water skiing, etc. Submerged aquatic weeds, such as milfoil, coontail, and pondweeds, can completely fill ponds that are 7 to 8 feet deep with aquatic growth, so that is impossible to even row a boat through the water. Bathers have become entangled in this type

[1]The Dow Chemical Company, Midland, Michigan

16501

(Paper presented at 1961 Michigan Forestry and Park Association Annual Conference, East Lansing, Michigan.)

DOW 331107

of growth and drown because of their inability to free themselves. Submerged aquatic weeds also tend to create a fish imbalance by providing protection for the small fingerlings, so that the large fish cannot consume them. This gives rise to a large population of small fish which consume much of the food supply. Fishermen often have difficulty in getting their hook and line through these weed masses.

The New Jersey Fisheries Laboratory at Milltown, New Jersey, conducted extensive tests on this problem and in 1956 demonstrated the activity of silvex for the control of submerged aquatic weeds (6). In their test, they found that silvex was effective on a broad spectrum of aquatic weeds using 2 ppm of the active ingredient. Since 1956, silvex has been tested and used commercially for submerged aquatic weed control throughout the United States. In 1958, Kuron* herbicide, Dow's propylene glycol butyl ether ester formulation of silvex, was registered for this use by the Pesticides Regulation Branch of the United States Department of Agriculture. Kuron, when used at the rate of 5 quarts per acre foot, will control certain submerged aquatic weeds such as water milfoil, fanwort, bladderwort, coontail and waterweed. Kuron should be applied in early summer when the water temperature is 65°-70°F. and weeds are approaching the water surface. To apply properly, first dilute the Kuron with enough water for uniform distribution over the water surface with the available equipment. Kuron has been especially effective when applied to water areas that have a minimum amount of water movement. Ponds with some water movement can be successfully treated if the water level can be lowered so there will be no overflow for at least three days after treatment. This rate of application of Kuron will also give control of some of the floating and emergent aquatic weeds, such as water lilies and spatterdock. Applications of Kuron ordinarily have not been harmful to fish; however, in certain situations where complete pond treatments have been made and where concentrations higher than the 5 quarts per acre foot have been used, some fish kill has been observed. Recent work in Massachusetts (1) has indicated that lower rates of Kuron have been effective on such weeds as water milfoil and bladderwort, and they are using 1-2 quarts per acre foot of water in some of their lakes. Kuron has a low mammalian toxicity, and normal precautions used in handling agricultural chemicals is sufficient for the use of this material. The Kuron formulation of silvex is an ester and an emulsion is formed when it is sprayed into the water.

Our company has been actively evaluating other formulations of silvex for aquatic vegetation control and has developed a salt formulation which forms a true solution with water. This has given very encouraging results. Silvex salt formulations will possibly be available in test market areas in Michigan this season as "Kurosal* G" and "Kurosal* SL". Kurosal G contains 20% silvex on a clay granule, while Kurosal SL contains 6 pounds of silvex per gallon. Kurosal G should be applied at 100 to 150 pounds per surface acre, depending upon the depth of the water. Kurosal SL is designed to be applied as a direct application into the water surface by a boom or by injecting a stream of the chemical in the wash of an outboard motor. Kurosal formulations appear particularly safe to use in waters inhabited by fish.

16502

* Trademark of The Dow Chemical Company

DOW 331108

Silvex is the most promising development in the aquatic herbicide field and offers the potential of solving many of the submerged and emergent aquatic weed control problems without the necessity of using materials which are toxic to animals.

2,4-D is available both as an ester and an amine salt and has been used to some extent for submerged aquatic vegetation control. In 1956, Dr. Buford Grigsby, from Michigan State University, reported on his results using 2,4-D on a granular carrier and there has been considerable interest in the use of granular 2,4-D for submerged aquatic vegetation control since that time. Esteron* 99 Granules herbicide, which contains 20% 2,4-D as the propylene glycol butyl ether ester on a clay granule, is one of several available commercial formulations of granular 2,4-D. 2,4-D granules applied to the surface of the water by hand or mechanical devices sink rapidly to the bottom of the water releasing 2,4-D. It was originally thought that 2,4-D adhered to the granule for long periods, releasing slowly and thus localizing the active ingredient around the roots of the submerged aquatic weeds. Some recent work has indicated that 2,4-D actually is removed from the granule fairly rapidly, and that it is dissolved into the water. However, the use of the granule carriers for distribution purposes is convenient for small scale applications around boat docks and in front of cottages, etc. Although some workers have indicated that granular 2,4-D was effective, regardless of water depth, it has become evident from later work that water depth is quite important to obtain satisfactory control. To insure effectiveness, 2,4-D should be applied at about 5 pounds acid equivalent per acre foot of water treated. Water movement may also decrease the effectiveness of 2,4-D granules, since the active ingredient can be removed from the site of application before it is active on the plant.

Esteron 99 Granules or other 2,4-D granules containing 20% 2,4-D should be applied at 100 pounds per surface acre in water that is 4 feet deep with an additional 25 pounds per surface acre for each foot of depth in excess of 4 feet. 2,4-D granules must be applied early in the growing season to be effective.

Later season treatments on weeds not actively growing have not been consistently effective. 2,4-D granules will control such weeds as water milfoil, coontail, and bladderwort, but is considerably less effective on pondweed (Potamogeton spp.). 2,4-D granules are not as effective on as broad a spectrum of weeds as sodium arsenite or silvex. In the granular form, 2,4-D is easy for the boat owner or cottage owner to use in small areas.

Control of Floating Weeds

Water lilies are a problem in recreational waters since they often interfere with boating, water skiing, and swimming. Water lilies can become so dense that it is nearly impossible to get through the area with a boat. Water lilies can be most effectively controlled by surface sprays with silvex. We recommend the use of Kuron at 2 quarts per 100 gallons

16503

* Trademark of The Dow Chemical Company

DOW 331109

of water to be sprayed as a wetting spray on the water lilies when they are in an active growth period, and the leaves have fully developed. Late season applications when the water lilies have completed their vegetative growth have not been consistently effective. 2,4-D esters have also been effective for the control of water lilies; however, the results have not been as consistent as with applications of Kuron. Duckweed (Lemna spp.) is a free-floating aquatic weed and is often a nuisance in sheltered areas and may completely cover the water surface. Duckweed has been very troublesome to control, since it can reinfest areas very rapidly. It has been reported that several materials will give immediate kill of the duckweed but reinfestation within three or four weeks is common.

Control of Emergent Aquatic Weeds

Emergent aquatic weeds have been a severe problem in waterfowl marshes and have decreased the waterfowl populations in some areas. Phragmites, cattails, and other weeds and grasses can cover an entire marsh, and thus open areas are not available for geese or ducks to land, and nesting and feeding areas are not attractive to them. Of course, emergent aquatic weeds are also a problem along the shoreline of recreational waters, cottages, etc. Cattails and other narrow-leafed plants can be controlled effectively by using sprays of Dowpon* or Radapon*. Radapon at 10 pounds per 100 gallons of water should be applied as a wetting spray when the weeds are in an active stage of growth. Aerial applications of Radapon at 10-15 pounds per acre in 5-10 gallons of water have been very effective for the control of cattails for opening up marsh areas. Radapon is particularly effective on narrow-leafed plants and has little effect on many broad-leafed plants such as smartweed, pokeweed, wild buckwheat, etc., which are desirable for waterfowl food. Radapon is less toxic than common table salt and has been used in water reservoirs for aquatic vegetation control without causing any deleterious effect on water quality.

Broadleaf emerged weeds often grow along the shoreline and may be a problem in some recreational areas. Some of these problem weeds are pickerelweed, arrowhead, water plantain, etc. Spray applications of Kuron using 2 quarts per 100 gallons of water have been effective in control of these weeds. Applications should be applied when the plants are in an active stage of growth. Emergent aquatic vegetation consisting of both narrow-leafed plants and broad-leafed plants can be effectively controlled by a mixture of silvex and dalapon which is sold commercially under the trademark, Garlon*. Garlon, used at 3 gallons per 100 gallons of water, has given effective control of cattails, burreed, arrowhead, pickerelweed, etc. Applications should be applied when the plants are in an active growing stage to be most effective.

* Trademark of The Dow Chemical Company

DOW 331110

Summary

Several herbicides are now available for the control of aquatic weeds and algae in recreational areas. New herbicides have shown some promise for the control of submerged aquatic weeds and additional research will probably develop new chemicals to be useful in this field. There are several chemicals presently available that can do a very effective job in the control of aquatic weeds and should be very useful in the management of recreational waters and marshes.

16505

Literature Reviewed

DOW 331111

1. Cortell, J. M. Re-evaluation of the Concentration Required for Effective Aquatic Weed Control with Silvex. PROCEEDINGS OF NORTHEASTERN WEED CONTROL CONFERENCE 14:478-482, 1960.

2. Heath, G. and L. C. Ruch. Aerial Control of Cattail with Radapon. DOWN TO EARTH, Winter 1957 Issue.

3. Mackenthun, K. M. The Chemical Control of Aquatic Nuisances. Committee on Water Pollution, Madison, Wisconsin, January 1958.

4. Palmer, C. M. Algae in Water Supplies. U. S. Department of Health, Education and Welfare. Public Health Service Publication No. 657, 1959.

5. Wiltse, M. G. Aquatic Vegetation Control with Kuron. (Presented at 1960 Meeting of Weed Society of America, Denver, Colorado.)

6. Younger, R. R. A Preliminary Report on Controlling Aquatic Vegetation in New Jersey with Kuron. DOWN TO EARTH, Spring 1958 Issue.

16506

2547

DOW 331100

AQUATIC VEGETATION CONTROL WITH KURON*

by

Mark G Wiltse**

Aquatic weed growth has become an increasing problem in recreational waters of the eastern half of the United States. Vegetation has restricted utilization of waters for such purposes as bathing, water skiing, boating, and in some instances has destroyed the normal beauty of aquatic areas. Fish production has often been limited by excessive growth of water weeds. A number of control methods have been employed with varying degrees of success.

Mechanical contrivances have ranged from under water cutting bars to hand rakes. Although these mechanical methods are often effective for a short time, regrowth from roots or rooting of stem fragments often increases the problem. The method is generally laborious and provides only temporary relief.

Sodium arsenite has been used as a herbicide for several years. In some areas, particularly static waters, this well-known chemical has given outstanding results. Principal limitations to its use have been its inherent toxicity, resistance to decomposition and failure to control a broad range of vegetation.

Several workers have been actively evaluating products which would control submerged aquatic weeds and overcome the limitations of previously existing methods. The New Jersey Division of Fish and Game inaugurated a five-year research program in 1951 financed by Dingell-Johnson funds to find an aquatic herbicide (8). In the course of this work, Kuron herbicide was found adaptable to this problem and seemed to offer control comparable to sodium arsenite (8). Kuron contains the active ingredient silvex 2(2,4,5-trichlorophenoxy) propionic acid as the propylene glycol butyl ether ester and acts as a plant growth regulator.

Aquatic Weeds Controlled

Early tests indicated that Kuron herbicide at 5 quarts per acre foot would control many of the troublesome submerged aquatic weeds in the northeastern United States (1,2,3,4,8). Experimental tests in 1957 and 1958 in several sections of the country evaluated the effectiveness of this concentration on several aquatic weeds. Some tests were conducted at reduced concentrations by certain workers (2). Commercial applications in 1958 and 1959 contributed additional performance information for controlling certain

* Trademark of The Dow Chemical Company

** The Dow Chemical Company, 916 Shoreham Building, Washington 5, D. C.

16507

(Paper presented at 1960 Meeting of the Weed Society of America, Denver, Colorado.)

species. The following list summarizes some of the weeds controlled by Kuron.

DOW 331101

Aquatic Weeds Controlled by Kuron at 1.25, 2.5 and 5 Quarts per Acre Foot

1.25 quarts/acre foot or less (0.5 ppm)

White water lily	(Nymphaea spp.)
Pickerelweed	(Pontederia cordata L.)

2.5 quarts/acre foot or less (1.0 ppm)

Yellow water lily	(Nuphar spp.)
Mud plantain	(Heteranthera spp.)
Water milfoil	(Myriophyllum heterophyllum)

5 quarts/acre foot or less (2 ppm)

Bladderwort	(Utricularia spp.)
Watershield	(Brasenia schreberi)
Coontail	(Ceratophyllum demersum)
Fanwort	(Cabomba caroliniana)
Waterweed	(Anacharis canadensis)
Variable pondweed	(Potamogeton diversifolius)
	(Potamogeton amplifolius)
Naiad	(Najas flexilis)
Spike rush	(Eleocharis spp.)
Tapegrass	(Vallisneria americana)
Waterstarwort	(Callitriche spp.)
Soft rush	(Juncus effusus)
Bulrush	(Scirpus americanus)
Burreed	(Sparganium spp.)

In some treatments, these weeds were controlled at lower concentrations, but there were not sufficient tests to indicate consistent effectiveness at the lower rates. Treated areas observed two years after treatment indicated a continued control over this period of time. In total treatments on ponds and lakes with Kuron at 5 quarts/acre foot two years control has been obtained in some instances.

Application Technique

Emerged species were controlled in many instances by using Kuron at one gallon per 100 gallons of spray solution applied to the emerged foliage as a thorough wetting spray. The applications for the control of the submerged aquatic weeds were generally applied by diluting the Kuron at one gallon in 10 to 40 gallons of water and directing this solution on or into the water. Proportioning pumps were often used to mix Kuron with lake water and pump it to the boom which would distribute the solution over a wide swath. Uniform distribution appeared to be desirable to allow a lethal concentration of the Kuron to come in immediate contact with all the vegetative growth.

Injection of the undiluted formulation of Kuron was used in some equipment, but on a limited basis.

DOW 331102

Environmental Effects

Applications of Kuron appeared to be most effective if treatments were made during periods of active growth. Treatments applied when water temperatures have been 65°F or higher have been most effective. The water temperature may not influence the reaction of the chemical to a significant degree, but the aquatic weeds may be in their most active growth at these higher temperatures and thus more susceptible. Undoubtedly, the most active growth periods vary for the various aquatic weed species.

Applications with Kuron have been most effective on submerged aquatic plants in lakes and ponds where there is no or limited water movement. Less satisfactory control of some plants has been observed where water currents replace the treated water soon after application. Translocation has been demonstrated in water lilies where applications to the leaf surface has caused a herbicidal effect on the rootstock. However, some aquatic plants have only primitive vascular systems which may not be functional and translocation to root areas probably is not important. In these plants, a lethal concentration of Kuron may be required to contact all the vegetative portions of the plant which are capable of growth or reproduction. Although the exact minimum contact or exposure time required is not clearly defined, 48 to 72 hours exposure to treated water has given control.

Crowell and Williams (7) compared the effectiveness of Kuron for controlling coontail (Ceratophyllum demersum) in greenhouse tank experiments at three pH levels and three concentrations of calcium ions. Kuron was most effective on coontail growing in slightly acid water. It was also more active on coontail growing in water with 250 and 500 ppm of calcium ion than in water with no calcium. In these tests, the effect of calcium was not differentiated from a possible effect of chloride ion which was added in equivalent amounts. Field plot treatments and commercial applications have been only slightly better under acid water conditions compared with treatments to neutral or slightly alkaline water. Perhaps the water pH is only important in water situations with a low level of calcium ions or similar dissolved ions.

Safety to Fish and Other Aquatic Organisms

The experimental treatments to plots and complete ponds have caused no fish injury at concentrations of Kuron of 1.25 to 5 quarts per acre foot. Commercial applications at concentrations of 5 quarts per acre foot or less have given no appreciable fish kill in numerous treatments throughout the country. Slight fish kill has occurred in some instances where applications are made at higher concentrations. The higher concentrations sometimes occurred in shallow areas where the spraying equipment could not operate properly and thus higher concentrations were developed. There has been no fish injury in over 99% of the treatments applied. Rapid incorporation of the spray solution particularly those associated with rates in excess of quarts per acre foot of Kuron should be avoided where fish kill cannot be tolerated.

Pierce (4,5,6) found that applications of Kuron had no influence on oxygen content in treated plots. Aquatic weeds treated with Kuron normally decompose slowly, in most situations 10 to 21 days are required for decomposition. This slow decomposition apparently does not lower the oxygen content of the water. This is a problem with some aquatic herbicides which give rapid kill of weeds. Pierce also reported that Kuron treatments showed no effect on population of benthic organisms represented by the following groups: Annelida, mostly leeches; Mollusca, including seven species of Gastropoda and one specie of Pelecypoda (Sphaerium); Amphipoda; Insecta, larvae of mayflies, damsel flies, dragon flies, midges and beetles. Applications of Kuron temporarily decreased populations of some plankton, but within two weeks after treatment the populations were equal to untreated areas. These plankton consisted of fifty identified species in the following large groups; Myxophyceae, Chlorophyceae, Desmidaceae, Diatomaceae, Flagellata, other Protozoa, Nematoda, Rotifera, Annelida, Crustacea. There was no deleterious effect on large aquatic vertebrates such as frogs and turtles in the treated areas.

DOW 331103

Prolific algae growth is often a problem soon after treating aquatic weeds with some aquatic herbicides. Algae growth has not normally occurred until three to four weeks after treatment with Kuron. The delay in appearance of algae apparently is associated with the slow kill of the aquatic weeds and thus the delay in release of nutrients into the water from the decaying vegetation.

Safety to User

Kuron herbicide is low in toxicity and presents a low degree of hazard in handling and use.

When Kuron herbicide was fed by intubation to groups of various animal species, the LD_{50} values were found to be as follows: rat, 1070 mg/kg; guinea pig, 850 mg/kg; rabbit, 850 mg/kg; mouse, 2140 mg/kg and chicks, 2000 mg/kg. From these data, it may be concluded that there should be no problem from ingestion incidental to the handling and use of this product. If large quantities are swallowed, accidentally or willfully, some injury might result. However, the likelihood of serious injury is remote. Nevertheless, Kuron herbicide should not be left where children or livestock have access to it.

Cattle, sheep, swine, ducks and chickens have consumed water containing Kuron herbicide at 50 ppm over a four week period with no adverse effects as determined by water consumption, body weight gains, and gross observations, including gestation and parturition in sheep. These data indicate that foliage or water treated with Kuron herbicide, as recommended, should not present a hazard from ingestion.

Skin irritation tests conducted upon rabbits have indicated that Kuron herbicide and its dilutions are slightly irritating, upon repeated prolonged contact. In these studies, there was no evidence of absorption through the skin in acutely toxic amounts. The observation of reasonable care and personal cleanliness practices should be adequate to avoid skin difficulties.

Kuron herbicide, when diluted to 1% with water, caused no primary irritation nor allergenic responses when applied to the skin of fifty human volunteers using a repeated insult technique. One subject did develop a mild fatiguing response. In addition, the product was "patch tested" on a second group of fifty human subjects at concentrations up to 50% without cutaneous reaction. Even when applied undiluted to the skin for two hours, it caused no skin response. Thus, it may be concluded with a high degree of assurance that when used as directed, this product should cause no skin irritation or skin sensitization responses.

When tested on the eyes of rabbits, Kuron herbicide and its solutions produced moderate pain but only slight irritation. Thus, this product presents no unusual hazards from eye contact. However, suitable eye protection, such as safety glasses, may be worn simply to avoid the discomfort that might result from contact. Swimmers should not experience any discomfort from water treated at recommended concentrations.

DOW 331104

Effect on Water Use

Kuron may remain in water for three to four months after treatment where no dilution occurs. An 88-acre lake was treated in New York after the water level was lowered two feet. No flow occurred through the outlet until three months after treatment. In analysis of the lake water thirty days after treatment, 2.4 ppm of silvex from Kuron was present, essentially the same amount that was applied. Analysis of the lake water seventy days after treatment showed 2.1 ppm of silvex present. Samples of water analyzed 100 days after treatment and thereafter showed no Kuron present. Chemical analysis of the bottom mud indicated no build-up of Kuron. Since Kuron herbicide is an organic compound, the components would be expected to be bacteriologically decomposed principally to the elements carbon, hydrogen and oxygen.

Silvex acts as a growth regulating compound on terrestrial broadleafed plants and is used as a stop-drop treatment prior to harvest of apples at concentrations of 10 to 20 ppm. Although 2 ppm of silvex as used for aquatic weed control might not cause injury if sprayed on some broadleafed plants, some susceptible species may be affected. Accordingly, it is suggested that water treated with Kuron should not be used for spray purposes or for irrigation during the season of treatment until more information is available on the possible hazards to crops.

Summary

Kuron aquatic herbicide is effective on several submerged and emerged aquatic weeds. Applications to still water at 5 quarts per acre foot are suggested for general aquatic weed control. Lower rates are effective on certain species. Conditions for growth, pH and dissolved ion content of water may influence the effectiveness of Kuron. The low mammalian toxicity, low action, and safety on fish food organisms are desirable properties of Kuron aquatic herbicide.

Literature Cited

1. Boschetti, M. M., "Field Testing of Kuron as an Aquatic Herbicide in Massachusetts," Proc. NEWCC, 13:315-321. 1959

2. Cortell, J. M., "Re-Evaluation of the Concentrations Required for Effective Aquatic Weed Control with Silvex", Proc. NEWCC 14:478-482. 1960

3. Hall, W. C., "Control of Various Aquatic Weeds with Silvex", Proc. NEWCC 14:476-477. 1960

4. Pierce, M. E., "The Effect of the Weedicide Kuron upon the Flora and Fauna of Two Experimental Areas of Long Pond, Dutchess County, New York", Proc. NEWCC 12:338-343. 1958

5. _____ "Further Studies of the Effect of Kuron upon the Flora and Fauna of Long Pond, Dutchess County, New York", Proc. NEWCC 13:310-314. 1959

6. _____ "Progress Report of the Effect of Kuron upon the Biota of Long Pond, Dutchess County, New York", Proc. NEWCC 14:472-475. 1960

7. Truchelut, G. B., and Williams, R. C., "Some Observations on the Influence of Dissolved Ions on the Effectiveness of Phenoxy-Type Herbicides on Submersed Aquatic Weeds", Proc. SWCC 13:221-227. 1960

8. Younger, R. R., "A Preliminary Report on Controlling Aquatic Vegetation in New Jersey with Kuron", DOWN TO EARTH, Spring 1958.

9. Younger, R. R., "Progress Report on the Use of Kuron, 2,4-D and 2,4,5-TP Granules on Aquatic Herbicides", Proc. NEWCC 13:322-329. 1959

DOW 331105

2543

KURON silvex herbicide continues to gain acceptance

DOW 331092

By Lawrence Southwick
Dow Chemical U.S.A.

For more than two decades, three chemicals in the phenoxy group of systemic herbicides, i.e. 2,4-D, 2,4,5-T and MCPA, have more or less dominated the attention of researchers, advisors, marketers and users. Generally speaking, 2,4-D and MCPA are still leaders in broadleaf herbaceous weed control, and 2,4,5-T, alone and in combination with 2,4-D, is widely used in woody plant control. The purpose of this brief paper is to review some of the herbicidal possibilities of silvex. This report does not and is not intended to encompass an exhaustive survey of the considerable literature generated over the past 20 years relative to this herbicide. Selected published papers are referenced to provide support for the thesis that silvex is indeed a very effective herbicide with a broad use spectrum.

Historical Background

Shortly following the introduction of 2,4-D in 1945, researchers began to characterize its herbicidal utility in several use areas. It rapidly found acceptance for controlling herbaceous broadleaf weeds in turf, small grains, corn and in non-cropland. It also showed promise for controlling woody species. However, as early as 1946, it became obvious that 2,4-D was not adequately effective on certain weeds, for example, chickweed in turf, and on many woody plants in non-cropland brush control.

By 1947, Dow research workers in Michigan, workers in Hawaii, and probably others reported significant differential species response to 2,4-D and 2,4,5-T. The latter soon found a place in the control of woody plants and also in selective weed control in rice. The idea of combining these two herbicides to broaden the spectrum of woody plant control was put into practice. Comme spraying of right-of-ways with mixtur 2,4-D and 2,4,5-T became a general pra and such combinations are in wide us the present time.

Early Dow studies in Michigan (1952 dicated that another related but chemi specific herbicide, 2(2,4,5-trichlorophen propionic acid, was very effective on a assortment of herbaceous weeds and w plants[2]. Silvex was coined as the com name. KURON® herbicide, a low voi ester formulation of silvex, was first keted in 1954.

Beginning around 1954, many rep have been presented at the regional w control conferences on the herbicidal c acteristics of silvex. For example, in Mi gan trials, it was reported very effectiv four species of oaks, and on maple[3]. In tensive tests in Pennsylvania, res checked three years after application sho silvex effective "—on mixed oaks and a ciated species"[1]. In Oklahoma, silvex excellent on "all oaks" which included p white, blackjack, black and chinquap Based on many field trials, silvex was fo to be an excellent overall turf herbicide controlling a wide assortment of weeds cluding many that are tolerant to 2,4-D[4] an aquatic herbicide, silvex was reporte be superior to others in Oklahoma[8], northeast[9, 18], and elsewhere.

An early report on basal treatment w KURON herbicide was made in 1956 on key oak in Florida[17]. Summer applicati were rated excellent while winter treatm was moderately effective. Work in Ore proved the effectiveness of KURON in o basal control of big leaf maple[15]. Curr

16513

OW 331093

LITERATURE CITED

(1) Arend, J. L. 1967. Some variables affecting hardwood control with stem injections of 2,4,5-T. North Central Forest Experiment Station, U.S.D.A. Forest Research Paper NC-18. 11 pp.

(2) Brinkman, K. A. 1970. Picloram in spaced stem injections to control Lake States hardwoods. North Central Forest Experiment Station, U.S.D.A. Forest Research Note NC-94. 4 pp.

(3) Carvel, K. L. and F. C. Cech. 1967. If used properly—tree injectors are effective! Science Serves Your Farm and Home. Agricultural Experiment Station, West Virginia University Bulletin 552: 6-10.

(4) Cope, J. A. and J. N. Spaeth. 1931. The killing of trees with sodium arsenite. J. Forestry 29. 775-783.

(5) Nation, H. A. 1967. Report on tree control via injection with Tordon 101 mixture. Down to Earth, Number 2: 24-27.

(6) Norris, L. A. 1971. Chemical brush control: assessing the hazard. J. Forestry 69: 715-720.

(7) Ryker, R. A. and L. S. Minckler. 1962. Methods and costs of killing hardwood culls. Central States Forest Experiment Station, U.S.D.A. Forest Service Technical Paper 191. 9 pp.

(8) Shipman, R. D. 1966. Soil-applied urea herbicides in the control of unwanted woody plants. Agricultural Science Review. First Quarter 1966. Vol. 4, No. 1: 21-27.

(9) _____. 1968. Penetration and accumulation of fenuron in forest soils. J. Forestry 66: 910-912.

(10) Shipman, R. D. 1969. Pelleted vs. liquid herbicides for control of low-grade white oak in central Pennsylvania. Proceedings, Northeastern Weed Control Conference. Vol. 23: 298-302.

(11) _____. 1969. Establishment and early release of underplanted Japanese larch with soil-applied fenuron. Tree Planter's Notes. Vol. 20, No. 1: 23-27.

(12) _____. 1970. Some variables affecting the response of unwanted hardwoods with tree-injected 2,4,5-T. Proceedings, Northeastern Weed Control Conference. Vol. 24: 250-256.

(13) Smith, J. L. 1959. Tests of injected chemicals for hardwood control in the Arkansas mountains. Southern Weed Conference, Shreveport, Louisiana. 3 pp.

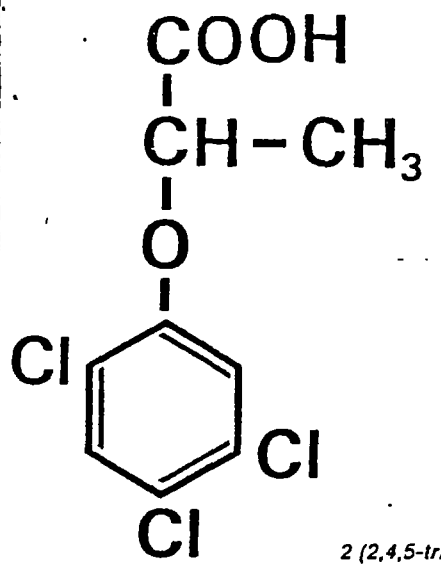
(14) Sterrett, J. P. 1967. Injector treatments on oaks, red maple and hickory with several water soluble herbicides. Proceedings, Southern Weed Conference. Vol. 20: 190 (abstract).

(15) Watson, A. J. and R. J. Mesler. 1964. Effect of Tordon herbicide as basal frill and tree injection treatments on certain hardwood trees.

(16) Down to Earth. Vol. 19 (4): 20-23.

Wiant, H. V. and L. C. Walker. 1964. Comparison of two silvicide injectors for killing oaks. The Consultant. Vol. 9, No. 4: 16.

16514



2 (2,4,5-trichlorophenoxy) propionic acid

DOW 331094

Oregon recommendations include silvex for this species as well as for vine maple[16]. Dormant treatments on salt cedar in New Mexico were moderately effective[10]. Commercial use of basal application of silvex has corroborated effectiveness on many species of woody plants. An effect commonly noted is delayed maximum response. Final control has been satisfactory.

Extensive aerial trials in Hawaii showed KURON herbicide to be the most promising of those tested to reclaim wetland brush jungle for cattle range[13]. Weedy species included melastoma, false staghorn fern, ohia, and lantana. Work in Oregon proved the superiority of silvex in air or ground applied foliage sprays on dalmation toadflax and this is the present state recommendation in Oregon[7] as well as in Montana. Control of prickly pear cactus with silvex is recommended in Montana, Nebraska, Wyoming and elsewhere. Data in the Research Progress Reports of the Western Weed Control Conference, even prior to 1962, indicated good control with KURON on several difficult species, including cholla cactus, common tansy, dalmation toadflax, lantana, larkspur, mesquite, milkweed, perennial peppergrass, poison oak, prickly pear cactus, Russian knapweed, salt cedar, snow berry, sorrel, turbinella oak, whitethorn, wild rose, yucca and others. For many years, silvex has been used extensively in turf and sugar cane and for aquatic weed control.

Weed and Brush Control with KURON

Two excellent references which characterize comparative effectiveness of phenoxy herbicides are: USDA Farmers' Bulletin No. 2183 (Jan. 1971 revision) "Using Phenoxy Herbicides Effectively"[11], and USDA Agriculture Handbook No. 269 (March 1965) "Herbicide Manual for Non-Cropland Weeds"[3]. Tables 1 to 8 were developed largely from these information sources.

Since the effectiveness of silvex has not been adequately reported on many species, the listings in the tables cannot be considered "complete". In Tables 1 through 4, effectiveness is given in terms of excellent, good, or fair which are characterized as follows:

excellent—one application at a normal rate (1 lb/acre) kills the plant in most cases.

good—more than one application at the normal rate or an application at a higher rate (2 to 4 lb/acre for weeds and 4 to 8 lb/acre for woody plants) needed for control.

fair—repeated applications at the normal or a higher rate often required for adequate control.

It is interesting to note in these tables the wide range of annual, biennial and perennial weeds, woody plants and aquatic weeds that are controlled with silvex. This relatively old, established herbicide obviously has attributes that may not have been fully evaluated and certainly were not fully developed due, in part, to the diversion of interest and research effort to the great influx of new families of herbicides which emanated from the research laboratories coincident with and shortly following the initial development of silvex. Yet the limited research studies with KURON herbicide and other silvex formulations as well as successful commercial usage have delineated specific use areas wherein the special attributes of silvex herbicide have become recognized. The infor-

16515

TABLE 1. Control of Annual and Biennial Weeds with Silvex

EXCELLENT	GOOD	FAI
amaranth	broomweed	buckwheat, t
bedstraw	coffeeweed	buckwheat, w
beggarticks	fieldpeppergrass	carrot, wild
bitterweed	geranium, Carolina	curlyindigo
bluemustard	goosefoot, oakleaf	jointvetch
burclover	groundsel, cressleaf	knotweed, pr
burdock	henbit	mayweed (ch
burhead	horseweed (marestail)	mullein
buttercups	knapweed, spotted	poorjoe
chickweed, com.	knotweed, japanese	puncturevine
coffeebean	medic, black	spurry, corn
corton	mexicantea	
dogfennel	mexicanweed	
ducksalad	mudplantain	
eveningprimrose	nightshade, black	
fiddleneck	pokeweed	
fleabanes	purslane	
galinsoga	rocket, London	
groundcherry	rocket, yellow	
hedgemustard	shepherd's purse	
hemlock, poison	smartweeds	
knawel, annual		
kochia		
lambsquarters		
marshelder		
morningglory		
mustards		
partridgepea		
passionflower		
pennycress, field		
pepperweeds		
pigweeds		
plantains		
poisonhemlock		
radish, wild		
ragweeds		
rape, bird		
redstem		
sesbania (coffeebean)		
sneezeweed		
spanishneedles		
sunflower		
thistle, bull, Russian		
tievine		
vetch, wild		
witchweed		
woodsorrel, yellow		
wormseed-mustard		

DOW 331095

16516

TABLE 4. Control of aquatic weeds with Silvex

EXCELLENT		GOOD
arrowhead	spattardock	alligatorweed
bladderwort	swamploosestrife	bulrush
cocotail	watercrowfoot	burreed
elodea	waterhyacinth	cattails
fanwort	waterlily	pondweeds
loosestrife	watermilfoil	
lotus	waterplantain	
needlerush	watershield	
parrotfeather	waterstargrass	
pickerelweed	waterstarwort	
primrosewillow	waterweed, Canada	
rush	wildcelery	

33109

mation in the tables is simply indicative of the acknowledged wide spectrum of species response to silvex. An exhaustive survey of literature and progress reports, and especially a compilation of user experiences undoubtedly would reveal additional susceptible species.

Comparison with 2,4,5-T

In Tables 5 and 6, two lists of weed and woody plant species are given to illustrate differential foliar responses to silvex and to 2,4,5-T. These ratings are taken from the information provided in the two performance references referred to above[5, 11] with assists from other data including California Bulletin 812.[12] In general, silvex often is considered more effective on the species listed thereunder in the tables and 2,4,5-T for those listed under it. However, this does not mean that control of some species listed under silvex is not possible with 2,4,5-T or vice versa. It simply means that reported field results on these particular species give the edge to one or the other herbicide. The differences in effectivity vary from moderate to very significant. However, it should be emphasized that other field data may not be in total agreement w that reported here, and likewise many c sired comparisons have not been reporte Considering the proven utility of combi tion treatments of 2,4,5-T with 2,4-D with picloram for broad spectrum and i proved woody plant control, it is likely th silvex plus 2,4-D or picloram will be effe tive combinations and commercial tri indicate that this is so.

Comparison with 2,4-D

In Tables 7 and 8, similar weed and woo plant lists are shown relative to silvex ve sus 2,4-D, again using information from th same published sources. Here again it obvious that there is a place for both herb cides since they do have certain differenti spectra of activity.

It is common knowledge that many herb ceous weeds and woody plants respon quite similarly to most of the phenoxy he bicides, including silvex, 2,4,5-T and 2,4-D On the other hand, it likewise is true th the response of many species to these thre chemicals varies significantly, which emph sizes the need for each of them in the herb cide arsenal. It also emphasizes the poss

16517

TABLE 2. Control of Perennial Weeds with Silvex

EXCELLENT	GOOD	FAIR
bedstraw	chicory	bindweed, field
blackeyedsusan	cutgrass	*cinquefoil*
broomweed	docks	daisy
bursage	groundivy	hoarycress
buttercup	kudzu	larkspur, duncecap
catsear	larkspur, tall	lettuce, blue
chickweed, mouse-ear	locoweed, white	skunkcabbage
coneflower	milkweeds	sorrel
coyotillo	pokeweed	sourdock
dandelion	sage, creeping	sowthistle
desertparsley	sheepsorrel	spikerush
fireweed	snakeweed, threadleaf	spurges
lupine, silvery	speedwell, purslane	strawberry, wild
milkvetch	violet	tansyragwort
mulesears	whitetop	thistle, Canada
norealbean		yarrow, common
pennywort, lawn		yellowrocket
ponyfoot (dichondra)		
vetch		
waterhemlock, spotted		
whiteclover		
woodsorrel		

TABLE 3. Control of woody plants with Silvex

EXCELLENT	GOOD	FAIR
alder	boxelder	blackberry, wild
aspen	buttonbush	cherry, wild
chittam	dogwood	chokecherry
chollacactus	honeysuckle	creosotebush
maples	locust, black	elm
oak, post	mesquite	grouncherry, clammy
oak, shinnery	oak, turkey	gum, black & sweet
palmetto, saw	persimmon, common	hickory
persimmon, Texas	raspberry, wild	manzanita
poisonivy	rose, McCartney	mulberry
poisonoak	sagebrush, sand	oak, blackjack
pricklypearcactus	saltcedar	oak, bluejack
redbud	sumac	oak, scrub
rose, cherokee	tree-of-heaven	oak, white
salmonberry	willows	osageorange
trumpetcreeper		rose, multiflora
willowweed		sagebrush, big
		tarbush
		toyon
		whitethorn
		yerba-santa
		yucca (soapweed)

DOW 3310997
16518

bility of herbicide rotation to forestall the development of weed spectra tolerant to a single phenoxy weed killer. It is obvious from the plant response tables that these herbicides are not equivalent and cannot completely replace one another.

Considering silvex in particular, it seems abundantly clear that this interesting herbicide, which has been around for many years, probably merits increased consideration not only on the part of those now advising on its present registered uses but also on the part of researchers to ferret out new possibilities, including combinations with other herbicides.

SUMMARY

KURON herbicide is a low volatile ester formulation of silvex, having wide utility in controlling broadleaf weeds and woody plants. Over the years, the product has gained acceptance for use mainly in aquatic and turf areas, but published literature affirms a broad range of utility for this herbicide and suggests possibilities for further use development. Many plant species show excellent response and can be controlled at moderate rates. The special utility of silvex in aquatic and turf weed control is noted.

Concerning the possible environmental impact of silvex use, it is known that breakdown and loss in soil occur via microbiological degradation. Silvex is degraded more slowly than 2,4-D but buildup in soil does not occur from annual increments. Used as recommended on the label, KURON herbicide presents no health hazard from ingestion or skin contact to humans, farm animals or wildlife. A toxicological summary was published in 1966[14].

SELECTED REFERENCES

1. Byrnes, W. R. *et al.* 1957. A test of 2,4,5-TP, 2,4,5-TA and ATA on mixed oaks and associated species. Proceedings 11th Northeastern Weed Control Conference:188-195.
2. Coulter, L. L. 1954. Silvex, a new selective herbicide. DOWN TO EARTH 9, No. 4:18-20.
3. Coulter, L. L. and J. W. Gibson. 1954. A progress report of field experiences with silvex. Proceedings 11th Annual North Central Weed Control Conference:90-91.
4. Coulter, L. L. and K. C. Barrons. 1956. KURON for turf weed control. DOWN TO EARTH 12, No. 2:24-25.
5. Dunham, R. S. 1965. Herbicide manual for noncropland weeds. USDA Agriculture Handbook No 269.

TABLE 5. Comparative control of certain herbaceous weeds with silvex and 2,4,5-T.

OFTEN BETTER CONTROL WITH SILVEX		
bedstraw (cleavers)	ground ivy	marshelder
blackeyedsusan	hemlock, poison	medic, black
chickweeds	henbit	milkweed, showy
evening primrose	knapweed, spotted	nightshade, black
fiddleneck	knawel	spurges
fireweed	knotweed, Japanese	spurry, corn

OFTEN BETTER CONTROL WITH 2,4,5-T		
buckwheat, wild	mayweed	poorjoe
coffeeweed	mesquite, velvet	purslane, common
daisy, oxeye	mexicantea	sage, creeping
goosefoot	mustards	shepherdspurse
indigo, curly	pennycress, field	yellowrocket
ironweed, western	pepperweed, field	

TABLE 6. Comparative control of certain woody species with silvex and 2,4,5-T.

OFTEN BETTER CONTROL WITH SILVEX	
cactus, cholla & prickly pear	poisonoak
honeysuckle	redbud
maples	rose, Cherokee
mulberry	sagebrush, fringed
oaks (live, blue, turkey, and others)	saltcedar
palmetto	trumpetvine
persimmon, Texas	yucca

OFTEN BETTER CONTROL WITH 2,4,5-T		
buckbrush	mesquite	sagebrush, big
chamise	oak, white	snowbrush
hawthorne	osage orange	sweetgum
	redbay	yerbasanta

16519

6. Elwell, H. M. *et al.* 1954. Aerial applications of herbicides on oak in Oklahoma. Proceedings 11th Annual North Central Weed Control Conference:91-95.
7. Gates, Dillard H. 1972. Rangeland weed control. Oregon Weed Control Handbook:126-127.
8. Gaylor, James and Alfred Houser. 1962. Three years' results with silvex for aquatic plant control in Oklahoma. DOWN TO EARTH 18, No. 3:2-4.
9. Hall, W. C. 1960. Control of various aquatic weeds with silvex. Proceedings 14th Northeastern Weed Control Conference: 476-482.
10. Hughes, E. E. 1967. Dormant boom-applied spray treatments for control of saltcedar. Research Progress Report, Western Weed Control Conference: 23-24.
11. Klingman, D. L. and W. C. Shaw. 1971. Using phenoxy herbicides effectively. USDA Farmers' Bulletin No. 2183.
12. Leonard, O. A. and W. A. Harvey. 1965. Chemical control of woody plants. California Agricultural Experiment Station Bulletin 812.
13. Motooka, P. S. *et al.* 1967. Control of Hawaiian jungle with aerially applied herbicides. DOWN TO EARTH 23, No. 1:18-22.
14. Mullison, W. R. 1966. Some toxicological aspects of silvex. Proceedings 19th Annual Meeting Southern Weed Conference: 420-435.
15. Newton, Michael. 1962. Control of bigleaf maple with silvex. DOWN TO EARTH 18, No. 1: 20-24.
16. Newton, Michael. 1972. Herbicides in forestry. Oregon Weed Control Handbook.
17. Owens, C. B. 1956. Chemical eradication of turkey oak. DOWN TO EARTH 11, No. 4:23-24.
18. Pierce, Madelene E. 1960. Progress report of the effect of KURON upon the biota of Long Pond, Dutchess County, Y.Y. Proceedings 14th Northeastern Weed Control Conference: 472-475.

TABLE 7. Comparative control of certain herbaceous weeds with silvex and 2,4-D.

OFTEN BETTER CONTROL WITH SILVEX		
bedstraw	groundcherry	medic, black
buttercups	groundivy	milkweed, common
blackeyedsusan	hemlock, poison	nightshade, black
chickweeds	henbit	purslane
dogfennel	horseweed	spurges
fiddleneck	knapweed, spotted	spurry, corn
fireweed	knawel	woodsorrel
fleabanes	lupines	yarrow
OFTEN BETTER CONTROL WITH 2,4-D		
blueweed, Texas	heal-all	pineappleweed
carpetweed	ironweed, western	poorjoe
cinquefoil	knapweed, diffuse	puncturevine
croton	mexicantea	sage, creeping
deathcamas	mudplantain	smartweed
garlic, wild	mustards	speedwell
	pennycress, field	yellowrocket

TABLE 8. Comparative control of certain woody species with silvex and 2,4-D.

OFTEN BETTER CONTROL WITH SILVEX		
alder	honeysuckle	persimmon, common
blackberry	locust, black	poisonivy
cactus	maples	poisonoak
cherry, wild	mesquite	raspberry
chokecherry	mulberry	redbud
elm	oaks	saltcedar
hickory	osage orange	tree of heaven
	palmetto	trumpetcreeper
OFTEN BETTER CONTROL WITH 2,4-D		
buckeye	cottonwood	snowbrush
buckbrush	manzanita	sagebrush, big
chamise	rabbitbrush	toyon

DOW 3131099

16520

HA-36

2549

DOW 331088

Program Your Aquatic Weed Control Needs

By

CHARLES T. LICHY

Plant Science Research and Development

The Dow Chemical Company

Winter Park, Florida

The title of this talk is PROGRAM YOUR AQUATIC WEED CONTROL NEEDS. What does it mean to program? Do we realize what the word program can imply? Webster defines program as a "plan of future procedures." An apt definition but one which does not completely describe all of the side effects of programming. Another definition listed for program is "a doctrine, theory or system whose validity can be tested only in practical application." This, perhaps, is even a better definition for us working in weed control. Basically, however, the word programming means "what are we trying to accomplish" and "how can we go about with the task at hand." One way to do this is to list what the problems might be in regard to the type of aquatic weed "problem" you have. "Problem" would include not only the species that are present, but which species you would like to have present, or perhaps what is the purpose of the weed control program, be it drainage ditch, irrigation canal, farm pond, water fowl refuge or what have you. Other points that need to be listed; what are the ramifications of your control program, is spray drift a problem, is it a water shed-area, how about fish population, is the area used for swimming, irrigation or what else? One factor that must be considered is that you obviously cannot use a different material for each species present. Therefore, you must use a material which has a broad enough spectrum so that the primary weed populations will be removed. Perhaps the next year, then, the material will have to be changed to pick up the resistant weeds that were not killed by the first year's application. Another factor which may be important is ecology. After you spray one weed population, what changes can be expected in the population? And, of course, last but not least, are the economics involved. Of course, they are always important but sometimes these are relatively important, sometimes they are relatively unimportant when looking at the total program. In short, programming your aquatic weed control needs actually becomes very complicated when all factors of control are considered.

Let us examine some of these factors in detail so that we can arrive at principles where these factors might be taken into consideration in programming our control needs. Perhaps the most obvious place to start is which weeds do we have to control or which weeds are our problem. It is not necessary to go into any discussion on identification of the various aquatic weeds since these have been discussed in some detail before the society on several occasions. One point, however, that must be considered is the considerable variation in the time when the aquatic weeds are most susceptible to chemical treatment. For some species like the Arrowarum (*Peltandra spp.*) the most susceptible period is during the flowering stage. For other plants such as the rose mallows, hibiscus species and cattails (*Typha spp.*) the time of greatest susceptibility is during the late flowering and the early stages of seed production. Giantcutgrass can be killed most easily during the period of maximum runner growth, which usually

16521

extends about a month after the initial flowering period. With the phragmites the most susceptible period is at the time of flowering or pollination, two to three weeks after the tassels. Cattails are most easily killed when in the late flowering or early fruiting stage. In the south the tropical cattail often flowers about two months after the narrow leaf cattail. For this reason multiple treatments sometimes have been required to control both species. Farther north, however, the period between flowering of the two species is much shorter. In Maryland, for example, tropical cattail flowers about one month after narrow leaf cattail and one treatment has given satisfactory control of both species. The best time for treatment of needlerush occurs during the flowering stage and lasts for three to four weeks. Regardless of all this, however, spraying can and should be done over a wide range of growth periods since satisfactory results can be obtained and frequently less chemical is required because of the smaller size of the plants. Whether the plant sites are flooded or non-flooded can also play an important role in determining effective control from a herbicide.

Aquatic plant control is also complicated by the need to consider the role of plants in different situations. Weeds are described as plants being out of place. Thus, plants that are undesirable in one location may be beneficial in another. To the waterfowl manager a weed is a plant that does not provide enough food or cover to justify the space it occupies. For example, buttonbush is responsible for 70% or more of the coppice growth which is a problem in the marshes and swamps of the lower Mississippi valley area. Yet in Maryland, Virginia and the Carolinas buttonbush contributes to valuable waterfowl habitat. Phragmites also can have a place in waterfowl management. The root growth will protect a na during storms. Also, the phragmites furnishes excellent cover for both the hunter and the hunted. For example, in past years it has helped to make the Thousand Acre Marsh in Delaware one of the best duck shooting areas along the Atlantic Coast. But solid stands are undesirable since landing areas are not available.

Since water is intimately associated with aquatic weed control many problems are unique to the water weed control field. Aquatic plants differ in physiology and habitat so significantly from terrestrial plants that one cannot confidently apply to aquatic plant control the information gained from experience solely with terrestrial plants. Water quality may be an extremely important factor. For example, work done by The Dow Chemical Company shows the effect of pH on coontail as shown in Table I. Similar results were also obtained with potamogeton and alligatorweed. Table II shows the effect of calcium as well as the effect of pH. Notice that the presence of calcium in the water, particularly at a pH of or above, improved kill of coontail. Water temperature may not influence the reaction of a chemical to a significant degree but the aquatic weeds may be in their most active growth at higher temperatures and thus would be more susceptible.

Contamination of water by herbicidal chemicals is of prime importance. Naturally, all due caution should be exercised when using herbicides. It is necessary to use those materials which are relatively non-toxic to humans and fish as well as possible desirable plant species along the edges of the aquatic area. It is thus very important to control spray drift; spray drift this instance being that physical drift, which through high pressure spray equipment or otherwise negligent use of spray equipment, in which particles of the spray material move onto desirable areas such as vegetables, citrus trees, home lawns, gardens, etc. Some of these problems can be avoided by using well trained and well equipped spray crews. Spray crews should be instructed or shown how to exercise care in proper chemical application. Materials should be used which have a non-volatile nature. It is important to use low pressure to provide largest sized droplets that are compatible with the type of application and control necessary. The end use of the water should be considered. That is, whether the water is used for swimming, irrigation, drinking, fishing; whether it is a water shed area, and some attention must be paid to this when selecting herbicides for weed control use.

Practical considerations must also be included in your choice of weed control materials. You obviously cannot use a different weed control material for each species you may find along a ditch bank or canal. You must, therefore, use a material which will control the primary species involved. This may require over a period of time that two or more herbicides be used if eradication is desired. It may even be desirable to make combinations of herbicides so that all the species are properly controlled. In this regard attention must be paid to the ecology, that is, the changing population after a herbicide application. In the case of waterfowl refuges, where certain plants are desirable, this can be made to work for you. Table III shows the effect of two chemicals on the ecology of two plots before and after chemical application. Note that before application the plant composition is approximately the same, yet after application, the plant composition is radically different.

A farm pond presents a somewhat different situation in aquatic weed control since you have a substantially stable water environment as contrasted to a ditch or canal. This means that chemicals applied to the water remain in contact for a long period of time. This has advantages in that it tends to make the chemical more effective in weed control but has a disadvantage in that it continues the water pollution problem for a long period of time unless after a period of weed control the pond can be flushed with fresh water. Ponds have an additional advantage in that construction of pond edges that drop off quite rapidly and fertilization which shades the aquatic weed serves as a reasonable means of weed control without chemicals. However, once weeds get into a pond, then chemicals must be used to eliminate them. Weeds may get into the pond from seeding upstream or birds scattering seeds or a number of other ways. Water weeds often grow in water stored for irrigation. They clog outlet and inlet pipes, spray nozzles and irrigation structures. They also harbor mosquitoes and interfere with fishing. Many irrigation reservoirs are shallow and have flat bottoms and are subject to frequent draw-down and refilling. In this case neither deep edges nor fertilization control weeds in such impoundments because of the fluctuating water levels. In deeper reservoirs or those in which draw-downs are infrequent, fertilization and deep edges may be practical. However, in irrigation ponds extreme care must be taken so that waters which have been contaminated with chemicals are not used for irrigating sensitive crops. Lakes have similar problems and normally not being equipped with deep edges and not being practical in most cases to fertilize, the problems are considerably magnified.

Let's discuss some of the chemicals that are available for aquatic weed control taking into account some of the principles and problems we have discussed above. I'm sure all of you have seen recommendations put out by Bob Blackburn and Lyle Weldon from Ft. Lauderdale. One of the chemicals you will notice mentioned in almost all of the aquatic weed

DOW 331089

... is The Dow Chemical Company
... tion of silvex as the propylene
... This is not a new material. It has
... erties for aquatic weed control.
... Kuron herbicide at 5 qts/acre
... of the troublesome submerged
... States. Experimental tests in
... sections of the country evaluated
... concentration on several aquatic
... ts in 1958 and subsequent years
... mance information. Kuron con-
... a in Table IV.
... cation can be used. Emergent
... ng Kuron at 1 gal./100 gal. of
... emerged foliage as a thorough
... for the control of submerged
... made by diluting Kuron at 1 gal.
... and directing this solution on or
... g pumps are also often used to
... and pump it to the boom which
... on over a wide swath. Uniform
... desirable to allow a lethal con-
... me into immediate contact with
... jections of undiluted formulations
... on a limited basis with varying

... t completely safe to fish. Experi-
... s in several ponds have caused
... areas where high concentrations
... lications at concentrations of 5
... ome fish kill in a limited number
... een no fish injury in over 99%
... Rapid incorporation of the spray
... associated with rates in excess
... e avoided where fish kill cannot
... Jersey found that applications
... on oxygen content in treated
... with Kuron normally decompose
... n to twenty days are required
... decomposition apparently does
... of the water. This is a problem
... which give a rapid kill of weeds.
... tments with Kuron showed no
... enthic organisms. Applications
... d populations of some plankton
... eatment the populations were
... plankton studied consisted of
... terious effect on large aquatic
... rtles in treated areas. Prolific
... n soon after treating aquatic
... ides. Algae growth does not
... four weeks after treatment
... rance of algae apparently is
... the aquatic weeds and thus
... nto the water from decaying
... toxicity and presents a low
... use. The Kuron herbicide
... l species and the following
... hown in Table V. From
... that there should be no
... to the handling and use
... cide should be left where
... it. Cattle, sheep, swine,
... water containing Kuron
... period with no adverse

effects as determined by water consumption, body weight gains and growth observations including gestation and parturition in sheep. These data indicate that foliage or water treated with Kuron herbicide as recommended should not present a hazard from ingestion. Skin irritation tests conducted upon rabbits have indicated that Kuron herbicide and its dilutions are slightly irritating upon repeated prolonged contact. In these studies there was no evidence of absorption through the skin in acutely toxic amounts. The observation of reasonable care and personal cleanliness practices should be adequate to avoid skin difficulties. Kuron herbicide when diluted to 1% with water or 1 gal. per 100 gal. caused no primary irritation or allergenic responses when applied to the skin of fifty human volunteers using a repeated insult technique. In addition, the product was patch tested on a second group of fifty human subjects at concentrations up to 50% without cutaneous reaction. Even when applied undiluted to the skin for two hours it caused no skin response. Thus it may be concluded with a high degree of assurance that when used as directed, this product should cause no skin irritation or skin sensitization responses.

Kuron may remain in water for three to four months after treatment where no dilution occurs. Accordingly it is suggested that water treated with Kuron should not be used for spray purposes or for irrigation.

Thus Kuron appears to present a possible solution to a wide variety of aquatic weed problems. It has a wide spectrum of activity on both submerged and emerged weeds, is relatively low in toxicity to fish and other aquatic animals and it has been exhaustively tested throughout the years. The scientists at Ft. Lauderdale and other places around the United States have had Kuron in test in the laboratory and in the field. The Corps of Engineers has put out a large scale field test for the control of alligatorweed. Since Kuron does have a wide spectrum of activity it is very useful where mixed stands of aquatic weeds occur. For example, in areas where alligatorweed and water hyacinth are intermingled, Kuron offers a solution for control of both species. Many chemicals such as 2,4-D and others will control water hyacinth but will not appreciably affect the alligatorweed. Kuron offers economic and efficient control of both.

For the control of aquatic and ditch bank grasses a material generally recommended is dalapon or the Dow trademarked product Dowpon or Radapon. This material, like Kuron, has been extensively field tested in a wide variety of locations and species. It is particularly useful in drainage ditches since grass and cattails can seriously reduce the flow of water. Cattails are particularly troublesome. They interfere with the proper utilization and maintenance of drainage and irrigation canals by reducing the velocity and volume of flow, and by causing deposition of silt and debris. Safety can also be an important consideration. In weed clogged drainage ditches water is held for a longer period of time after storms, increasing the chance that small children may tumble in and drown. In reservoirs, farm ponds, marshes and lake margins cattails waste large quantities of water, crowd out plants which provide food for wildlife and often interfere with fishing, boating and other uses of these bodies of water. An excellent bulletin on this has been issued by the U. S. Department of Agriculture, July 1963, entitled "Studies on the Control of Common Cattail in Drainage Channels and Ditches." These studies indicated that Dowpon herbicide was extremely effective in controlling cattail. Additional work has been reported in Michigan, Oregon and other states which shows the effectiveness of Dowpon in controlling cat-

DOW 331090

tails. Ted Ball of the U. S. Fish and Wildlife Service reported the results applying Dowpon by aircraft in the southeast. In this test Dowpon was applied to control cattail, maidencane and giantcutgrass. All showed good response. In addition, Dowpon is non-toxic to fish and wildlife species as well as domestic animals.

Brush control can also be important in certain cases of aquatic weeds, particularly in ponds and ditch banks. Brush control has been done by 2,4-D, 2,4,5-T or combinations of both. All of you are familiar with these results. Recently The Dow Chemical Company has discovered a new brush control material trademarked Tordon. Although this material is not yet in the marketing stage for aquatic weed control, excellen brus control results have been obtained on utility rights-of-way. The material is also currently under test throughout the United States with aquatic weed workers.

In summary—remember that aquatic weed control is perhaps more complicated and has more ramifications than terrestrial weed control. Be sure to take into consideration all the problems and possibilities of problems in controlling aquatic weeds by chemicals. The chemical companies are aware of your complicated problems and are constantly striving in research and development to offer better solutions to those problems. It is up to each of you to be aware not only of the problems of aquatic weed control but also the latest solutions to these problems.

TABLE I

Effect of pH of Water on Silvex Effectiveness

pH	Silvex ppm	Kill rating
5.0	0	0
7.	0	0
9.0	0	0
5.0	3.5	10
7.0	3.5	8
9.0	3.5	3

TABLE II

Effect of Ca and pH on Response of Coontail to Silvex at 2.5 ppm

Ca ppm	pH	Kill rating
0	5	10
0	7	7
0	9	2
250	5	10
250	7	9
250	9	8
500	5	10
500	7	9
500	9	8

TABLE III

Plant Composition as Affected by Chemical Treatment

	Plot 1		Plot 2	
	Before	After	Before	After
Cordgrass	96%	1%	95%	20%
Hibiscus	3%		3%	70%
Dock	1%	98%	2%	
Cypress	Trace	1%	Trace	2%
Smartweed			Trace	5%

TABLE IV

Aquatic Weeds Controlled by Kuron at 1.25, 2.5 and 5 Qts./acre foot.

1.25 quarts/acre foot or less (0.5 ppm)

White water lily ..... (*Nymphaea spp.*)
Pickerelweed ..... (*Pontederia cordata L.*)

2.5 quarts/acre foot or less (1.0 ppm)

Yellow water lily ..... (*Nuphar spp.*)
Mud plantain ..... (*Heteranthera spp.*)
Water milfoil ..... (*Myriophyllum heterophyllum*)

5 quarts/acre foot or less (2 ppm)

Bladderwort ..... (*Utricularia spp.*)
Watershield ..... (*Brasenia schreberi*)
Coontail ..... (*Ceratophyllum demersum*)
Fanwort ..... (*Cabomba caroliniana*)
Waterweed ..... (*Anacharis canadensis*)
Variable pondweed ..... (*Potamogeton diversifolius*)
(*Potamogeton amplifolius*)
Naiad ..... (*Najas flexilis*)
Spike rush ..... (*Eleocharis spp.*)
Tapegrass ..... (*Vallisneria americana*)
Waterstarwort ..... (*Callitriche spp.*)
Soft rush ..... (*Juncus effusus*)
Bulrush ..... (*Scirpus americanus*)
Burreed ..... (*Sparganium spp.*)

TABLE V

LD 50 Values

Animal Species	mg/kg
Rat	1070
Guinea pig	850
Rabbit	850
Mouse	2140
Chicks	2000

Bibliography

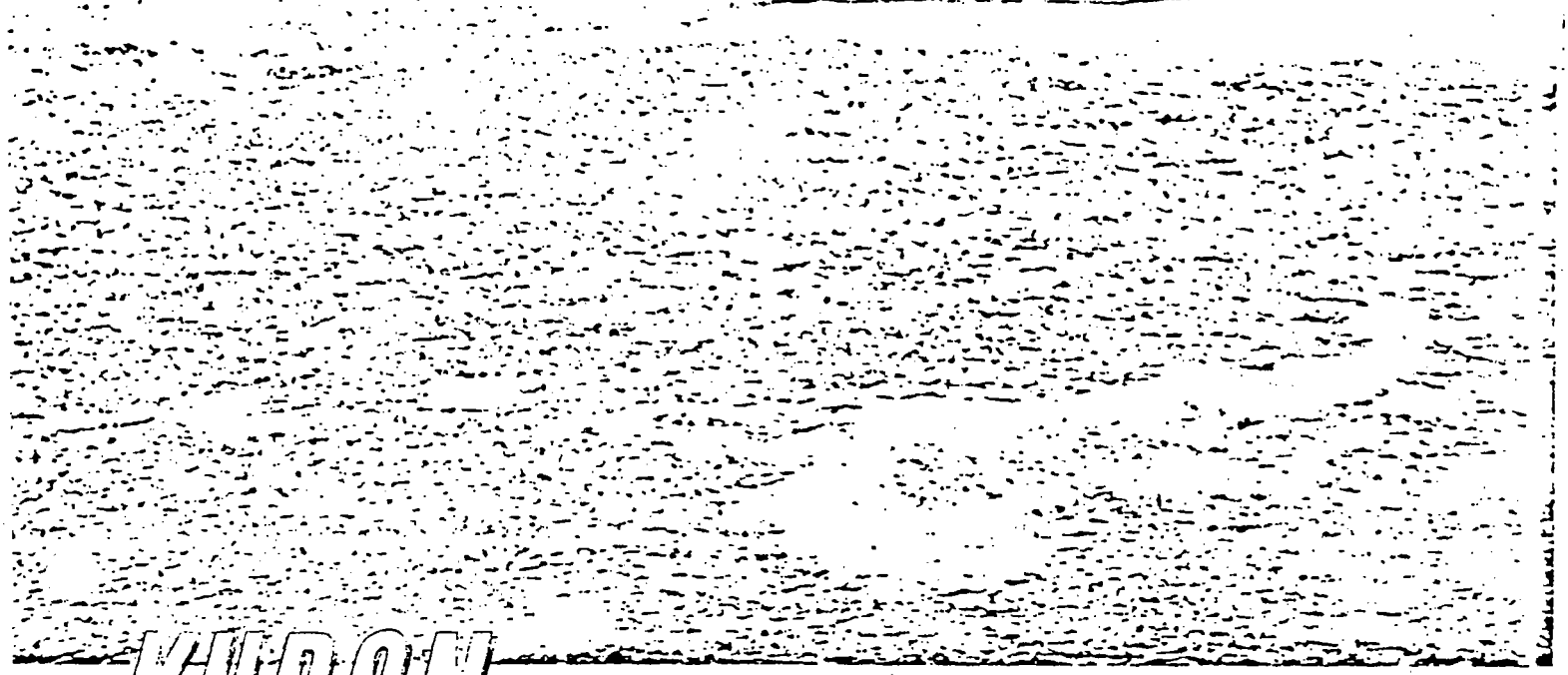
1. Ball, Edwin, "Results in Applying Dalapon by Aircraft to Pest Plants in Waterfowl Areas of the Southeast," DOWN TO EARTH, Summer, 1958.
2. Day, B. E. and Swezey, A. W., "Control of Phragmites with Dalapon," DOWN TO EARTH, Spring 1956.
3. Goodrum, Phil D. and Gray, Charles E., "Experimental Control of Giant Cutgrass in Eastern Texas," DOWN TO EARTH, Winter 1958.
4. Grigsby, B. H., Reimer, C. H. and Cutler, W. A., "Observations on the control of Cattail, Typha Spp., by Chemical Sprays," Quarterly Bulletin of the Michigan Agricultural Experiment Station, Vol. 37 (3) pp 400-406, February 1955.
5. Heath, R. G. and Ruch, L. C., "Aerial Control of Cattail With Radapon," DOWN TO EARTH, Winter 1957.
6. Pierce, M. E., "The Effect of the Weedicide Kuron on the Flora and Fauna of Two Experimental Areas of Long Pond, Duchess County, New York," Proc. N.E.W.C.C. 12: 338-343, 1958.
7. Pierce, M. E., "Progress Report of the Effect of Kuron Upon the Biota of Long Pond, Duchess County, N. Y.," Proc. N.E.W.C.C. 14: 472-375, 1960.
8. Steenis, J. H., "Aquatic Weed Control," Proceedings Forestry Symposium, August 30 and 31, 1960, The Pennsylvania State University.
9. Timmons, F. L. et al "Studies on the Control of Common Cattail in Drainage Channels and Ditches," USDA Technical Bulletin 1286, July 1963.
10. Weldon, L. W., "A Summary Review of Investigations on Alligatorweed and its Control," USDA, Corps of Engineers, CR 33-60 September 1960.
11. Wiltse, M. G., "Aquatic Vegetation Control With Kuron," Presented at 1960 meeting of the Weed Society of America.

DOW 331091

16524

2550
DOW 331085

END TO WATER WEEDS



KURON OFFERS A CONTROL FOR UNWANTED VEGETATION IN LAKES AND PONDS

THE DEFINITION-OF-THUMB says a weed is "a plant out of place." A weed can be out of place in a garden, in a lawn, in a farm field . . . or in a lake. Finding suitable methods of controlling unwanted aquatic vegetation has been a goal of agricultural chemists, conservationists and water sports enthusiasts for years.

The nature of aquatic weed problems depends largely on one's aquatic interests. Odors that accompany excessive aquatic weed infestations are offensive to sightseers. Vegetation can jam boat propellers, trip water skiers or ruin swimming spots. Anglers know that excessive vegetation can destroy fish spawning beds or unbalance fish populations. And to industry, excess aquatic growth in cooling ponds can interfere with circulation of cooling water.

To qualify for aquatic use any herbicide chemical must meet rigid specifications. First, it must do a good job of controlling aquatic plants. Secondly, the chemical must have a wide margin of safety to birds, animals, fish and humans.

The recent acceptance of *Kuron* as an aquatic weed control agent marks a big step forward in this field. *Kuron* has been known for years as a brush killer, especially effective on hard-to-kill species such as oak and maple. The new use registration has been granted by government regulatory agencies on the basis of performance coupled with substantial safety in use.

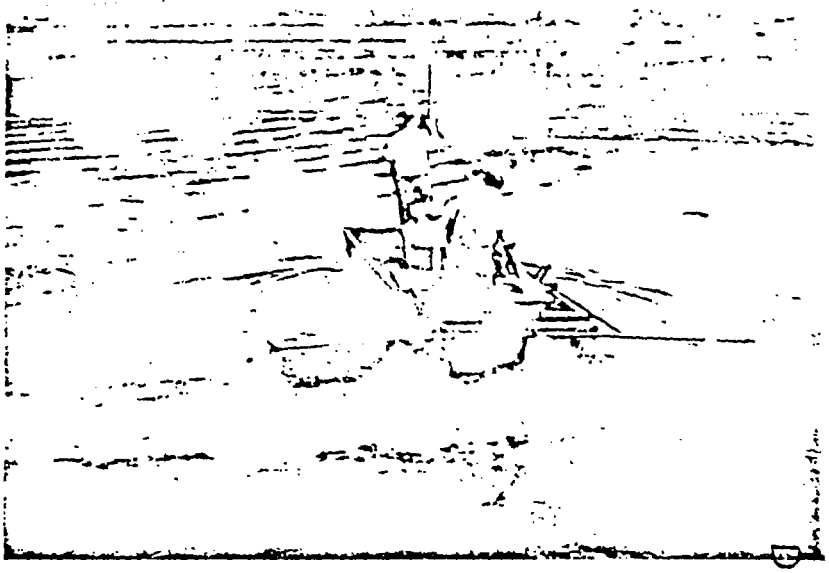
The story of Truesdale Lake shows the possibilities for *Kuron* in this new field.

Truesdale Lake is an attractive body of water in New York State. The lake is ringed with summer homes and was a popular watering spot until it became choked with weeds which virtually ruled out swimming, boating and fishing. Members of the Truesdale Lake Property Owners Association faced decreasing fun and declining property values and

Reprinted from April 1959 Dow Diamond

16525

of Truesdale Lake in New York before treatment
move vegetation. The lake has an area of 88
and its shores are populated with summer homes.
ie past several years weeds had interfered
reasingly with swimming, boating and fishing



Treating the lake with *Kuron* to remove vegetation. A mixture of the chemical and water was applied as a coarse spray over the surface of the lake. *Kuron* formed a white emulsion in the water, persisting for about 20 minutes and eliminating the need for marker buoys. *Kuron* is effective in controlling some of the most obnoxious aquatic weeds when as little as two parts per million are applied in lake water. The treatment was made by Robert Sheridan

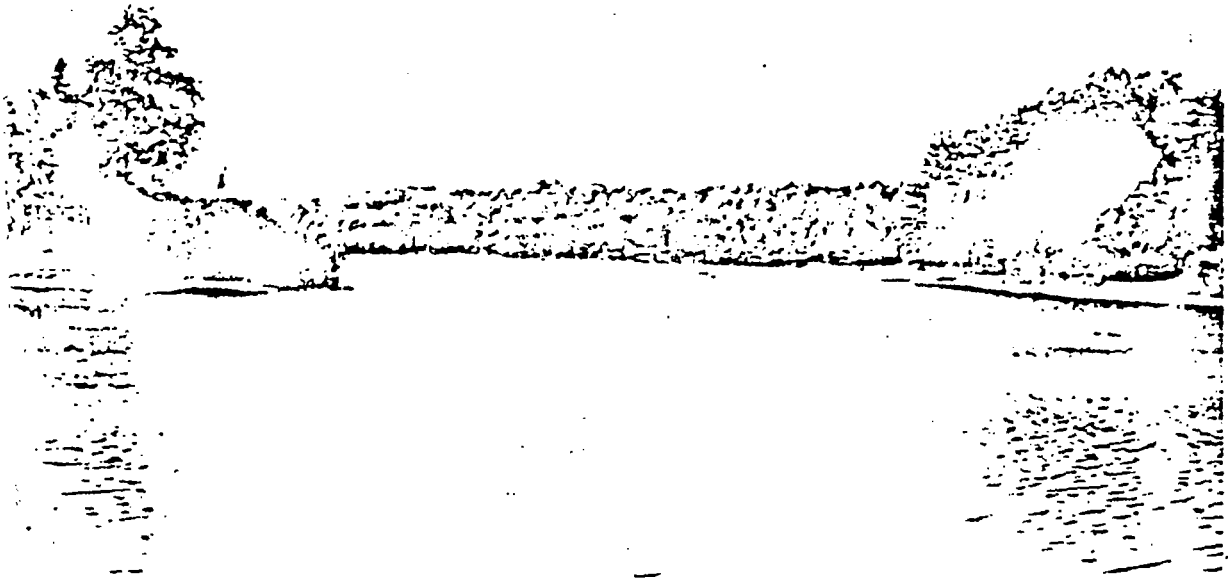
knew that something had to be done. "Something" proved to be a big order. Various materials were tried with discouraging results. When association members heard of successful tests in New Jersey using *Kuron* to control aquatic weeds, they determined to try the method on Truesdale Lake.

Necessary arrangements were made with the City and State of New York to make the application last summer. Since the lake is a part of the watershed serving New York City, special arrangements were made to measure chemical concentrations in the water.

The program was conducted under the general supervision of Dr. E. C. Raney, professor of fisheries biology and zoology, Cornell University, with assistance from Roy R. Younger of the New Jersey Department of Conservation. Robert Sheridan, a custom spray operator from Dover, New Jersey, was engaged to make the treatment.

The entire operation came off with no adverse effects, save a minor kill of fish. This seems almost inevitable in aquatic vegetation control work since decaying vegetation ties up oxygen in the water. However, *Kuron* kills aquatic plants gradually, holding this problem to a minimum. At Truesdale Lake, cottage owners were glad to overlook the slight fish kill in favor of greatly improved fishing conditions. And this past summer, for the first time in five years, boating, swimming and fishing were possible and pleasant in July.

OW 331086



Four weeks after application the lake had become so clear that the bottom could be seen in many areas. Observers watching the operation reported a gradual kill of water weeds. In about a week after application plants began turning down, with most weeds going to the bottom two weeks or so after application

16526

occurs, even when doses far superior to the LD_{50} are administered), and high potency as an enzyme inducer [4], suggest that this could be of a cascade-type involving a series of biochemical lesions. We have therefore investigated the effect on the enzyme equipment of rats intoxicated with (I), so as to define the targets of this biological damage.

Adult Wistar rats (10♂ and 10♀) weighing 210 ± 20 g were given a single dose of 10 mg of (I)/kg in olive oil by intraperitoneal injection: the controls (10♂ and 10♀) received the solvent alone. Both treated rats and controls received a normal diet and water *ad libitum*. After 10 days, blood samples were taken by cardiac puncture and the levels of the following serum enzymes were measured: glutamic oxaloacetic transaminase (G.O.T.) [5], glutamic pyruvic transaminase (G.P.T.) [5], aldolase (Ald.) [6], lactic dehydrogenase (L.DH.) [7], hydroxybutyric dehydrogenase (Hb.DH.) [8], alkaline phosphatase (A.P.) [9], arylesterase (A.E.) [10], and cholinesterase (Ch.E.) [10]. The results, analyzed statistically by Student's t-test, are given in Table 1.

Table 1. Effect of (I) on serum enzyme levels

Enzyme	Sex[g]	Controls	Treated
S.G.O.T.[a]	♂ + ♀	65.21 ± 4.19	344.38 ± 40.08[d]
S.G.P.T.[a]	♂ + ♀	32.83 ± 1.55	43.64 ± 3.87[d]
Ald.[a]	♂ + ♀	27.48 ± 1.74	29.30 ± 2.85[e]
L.DH.[a]	♂ + ♀	473.60 ± 31.73	716.83 ± 76.84[d]
Hb.DH.[a]	♂ + ♀	250.44 ± 28.01	389.92 ± 41.02[f]
A.P.[b]	♂ + ♀	17.86 ± 1.22	17.14 ± 1.26[e]
A.E.[c]	♂	333.20 ± 19.05	165.60 ± 28.81[d]
	♀	316.80 ± 14.50	213.60 ± 6.73[d]
Ch.E.[c]	♂	16.88 ± 2.13	4.40 ± 0.64[d]
	♀	18.48 ± 1.61	3.22 ± 0.85[d]

[a] Expressed in mIU/ml. [b] Expressed in Bodansky units. [c] For A.E., results expressed in mg of phenyl acetate hydrolyzed in 1 h by 1 ml of serum; for Ch.E., results expressed in mg of acetylcholine hydrolyzed in 1 h by 1 ml of serum. [d] Statistically highly significant. [e] Non-significant. [f] $P < 0.02$. [g] Separate figures for ♂ and ♀ given only when there are statistically significant differences.

Table 2. Effects of (I) on blood serum components in rats (♂ and ♀)

Component	Unit	Controls	Treated
Glucose	g/l	1.17 ± 0.04	0.68 ± 0.03[a]
Cholesterol	g/l	2.37 ± 0.1	2.65 ± 0.15[b]
Urea	g/l	0.34 ± 0.02	0.43 ± 0.03[c]
Total lipids	g/l	3.79 ± 0.15	6.25 ± 0.43[d]
Protein	g/l	69.79 ± 1.58	57.1 ± 1.95[a]
Bilirubin	mg/100 ml	3.16 ± 0.3	23.57 ± 4.89[d]
Sodium	mEq/l	146.0 ± 2.41	139.25 ± 3.31[c]
Potassium	mEq/l	4.87 ± 0.30	5.42 ± 0.41[b]
Chloride	mEq/l	102.4 ± 0.71	99.6 ± 1.5[b]

[a] $P < 0.001$. [b] Non-significant. [c] $P < 0.02$. [d] Highly significant.

It is evident from Table 1 that "dioxin" produces deep perturbations in several enzymatic systems in rats; the disturbance may be a decrease (particularly remarkable in the case of cholinesterase) or an increase (particularly considerable in the case of S.G.O.T.).

Table 2, which records the effect of (I) on the main components of blood serum, also shows a profound disturbance in the homeostasis indicating, *inter alia*, that the liver is one of the main targets of "dioxin" intoxication. Histopathologic evidence of this damage to the liver and to other target organs (notably the thymus and heart) will be presented in a separate paper.

Received January 17, 1972

[1] Buu-Hoï, N. P., *et al.*: C.R. Acad. Sci. (Paris), Sér. D 273, 708 (1971). — [2] LD_{50} *ca.* 22.5 μg/kg in the rat, see Verrett, J., in: Effects of 2,4,5-T on Man and the Environment. Hearings of the Committee on Commerce (U.S. Senate), April 7 and 15, 1970. U.S. Govt. Printing Office, Washington, D.C., 1970, p. 190. — [3] Higginbotham, G. R., *et al.*: Nature 220, 702 (1968). — [4] Buu-Hoï, N. P., *et al.*: C.R. Acad. Sci. (Paris), Sér. D 272, 1447 (1971). — [5] Reitman, S., Frankel, S.: Amer. J. Clin. Path. 28, 56 (1957). — [6] Shapira, G., *et al.*: Sem. Hôp. Paris 29, 1917 (1953). — [7] Wroblewski, F., *et al.*: Proc. Soc. Exper. Biol. Med. 90, 210 (1953). — [8] Rosalski, S. B., Wolkinson, J. H.: Nature 188, 1110 (1960). — [9] Bodansky, O.: J. Biol. Chem. 99, 197 (1932). — [10] Vincent, D., *et al.*: Ann. Biol. Clin. 18, 489 (1960).

2551

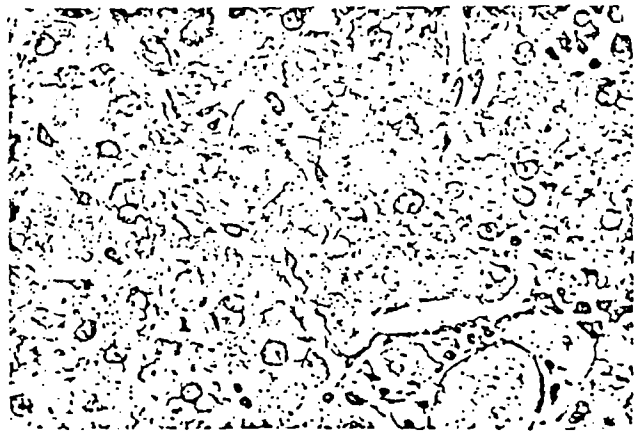
Organs as Targets of "Dioxin" (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin) Intoxication

N. P. Buu-Hoï†, Pham-Huu Chanh, (Miss) G. Sesqué, (Mrs.) M. C. Azum-Gelade, and G. Saint-Ruf

Centre National de la Recherche Scientifique, Paris, France

In previous papers [1, 2], we showed by means of enzyme studies that the liver is a main target of intoxication by the extraordinarily potent toxic and teratogenic agent "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (I). The present paper supplies anatomo- and histopathologic evidence of damage to this organ, and to other organs which we have also found to be profoundly affected within the short duration (10 days) of our experiment.

Wistar rats (10♂ and 10♀) weighing 210 ± 20 g were given a single dose (10 mg/kg) of (I) by intraperitoneal injection in olive oil; the controls (10♂ and 10♀) received the solvent only. All the animals received a normal diet and water *ad libitum*. The treated animals lost considerable weight as from the



a



b

Fig. 1 a and b. Effect of "dioxin" intoxication on liver and thymus. a) Hepatocyte deterioration: steatosis, karyoclasia, voluminous plasmodial forms (×250). b) Inverted picture of thymus structure (×62.5)

† Deceased January 28, 1972.

DOW 1 037273

16527

2nd day of the experiment, and by the 10th day they weighed (at 50 g less than at the start, whereas the controls had gained (at 70 g. Haematologic examination showed a highly significant increase (Student's *t*-test) in the haematocrit (controls: 39.50 ± 0.9; treated: 49.25 ± 2.37), significant increase in the leukocyte count (controls: 5912 ± 308; treated: 7315 ± 451) with a decrease of lymphocytes (controls: 79% ± 1.64; treated: 61% ± 11) and augmentation of polynuclear neutrophils (controls: 17.9% ± 1.8; treated: 37.3 ± 11). However, no significant effect was made on the bone marrow within this brief period.

All the treated animals had *hepatic* lesions, the females being more severely affected. The lobules were reshaped by important centrilobular stasis dissociating the Remocke trabeculae (pronounced anisokaryosis, frequent binucleation, cytoplasm loaded with chromophobe vacuoles and hyaline inclusions), and by a focal hyperplasia of the Kupffer cells; alongside the binucleated hepatocytes, voluminous plasmodes were often present. These findings were already prominent by the 6th day (Fig. 1a).

Apart from these hepatic lesions, which confirm the biochemical findings reported [1, 2], two other organs manifested considerable involvement. The modifications sustained by the *thymus* ranged from diffused pyknosis of the thymocytes to an inverted picture of the structure (migration of thymocytes towards the medulla) or total involution; these lesions were manifest as early as the 5th day (Fig. 1b). This thymus involvement explains the particularly pronounced toxicity of (I) in *young* animals. The third organ to undergo considerable change was the *heart*, the animals presenting a valvulitis with erosion of the endocardium on the ventricular face of the valves, fibrinoid thrombi, and oedema of the valves which were thickened by fibroblastic proliferation. Focal lesions located deep in the myocardium were also observed, with a disappearance of myofibrillae and interstitial polynuclear infiltration. At the same time, intra-arterial fibrino-leukocyti-thrombi, periarteriolar fibroblastic reactions, and foci of alveolitis were seen in the *lungs*.

These target organs suffered qualitatively the same damage when a considerably smaller dose, i.e., 1 mg/kg, of (I) was administered.

The pathological deterioration observed in the liver and heart, and, to a lesser degree, the lungs, of rats, is similar to that reported by Allen and Carstens [3] in an experiment with monkeys on a long-term diet which included a toxic fat contaminated with a "chick edema factor" which was later found to contain chloro derivatives of dibenzo-*p*-dioxin including (I) itself [4]; the thymus was not included in their study.

We thank Dr. S. S. Epstein, Cleveland, Ohio, for helpful discussions and advice.

Received January 18, 1972

[1] Buu-Hoï, N. P., *et al.*: C.R. Acad. Sci (Paris), Sér D 272, 1447 (1971). — [2] Buu-Hoï, N. P., *et al.*: Naturwissenschaften 59, 173 (1972). — [3] Allen, J. R., Carstens, L. A.: Amer. J. Vet. Res. 28, 1513 (1967); Lab. Invest 15, 970 (1966). — [4] Higginbotham, G. R., *et al.*: Nature 221, 702 (1968); Cantrell, J. S., *et al.*: Acta Cryst. 25 B, 150 (1969).

DGM 1037271

Buchbesprechungen

Otto Hahn — Eine Bilddokumentation. Von Ernst Berninger. München: Heinz Moos 1969. 106 S., 137 Abb., DM 18,—.

Das Buch ist ein dokumentarischer Bericht über einen außergewöhnlichen Menschen und sehr erfolgreichen Forscher. Die bebilderte Biographie ist in sich vollständig und umfassend, wenn es auch zu beklagen ist, daß historische, wissenschaftlich interessante Dokumente und ein Teil des Schriftwechsels im Arbeitszimmer Otto Hahns durch Kriegseinwirkung vernichtet worden sind. Hahns Charakter, sein Charme, seine Klugkeit — Weisheit — sprechen aus dem Buch, wie wir, seine Schüler, ihn kannten. Ein Wissenschaftler hohen Ranges, sicher im Leben und seiner Umwelt stehend, kontaktfreudig und heiter erscheint er uns als eine Persönlichkeit, die in ihrer Art einzigartig war und bleiben wird. Ein interessantes Buch über einen Deutschen, aber zugleich auch über eine vergangene und vielseitige Epoche der hohen Zeit der Wissenschaft in Deutschland. W. Seelmann-Eggebert (Karlsruhe)

Elastische, piezoelektrische, piezooptische, elektrooptische Konstanten und nichtlineare dielektrische Suszeptibilitäten von Kristallen. Von R. Bechmann, R. F. S. Hearmon und S. K. Kurtz (Landolt-Börnstein, Neue Serie, Gruppe III, Bd. 2. Hrsg. K.-H. Hellwege und A. M. Hellwege). Berlin-Heidelberg-New York: Springer 1969. 232 S., 196 Abb., DM 136.—, $ 37.40.

Nur 3 Jahre nach dem Erscheinen des 1. Bandes der Gruppe III „Kristall- und Festkörperphysik" (Neue Serie) hat sich ein Ergänzungsband als notwendig erwiesen. Er bringt Neubestimmungen und Ergänzungen zu den elastischen, piezoelektrischen, piezooptischen und elektrooptischen Konstanten, sowie zu deren Temperatur- und Druckkoeffizienten. Die Literatur ist bis etwa Mitte 1968 erfaßt. Der vorliegende 2. Band ist außerdem stofflich erweitert um Angaben über die Elastizitätskonstanten dritter Ordnung, über die Erzeugung optischer Oberwellen von Licht in Kristallen (unter Einschluß einzelner sehr genauer Messungen von Brechungsindizes) und über elektrooptische Konstanten zweiter Ordnung. Die Kapitel werden zugänglich gemacht durch klar abgefaßte ausführliche Einführungen, die aus der ersten Ausgabe (gestrafft) übernommen sind. Der Zusammenhang zwischen den kristallographischen Eigenschaften wird durch vielfache Querverweise hergestellt. Ein gemeinsames, nach Bruttoformeln alphabetisch geordnetes Substanzverzeichnis erschließt und verbindet den Inhalt beider Bände. Leider sind hierin keine Hinweise auf die Art der nachgewiesenen Daten enthalten. — Beide Bände gehören zusammen und sollten in keinem Institut fehlen, das sich mit Festkörperphysik oder Kristallographie beschäftigt, sei es grundlagemäßig, sei es hinsichtlich der Entwicklung neuer physikalischer Bauaggregate. Gerade die neu zugefügten Kapitel sind in diesem Arbeitsbereich von besonderer Aktualität.

A. Neuhaus (Bonn)

Theorie und praktische Anwendung von Komplexbildnern. (Methoden der Analyse in der Chemie, Bd. 9). Von F. Umland, A. Janssen, D. Thierig und G. Wünsch. Frankfurt/Main: Akademische Verlagsgesellschaft 1971. 760 S., DM 150.

This treatise has 2497 references and offers an extensive discussion of complexes, mainly with multidentate organic ligands, in gravimetric, volumetric, photometric and fluorometric quantitative analysis of virtually all elements. The theoretical introduction is rather weak and relies on the archaic hybridization theory; thus, on page 13, the possibility is left open that square-planar (this redundant word ought to be "quadratic") copper (II) complexes contain one 4p electron. On the other hand, the general description of what ligating atoms are preferentially bound to a given central atom is quite instructive. The text contains detailed prescriptions for a large number of specific methods, and highly useful tables. The sources of errors and interference are carefully discussed. This book, representing a tremendous amount of critical study of the literature, can be recommended to everybody interested in inorganic analytical chemistry. A specific aspect is the discussion of diphenylborate derivatives.

C. K. Jorgensen (Genf)

Colour Chemistry. By R. L. M. Allen. London: Nelson 1971. 336 S., £ 5.

Ungeachtet der lawinenartig zunehmenden Bedeutung der Kunststoffe bleiben die technischen Farbstoffe ein wichtiges,

16528

1. M. F. Holick, J. E. Frommer, S. C. McNeill, N. M. Richtand, J. W. Henley, J. T. Potts, Jr., *Biochem. Biophys. Res. Commun.* 76, 107 (1977).
2. M. F. Holick, N. M. Richtand, S. C. McNeill, S. A. Holick, J. E. Frommer, J. W. Henley, J. T. Potts, Jr., *Biochemistry* 18, 1003 (1979).
3. M. F. Holick, J. A. MacLaughlin, M. B. Clark, J. T. Potts, Jr., R. R. Anderson, I. H. Blank, J. A. Parrish, P. Elias, *Science* 210, 203 (1980).
4. T. Okano, K. Mizuno, N. Matsuyama, N. Nobuhara, T. Kobayashi, *Recl. Trav. Chim. Pays-Bas*, 98, 253 (1979).
5. J. W. Melski, L. Tanenbaum, J. A. Parrish, T. B. Fitzpatrick, H. L. Bleich, *J. Invest. Dermatol.* 68, 328 (1977). "The criteria for patients with skin types I, II, III, and IV, generally of European extraction, are based on the history of the usual reaction to the first hour of full sun exposure in early summer. Skin type V generally includes patients of Asiatic, American Indian, Mexican, and Puerto Rican extraction. Skin type VI generally includes patients of African extraction."
6. I. H. Blank, R. D. Griesemer, E. Gould, *J. Invest. Dermatol.* 29, 292 (1957).
7. R. Belsey, M. B. Clark, M. Bernat, J. Glowacki, M. F. Holick, H. F. DeLuca, J. T. Potts, Jr., *Am. J. Med.* 57, 50 (1974).
8. D. Schacter, D. V. Kimber, H. Schenker, *Am. J. Physiol.* 200, 1263 (1961). Male weanling Holtzman rats were fed a diet deficient in vitamin D but containing 0.47 percent calcium and 0.3 percent phosphorus for 4 weeks. *SIM* refers to the ratio of ^{45}Ca on the inside of the gut sac (serosal side) to the incubation medium on the outside of the gut sac (mucosal side). There were six animals in each group; the values are reported as the mean ± standard error.
9. W. F. Loomis, *Science* 157, 501 (1967).
10. F. Daniels, Jr., P. W. Post, B. E. Johnson, in *Pigmentation: Its Genesis and Biological Control*, V. Riley, Ed. (Appleton-Century-Crofts, New York, 1972), p. 13.
11. At the time that Loomis proposed this theory, it was not known that during exposure to sunlight, vitamin D_3 is not directly formed in the skin (*1–4*) and that vitamin D_3 must undergo a series of regulated metabolic hydroxylations to 1,25-dihydroxyvitamin D_3 before it is biologically active (*12*).
12. E. Havinga, *Experientia* 29, 1181 (1973).
13. M. F. Holick and J. T. Potts, Jr., in *Harrison's Principles of Internal Medicine*, K. J. Isselbacher, R. D. Adams, E. Braunwald, R. G. Petersdorf, J. D. Wilson, Eds. (McGraw-Hill, New York, 1980).
14. S. A. Levinsohn, *Am. J. Dis. Child.* 34, 955 (1927).
15. International Commission on Illumination, *CIE Publication No. 20 (TC-2.2), 1972* (Paris, 1972).

16. We thank R. R. Anderson, J. A. Parrish, and J. T. Potts, Jr., for helpful advice and assistance, and L. B. Fred, J. S. Adams, and S. A. Holick for editorial assistance. Supported in part by a NASA grant (NAS9-15205) and NIH grants (AM25395-01 and R01-AM27334-01).

8 July 1980; revised 1 October 1980

2,4,5-Trichlorophenoxyacetic Acid Causes Behavioral Effects in Chickens at Environmentally Relevant Doses

Abstract. *Administration of the herbicide 2,4,5-trichlorophenoxyacetic acid to incubating chicken eggs alters behavior after hatching. Single doses, with no morphological effects, retard learning (lowest dose, 7 milligrams per kilogram of body weight) and increase general activity (27 milligrams per kilogram) and jumping (13 milligrams per kilogram). Day 15 of incubation is the most susceptible stage of development.*

As far as we know, there has been only one earlier study reporting teratological effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on behavior (*1*). Administration of 2,4,5-T (100 mg/kg) to pregnant rats produced no morphological deformities in the offspring, but it did cause them to have learning deficits and to exhibit increased activity in the open field. We have shown that even lower doses can produce behavioral abnormalities in the chicken. A single dose of 2,4,5-T [Sigma; containing 0.03 part per million of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), according to the Australian Government Analytical Laboratory] was administered to the chicken egg on either day 8 or day 15 of incubation (*2*). A third group received 2,4,5-T by intraperitoneal injection on day 2 after hatching. The 2,4,5-T was suspended in a 0.5 percent solution of gum tragacanth made with sterilized, distilled water. Control animals (matched for each separate hatching) received the vehicle alone. Doses were calculated according to the weight of the chicken or egg at the time of injection and ranged from 7 to 53 mg/kg for eggs and 75 to 225 mg/kg for chicks after hatching.

The median lethal dose (LD_{50}) on day 2 after hatching was 200 mg/kg, and on day 15 of incubation it was 53 mg/kg. There was 70 percent hatching in eggs given 2,4,5-T on day 15 of incubation at doses up to 27 mg/kg. Some 5 to 10 percent of the hatched chicks, irrespective of dose, showed abnormal leg development; they either dragged one leg or held it off the ground. Approximately 5 percent of the treated chicks showed depigmentation of feathers and down. Both of these effects have been reported (*3*). No differences in body weight gain were observed in chicks receiving 2,4,5-T during incubation or at doses less than 150 mg/kg on day 2 after hatching.

The chicks without apparent morphological deformities (after doses shown in Fig. 1) were given behavioral tests in week 2 after hatching. The tester was unaware of the treatment that each chick had received. There were 8 to 20 chicks in each group tested. We measured the activity in an unfamiliar environment on day 7 of life by placing the chick in a gray-walled box (30 cm by 30 cm with 25-cm walls). Cumulative activity for a 3-minute interval ("general activity") was scored by an Animex activity meter, and the number of jumps made was counted by eye. We scored ambulation for some groups by dividing the floor of the box into quadrants and counting the number of times the chick crossed into a new quadrant. We tested visual discrimination learning on day 9 of life by using the "pebble floor" test (*4*), which consists of a search for grains of food scattered over a background of small pebbles adhering to the floor. Within a 60-peck trial, a measure of visual learning is generated by recording the number of incorrect responses (pecks at pebbles) within consecutive 20-peck intervals.

General activity, jumping, and visual learning rate were altered by 2,4,5-T treatment (Fig. 1). Since the same doses were administered on day 8 and day 15 of incubation, these data could be examined by a two-way analysis of variance (that is, dose, age, and their interaction). A one-way analysis of variance was used for the data from day 2 after hatching. General activity scores conformed to a parametric distribution, but jumping and learning scores required logarithmic transformation.

Jumping behavior proved to be the most sensitive parameter affected by 2,4,5-T. For those treated on days 8 and 15 of incubation, jumping showed both a significant dose effect ($F = 2.78$; d.f. = 3, 90; $P < .05$) and significant interaction between age and dose ($F = 3.33$; d.f. = 3, 90; $P < .05$). Comparisons of the groups given the two highest doses with their control groups (*t*-test) showed a significant increase in jumping in the groups treated with 13 mg/kg ($P < .05$) and 27 mg/kg ($P < .01$) on day 15 of incubation, but there were no significant differences in those treated on day 8 (see Fig. 1). There was also a significant dose effect for jumping in the groups treated on day 2 after hatching ($F = 4.06$; d.f. = 2, 20; $P < .05$), and a *t*-test of data for the control group and the group receiving a dose of 150 mg/kg revealed a significant increase in jumping after 2,4,5-T treatment ($P < .025$), this dose being tenfold higher than the lowest dose found to be effective on day 15 of incubation.

General activity, which includes measures of jumping, ambulation, and other movements, shows a similar tendency to increase after 2,4,5-T treatment on day 15 of incubation and day 2 after hatching (Fig. 1). Analyses of variance just failed to reveal significant effects, but *t*-tests of data for the control groups and those groups that had received the highest doses at each age revealed a significant elevation of general activity in the groups treated with 27 mg/kg on day 15

0036-8075/81/0206-0593$00.50/0
Copyright © 1981 AAAS

MN 0533033 DOH 2123634 16529

incubation ($P < .01$) and those receiving 150 mg/kg on day 2 after hatching ($P < .025$). There were no significant effects after treatment on day 8.

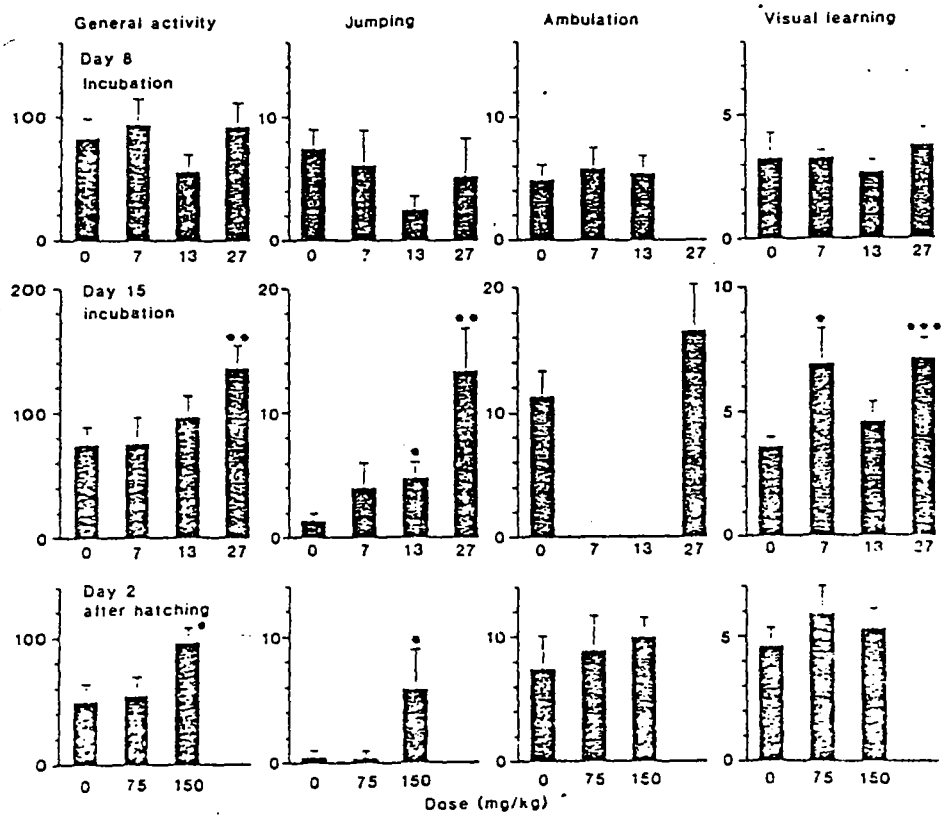
Ambulation, the other component of general activity that we measured, did not show any dependence on dose and no significant differences were found as a result of examination of the data by *t*-tests, even though there was a tendency for ambulation to increase with increasing dose after treatment on day 15 of incubation and day 2 after hatching (Fig. 1). Thus, as found in rats, 2,4,5-T has long-lasting effects on activity, but in chicks the most sensitive parameter contributing to the increased activity is jumping rather than ambulation, which may indicate increased fear responsiveness to novelty.

There was a significant dose effect for visual discrimination learning in those groups treated on days 8 and 15 of incubation ($F = 4.19$; d.f. = 3, 86; $.005 < P < .01$) but not for those treated on day 2 after hatching. Comparison of treated animals with their controls at each age revealed a significant slowing of learning in those treated on day 15 of incubation with 27 mg/kg ($P < .001$) and 7 mg/kg ($.01 < P < .025$), but no significant effects were present after treatment at the other two ages.

Day 15 of incubation appears to be a stage of maximum sensitivity to the behavioral effects of 2,4,5-T. Neuronal cell division is at a peak on day 8 of incubation and is largely completed by day 15 when synaptic proliferation is occurring (*5*). Synaptic proliferation is completed before day 2 after hatching (*4*). The brain therefore seems to be most sensitive to 2,4,5-T at a time of maximum synaptic formation. The fact that 2,4,5-T caused adverse effects at lower doses than reported previously may indicate an extreme susceptibility of the developing nervous system and hence behavior. This is consistent with one report of the possible adverse effects of 2,4,5-T in the human species (*6*).

The extent to which our results are due to 2,4,5-T or to very small amounts of its highly toxic contaminant, TCDD, remains unresolved. However, it is important to remember that in this study we used 2,4,5-T of greater purity, particularly with respect to TCDD content, than that available commercially and sprayed in the environment. Based on the concentration recommended in Australia for spraying blackberries (*7*), which is 2.8 liter/ha of commercial 80 percent 2,4,5-T (weight to volume), 1 m^2 would receive 224 mg of 2,4,5-T. Since the surface area of an egg is approximately 50 cm^2, the dose of 2,4,5-T received by one egg after spraying would be 1.1 mg or 22 mg/kg, which is greater than the lowest dose found to be behaviorally teratogenic. As 2,4,5-T has been found to pass through the egg shell (*8*), it may therefore present a real risk to bird species. It is questionable whether there is a dose below which 2,4,5-T can be said to have no effect since some individuals appear to be highly susceptible. At all the doses we used, morphological deformations were seen in some of the treated chicks, and, as the raw data for jumping in Fig. 2 illustrate, some individuals with no morphological deformations were highly susceptible to the behavioral effects of 2,4,5-T, even at the lowest dose (7 mg/kg) that we administered.

There is considerable species variation in sensitivity to the toxic effects of 2,4,5-T. At present one of the best indicators of interspecies toxicity is the rate at which 2,4,5-T is metabolized and excreted (*9*). Based on such measures, chicks and rats appear to be of roughly similar sensitivity whereas humans have



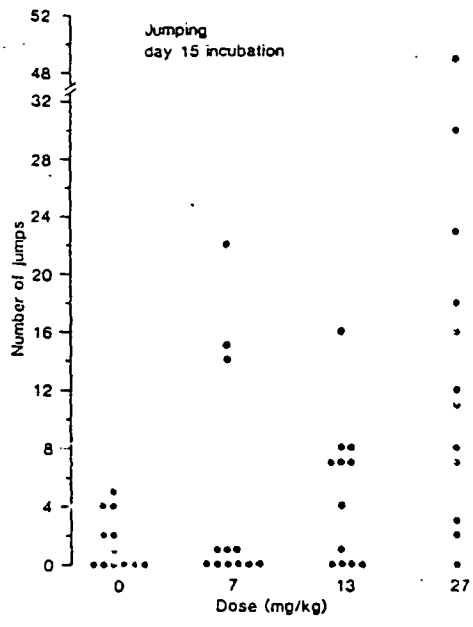


Fig. 1 (left). Plots of means and standard errors for four behavioral measures after the administration of 2,4,5-T at the doses indicated on day 8 or 15 of incubation or day 2 after hatching. Behavior has been tested in week 2 of life. The ordinate scales are as follows: for general activity, counts on the electronic activity meter in the total 3 minutes of scoring; for jumping, the number of jumps in 3 minutes; for ambulation, the number of times the chick crossed with both feet into a new quadrant of the box; and for visual learning, the number of errors (pecks at pebbles) in the last 20 pecks of the shock-floor learning task. In the latter test, a higher error score indicates slower learning. The asterisks indicate significant differences from the control groups as determined by one-tailed *t*-tests: *, $P < .05$; **, $P < .01$; and ***, $P < .001$. Fig. 2 (right). The raw data for jumping scores obtained during 3 minutes in a novel environment after treatment with 2,4,5-T on day 15 of incubation. Each point represents the score for a single individual. The fact that some individuals are highly susceptible to the behavioral teratological effects of 2,4,5-T is demonstrated by the fact that, even after treatment with the lowest dose (7 mg/kg), some individuals show a marked increase in jumping.

DOH 2123635

16530

at least a threefold greater sensitivity (*10*). Our findings therefore imply a risk to the human species.

C. A. SANDERSON
L. J. ROGERS

Pharmacology Department,
Monash University,
Clayton, 3168, Victoria, Australia

References and Notes

1. P.-O. Sjöden and U. Soderberg, *Physiol. Psychol.* 3, 175 (1972); in *Chlorinated Phenoxy Acids and Their Dioxins*. C. Ramel, Ed. (Swedish Natural Science Research Council, Stockholm, 1978), p. 149.
2. J. McLaughlin *et al.*, *Toxicol. Appl. Pharmacol.* 5, 760 (1963).
3. H. Lutz and Y. Lutz-Ostertag, *Adv. Exp. Med. Biol.* 27, 127 (1972); J. F. Dunachie and W. Fletcher, *Nature (London)* 215, 1406 (1967).
4. L. J. Rogers, H. D. Drennen, R. F. Mark, *Brain Res.* 79, 213 (1974).
5. B. M. Freeman and M. A. Vince, *Development of the Avian Embryo* (Chapman and Hall, London, 1974); J. Sedlacek, in *Advances in Psychobiology*, G. Newton and A. Riesen, Eds. (Wiley, New York, 1972), vol. 1, pp. 129–170.
6. B. Field and C. Kerr, *Lancet* 1979–I, 1341 (1979).
7. Vermin and Noxious Weeds Destruction Board, *Keith Turnbull Research Institute Bulletin 3E* (Department of Crown Lands and Survey, Victoria, 1977).
8. N. Gyrd-Hansen and S. V. Dalgaard-Mikkelsen, *Acta Pharmacol. Toxicol.* 35, 300 (1974).
9. F. J. Koschier and M. Acara, *J. Pharmacol. Exp. Ther.* 208, 287 (1978).
10. W. N. Piper, J. Q. Rose, M. L. Leng, P. J. Gehring, *Toxicol. Appl. Pharmacol.* 26, 339 (1973); K. Erne, *Acta Vet. Scand.* 7, 240 (1966).
11. We thank Drs. G. Bentley and M. Cullen for valuable advice.

23 July 1980; revised 29 September 1980

Neurosecretory Granules: Evidence for an Aging Process Within the Neurohypophysis

Abstract. *When cysteine labeled with sulfur-35 is injected into the third ventricle of the rat brain, it is first incorporated into only one of the two populations of neurosecretory granules that can be isolated on an isosmotic gradient. The second population of granules is labeled much later. Stimulation of hormone release from isolated labeled neural lobes and subsequent isolation of neurosecretory granules at different times after the injection of labeled cysteine shows that the radioactivity decreases in only one population of granules. One of the fractions of the gradient represents the granules found near the release site; the second population is probably located deeper in the nerve endings or in the nerve swellings. Whereas neurophysins are found in both populations, smaller proteins can only be detected in one. Thus it appears that neurosecretory granules undergo an aging process and that isosmotic gradients can separate the aged granules from those newly formed.*

Neurosecretory granules (NSG) in the hypothalamo-neurohypophysial tract are formed in the magnocellular neuron of the supraoptic and paraventricular nuclei (*1*). Labeling with [^{35}S]cysteine indicates that the magnocellular neurons mainly contain a large precursor protein (~ 20,000 daltons), which presumably is packaged into granules. The contents of the granules mature during transport toward the neurosecretory nerve terminals. When the granules reach the neural lobe, almost all the precursor has been converted into proteins of 12,000 ± 2,000 daltons, neurophysins, and peptides that may include the hormones oxytocin and facopressin (*2*).

The neurosecretory axons in the neurohypophysis can be subdivided into three compartments, all of which contain NSG (*3*): undilated axons containing few NSG, nerve endings characterized by the presence of microvesicles, and nerve swellings containing secretory granules but few if any microvesicles. We previously showed that the mean diameter of NSG in the endings differs from that of NSG in the swellings (*4*). Furthermore, we showed that fractionation of NSG from the neural lobe of rats on an isosmotic gradient yields two populations of NSG, each of which contains neurophysins, oxytocin, and vasopressin. One population has an isopycnic density of 1.13 g/cm^3 and is insensitive to osmotic changes in the surrounding medium, whereas the other sediments at a density of 1.11 g/cm^3 and is much affected by the osmotic pressure of the surrounding medium. Furthermore, the mean diameter of osmotically insensitive NSG after fixation is very similar to that of the NSG in the nerve endings, whereas the mean diameter of the osmotically sensitive NSG resembles that of the granules in the swellings.

In this report we present evidence that the osmotically insensitive granules are newly formed NSG (NF-NSG) that have recently arrived at the neural lobe and that the osmotically sensitive NSG are probably derived from the former. Furthermore, we show that the NF-NSG are located in the nerve endings, whereas most of the aged granules (A-NSG) cannot immediately release their contents and are thus likely to be found in the swellings. Protein species of a molecular weight lower than that of neurophysins can be demonstrated in the A-NSG. This suggests possible postmaturational cleavage of intragranular proteins.

Albino (Wistar) male rats weighing 250 to 300 g were anesthetized with ketamine chlorohydrate (10 mg per 100 g of body weight) and given intracisternal injections of [^{35}S]cysteine that was obtained from the supplier (New England Nuclear, 500 Ci/mmole) or by reduction of [^{35}S]cystine (470 Ci/mmole) with 10 m*M* dithiothreitol in 0.1*M* phosphate buffer (*p*H 7.0). Each rat was injected with 10 μl of the final solution (50 μCi of radioactive cysteine). The rats were decapitated at different times after the injection and their neural lobes were dissected out in less than 1 minute. Immediately after their isolation, ten of the radioactive lobes were homogenized in 1.0 ml of 0.3*M* sucrose buffered at *p*H 6.8 with 10 m*M* Hepes, and mixed with 20 nonradioactive neural lobes homogenized under the same conditions. The NSG were isolated as described by Nordmann *et al.* (*4*). The gradient fractions were collected with a Buchler device, and a portion of each fraction was kept for a determination of density. Scintillation fluid was added to the fractions and their total radioactivity was measured with a

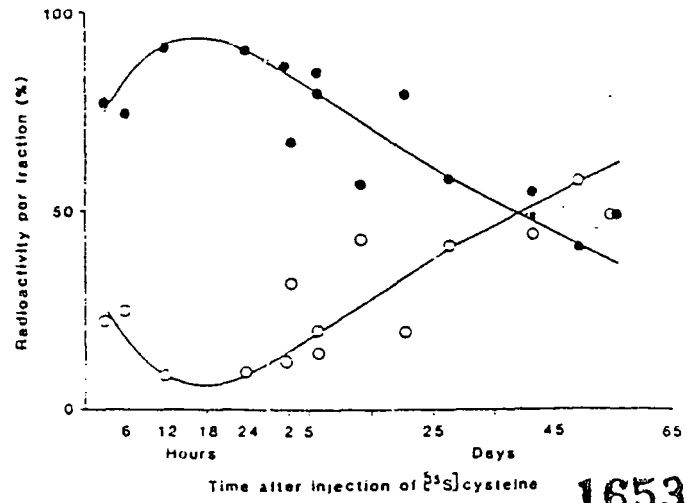


Fig. 1. Time course of the appearance of [^{35}S]cysteine in NSG fractions isolated on a sucrose-metrizamide isosmotic gradient at different times after the injection of the isotope. The radioactivity in NF-NSG (●) and A-NSG (○) was measured and the results are given as the percentage of the total radioactivity found in both fractions.

DOE 2123636

16531

0036-8075/81/0206-0595$00.50/0 Copyright © 1981 AAAS

Monitor

NO 5330

Herbicide is 'real-risk' to birds and people

Human beings are at risk from the controversial herbicide 2,4,5-T, according to two Australian scientists writing in the current issue of *Science* (vol 211, p 593). The scientists are seriously worried by the results of a recent series of experiments in which they found that eggs exposed even to small doses of the chemical often developed into chicks with impaired learning abilities. As expected, the chemical killed some of the embryos outright. Humans are approximately three times more sensitive to 2,4,5-T than chicks.

Previous research on 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) had shown that rates in the uterus given fairly big doses—100 milligrams of the chemical for every kilogram of the mother's body weight—were more active and learnt more slowly than other rats. These doses are higher than those that are likely to occur when farmers spray crops or pastures with the herbicide. So C. A. Sanderson and L. J. Rogers of Monash University in Australia set out to discover what happens to chicks given smaller doses before they hatch.

The scientists gave single doses of 2,4,5-T (containing 0·03 parts per million of the highly toxic contaminant 2,3,7,8-tetrachlorodibenzo-para-dioxin) to chick eggs at two points during incubation: the 8th or the 15th days. The doses ranged from 7 to 53 mg/kg body weight.

These doses were high enough to kill some of the embryos: for example, half the chicks given 53 mg/kg on day 15 of incubation died. The herbicide caused birth defects—such as depigmentation of the feathers or abnormal development of the legs—in another 10 per cent. Sanderson and Rogers tested the chicks that were not obviously damaged to discover any effects the herbicide may have had on their behaviour. They found that 2,4,5-T affected three measures of the chicks' behaviour: their general activity in an unfamiliar environment; the number of times they jump; and how well they learnt to discriminate visually between food grains and small pebbles.



The herbicide 2,4,5-T hits chick embryos hardest when their nerve cells are beginning to link up

However, the 2,4,5-T exerted these teratogenic effects on behaviour *only* when the chick embryos received the dose on the 15th day of incubation—giving 2,4,5-T on the 8th day of incubation had no statistically significant effect. On the 8th day of incubation, the chick embryo is rapidly forming its nerve cells. By the 15th day, this process is nearly completed and the nerve cells are making connections—synapses—with other nerve cells. Sanderson and Rogers say "the brain therefore seems to be most sensitive to 2,4,5-T at a time of maximum synaptic formation".

The scientists admit that they do not know whether the 2,4,5-T itself or its dioxin contaminant causes the damage. However, the 2,4,5-T they administered contained less dioxin than commercial Australian 2,4,5-T. (But not British 2,4,5-T which cannot, by law, contain more than 0·01 ppm of dioxin.) Spraying Australian 2,4,5-T on blackberries at the recommended rates of application, the researchers say, could expose the eggs of wild birds to 22 mg/kg—higher than the smallest dose they found to damage the unborn chick. As the chemical passes through the shells of eggs, Sanderson and Rogers conclude that "it may therefore present a real risk to bird species".

Because the formulation of 2,4,5-T sprays in Britain is different, doses to the eggs of British wild birds from 2,4,5-T would be smaller—about 2 mg/kg. But the Australian researchers point out that some individual chicks were especially susceptible to the herbicide.

They believe that humans may be at risk too. They cite previous, inconclusive, research by Barbara Field and Charles Kerr of Sydney University which showed that the yearly birth rate of babies with defects in their neural tubes in New South Wales correlated well with how much 2,4,5-T farmers used in the previous year. What is more, the highest rates of these abnormalities occurred nine months after the peak spraying periods in the antipodean summer. This evidence—together with the rough estimate that humans are three times more sensitive to 2,4,5-T than chicks and rats—leads Sanderson and Rogers to warn that "our findings therefore imply a risk to the human species". □

A plant cell that can store electricity

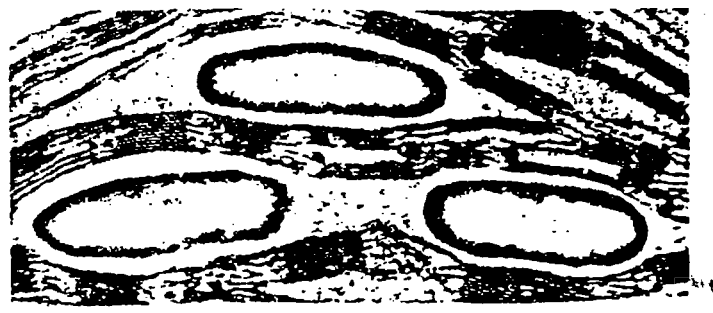
Biochemists at Ohio State University have combined "living" cell components with synthetic biochemicals to make what is effectively a giant specialised leaf cell that makes and stores electricity with an efficiency approaching what would be practically useful. Chemical and biological systems cooperate to make the new cell work this way.

Elizabeth Gross and her colleagues at Ohio first made successful plant-based photocells from particles carefully isolated from chloroplasts—the organelles responsible for photosynthesis inside living cells. Now Gross, Rong Pan, and Ravindra Bhardwaj have used the same chemical support system to drive cells based on whole chloroplasts, which are easier to separate from the rest of the plant tissue (*Nature*, vol 289, p 396).

The researchers deposited the chloroplasts onto a microfilter membrane separating two solutions. One solution contained an electron-donating chemical, and the other contained an electron acceptor. When light is shone through the acceptor solution onto the chloroplasts, both become excited, and electrons flow from the donor solution via the chloroplasts into the acceptor solution. Because the acceptor molecule that they used is sensitive to light, the scientists found that their cell produced a voltage even when they used dead chloroplasts. Taking into account the proportion of the total membrane area covered by chloroplasts, the researchers calculate that a maximum of about 3 per cent of the total light power is converted instantaneously into electric power; the chloroplasts supply about two-thirds.

There are two important factors which make these figures a rather poor measure of the new cell's performance. First, it is difficult to measure exactly how much the chloroplasts contribute to the cell efficiency, because the biological and chemical systems in the cell probably act synergistically—that is, the whole is greater than the simple sum of the individual contributions. Second — and much more important — the cell also *stores* large amounts of the light energy, so that instantaneous power-conversion efficiency measurements are misleading.

Gross and her colleagues found that after about half an hour's illumination, the cell went on producing much the same voltage in the dark for another hour. Cells that can store solar energy in this way are obviously much more useful than conventional photocells which only produce electricity when illuminated. □



The Cell Concept/Macmillan

Cells based on whole chloroplasts have been used to convert the Sun's energy into electricity. Part of a chloroplast, ×72 000, is shown. The plant's use of light energy begins in the thin membranes

16532

ISSN 0036-8075
12 March 1982
Volume 215, No. 4538

SCIENCE

2530

LETTERS Nuclear Weapons Materials: *T. B. Cochran, M. M. Hoenig, W. M. Arkin*; Assessing Agricultural Research: *W. W. Konkle*; Transamazon Highway: Impact on Indians: *D. C. P. Grossman*; X-ray Holography: *D. Sayre*; The Cray-1: *W. R. Smith*; Synthetic Vaccines: *H. Langbeheim*; Damaging Stereotype: *B. Hansen* 1344

EDITORIAL Energy and Chemicals from Trees 1349

MN 053305
DOE 21233631

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

REPORTS Recent Volcanic Activity at Glacier Peak: *J. E. Beget* 1389

Kinetics of Delignification: A Molecular Approach: *J. F. Yan* 1390

Dating of a Fault by Electron Spin Resonance on Intrafault Materials: *M. Ikeya, T. Miki, K. Tanaka* 1392

α_2-Adrenoceptor–Mediated Hyperpolarization of Locus Coeruleus Neurons: Intracellular Studies in vivo: *G. K. Aghajanian* and *C. P. VanderMaelen* .

Oxytocin Receptors and Human Parturition: A Dual Role for Ox... Initiation of Labor: *A.-R. Fuchs* et al. .....

Rapid Electronic Autofluorography of ... Dimensional Gels: *J. B. D...* ...411

Human Liver MAO ... with M... 1413

P... ...ffect on Life-Span ...uch and *R. L. Walford* 1415

...ed Retinotectal Projection During Chick ...Loon 1418

...steroid Regulation of Muscle Degeneration in an Insect: *L. M. Schwartz* and *J. W. Truman* 1420

Technical Comments: Jumping Chickens: Relevance to Hazard in Humans: *M. L. Leng*; *L. J. Rogers* and *C. A. Sanderson* 1421

PRODUCTS AND MATERIALS Personal Computer; Cell Culture Chamber; Refrigerators; Sample Preparation; Human T Cell Growth Factor; Infrared Spectrometer; Literature 1424

Jumping Chickens: Relevance to Hazard in Humans

Sanderson and Rogers have reported the results of a study on the behavioral effects in chickens given "environmentally relevant doses" of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and they postulated that humans would be three times more sensitive to this herbicide than chicks (*1*). The conclusions drawn from this study do not reflect a valid evaluation of the data because (i) the doses were not environmentally relevant for bird eggs, much less for humans, and (ii) chick embryo data are not a suitable model for safety assessment in humans and are not used by any government agencies for this purpose.

Recent studies by Lavy *et al.* (*2*) have shown that the maximum exposure to

0036-8075/82/0312-1421$01.00/0 Copyright © 1982 AAAS
16533

2,4,5-T likely to be encountered in humans is about 0.1 mg per kilogram of body weight, which is orders of magnitudes less than in the chicken studies. Furthermore, such exposure would be likely to occur only in applicators who are using knapsack sprayers and wearing contaminated clothing or in mixers or loaders who are not careful to avoid contact with the concentrate and spray solutions. Measurable exposure to 2,4,5-T is extremely unlikely in bystanders or the general population (*3*).

In the study of Sanderson and Rogers, the doses ranged from 7 to 53 mg of 2,4,5-T per kilogram of body weight injected into the yolk sac of fertile chicken eggs on day 8 or day 15 of incubation, and from 75 to 225 mg/kg injected intraperitoneally into chicks on day 2 after hatching. The 2,4,5-T contained 0.03 part per million of the highly toxic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is representative of the level in 2,4,5-T produced worldwide today.

"Environmental relevance" was based on an estimated dose of 22 mg/kg for an egg contaminated with 1.1 mg of 2,4,5-T over the entire shell surface (area, 50 cm^2), presumed to be equivalent to spraying an 80 percent 2,4,5-T product (derivative not specified) at a rate of 2.8 liter/ha. The latter is equivalent to 224 mg of 2,4,5-T (or derivative) per square meter of horizontal surface area, not the entire egg surface, and 2,4,5-T is generally applied as a 2 to 5 percent solution in oil, oil-water, or water emulsion. Injection of 2,4,5-T into the egg was also assumed to be equivalent to contamination of the shell surface. However, only a small fraction of the herbicide applied to the surface of an egg penetrates the shell and reaches the chick embryo (*4*).

Sanderson and Rogers concluded that humans would be at least three times as sensitive to 2,4,5-T exposure as rats or chickens. This conclusion was based on studies by Piper *et al.* (*5*) and Erne (*6*), although neither study implied such a relationship. What happens as a result of the injection of a large dose of a chemical into an isolated egg is not representative of what might happen in a human fetus connected to its mother's efficient detoxification system, particularly for a chemical such as 2,4,5-T which is rapidly excreted in the urine of humans (*7*). Even more questionable is their extrapolation of effects from the injection of a nearly lethal dose of 2,4,5-T into newly hatched chicks to the potential effects from low-level exposure in adult humans. Unfortunately, reports of such studies done under highly exaggerated conditions continue to add to the confusion surrounding the use of beneficial agricultural chemicals such as 2,4,5-T.

MARGUERITE L. LENG
Health and Environmental Sciences,
Dow Chemical Company,
Midland, Michigan 48640

References and Notes

1. C. A. Sanderson and L. J. Rogers, *Science* 211, 593 (1981).
2. T. L. Lavy, J. S. Shepard, J. D. Mattice, *J. Agric. Food Chem.* 28, 626 (1980); T. L. Lavy, J. S. Shepard, D. C. Bouchard, *Bull. Environ. Contam. Toxicol.* 24, 90 (1980); T. L. Lavy, paper presented at the meeting of the Weed Science Society of America, Toronto, Canada, February 1980.
3. M. L. Leng, T. L. Lavy, J. C. Ramsey, W. H. Braun, in *Pesticide Residues and Exposure* (Symposium Series 182, American Chemical Society, Washington, D.C., 1982), pp. 133–156.
4. J. D. Somers, E. T. Moran, Jr., B. S. Reinhart, *Bull. Environ. Contam. Toxicol.* 11, 511 (1974).
5. W. N. Piper, J. Q. Rose, M. L. Leng, P. J. Gehring, *Toxicol. Appl. Pharmacol.* 26, 339 (1973).
6. K. Erne, *Acta Vet. Scand.* 7, 240 (1966).
7. P. J. Gehring, C. G. Kramer, B. A. Schwetz, J. Q. Rose, V. K. Rowe, *Toxicol. Appl. Pharmacol.* 26, 352 (1973).

30 March 1981; revised 1 June 1981

Several aspects of Leng's technical comment require correction. We found that, for chicken eggs, the most sensitive period to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was day 15 of incubation, when the injection of as little as 7 mg per kilogram of body weight caused subsequent behavioral abnormalities (*1*). This amount does not cause marked morphological abnormalities and is well below the LD_{50} of 53 mg/kg (the dose lethal to 50 percent of the animals tested) [see (*1*)].

The question of the amount of 2,4,5-T absorbed through the eggshell is, of course, controversial. If we accept that only 30 to 50 percent of an egg may be covered by the spray and if we use the concentration recommended by the government of Victoria, Australia, for spraying blackberries, an egg would receive 7 to 11 mg/kg. Even if only some of this is absorbed, it is still uncomfortably close to the amounts that caused behavioral abnormalities in our sample. Leng does not allow for a safety factor. Attempts to extrapolate from these results to the calculation of the "no-effect" dose in humans are complicated by individual variations in sensitivity to 2,4,5-T [see our jumping data in (*1*)].

We used the acid form of 2,4,5-T, which is less toxic than its esters, usually used for spraying. Moreover, we gave only one dose to each egg. Repeated dosing may well be toxic at lower daily rates of administration. Leng quotes a maximum exposure to 2,4,5-T of 0.1 mg/kg for knapsack sprayers but fails to say that this value was calculated for one operation lasting 180 minutes. Most sprayers are repeatedly exposed and for longer periods. The maximum exposure measured by Lavy *et al.* (*2*) was actually 1.85 mg/kg per spraying operation, and this did not include spray inhalation, which is probably a more important route of exposure, particularly in the vicinity of aerial spraying.

We agree that other species must be tested with 2,4,5-T. We have in fact shown that the oral dosing of pregnant rats with as little as 3 or 6 mg/kg on day 8 of pregnancy causes behavioral abnormalities in the pups tested several months later (*3*). This dose is one-hundredth of the LD_{50} in this species and one-tenth of the lowest dose reported to cause physical deformities (*4*). Phenoxyacetic acids themselves appear to be able to cause these effects; we have found similar effects with 2,4-dichlorophenoxyacetic acid (2,4-D), which does not contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (*3*).

Our statement that humans are likely to be more sensitive than rats and chicks is based on deductions from the plasma half-life, which for an oral dose of 2,4,5-T of 5 mg/kg is 23.1 hours in humans and 4.7 hours in rats (*5*). The results for chicks appear to be similar to those for rats (*5*). We therefore maintain that 2,4,5-T (and 2,4-D) may present risks to brain development and function in humans and other species.

L. J. ROGERS
C. A. SANDERSON
Pharmacology Department,
Monash University,
Clayton, Victoria 3168, Australia

References

1. C. A. Sanderson and L. J. Rogers, *Science* 211, 593 (1981).
2. T. L. Lavy, J. S. Shepard, D. C. Bouchard, *Bull. Environ. Contam. Toxicol.* 24, 90 (1980).
3. M. A. Crampton and L. J. Rogers, in preparation; P. J. Booth and L. J. Rogers, in preparation.
4. K. S. Khera and W. P. McKinley, *Toxicol. Appl. Pharmacol.* 22, 14 (1972).
5. P. J. Gehring, C. G. Kramer, B. A. Schwetz, J. Q. Rose, V. K. Rowe, *ibid.* 26, 352 (1973); W. N. Piper, J. Q. Rose, M. L. Leng, P. J. Gehring, *ibid.*, p. 339; M. W. Saueroff, W. H. Braun, G. E. Blau, J. E. LeBeau, *ibid.* 37, 136 (1976); K. Erne, *Acta Vet. Scand.* 7, 240 (1966).

23 November 1981

DOH 2123932

16534

Hobson, L.B. 1983.
2501
HUMAN
Phenoxy Herbicide Soft Tissue Sarcom

EPIDEMIOLOGY OF SOFT-TISSUE SARCOMA AND RELATED HUMAN RESEARCH

(as related to herbicide exposure)

M07866

LAWRENCE B. HOBSON, M.D., Ph.D.
Deputy Director
Agent Orange Projects Office
Veterans Administration
March 1983

DOH 2120649

16535

EPIDEMIOLOGY OF SOFT-TISSUE SARCOMA AND RELATED HUMAN RESEARCH

Initiation of Swedish Studies of Herbicides and Cancer

In 1972 Swedish newspapers published rumors that railroad workers were dying from lung cancer as a result of exposure to herbicides used in their work. The National Board of Occupational Safety, as a result, requested Professor Olav Axelson, a specialist in occupational medicine, to undertake an epidemiological investigation of the matter.

The results of this investigation have been reported in a series of four papers, 1-4. Another series, 5-7, was prompted by criticism of the epidemiological and statistical methods employed in this and related studies. Attention in the United States has focussed on the pre-publication manuscript of the 1980 paper by Axelson, Sundell, Andersson, Edling, Hogstedt, and Kling, 4.

This paper dealt with two aspects of the study of railroad workers. The initial phase was a cohort study of 348 men who had been exposed, individually rather than as a group, to herbicides for more than 45 days during 1957 to 1972 and who were followed through October 1978. Exposure information was incomplete but the workers were divided into subcohorts with exposure to phenoxy acids (which include the ingredients of Agent Orange), amitrol, or to both herbicides. The mortality rates for these exposed subcohorts were compared to the age-specific national death rates for Swedish men, the latter serving as the control cohort.

Overall 49 deaths were expected in the exposed cohort; 45 occurred, a result attributed to the "healthy worker effect." There were, however, 17 tumors found where 11.85 were expected. Among the deaths occurring at least ten years after the first exposure, 6 cancers were found although only 1.78 were expected. Dr. Axelson, 8, later increased this to 7 tumor cases. Each subcohort had an excessive number of tumor deaths, the greatest being in the group exposed to both phenoxy herbicides and amitrol.

Although initially, 2, amitrol was associated with an increased tumor mortality, somewhat different results were found in a second phase of the examination, described as a case-referent study (identical to a case-control study). The data indicated a "statistical association" of phenoxy herbicides and excess tumor mortality, 3. Suspicion was increased by finding that workers exposed to phenoxy acids

DOW 2120650

alone had a "statistically significant excess of stomach cancer", specifically 2 cases compared to 0.33 expected when this type of herbicide was used alone and 3 cases as compared to 5.1 expected (increased from 4.1, 8) for all workers exposed to phenoxy herbicides, alone or with amitrol.

The series of papers on railroad workers has been criticized on several methodological grounds, 9, and Axelson has replied to these criticisms, 6. Richard D. Remington, Dean of the School of Public Health, University of Michigan, reviewed this and other Swedish studies for the Office of Technology Assessment. His evaluation, 10, was that the Axelson investigations had been "carefully conducted" and "well reported." He pointed out the limitations of the statistical methods used and found that "the numbers available ... are inadequate to permit definite conclusions" although "the results ... are suggestive."

Of interest in connection with the question of soft-tissue sarcomas and phenoxy herbicides is the type of tumors found by Axelson's group, 4. One case of Hodgkin's lymphoma occurred among the eight tumors in men exposed to phenoxy herbicides alone and no soft-tissue sarcomas or non-Hodgkin's lymphomas was diagnosed among the eight tumors appearing in workers exposed to both amitrol and phenoxy herbicides. In other words, no sarcomas were reported for the total of 207 men exposed to phenoxy compounds, 2. A reticulum cell sarcoma and a Hodgkin's lymphoma were found among the 7 tumors of workers exposed to amitrol alone. Thus, there was one soft-tissue sarcoma reported for 152 men exposed to amitrol, 2. Another 28 persons described as exposed to "other herbicides and combinations" cannot be identified as to exposure to specific herbicides but apparently none developed a tumor.

Axelson's work is directly related to the later work on soft-tissue sarcomas and lymphomas by Lennart Hardell. Indeed, Axelson suggested to Hardell in 1976, that he conduct a case-control study of soft-tissue sarcomas and has actively assisted in Hardell's work since then.

Swedish Investigation of Soft-Tissue Sarcoma

That work began when Hardell admitted for treatment 3 patients in the autumn of 1976, each with a soft-tissue sarcoma, and a history of exposure to phenoxy herbicides. He then found a total of 7 patients with "malignant mesenchymal tumors" (soft-tissue sarcomas) who gave a history of having worked with phenoxy herbicides 10 to 20 years earlier. The cases were among 87 patients with soft-tissue sarcomas, 55 of

DOH 2120651

whom were men. Of these men, 9 were forestry workers, 6 worked in forestry and on farms, and 6 were employed in saw mills or pulp plants. Another two tumors appeared in men whose connection with forestry was less direct. The malignancies found were two leiomyosarcomas, two rhabdomyosarcomas, two neurofibrosarcomas, with one each of fibroid liposarcoma, myxofibrosarcoma, and polymorphocellular sarcoma, 12.

Following Axelson's advice, Hardell began a case-control study that was published in two journals, 13, 14, a common practice of reporting in Swedish with an almost identical paper in English. The second paper in English aroused much interest in the United States.

Hardell and Sandstrom found 21 living and 31 dead men who were diagnosed as having soft-tissue sarcomas in Hardell's oncology department in northern Sweden. They were matched for age and place of residence, as well as date of death for the deceased, with other men selected from the Swedish National Population Registry or from the National Registry for Causes of Death. Each living patient had 4 living controls; each dead man had 4 deceased controls. Exposure information was sought by the use of a mailed questionnaire that has never been published. It contained 130 questions, including 16 about the use of organic solvents, 4 about plastics, 3 about glues, 4 about drugs, "several" about smoking habits and an unstated number about exposure to phenoxy herbicides and chlorophenol as used in the lumber and paper mills. This questionnaire was mailed to the patient or to his next of kin if he were dead, 15.

When the answers to the questionnaire were less than clear, a supplementary interview was obtained, usually by telephone, with the interviewer unaware of the health status of the person in question. Employers, neighbors, and others were consulted "if necessary to verify and monitor the accuracy of the exposure information," 15.

Using the criteria for exposure established for the study, 36.5% of the 52 patients and 9.2% of the 208 controls had been exposed to phenoxy herbicides and/or chlorophenols. The "relative risk" of developing soft-tissue sarcomas was calculated as 5.7, i.e. men exposed to the chemicals had almost six times as great a change of developing a sarcoma as did those who were not exposed. The relative risk was 5.3 for the 46 men exposed to phenoxy herbicides alone, and 6.6 for the 40 men exposed only to chlorophenols. It was thought that confounding factors had an insignificant effect.

DOH 2120652

The authors concluded that "the investigation showed an increased risk for soft-tissue sarcomas" but "a specific evaluation of the effect of separate chemical substances was not possible," 14.

The study's methods have been criticized and doubts have been expressed about the 100% response rate to the questionnaire approach, 9. (Actually, 2 of 208 controls did not answer, 14.) The statistical approach was described as slightly misrepresented and a major criticism was leveled because of the possibility of "selective recall," the greater tendency for an ill person to remember a supposed "cause" for the illness than a well person would have to remember the same "causal" event.

The criticisms evoked several replies. Axelson defended the case-control design, the objectivity of obtaining exposure data retrospectively, and the statistical techniques, 6. He concluded that the use of interviews for determining exposure is justified, 7, and defended in principle the treatment of confounding factors, 16. Hardell recalculated the 1979 results and his subsequent papers to substantiate his earlier findings and performed a separate investigation in support of his confidence that "no substantial observational bias could exist in the studies," 15.

Remington, 10, expressed the view that "the findings of this particular investigation are suggestive" and that "a relative risk of 5.3 for exposure to phenoxyacetic acids must be taken seriously." However, "case-control studies are uniquely susceptible to hidden sources of bias" even when the investigators are "unusually careful" as they are in this "excellent investigation."

Hardell's group also undertook a second case-control study of identical design in southern Sweden which is more devoted to agriculture than to forestry, 17, 18. In this investigation each of 72 living and 38 dead patients was matched with two controls. Among the 110 cases, 22.7% reported exposure to phenoxy herbicides or chlorophenols and, among the 219 controls, 5.9% were so exposed. This gave a relative risk of 5.1 with matching and 4.7 when the matching was dissolved, i.e. when sorting by age was ignored during statistical calculations. The relative risk from exposure to phenoxy herbicides was calculated to be 6.8, and that from chlorophenols to be 3.3. Exposure to more than a dozen other noxious materials, e.g. asbestos, smoking, DDT, and lindane, were considered as possible confounding factors although none was found to be clearly associated with an increased risk by itself.

DOW 2120653

16539

The reports list the diagnoses of all 110 cases of soft-tissue sarcoma as: leiomyosarcoma, 33; malignant fibrous histiocytoma, 19; liposarcoma, 15; neurogenic sarcoma, 11; angiosarcoma, 9; myxofibrosarcoma, 7; fibrosarcoma, 5; dermatofibrosarcoma, 3; atypical fibroxanthoma, synovial sarcoma, sarcoma NOS, 2 each; Ewings's sarcoma (extraskeletal) and rhabdomyosarcoma, 1 each. No statement is made as to which of these tumors was found in the 25 cases with identified exposures and no histological diagnoses are reported for the northern Swedish series, 13, 14.

The authors of the southern Swedish study conclude that "exposure to phenoxy acids and chlorophenols might constitute a risk factor in the development of soft tissue sarcomas," 18. The investigation has been the subject of the same criticisms and refutations as the earlier study.

Remington concludes that "the results are consistent with the hypothesis that phenoxy acid exposure increases the risk of tumors of this type" but adds that "case-control methodology is intrinsically susceptible to subtle and unmeasurable biases."

Swedish Investigation of Lymphoma

In May, 1978, Hardell was prompted to a new study by a patient with a malignant histiocytic lymphoma and a history of "massive exposure to phenoxyacetic acids." All men admitted to the oncology department with this type of tumor during the first nine months of 1978 were asked about their occupation and possible chemical exposure. Of 17 patients, 14 reported an occupation consistent with exposure and 11 of them had had contact with phenoxy herbicides or chlorophenols ten or more years earlier, 19.

These observations led to a case-control study, the report of which in 1981, 21, differs considerably from that in 1980, 20. The earlier report was commented upon in manuscript form by various experts but the later version will be used here.

The investigation, in collaboration with Axelson, 20, included both Hodgkin's disease and non-Hodgkin lymphomas. The 169 cases consisted of 60 Hodgkin's disease patients (lymphocyte predominance, 20; nodular sclerosis, 3; mixed cellularity, 27; lymphocyte depletion, 10), 105 men with non-Hodgkin's lymphomas (follicular center cell (FCC) type, 53; non-FCC type, 52), and 4 individuals with unclassifiable lymphomas. Each case had two matched controls, 338 in all. Of the cases, 62 had died as had 124 of the controls.

DOH 2120654

Questionnaires and interviews were used to determine exposure to phenoxy herbicides, chlorophenols, organic solvents, or medicines and to characterize jobs, hobbies, and smoking as they were determined in the soft-tissue sarcoma investigations, 20. All cases and controls were from northern Sweden.

Cases in which exposure was reported to chlorophenol, or to "mutagenic" solvents (benzene, trichloroethylene, perchloroethylene and styrene) were divided into high-grade and low-grade exposure groups. Continuous exposure for a week or less or repeated exposures totaling less than a month were considered low-grade. Analyses also divided cases into two groups depending on whether 5 years had elapsed as a latency period between the first exposure to the chemical and the tumor diagnosis.

Of the cases, 36.1% had been exposed to phenoxy herbicides or chlorophenols; 9.6% of the controls had been so exposed. The relative risk for these exposures was 6.0 with matching and 5.3 without it. Phenoxy herbicides gave a relative risk of 4.8 although it was greater if exposure was for 90 days or more. Chlorophenols gave relative risks of 8.4 for high-grade exposure, 2.9 for low-grade. High- and low-grade exposure to organic solvents gave relative risks of 2.8 and 1.2 respectively. On the other hand the few cases with both phenoxy herbicide and high-grade organic solvent exposure was calculated to have a relative risk of 11.2 and some other combinations also gave large relative risks. The length of the latency period, however, seemed to have no effect.

The authors conclude that "this investigation suggests that exposure to organic solvents, chlorophenols, and/or phenoxy acids constitutes a risk factor for malignant lymphoma," 21. Dr. Remington commented that "a substantial and statistically significant relative risk is found for this group of tumors. And again, phenoxy acid exposure is specifically incriminated." He continues, however, that the limitations of case-control methods have to be considered as well.

Swedish Investigation of Carcinoma of the Colon

Hardell undertook to answer doubts that his questionnaire and interview methods allowed observational bias in assessing exposure by conducting a case-control study of "colon cancer." The condition is not suspected of having any association with phenoxy herbicides or chlorophenols. In consequence, if the previously used exposure determination

DOH 2120655

resulted in a relative risk of 1.0 or near it, there had been no observational bias in the questionnaire-interview procedure used in the earlier studies of soft-tissue sarcomas and lymphomas.

Of the 157 men with colon cancer all but 3 answered the questionnaire. The controls consisted of the control groups from the soft-tissue sarcoma study (206 men) and the malignant lymphoma study (335 men). In all, 41% of the cases and 45% of the controls were dead. Of the cases and controls, 11.0 and 10.4% respectively had been exposed to phenoxy herbicides or chlorophenols. For phenoxy herbicides, the relative risk was calculated to be 1.3 and for chlorophenol it was 1.8. Neither was significantly above 1.0. The conclusion was that "the previously reported associations between exposure to phenoxy acids or chlorophenols and soft-tissue sarcoma and malignant lymphoma cannot to any essential degree be explained by observational bias," 15.

Later Criticism of Swedish Studies

There remain, however, doubts about the practical significance of the Swedish epidemiological studies stemming from several of their characteristics. The main criticism is the reliance on recall of the men or their relatives, employers and associates for undramatic events years earlier as well as the possibility of unconscious bias on the part of the interviewer, the "observational bias" discussed above. Coggan and Acheson point out that the positive association between exposure to phenoxy herbicides and the development of several or many soft-tissue sarcomas, Hodgkin's disease and non-Hodgkin's lymphoma may indicate "a serious undetected bias" even though the explanation has been offered that all these tumors are embryologically related, 22. These authors conclude that "it is as yet impossible to estimate with any precision the risk of soft-tissue sarcoma due to phenoxy herbicides" but add that "there is suggestive evidence of a biological association between phenoxy herbicides (or their contaminants) and soft-tissue sarcomas." They feel that there is weaker evidence for an association between herbicides and lymphomas.

Hardell and Axelson reject the idea of observational bias, citing the colon cancer study as evidence, 23. They also defend the aggregation of tumors because of the "so-called addition theorum for chi-square and Poisson distributions" as well as the embryological relationship of the neoplastic tissues.

DOW 02120656

American Support for Swedish Conclusions

Support for the connection between soft-tissue sarcomas and exposure to phenoxy compounds has been reported in several papers from outside Sweden. The data most often cited as favoring the relationship are derived from observations in the American chemical industry.* The first was a note by Honchar and Halperin in which they pointed out that of 105 deaths in four exposed industrial "cohorts" 3 (2.9%) were due to soft-tissue sarcoma, whereas only 0.07% of deaths among adult American men are so caused. The three cases were malignant fibrous histiocytoma, fibrosarcoma, and liposarcoma. The authors felt that these "suggest a common pattern," 24. Cook added a fourth case, another malignant fibrous histiocytoma and noted that all four were smokers and two had chloracne, 25.

Moses and Selikoff reported a fifth case, a non-smoker, with neurogenic sarcoma (malignant schwannoma). They give the total annual incidence of soft-tissue sarcomas as 4500 (less than 1% of newly diagnosed cancers) in the U.S. and quote 4.9% of soft-tissue sarcomas as malignant schwannoma, 26.

Johnson and his co-workers briefly described a young man who died of fibrosarcomatous mesothelioma some four years after first being exposed to phenol. His father had a liposarcoma after "prolonged exposure" in a plant manufacturing chlorinated phenols among other chemicals, 27.

Hardell and Ericksson accepted the two additional cases to total 7 deaths from soft-tissue sarcoma among 105 deaths among American industrial workers, the expected number being 0.07%. This would "fit in with" the Swedish investigations, they believe, 28.

To date no critical review has been made of the cases and the industrial population in which they were detected. The reports have been brief "Letters to the Editor" and each discusses one to three cases. The total of 105 deaths used as the number of dead workers has not been kept current as new soft-tissue sarcoma cases were added and the total number

*Data given by Honchar and Halperin, Cook, Moses and Selikoff, and Johnson et al pertain to workers at Monsanto Company and Dow Chmical Company. For details of studies of these workers see 24a and 25a.

DOW 2120657

16543

of exposed workers has not been given. No use has been made of controls, even in the form of a retrospective cohort comparison.

A case report without statistical data briefly described three soft-tissue sarcomas among Vietnam veterans who reported exposure to phenoxy herbicides in that country. One man had an inflammatory histiocytoma, another suffered from a fibrosarcoma, and the third had a leiomyosarcoma, 29.

European Support for Swedish Conclusions

Barthel determined the frequency of malignant neoplasms among 1791 pesticide sprayers and agricultural technicians in East Germany during 1976 to 1979. He states the retrospective cohort study used police as controls but gives no data for them. After eliminating "on statistical grounds" 133 cases who died before 1970, he compared the mortality rate and cancer incidence with corresponding figures from the death statistics and the cancer registry of the Health and Social Welfare. The "case" group had multiple exposures over the years to fungicides, insecticides, and herbicides including phenocyacetic acids. Among 169 malignant neoplasms in 1658 exposed men were 1 lymphosarcoma, 3 plasmacytomas, 1 described as a malignancy of lymphoid tissue, and 1 of soft-tissue, not otherwise characterized. Bronchogenic carcinoma was the most common malignancy with 59 cases, double the expected occurrence, although the cases had smoking habits like those of the general population, 30. A brief report describes a case of non-Hodgkin's lymphoma and a second of malignant lymphoma among 158 workers with pentachlorophenol. This type of neoplasm would have an expected occurance of 0.28, 31.

Studies Not Supporting Swedish Conclusions

In contrast to the reports of an association between phenoxy herbicides or related compounds and soft-tissue sarcomas and malignant lymphomas, some investigators have found no association. Some of these investigated a possible relation, others were "follow-up" studies of industrial workers in whom no sarcomas or lymphomas were found.

Dr. Riikimaki and his collaborators have completed nine years of mortality study following 1,926 persons who worked with phenoxy herbicides in Finland during the 1955-1971 period. All had at least two weeks of exposure and a quarter of the men totalled eight weeks or more as of 1971. The mortality rates among the workers were compared with the national death rates. As of 1980, there had been 82 deaths

DOW 2120658

of exposed men as compared to 91 expected and, of these, 17 were cancer deaths with 18.4 expected. There were no cases of soft-tissue sarcoma nor of lymphomas although 0.1 and 0.8 would have been expected. The authors believe that "the investigation cannot be regarded as a conclusive negative study" but point out that the "results do not confirm the ... association between mixed herbicide exposures" and cancer risk, 32.

Hogstedt and Westerlund compared the mortality rate of Swedish supervisors and workers in forestry. The supervisors were fewer in number (142) than the workers (244) but the former were judged to have been more heavily exposed. The relative risk of death was about the expected but, after a 10-year latent period, the relative risk for cancer was about 4 for the supervisors and only about 0.4 for the workers. The fatal tumors were of various types but there was no soft-tissue sarcoma or lymphoma, 33.

Two case-control studies in New Zealand have been initiated by Smith et al to examine the association suggested by Swedish studies of phenoxy herbicides with soft-tissue sarcomas and malignant lymphomas. In the first investigation, 102 cases of soft-tissue sarcoma have been identified in men from the New Zealand Cancer Registry between 1976 anmd 1980. An equal number of matched controls with other forms of cancer were selected for comparison. The sarcomas are fibrosarcomas, 25; liposarcomas, 20; rhabdomyosarcomas, 9; leiomyosarcomas, 7; malignant histiocytomas, 6; other types, 22; and unspecified, 13. The preliminary report compares cases and controls as to the occupation shown on the Registry enrollment. There was no significant difference betwen the groups as to the number of men working in agriculture, forestry, and fishing, the occupations with the greatest likelihood of exposure to phenoxy herbicides and chlorophenols. The only occupations associated with soft-tissue sarcomas exclusively are blacksmiths, machine tool operators, electrical fitters, and electrical workers. The investigators are now obtaining work histories for cases and controls by telephone interviews and warn that later results may change their conclusions. The data at present "do not give evidence for a relationship (of soft-tissue sarcoma) with occupational exposure to phenoxy herbicides and chlorophenols" but "should not be taken as substantive evidence against the hypothesis", 34.

A second report by Smith et al includes the results of the telephone interviews regarding 80 cases and 92 controls already completed. Probable or definite exposure to phenoxy herbicides for more than one day earlier than five years before cancer registration was found in 17 cases and 13 con-

DOW 2120659

trols, giving an odds ratio of 1.6. This would be expected to increase when the exposure criteria were more stringent but, when exposure was at least five days and more than ten years before registration, there were 13 cases and 12 controls included reducing odds ratio of 1.3. Neither ratio is statistically significant and there have been no soft-tissue sarcomas reported among the most highly exposed group of 2000 aerial and ground sprayers. The results, the authors believe, "do not generally support the hypothesis that exposure to phenoxy acid herbicides cause soft-tissue sarcoma," 35.

A brief initial report by Edling and Granstam compared the causes of death for 375 Swedish forestry workers, aged 25 to 69 years, who died during 1968 to 1977, with the mortality figures from the Swedish national statistics. There were 75 deaths from all malignant tumors, as compared to 86 expected. Renal tumors killed 8 with 3.84 expected and "tumors of lymphatic and hematopoetic systems" were responsible for 14 deaths with 7.5 expected. No deaths were attributed to soft-tissue sarcoma, 36.

In addition to these studies, several small industrial groups have been followed well into the latent period for solid tumors. None has been reported to include cases of soft-tissue sarcoma or malignant lymphoma. May examined 41 of 79 workers who developed chloracne following accidental exposure to trichlorophenol in 1968 at the Coalite Company in Great Britain. Another 54 employees were possibly exposed. None of the workers had significant changes ten years after the accident and neither death from nor evidence of neoplasm was found, 37. Jirasek's group has closely followed 55 men who were intensely exposed during the manufacture of 2,4,5-trichlorophenoxyacetate from 1965 to 1968 in Spolana, Czechoslovakia, and who developed evidence of acute intoxication. Two workers died of bronchogenic carcinoma 5 to 5.5 years after the first exposure. There was no other evidence of malignant neoplasms during a ten-year follow-up, 38.

In 1963 an explosion at Philips-Duphar, Amsterdam, exposed 106 workers involved in manufacturing 2,4,5-tetrachlorophenyoxy acewtate. Among the 93 workers followed to 1977, only one death 14 months after the accident was due to cancer and the pancreatic carcinoma involved was apparently symtomatic before the explosion. No case of soft-tissue sarcoma or malignant lymphoma was reported, 39.

One study is often cited with the Swedish studies although it did not deal with soft-tissue sarcomas and malignant lymphomas, 40. A more recent review by Thiess et al reports that all 74 exposed persons are still being followed

DOW-2120660

after 26 years. There have been 21 deaths, about equal to the 18 to 20 deaths expected from major comparative populations and 18 and 19 deaths expected among matched unexposed controls. Cancer was responsible for 7 deaths as compared to 4.1 expected from the comparative populations and 5 in each internal control group. Gastric carcinoma in 3 exposed persons exceeds the expected 0.61 to 0.70 expected cases. There were, however, no soft-tissue sarcomas or malignant lymphomas among these chemical workers at BASF, 41.

A number of other industrial exposures to phenoxy herbicides, their precursors or contaminants were reported before 1973, 42. The populations were small but generally heavily exposed. Unfortunately it has not been possible to locate late reports on the exposed populations although ten years or more have elapsed since exposure.

The accident at the ICMESA factory in Seveso, Italy, in July 1976 exposed many people to trichlorphenol; more than 5400 adults and children of both sexes are known to have been in contact with the chemicals for several days, 43. Although only about six years have elapsed since the exposure, the population has been under surveillance and the rate and causes of death are being followed. To date no soft-tissue sarcomas or malignant lymphomas have been reported.

Another less systematic observation bears on the situation. The phenoxy herbicides have been used frequently and extensively in agriculture and forestry in the United States since the late 1940's. They were used on lawns in cities, as well, for most of that period. If the relative risk of developing so distinctive a group of tumors as the soft-tissue sarcomas and the malignant lymphomas had increased by 5 of 6 fold over that before 1945 as the Swedish studies would predict, it almost certainly would have been evident to clinicians and pathologists, especially in the rural areas, even without systematic studies. No such increase was noted.

Critical Evaluations

The Swedish investigators have been cautious in interpreting their results. In his medical dissertation based on his epidemiological studies, Hardell judges that the similar results in the two case-control investigations (12, 13, 14, 16) "seem to increase the confidence that the observed association of exposure to phenoxy acids and soft-tissue sarcoma was not spurious" and did not believe that confounding factors "could account for the observed relation." In summary, he concluded that "it is suggested that exposure to phenoxy

DOH 2120661

16547

acids should be looked upon as an occupational cancer hazard," 44.

Other reviewers have been more skeptical as to the significance of the work. Remington's overall opinion was that "in toto, the Swedish work is credible if not fully conclusive. Certainly this work would seem to justify further investigation," 10. Coggan and Acheson, after reviewing other work as well as the Swedish studies, state that "on the present evidence it seems possible that soft-tissue sarcomas have arisen in association with exposure to phenoxy herbicides" but continue that "it is as yet impossible to estimate with any precision the risk of soft-tissue sarcoma due to phenoxy herbicides." They conclude that "there is suggestive evidence of a biological association between phenoxy herbicides (or their contaminants) and soft-tissue sarcoma. The evidence relating these products to the occurance of lymphoma is weaker," 22. An unsigned editorial in Lancet commenting on the opinions of Coggan and Acheson seems to agree with their conclusions with regard to soft-tissue sarcomas, 45.

Hardell and Axelson disagreed with both the Coggan and Acheson's opinions and the Lancet editorial, 23. They have been at some pains to counter charges of "observational bias," 15, but have not convinced everyone that faulty memories do not result in significant errors in evaluating exposure, 45.

The causal connection between phenoxy herbicides and soft-tissue sarcomas would be much more likely if there were a unique preponderance of one type or even of a few types in the exposed men. The Swedish reports never compare the morphological types or location of the malignant tumors in cases with those in controls, 45. Their only justification for aggregating the types, and presumably for omitting the data from their reports is "the uncertainty of relations between the various histological groups in terms of causal mechanisms" and "the so-called addition theorem for chi-square and Poisson distribution," 23. The uncertainty of causal relations is precisely the reason for reporting the groups and the addition theorem cannot justify the aggregation of unlike

*2,3,7,8-tetrachlorodibenzo-p-dioxin has been suggested as the principal carcinogen in the phenoxy herbicide 2,4,5-T and trichlorophenols but this has been disputed. See 22, 23, 45, 46. The controversy is not considered in this discussion.

DOH 2120692

16548

entities unless significant common factors have been demonstrated.

Scientific results are strengthened greatly when independent investigators substantiate them. The Swedish studies have been said to be independent and confirmatory. The two soft-tissue sarcoma investigations do support one another (12, 13, 14, 16) but they are the work of the same group of investigators. The investigation of malignant histiocytic lymphoma was also conducted by the same group but was a case-control study of a separate entity, (19-21). Axelson's work on herbicide exposure and cancer (1-4) was not truly independent from Hardell's efforts since Hardell has recognized his indebtedness to Axelson for his assistance in the first case-control study, (13, 14). More important Axelson did not associate phenoxy herbicides or chlorophenolic compounds with soft-tissue sarcomas nor with malignant lymphomas among railroad workers, 1-4.

The reports of soft-tissue sarcomas among chlorphenol workers in the United States (24-27) have been cited as supporting Hardell's conclusions, 24, 28, 44, 46. The data have been reported piecemeal without a clearly enumerated total population from which they were drawn. The comparison was made to mortality data for the general population of the appropriate age and sex. The type of soft-tissue sarcoma is known for each case; among the 7 men were 2 malignant fibrous histiocytomas, 2 liposarcomas, as well as one each of fibrosarcoma, malignant schwannoma, and fibrosarcomatous mesothelioma. As before, the tumors are not of a uniform type.

Coggan and Acheson comment that the Swedish studies and the American reports taken separately do not "provide convincing evidence that the incidence of soft-tissue sarcomas is increased after exposure to phenoxy acids and chlorophenols, -- Considered together the whole becomes more persuasive." They add that "it is surprising that the association should apply to tumors of such a variety of tissues," 22. The Lancet editorial finds only that "the number of deaths due to soft-tissue sarcomas [in the American data] is disturbing;" 45.

In addition to the American experience, the British (37), European (30, 32, 33, 36, 38-41) and New Zealand (34, 35) medical and scientific writers have studied populations five years or longer after exposure to phenoxy herbicides and/or chlorophenols in a variety of situations, some intense and acute, others prolonged. Only one observer (30) reported a case described as a soft-tissue malignant neoplasm without

DOW 02206963

further characterization. The same report included a lymphosarcoma, a malignant neoplasm of lymphoid tissue and 3 plasmocytomas. No other study found a soft-tissue tumor.

In summary, the Swedish studies of soft-tissue sarcomas cannot be considered to have proved that exposure to phenoxy herbicides is the cause of one or more types of this varied group of malignant tumors. There are no fully reported systematic studies to confirm what the Swedish investigators describe as an association. There are an epidemiological study (32) and observations of exposed populations that do not support the finding as opposed to uncorrelated American observations and an East German study (30) that do strengthen the case for such an association.

At best, the Scottish verdict of "Not proven" seems most realistic at this time. The Advisory Panel on Toxic Substances of the American Medical Association says that "while 2,4,5-T and 2,4-D pesticides (phenoxy herbicides in Agent Orange) have been used in agriculture, forest management and residential landscaping for over 30 years, there is still no conclusive evidence that they and/or TCDD (a contaminant of Agent Orange) are mutagenic, carcinogenic, or teratogenic in man, nor that they have caused reproductive difficulties in the human," 47.

L. B. HOBSON 3/5/83

DOW 2120664

BIBLIOGRAPHY

1. Sundell, L., Rehn, M., and Axelson, O. Exposure to herbicides - mortality and tumor incidence. An epidemiological investigation in Swedish railway workers. Lakartidningen 71:2466-2470. 1974

2. Axelson, O. and Sundell, L. Herbicide exposure, mortality, and tumor incidence. An epidemiological investigation on Swedish railroad workers. Work Environ. Health 11:21-28. 1974

3. Axelson, O. and Sundell, L. Phenoxy acids and cancer. Lakartidningen 74:2887-88. 1977

4. Axelson, O., Sundell, L., Andersson, K., Edling, C., Hogstedt, C., and Kling, H. Herbicide exposure and tumor mortality. An updated epidemiological investigation on Swedish railroad workers. Scand. G. Work Envirn. & Health 6:73-79. 1980

5. Axelson, O. Aspects on confounding in occupational health epidemiology (Letter.) Scand. J. Work Environ. & Health 4:85-89. 1978

6. Axelson, O. Views on criticism of pesticide studies (Reply to note by Jannerfeldt, E.). Lakartidningen 77(12):1096-1099. 1980

7. Axelson, O. A note on observational bias in case-referent studies in occupational health epidemiology. Scand. J. Work Environ. & Health 6:80-82. 1980

8. Axelson, O. Testimony before EPA Hearing, EPA Exhibit No. 587. 1980

9. Jannerfeldt, E. Epidemiologic methodology and pesticide studies. Lakartidningen 77(12): 1096. 1980

10. Remington, R. D. Specific summary critique of five investigations related to concerns about Agent Orange. Congressional Record, pp. S 10911, S 10912, August 6, 1980.

11. Hardell, L. Verbal testimony at EPA Hearing. September 29, 1980.

DOW 212065

16551

12. Hardell, L. Malignant mesenchymal tumors and exposure to phenoxy acids - A clinical observation. Lakartidningen 74(33):2753-2754. 1977

13. Hardell, L. and Sandstrom, A. Malignant mesenchymal soft tissue tumors and exposure to phenoxy acids or chloramphenacol. Lakartidningen 75:3535-3536. 1978

14. Hardell, L. and Sandstrom, A. Case-control study: Soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Brit. G. Cancer 39:711. 1979

15. Hardell, L. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand. G. Work Environ. & Health 7(119-130). 1981

16. Axelson, O. Aspects of confounding and effect modification in the assessment of occupational cancer risk. J. Toxicol. & Environ. Health 6(5-6):1127-1131. 1980

17. Eriksson, M., Hardell, L., Berg, N. O., Moller, T. and Alexson, O. Case-control study of malignant mesenchymal tumors of the soft tissue and exposure to chemical substances. Lakartidningen 76:3872-3875. 1979

18. Eriksson, M., Hardell, L., Berg, N. O., Moller, T., and Axelson, O. Soft-tissue sarcomas and exposure to chemical substances: a case-referant study. Brit. J. Industr. Med. 38:27-33. 1981

19. Hardell, L. Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet 1(8106):55-56. 1979

20. Hardell, L., Erikson, M., Lenner, P. Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols and phenoxy acids. Lakartidningen 77(4):208-210. 1980

21. Hardell, L., Erikson, M., Lenner P., and Lundgren, E. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Brit. J. Cancer 43:169. 1981

DOH 2120666

22. Coggon, D. and Acheson, E. D. Do phenoxy herbicides cause cancer in man? Lancet 1(8278)1057-1059. 1982

23. Hardell, L. and Axelson, O. Soft-tissue sarcoma, malignant lymphoma, and exposure to phenoxy acids on chlorophenols. Lancet 1(8286):1408-1409. 1982

24. Honchar, P. A. and Halperin, W. E. 2,4,5-T, trichlorophenol, and soft-tissue sarcoma. Lancet 1(8214):268-269. 1981

24a. Ott, M. G., Holder, B. B., and Olson, R. D. A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J. Occup. Med. 22:47-50. 1980

24b. Cook, kR. R., Townsend, J. C., Ott, M. G., and Silverstein, L. G. Mortality experience of employees exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Occup. Med. 22:530-532. 1980

25. Cook, R. R. Dioxin, chloracne, and soft-tissue sarcoma. Lancet 1(8220)618-619. 1981

25a. Zack, J. A. and Suskind, R. R. The mortality experience of workers exposed to tetrachlorodibenzo-dioxin in a trichlorophenol process accident. J. Occup. Med. 22:11-44. 1980

25b. Zack, J. A. A mortality study of workers employed at the Monsant Plant in Nitro, W.Va. Unpublished.

26. Moses, M. and Selikoff, I. J. Soft-tissue sarcomas, phenoxy herbicides, and chlorinated phenols. Lancet 1(8234):1370. 1981

27. Johnson, F. E., Kugler, M. A., and Brown, S. M. Soft tissue sarcomas and chlorinated phenols. Lancet 2(8236):40. 1981

28. Hardell, L. and Erikson, M. Soft-tissue sarcomas, phenoxy herbicides and chlorinated phenols. Lancet 2(8240):250. 1981

29. Sarma, P. R. and Jacobs, G. Thoracic soft-tissue sarcoma in Vietnam veterans exposed to Agent Orange. New Engl. J. Med. 306(18):1109. 1981

DOW 2120667

30. Barthel, E. Cancer risk in agricultural workers exposed to pesticides. Arch. Geschwulstforsch. 51(7):579-585. 1981

31. Bishop, C. M. and Jones, A. H. Non-Hodgkin's lymphoma of the scalp in workers exposed to dioxin. Lancet 2(8242):369. 1981

32. Riikimaki, V., Asp, S., and Hernberg, S. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland. Scand. J. Work Environ. & Health 8:37-42. 1982

33. Hogstedt, C. and Westerlund, B. Cohort study of mortality rates of forestry workers with and without exposure to phenoxy compounds. (No translation available) Lakartidningen 77(19):1828-1830. 1980

34. Smith, A. H., Fisher, D. O., Pearce, N. and Teague, C. A. Do agricultural chemicals cause soft-tissue sarcoma? Initial findings of a case-control study in New Zealand. Unpublished report at ANZSEARCH Annual Conference, May, 1982

35. Smith, A. H., Fisher, D. O., Giles, H. J., and Pearce, N. The New Zealand soft-tissue sarcoma case-control study: interview findings concerning phenoxyacetic acid exposure. Unpublished report at Third International Symposium on Chlorinated Dioxins and Related Compounds. October 1982

36. Edling, C. and Granstam, S. Pattern of causes of death among forestry workers, a pilot study. Hygiea 88(3):74. 1979

37. May, G. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. Brit. J. Industr. Med. 39:128-135. 1982

38. Pazderova-Vejlupkova, J., Nemcova, N., Pickova, J., Lukas, E., and Jirasek, L. The development and prognosis of chronic intoxication by tetrachlorodibenzo-dioxin in man. Arch. Environ. Health 36(1):5-11. 1981

DOH 2120668

39. Cited in Huff, J. E., Moore, J. A., Saracci, R. and Tomatis, L. Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ. Health Prespectives 36:221-240. 1980; and Hay, A. Accidents in trichlorophenol plants: a need for realistic surveys to ascertain risks to health. Ann. N.Y. Acad. Sci. 320:324. 1979

40. Thiess, A. M. and Frentzel-Beyme, R. Mortality study of persons exposed to dioxin following an accident which occurred in the BASF on November 13, 1953. Presented at the Fifth International Conference on Medichem. September 1977

41. Thiess, A. M., Frentzel-Beyme, R., and Link, R. Mortality study of persons exposed to dioxins in a trichlorophenol process accident that occurred in the BASF AG on November 17, 1953. Am. J. Industr. Med. 3:179-189. 1982

42. Young, A. L., Calcagni, J. A., Thalken, C. E., and Tremblay, J. W. The toxicology, environmental fate, and human risk of Herbicide Orange and its associated dioxin. USAF OEHL Technical Report (Report OEHL TR 78-92) pp. V-7, V-8

43. Caramaschi, F., del Corna, G., Favaretti, C., Giambelluca, S. E., Montesarchio, E., and Fara, G. M. Chloracne following environmental contamination by TCDD in Seveso, Italy. Intern. J. Epidemiol. 10(2):135-143. 1981

44. Hardell, L. Epidemiological studies on soft-tissue sarcoma and malignant lymphoma and their relation to phenoxy acid or chlorophenol exposure. Umea University Medical Dissertations, New Series No. 65. 1981

45. Anonymous. Phenoxy herbicides, trichlorophenols, and soft-tissue sarcomas (Editorial). Lancet 1(8278):1051-1052. 1982

46. Hardell, L. and Axelson, O. Phenoxy acids, chlorophenols, and cancer. Lakartidningen 78(34):2862-2863. 1981

47. Council on Scientific Affairs' Advisory Panel on Toxic Substances (American Medical Association). The health effects of "Agent Orange" and polychlorinated dioxin contaminants. October 1, 1981

DOW 02120669

*DIOXIN AND HUMAN HEALTH

During the past decade, considerable controversy has developed over the human health effects of exposure to TCDD, an unintentional contaminant from the production of some herbicides and antibacterial agents and a substance created by the process of combustion.

This controversy has been fueled by reports on the effects of Agent Orange exposure on returning Vietnam veterans, the Love Canal situation, the explosion of a 2,4,5-trichlorphenol plant in Seveso, Italy, and, most recently, the buy-out by the U.S. government of Times Beach, Missouri, a town contaminated with dioxin, (i.e., 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]).

During this time period, much speculation and misinformation regarding this issue have been generated, with resulting confusion and apprehension in the public mind. It is the purpose of this paper to state the facts regarding TCDD and human health and to place the issue in its proper perspective.

This paper will document the following key points:

- Certainly 2,3,7,8-TCDD is a potent and toxic compound that can cause a variety of ill health effects at relatively low doses in laboratory animals. We should be concerned with reducing any unreasonable risk of human TCDD exposure in our environment. Based on the preponderance of scientific studies, the trace environmental presence of TCDD does not pose a health hazard to people.

- There is a wealth of epidemiological data supporting the position that humans are far less susceptible to TCDD than several of the animal species which have been studied.

- The mere presence of TCDD in the environment (soil, water, fish, etc.) is not proof of contamination from industrial chemical sources.

- The scientific evidence does not demonstrate that TCDD exposure causes cancer in humans.

- There is no valid epidemiologic evidence linking TCDD exposure in humans with a higher-than-normal rate of birth defects.

DOW 2113892

---

*2,3,7,8-tetrachlorodibenzo-p-dioxin

TABLE OF CONTENTS

What is Dioxin? 2

The American Medical Association Report 4

Seveso. 7

Epidemiological Studies 9

Soft Tissue Sarcoma 10

The Dow Mortality Study 17

Birth Defects 19

The Toxicity of TCDD in Animal Species. 21

Environmental Hazard Evaluation (Soil/Fish) 24

References. 28

DOW 21138893

What is Dioxin?

The term "dioxins" (or more scientifically, "chlorinated dibenzo-p-dioxins") refers to 75 similar, but different substances.[1]

Each of the 75 dioxins that has been studied has its own unique identity and toxicological properties.

On the basis of animal studies, 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) is considered to be the most toxic of the dioxins, and it has been comprehensively examined for its toxicological effects. This report will generally address the health impact of TCDD exposure.

TCDD is a highly toxic compound that can cause a variety of ill health effects at relatively low doses in laboratory animals. We must be concerned with establishing safe levels of human TCDD exposure in our environment.

TCDD is formed as a trace unwanted contaminant in the production of 2,4,5-trichlorophenol from tetrachlorobenzene. The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), other trichlorophenoxyacids, and the germicide hexachlorophene are synthesized from 2,4,5-trichlorophenol and may contain trace amounts of TCDD and other polychlorinated dioxins.[2]

Dow has not produced the herbicide 2,4,5-T in the United States since 1979 due to reduced market demand from an Environmental Protection

DOW 2113894

Agency (EPA) emergency suspension which restricted some applications of the product. There are no manufacturers currently producing 2,4,5-T in the United States. A Dow joint venture company produces 2,4,5-T in New Zealand. Dow currently supplies 2,4,5-T from inventories for the rice and rangeland applications presently approved by EPA. The trace amount of TCDD present in Dow-inventoried 2,4,5-T is less than 10 parts per billion.

TCDD can also be created by combustion. Data indicate that chlorinated dioxins may arise from the combustion of most types of organic material, and suggest that chlorinated dioxins result from trace chemical reactions occurring in fire.[3]

Many chemical reactions occur during combustion at very low concentrations -- parts per billion and lower. With more advanced analytical capabilities, scientists have discovered numerous sources where various dioxins have been formed, including TCDD.

Some of these sources include refuse incinerators, wood-burning stoves, gasoline- and diesel-powered automobiles and trucks, fireplaces, charcoal grills and cigarettes. TCDD and the other dioxin compounds have been entering the atmosphere via airborne particulates from these various sources and settling on soil and in bodies of water.

There have been a number of industrial incidents throughout the world where people have been exposed to TCDD. Epidemiological studies have

DOW 2113895

evaluated these incidents and all of the evidence so far tends to reduce the initial concern about the health effects of TCDD on humans. An overall look at the mounting data has been undertaken by the American Medical Association. These findings and conclusions are of interest and bear mention.

The American Medical Association Report

In 1981 the American Medical Association (AMA) published a technical report reviewing the medical evidence regarding the toxicity and long-term health effects of the TCDD contaminant in Agent Orange*.

The report's preface stated:

> "In spite of the voluminous data...there is still very little substantive evidence for many of the alleged claims that have been made against these compounds (dioxins). The most serious of these allegations assert that Agent Orange, or compounds of a like nature, have caused malignant tumors, spontaneous abortions and birth defects. Although data from studies on experimental animals tend to support some of these claims, it is not certain that the animal data are extrapolatable to man. No laboratory animal can fully substitute for man; we must, therefore, depend on the results of ongoing epidemiological studies on persons who are known to have been exposed."

*(Agent Orange was a 50/50 herbicide mixture of 2,4,5-T and 2,4-D produced according to specifications set by the U.S. government for use as a defoliant in Vietnam to protect American troops from enemy ambush.)

D04 2113896

A close look at the summary of the report gives a clearer insight into what the AMA's Advisory Panel on Toxic Substances found.

The AMA Panel reported that, in addition to the marked variations in the sensitivity and susceptibility of animal species to all toxic substances, there are significant differences between some of the toxic effects of TCDD in experimental animals and the human experience. People appear to be less sensitive to the health effects of TCDD than some of the animal species studied.

One of the more pronounced biological effects of heavy exposure to TCDD, as well as a number of other chlorinated aromatic compounds, is a tendency to cause chloracne in certain animals and humans, the AMA Panel reported. This particular skin condition is regarded as the clinical marker for TCDD over-exposure in people. The Panel noted that human systemic disorders from exposures to TCDD are unlikely to occur in the absence of chloracne. Such findings as impaired liver and kidney function, gastrointestinal irritation, muscle and nerve disorders, and depression and irritation of the central nervous system have been reported after exposure to relatively large amounts of TCDD. However, these disorders have not been progressive, and they have disappeared with time, the AMA report stated. In short, if there is no chloracne, there are unlikely to be other persistent toxic effects from exposure to TCDD.

DOW 2113897

The AMA Panel also reported that other toxic effects of TCDD, when experimentally produced in test animals, appear as pathological changes in the liver, lymphoid and epithelial tissue.

TCDD is a powerful enzyme inducer. In addition to altering normal enzyme activity, it may potentiate or inhibit the action of other toxins.

The AMA report stated that TCDD may act as a promoter of carcinogenesis in some strains of rats and mice. For cancer to start, it must go through several distinct steps. The first stage, called initiation, is produced by a carcinogen (an initiator) and probably involves irreversible mutational changes. Subsequent growth and development of an actual cancer may require promotion, which may be the result of additional applications of a carcinogen, or of other, non-carcinogenic materials. Promotion may involve additional unknown processes which could aid the growth of the cancer or reduce resistance to the developing cancer.

TCDD is among the group of chemicals that may secondarily influence the formation of cancer by promotion. The carcinogenicity associated with TCDD in laboratory animals is typically accompanied by other signs of systemic toxicity in contrast to some other chemical carcinogens for which cancer is the only observable toxic effect.

TCDD has reportedly induced genetic changes in some forms of bacteria, but evaluations in rodents and humans do not indicate that these mutational changes will occur in higher animals, let alone people.

The AMA Panel made clear in its summary that the extensive information collected for more than 30 years provides no conclusive evidence that

DOE 2113898

2,4,5-T and/or TCDD are mutagenic, carcinogenic or teratogenic in humans, nor that they have caused reproductive difficulties in people.

Furthermore, the AMA report stated that 2,4,5-T undergoes relatively rapid decomposition in the soil. However, TCDD does persist in soil longer than 2,4,5-T, but, in general, the half-life of TCDD in soil may be no longer than one year. TCDD exposed to ultraviolet light is broken down rapidly when present as a thin film on plants, water and the surface of soil.[4]

Seveso

The most well-known and widely studied incident of community exposure to TCDD followed the July 10, 1976, explosion at the ICMESA trichlorophenol plant at Seveso, Italy. As a result of that accident, approximately seven square miles of the countryside were contaminated by chemicals, including TCDD, exposing as many as 30,000 people.[5,6] The heaviest contamination involved an area of 180 acres (Zone A), occupied by 736 people. TCDD was reportedly detected on soil at levels in excess of 5.5 parts per million (ppm).[7,5] In general, little effect was noted on plant life, but wild animals did die, particularly in Zone A (nearest the chemical plant). About four percent of the domestic animals in contaminated zones died, and virtually all of these were small animals.[5] Some of these deaths may have been due to other chemicals released at the same time.

DOW 211389

Chloracne, which has generally been regarded as the hallmark or sentinel sign of TCDD exposure[8], was observed in this population, although at generally low rates and primarily in children.[9,10,5,6] The highest incidence of chloracne reported was about 13-14 percent among elementary school children in Seveso, and this was generally mild and rapidly resolved in most affected people.[5,6,11] Other than chloracne (of which there were ultimately 100-200 cases), nausea, vomiting, itching and headache were the most commonly observed symptoms[6] readily attributable to the exposure which may have involved chemicals other than TCDD. Headache, stomach and intestinal upset were more commonly noted in individuals with chloracne.[9,6]

Bisanti[12] concluded that no "major event" had taken place with respect to birth defects, miscarriages (spontaneous abortions), births and deaths. While this seems to be the consensus, changes in birth defect reporting and in the reporting of "spontaneous" (sometimes "therapeutic") abortions have obscured any small changes which might have occurred.[13,5,6,14,11] Data on reproductive outcome are limited in number, but Reggiani[11] reports that examination of fetuses in 34 cases of medically-induced abortion revealed no evidence of injury attributable to TCDD. Pregnancy outcome in the years following 1976 appears to be comparable with Western experience.[6]

Of particular interest is the fact that no birth defects were observed during 1977 among the 70 births in Zones A and B. Only those pregnancies coming to term after January 1977 would have been relevant, i.e.,

DOW 2113900

those in which July 1976 maternal exposure would have taken place during the critical period for the developing fetus.

Many factors are known to cause birth defects in humans, which occur in three to six percent of live births: viral infections, genetic predisposition, smoking, alcohol, and even medications, including excess quantities of certain vitamins.

Epidemiological Studies

In recent years a number of investigations have been conducted concerning the human health effects associated with exposures to TCDD.

The following brief review of the human exposure experience with TCDD and the epidemiological studies of those people are taken from a recent report by the Advisory Committee on Pesticides, which is charged with making recommendations concerning the safety and safe use of pesticides in the United Kingdom.

- Monsanto's plant at Nitro, West Virginia: 121 workers were followed up for 30 years after exposure to TCDD. No apparent excess in total mortality or in deaths from cancer or diseases of the circulatory system was observed.[15]
- Coalite plant (1968) in the United Kingdom: Chloracne was initially seen in 79 workers and some had initial abnormalities in the liver but these returned to normal limits within 10 days. No clinically recognizable disease has been demonstrated. There is no evidence of liver or cardiovascular disease.[15]

DOW 2113901

- Dow Chemical Company workers exposed to 2,4,5-T: No adverse effects have been noted in an epidemiological study in these workers (mortality was actually less than expected).[15]

- Vietnam: Exposure to Agent Orange is blamed for a host of illnesses. The U.S. Veterans Administration found that no cause-effect relationship was established between TCDD exposure and alleged medical complications.[15]

- Finnish railroad workers using 2,4,5-T and other phenoxyacetic acid herbicides in forestry and on roads and railways: 1,960 men were studied and no increased mortality from cancer was demonstrated. There were no deaths from cancer in forestry workers although six deaths would have been statistically predicted.[15]

Soft Tissue Sarcoma

The most persistent hypothesis deals with the possible causal association between TCDD and soft tissue sarcomas. This hypothesis is derived from the epidemiological work done by Dr. Hardell and his associates in Sweden.[16]

Soft tissue sarcoma is a generic term for a group of lesions that include more than 100 different types of tumors.[17] These tumors are relatively rare; thus few pathologists have been able to achieve a high degree of consistency in their diagnosis.[18] Most experts believe that if TCDD were acting as a carcinogen it would cause an increase in one type of soft tissue sarcoma, but would be unlikely to raise the risk all across the board. One of the reasons vinyl chloride is accepted as a human carcinogen is the specific way it acts. At high levels, it produces a single type of cancer: angiosarcoma of the liver.

DOW 2113902

The question which arises is: If there is a common exposure link between various reports of soft tissue sarcomas in occupational groups, is TCDD the link?

The exposure documentation differs from study to study. In some, based on detailed technical knowledge of the chemical process, analyses of reaction contaminants, and clinical evidence of excessive group exposure (such as chloracne), there is evidence that TCDD is at least one of the common exposure links. In other reports, the evidence is more presumptive, and in some cases it is totally lacking.

Epidemiologists from Dow Chemical and Monsanto have reported on the soft tissue sarcoma cases occurring in their occupational groups presumably exposed to high levels of 2,3,7,8-TCDD.[19,20,21]

In 1964, a group of Dow trichlorophenol production workers experienced an outbreak of chloracne. Sixty-one workers were identified as being involved with the trichlorophenol process. Forty-nine of these workers developed a rash diagnosed as chloracne. Epidemiologists followed up on the status of all 61 men through 1978, 14 years after the incident. Statistically, 7.8 deaths would have been expected in this group, but only four had occurred. Among the causes of death, one was from cardiovascular disease when 3.8 would have been expected. Cancer was identified as the cause of three deaths when 1.6 were expected. None of these findings represent a statistically significant deviation from the expected experience.[20] One of the cancer deaths in the Dow group was

DOW 2113903

due to a type of soft tissue sarcoma called fibrosarcoma. In 1981 another employee in the Dow trichlorophenol worker group developed another type of soft tissue sarcoma, malignant fibrous histiocytoma, one of a multitude of tumors which comprise the soft tissue sarcoma category. The employee died in 1983.

In the 1978 Monsanto study, no link between exposure to TCDD and excess deaths due to cancer or other causes was found among 121 current and former workers employed at a plant in Nitro, West Virginia. The employees who participated in the study included workers who developed chloracne as a result of over-exposure to TCDD in a manufacturing accident in 1949.

Among the Monsanto workers studied, 32 deaths were reported versus 46 which would be expected. Cancer accounted for nine deaths among the Monsanto group compared with slightly more than nine deaths expected. One of these cancer deaths was attributed to soft tissue sarcoma, a malignant fibrous histiocytoma.

DOW 2113904

A second Monsanto study evaluated the mortality experience of male hourly workers employed at the Nitro plant during or after 1955 and followed through 1977.[22] When adjustments were made for prior exposures to a known bladder carcinogen, the observed deaths due to all causes and due to cancer were less than expected. Among the subset of employees who had worked in 2,4,5-T production, the proportion of deaths due to cancer was lower than that found in the rest of the plant population. In this subset one individual died as a result of a soft tissue sarcoma, a liposarcoma.

16568

These four soft tissue sarcoma cases were summarized in the literature in 1981.[23,24] All four had occupational exposures to trichlorophenol. Only one, the second Monsanto case, had also been exposed to 2,4,5-T. Three had frank chloracne and the fourth, a Dow employee, a facial rash during his assignment in the trichlorophenol plant. All four had a history of cigarette smoking. It was suggested that the hypothesis of soft tissue sarcomas being caused by heavy TCDD exposure among cigarette smokers needed to be tested.

Three other cases of soft tissue sarcoma were also reported in 1981.[25,26] Exposure to 2,4,5-T, trichlorophenol or TCDD was not well documented. In one case, the individual was considered unexposed until his disease was discovered. The other two occurred in a father and son.

In a group of Dow 2,4,5-T employees (none of whom had chloracne)[27] there have been no cases of soft tissue sarcoma.

At Dow, the mortality experience of 204 persons exposed to 2,4,5-T during its manufacture from 1950 to 1971 was studied through 1978. No adverse effects were observed with respect to occupational exposure to either 2,4,5-T or its feedstock, 2,4,5-trichlorophenol. Mortality of the studied workers was favorable compared to that of U.S. white males.

There have been no cases of soft tissue sarcoma reported in a BASF worker group, many of whom experienced severe chloracne. Seventy-four

DOW 2113905

members of the BASF group were exposed to TCDD during a 1953 accident in a trichlorophenol production unit or during cleanup and repair following the accident. Exposures were heavy. Twenty-seven years after the accident a mortality study of those exposed to TCDD in the accident was undertaken. Overall mortality did not differ in this group from the rate expected in three external reference populations or from that observed in two internal comparison groups.[28]

Dow is cooperating with the National Institute for Occupational Safety and Health (NIOSH) to prepare a study of more than 4,000 industrial workers potentially exposed to dioxins.

DOW 02113906

Swedish Epidemiological Studies

Dr. Hardell's work, which has lead to the question about soft tissue sarcomas, uses a different methodology than in the foregoing studies -- the case control approach -- and reports on different patterns and levels of exposure.[16,29] In his first study, he reported a five- to six-fold increased risk of soft tissue sarcomas for those presumably exposed to phenoxyacetic acids, predominantly 2,4,5-T, and chlorophenols ("presumably" because the exposure information was subjectively reported by the study participants and/or next of kin). Objective records were incomplete and were difficult to interpret. In the second study, he also estimated the risk associated with exposure to phenoxyacetic acids not contaminated with TCDD and found the same magnitude of risk.

Some epidemiologists reviewing the information find this confusing. The justification for combining chlorophenols and 2,4,5-T was the presumption of a common contaminant, TCDD.[29] If that were the case, increased risk would not be expected for the phenoxyacetic acids without this contaminant.

In both of his studies, Dr. Hardell first administered mail questionnaires and then followed up with telephone interviews on a selected subset of subjects. While the questionnaire was designed with the idea of masking the intent of the investigation, the telephone inquiry specifically focused on the exposures of pertinent interest: phenoxyacetic acids and chlorophenols.

Interestingly, in his second study, Dr. Hardell reported the frequency of the various exposures among both cases and controls and most of the risk estimates were elevated. The risk from only one out of 15 chemicals, sodium chlorate, was not elevated. Nicotine had a high risk. Unfortunately, the risk estimates derived from the questionnaire alone are not known, nor is it known what the risk estimates would have been for other agents, if they had been probed in the same depth as were the phenoxyacetic acids and chlorophenols.

DOW 211390

There are a number of other concerns relating to Hardell's work. The accuracy with which exposure to the chemicals is determined is of vital importance, and the memory recall techniques Dr. Hardell used are subject to error. Workers are unlikely to remember with accuracy the chemicals they used some years in the past. It is also extremely difficult to estimate the extent or duration of the exposures. Furthermore, the

16571

identification and classification of the various soft tissue sarcomas have not been consistent. The diversity of tumors is particularly difficult to explain. If all the tumors had been of a single type, the data would be much more convincing.

Furthermore, research currently underway does not support Hardell's hypothesis concerning soft tissue sarcoma:

- The U.K. Government Advisory Committee on Pesticides noted in February 1983: "It is our view that the procedures used for establishing the Swedish study groups, and some aspects of the collection of the exposure data, were not reliable."
- Smith and associates[30] are conducting a case-control study in New Zealand. Herbicide application is a registered profession in New Zealand, and phenoxyacetic acids have been used in bulk since the late 1940s. In a preliminary report presented at the 1982 International Symposium on Chlorinated Dioxins and Related Compounds, Smith noted he was unable to establish an association between the use of phenoxyacetic acids and soft tissue sarcoma. In fact, not one of the soft tissue sarcoma patients had ever worked as a licensed phenoxyacetic acid herbicide applicator.
- Milham[31] explored this issue in the state of Washington using death certificates. While there has been considerable exposure over the years to phenoxyacetic acids and chlorophenols in Washington state, occupations related to their use did not show consistent patterns of death due to soft tissue sarcomas. Surprisingly, the two occupations with the highest proportional mortality ratios were marine engineers and bankers.
- Among the deaths in the U.S. Air Force "Ranch Hand" Study, none were due to soft tissue sarcomas.[32]
- Riihimaki studied 1,960 Finnish herbicide applicators and found no difference in death rate from any natural cause, including cancer, compared with the total Finnish male population. There were no soft tissue sarcomas reported in this group of workers.[33]
- The Michigan Department of Public Health (MDPH) is investigating the possible relationship between dioxin and a small number of soft and connective tissue cancer deaths among women in Midland County (where Dow has facilities).[34] In a recent report (May 1983) MDPH

DOW 2113908

did not identify a specific association between any environmental factor and the reported increase of soft tissue cancer in the county. While overall age-adjusted cancer mortality rates in Midland County were below the state of Michigan average, soft and connective tissue cancers among white females in the county were elevated. As part of the initial study, MDPH reviewed data for 28 other U.S. counties in which TCDD or other dioxins were likely produced as a chemical manufacturing contaminant. They found no increase of soft tissue cancer in these counties versus counties that did not have industrial manufacturing sources suspected of generating such dioxins.

- Various animal species did not develop soft tissue sarcomas when fed TCDD during laboratory experiments.

Additional research is under way in the United States and elsewhere. The results of these studies should offer a better perspective on this issue. But right now, the preponderance of evidence does not demonstrate a link between TCDD exposure and soft tissue sarcomas.

DOW 2113909

The Dow Mortality Study

In 1976 a research study was published that summarized the mortality experience of over 8,000 men employed by The Dow Chemical Company. The population of interest was defined based on a March 1, 1954, employee census of the Midland, Michigan, manufacturing location of the Company.[35]

The study originally developed background mortality data on the Dow worker population so that findings obtained from smaller groups directed at more specific questions, (e.g. questions regarding particular chemical exposures or processes), could be placed in perspective. The observation period for mortality follow-up was 1954 through 1972, and

comparisons were made to the mortality experience of the corresponding United States white male population. At that time, mortality among Dow employees was found to compare favorably with that of the general U.S. population.

The objectives of the most recent update of this group of 8,181 employees were to extend the observation period through 1978, and to include comparisons with a community-based group of employed men not engaged in chemical manufacturing activities. In addition, the effects of cigarette smoking on mortality were investigated.

The Dow group consisted of personnel from research departments, the corporate headquarters, and a major manufacturing division of the Company. It has been estimated that as many as 500 distinct chemical processes ranging from small batch operations to large continuous and highly-automated production units have been onstream at the Midland manufacturing site, including production of trichlorophenol and related products.[36]

For the most part, the men in the study represent long-term Company employees. Some were hired prior to the 1940s.

Cause of death was obtained from death certificates for all but nine of the 1,932 deceased members of the study population.

DOW 2113910

Overall mortality in the Dow group was 19 percent less than that expected for the corresponding United States population. Expressed in another way, a Dow worker of age 25 in 1972 could expect to live 2.2 years longer than a non-Dow worker of the same age.

There were no statistical findings of excess mortality among Dow employees for any cause of death category. For total malignant neoplasms, there were five percent fewer deaths observed than expected based on the United States white male population. Significantly fewer deaths were observed than were expected for all categories. Presumably, if low-level TCDD exposure caused a variety of increased illnesses, the opposite from what is stated above would have been found.

Comparing the Dow workers with a comparable employee population in Michigan, the overall mortality risk was about 10 percent lower in the Dow group than the corresponding non-Dow group.[37]

DOW 2113911

Birth Defects

Concerns that TCDD may cause birth defects are not substantiated by epidemiological science. A brief review of some areas of concern and the corresponding results, and a more specific look at the Dow situation in Midland, Michigan, are presented:

- Aerial spraying of 2,4,5-T in Oregon: Claims of a link between incidence of miscarriages and 2,4,5-T herbicide could not be established when all the data were available.[15]

16575

- A six-year study of agriculture and forestry workers in Hungary exposed to 2,4,5-T: Data do not suggest that there has been any significant increase of birth defects.[15]

- New Zealand workers exposed to 2,4,5-T: An alleged abnormally high incidence of neural tube defects (such as spina bifida) were chance occurrences, and there is "a very high assurance of safety in normal use" of 2,4,5-T.[15]

- Australian worker exposure to 2,4-D and 2,4,5-T: Claims of high incidence of birth defects were investigated, and available information revealed no evidence that the birth defects were caused by exposure to these two compounds.[15]

- Facial cleft defects in Arkansas: Study of exposure to 2,4,5-T from 1948-74 reveals these defects to have no association with 2,4,5-T.[15]

About five years ago it was reported that the birth defect rates in Midland County (Michigan) were three times above those for the state of Michigan. At that time, the Michigan Department of Public Health (MDPH) examined this issue. It found an increase had occurred between 1971 and 1974 due to additional reporting of minor congenital malformations. Before 1971 and after 1974, the birth defect rates in Midland County were either at or below state rates. If widespread chemical contamination of the environment was responsible for birth defects, the rates should have gone up and stayed up. They did not. The changes may have been due to reporting techniques, not additional cases of birth defects.

It is very important to note that the numbers of county births and birth defects were small, and thus, rates calculated from them tended to be more variable from year to year than the comparable rates for the entire state. The MDPH recently announced (May 1983) an updated report which concludes that the birth defect elevations noted in the early 1970s were not unique and that no additional studies are warranted at this time.[38]

DOW 2113912

16576

In 1982 Dow scientists published a report of a survey of reproductive events among 370 wives of Dow Michigan Division employees exposed to dioxins.[39] They analyzed the associations between exposure and spontaneous abortion (miscarriage), stillbirths, infant deaths, and several categories of congenital malformations before and after controlling for multiple confounders (i.e., sources of error or confusion) independently and in various combinations. They did trend analysis to determine if duration of paternal exposure was related to adverse pregnancy outcome. There were no associations between any exposure and adverse pregnancy outcomes. These findings have recently been confirmed in another study conducted by Australian scientists[40] who examined the impact on birth rates involving Vietnam veterans potentially exposed to Agent Orange.

UOM 211 3913

The Toxicity of TCDD in Animal Species

There are wide variations in how different animal species are affected by TCDD. For example, the single oral dose LD_{50} (the amount that it would take to kill 50 percent of the test animals) in guinea pigs is 0.6-2.0 ug/kg (micrograms per kilogram or every 2.2 pounds of body weight [a microgram is one millionth of a gram; a kilogram is 1,000 grams, or 2.2 pounds]).[41,42] In hamsters, however, TCDD is much less toxic, with an oral LD_{50} of 1157-5051 ug/kg.[43,44]

Therefore, the guinea pig is approximately 5,000 times more sensitive than the hamster, although both species are closely related biologically. The direct relevance of any single animal experiment to humans must be evaluated with respect to all other available data.

16577

In acute and subchronic studies, liver toxicity is a prominent component of TCDD toxicity in rats, mice and rabbits, but not in monkeys, where effects on the bone marrow and epithelial tissue are more prominent.

Thymic atrophy in early toxicity studies suggested that TCDD might decrease the immune response. In laboratory animals, scientists can induce immunotoxic effects with high doses of TCDD. However, as the Seveso incident shows, these effects have not been demonstrated in exposed humans.[45,46]

DOH 2113914

Teratogenicity/Animal Species

Of the various toxic responses of animals to TCDD, the potential for teratogenicity (birth defects) has perhaps received the most attention. Teratogenic effects resulting from TCDD have been realized only in mice, which show an increased frequency of cleft palate, along with an abnormality of the central collection system of the kidney. It is well known that many factors can cause birth defects in mice, including the stress of being transported by air during pregnancy, or being deprived of drinking water overnight. In both rats and mice, dose levels of TCDD have been identified at which these effects do not occur.

In rats, TCDD does not cause a teratogenic effect but sufficiently high doses can cause embryo- and fetotoxicity.

Many of the studies with TCDD in monkeys have been conducted at the University of Wisconsin. In experiments where the high dose level of about 0.011 ug/kg/day TCDD in the diet was given to the monkeys for up to 9.3 months, there were substantial toxic effects.[47,48] A preliminary abstract of a follow-up study[49] showed that monkeys given diets containing approximately 0.0017 ug/kg/day of TCDD had only slight toxicity. There are studies currently in progress at the University of Wisconsin at lower doses.

In a three-generation reproduction study[50] of rats at incremental dose levels of TCDD in the diet, high doses caused decreased fertility and neonatal survival. The intermediate dose level caused decreased fertility and other effects in the first two generations, but not in the third. At the low-dose level, there was no impairment of reproductive capacity through the three consecutive generations, indicating that 0.001 ug/kg/day was a no-adverse-effect level over multiple generations.

DOW 2113915

Mutagenicity/Animal Species

A number of mutagenic studies have been conducted with TCDD. The majority of the tests have been negative or uninterpretable. Only two of the tests were positive in one strain of Salmonella bacteria, showing that there is low possibility for mutagenesis with TCDD in bacteria.

In studies[46,51,52,53,54,55,56,57] with rats or human cells, there is little indication that TCDD elicits a mutagenic response.

16579

Thus, while a few positive or questionable mutagenic responses have been observed in certain plant or microbial test systems, there appear to be no definitive correlates of mutagenicity in higher animals or humans.

Carcinogenicity/Animal Species

Carcinogenic studies following ingestion of TCDD have been conducted in rats and mice.[58,59,60] Review of these data indicates good correlation of the results in rats and mice, with a carcinogenic response associated only with lifetime ingestion of higher dose levels that also induce other toxicity. The liver was the primary target for cancer in both rats and mice. No cancer response occurred at dose levels of 0.001-0.0014 ug/kg/day in the rats and 0.001-0.03 ug/kg/day in mice.

TCDD does cause cancer in animals. However, it is only at very high dose levels -- dose levels that are higher than those that elicit other kinds of toxicity. Cancer induced by exposure to TCDD would be preceded by substantial signs of toxicity. This toxicity (which is reversible when exposure stops) would act as a sentinel, or warning at dose levels below those that might cause cancer.

Environmental Hazard Evaluation

Soil

Potential human exposure to TCDD from environmental contamination concerns many people. The following factors should be considered when evaluating potential human health hazards due to TCDD in soil:

DOH 2113916

- TCDD is tenaciously bound by soils and other materials (carbon, charcoal), and studies have shown that plants grown in soils containing TCDD do not accumulate or translocate the material.

- The amount of material absorbed depends on contact with the skin, or ingestion.

- Soil binding of TCDD reduces the amount that can be absorbed through the skin or internally from ingestion.

- Animal studies show that only a small amount of TCDD is absorbed from contaminated soil. In animal studies, when such soil contained about 500 parts per billion (ppb) ppb TCDD and when the soil was held in contact with skin for 24 hours (covered with aluminum foil), about 1/1000 of the TCDD in soil was absorbed.

- Estimates of absorption from the Seveso incident suggest that people would be expected to absorb about 1/2000 of the average amount of material present in 1 square meter (approximately 1.2 square yards) of their environment each day. If one uses the laboratory or Seveso data, one can estimate that 100-1000 ppb TCDD in soil could result in human absorption of as much as a dose equivalent to that which produced no effect when fed to laboratory rats for a lifetime. If one assumes that soil is eaten in significant amounts by children, the permissible level is less, perhaps 10 ppb.

DOW 2113917

Based on the assumptions and calculations presented here, (particularly those based on the work of Dr. Kingsley Stevens),[61] it appears that general environmental soil contamination in the range of low parts per billion would not pose a health hazard to the general population, including the unborn, children, pregnant women or the old and infirm. For the general population, excluding children with intimate soil contact or those who ingest soil, a level of 100 ppb would still pose no hazard. In occupational settings, where workers are protected by standard industrial hygiene practices, higher levels of contamination would not be accompanied by greater exposure or hazard.

DOW 2113918

Dioxin and Fish

Rats consuming a diet containing 22 parts per trillion (ppt) ppt TCDD (1 nanogram TCDD/kg of body weight per day) for a lifetime showed no adverse health effects due to TCDD. Rats consume about 10-20 percent of their body weight in food each day, while people daily consume about 2-3 percent of their body weight. Thus, for a given TCDD concentration, rats will ingest more of the compound than people. A person eating about 2-3 pounds of food per day uniformly contaminated with 25 ppt TCDD would ingest about 0.5 nanogram/kg body weight/day or about one-half the dose (weight of TCDD per unit of body weight) shown to produce no adverse health effects when fed to rats for a lifetime. Again, this is true because people eat less food in proportion to their body weight compared to laboratory rats.

The dose received by eating contaminated fish is much less. Most people in the United States (99 percent according to U.S. government figures) eat less than 0.6 pound of fish per week. Nine out of ten people in the United States eat less than 0.3 pound of fish per week, while the "average" person eats still less. Since fish is such a small part of the American diet, 99 percent of the American public would receive less than 1/70 of the amount of TCDD shown to produce no effect in laboratory rats fed TCDD daily for their lifetimes, if those people ate fish containing 25 ppt of TCDD. Or said another way, a person could eat nearly one ton of fish per year containing 25 ppt of TCDD and not exceed the no-effect level established by laboratory animal experiments.

The fish consumption guideline of 25 ppt established by the Food and Drug Administration assures an adequate margin of safety to protect people from overexposure to TCDD.[62]

* * *

DOW 2113919

REFERENCES

1. Kociba, R. J. and Schwetz, B. A., (1982): Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). **Drug Metabolism Reviews**, 13(3):387-406.

2. Garattini, S., (1982): TCDD Toxicology with Particular Reference to Seveso: Introductory Remarks. **Drug Metabolism Reviews**, 13(3):345-353.

3. Bumb, R. R., Crummett, W. B., et al., (1980): Trace Chemistries of Fire: A Source of Chlorinated Dioxins. **Science**, 210, pp. 385-390. October 24, 1980.

4. Beljan, J. R., MD, et al., (1981): The Health Effects of "Agent Orange" and Polychlorinated Dioxin Contaminants, Technical Report, Prepared by the Council of Scientific Affairs' Advisory Panel on Toxic Substances, Dept. of Environmental, Public and Occupational Health, American Medical Association, Chicago, IL, October 1, 1981.

5. Homberger, E., et al., (1979): The Seveso Accident: Its Nature, Extent and Consequences. **Ann. Occup. Hyg.**, 22:327-370.

6. Pocchiari, F., et al., (1979): Human Health Effects from Accidental Release of Tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. **Ann. N.Y. Acad. Sci., vv**:311-320.

7. Fanelli, R., et al., (1982): TCDD Contamination in the Seveso Incident. **Drug Metab. Rev.** 13(3)407-422.

8. Crow, K., (1978): The Chemical Disease. **New Scientist.** April 13, 1978.

9. Caramaschi, F., et al., (1981): Chloracne Following Environmental Contamination by TCDD in Seveso, Italy. **International J. Epidemiology, 10(2)**:135-143.

10. Fara, G. M., et al., (1980): Chloracne After Release of TCDD at Seveso, Italy. In: **Chlorinated Dioxins and Related Compounds: Impact on the Environment**, Oxford: Pergamon Press, pp. 545-559.

11. Reggiani, G., (1979): Estimation of the TCDD Toxic Potential in the Light of the Seveso Accident. **Arch. Toxicol. Suppl.**, 2:291-302.

12. Bisanti, L., et al., (1979): Experience of the Accident of Seveso. Proceedings of the 6th European Teratology Society Conference, September 4-7, 1978, Budapest, Hungary, 1979.

13. Abate, L., et al., (1980): Mortality and Birth Defects From 1976 to 1979 in the Population Living in the TCDD Polluted Area of Seveso. In: **Chlorinated Dioxins and Related Compounds: Impact on the Environment**, pp. 571-587, Pergamon Press, Oxford.

DOH 2113920

14. Reggiani, G., (1977): Toxic Effects of TCDD in Man. NATO Workshop on Ecotoxicology, Guilford, England, July-August, 1977.

15. Kilpatrick, R., et al., (1980): Further Review of the Safety for Use in the U.K. of the Herbicide 2,4,5-T, Report of the Advisory Committee on Pesticides, Ministry of Agriculture, Fisheries and Food, United Kingdom, December, 1980.

16. Hardell, L. and Sandstrom, A., (1979): Case-Control Study: Soft Tissue Sarcomas and Exposure to Phenoxyacetic Acids or Chlorophenol. **British Journal of Cancer**, 39:711-717.

17. Hajdu, S. I., (1981): Soft Tissue Sarcomas: Classification and Natural History. **CA-A Cancer Journal for Clinicians**, 31(5):271-280.

18. Rubin, P., Ed., and Bakemeier, R. F., Assoc. ed., (1978): Soft Tissue Sarcoma, W. B. Patterson, In: **Clinical Oncology for Medical Students and Physicians.** Chapter XIX, American Cancer Society, pp. 210-217.

19. Zack, J. A. and Suskind, R. D., (1980): The Mortality Experience of Workers Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident. **Journal of Occupational Medicine**, 22:11-20.

20. Cook, R., et al., (1980): Mortality Experience of Employees Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). **Journal of Occupational Medicine**, 22:530-32.

21. Zack, J. A., Gaffey, W., (1980): A Mortality Study of Workers Employed at the Monsanto Chemical Plant in Nitro, West Virginia, Unpublished.

22. Zack, J. A. Gaffey, W. R., (1983): A Mortality Study of Workers Employed at the Monsanto Company Plant in Nitro, West Virginia. In: **Human and Environmental Risks of Dioxin and Related Compounds**, R. E. Tucker, Ed.; Plenum Publishing, pp. 575-591.

23. Cook, R. R., (1981): Dioxin, Chloracne, and Soft Tissue Sarcoma. Letter to the editor. **The Lancet**, March 14, 1981, p. 618.

24. Cook, R. R., (1983): Soft Tissue Sarcoma: Clues and Cautions. In: **Human and Environmental Risks of Dioxin and Related Compounds**, R. E. Tucker, Ed.; Plenum Publishing, pp. 613-618.

25. Moses, M., and Selikoff, E. J., (1981): Soft Tissue Sarcoma, Phenoxy Herbicides, and Chlorinated Phenols. Letter to the editor. **The Lancet**, June 20, 1981, p. 1370.

26. Johnson, F. E., Kugler, M. A., and Brown, S. M., (1981): Letter to the editor. **The Lancet**, July 4, 1981, p. 40.

DOH 2113921

27. Ott, M. G., et al., (1980): A Mortality Analysis of Employees Engaged in the Manufacture of 2,4,5-Trichlorophenoxyacetic Acid. **Journal of Occupational Medicine**, 22:47-50.

28. Thiess, A. M., et al., (1982): Mortality Study of Persons Exposed to Dioxin in a Trichlorophenol-Process Accident That Occurred in the BASF on 13 November 1953. **American Journal of Industrial Medicine**, 3:179-189.

29. Eriksson, M., et al., (1979): Case-Control Study on Malignant Mesenchymal Tumors and Exposure to Chemical Substances. **Lakartidningen** 76:3872-75, (EPA Translation).

30. Smith, A. H., et al., (1982): The New Zealand Soft Tissue Sarcoma Case-Control Study: Interview Findings Concerning Phenoxyacetic Acid Exposure, Third International Symposium on Chlorinated Dioxins and Related Compounds, Salzburg, Austria, October 12-14, 1982.

31. Milham, S., (1982): Herbicides, Occupation and Cancer. **Lancet**, 1:1464-1465, 1982.

32. Chesney, M., (1982): Status of the Ranch Hand Study Concerning Agent Orange, Presentation to the House Committee on Veteran's Affairs Subcommittee on Oversight and Investigations.

33. Riihimaki, V., et al., (1982): Mortality of 2,4-Dichlorophenoxy-acetic Acid and 2,4,5-Trichlorophenoxyacetic Acid Herbicide Applicators in Finland. **Scand. J. Work. Environ. Health**, 8:37-42.

34. "Evaluation of Soft and Connective Tissue Cancer Mortality Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide," Michigan Department of Public Health, May 4, 1983.

35. Ott, M. G., et al., (1976): Determinants of Mortality in an Industrial Population. **J. Occup. Med.**, 18:171-77.

36. Ott, M. G., et al., (1975): Linking Industrial Hygiene and Health Records. **Am. Ind. Hyg. Assoc. J.**, 36:760-766.

37. Ott, M. G., (1982): Effects of Selection and Confounding on Mortality in an Occupational Cohort (Doctoral Dissertation) (Mortality Among Men In A Chemical Manufacturing Company -- Executive Summary), University Microfilms, Ann Arbor (In Press).

38. "Evaluation of Congenital Malformation Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide", 1979-1981, Michigan Department of Public Health, May 4, 1983.

39. Townsend, J. C., et al., (1982): Survey of Reproductive Events of Wives of Employees Exposed to Chlorinated Dioxins. **Am. J. Epidem.**, 115:695-713.

DOW 2113922

40. Armstrong, B., (1983): Australia Reports No Link Between Service in Vietnam and Birth Defects Among Offspring. **The Epidm. Monitor, 4**(3):1.

41. Schwetz, B. A., et al., (1973): **Environ. Health Perspect. Exp.**. Issue No. 5:87.

42. McConnel, E. E., et al., (1978): **Toxicol. Appl. Pharmacol., 44**:335.

43. Olson, J. R., et al., (1980): **Toxicol. Appl. Pharmacol., 55**:67.

44. Henck, J. W., et al., (1981): **Toxicol. Appl. Pharmacol., 59**:405.

45. Homberger, E., et al., (1979): The Seveso Accident: Its Nature, Extent and Consequences, Report of the Givaudan Research Company.

46. Reggiani, G., (1980): **J. Toxicol. Environ. Health, 6**:27.

47. Allen, J. R., et al., (1977): **Toxicol. Appl. Pharmacol., 41**:177.

48. Allen, J. R., et al., (1977): **Food Cosmet. Toxicol., 15**:401.

49. Schantz, S. L., (1979): **Toxicol. Appl. Pharmacol., 48**:A180.

50. Murray, F. J., et al., (1979): **Toxicol. Appl. Pharmacol., 50**:241.

51. Green, S., Moreland, F. S., (1975): **Toxicol. Appl. Pharmacol., 33**:161.

52. Green, S., et al., (1977): **DA By-Lines, 6**:292.

53. Wassom, J. S., et al., (1977-78): **Mut. Res., 47**:141.

54. Khera, K. S., Ruddick, J. A., (1973): "Polychlorodibenzo-p-dicxins: Perinatal Effects and the Dominant Lethal Test in Wistar Rats", in Chlorodioxins - Origin and Fate. **Advances in Chemistry Series, No. 120** (Etcyl H. Blair, ed.), American Chemistry Society, Washington, DC.

55. Beatty, P. W., et al., (1975): **Toxicol. Appl. Pharmacol., 31**:309.

56. Tenchini, M. L., et al., (1977): Approaches to Examination of Genetic Damage After a Major Hazard in Chemical Industry: Preliminary Cytogenic Findings on TCDD-Exposed Subjects After Seveso Accident, Special Project of Investigations on TCDD-Exposed Pregnancies (Prof. G. B. Candiani and Prof. L. DeCarli), University of Milan, Italy.

57. Rehder, H., et al., (1978): **Schweiz. Med. Wochenschr., 108**:1617.

58. Kociba, R. J., et al., (1978): **Toxicol. Appl. Pharmacol., 46**:279.

59. National Cancer Institute, (1980): DHHS Public. No. NIH80-1765.

DOW 2113923

60. Toth, K., et al., (1979): **Nature, 278:548.**

61. Stevens, K. M., (1981): Agent Orange Toxicity: The Quantitative Perspective. **Human Toxicology, 1, pp. 31-39.**

62. Cordle, F., (1981): The Use of Epidemiology in the Regulation of Dioxins in the Food Supply. **Regulatory Toxicology and Pharmacology 1,** Academic Press, pp. 379-387.

DOH 2113924

16588

MN72283

Health & Environmental Information

For Further Information Contact:
R. W. (Bob) Charlton
(517) 636-9303

*DIOXIN AND HUMAN HEALTH

Executive Summary

The issue of dioxin in the environment and the possible human health effects of this compound has been the subject of much speculation over the past decade.

This paper discusses scientific research and makes the following major points:

- The word "dioxin" refers to a family of 75 compounds of which the one called TCDD* is the most toxic based on animal tests. Dioxins can be produced as an unwanted contaminant of some manufacturing processes and through some combustion sources.

- An American Medical Association review of available research has concluded that there is no convincing support for allegations that TCDD causes cancer, birth defects or other reproductive difficulties in humans.

- Studies of some 30,000 people exposed to TCDD after an industrial accident in Seveso, Italy, found a chemically-induced acne that occurred in approximately 200 people and no persistent effects which could be verified. There was no excess of birth defects, abortions or deaths in the population.

- A number of studies of workers accidentally exposed to large amounts of TCDD as long as 30 years ago have identified cases of chloracne in some of those workers. However, no long-term health effects have been demonstrated among this worker group. The studies have looked for cancer, heart disease, liver damage and other ailments.

- The work of a Swedish scientist who purports to find a link between TCDD exposure and soft tissue sarcoma (a relatively rare cancer) is not consistent with other studies. This research is not supported by the bulk of studies of exposed industrial employees, herbicide applicators and others which show no excess of this type of cancer.

- A study of 8,000 Dow employees in Midland, Michigan, where herbicides were produced, found their death rate to be 19 percent less than the general U.S. population. There was five percent less cancer than predicted.

DOW 2113890

*2,3,7,8-tetrachlorodibenzo-p-dioxin

16569

- Investigations of herbicide applicators, forest workers and industrial workers and their wives found no link between TCDD exposure and birth defects.

- Studies of animals exposed to TCDD show a variety of adverse health effects that vary widely from species to species. In most studies, safe levels of dioxin exposure have been identified. Cancer occurred only at levels where animals were suffering from obvious TCDD poisoning.

A review of the scientific literature suggests that humans are less susceptible to TCDD than various animal species.

As is the case with other toxins, TCDD exposure should be carefully controlled. The scientific evidence to date does not warrant undue concern about individual exposure to trace amounts of TCDD presently measured in the environment. Government environmental guidelines currently provide for more than adequate margins of safety.

DOW 2113891

16590

2616

D0012044587

Analytical Studies
for the U.S.
Environmental Protection Agency

VOLUME IIa

Decision Making in the Environmental Protection Agency

CASE STUDIES

Prepared by
Robert E. Burt, Nathan J. Karch,
Raphael G. Kasper, Richard W. Lowerre,
Lawrence E. McCray, and *J. Roxanne Arnold*
for the
Committee on Environmental Decision Making

Commission on Natural Resources
National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D.C. 1977

16591

EXPLICIT CRITERIA AND PRINCIPLES FOR IDENTIFYING CARCINOGENS: A FOCUS OF CONTROVERSY AT THE ENVIRONMENTAL PROTECTION AGENCY

Nathan J. Karch[1]

00120458

CONTENTS

I.	DESCRIPTION OF THE DECISIONS	121
II.	INTRODUCTION	121
	A. The Nature of the Disease	122
	B. Cost and Extent of Cancer	123
	C. Environmental Origins of Cancer	124
	D. Strategy for Control: New Emphasis on Prevention	124
III.	CANCER PRINCIPLES	126
	A. Identification of Carcinogenic Substances	126
	B. Early Development of Principles	128
	C. Statutory Framework	130
	D. Use of Cancer Principles in the DDT Cancellation Proceeding	131
	E. Use of Cancer Principles in the Aldrin/Dieldrin Suspension Proceeding	133
	F. Use of Cancer Principles in the Heptachlor/ Chlordane Cancellation and Suspension Proceedings	137
	1. Magnitude of the Cancer Problem	142
	2. Uncertainty in the Mechanism of Cancer Induction	142
	3. Establishment of Carcinogenicity and Assessment of Risk	142
	4. Problems with Experiments in Animals	143
	G. Use of Cancer Principles in the Mirex Fact-Finding Proceeding	153
	H. Use of Cancer Principles in Rule Making	159
	I. Origins of the Cancer Principles	161
	1. Extrapolation from Rodents to Humans	162
	2. Can All Chemicals Cause Cancer?	164

16592

3. Do Benign Tumors Characterize a Carcinogenic Response? 165
4. Can "No-Effect" Levels be Set for Chemical Carcinogens? 168

J. Organized Opposition 172
K. Attempt at Peer Review 176

IV. CONCLUSIONS 184

A. Nature of Controversy 184
B. How the Controversy Might Have Been Averted 186

V. NOTES 188

VI. APPENDICES 204

DOH2004589

16593

many scientists in OPP had extensive toxicological experience in industry, USDA, or FDA with substances that induce reversible effects below a certain "threshold" of action. Thus, they believed "no-effect" levels should be set for carcinogens as they are for substances posing acute and certain other chronic hazards. Furthermore, many in OPP believed rodents (mice especially) were inappropriate animal models for cancer testing.

Because cancer is such a poorly understood disease, there are no unequivocal answers to a great many questions. The principles were an attempt to summarize reasonable presumptions concerning the design and interpretation of studies to detect carcinogens. They sought to present in simple terms the thinking of expert committees that had addressed the problems of carcinogenicity testing for over 25 years. Not surprisingly, many scientists in OPP opposed the use of the principles, and OGC took the lead in preparing the scientific and technical basis for the Agency's position on carcinogenicity in cancellation actions. Under other circumstances, OPP would have had a large hand in the cancer issue during cancellation actions; OPP and other Agency scientists testified on many issues other than cancer.

DOH2044590

I. Origins of the Cancer Principles[109]

Although it is not the intention in this case study to evaluate the scientific validity of the cancer principles, it seems appropriate to examine in detail some of the underlying documentation. For this reason, some of the reports and articles that were entered into the record of various fact-finding, cancellation, and suspension proceedings, and that form the basis for the principles will be discussed. The following discussion is intended to illustrate the surprising consensus--short of unanimity--on the concepts embodied in the cancer principles and is limited to four such concepts:

-- It is necessary to extrapolate from animal studies, particularly in rodent species, to humans in order to evaluate the risk of cancer.

-- Not all chemicals are capable of causing cancer, even at maximum doses of administration.

-- Although a distinction can frequently be made between benign and malignant tumors, for the purposes of establishing the carcinogenicity of a substance, they should be considered synonymous.

-- There is no practical method for determining a "no-effect" or threshold exposure level in humans for carcinogens.

1. Extrapolation from Rodents to Humans

The first issue of reliance on studies in rodents has already been discussed earlier to some extent. It should be recalled that the various expert committees agree that reliance on animal studies is necessary because of the practical and ethical limitations in reliance on epidemiological studies to identify carcinogenic agents and because of the evidence of the validity of studies in experimental animals as the basis for establishing the carcinogenicity of a substance.

DOI120459I

Discussions by expert committees of the optimal conditions for carcinogenicity testing has included consideration of appropriate mammalian species for testing. After a thorough review of carcinogenicity testing, the Mrak Commission reported in 1969:

> The species most practical for testing are rats, mice, and--as more recently shown--hamsters. Strains and colonies should be selected to provide adequate sensitivity to tumor induction, as revealed by positive control tests with known carcinogens.... The use of nonrodent species, recommended in the earlier reports, has now been substantially dropped. A suitable, practical nonrodent species would be useful but it is not available at this time.... While dogs have been employed for tests of carcinogenicity, with noteworthy success in selected cases (bladder carcinogenicity of aromatic amines), the requirement of lifetime feeding makes this species too expensive, in terms of time and funds, to be employed routinely.[110]

Again in 1975, an NRC committee confirmed the views expressed in many of the earlier reports:

> Rodents are the animal of choice for carcinogenesis tests because of their convenience, comparatively short life span and proven susceptibility to a broad range of carcinogenic agents.[111]

Another NRC report published in 1975 concluded:

> ...the carcinogenic risks, and other health hazards, of pesticides require continuing evaluation by testing with laboratory mammals; and...despite the problems involved in translating

16595

103 Ibid.

104 Federal Register, Vol. 40, (July 3, 1975), pp. 28242-28296.

105 Ibid., p. 28256.

106 Ibid., p. 28257.

107 Ibid., p. 28263.

108 Bob Wyrick, "EPA Officials Devised Cancer Tests on People," Washington Post, Thursday, June 23, 1977, p. A1; "EPA Proposed Carcinogen Test on Mexicans," Ibid. Wednesday, May 11, 1977, p. A1.

D0112045392

109 I wish to acknowledge the contribution made to the writing of this section by John E. Bonine, Office of General Counsel, EPA. Bonine gave me a preliminary draft of a paper tracing the term "principles" and reviewing some of the reports of expert committees.

110 Mrak Commission (1969), supra note 37, p. 465, text and footnote 2.

111 National Research Council (1975) Evaluating Chemicals, supra note 37, p. 150.

112 National Research Council (1975) Pest Control, supra note 34, p. 66.

113 Mrak Commission (1969), supra note 37, p. 11.

114 National Cancer Advisory Board (1977), supra note 31, p. 463.

115 Tomatis, supra note 37, p. 1.

116 U.S. Department of Health, Education and Welfare, Survey of Compounds Which Have been Tested for Carcinogenic Activity, Volumes I-III, (1974-1976), (Washington, D.C.: Government Printing Office).

117 The study was performed by the Litton-Bionetics Research Laboratories under contracts PH 43-64-57 and PH 43-67-735; see U.S. Department of Health, Education, and Welfare (1969), supra note 31, pp. 461-506; J.R.M. Innes, B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta, R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell, and J. Peters, "Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note," J. National Cancer Institute, Vol. 42 (1969), pp. 1101-1114.

16596

Black No. 12

2626

DOW 21237733

MN 053305

SUMMARY OF DOW'S RESPONSE TO EPA'S RISK ANALYSIS ON THE USE OF 2,4,5-T HERBICIDE

compiled by

Marguerite L. Leng
Product Registrations
Health & Environmental Sciences
THE DOW CHEMICAL COMPANY
Midland, Michigan USA

February 1979

Excerpts from Volume I of Dow risk rebuttal
and from Dow legal brief
submitted to U.S. Environmental Protection Agency
on August 4, 1978

16597

Block No. 12

list of names compiled by N. Long from responses to RPAR submitted to EPA during hearing as exhibit in cross examination of Jim Boland of EPA who reported "incidents" with 2,4,5-T mostly effects on crops or trees. Human "incidents" were not related to T as he later admitted in cross examination.

APPENDIX II

Letters Submitted to EPA in Response to 2,4,5-T RPAR: No Ill Effects from Registered Uses.

EPA #	Name of Submitter	Town	State	Occupation	Years of use
5	James Erwin	Roseburg	OR	Secretary, State Sprayers	400 man years
6	Mack L. Little	Lufkin	TX	rancher/sprayer	since 1958
7	C. Eddie Meeks	Thomaston	GA	County agent coop extension service	
13	Donald W. Castellaw	Moscow	ID	pest control consultant	20 years
18	Howard G. McDowell	Missoula	MT	Exec. V.P. Inland Forest Resource Council	
28	J. F. Howell	Clarendon	TX	rancher	years
34	John W. Hinrichs	Grant County	NM	County extension agent	years
37	Frederick Drummond	Pawhuska	OK	uprayer/rancher	years
43	Wm. J. Waldrip	Lubbock	TX	research and range manager	30 years
77B	Richard A. McDougal	National Cattleman's Assoc. . (280,000 professionals)			
86	James L. Cole	Leavenworth	IN	small ranch and woods	over 25 years
90A	Barry F. Malac	Rincon	GA	manager, woodlands research	years
91	James R. Floyd	Liberal	KS	aerial applicator	200.000 acres
93	Patrick J. Kenny	Westborough	MA	New England Power Service Co.	20 years
102	Ronald A. Michieli	Washington	D.C.	Exec. Director Public Lands Council	

DOW 2123734

16598

Block No. 13

DP/Dr.Le/th

2631

TRANSLATION

GERMAN PARLIAMENT
9th Election Period

Publication 9/379
April 29, 1981

UOH 21237335
MN053305

R E P L Y

of the Federal Government
to

the inquiry of members of the parliament, Frau Dr. Hartenstein, Schäfer (Offenburg), Catenhusen, Duve, Jansen, Kiehm, Dr. Kübler, Marschall, Frau Dr. Martiny-Glotz, Reuter, Dr. Schwenk (Stade), Frau Weyel, Witek, Frau Zutt, Ibrügger, Bergerowski, Engelhard, Dr.Hirsch, Kleinert, Dr. Wendig, Wolfgramm (Göttingen) and the parliamentary groups of SPD and FDP

- Publication 9/292 - 4/1/81

see different translation attached as received from John Davidson 11/6/81

2,4,5-T - H E R B I C I D E S

The Secretary of the Dept. of Food, Agriculture and Forestry - 313-3320-1/1 - has answered the inquiry on behalf of the Federal Government by letter of April 14, 1981, as follows:

The Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) and the Bundesgesundheitsamt (BGA) which are responsible for the registration of pesticides, have repeatedly expressed their expert opinion on the herbicide 2,4,5-T. It was established in a joint statement of the year 1975 that a hazard does not exist if it is used properly and according to the regulations. Necessary restrictions for the use are ruled by the decree about prohibitions and restrictions of the use of pesticides, dated December 19, 1980 BGBl. I p. 2355) or by requirements.

2,4,5-t containing herbicides as registered in the Federal Republic of Germany today, cannot be put in a relation to the "agent orange" which was used as a defoliant for forests in the Vietnam war. These products were contaminated very much with the toxic TCDD (Dioxin), which also reached the environment during the accident of Seveso. The defoliant used in Vietnam contained up to 5000-times more TCDD than the formulations registered in the Federal Republic of Germany today. The BBA has required from the manufacturers in the registration that a TCDD content of 0.01 mg/kg 2,4,5-T is not exceeded, i.e. 100 g/t a.i.. In contrast to this, the guideline level of the World Health Organization amounts to 0.5 mg/kg, i.e. 50-times higher than the German guideline level.

16599

AIO 00/100

2632

DOH 2123744

THE RESPONSIBLITY OF THE CHEMIST TO SOCIETY

A symposium sponsored primarily by the American Chemical Society's Division of Pesticide Chemistry, with the cooperation of the Committee on Chemistry and Public Affairs, and the Divisions of Agricultural and Food Chemistry, Chemical Health and Safety, and Professional Relations. Held on ~~March~~ April 26, 1980, in Las Vegas, Nevada, during the 180th national meeting of the Society.

American Chemical Society

1155 16th St., N.W.
Washington, D.C. 20036

16600

CCH 4/1/717

PROPOSALS FOR EPIDEMIOLOGICAL INVESTIGATIONS OF MORBIDITY ASSOCIATED WITH HERBICIDES

1 The assessment of the available data concerning a possible biological association between exposure to phenoxy acid herbicides and soft tissue sarcoma has led to the conclusion that there is a need for further research to permit the confirmation or refutation of this suggestion. It has also been noted that a number of studies are already in progress or are about to be initiated in several countries around the world using data which are available on both a national and international basis. These include systematic epidemiological studies of both a retrospective and a prospective nature which constitute the only appropriate methods available for this purpose. In view of the known temporal and geographical variations in the incidence of a number of malignant conditions and of the importance of the phenoxy acid herbicides to agriculture and forestry in this country it is considered advisable to investigate the situation as it exists in the United Kingdom. Appropriate information is available to enable epidemiological studies of both a retrospective and a prospective nature to be carried out. In addition to being the only way of obtaining information of direct relevance to the UK workforce it should be possible, from the data available, to obtain evidence which is less open to the criticisms that have been levelled against some of the reports that have been published so far. There would also be an opportunity to extend the scope of the investigations to include aspects other than the occurrence of soft tissue sarcoma and the possible involvement of other chemicals such as the chlorophenols.

Retrospective Study

2 This would examine whether patients suffering from soft tissue sarcomas (or other specified malignant conditions) had an unusual history of exposure to phenoxy acids during their manufacture or use as herbicides. The first step would be to establish a group of sarcoma patients and an appropriate control group. Since comprehensive cancer registration data are available covering the whole country, basic information eg age, sex, type of malignancy, when and where registered, for all identified cases in specified categories of soft tissue sarcoma is readily accessible. However, these are relatively rare tumours, and a decision would have to be taken whether to study all of them in relevant age groups, or only those in parts of the country where herbicide use is likely to be more common. The control group, which would have to be matched at least by age and sex, could be cancer registrations for body sites where there are no reasons to believe that tumour occurrence could be associated with herbicides.

3 The major problem with retrospective studies is in obtaining the history of herbicide exposure. For most patients the occupational exposure will not have been documented precisely, if at all. Memories are notoriously fallible, even in the short term and recall concerning events many years

previously will probably be uncertain. For patients who have died recourse might be had to their families to obtain the history, as in some of the published reports, but this would be even more unreliable. Furthermore, whether obtained from the patient or a relative, there is the possibility of a bias in the histories of herbicide use from cases compared with controls because of the publicity on possible hazards.

4 A retrospective study would have to be conducted in the expectation that only in a small proportion of cases and controls could the histories of herbicide use be validated. In such an investigation particular attention would have to be paid to ethical considerations. Information in cancer registries is confidential, and a proper procedure would have to be established before any approach could be made to consultants, general practitioners, to the patients and their families, or to any other source of data.

5 Despite these drawbacks a retrospective study has certain advantages. It can be mounted fairly quickly and is relatively cheap. It does not take long, the duration being governed largely by the time required to obtain occupational histories.

DOH 2127145

Prospective Study

6 In this study a cohort with known exposure to herbicides would be identified and monitored to determine whether they subsequently suffered any excess of soft tissue sarcomas or morbidity of other kinds. There would be a need for a control cohort comprised of individuals not exposed to herbicides. The subsequent morbidity of this group would be recorded in exactly the same way as for the herbicide user cohort.

7 Since reliable information concerning exposure is of paramount importance in such a study an essential pre-requisite is that there should be good documentation available concerning herbicide use. Organisations such as the Forestry Commission or commercial sprayers who keep routine records should therefore be selected in preference to farmers whose occasional use of herbicides may go unrecorded. The documentation system would have to be reviewed to assess the need for possible changes to ensure that complete, uniform and appropriate data are obtained.

8 The essence of the prospective study would be the collection of morbidity information in cohorts defined as either using or not using herbicides. Leaving aside the choice of the control cohort, the non-users, there are different problems depending on what aspect of morbidity is selected for study. The extreme event, death, could be readily ascertained, even if members of the cohort moved or changed their occupation, by tagging their entries in the NHS Central Register. Cancer registrations, as well as deaths from any cause, are reported to this Register so that the cancer morbidity as well as mortality can be obtained for the defined cohort.

9 Other expressions of morbidity, leading to consultation with a general practitioner, out-patient referral or admission to hospital, are not obtainable from a central register. If there was a case for seeking evidence on anything besides general mortality and cancer morbidity,

16602

eg on liver disease, this would need a separate organisation such as a questionnaire to the patient or his doctor to determine whether there was any episode of illness worth further enquiry. It would add considerably to the cost and complexity of the study to extend the range of outcomes in this way, and there would need to be a strong case for such addition.

10 It could be difficult in a prospective study to establish a satisfactory control cohort. Ideally it should comprise individuals of similar age, sex, and social class distribution, and probably from the same areas. In occupational studies of this kind it is sometimes possible to find groups within the same organisations, such as administrative and clerical staff, who are not in contact with the process, but their numbers may well be too small in this instance. It is always possible to use national or Regional data on mortality and cancer registration as a standard of comparison for the cohort of users, and this compromise may be a reasonable one to adopt.

11 The main disadvantage of a prospective study is the length of time before sufficient events have occurred to warrant comparisons betw users and non-users particularly where rare events such as the occurrence of soft tissue sarcomas is concerned. Furthermore, the cohorts defined initially will not remain 'pure' in the sense that additional exposures to similar or different agents may occur. Nevertheless this type of study is more likely to lead eventually to a valid measure of the risk, if any, of exposure to specific herbicides and should, therefore, be given serious consideration, although the difficulties which have been outlined above should not be underestimated.

12 A cohort, prospective, study set up now could provide evidence only on phenoxy acid herbicides currently produced. However, there may be scope for conducting a cohort study retrospectively. If in selected organisations, such as the Forestry Commission, it was possible to prepare nominal lists of all employees in some period in the past, say the decades 1945-54, 1955-64, and to categorise them by pesticide use, their subsequent experience up to the present day of mortality and cancer registration might be retrievable. The mortality picture could be virtually complete, but cancer registration was not fully established in the earlier years and would be reliable only for the last 10-15 years. The study would not be easy, but might be valuable because it would provide information on exposure to herbicides with a relatively high dioxin contamination. Unlike a new cohort study established now, some measure of the outcome 20-30 years after exposure could be obtained relatively quickly.

DOH 2127146

16603

DOH 2127138

SUMMARY OF EPIDEMIOLOGICAL AND CLINICAL STUDIES CONTRIBUTING ORIGINAL OBSERVATIONS CONCERNING A POSSIBLE ASSOCIATION BETWEEN CHEMICAL EXPOSURE AND SOFT TISSUE SARCOMAS

Ref	Country of Origin	Study Population	Summary of findings
	CASE CONTROL STUDIES		
1	Sweden	52 cases of soft tissue sarcoma and 208 matched controls from Northern Sweden	Exposure to phenoxy acids reported by 25% of cases and 7% of controls. Exposure to chlorophenols reported by 13% of cases and 3% of controls. Relative risk* of developing a soft tissue sarcoma associated with exposure to phenoxy acids = 5.3. (Relative risk associated with exposure to chlorophenols = 6.6). Chlorinated dibenzodioxins and dibenzofurans possibly involved.
2	Sweden	110 cases of soft tissue sarcoma and 219 matched controls from Southern Sweden.	Exposure to phenoxy acids reported by 13% of cases and 3.2% of controls Exposure to chlorophenols reported by 10% of cases and 3.7% of controls Relative risk of developing a soft tissue sarcoma associated with exposure to phenoxy acids = 5.8. (Relative risk associated with exposure to chlorophenols = 3.3).
3	Sweden	169 cases of malignant lymphoma (60 Hodgkin's disease and 109 non-Hodgkin's lymphoma) and 338 matched controls.	Exposure to phenoxy acids reported by 24% of cases and 7% of controls. Exposure to chlorophenols reported by 30% of cases and 10% of controls. Relative risk of developing a lymphoma associated with exposure to phenoxy acids = 4.8. (Relative risk associated with exposure to chlorophenols = 4.3).
4	Sweden	154 cases of colon cancer and 541 unmatched controls. (Supplementary study to 1, 2 and 3 above).	A re-examination of the data presented in 1, 2 and 3 above confirms relative risk factors of 4.2, 5.6 and 4.1 respectively for an association between the development of soft tissue sarcoma and malignant lymphoma and exposure to phenoxy acids following a 5 year latency period. No association was established between exposure to such chemicals and the development of colon cancer, relative risk = 1.1.

*Relative risk is the statistically derived expression of the ratio between the incidence of exposure reported by cancer patients and that reported by the control group.

16804

Ref	Country of Origin	Study Population	Summary of findings
5	New Zealand	102 cases of soft tissue sarcoma first diagnosed between 1976 and 1980 and 306 other cancer patients as matched controls.	Preliminary report. On the basis of Cancer Registry Records there is no evidence of an excess of soft tissue sarcomas among the occupational group involving agriculture and forestry.
6	New Zealand	80 cases of soft tissue sarcoma and 92 other cancer patients as controls. (Further data from the study reported at 5 above).	Further preliminary report. Exposure to phenoxy acids reported by 26% of soft tissue sarcoma cases and 21% of controls. Estimat for the relative risk of developing a soft tissue sarcoma following exposure to phenoxy acids varied from 1.3 for potential exposure of more than 1 day at least 5 years before cancer registration to 1.6 for probable exposu No soft tissue sarcoma patient had worked full time on phenoxy acid herbicide spraying.
	COHORT STUDIES		
7	West Germany	75 chemical workers involved in a trichlorophenol plant accident in 1953	42 cases of severe chloracne associated with exposure during the accident or subsequent cleaning work. By 1978 17 deaths had occurred (11 to 25 expected), 6 due to cancer (4 expected). The incidence of stomach cancer (3 cases was higher than expected. There were no cases of soft tissue sarcoma
8	Finland	1,971 herbicide sprayers (Railway, forestry, highway and electric company workers)	Retrospective study 1955-1971, 49 de occurred (87.8 expected), 13 due to cancer (16.4 expected). Prospective follow-up, 1972-1976, 62 deaths occu (92.9 expected) 9 due to cancer (18. expected). Prospective follow-up, 1977-1980, 82 deaths occurred, (91.1 expected), 17 due to cancer, 18.4 expected. No cases of chloracne. N cases of soft tissue sarcoma.
9	USA	61 chemical workers involved in trichlorophenol production and exposed to 2,3,7,8-TCDD in 1964	49 cases of chloracne were reported ranging from mild to severe. By 1978 4 deaths had occurred (7.8 expected), 1 due to cancer (1.6 expected). One case was a soft tissue sarcoma (fibrosarcoma).

EOH 2127139

Ref	Country of Origin	Study Population	Summary of findings
10	USA	204 chemical workers involved in 2,4,5-T production between 1950 and 1971	No case of chloracne. By 1976 11 deaths had occurred (20.3 expected) only 1 due to cancer (3.6 expected). This was not a case of soft tissue sarcoma.
11	USA	121 chemical workers involved in a trichlorophenol plant accident in 1949	All members of the selected cohort exhibited chloracne attributable to exposure during the accident. By 1978 32 deaths had occurred (46.6 expected), 9 due to cancer (9 expected). One case was a soft tissue sarcoma (malignant fibrous histiocytoma).
12	Sweden	142 forestry workers exposed to phenoxyacetic acid herbicides between 1954 and 1978 and 244 forestry workers not so exposed	The pattern of deaths among both exposed and unexposed cohorts is in accord with Swedish national mortality statistics. However among foremen, the most extensively exposed workers, there is an excess of mortality (8 observed/4.1 expected) due to an excess of both cancer (3 observed/1.0 expected) and cardiovascular disease (4 observed/ 1.9 expected). There were no soft tissue sarcomas.
13	Sweden	348 railway workers (herbicide sprayers between 1957 and 1972)	No cases of chloracne. By 1978 45 deaths had occurred (49 expected), 17 due to cancer (11.9 expected). No specific type of tumour predominates and no soft tissue sarcoma was reported.
14	Czecho-slovakia	55 chemical workers exposed to 2,3,7,8-TCDD over a 10 year period during the production of 2,4,5-T from trichlorophenol	52 cases of chloracne. By 1980 6 deaths had occurred, 2 due to cancer. There were no cases of soft tissue sarcoma.
15	USA	Review of 9, 10 and 11 above plus additional data on chemical workers involved in the production of 2,4,5-T from trichlorophenol	An additional case of soft tissue sarcoma (generalised liposarcoma) is reported in a worker involved in the production of 2,4,5-T from trichlorophenol. There was no prior history of chloracne.
16	USA	Supplementary data concerning the cohort studied at 9 above	An additional case of soft tissue sarcoma (malignant fibrous histiocytoma) in a trichlorophenol production worker is reported. There was a previous history of chloracne.

DOE 2127140

16606

Ref	Country of Origin	Study Population	Summary of findings
17	United Kingdom	126 chemical workers involved in a trichlorophenol plant accident in 1968	79 cases of chloracne were reported after the accident. By 1981 no deaths had occurred nor had any case of cancer been identified. No abnormal biochemical or clinical effects attributable to exposure.
18	USA	Retrospective analysis of mortality data - Washington State (1950-1979)	In occupations with exposure to phenoxy acid herbicides or chlorophenols 49 soft tissue sarcomas were observed (41 expected). In those occupations with the greatest excess of deaths from soft tissue sarcoma there was no obvious association with exposure to phenoxy acid herbicides or chlorophenols.
19	USA	Retrospective analysis of mortality data - butchers and slaughterhouse workers (1965-1980)	An analysis of cancer mortality among butchers and slaughterhouse workers revealed an increased risk relative for soft tissue sarcoma of 5.6 (1 observed case/ 0.18 expected).
	CLINICAL REPORTS		
20	Sweden	7 cases of soft tissue sarcoma (included in the case control study reported at 1 above)	Exposure to phenoxy acids is reported in all 7 cases. In 5 cases the exposure was direct and heavy.
21	Sweden	17 cases of malignant lymphoma (included in the case control study reported at 3 above)	Exposure to phenoxy acids, is reported in 8 cases.
22	USA	1 case of soft tissue sarcoma in a chemical worker	A case of retroperitoneal neurogenic sarcoma is reported in a truck driver and maintenance worker in a chemical plant producing trichlorophenol or 2,4,5-T. No history of prior chloracne.
23	USA	2 cases of soft tissue sarcoma in chemical workers (father and son)	Two cases of soft tissue sarcoma are reported (a fibro-sarcomatous mesothelioma and a liposarcoma). Both individuals had been employed at a chemical plant producing chlorophenols. No history of prior chloracne.

DOE 2127141

16607

REFERENCES

1. Hardell, L., Sandström, A., (1979), British Journal of Cancer 39 711-717 "Case-control study: soft-tissue sarcomas and exposure to phenoxy acetic acids or chlorophenols".

2. Eriksson, M., et al., (1981), British Journal of Industrial Medicine 38 27-33, "Soft-tissue sarcomas and exposure to chemical substances: a case-referent study".

3. Hardell, L., et al., (1981), British Journal of Cancer 43 169-176, "Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study".

4. Hardell, L., (1981) Scandinavian Journal of Work Environment and Health 7 119-130 "Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents".

5. Smith, A.H., et al., (1982) Community Health Studies 6 114-119, "Do agricultural chemical cause soft tissue sarcoma? Initial findings of a case-control study in New Zealand".

6. Smith, A.H., et al., (1982) Proceedings of the 3rd International Symposium on Chlorinated Dioxins and Related Compounds. In Press. "The New Zealand soft tissue sarcoma case-control study: interview findings concerning phenoxyacetic acid exposure".

7. Thiess, A.M., Frentzel-Beyme, R., (1978) Working paper, quoted in Internation Agency for Research on Cancer, (1978). "Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans" Internal Technical Report No 78/001, Lyon. "Mortality study of persons exposed to dioxin after an accident which occurred in the BASF on 13th November 1953".

8. Riihimaki, V., et al., (1982) Scandinavian Journal of Work Environment and Health 8 37-42 "Mortality of 2,4-trichlorophenoxyacetic acid herbicide applicators in Finland".

9. Cook, R., et al., (1980), Journal of Occupational Medicine 22 530-532 "Mortality experience of employees exposed to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD)".

10. Ott, MG., et al., (1980) Journal of Occupational Medicine 22 47-50 "A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorphenoxyacetic acid".

11. Zack, J.A., Suskind, R.R., (1980) Journal of Occupational Medicine 22 11-14 "The mortality experience of workers exposed to tetrachloro-dibenzodioxin in a trichlorophenol process accident".

DOH 2127142

16608

12. Hogstedt, C., Westerland, B., (1980) Läkartidningen 19 1829-1831 "Cohort study of causes of death of forestry workers with and without exposure to phenoxy acid preparations".

13. Axelson, O., et al., (1980) Scandinavian Journal of Work Environment and Health 6 73-79 "Herbicide exposure and tumour mortality. An up-dated epidemiological investigation on Swedish railroad workers".

14. Pazderova-Vejlapkova, J., et al., (1980) Pracovni Lekanstri 32 204-209 "Chronic poisoning by 2,3,7,8-tetrachlorodibenzo-p-dioxin".

15. Honchar, P.A., Halperin, W.E., (1981) Lancet i 268-269 "2,4,5-T, trichlorophenol, and soft-tissue sarcoma".

16. Cook, R.R., (1981) Lancet i 618-619 "Dioxin, chloracne, and soft-tissue sarcoma".

17. May, G., (1982) British Journal of Industrial Medicine 39 128-135 "Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure".

18. Milham, S., (1982) Lancet i 1464-1645 "Herbicides, occupation, and cancer".

19. Johnson, E.S., Fischman, H.R., (1982) Lancet i 913 "Cancer mortality among butchers and slaughterhouse workers".

20. Hardell, L., (1977) Läkartidningen 33 2753-2754 "Malignant mesenchymal tumours and exposure to phenoxy acids - a clinical observation".

21. Hardell, L., (1979) Lancet i 55-56 "Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorphenols.

22. Moses, M., Selikoff, I.J., (1981) Lancet i 1370 "Soft tissue sarcomas, phenoxy herbicides and chlorinated phenols".

23. Johnson, F.E., et al., (1981) Lancet ii 340 "Soft tissue sarcomas and chlorinated phenols".

DOW 01127143

RK/JAK 13th December, 1982

The Rt Hon Peter Walker, MBE, MP,
Minister of Agriculture, Fisheries and Food,
Whitehall Place,
London SW1A 2HH.

2646

Dear Minister,

1. In your letter of 8 May 1982, you sought the Advisory Committee on Pesticides' observations on new evidence concerning the safety of 2,4,5-T herbicides, with particular reference to a review article published in the Lancet on that same date. We have now reached a point in our enquiries at which we think it would be helpful to report to you and the other Departmental Ministers with an interest in the safety of these herbicides.

DOH 2127134

Background to Study

2. The Committee decided that a panel of independent members should be set up under my chairmanship to consider the latest evidence, and the advice in this letter reflects their detailed examination of all the relevant studies known to us which are summarised in Appendix A to this letter. The panel included all areas of expertise needed for a study of a postulated association between use of a herbicide and a particular type of cancer, and consisted of:-

Professor C.L. Berry
Dr R.L. Carter
Dr R. Goulding
Professor J. Knowelden
Professor R.I. McCallum
Professor G.R. Sagar

3. Before giving you our conclusions, I think that a brief review of the evidence we have considered since publication of our December 1980 report might help you and your colleagues to assess our advice. You will recall that much of our 1980 enquiry was concerned with the question of whether the use of 2,4,5-T herbicides might be associated with miscarriages and birth deformities among humans and animals, and the evidence included a number of specific cases in which such an association was claimed. Each of these was investigated in detail by the appropriate Government Departments and the Health and Safety Executive in consultation with the general practitioners, hospital consultants and veterinary surgeons concerned, before the Committee reached its conclusion. Our examination revealed no evidence linking any of the cases with use of 2,4,5-T herbicides; and we could find no other evidence which established any association between use of these products and harmful effects on humans, other living creatures or the environment. We were thus able to conclude that: "2,4,5-T herbicides can safely be used in the UK in the recommended way and for the recommended purposes".

4. Our present enquiry arose out of a separate and specific question - that there might be an association between exposure to phenoxy acid herbicides, chlorophenols or the contaminants that may be present in these products and substances, and an increased incidence of a group of tumours classified as soft tissue sarcomas. The term soft tissue sarcomas includes some 50 varieties of tumour. As a group they are rare, in that they represent approximately one in every 1500 individuals who die each year, and three from every 1,000 people who die from cancer each year. Obviously such a low overall incidence makes assessment of changes related to possible causal agents a most difficult exercise in statistical significance.

16610

5. The evidence we have reviewed is based on studies of two quite distinct types of populations. The first is of users of various herbicides and wood preservative products, and the second is of factory workers engaged in the production of chemicals. The ACP is concerned with advising on all risks that may arise from the use of pesticides, as distinct from any risks which may arise in the course of manufacture, and which are strictly speaking outside our remit. We, therefore, concentrated on the first type of population - pesticide users - and our advice is based on the weight of evidence emerging for this type. Nevertheless, we also examined factory studies to ensure that no evidence which might have a bearing on our task had been overlooked.

Discussion of Evidence - Herbicides and Wood Preservatives

6. Studies of pesticide user groups have been undertaken in various countries and consist both of case control and mortality investigations. The case control studies involved a comparison of individuals with soft tissue sarcomas whose exposure to herbicides and wood preservatives had been assessed mainly by memory recall, with control groups who either had no cancer or other types of cancer. In the mortality studies the incidence of death among groups of workers largely identified on the basis of occupational histories was compared with national or regional mortality statistics.

DOH 2127135

7. For users of herbicides and wood preservatives, a positive statistical association between reported exposure and soft tissue sarcomas had been suggested from two studies carried out in Sweden. Successive reports on a similar case control study have also become available from New Zealand. These showed no correlation between exposure to phenoxy acid herbicides and soft tissue sarcomas. It is our view that the procedures used for establishing the Swedish study groups, and some aspects of the collection of the exposure data, were not reliable. In that country, separate case control studies were undertaken in Northern and Southern Sweden following publication of a previous clinical report in which 7 individuals with soft tissue sarcomas recalled being exposed occupationally to phenoxy acid herbicides at some time during the 1950's and 1960's. I should explain that whenever clinical evidence of that kind becomes available in any area, it is normal epidemiological practice to follow up the findings by means of investigations among totally different groups, thus preventing the possibility of bias. In the follow up study in Northern Sweden, however, the 13 cases in which exposure to phenoxy acid herbicides was claimed (amongst a total of 52 cases of soft tissue sarcomas) included the 7 cases identified during the earlier clinical investigations. We consider this to be unorthodox epidemiological practice; if these 7 cases are omitted from the study, the relative risk is reduced to within error limits. This was not the only defect in the study. For example, patients and controls who reported exposure only to chlorophenols were excluded from the calculation of the relative risk factors associated with phenoxy acid herbicides while the control group excluded patients with any malignancies. The relative risk factor for chlorophenols was similarly artificially raised by excluding patients and controls who reported exposure only to phenoxy acid herbicides. Moreover, that study, and the further one in Southern Sweden, were conducted at least one year after a publication drawing attention to the possibility of the suggested association from clinical experience. Because public awareness of alleged risks from 2,4,5-T herbicides had been acute for some years, there is a probability that a bias from exposure recall occurred in both studies.

8. Although they may be valuable, case control studies are not a precise tool for establishing a causal relationship. They can be very unreliable and misleading unless supported by sound documented information on exposure. Such information was not available in either of the Swedish studies, where it was largely based on the memory recall of individual patients or their relatives. We have, therefore, looked for corroborative evidence from mortality studies of cohorts of herbicide users. We have found none. Two such cohorts have been studied in Sweden and one in Finland. A total of 2,461 individuals were included in them. There was no overall increase in deaths from all causes or from cancer. No case of soft tissue sarcomas was identified. Similarly, no correlation between soft tissue sarcomas and occupations involving the use of phenoxy acid herbicides emerged from a study of deaths in Washington State between 1950-1979. We comment further on this study in paragraph 13 below.

9. One feature of our 1980 enquiry again became evident during the current one and has already been referred to above. This is that much of the evidence depends on personal recollection of what took place well before the association was claimed, and that recollection may have been influenced by later publicity. In our 1980 report we argued, in relation to miscarriages and birth deformities, that it can be relatively easy to select some products, or other factor, and without enquiring very closely, to assume some connection with family misfortunes of that kind. This tendency has again been illustrated by a recent publication in this country ("Portrait of a Poison: The 2,4,5-T Story") in which two deaths were attributed to soft tissue sarcomas developing after alleged exposure to 2,4,5-T herbicides. We have enquired carefully into these cases, which have been given some publicity. In addition to the lack of reliable exposure data, we have established beyond doubt that in neither case was the tumour involved a soft tissue sarcoma.

Conclusions - Herbicides and Wood Preservatives

10. In summary, a number of factors have emerged from our analysis of studies of user groups that are relevant to the assessment of the safety of 2,4,5-T herbicides in this country. Firstly, none of the reports we have examined is concerned only with use of formulated 2,4,5-T herbicides, or indeed, only with production of 2,4,5-T. These studies relate to the phenoxy acid group of herbicides as a whole, as well as to wood preservatives. In this country, use of 2,4,5-T herbicides constitutes only a small fraction of the total use of all these products. Secondly, deficiencies in those studies that gave positive results are such as artificially to raise the calculated relative risk factor. And thirdly, we are not aware of any instance in this country where an individual has used 2,4,5-T herbicides and developed a soft tissue sarcoma. We conclude that there continue to be no grounds for changing our previous advice that formulations of 2,4,5-T herbicides as presently cleared, or, for that matter, other phenoxy acid herbicides and related wood preservatives, do not pose a safety hazard, whether used in agriculture, forestry, the home and garden, or elsewhere.

11. However, it has always been our normal practice to keep the safety of any pesticide under continuous review, and to take account of any additional relevant evidence that may become available. In the present case, we believe it would be practicable and desirable to pursue some further enquiries, based on available UK cancer mortality data, in order to establish whether there has been any change in the incidence of soft tissue sarcomas over the past 20 years, and whether there has been any correlation between such sarcomas and particular occupations. This will take a little more time, but without such an analysis the examination we have made of all known relevant studies would not be complete. We will, therefore, be reporting further.

12. Separately from this we suggest that the Government should consider arranging for systematic and complementary retrospective and prospective case control investigations to be undertaken as a contribution to the work being done internationally in this area. To this end we set out, in Appendix B, details of the kind of enquiries which would be appropriate and comment on the problems involved in setting them up, and on their potential value.

Discussion of Evidence - Production of Chemicals

13. The second type of evidence we have reviewed (paragraph 5 above) comes from mortality and disease data of cohorts of chemical workers in the USA, West Germany, Czechoslovakia and the UK. Whereas in the last three of these countries the findings were negative, positive evidence has emerged only from the USA, where 7 individual cases of soft tissue sarcomas have been identified in various studies. Three of these cases were the subject of individual clinical reports where neither the size of the exposed population nor the nature and extent of exposure, if any, of the patients was established. Another case occurred among an undefined group of chemical production workers. The remaining three cases, each of which had a prior history of chloracne testifying to the fact and intensity of toxicologically significant exposure to dioxins, were drawn from two cohorts comprising a total of 182 chemical production workers. Exposure to chlorophenols was common to the latter 4 cases. Other cohort studies in the USA and elsewhere among workers similarly exposed to chlorophenols have revealed no cases of soft tissue sarcomas among a total of 460 workers. Two further American studies, involving the retrospective analysis of mortality data, did not establish any association betwen the incidence of soft tissue sarcomas and likely occupational exposure to either phenoxy acid herbicides or chlorophenols.

DOH 2127137

14. We do not consider these studies to be relevant to any assessment of the safety in use of the phenoxy acid group of herbicides, because the circumstances, nature and extent of possible exposure differ greatly between the factory and the field, the forest or the garden. The studies of cohorts of chemical production workers which we have examined, and which identified the occurrence of chloracne among some of the individual cases, indicated that exposure was to both a wider range of substances and to higher levels than was the case with users of formulated products, and that it included exposure to contaminants which have now been virtually eliminated from formulated products. There was no such evidence among the populations who had merely used the formulated products.

Conclusion - Production of Chemicals

15. In this country, chlorophenols are produced and used in many situations outside agriculture and wood preservation. A few have limited use in their own right, but the majority are used, or have been used, in the production of a wide range of products such as adhesives, dyes, paints, inks, disinfectants, wax emulsions, cutting oils, fire extinguishers, ropes, tents, pharmaceuticals, toothpastes and cosmetics, as well as in the leather, textile and paper and board industries. The evidence we have examined concerning chemical production workers leads us to the conclusion that it would be prudent for the Government to undertake further relevant investigations in factory situations in this country. Neither the factory processing of chlorophenols nor the safety of the products and practices described above is within the remit of the Advisory Committee on Pesticides; but we would wish to be involved in any such wider investigations in so far as these might relate to the specific area of the production of herbicides and wood preservatives.

Yours sincerely,

Robert Kilpatrick
Chairman
Advisory Committee on Pesticides

DOW CHEMICAL U.S.A.

ROUTE 1, BOX 1313
DAVIS, CALIFORNIA 95616
916 · 753-6608

August 28, 1980

J. B. SWEELEY
MASONITE CORPORATION
WESTERN WOODLANDS DIV.
P.O. BOX 97
CALPELLA CA 95418

bcc: W. E. Allison
J. H. Davidson
D. D. Hunt
M. G. Norris
A. J. Watson

MN3011159 UUM 2124046

Dear Jack,

Regarding your letter of August 12, I just returned from vacation -- hence the belated reply.

Based on our knowledge of 2,4-D and 2,4,5-T, the claim or insinuation that these materials could cause any adverse effect on humans or animals at the low exposure levels resulting from applications several years previously is spurious. Hundreds of water samples have shown that the levels immediately below the treated area are less than 10-15 ppb. Data from Norris and others indicates that the levels are reduced to non-detectable ones in ½ mile or so downstream. There is a tolerance for 2,4-D in potable water for 100 ppb.

At the rate 2,4,5-T was applied, there would have been very little in the water immediately after application and only if run-off occurred in the spring after treatment. If there was some TCDD in the 2,4,5-T it would have been decomposed mostly by sunlight and if it did get to the soil or duff it is so insoluble that it would be extremely unlikely to get to the water by run-off.

It is interesting that workers in the Dow 2,4,5-T plant have not reported chloracne during the 25 years of operation. Chloracne is the earliest expression of reaction to TCDD. Nor have any adverse effects been reported by workers in the 2,4-D plants. These groups have been observed very carefully.

You may want some references to support the above statements; a copy of the CAST report on Phenoxy Herbicides and several others concerned with phenoxy herbicides that may be of interest are enclosed.

Since it is very unlikely that the "itchy back" resulted from exposure to the forest herbicides, we can look for other causes. If Mr. Johnson "swims" in the creek that is too shallow to swim in, he may be exposed to poison oak or a number of other plants that can cause skin problems. Of course, many other things, like soap, cleaners, oils, etc., are known irritants.

LIFE IS FRAGILE HANDLE WITH CARE

It seems odd that the "itchy back" has only now become serious enough to cause a complaint -- 7 years after the last application. You may recall the Seveso incident where 2-4 lbs. of TCDD was distributed over 200 to 400 acres in Italy. Children and adults were not moved out for 2 weeks. Many cases of serious chloracne were treated, but after 12-18 months all cases had healed up; there have been no other observed adverse effects above those in the surrounding populations. The 2,4-D may last for 2-3 months and the 2,4,5-T about 4 to 6 months. It's quite impossible for these materials to last more than a year or 2 in the trees and it disappears in 2-4 weeks from soil. The spring treatments would have allowed these or faster rates of decomposition because of warming conditions and good soil moisture. The previous treatments should have enriched the microorganisms and enhanced 2,4-D breakdown.

I will pass this on to other members of our staff who may have added comments of value. I hope this analysis and the reports will help refute the allusion of Mr. Johnson.

Yours very truly,

Jack

L. E. Warren
Agricultural Products Department
Technical Service and Development

Enc.

cc: J. Jones, DOW CHEMICAL U.S.A., Sacramento.
Matt Anderson, CFPA, Sacramento
Norman Parker, Western Helicopters, Newberg, OR.

kim

DOW 2124047

Dow Chemical Co.
EPA Hearing
Submissions Re: Cancellation - Volume 2

16. "Petition for the Establishment of Tolerances for the Pesticide Chemical, (2,4,5-T) on Raw Agricultural Commodities", Sec. C: Full Reports of Investigations re: 2,4,5-T, 12/8/67:

(1) Summary of 11 tests on safety of 2,4,5-T (A-K below)

(2) Conclusion:

-more data on 2,4,-D than 2,4,5-T is available, but indications are that two materials are comparable; acute oral toxicity of both in lab animals is in same range, 300-1000 mg/kg.

-long history of usefulness not produced evidence of unusual toxicity.

A. "Acute Oral Toxicity of 2,4,5-T to Rats, Mice, Guinea Pigs and Chicks", Rowe, McCollister and Spencer, (Dow, 1950).

(1) Single oral doses (administered by stomach tube) of 2,4,5-T as a 10% suspension in olive oil, to mixed sex group of rats, mice and guinea pigs, 3% solution in olive oil given to chicks.

(2) Symptons of poisoning = ataxia, myotonia; death at highest dosage levels; doses ranged from 0.1 - 1.0 grams/kg.

(3) LD 50's:

rats	=	0.500	grams/kg.
mice	=	0.389	"
guinea pigs	=	0.381	"
chicks	=	0.310	"

(4) 2,4,5-T significantly more toxic to chicks than to rats.

B. "Toxicity of 2.4-D and 2,4,5-T - a report on their acute and chronic toxicity in dogs", Drill and Hiratzka, 1953. ("There have been no reports concerning the toxic properties of 2,4,5-T").

(1) Experimental Procedures: adult mongrel dogs of both sexes

used, fed "Friskies" ad lib, commercial 2,4-D and 2,4,5-T used.

-single oral dose administered in capsules. Survivors autopsied after 14 days (acute studies).

-oral administration in capsules imbedded in 4-5 gram piece of commercial canned dog food, 5 days/wk for 13 weeks (chronic studies).

(2) Results of Acute Studies:

(a) 2,4-D:

-400 mg/kg caused death in 2/2 dogs in 2-3 days following administration.

-250 mg/kg - death in 3/3 in 4-8 days

-100 mg/kg - 2/4 died in 9 days, 2 survived to 14th da

- 25 mg/kg - no deaths, all 3 survived to day 14

(b) 2,4,5-T:

-400 mg/kg - 1/1 died on day 2

-250 mg/kg - 1/1 died on day 3

-100 mg/kg - 1/4 died on day 7, 3 survived to day 14

- 50 mg/kg - no deaths, all survived to day 14

(c) Oral LD_{50} for 2,4-D = approx. 100 mg/kg; for 2,4,5-T, = 100 mg/kg or higher

(d) Body weight - decrease with larger doses of both 2,4-D and 2,4,5-T; these animals developed anorexia, those that died refused food towards end.

(e) Symptoms: ataxia, stiffness in hind legs, myotonia

(f) Pathology: gastrointestinal changes - redness in small intestine, some necrosis and inflamma hepatic necrosis, mild renal tubular degenerati deaths due to nonspecific changes - hepatic con many cases of death due to pneumonia.

(3) Results of Chronic Studies:

(a) Mortality -

-2,5,10 mg 2,4-D or 2,4,5-T/kg: all dogs survi 90-day test period

-20 mg/kg of compounds: all dogs died during st

(b) Body Weight - loss in weight occurred signific only in those animals that died

(c) Symptoms - survivors were free of any symptoms; both 2,4-D and 2,4,5-T dead, weakness, leg stiff difficulty in swallowing food, bleeding from gu

(d) Blood Count - only change observed = fall in % c lymphocytes in 3 animals that died.

(e) Organ Weights - no change in survivors; in 2 of 4-D deaths, slight increase in heart and kidney similar change in 2 of 4 2,4,5-T deaths.

(f) Gross Pathology - 2,4-D:2 dogs showed areas of ness in duodenum; 2,4,5-T : one dog showed di reddened duodenum and jejunum, another demonstr a generalized icterus.

(g) Microscopic Changes - 5 cases of focal necrosis liver (not related to dosages nor death, thus, significance). Some duodenal hyperemia, some infiltration of cells in the mucosa. Submucosa serosa were normal. Slight increase in # of c in kidney sections in some dogs (unrelated to d and of doubtful significance).

(4) Comment:

-effects of 2,4,5-T not as severe as those of 2,4-D in acute studies.

-delayed death in chronic studies with 2,4-D may indicat a cumulative effect of 2,4-D.

-signs observed in dying animals in acute study differe from those in chronic study.

(5) Summary: 2,4-D acute oral LD_{50} in dogs = 100 mg/kg, doses at this level or higher produced a definite myotonia with anorexia and weight loss.

2,4,5-T acute oral LD_{50} in dogs = 100 mg/kg or higher; such doses produced only signs of a mild spasticity.

Death during the repeated administration of 2,4-D or 2,4 5-T was not related to pathological changes in the liver, kidney, or other organs examined.

C. "Summary of Toxicological Information on 2,4-D and 2,4,5-T Type Herbicides and an Evaluation of the Hazards to livestock associated with their use", (Rowe and Hymas, 1954).

(1) Early in 1950, Grigsby and Farwell published results of experiments with 2,4-D and 2,4,5-T involving domestic livestock, concluded "it seems that the farm use of these materials for pasture weed control is a reasonably safe procedure."

(2) Commercial Herbicides - formulations of active ingredients appearing as a salt (water-soluble) or an ester (soluble in oil); most formulations also contain a dispersant, a solvent, a wetting agent, perhaps a diluent (usually considered inert ingredients though they may affect herbicidal effectiveness).

(3) Oral Administration of Single Doses:

(a) test materials administered primarily by intubation.

(b) aqueous, or olive oil or corn oil, solutions used

(c) LD_{50}'s:

	2,4,5-T	2,4-D
rats =	500 mg/kg	375 mg/kg
mice =	389 mg/kg	368 mg/kg
guinea pigs =	381 mg/kg	469 mg/kg
chicks =	310 mg/kg	541 mg/kg
dogs =	100 mg/kg	100 mg/kg

(d) toxic symptons of LD_{50}'s include loss of appetite, weight loss, depression, roughness of coat, general tenseness, muscular weakness; post mortem findings - stomach irritation, minor liver and kidney injury, lung congestion.

(4) Oral Administration of Repeated Doses of 2,4-D:

(a) Rats - fed 2,4-D 5 times/week by intubation, in olive oil solutions. Dosages were 0.0, 3.0, 10.0, 30.0, 100.0, 300 mg/kg.

Results - no adverse effects at control, 3, 10, 30 mg/kg levels; 100 mg/kg group - gastrointestinal irritation, swelling in liver, depressed growth rate; 300 mg/kg group failed rapidly & died, severe gastro-irritation observed.

(b) Another Rat Study - fed 2,4-D for 113 days at levels of 0, 100, 300, 1000, 3000 and 10,000 ppm.

Results - no adverse effects in control, 100 and 300 ppm groups; 1000 ppm group suffered depressed growth rate, excessive mortality, slightly increased liver weights, liver swelling; 3000 and 10,000 ppm groups were destroyed after 12 days since they were not eating and were losing weight; examination revealed increased liver and kidney weight, l. and k. pathological changes.

(c) Chicks - fed 2,4-D for 7 days at levels of 0,500, 1,000 and 3000 ppm.

Results - only adverse effects in 3000 ppm group - reduction in food intake and retarded growth rate (no histopathological exams made).

(5) Oral Administration to Steers - (experiments performed to see if extrapolation of data on small animals to larger animals is justified).

(a) "Esteron Brush Killer" used (formulation of esters of 2,4-D and 2,4,5-T); administrations made by intubatio

-experiment #1: 291 kg steer given single dose of 1000 mg/kg - no toxic symptoms.

-experiment #2: 295 kg steer given 1000 mg/kg estero for 3 days.

results: general depression, decreased food and water intake, decreased rumen motility; animal died on 3rd day after last dose (death was undra matic). Necropsy revealed dry rumen contents with strong smell of esteron, abomasum was impacted, intestinal contents entirely fluid, congested mesenteric vessels, spleen dark and shrunken.

-experiment #3: 295 kg steer given 500 mg/kg for 2 days.

results: on 3rd day, animal was off feed, rumen motility ceased. On 4th day, steer appeared perfectly normal, no discernible after-effects.

-experiment #4: 336 kg steer given 500 mg/kg for 3 days.

results: no toxic symptoms observed, animal remained on full feed.

-experiment #5: 250 kg steer given 100 mg/kg for 15 days.

results: outward appearance normal. Killed 48 hrs after last dose for study. Duodenal hemorrhage, abomasum irritation, hemorrhagic necrosis of liver, fatty degeneration, slight edema and congestion in kidneys were found.

(b) "Kuron" used in another experiment: 250 kg steer given 100 mg/kg Kuron for 15 days.

results: outward appearance normal. Killed 48 hours after last dose for examination. Very slight edema in kidney, but this is frequently observed in untreated animals; its significance is questionable.

(6) Discussion:

(a) Dogs more susceptible to 2,4-D and 2,4,5-T than other species studied; chicks appear tolerant.

(b) "Inert" ingredients in commercial formulations have no effect on toxicity of active materials.

(c) 2,4-D and 2,4,5-T can be tolerated without adverse effects in doses only slightly smaller than those which cause toxic effects when given only once - these materials have a low degree of chronicity, therefore.

(d) There is a similarity in susceptibility of cattle and small lab animals to these herbicides.

(e) If esteron were applied at the normal rate of no more than 47 mg/sq. ft. (or 2 qts. per acre) a 350 kg (770 lb.) animal would have to consume all forage on 744 sq. ft.to acquire a 100 mg/kg dose of herbicide, a level at which ill effects would not be expected.

(f) ". . . there is little, if any, direct hazard to livestock or wild-life foraging areas treated with herbicides of the type described herein. This has been confirmed by the extensive use of these materials over a number of years without any proved cases of adverse effects."

(g) Spraying of certain plants with 2,4-D can increase their nitrite content to hazardous levels, but some of these plants were sprayed accidentally, and others are not ordinarily eaten by livestock. Toxic amounts of these materials can be obtained if animals have access to spray tanks or other containers of the material.

D. "Results of Toxicological Tests on (2,4,5-T)", Dow, August 1950. (data submitted to FDA Hearing on residue tolerances).

(1) Because of similarities between 2,4-D and 2,4,5-T, residue tolerances for both should be same - 20 ppm "would provide a wide margin of safety."

(2) 10% solution of 2,4,5-T in propylene glycol introduced into rabbit eye caused pain and irritation immediately, severe conjunctivitis and moderate corneal damage apparent 5 hours later, continued for 2 days before healing began.

(3) 10% solution of 2,4,5-T in butyl carbitol acetate applied to rabbit ear and abdomen caused slight irritation.

(4) 2,4,5-T is moderate in acute oral toxicity for the four species, rat, mouse, cavie, and chick (rat more resistant, chick most susceptible).

(5) Handling hazards and precautions:

-swallowing, although unlikely, could cause serious ill effects; induce vomiting.

-avoid contact with eyes and skin, don't wear clothing or shoes contaminated with material.

-no inhalation studies to date, but doubtful that toxic concentrations would be inhaled due to irritation it will produce in upper respiratory passages.

E. "Toxicity tests in NuAmine D/T", Report to Diamond Alkali Company by Hazleton labs, February 1962.

(1) NuAmine D/T = 1,3 - propylene diamine esters of 2,4-D and 2,4,5-T.

(2) Acute Oral Administration - NuAmine D/T given to Sprague-Dawley rats (6 groups of 5 each) by stomach tube, either in water emulsion or undiluted dosages of 10, 31.6, 100, 316, 1000 or 3160 micrograms/kg body weight. Concentrations of material varied from 1%, 10%, or 100%. Observation period of 14 days following dosing.

(a) Mortality: After 24 hrs., 2 in 1000 microgram/kg group, and all (5/5) in 3160 microgram/kg group died. On day 12, another in 1000 microgram group died, and another on day 14. These were only deaths recorded.

(b) Other Effects:

-10, 31.6, and 100 micrograms/kg groups: normal appearance and behavior, increase in body weight, no pathological findings.

-316 micrograms/kg: 4/5 seemed normal, 1 was depressed on days 12-14. Slight increase in average body weight 2/5 showed congestion and hemorrhage of lungs.

-1000 micrograms/kg: 4/5 normal at first, 1 showed depression, ataxia, and labored respiration after 4 hrs. 2 died after 24 hrs., survivors showed depression and labored respiration. After 48 hrs., all survivors appeared normal; at 2nd week, all showed unthriftiness, depression, labored respiration and ataxia. 2 more died on days 12-14.

Autopsies showed lung, adrenal congestion, kidney and stomach hemorrhage, hematuria, intestinal inflamation, discoloration and/or reduced size in kidneys spleen and liver, stomach inflammation, congestion of fat around tests.

-3160 micrograms/kg: appeared normal following intubation; depression and ataxia after 1 hr.; prostration, ptosis, salivation, labored respiration, poor reflexes after 4 hours; 1 death at 5 hours, survivors showed hematuria. Total mortality by 24 hours.

Autopsies revealed congestion of lungs, adrenals, liver and spleen; kidney and stomach mucosa hemorrhage intestinal inflammation; bloody fluid in stomach and urinary bladder.

(3) Acute Eye Application - NuAmine D/T applied to eyes of 3 albino rabbits (left eye only, right served as control).

Single application of 0.05 ml of undiluted test material made into conjunctival sac. Periodic observations for 1 week. Food (Purina rabbit pellets) and water ad lib.

(a) Results: Animals seemed normal throughout study, slight decrease in body weights in some.

Eyes showed erythema, film over cornea, slight vascularization of sclera and nictitating membrane, lacrimation, edema of lids (obscuring eye and preventing thorough exam after a few hours), exudation, corneal opacity, and corneal lesion (1 animal).

(4) Acute Dermal Application - albino rabbits used; single application of 50% volume/volume aqueous emulsion of NuAmine D/T at dose of 0.1 mg/kg body weight, and single application of undiluted test material at doses of 0.1, 0.5, 2.0 ml/kg. Test material applied to abdominal skin. "Purina Rabbit Pellets" and water ad lib.

(a) Results: 3 deaths at 2 ml/kg level, no deaths at any other level. Estimated acute dermal LD_{50} of NuAmine D/T for albino rabbits = 1.2 ml/kg.

1. 50% emulsion: application(0.1 ml/kg) produced no signs of systemic toxicity, no pathological finding 24 hours after application - moderate erythema and edema. At fourth and fifth days, erythema and edem subsided, but animals showed slight or moderate atonia and desquamation. By 12th day, almost all signs of irritation gone.

2. Undiluted:

-0.1 ml/kg - normal appearance and behavior. Autopsy showed kidney congestion, mottled and blanched livers.

-0.5 ml/kg - normal appearance and behavior. Autopsy showed kidney congestion, and granular appearance of liver.

-2.0 ml/kg - 3 deaths at this level, on 4th, 5th, and 7th days of observation. 24 hrs. post application, 3 animals showed depression and labored respiration, 4th seemed normal. Toxic signs gradually became more severe: depressed reflexes and coordination, labored respiration, ptosis, ataxia, coma phonation, salivation. Autopsies showed congestion or hemorrhage of lungs and kidney; bloody urine in 1 bladder, bright yellow urine in another; liver damage (blanching and discoloration) and toughened liver surface; spongy brain tissue with excessive fluid in skull; extremely congested or hemorrhagic appearance of skull cap and bone marrow. One survivor was sacrificed; showed congestion of kidneys. Subcutaneous edema, damage to all layers of the skin.

Dermal effects - 24 hrs. after application, skin at all dosage levels showed edema and blanched areas surrounded by moderate erythema. In 3 animal that died at highest dosage, dermal irritation was relatively unchanged at time of death. Survivors in all levels - edema subsided after 3-5 days, scab formations or marked desquamation, atonia, coriaceousness, and fissuring developed to end of observation.

(5) Repeated Dermal Applications - albino rabbits given 1 ml/k NuAmine D/T in varying strengths (4, 12, 20 oz./gal. aqueous emulsions) 5 days a week for 4 weeks.

Results: each group showed moderate degree of dermal irritation - erythema, edema, atonia, desquamation. Several in 20 oz./gal. group showed fissuring. One animal in each of the test groups died, all showed systemic toxicity - depression, labored respiration, sprawling of limbs, weakness, weight loss. Moribund animals also showed prostration, poor coordination, depressed reflexes, clonic convulsions. Abnormal blood and/or urine findings in each group, severe liver, kidney and skin damage in 4 and 20 oz./gal. groups.

E-1 "Microscopic examination of bone marrow sections" from NuAmine D/T study, Hazleton Labs report to Diamond Alkali Co., 12/16/65. - supplement to report of 2/23/62; tests on bone marrow taken from rabbits receiving repeated dermal applications of NuAmine-D/T.

Results: No distinctive effect on bone marrow at 2 concentrations (4 and 12 oz./gal.). Higher concentration (20 oz./gal.) group showed somewhat atrophied adipose tissue. A possible effect - megakaryocytes (in 20 oz./gal. group) underwent degenerative changes, more so than in controls.

F. "Results of 90-day Dietary Feeding Studies of Dowanol 97B (ester of 2,4,5-T) in Rats", Dow, 11/27/61

(1) Dowanol 97B ester of 2,4,5-T is an agricultural herbicide (amber liquid)

(2) experimental procedure: 5 groups of 20 m & f rats were maintained for 90 days on diets containing 0.0(control), 0.3, 0.1, 0.03, and 0.01% of the ester. Food (ground Famo Laboratory Chow) and water ad lib.

(3) Results:

0.03 and 0.01% levels: no evidence of adverse effects increase in average weight of spleen in males, increase in body weight of females - believed due merely to chance.

0.3% level: growth retardation in males, liver and kidney/body weight ratio increased in males. M and F livers were large and light in color, some swelling and necrosis. Some swelling in male kidneys; female kidneys appeared greenish, some swelling and necrosis. Increase in serum alkaline-phosphatase determinations in males.

0.1% level: livers (m and f) showed some swelling and necrosis, females showed hypercellularity of glomerular tuft, swelling of renal tubular epithelium. Increase in average male kidney weight.

(4) Conclusions: the Dowanol 97B ester of 2,4,5-T is moderate in repeated oral toxicity in 90 day feedings. Levels of 0.03% and below were tolerated without evidence of adverse effect. Male and female rats showed some injurious effects (liver & kidney pathology) at levels of 0.1% and above.

Long-term dietary studies (2 years, with rats and dogs) may be required if ester is found in significant amounts as residue in food.

G. "The Toxicologic Effects of Certain Fungicides and Herbicides on Sheep and Cattle", Palmer and Radeleff, 1964

(1) "In contrast to insecticides, organic fungicides and herbicides (with only a few exceptions) have generally been considered to provide comfortable margins of safety for grazing livestock when the compounds are used according to directions."

(2) Sheep and cattle maintained on grain concentrates with hay supplements.

(3) Commercially available formulations used, as fluid dilutions or in gelatin capsules; number of doses varies.

Results:

(1) Fungicides -

a) "Captan": sheep are regularly poisoned by doses of 250 mg./kg or higher; reduction to 50 or 100 mg/kg permits animals to eliminate most of material without being harmed. Cattle more tolerant of Captan than sheep. Depression and anorexia, tendency to stay away from group were only toxic signs in sheep; in one heifer, diarrhea and abortion observed. At necropsy, hepatic and renal changes noted but not remarkable.

b) "Zineb": Single doses of 500 mg/kg. tolerated by sheep and cattle. 90 daily doses of 100 or 250 mg./kg. did not poison sheep. In single poisoned sheep (15 doses of 500 mg./kg.), anorexia and bright yellow diarrhea observed. Necropsy showed congestion of the lungs, hepatitis, and nephritis.

c) "Ceresan M": Sheep fed 1, 1.5, or 2 lbs. of treated grain per day. Lethal to all, at doses of 1 lb./day x 33 doses, 1.5 lb./day x 22 doses, or 2 lb./day x 6 doses. Signs of poisoning included anorexia, diarrhea, weight loss, nasal discharge, stilted gait and lameness. Necropsy showed lesions characteristic for mercurial poisoning. (Ceresan contains mercury).

(2) Herbicides -

a) 2,4-D: administered as alkanolamine salt and as propylene glycol butyl ether ester to sheep and cattle. Very little difference in toxicity of 2 derivatives. "Remarkable tolerance" of 481 daily doses of 100 mg./kg. by sheep indicates little probability of acute poisoning in normal applications of the compounds. Toxic signs in sheep - anorexia, depression, weight loss; in cattle - muscular weakness, ataxia, dry, cracked muzzle, ulceration of nasal mucous membrane. Necropsy - lesions varied usually liver and kidney degeneration; rumen stasis with bright, undigested ingesta; hemorrhages in heart and excess of pericardial fluid.

b) 2, 4, 5-T: administered as triethylamine salt and as propylene glycol butyl ether ester to sheep and cattle. Salt appeared least toxic (sheep tolerated 481 doses of 100 mg./kg.) while esters were lethal at 369 doses of 100 mg./kg. Esters were lethal to sheep and cattle after 7 doses of 250 mg./kg. 2, 4, 5-T somewhat more toxic to sheep and cattle than 2, 4-D, "although poisoning under ordinary circumstances of use would be most unlikely."

c) 2, 4, 5-TP (2 - (2, 4, 5-trichlorophenoxy) - propionic acid) administered as butyl ether esters of propionic acid derivative to sheep and cattle. Markedly more toxic than 2,4-D or 2, 4, 5-T (sheep succumbed to 11 doses of 100 mg./kg., cattle to 29 such doses). Signs of poisoning: anorexia & progressive weight loss. Necropsy showed degeneration of liver and kidneys, hemorrhages on heart surface, congestion of visceral blood vessels, swelling of lymph glands, rumen atony with large quantities of ingesta.

(3) Benzoic Acid Compounds - dimethylamine salts of 2, 3, 6-trichloro- (Trysben) and 2-methoxy - 3,6-dichloro enzoic acids (Banvel D) tested. Both compounds slightly more toxic than phenoxyacetic acids studies. Trysben at dosage of 250 mg./kg. x 10 doses was lethal to the one sheep, but the cow was unaffected; at 500 mg./kg. x 2 doses, the compound produced mild poisoning in one other cow. Banvel D in doses of 250 mg./kg. x 10 doses did not affect a sheep, but killed another sheep at 500 mg./kg. x 2 doses. A cow was not affected by 5 doses of 250mg/kg. Signs of poisoning: salivation, trembling, depression, tympanites. Necropsy showed congested lungs, hemorrhages on heart, pericardium and larger blood vessels, undigested feed in rumen, edema in respiratory system, inflamed kidney liver engorged with blood.

(4) Triazines - (Propazine, Simazine, and Atrazine): administered to sheep and cattle; Atrazine was lethal at all doses tested (50 mg./kg. x 199 doses, 100 mg. x 16, 250 x 2) for sheep, and lethal to the cow tested at 250 mg./kg. x 2 doses. At the 250 mg./kg. level in sheep, Simazine was more toxic than Propazine; at 50 mg./kg. Simazine was more toxic than Atrazine. Signs of poisoning (Propazine anorexia and depression. Signs of poisoning (Atrazine and Simazine): muscular spasms and fasciculation, stiff gait, increased respiratory rates. Necropsy in sheep and cattle given Atrazine showed degeneration and discoloration of the adrenal glands; congestion of lungs, liver and kidneys; presence of undigested food in rumens.

DOH2060425

(5) Miscellaneous compounds:

a) Dalapon (2, 2-dichloropropionic acid) - sodium salt is of a low order of toxicity (500 mg./kg. x 10 doses not toxic to sheep or cattle, one sheep tolerated 481 doses of 100 mg./kg.) No poisonous signs.

b) MCP Amine (2-methyl (4-chloro-o-toloxyacetic acid) - alkanolamine salts poisoned one sheep lethally at 383 doses of 100 mg./kg.; a cow was poisoned by 8 doses of 500 mg./kg. but survived. Signs of poisoning in cattle: anorexia, chronic tympanites; in sheep, anorexia, depression and ataxia before death.

c) Fenuron (3-phenol-1, 1-dimethyl urea) - appeared more toxic than most herbicides discussed, but high dosages still required to produce poisoning (e.g., 250-500 mg./kg. x 2-10 doses). Toxic signs: depression, anorexia, complete incoordination (heifer - abortion); slow recovery. Necropsy: lung congestion, heart hemorrhages.

d) Bandane (polychlorodicyclo pentadiene isomers) - sheep poisoned by 3 doses of 250 mg./kg. but survived, while same dosage was lethal for cattle. Toxic signs in sheep: salivation, anorexia, tympanites, dyspnea and depression. Toxic signs in cattle: tense muscles, muscular spasms. (Even when dosing stopped, signs progressed, duplicating tetanus). Death preceded by convulsions. Necropsy: hemorrhages of cerebrum and medulla oblongata, hemorrhage of right psoas muscle, skeletal fat replaced by gelatinous material, lung congestion.

(6) Discussion: studies offer no "reason to alter the already generally accepted concept of the hazards presented by these herbicides." Further study needed re toxic signs.

(7) Conclusions: feeding Ceresan M (13-38 mg./kg.) on treated grain by mistake can easily poison sheep; other materials studied are safe when used as recommended.

H. "Commercial Fishery Investigations", Philip A. Butler - Acute and Chronic Toxicity Studies, 1964

(1) purpose of project: to learn how to protect and preserve marine environment from possibly adverse effects of agricultural chemicals; discovery of pesticides that may be useful in improving fish harvests

16630

(2) "The need for this research does not imply that the widespread use of pesticide formulations automatically constitutes a serious threat to marine life."

(3) "Even the most harmless of chemicals, including water, is toxic when present in sufficient quantity."

(4) different forms of marine life may react quite differently to the same chemical

(5) four bioassay groups (phytoplankton metabolism, oyster shell growth, shrimp survival, fish survival) tested for sensitivity to four pesticides (dyrene, methyl parathion, phosdrin, phosphamidon). Phosdrin and phosphamidon produced the least reaction; dyrene had some effect on all 4 groups, especially fish survival; shrimp survival was very sensitive to methyl parathion.

(6) phytoplankton - microscopic sea plants, essential link in conversion of solar energy into food for more complex marine animals. Using decrease in growth, or decrease in carbon fixation as a standard, toxicities of pesticides may be compared.

(7) crustacea - shrimp is most valuable fishery; most pesticides kill terrestial members of shrimp's phylum (arthropod). Some pesticides paralyze shrimp and other crustaceans, rather than kill; toxic criteria for this group is effective concentration causing paralysis or death to half the samples within a stated period (EC_{50})

(8) mollusks - clams and oysters store high concentrations of chemicals that exist in only trace amounts in the surrounding sea water. Mollusks, however, are able to close their valves and protect themselves from toxic substances in the environment. Thus, tests must be conducted at sublethal concentrations; growth is an objective index to measure, using EC_{50} measure of effect

(9) fish - younger specimens usually most quickly affected by pesticides.

(10) 2, 4, 5-T (polyglycol butyl ether ester):

- EC_{50} for oyster shell growth = 0.14 mg./liter (96 hrs. 1 week recovery period.

I. "Commercial Fisheries Investigations," Philip A. Butler - Laboratory Studies and Toxicology

(1) phytoplankton - majority of chemicals tested caused significant decrease in productivity at 1 ppm, although at lower concentrations an increase in productivity rate was frequently observed (probably because pesticide was toxic to animal part of natural phytoplankton community). Herbicides and DDT very toxic to some plant species.

(2) crustacea - post-larvae of brown shrimp and blue crabs are quite sensitive to chlorinated hydrocarbon insecticides. All shrimp and most crab tests conducted in sea water with acetone stock solution of pesticide (constant flow systems, in which test solutions are renewed continually, have advantage of insuring sufficient oxygen and desired concentration of pesticide). After exposure to lethal or near lethal concentrations, crabs and shrimps often moribund for hours.

(3) mollusks - oyster and clam tested, constant flow system with acetone-stock solutions of pesticides. Oyster closes its shell when sufficiently irritated by contaminated water - does not feed while closed and therefore does not grow; therefore relative chemical toxicity is indicated by changes in growth rate (shell growth). Controls used to compare growth rates. Chlorinated hydrocarbon insecticides were most toxic pesticides to oysters - most inhibited shell growth at well below 1 ppm; other pesticides (organophosphorus and carbamate insecticides) had little or no effect on oyster growth - they decomposed in water. Metabolism increased at summer temperatures; therefore, at higher temperatures some chlorinated hydrocarbon insecticides killed oysters at concentrations which at lower winter temperatures only stopped growth. 1-4 week recovery period for reduced growth rate; data indicates that "a single field application which might temporarily affect oyster growth would probably not have a lasting effect." Chronic, rather than acute, exposure to pollutants is more likely.

Chemicals tested included aldrin, dieldrin, DDT, toxaphene, malathion and acetone (acetone used as a solvent in program, so tested for possible toxicity). In general, no decrease in growth rate; treated groups sometimes grew slightly better than controls. Oysters did not acquire tolerance to pesticides.

- EC_{50} for decrease in shell growth:
  2,4,5-T acid (temps. of 16°, 30°C) - no decrease at 2 ppm
  2,4-D acid (temps: 9°, 30°C) - no decrease at 2 p

(4) fish - juvenile white mullet & longnose killifish used as test animals in running sea water aquaria. 24 and 48 hr. median tolerated limit values (TLm) obtained. Chronic low-level pollution studies important, since many marine fish pass their early growth stages in estuaries.

3 groups of Spot (leiostomus xanthurus) exposed 3 mos. to sublethal concentrations of dieldrin (0.1, 0.01, 0.001 ppb), in running sea water. High mortalities, 30-37%, but also in controls. No differences in mean lengths, though some experimental fish had axial skeletal distortions, not in controls.

Survivors exposed to 2 ppb dieldrin to determine if earlier low-level exposure had created any resistance: 80% of experimental fish survived 24-hr. exposure, while previously unexposed fish all died.

-2,4,5-T acid tested on longnose killifish showed no effect at 50 ppm (24 and 48 hrs. TLm) with 19° C water temperature.

-Esteron 99 (formulation): TLm for 50% mortality in mullet - 1.5 ppm (24 and 48 hrs.) at 20°C; in killifish = 3.5 ppm (24 hrs.) and 3.0 ppm (48 hrs. at 19°C.

(5) Future considerations: "... to determine which pesticid now considered necessary may be expected to cause the least damage to marine resources. . . careful selection of the control agent and method of application can lessen the current pesticide hazards."

J. "Commercial Fishery Investigations", P.A. Butler - Acute Toxicity, 1965

(1) Crustacea - shrimp - most valuable commercial fishery resource, spend part of life span in estuaries susceptible to pesticide pollution. Since they are rather closely related to insects, insecticides are particularly toxic to them - either kill quickly or paralyze them so they lose sense of balance. Tests conducted 24, 48 hrs. in flowing seawater.

Results: 2,4,5-T acid had no effect on brown shrimp mortality or equilibrium at 1 ppm after 24, 48 hrs. (avg. water temp. = 28°C)

2,4,5-T polyglycol butyl ether ester tested on brown shrimp in 28°C water caused 10% mortality (or loss of equilibrium) at 1 ppm after 24 hrs., and 20% mortality at 1 ppm after 48 hrs.

Veon 245 tested on brown shrimp in 26°C water had no effect on mortality of Penaeus aztecus after 24 hr. and 48 hr. exposure at 1 ppm level.

00H20604 29

(2) Fish - younger specimens most quickly affected, therefore juveniles used in tests. Sea water used.

Results:

-2,4,5-T polyglycol butyl ether ester tested on Spot in 16°C water temp.; had 24 and 48 hr. EC_{50} of 0.32 ppm for mortality.

-Veon 245, also tested on Spot, in 27°C, had no effect after 24 and 48-hr. concentrations at 1 ppm.

K. "Recommendations for Minimizing Dangers of Pest Control and Pesticides to Fish and Wildlife", John L. George, June, 1963

(1) be sure there is real need for pesticide use

(2) discuss possible hazards with biologists prior to use

(3) avoid treatment of any significant portion of range of a rare species or one with specialized habits - consult with conservation officials

(4) treat minimum necessary area; avoid direct treatment of streams, etc., consider danger of runoff contamination

(5) judge danger of chemical both by its toxicity and its disappearance rate

(6) use method of application which will minimize contact with wildlife. Emulsions are more toxic to water environments than oil solutions or suspensions. Granules often concentrate the effect of the toxicant in one stratum.

(7) no double-dosing

(8) avoid main spring migration, nesting periods of birds, etc. when choosing time of treatment - consider possibility of using repellants or scare devices to move mobile wildlife from area.

(9) physical or biological control methods have advantage over chemical methods - more specific, reduce pests without loss of beneficial population.

(10) susceptibility to different pesticides varies greatly among animals

00H20604 29

16634

(11) DDT susceptibility: 0.1 - 5 lbs./acre -

heavy kill effect on crustaceans and fish, moderate kill to amphibians and reptiles, some kill to birds, no effect on mammals until 5 lbs./acre level

(12) table with toxicities of other chemicals as compared to DDT: 2,4,5-T derivatives (DDT=1):

rats - LD_{50} of 0.4 (single oral dose)

bobwhites - 0.2 chronic toxicity LD_{50} (10-100 days)

pheasants - 0.1 " " LD_{50} "

mallards - 0.2 " " LD_{50} "

bluegills - LD_{50} of 0.1 (96 hr. test)

(13) disposal of pesticides always difficult procedure - unopened containers should be returned to wholesaler or sold; disposal of opened containers:

- burned in a h o t flame (liquid organic chemicals)
- addition of lime (wettable powders)
- dumping into sanitary landfill-type dump, containerized if soluble
- empty containers - burned or dumped - do not clean equipment at creeks or waterways

D0112060430

16635

17. Dow, "The Analysis of Rice for Residues of [2, 5, 5-T]", 4/25/5

(1) ingredients of Dow, 2, 4, 5-T Amine weed killer

(2) directions for use - 4-8 weeks after emergence of rice (7-9 weeks when flooded), treat with 1 - 2.5 pts. product in approx. 5-7 gallons water per acre (= 0.5-1.25 lbs. 2, 4, 5-T acid)

(3) with some weeds, up to 3 pts./acre may be necessary, but yield may be reduced

(4) treatment after flooding is safer than treatment before flooding

(5) warning: do not apply to vegetables, flowers, grapes, fruit trees, ornamentals, cotton or other desirable plants which are sensitive to 2, 4, 5-T; do not permit spray mist to drift since even minute quantities of the spray may cause injury to them.

(6) residues - methods for determination - extraction, chromatography, colorimetric determination, standard curve. Method capable of determining residues at level of 0.1 ppm.

(7) results indicate residues less than 0.1 ppm when applied at levels of 0.75 and 1.25 lbs./acre.
(2 locations: Beaumont, Texas and South Dos Palos, Texas

00120604 31

18. Devine, J.M., Syracuse University Research Corporation Report on 2,4,5-T Residues in Rough Rice and Rice Straw"

A. Purpose: to determine residues of 2,4,5-T in rough rice and rice straw from Arkansas, Louisiana and Texas

(1) no detectable residues found in any rough rice sample

(2) Louisiana straw samples contained 1.04 ppm residue after 0.75 and 1.5 lb./acre treatment; 0.85 ppm found after a single 1.5 lb./acre treatment

(3) Texas straw contained 0.18 ppm after double treatment (0.75 and 1.5 lb./acre), no detectable residue after single treatment (1.5 lb/acre)

(4) Arkansas straw - detectable residues of 0.02 ppm 2,4,5-T only after single treatment (this level residue is close to limit of detection and may be due to background interferences)

(5) no detectable residues of "bound" 2,4,5-T found (both double and single treatment) in rough rice from all 3 states

(6) 1.5 lb 2,4,5-T/acre mistakenly applied over a Silvex treatment; analysis revealed 0.02 ppm 2,4,5-T.

(7) average recovery of 2,4,5-T from rice and straw was 84±10.5%

B. Supplements, etc.

(1) procedure for free chlorophenoxy acids

(2) procedure for bound chlorophenoxy acids

(3) appendix:

Arkansas - rice seeded 5/6/69, treated 6/16 and 7/11 harvested 10/3-6

Louisiana - rice seeded 5/12/69, treated 6/19 and 7/ harvested 8/28

Texas - rice emerged 5/4/69, treated 5/28 and 6/23, harvested 8/15

C. Letter from Woodbury to Dunn (both Hercules, Inc.) 6/3/69 re cooperators in phenoxy rice residue project, samples they are using and approximate harvest dates.

D. Suggested Protocol - Phenoxy Herbicide Residues - Rice:

- objective: to obtain samples of rough rice, straw, mill rice, rice hulls and rice polish and bran of adequate size for residue analyses - data to be used in supplementing a petition for establishing tolerances for 2, 4-D, 2,4,5-T, silvex and MCPA in or on rice and milled fractions thereof.

- general consideration: conditions for growing should conform to those typical of the area

- rice variety: should be typical of those used in the state.

- formulations: amine salts of 2,4-D, 2,4,5-T, low volatile ester of silvex, and MCPA to be used - this form is most widely used and recommended

- rates and timing of application: highest registered rates of application and shortest interval between applications and harvest consistent with registration limitations should be used, to obtain highest residues. Single treatment, unless two applications are required.

- Suggested treatments:

  (1) 2,4, 5-T - 0.75, followed by 1.5 lb./acre, 3-5 weeks after emergence

  (2) 2, 4, 5-T - 1.5 lb./acre, just before panicle initiation (when internode length is 1/2 in. or slightly less)

- size of plot: 4' x 20' minimum, replicated 4 times, is suggested

- rice products for analyses: about 10 lbs. straw and 20 lbs. rough (unmilled) rice from each treatment will be required.

E. Rice - USDA registered uses:

2,4,5-T:

1.25 lb./acre - tiller to boot and before flooding (4-8 weeks after rice emerges)

1.5 lb./acre - apply after flooding (2-3 weeks) or 7-10 weeks after planting

16638

00120604433

F. Rice Production and Acreage

Arkansas = 430,061 acres (24.89% of US production)
California = 323,630 acres (24.12% of US production)
Louisiana = 512,884 (24.26% of US production)
Texas = 464,732 (23.99% of US production)

Average US yield/acre = 3,563 lb (5 year average)

00120604 34

G. Letter from Woodbury (Hercules) to Baker (Louisiana State Univ.), 3/11/69 re: rice residue project, enclosing copy of suggested protocol.

Letters also sent to:

- Flinchum (Texas A & M Univ., Beaumont, Texas)
- Smith (USDA Rice Branch Experiment Station, Stuttgart, Arkansas)

H. Letter from Flinchum to Devine, 11/14/69, notifying of shipment of samples from project and listing treatments. Treatment D (1.5 lb/Acre Silvex) mistakenly applied over Treatment B (0.75 + 1.5 lb/Acre Silvex). Each treatment replicated 4 times.

I. Letter from Smith to Woodbury, 11/25/69, notifying of shipment of samples from project and listing treatments.

J. Letter from Baker to Woodbury, 11/24/69, notifying of shipment of samples from project and listing treatments.

K. Letter from Flinchum to Woodbury, 2/5/70, describing project at Beaumont.

16639

Other pesticides used besides 2,4-D, 2,4,5-T, Silvex and MCPA:

Arasan, 42-S, Aldrin; propanil

Yield Data:

-2,4,5-T (0.75 + 1.5)	=	2830 lb/acre (lowest in grou
-2,4,5-T (1.5)	=	3031 lb/acre
no herbicide	=	3579 lb/acre (highest in gro

D0H20060435

16640

19. Dow, effect of irradiation upon TCDD - 5/25/70:

-1.02 mg TCDD, in 100 ml. chloroform saturated with water, exposed for 2-1/2 hrs to UV light (GE sunlamp) at 35°C.

-50 to 100% degraded into other compounds (not identified). "a dramatic change"

DOH2060436

20. Dow (12/8/64, 4/13/70) "Solubility of [TCDD] in various solvents at 25°C.

-least soluble in water

-most soluble in O-dichlorobenzene

00120604 37

21. USDA Notice, NRC Pesticide Residues Committee "Statement for Implementation of Report on No Residue and Zero Tolerance" (approved by Orville Freeman, Sec'y, 4/1/66).

(1) 11 recommendations of NRC committee, from June 1965 study and subsequent report:

(a) "No residue" and "zero tolerance" concepts are scientifically and administratively untenable and should be abandoned.

(b) "Negligible residue" or "permissible residue" registration suggested.

(c) Registration for residue-producing pesticides only if negligible residue in line with FDA regulations.

(d) "Negligible residue" figure should be published, as well as analytical method for determining residues.

(e) If pesticide is known to be too hazardous for a particular use, registration for such use should be refused.

(f) Responsibility for registration properly rests with USDA.

(g) Schedule for transition to new procedure to be decided by USDA and HEW.

(h) Manuals, mutual assistance encouraged.

(i) Formal program for education in residue analysis is needed - agencies should cooperatively sponsor program.

(j) Expanded research program needed in this area.

(2) After considering report, Agricultural Research Service and FDA agreed on certain general principles and procedures.

(3) Federal Food, Drug and Cosmetic Act: any pesticide in or on food shall be deemed unsafe unless a "tolerance" has been prescribed.

(4) Both agencies agree in establishing finite tolerances concept.

(5) Residue-forming pesticides shall not be registered under FIFRA unless a finite residue level is provided for by FF,D,&C Act.

(6) "Tolerances should be established on the basis of data in petitions presented by proponents to establish that such uses will be safe".

(7) "Agricultural uses of pesticides for which it can be concluded there is no reasonable expectation of any residues on the food will be considered as non food uses and can continue to be registered in the absence of a finite tolerance" (until or unless residues are discovered later on).

(8) For tolerance purposes, chronic feeding studies in 2 species of animals and reproduction studies required (unless only negligible levels are involved, when 90 day feeding studies on 2 species may be sufficient to provisional tolerance).

(9) "No procedure or formula is to be employed which will serve to override scientific judgement based on adequate safety data".

(10) "Prompt action will be taken on petitions for tolerances for negligible residues of such pesticides".

(11) All petitions should supply an analytical method.

(12) "It is reasonable to expect that uses of persistent pesticides on crops or in soil in which crops to be grown may result in residues on the crop at harvest".

(13) "Zero tolerance" or "no tolerance" registrations should be discontinued as of 12/31/67 unless finite tolerances established or in process of being established; in no case should former registrations extend past 12/31/70.

(14) Both agencies (FDA and USDA) can receive and process petitions.

(15) Comparable problem with inadvertent and unavoidable residues in foods which needs resolution.

(16) Public Health Service of HEW is available for consultation and correlating human experience with animal experience.

DOH2060139

22. A Statement by Julius E. Johnson (Dow) before Senate Subcommittee on Energy, National Resources, and the Environment, 4/15/70.

DOH2060440

(1) 10/29/69 announcement by Dr. Dubridge (Office of Science and Technology) referring to birth defects observed in Bionetics test. This preceded final report of Panel on Teratology to Mrak Commission (Commission on Pesticides and their Relationship to Environment and Health, appointed 5/69 by Secretary of HEW). Commission members had not seen Bionetics report at that time.

(2) 2,4,5-T sample in Bionetics tests came from Diamond Alkali Co. (no longer makes 2,4,5-T); 2,4,5-trichlorophenol came from Coleman Mathison Bell, via McKesson Robbins, via Dow. Quality of 2,4,5-T is related to quality of its intermediate 2,4,5-trichlorophenol; possibility of a highly toxic impurity being formed in 2,4,5-trichlorophenol as a side reaction under conditions of elevated processing temperatures. Most sensitive toxic reaction observed in humans - chloracne, mostly prevalent on face, neck and back. Impurity not formed during manufacture of Dow 2,4,5-T from the trichlorophenol, nor does it form on storage even at high temperatures. Impurities concentrated in waste oils from 2,4,5-trichlorophenol process.

(3) Early control test - rabbit ear test for dermatitis.

(4) 1964 - Some Dow workmen developed chloracne; rabbit ear test showed chloracne potential of waste oil from 2,4,5-trichlorophenol process was building up to a danger point, caused by operating changes made to improve production capacity. Waste is routinely destroyed by incineration at high temperature. Exposure to waste oil caused acne in workmen. Plant shut down; it was discovered that principal offending impurity was TCDD (found by gas chromatography). Plant was redesigned to ensure minimal TCDD production. Since

1966 Dow 2,4,5-T and 2,4,5-trichlorophenol have met specification of less than 1 ppm (most were less than 0.5 ppm) TCDD.

(5) In Bionetics test, 2,4,5-trichlorophenol showed no significant increase of anomalies, but 2,4,5-T sample did display a significant increase of anomalies. Samples of Diamond Alkali 2,4,5-T contained TCDD up to levels of 16 ppm, according to records.

(6) Above information presented to Mrak Commission on 11/7/69.

(7) Low mammalian toxicity of 2,4,5-T, and absence of reports of increased birth defects in cattle or sheep grazing 2,4,5-T treated ranges caused Dow to doubt Bionetics study's reliability.

(8) Tests performed at National Institute of Environmental Health Science (NIEHS) labs at Research Triangle, North Carolina. Previously agreed to try to duplicate Bionetics study, using Sprague-Dowley rats (superior to mice tests) and using Dow 2,4,5-T of regular production grade. Dow would provide samples of 2,4,5-T and TCDD to NIEHS labs.

(9) On 12/11/69, Dr. Courtney (NIEHS) provided sample of 2,4,5-T used by Bionetics labs; examined at Dow and showed to cause positive rabbit ear reaction characteristic of the contaminant, and gas liquid chromatography indicated presence of 27± 8 ppm TCDD.

(10) 1/12/70 report to HEW showed that Dow 2,4,5-T of regular production grade did not cause birth defects in rats as determined by gross examination of fetuses. Dosage levels used were selected in consultation with Drs. Falk, Courtney and Gaylo of NIEHS. Pilot study with pregnant rabbits also showed 2,4,5-T had not caused birth defects. Later

microscopic examination of tissues and skeltons confirmed preliminary findings of no causality.

(11) TCDD teratology study in rats showed high level of maternal and fetal toxicity; concluded that presence of TCDD in Bionetics sample could well have accounted for observations reported and attributed to 2,4,5-T.

(12) Dow physicians have made in-depth evaluation of health of 130 male employees exposed to 2,4,5-T in manufacturing operations for from 6 months to approximately 20 years; 50 clinical parameters evaluated; control population = 4,600 individuals for whom similar data were available. Conclusion: no evidence of adverse effects.

(13) Dow's belief : 2,4,5-T with less than 1 ppm TCDD presents no practical hazard when used in accordance with good agricultural practices.

(14) Industry Task Force on Phenoxy Herbicide Tolerances established by National Agricultural Chemica Association, pooled information, submitted petitio for negligible residue tolerances of 2,4,5-T in Dec. '67; in April of 1968, FDA said more information needed on metabolism and residues.

(15) 2,4,5-T originally registered under "no residue" tolerance; on 4/13/66 , Secy's of USDA and HEW changed policy to finite residue tolerances. Since Task Force data would not be ready before Jan. 1, 1970 deadline for new registration, the Task Force withdrew its petition without prejudice to future filing, and requested extension of 2,4,5-T registrations since no imminent hazard from continued use was anticipated. In response, USDA extended registrations to 1/1/71; Industry Task Force to refile before 1/1/71.

B "The Response of Rabbit Skin to Compounds Reported to Have Caused Acneform Dermatitis". Adams, Irish, Spencer and Rowe (Dow), from Jan. 1941 issue of Industrial Medicine.

(1) Materials have been applied to inner surface of the ear of albino rabbits and to the shaven belly both undiluted materials and solutions of various concentrations in olive oil, paraffin oil U.S.P., propylene glycol, ethanol and water. Applications made once a day, 5 days a week, for 4 weeks or until a marked reaction resulted.

(2) Some of strongest irritants produce a rapid destruction of the tissue (necrosis) without the skin having an opportunity to show an active response; milder irritants have some effect upon the tissues Most irritants resulted in responses which tend to develop rapidly and to subside in a short time; response may include hyperemia, congestion, inflammation, exfoliation, edema, blistering, sloughing, exudation, crustation, necrosis, induration, hair loss. One type of response has a latent reaction and a much more prolonged course - epithelial hyperplasia, with its resultant thickening of the skin, follicle enlargement and sequellae (varying degrees possible, from slight to severe).

(3) Indications of a relationship between reaction observed in rabbit and acneform dermatitis of man: reaction in rabbit produced by 5 types of substances known to cause an acneform dematitis in man (chlorinated diphenyls, chlorinated naphthalenes, chlorinated diphenyloxides, crude chlorinated phenols, and petroleum oils).

(4) Other reports: epithelial hyperplasia from various oils, fats, paraffin, dyes; eczema from aniline and coal tar exposure (also warts and acneform dermatitis (chloracne)).

(5) Chloracne = visible response of skin to irritant, taking form of (1) epithelial changes, (2) inflammatory and degenerative changes, and (3) regenerative processes, (in order of appearance).

C Dow: "Determination of (TCDD) in (2,4,5-T) by gas-liquid Chromatography" 6/22/65 - Analytical Method.

(1) Dioxin can be detected at 1 ppm level with a lower limit of 0.5 ppm possible at optimum operation conditions.

(2) Accuracy of this method = ± 5% or less.

D Abstract from "Teratogenic Study of (2,4,5-T) in the Rat", Emerson, Thompson, Gerbig and Robinson, Dow (presented 3/17/70).

(1) Study to determine embryotoxicity or teratogenicit of 2,4,5-T containing less than 1 ppm TCDD, tested on Sprague-Dawley rats.

(2) 5 treatments groups, 25 females each

(3) Given 1,3,6,12 or 24 mg/kg/day of compound via gavage in 0.25% METHOCEL on days 6-15 of gestation Control group (50 females) received vehicle alone.

(4) No clinical or gross pathologic signs of adverse chemical effect were observed in treated dams; no teratogenic or embryotoxic effects.

(5) Results of study failed to substantiate Bionetics Study.

E Abstract from "Teratogenic Study of (TCDD) in the Rat", Sparschu, Dunn, and Rowe, Dow (presented 3/17/70).

(1) Study to determine if TCDD impurity could account for fetal abnormalities in test animals in Bionetics tests.

(2) Dioxin given by gavage in 9:1 corn oil - acetone solution in doses of 0(control), 0.03, 0.125, 0.5, 2.0, and 8.0 micrograms/kg/day to groups of 24 (control) and 12 (treatment) pregnant Sprague-Dawley rats on days 6-15 of gestatio dams sacrificed on day 20.

(3) No differences in fetuses from control and 0.03 micrograms group.

(4) 0.125 microgram group showed slight decrease in average weight and intestinal hemorrhage (18/127) and subcutaneous edema (22/80) in fetuses.

(5) 0.5 microgram level - number of fetuses reduced, number of resorptions and fetal deaths increased; average weight of viable fetuses slightly decrease intestinal hemorrhage (36/99) and subcutaneous ede (31/65).

(6) 2.0 microgram level - only 7 viable fetuses, from 4 of 11 litters, numerous resorptions, intestinal hemorrhage in 4 of the 7; subcutaneous edema in 4; 1 fetus had kinked tail and 2 misshapen feet.

(7) 8.0 microgram level - toxic to dams; no viable fetuses; all resorptions were early and no fetal tissue found.

(8) Delayed ossification of some sternebrae and skull bones occurred throughout various groups, including controls - not considered to be of practical significance.

(9) Results indicate high level of maternal and fetal toxicity to be associated with TCDD; could account for Bionetics observations attributed to 2,4,5-T.

23. USDA notice to Manufacturers, Formulators, Distributors, and Registrants of Economic Poisons re "extension of cert 'no residue' and 'zero tolerance' registrations beyond Dec. 31, 1969" (dated April 9, 1970).

(1) Lists were extended until Jan. 1, 1971.

(2) 2,4,5-T uses on McIntosh apples, low bush blueberries, grains (cereal-undesignated), pastures (grasses), rangeland clearances, rice and sugarcane - extended by this notice.

(3) Signed by Harold G. Alford, Ass't. Dir. for Registration.

24. Dow, "Product Specification for 2,4,5-T", 2/27/69, released for use by USDA etc. by G. E. Lynn.

(1) Lists TCDD to be at a non-detectable level, whether produced by Dow itself or purchased by Dow for resale, etc.

25. Dow, "Sales Specification for 2,4,5-T", 2/13/70

(1) Shows TCDD to be less than 1 ppm.

DOH2060448

26. Dow; "Analytical Method for quality testing of 2,4,5-T", giving procedures for determining assay and freezing poi 6/5/65.

DOH2060449

27. Dow, "Analytical Method for determination of TCDD in 2,4,5-T by gas-liquid Chromatography", 6/22/65.

DOH20600450

END OF VOLUME TWO

16655

Dr. BAYLEY. I understand.

It is simply a matter of the care and sophistication of the method, the time required to get the job done. We have built, Dr. Bayley has pointed out, an isolation laboratory—I say built, I change that to equipped an isolation laboratory. We have the scientists. We are, in fact, ready to proceed. The time, therefore, would be the time required to do the analyses and verify them.

Mr. BICKWIT. Thank you very much.

Senator HART. Gentlemen, thank you. It has been an interesting and informative morning. Congratulations again for the effort that you, I am sure, put into developing and then persuading departmental acceptance of the suggested amendments. I hope improvement in that basic law soon will be written.

Dr. BAYLEY. Thank you, Mr. Chairman.

Senator HART. We adjourn, to resume tomorrow at 11 a.m. in the morning in the hearing room of the Committee on Commerce 5110.

(Whereupon, at 12:40 p.m., the hearing recessed to reconvene at 11 a.m. on Thursday, June 18, 1970.)

EFFECTS OF 2,4,5-T AND RELATED HERBICIDES ON MAN AND THE ENVIRONMENT

THURSDAY, JUNE 18, 1970

U.S. SENATE,
COMMITTEE ON COMMERCE,
SUBCOMMITTEE ON ENERGY, NATURAL RESOURCES,
AND THE ENVIRONMENT,
Washington, D.C.

The subcommittee met, pursuant to adjournment, at 11:35 a.m. in room 5110, New Senate Office Building, Hon. Philip A. Hart (chairman of the subcommittee) presiding.

Present: Senator Hart.

Senator HART. The committee will be in order.

Let me attempt to apologize to the witnesses who have been inconvenienced by this 35 minute delay. A meeting was called yesterday of the Democratic caucus for 10 a.m. and I felt compelled to participate. I wish we could manage things a little more responsibly around here, a body in Congress is running everybody else's business.

The first witness today is the distinguished science advisor to the President, Dr. DuBridge.

STATEMENT OF DR. LEE A. DuBRIDGE, SCIENCE ADVISOR TO THE PRESIDENT AND DIRECTOR, OFFICE OF SCIENCE AND TECHNOLOGY; ACCOMPANIED BY DR. EDWARD J. BURGER, JR., TECHNICAL ASSISTANT

Dr. DuBRIDGE. Mr. Chairman, I have asked my associate, Dr. Burger, of my office, to accompany me. He is an M.D. who has been following the matters related to health and the other effects of pesticides.

Mr. Chairman, I have testified before this committee on April 15 on the 2,4,5-T subject, and I am not sure there is very much to add to what I said at that time, but there are a few points I would like to review and emphasize.

I reviewed then something about the history and development and value of the use of this herbicide and I used that review as a text from which to draw what I considered to be some important generalizations about pesticides, and these are some of the matters I would like to repeat.

Let me begin by pointing out that 2,4,5-T is a pesticidal chemical which has been introduced intentionally into man's surroundings because of the benefits presumed to follow. Its purpose was to serve as an adjunct to other means of weed and brush control land and

waterway and agricultural management. Over a period of 20 years it has proved its utility so that we are now in a position of relative dependence on this material.

However, especially in recent months, we have begun to question in greater and greater depth the possible human health effects of pesticides like 2,4,5-T and this has required a greater sophistication in research and testing than was previously thought adequate.

The example, of course, was the Bionetics study for the National Cancer Institute. Previous research on 2,4,5-T had concentrated on the acute toxicity of that compound and had shown this to be of a low level.

The Bionetics study represented a departure in that it investigated the potential of the herbicide to provoke tumors, birth defects and genetic alteration in appropriately exposed experimental animals. 2,4,5-T emerged from this study as a possible teratogenic agent.

As I have said, this study was a departure in several ways. Whereas nearly all of the background toxicology on pesticides had been performed as part of the development process by the developing company or industry, this study was launched and paid for by the Government.

I hinted that this might represent a precedent. If our society demands a very high level of sophistication in this type of research, industry may not be able to afford the increased cost of development and further development of valuable new products may be discouraged or prevented. Hence I suggested that new ways of distributing the costs of this work may have to be found. Expenditures of public funds and Government participation in this research may be desirable.

I emphasized that at any point in time, we find it difficult to get complete information about the true hazards of any pesticide or any other chemical substance. That is, research in this area (as in any other) has no finite end points. It may take long experiments with all kinds of levels and all kinds of circumstances to make any such assertion and one can never be sure what new research results will turn out.

As one performs more research to investigate various hypotheses, one inevitably raises additional questions—as well as answers. It follows from this that any regulatory system for pesticides must be able to accommodate new and unexpected information.

I pointed out that our present arrangement for regulation is not sufficiently flexible to reflect new information as it emanates from research. Again, these points were clearly illustrated by the case of 2,4,5-T.

What I said in April was that there does not exist a mechanism whereby the Government may exercise prudent and unequivocally effective restraint temporarily on the receipt of new, unexpected information and possibly preliminary results and while awaiting more definitive conclusions.

In many ways the Federal Government did act with dispatch in the case of 2,4,5-T. After the October 29 announcement about restrictions imposed on 2,4,5-T additional research studies were begun in a number of agencies. These studies were initiated both by the Government and by industry. The aim in every case was to confirm

1657

or extend the unexpected results obtained from the Bionetics-National Cancer Institute studies.

One of the new issues examined in the new set of investigations was the importance of impurities present in many samples of 2,4,5-T. It had been discovered that over a period of years commercial 2,4,5-T contained varying amounts of a highly toxic impurity which was a member of a family of polychlorinated dioxins. It was of obvious importance to ascertain the relative contributions of the 2,4,5-T and the dioxin impurity as potential teratogenic agents. The dioxin was known to be very toxic. Hence this question became part of the experimental aim.

Fortunately, teratogenesis is a relatively acute affair and experiments necessary to investigate this phenomenon are short-term experiments. Answers were expected in a fairly short period of time.

Some of these confirmatory experiments were undertaken by one of the National Institutes of Health—the National Institute of Environmental Health Sciences. The results of these experiments were reported to you as fresh out of the laboratory at the time of the last hearings.

In brief, you may recall, these results implicated both 2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin as potentially teratogenic in nature. In rats, over the same dose range, only the dioxin appeared to produce birth defects.

It was principally on the basis of these results that Secretary Hardin, Secretary Finch and Secretary Hickel jointly announced the series of restrictions on the use of 2,4,5-T. These were related to you by the Surgeon General, Dr. Steinfeld.

In brief, the philosophy behind these restrictions was hoped for protection of women of childbearing age. Thus the Department of Agriculture suspended the registration of liquid formulations of the weedkiller for uses around the home and of all formulations for use on lakes, ponds and ditch banks.

In addition, registrations were cancelled for uses of nonliquid formulations around the home and of all formulations for use on food crops intended for public consumption.

Of the total amounts of 2,4,5-T used in this country for all purposes it was estimated that these restrictions applied to about 20 percent—the 20 percent of the cases where human exposure was possible.

I firmly believe that the issues raised by the case history which I outlined in April continue to be prominent. In a way, I suppose, we can thank the existence of the questioning about 2,4,5-T for bringing to our attention matters such as the ones I have described.

This study has served as a most useful vehicle and we may learn some lessons for future studies. However, as I warned, any attempt to answer the research questions raised will inevitably raise some additional questions.

I suggested that in some ways we were fairly lucky in our investigations of 2,4,5-T. The issues have appeared fairly straightforward and it was possible to start confirmatory experiments fairly quickly and to get confirmatory results quickly.

Yet, while this appears to have been a modest succ story, some may rightly ask: Shouldn't the kinds of experiments which were

47-109

mobilized on the spur of the moment for 2,4,5-T have been accomplished on a more systematic basis and without the spirit of a crisis necessary to urge them on?

Further, one might ask whether or not it might be desirable to support a fairly sophisticated level of investigation for a large number of pesticidal chemicals—not just 2,4,5-T.

Now, I and others have outlined the research work on 2,4,5-T and I have termed it relatively sophisticated. Yet I will have to admit that there has been almost no work done to elucidate the metabolic handling of this herbicide in the animal organism. There is little known in biochemical terms of the mechanism of its actions and there is essentially no knowledge of any possible interactions between this chemical and other materials.

In similar fashion we are poorly informed about the characteristics of the dose-response relationship for very low dose levels. This, of course, is the situation which we face in real life in the case of a variety of environmental agents—including pesticide residues. Here the problem is a statistical one. In order to derive meaningful answers with any useful level of confidence very large colonies of experimental animals must be tested, often over a long period of time.

I suggest these comments to illustrate that there are various levels of sophistication in research.

In the realm of pesticides the level of our research activities may not have kept up with the state of that art nor with a corresponding level of questioning to which policy makers and the public are now seeking answers.

The very excellent report on research needs compiled by an advisory task force to the National Institute of Environmental Health Sciences outlined these research areas very well and very explicitly. This report, I am informed, is just now being published.

All of this—more sophisticated research, more expensive research, research sponsored by the Government—will cost money. Again, I repeat, if a really serious thrust is taken in this direction we may be obligated to find new institutional avenues for accommodating this research since, as a part of the cost of development, the bill to industry may be higher than we might desire.

All of this discussion brings me once again to a point which I made in my previous testimony and which I feel is worth emphasizing. While we as a society have recently begun to ask more penetrating questions about the possible adverse health effects of environmental agents it is not clear that we know how penetrating this questioning should be or must be.

What I said before was that we had set our sights higher. What I should add is that we are not sure how high they should be set. For example, up to the present time we have been willing to live with a system under which the amount of toxicological research performed on a pesticide was to some extent related to the probability of human exposure. With a low or seemingly negligible probability of exposure, relatively little understanding was sought and little research was undertaken. In fact, one could argue that since the appearance of residues of 2,4,5-T have been rare events—it is very rare to find measurable residues of 2,4,5-T on food—therefore one

8008661

1668

did not have to know too much about the toxicology of the herbicide. Now we are more particular.

I feel that we should be more explicit about assumptions such as these. If they are valid they will stand on their own merit. If they are not valid we should change them. I am happy to say that my office is examining questions such as these at the present time.

Thank you, Senator Hart.

Senator HART. Thank you.

You suggest the possibility that the Government may have to assume a greater role in any testing area but specifically in the matter of pesticides.

Dr. DuBRIDGE. Yes.

Senator HART. You indicate that otherwise the bill to industry might be higher than we might desire, which I suppose is another way of saying an industry could not afford it. How should we read that?

Dr. DuBRIDGE. What I meant to say is if industry is required to carry on years of very expensive research before any new product can be manufactured, obviously industry will no longer be interested in manufacturing new products because they could not recover the loss. Thus the community, the society would be robbed of many future very valuable chemicals which society might find extremely important for health and other reasons. To impose the burden on a particular company that before it can market a product it must undertake millions and millions of dollars more worth of research than it has in the past would simply stop the development of new products. Therefore it seems to me only fair, since we want to protect society as a whole but also to encourage benefits to society, that society as a whole ought to participate in the cost of determining what the damages may be as well as what the benefits may be.

Senator HART. That then would be your basic answer to the suggestion or argument that research is just another element of the cost of production.

Dr. DuBRIDGE. Yes.

Senator HART. That the user of the product should bear that cost along with other costs.

Dr. DuBRIDGE. Yes. Exactly. I am not saying that the manufacturer should not also bear substantial costs. He should make sure that the product that he is proposing to market is not dangerously poisonous, does not have obvious adverse human health effects. Industry should be required to undertake a reasonable set of experiments, and they always do, to assure that this product has a relatively good safety factor. But it may take years to find out low level and easily hidden dangers which sometimes may become obvious only when mass use is undertaken. To help avoid these dangers I think some Federal participation in the research program would be desirable.

Senator HART. Do you know whether the administration intends to embark on broad new research programs in this area?

Dr. DuBRIDGE. There are several agencies which are developing plans, pursuing research in this area, and I believe an advisory committee of the Department of HEW headed by Dr. Emil Mrak

is making some proposals to HEW about substantially extending its research and testing activities in this field of pesticides, and about developing extensive facilities, large animal colonies and large-scale testing equipment and personnel, to carry out extended tests in this field.

These are recommendations that are being formulated and are being proposed to HEW. I do not know what the status of them is at the moment.

Senator HART. It is likely you would not have the figure with you, but let me ask the question. Perhaps it could be provided for the record and I have no idea what it will show. But would it be possible to identify those activities which are not undertaken by the Department of Defense that are comparable to the line item research and development that is done by the Department of Defense? The total figure for research by the Department of Defense is in the range of $7 billion a year. How much are we spending in other research?

Dr. DUBRIDGE. The total Federal budget for research and development in this current year is close to $17 billion.

Senator HART. Including the DOD?

Dr. DUBRIDGE. Including the DOD. If you take out somewhat over $7 billion of DOD funds, it is $9 billion to $10 billion in all other branches of the Government.

Senator HART. We will let others judge whether the allocation of the resources is or is not prudent. I think it is good to have it in the record.

Yesterday we received testimony from the Department of Agriculture. When we think about changes in the system of pesticide research and control we have to think about changes in the basic law of pesticide regulations. Included in the testimony yesterday from the Department of Agriculture were a number of suggestions and changes that they recommend be made in the basic act. Are you familiar with those suggestions?

Dr. DUBRIDGE. Dr. Burger told me about them this morning and I have a copy of them here, and we have discussed them. These are in line with some of the suggestions we have had with Agriculture and other agencies; namely, that there is not sufficient flexibility in the present statute to take a suitable action in all cases where new information becomes available.

Sometimes new information like the Bionetics study is very suggestive but cannot be said to be finally conclusive because of the small number of animals and small number of circumstances involved and the separation of an impurity may not be taken care of.

It will frequently happen that you will get preliminary suggestive results not sufficient to abolish the use of a chemical but sufficient to take some prudent action until more final results have been obtained.

I think the Agriculture suggestions do move in this direction to give, for example, what they call preliminary suspension authority. So if a danger sign is raised you take prudent temporary action and continue further research. If the further research confirms that safety is there after all you can remove the suspension. If further resea[illegible] proves the danger, then permanent suspension can be achie[illegible]

T6659

Senator HART. I did not quarrel yesterday with the Department of Agriculture's interpretation of the basic act. Overnight I have tried, as we always do, to justify what we did, namely, those of us who wrote the law. I am not convinced that that statute prohibits the Department of Agriculture, when, as you say, the signal goes up from suspending the marketing of the product—I am not sure that Congress should be held to have said that we excluded the possibility of a temporary suspension.

Just as I was not yesterday, I am not sure you are not equipped, and did not plan to debate what limits there are under the existing law.

Dr. DUBRIDGE. No, I am not an expert on the question of interpretation of the law, but it has simply been taken for granted, maybe not properly and maybe under an interpretation that should be further developed, but it has been taken for granted that preliminary or temporary suspension was not provided for, explicitly at least, in the statute.

If the law were interpreted to allow this, it would be fine.

Senator HART. I cannot imagine there would be any different criteria for a temporary suspension than a suspension. I still have the feeling that the suspension is not for a thousand years. If you suspend and then discover that your alarms were groundless, surely you can unsuspend. That would argue that you can temporarily suspend.

Dr. DUBRIDGE. If that is the case I think that is fine. It apparently needs to be made more explicit to the people who are doing the suspension because they do not feel that they have this authority.

Senator HART. Clearly they do not.

Are you yet in a position to advise whether you would support or recommend support of the suggestions for law changes made yesterday by Agriculture?

Dr. DUBRIDGE. I think it is fair to say that we would believe that these are proper moves. Again, as I say, we are not experts in the regulatory field and the legal field. What we tried to do is to find the science and technology that is applicable, and we leave it to the Congress and the legal authorities to determine what specific regulations and statutes are required.

But I think this additional flexibility does in principle sound very desirable to us, simply because research results, you know, are never the final answer unless they are extremely conclusive results of extreme danger.

It is almost never possible to say that all the research has been done and it proves that the thing is forever safe.

Senator HART. Conversely, forever unsafe.

There were several other suggestions that Agriculture made. One regarded the ineffectiveness of handling pesticide regulation by labeling. They were going to recommend restructuring the law to require a grading by degree of the hazards of a pesticide and to ensure that extremely hazardous products would be permitted to be handled only by individuals or institutions licensed to do so. Do you have any comment on that one?

Dr. DUBRIDGE. I think I have no very expert comment except to note the experience within my own family that labels on packages

are often not adequately read and that the labeling problem is a difficult one.

Senator HART. As Agriculture indicated, not a very reliable handle for protection against injury to health or environment.

Well, we would hope that you will lend your distinguished scientific support to the recommendations that Agriculture has made.

A couple of other questions, and this goes to the different actions that have been taken with respect to pesticides or herbicides by different departments of the Government.

Agriculture suspended I think you said about 20 percent of the use of 2,4,5-T.

Dr. DUBRIDGE. I did not intend to imply that was all Agriculture. I said the total suspension amounted to 20 percent of the total use.

Senator HART. It is my understanding that the Department of Defense has suspended it entirely for use in Vietnam.

Dr. DUBRIDGE. That is correct.

Senator HART. And the Department of Interior very recently suspended it for use on public lands; lands in its ownership.

Dr. DUBRIDGE. Yes.

Senator HART. How do we explain the different reactions from the several departments with respect to the same product?

Dr. DUBRIDGE. I do not know that I can fully explain it, but the situation in military operations is not necessarily the situation in normal peaceful operations in this country.

In military operations, by necessity, these chemicals are distributed by aircraft, and it is not always easy to control where they go and how far away they blow or even that the aircraft is hitting the right target. Therefore I think somewhat more prudence may be required.

When you are doing it in a normal way, in agricultural practice or land management, you can be much more careful and make sure that the material does not get on food crops or in your waters, which will contaminate the waters, or get on grazing lands on which animals will be grazing and therefore get into milk or meat.

Fortunately 2,4,5-T degrades quite rapidly. It is not like DDT. If you spray a pasture with 2,4,5-T, essentially all traces of it are gone after about three months' exposure to wind, rain, and sun. Therefore cattle can quite safely graze on grazing land treated with 2,4,5-T after this period.

Also, if it gets on food it is likely to degrade pretty rapidly, though it is desirable to have no tolerance for food. But where it is used in areas where there is no human population and where there is no danger of contaminating food or water, then a controlled use can be extremely valuable, and since no dangers would be resulting I think it is perfectly proper to have controlled use in this country.

Senator HART. What do you say, then, to Interior's prohibition for use on its lands?

Dr. DUBRIDGE. I guess I am not familiar with how extensive that use is.

Senator HART. I am told that Interior's action was taken only yesterday.

Dr. DUBRIDGE. I see. I am not familiar with the background for that action or the extent of it; or maybe they found that other ma-

U.S. GOVERNMENT PRINTING OFFICE 99831

1681

terials could be used on public lands other than 2,4,5-T which would serve the purpose. I just do not know.

Senator HART. But it contributes to the uneasiness of the public when we see these seemingly conflicting reactions.

Dr. DUBRIDGE. This is one of the very delicate things that the Government faces; that is, not to overreact to situations which will do damage by overreaction. But there is an opposite injury of under reaction and not taking prompt or adequate action when dangers are evident. I would like to defer to the Department of the Interior in this case, and with your permission, have that Department submit a statement for the record.

(The information follows:)

STATEMENT ON INTERIOR PESTICIDE POLICY

The Department of the Interior policy statement, issued by Secretary Hickel on June 18, does not differ markedly from the one that has been followed for several years. Nevertheless, it adds some chemicals about which we have received additional data within the last year and recognizes the concern expressed by cooperating Departments on some others.

The prohibition against most of the chlorinated hydrocarbon insecticides had been in effect for several years and that on 2,4,5-T since October 1969. The hazards of mercury have been recognized for some time but were accentuated by monitoring results in the last six or eight months. Amitrol was included because of the objections of the Food and Drug Administration to the use of carcinogens.

The Department of the Interior policy is not intended as a Federal pesticide policy. It has long been the policy of this Department to set a standard in its use of pesticides that is beyond reproach from the standpoint of safety. Consequently, our position may be more strict than some others will wish to set. If research demonstrates that some of the pesticides listed may be less hazardous than we suspect then they may be restored for use on Interior lands.

Senator HART. You reminded me of a question I kicked myself yesterday for not having asked the Department of Agriculture witnesses. Perhaps you can help.

You mentioned DDT. As I recall the testimony yesterday, under the FIFRA Act, a science advisory committee is established when there is a cancellation procedure aimed at a product. They explained that in the case of DDT that some six months have passed since the cancellation procedure was initiated and no committees have been formed; hence the passage of time has been extended at last by this amount during which, under the cancellation procedures, continued marketing of the product goes on. Why the 6-month delay?

Dr. DUBRIDGE. I cannot explain that. I do not know why there should be a long lag between these two events. The only thing I can think of is since the Secretary's pesticide advisory commission has been continuously at work on the pesticide problem, especially with attention to DDT, they were depending on it to examine this particular problem.

Senator HART. I repeat, I should have asked them yesterday and I did not. But if they read so strictly the statute with respect to suspension, I would assume the same strict reading would tell them that they cannot substitute the Mrak Commission for the explicit statutory requirement that there be a science board established for each of these products.

MOO

Dr. DuBridge. I would agree. I think maybe the law does not set ime at which the science advisory commission shall be established.
Senator Hart. No; it does not. It was assumed that it would be ablished at least with all deliberate speed, and 6 months seems to undue delay.
Dr. DuBridge. Yes, I agree. With your permission, I would like ask the Department of Agriculture to provide an explanation of s apparent delay.
(The information follows:)

DEPARTMENT OF AGRICULTURE,
OFFICE OF THE SECRETARY,
Washington, D. C. July 7, 1970.

LEE A. DUBRIDGE,
cutive Secretary, Council on Environmental Quality, Executive Office of the President, Washington, D.C.

EAR DR. DUBRIDGE: In reference to the question raised at the Hart mittee hearing as to why it has taken so long to establish an advisory mittee on DDT, we submit the following explanation:

a) After the announcement of cancellation, the companies involved did request an advisory committee or public hearing for the four uses of DDT e cancelled until the latter part of the 30-day period provided for appeals the Federal Insecticide, Fungicide, and Rodenticide Act.

b) Meetings and discussions were held with representatives of the National demy of Sciences on makeup of the committee.

c) Previously we had asked for lists of names of persons to serve on ad- ry committees for other cancelled products, and they were already in the ess of compiling them.

d) Due to unfavorable publicity related to conflict-of-interest charges re- ling authorities that served as consultants to the Department in the past, experts wre not willing to serve.

e) Two additional requests to NAS for candidates to serve, besides the inal, were necessary in order to complete the committee.

f) Two of three companies requesting advisory committees withdrew, ing only one for a tracking powder use.

g) Getting written position from DHEW as to whether tracking powder considered an essential use from a public health standpoint.

h) Contacting and getting approval of proposed candidates to serve on the mittee.

i) Notifying committee members that they were selected to serve on the mittee.

j) Conflict-of-interest review and evaluation within the USDA.

Sincerely,

NED D. BAYLEY,
Director, Science and Education.

enator Hart. I would hope in connection with the cancellation ceeding on 2,4,5-T an advisory committee will be formed with delay.
his next question bears directly on your broad background. One he difficulties in this 2,4,5-T story was the difficulty experienced btaining information which might have been of public health sig- ance.
nder what conditions do you suggest scientific information ought e kept secret when a question is raised as to the safety of the uct?
r. DuBridge. If you are talking about scientific information, not think it should be kept secret. When you are talking about rmation having to do with the manufacture of commercial prod- , that is a very different situation because the costs of develop- t, testing and g ing a commercial product into production are

very high. It is quite proper that the manufacturer or the [illegible] be protected so that he will have an opportunity to recover [illegible] costs. He should have a patent or a protection for his invention or his product so that he can recover the very large costs to develop it. Whereas, if he instantly published all the information about how to make this product so that others would instantly start making it without the expense of development, this would obviously be an unfair kind of competition.

Our whole system is based upon the fact that inventors of new processes and products have protection to regain their investment and recover their costs. Therefore I think the publication and the distribution of information with regard to the manufacture of products and materials is a proper trade secret.

On the other hand, when it is clear that human health is at stake I would assume there should be mechanisms by which Government agencies in proper authority could be told something about the composition of the product so that they would be able to determine whether or not there might be materials in the product which ought to be investigated for their harm.

I do not know exactly what the law is on this, but it would seem sensible.

Senator HART. Your suggestion is that when a question of health is raised with respect to a product, data and information on the product should be made available to the appropriate Government agency for its determination as to a question of health and safety?

Dr. DUBRIDGE. Yes, sir.

Senator HART. That excludes, of necessity, the judgment and the comment of perhaps very gifted men and women of science in arriving at the determination of whether public health is or is not in jeopardy. This is not to suggest that the appropriate agency lacks qualified and competent people but surely they do not have a monopoly on that.

Is there some way, notwithstanding the obligation to protect trade secrets and encourage invention and discovery, we can do a better job of permitting the outsider, whether it is the head of the chemistry department at Cal Tech or someplace else, being brought in and having an opportunity to sharpen the judgment of everybody?

Dr. DUBRIDGE. Well, I think that the various advisory mechanisms available to the various Government agencies ought in general to accomplish that objective. A science advisory group can be called in to consult on a particular problem, on the possible dangers of the particular chemicals that happen to be in a particular commercial product.

I am sure that they could tap the rest of the scientific community to find out whether chemical A or chemical B is of a nature that it would likely be harmful. They do not have to reveal the whole composition of the product in order to say this product happens to contain a certain amount of compound A, is there any evidence or any chance or any reason to believe that this compound A is harmful. I think the knowledge of the scientific community could be obtained.

Senator HART. Should be obtained?

Dr. DUBRIDGE. And should be, of course.

Senator HART. The suggestion has been made that all of us as a people would be better off if we had some centralized clearinghouse or data bank into which could be fed all of the information not subject to trade secrets. Have you given any thought to that suggestion?

Dr. DUBRIDGE. I understand that Dr. Steinfold is going to discuss that question when he appears.

Senator HART. Your reaction would not stop him.

Dr. DUBRIDGE. He knows so much more about it. I do not want to anticipate it. It is this question of the dissemination of scientific information which is one of our most difficult problems.

In past years we always assumed that if you published in scientific journals and books that anybody had access to, anyone would just go to the library and look up what he wanted to know. The volume of scientific information has become great, the urgency of finding pieces of scientific information quickly has become great, and this has led to the question as to whether one can or cannot use modern scientific equipment to store and retrieve scientific information more expeditiously.

The only trouble is it is very expensive, and the development of techniques for putting it in suitable form for data processing, the question of how many agencies should be involved in feeding the information in and how to get it out these are complex technological problems which together with the expense have not been worked out. I think it is a very urgent problem.

There are people in our office that are working on this and I hope we can find ways to have a storage of needed technical information, particularly in the health field.

Senator HART. I share in that feeling.

My last question, again, is a general one. We had an exchange yesterday with the Department of Agriculture witnesses on the old problem that is created in the minds of some when you have a department that is charged with the promotion of an activity undertaking also to regulate it. In this case, expanding agricultural production is a responsibility of the department, and at the same time we say make sure that herbicides are not permitted that do damage. Do you have any general rule as to whether promotional and regulatory functions should be separated?

Dr. DUBRIDGE. I do not think I would propose a general rule on that. In the particular case of pesticides it is true that Agriculture is involved. But under the interagency agreement, Health, Education, and Welfare and Interior act jointly with Agriculture on these questions. If there is an agricultural product that is in use and HEW has information that there may be health effects from this, they can immediately bring it to the attention of Agriculture and action can be taken then by joint agreement among these three departments.

I think this is one purpose of having this interdepartmental arrangement. I am sure that no Department is anxious to promote something that has danger to human health, and as soon as human health aspects are brought out HEW has the obligation and the authority to bring this to the attention of any other Department. In the case of pesticides they meet and take action jointly.

Box 1998313

Senator HART. As I understood it, all [illegible] voice but only one fellow votes, and that is the Department of Agriculture.

Dr. DUBRIDGE. No, I do not think that is quite correct. I think it has been agreed that the unanimous consent of the three will be sought for specific action. I think this is a private agreement. I do not know whether it has been made public or not.

Senator HART. It is my understanding if there were disagreement the decision would be Agriculture's.

Mr. Bickwit?

Mr. BICKWIT. You have told us that the Department of Agriculture does not believe that they have certain authority that you would like to see them have in terms of temporary suspensions and the like. Have they communicated to you, as they communicated to us yesterday, that they would never have authority to ban the use of a hazardous pesticide on food crops when it was known that the ingestion of food which had been treated by that pesticide, was dangerous to man?

Dr. DUBRIDGE. When there is clear evidence of danger to man they have full authority, of course, to cancel or suspend the use of it. The problem comes when the evidence is not clear or is not conclusive or definitive.

Mr. BICKWIT. That was my impression as well. But they contend that the use of a pesticide on food crops will never create an imminent hazard to the public because it will take several months for that food to arrive on the tables of those people who ingest it, and therefore that the hazard created is not in fact imminent.

Dr. DUBRIDGE. Well, there is a legal determination and interpretation of the word "imminent." Sometimes food reaches your table promptly, sometimes it does not.

Mr. BICKWIT. Can we impute to Congress the intent to leave the public unprotected in such a case? As I understand it, there is no legislative history on the use of the terminology "imminent hazard" in the relevant act, and Congress has not defined that terminology in that act.

Dr. DUBRIDGE. As I understand it, imminent hazard leads to one kind of action but hazard present but not imminent leads to a different kind of action. One can have either suspension or cancellation in either case. Suspension occurs when there is imminent hazard, and suspension, though it sounds more temporary, really is not.

Mr. BICKWIT. It certainly is not, given the cancellation procedures may take as long as 3 years.

Dr. DUBRIDGE. They take time. So, there can be a suspension in the case of imminent hazard, there can be cancellation if there is hazard but not imminent. I do not know how you interpret imminent, whether it is a day, a week or a month or what.

Mr. BICKWIT. They interpreted it so that they would not be allowed to move in the case of known hazards to human health when the hazards result from the use of a hazardous pesticide on food crops.

Dr. DUBRIDGE. Then possibly a clarification of the statute would be desirable.

Mr. BICKWIT. I just wonder whether it is needed in view of the fact that it seems perverse to me to assume that Congress would intend to exclude protective action in cases such as that.

Dr. DUBRIDGE. Please do not ask me to understand the views of legal counsels for the various departments, or how they come to the various conclusions as to what their departments can or cannot do under the law.

Mr. BICKWIT. I will not if you do not ask me to understand it.

I have just one other question which relates to a legal term, but I would like to hear your reaction to it from a scientific standpoint without legal context attached to it.

Do you have any reasonable doubt about the safety of pesticides such as 2,4-D and Silvex?

Dr. DUBRIDGE. I have reasonable doubt about anything in which the research and testing have not been adequately carried out. The questions of substantial dangers in those cases I think have not been proved so I would be much more comfortable about the use of those than I would about 2,4,5-T where the teratogenic effect is now clearly established. For these others, I think imminent or serious hazards have not been found.

Mr. BICKWIT. Thank you very much.

Senator HART. Thank you.

Dr. Burger, is there anything you want to add in light of our exchange?

Dr. BURGER. No, I do not believe so, Senator.

Senator HART. I renew my apologies as I ask Dr. Steinfeld to come up. I know there are many things he would hope to be able to do this morning, which have been delayed.

Dr. Jesse Steinfeld, the Surgeon General.

STATEMENT OF DR. JESSE STEINFELD, SURGEON GENERAL, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; ACCOMPANIED BY DR. PAUL KOTIN, DIRECTOR, NATIONAL INSTITUTE OF ENVIRONMENT HEALTH SCIENCE; AND DR. WILLIAM M. UPHOLT, ACTING STAFF DIRECTOR, SECRETARY'S PESTICIDE ADVISORY COMMITTEE

Dr. STEINFELD. Thank you, Senator Hart.

With me this morning are Dr. Paul Kotin, who is the Director of the National Institute of Environmental Health Sciences. He is on my left. And on my right is Dr. William Upholt, who is the executive secretary of the Secretary's Pesticide Advisory Committee.

Before I begin, I would like to apologize for a number of misspelled words, run-on sentences and so forth in the statement of which you have a copy. It will be somewhat different than it is before you as I read it, if I may be permitted to read it.

Senator HART. You may be almost certain I won't spot the misspellings.

Dr. STEINFELD. I am pleased to appear before you today to discuss the actions that have been taken to protect the public health by the Department of Health, Education, and Welfare regarding the chlorophenoxyacid herbicides, particularly 2,4,5-T and 2,4-D.

5983214

During our last appearance before this committee, we took note of certain needs to increase the Federal Government's effectiveness in dealing with questions of hazards to the public health presented by the pesticides and pledged ourselves to action. We have made progress, even in the extremely short time since our April 15, 1970, appearance.

We are now defining how best to undertake to study the means for predicting, in laboratory animal systems, the potential hazards posed for man by chemical pesticides. It is certainly desirable, and may prove essential that we find some means of extrapolating the results from feeding animals very large doeses of pesticides to the real life situation in which man is exposed for a long period to very small amounts of these chemicals. However, I must emphasize that even with results based on studies in two species of mammals, uncertainties remain as to the significance of those studies when applied to man.

Complete information on the pesticides is essential to the efficient performance of all agencies concerned with the public health aspects of pesticides, be they Federal, State, or local. A centralized clearinghouse for information on all types of pesticides is being established jointly by the National Library of Medicine and the Food and Drug Administration. It is now being established. The Division of Toxicology of FDA and the National Library of Medicine are now sharing toxicological information and are building on this base to form the clearinghouse. I am very pleased with the progress on this information center to date.

The Food and Drug Administration has issued instructions that special attention to the extent of available resources is to be given to the analyses for residues of 2,4,5-T on food crops for which this herbicide was formerly registered for use. This step was taken as an additional precaution to prevent accidental exposure to residues of 2,4,5-T even though our surveillance activities had not detected significant residues of 2,4,5-T on these food crops.

The scientific research on which the April 15 announcement was based has continued.

The National Institute of Environmental Health Sciences is conducting further research on 2,4,5-T, certain related herbicide compounds and 2,3,7,8 tetrachlorodibenzoparadioxin which is the dioxin we referred to in our previous testimony.

Additional studies on the teratology of 2,4,5-T and tetrachlorodioxin in the random bred mouse have confirmed the earlier studies that, 2,4,5-T produces cleft palate in the mouse. One study which utilized a combination of tetrachlorodioxin and the purest 2,4,5-T available indicates that there is no synergistic effect of these two compounds on the production of cleft palate in the mouse.

Preliminary studies have been initiated with three esters of 2,4,5-T, namely, the isobutyl-ester, the isooctyl-ester, and the propylene-glycol-butyl-ester. The experiment design is the same as that used to study the acid form of 2,4,5-T earlier.

The results that are available to date are suggestive that at least some esters may be comparable in teratogenic activity to that of 2,4,5-T. At this time more definitive studies on these esters are underway.

I'm no toxicologist. People here are emotional and each morning wake up with new nails pounded into their palms. What's needed is solid scientific investigation. All I hope is they don't leave us hanging in the air for the next 20 years."

Appendix 3

[From the New Yorker, Feb. 7, 1970]

A Reporter at Large: Defoliation

By Thomas Whiteside

Late in 1961, the United States Military Advisory Group in Vietnam began, as a minor test operation, the defoliation, by aerial spraying, of trees along the sides of roads and canals east of Saigon. The purpose of the operation was to increase visibility and thus safeguard against ambushes of allied troops and make more vulnerable any Vietcong who might be concealed under cover of the dense foliage. The number of acres sprayed does not appear to have been publicly recorded, but the test was adjudged a success militarily. In January, 1962 following a formal announcement by South Vietnamese and American officials that a program of such spraying was to be put into effect, and that it was intended "to improve the country's economy by permitting freer communication as well as to facilitate the Vietnamese Army's task of keeping these avenues free of Vietcong harassments," military defoliation operations really got under way. According to an article that month in the New York Times, "a high South Vietnamese official" announced that a seventy-mile stretch of road between Saigon and the coast was sprayed "to remove foliage hiding Communist guerrillas." The South Vietnamese spokesman also announced that defoliant chemicals would be sprayed on Vietcong plantations of manioc and sweet potatoes in the Highlands. The program was gathering momentum. It was doing so in spite of certain private misgivings among American officials, particularly in the State Department, who feared, first, that the operations might open the United States to charges of engaging in chemical and biological warfare, and second, that they were not all that militarily effective. Roger Hilsman, now a professor of government at Columbia University, and then Director of Intelligence and Research for the State Department, reported, after a trip to Vietnam, that defoliation operations "had political disadvantages" and, furthermore, that they were of questionable military value, particularly in accomplishing their supposed purpose of reducing cover for ambushes. Hilsman later recalled in his book, "To Move a Nation," his visit to Vietnam, in March, 1962: "I had flown down a stretch of road that had been used for a test and found that the results were not very impressive. . . . Later, the senior Australian military representative in Saigon, Colonel Serong, also pointed out that defoliation actually aided the ambushers—if the vegetation was close to the road those who were ambushed could take cover quickly; when it was removed the guerrillas had a better field of fire." According to Hilsman, "The National Security Council spent tense sessions debating the matter."

Nonetheless, the Joint Chiefs of Staff and their Chairman, General Maxwell Taylor, agreed that chemical defoliation was a useful military weapon. In 1962, the American military "treated" 4,940 acres of the Vietnamese countryside with herbicides. In 1963, the area sprayed increased five-fold to a total of 24,700 acres. In 1964, the defoliated area was more than tripled. In 1965, the 1964 figure was doubled, increasing to 155,610 acres. In 1966, the sprayed area was again increased fivefold, to 741,247 acres, and in 1967 it was doubled once again over the previous year, to 1,486,446 acres. Thus, the areas defoliated in Vietnam had increased approximately three hundredfold in five years, but now adverse opinion among scientists and other people who were concerned about the effects of defoliation on the Vietnamese ecology at last began to have a braking effect on the program. In 1968, 1,267,110 acres were sprayed, and in 1969 perhaps a million acres. Since 1962, the defoliation operations have covered almost five million acres, an area equivalent to about twelve per cent of the entire territory of South Vietnam, and about the size of the state of Massachusetts. Between 1962 and 1967, the deliberate destruction of plots of rice, manioc, beans, and other foodstuffs through herbicidal spraying—the word "deliberate" is used here to exclude the many reported instances of accidental

DOW 1 129055

16663A

DOW-1 129057

since 1963, their use had risen two hundred and seventy-one percent—more than double the rate of increase in the use of pesticides, though pesticides are still far more extensively used. By 1960, an area equivalent to more than three per cent of the entire United States was being sprayed each year with herbicides.

Considering the rapidly growing civilian use of these products, it is perhaps not surprising that the defoliation operations in Vietnam escaped any significant comment in the press, and that the American public remained unaware of the extent to which these uses had their origin in planning for chemical and biological warfare. Nevertheless, between 1941 and the present, testing and experimentation in the use of 2,4-D, 2,4,5-T, and other herbicides as military weapons were going forward very actively at Fort Detrick. While homeowners were using herbicidal mixtures to keep their lawns free of weeds, the military were screening some twelve hundred compounds for their usefulness in biological-warfare operations. The most promising of these compounds were test-sprayed on tropical vegetation in Puerto Rico and Thailand, and by the time fullscale defoliation operations got under way in Vietnam the U.S. military had settled on the use of four herbicidal spray materials there. These went under the names Agent Orange, Agent Purple, Agent White, and Agent Blue—designations derived from color-coded stripes girdling the shipping drums of each type of material. Of these materials, Agent Orange, the most widely used as a general defoliant, consists of a fifty-fifty mixture of *n* butyl esters and of 2,4-D and 2,4,5-T. Agent Purple, which is interchangeable with Agent Orange, consists of the same substances with slight molecular variations. Agent White, which is used mostly for forest defoliation, is a combination of 2,4-D and Picloram, produced by the Dow Chemical Company. Unlike 2,4-D or 2,4,5-T, which, after application, is said to be decomposable by micro-organisms in soil over a period of weeks or months (one field test of 2,4,5-T in this country showed that significant quantities persisted in soil for ninety-three days after application), Picloram—whose use the Department of Agriculture has not authorized in the cultivation of any American crop—is one of the most persistent herbicides known. Dr. Arthur W. Galston, professor of biology at Yale, has described Picloram as "a herbicidal analog of DDT," and an article in a Dow Chemical Company publication called "Down to Earth" reported that in field trials of Picloram in various California soils between eighty and ninety-six and a half per cent of the substance remained in the soils four hundred and sixty-seven days after application. (The rate at which Picloram decomposes in tropical soils may, however, be higher.) Agent Blue consists of a solution of cacodylic acid, a substance that contains fifty-four per cent arsenic, and it is used in Vietnam to destroy rice crops. According to the authoritative "Merck Index," a source book on chemicals, this material is "poisonous." It can be used on agricultural crops in this country only under certain restrictions imposed by the Department of Agriculture. It is being used herbicidally on Vietnamese rice fields at seven and a half times the concentration permitted for weed-killing purposes in this country, and so far in Vietnam something like five thousand tons is estimated to have been sprayed on paddles and vegetable fields.

Defoliation operations in Vietnam are carried out by a special flight of the 12th Air Commando Squadron of the United States Air Force, from a base at Bien Hoa, just outside Saigon, with specially equipped C–123 cargo planes. Each of these aircraft has been fitted out with tanks capable of holding a thousand gallons. On defoliation missions, the herbicide carried in these tanks is sprayed from an altitude of around a hundred and fifty feet, under pressure, from thirty-six nozzles on the wings and tail of the plane, and usually several spray planes work in formation, laying down broad blankets of spray. The normal crew of a military herbicidal-spray plane consists of a pilot, a co-pilot, and a technician, who sits in the tail area and operates a console regulating the spray. The equipment is calibrated to spray a thousand gallons of herbicidal mixture at a rate that works out, when all goes well, to about three gallons per acre. Spraying a thousand-gallon tankload takes five minutes. In an emergency, the tank can be emptied in thirty seconds—a fact that has particular significance because of what has recently been learned about the nature of at least one of the herbicidal substances.

The official code name for the program is Operation Hades, but a more friendly code name, Operation Ranch Hand, is commonly used. In similar fashion, military public-relations men refer to the herbicidal spraying of crops sup-

16664

President Johnson from twenty-two scientists, including seven Nobel laureates. The petition pointed out that the "large-scale use of anticrop and 'nonlethal' antipersonnel chemical weapons in Vietnam" constituted "dangerous precedent" in chemical and biological warfare, and it asked the President to order it stopped. Before the end of that year, Dr. Edsall and Dr. Matthew S. Meselson, a Harvard professor of biology, obtained the signatures of five thousand scientists to co-sponsor the petition. Despite these protests, the area covered by defoliation operations in Vietnam in 1967 was double that covered in 1966, and the acreage of crops destroyed was nearly doubled.

These figures relate only to areas that were sprayed intentionally. There is no known way of spraying an area with herbicides from the air in a really accurate manner, because the material used is so highly volatile, especially under tropical conditions, that even light wind drift can cause extensive damage to foliage and crops outside the deliberately sprayed area. Crops are so sensitive to the herbicidal spray that it can cause damage to fields and gardens as much as fifteen miles away from the target zone. Particularly severe accidental damage is reported, from time to time, to so-called "friendly" crops in the III Corps area, which all but surrounds Saigon and extends in a rough square from the coastline to the Cambodian border. Most of the spraying in III Corps is now done in War Zones C and D, which are classified as free fire zones, where, as one American official has put it, "everything that moves in Zones C and D is considered Charlie." A press dispatch from Saigon in 1967 quoted another American official as saying that every Vietnamese farmer in that corps area knew of the defoliation program and disapproved of it. Dr. Galston, the Yale biologist, who is one of the most persistent critics of American policy concerning herbicidal operations in Vietnam, recently said in an interview, "We know that most of the truck crops grown along roads, canals, and trails and formerly brought into Saigon have been essentially abandoned because of the deliberate or inadvertent falling of these defoliant sprays; many crops in the Saigon area are simply not being harvested." He also cited reports that in some instances in which the inhabitants of Vietnamese villages have been suspected of being Vietcong sympathizers the destruction of food crops has brought about complete abandonment of the villages. In 1966, herbicidal operations caused extensive inadvertent damage, through wind drift, to a very large rubber plantation northwest of Saigon owned by the Michelin rubber interests. As the result of claims made for this damage, the South Vietnamese authorities paid the corporate owners, through the American military, nearly a million dollars. The extent of the known inadvertent damage to crops in Vietnam can be inferred from the South Vietnamese budget—in reality, the American military budget—for settling such claims. In 1967, the budget for this compensation was three million six hundred thousand dollars. This sum, however, probably reflects only the barest emergency claims of the people affected.

According to Representative Richard D. McCarthy, a Democrat from upstate New York who has been a strong critic of the program, the policy of allowing applications for defoliation operations to flow, usually without question, from the level of the South Vietnamese provincial or district chiefs has meant that these local functionaries would order repeated sprayings of areas that they had not visited in months, or even years. The thought that a Vietnamese district chief can initiate such wholesale spraying, in effect without much likelihood of serious hindrance by American military advisers, is a disquieting one to a number of biologists. Something that disquiets many of them even more is what they believe the long-range effects of nine years of defoliation operations will be on the ecology of South Vietnam. Dr. Galston, testifying recently before a congressional subcommittee on chemical and biological warfare, made these observations:

"It has already been well documented that some kinds of plant associations subject to spray, especially by Agent Orange, containing 2,4-D and 2,4,5-T, have been irreversibly damaged. I refer specifically to the mangrove associations that line the estuaries, especially around the Saigon River. Up to a hundred thousand acres of these mangroves have been sprayed. . . . Some (mangrove areas) had been sprayed as early as 1961 and have shown no substantial signs of recovery. . . . Ecologists have known for a long time that the mangroves lining estuaries furnish one of the most important ecological niches for the completion of the life cycle of certain shellfish and migratory fish. If these plant communities are not in a healthy state, secondary effects on the whole

DOW 1 129059

16665

latex production in the affected plantations fell off by an average of between thirty-five and forty per cent. According to a report by the two scientists, "A large variety of garden crops were devastated in the seemingly endless number of small villages scattered throughout the affected area. Virtually all of the . . . local inhabitants . . . depend for their wellbeing upon their own local produce. These people saw their crops . . . literally wither before their eyes." The Cambodian claim is still pending.

Until the end of last year, the criticism by biologists of the dangers involved in the use of herbicides centered on their use in what were increasingly construed as biological-warfare operations, and on the disruptive effects of these chemicals upon civilian populations and upon the ecology of the regions in which they were used. Last year, however, certain biologists began to raise serious questions on another score—possible direct hazards to life from 2,4,5-T. On October 29th, as a result of these questions, a statement was publicly issued by Dr. Lee DuBridge, President Nixon's science adviser. In summary, the statement said that because a laboratory study of mice and rats that had been given relatively high oral doses of 2,4,5-T in early stages of pregnancy "showed a higher than expected number of deformities" in the offspring, the government would, as a precautionary measure, undertake a series of coordinated actions to restrict the use of 2,4,5-T in both domestic civilian applications and military herbicidal operations. The DuBridge statement identified the laboratory study as having been made by an organization called the Bionetics Research Laboratories, in Bethesda, Maryland, but gave no details of either the findings or the data on which they were based. This absence of specific information turned out to be characteristic of what has been made available to the public concerning this particular research project. From the beginning, it seems, there was an extraordinary reluctance to discuss details of the purported ill effects of 2,4,5-T on animals. Six weeks after the publication of the DuBridge statement, a journalist who was attempting to obtain a copy of the full report made by Bionetics and to discuss its details with some of the government officials concerned encountered hard going. At the Bionetics Laboratories, an official said that he couldn't talk about the study, because "we're under wraps to the National Institutes of Health"—the government agency that commissioned the study. Then, having been asked what the specific doses of 2,4,5-T were that were said to have increased birth defects in the fetuses of experimental animals, the Bionetics official cut off discussion by saying, "You're asking sophisticated questions that as a layman you don't have the equipment to understand the answers to." At the National Institutes of Health, an official who was asked for details of or a copy of the study on 2,4,5-T replied, "The position I'm in is that I have been requested not to distribute this information." He did say, however, that a continuing evaluation of the study was under way at the National Institute of Environmental Health Sciences, at Research Triangle Park, North Carolina. A telephone call to an officer of this organization brought a response whose tone varied from wariness of downright hostility and made it clear that the official had no intention of discussing details or results of the study with the press.

The Bionetics study on 2,4,5-T was part of a series carried out under contract to the National Cancer Institute, which is an arm of the National Institutes of Health, to investigate more than two hundred compounds, most of them pesticides, in order to determine whether they induced cancer-causing changes, fetus-deforming changes, or mutation-causing changes in experimental animals. The contract was a large one, involving more than two and a half million dollars' worth of research, and its primary purpose was to screen out suspicious-looking substances for further study. The first visible fruits of the Bionetics research were presented in March of last year before a convention of the American Association for the Advancement of Science, in the form of a study of possible carcinogenic properties of the fifty-three compounds; the findings on 2,4,5-T were that it did not appear to cause carcinogenic changes in the animals studied.

By the time the report on the carcinogenic properties of the substances was presented, the results of another part of the Bionetics studies, concerning the teratogenic, or fetus-deforming, properties of the substances, were being compiled, but these results were not immediately made available to biologists outside the government. The data remained—somewhat frustratingly, in the view of some scientists who had been most curious about the effects of herbicides—out of sight, and a number of attempts by biologists who had heard about the

DOW_1 129061

16666

Harvard. In early October, Miss Johnson's friend, in a conversation with Professor Matthew Meselson, mentioned Miss Johnson's report on the preliminary Bionetics findings. This was the first that Dr. Meselson had heard of the existence of the Bionetics study. A few days previously, he had received a call from a scientist friend of his asking whether Dr. Meselson had heard of certain stories, originating with South Vietnamese journalists and other South Vietnamese, of an unusual incidence of birth defects in South Vietnam, which were alleged to be connected with defoliation operations there.

A few days later, after his friend sent him further information, Mr. Meselson decided to obtain a copy of the Bionetics report, and he called up an acquaintance in a government agency and asked for it. He was told that the report was "confidential and classified," and inaccessible to outsiders. Actually, in addition to the preliminary report there were now in existence the full Bionetics report and a statistical summary prepared by the National Institute of Environmental Health Sciences, and, by nagging various Washington friends, Dr. Meselson obtained bootlegged copies of the two latest reports. What he read seemed to him to have such serious implications that he got in touch with acquaintances in the White House and also with someone in the Army to alert them to the problems of 2,4,5-T, in the hope that some new restriction would be placed on its use. According to Dr. Meselson, the White House people apparently didn't know until that moment that the reports on the adverse effects of 2,4,5-T even existed. (Around that time, according to a member of Nader's Raiders, "a tremendous lid was put on this thing" within government agencies, and on the subject of the Bionetics work and 2,4,5-T "people in government whom we'd been talking to freely for years just shut up and wouldn't say a word.") While Dr. Meselson awaited word on the matter, a colleague of his informed the press about the findings of the Bionetics report. Very shortly thereafter, Dr. DuBridge made his public announcement of the proposed restrictions on the use of 2,4,5-T.

In certain respects, the DuBridge announcement is a curious document. In its approach to the facts about 2,4,5-T that were set forth in the Bionetics report, it reflects considerable sensitivity to the political and international issues that lie behind the widespread use of this powerful herbicide for civilian and military purposes, and the words in which it describes the reasons for restricting its use appear to have been very carefully chosen:

"The actions to control the use of the chemical were taken as a result of findings from a laboratory study conducted by Bionetics Research Laboratories which indicated that offspring of mice and rats given relatively large oral doses of the herbicide during early stages of pregnancy showed a higher than expected number of deformities.

"Although it seems improbable that any person could receive harmful amounts of this chemical from any of the existing uses of 2,4,5-T, and while the relationships of these effects in laboratory animals to effects in man are not entirely clear at this time, the actions taken will assure safety of the public while further evidence is being sought."

These actions, according to the statement, included decisions that the Department of Agriculture would cancel manufacturers' registrations of 2,4,5-T for use on food crops, effective at the beginning of 1970, "unless by that time the Food and Drug Administration has found a basis for establishing a safe legal tolerance in and on foods," and that the Departments of Agriculture and the Interior, in their own programs, would stop the use of 2,4,5-T in populated areas and in all other areas where residues of the substance could reach man. As for military uses of 2,4,5-T, the statement said, "The chemical is effective in defoliating trees and shrubs and its use in South Vietnam has resulted in reducing greatly the number of ambushes, thus saving lives." However, the statement continued, "the Department of Defense will [henceforth] restrict the use of 2,4,5-T to areas remote from the population."

All this sounds eminently fair and sensible, but whether it represents a candid exposition of the facts about 2,4,5-T and the Bionetics report is debatable. The White House statement that the Bionetics findings "indicated that offspring of mice and rats given relatively large oral doses of the herbicide during early stages of pregnancy showed a higher than expected number of deformities" is, in the words of one eminent biologist who has studied the Bionetics data, "an understatement." He went on to say that "if the effects on experimental animals are applicable to people it's a very sad and serious situa-

their total number was, in fact, greater, not less, than this average. Including controls but excluding litters, the total number of animals used in the 2,4,5-T studies was two hundred and twenty-five. Analysis of the results by the National Institute of Environmental Health Sciences found them statistically "significant," and this is the real purpose of such a study: it is meant to act as a coarse screen to shake out of the data the larger lumps of bad news. Such a study is usually incapable of shaking out anything smaller; another kind of study is needed to do that.

Thus, the DuBridge statement seems to give rise to this question: If the Bionetics study, based on the effects of 2,4,5-T on two hundred and twenty-five experimental animals of two species, appears to be less than conclusive, on the ground that "the study involved relatively small numbers of laboratory rats and mice," what is one to think of the adequacy of the tests that the manufacturers of pesticides make? If, as the DuBridge statement says, "at best it is difficult to extrapolate results obtained with laboratory animals to man," what is one to say of the protection that the government affords the consumer when the results of tests of pesticidal substances on perhaps a hundred and twenty rats are officially extrapolated to justify the use of the substances by a population of two hundred million people—not to mention one to two million unborn babies being carried in their mothers' wombs?

The very coarseness of the screen used in all these tests—that is, the relatively small number of animals involved—means that the bad news that shows up in the data has to be taken with particular seriousness, because lesser effects tend not to be demonstrable at all. The inadequacy of the scale on which animal tests with, for instance, pesticides are currently being made in this country to gain F.D.A. approval is further indicated by the fact that a fetus-deforming effect that might show up if a thousand test animals were used is almost never picked up, since the studies are not conducted on that scale; yet if the material being tested turned out to have the same effect, quantitatively, on human beings, this would mean that it would cause between three and four thousand malformed babies to be produced each year. The teratogenic effects of 2,4,5-T on experimental animals used by the Bionetics people, however, were not on the order of one in a thousand. Even in the case of the lowest oral dose given rats, they were on the order of one in three.

Again, it is fair to say that what is applicable to rats in such tests may not be applicable to human beings. But it is also fair to say that studies involving rats are conducted not for the welfare of the rat kingdom but for the ultimate protection of human beings. In the opinion of Dr. Epstein, the fact that the 2,4,5-T used in the Bionetics study produced teratogenic effects in *both* mice and rats underlines the seriousness of the study's implications. In the opinion of Dr. McLaughlin, this is even further underlined by another circumstance—that the rat, as a test animal, tends to be relatively resistant to teratogenic effects of chemicals. For example, in the late nineteen-fifties, when thalidomide, that disastrously teratogenic compound, was being tested on rats in oral dosages ranging from low to very high, no discernible fetus-deforming effects were produced. And Dr. McLaughlin says that as far as thalidomide tests on rabbits were concerned, "You could give thalidomide to rabbits in oral doses at between fifty and two hundred times the comparable human level to show any comparable teratogenic effects." In babies born to women who took thalidomide, whether in small or large dosages and whether in single or multiple dosages, between the sixth and seventh weeks of pregnancy, the rate of deformation was estimated to be one in ten.

Because of the relatively coarse testing screen through which compounds like pesticides—and food additives as well—are sifted before they are approved for general or specialized use in this country, the Food and Drug Administration theoretically maintains a policy of stipulating, as a safety factor, that the maximum amount of such a substance allowable in the human diet range from one two-thousandth to one one-hundredth of the highest dosage level of the substance that produces no harmful effects in experimental animals. (In the case of pesticides, the World Health Organization takes a more conservative view, considering one two-thousandth of the "no-effect" level in animal studies to be a resaonsable safety level for human exposure.) According to the standards of safety established by F.D.A. policy, then, no human being anywhere should ever have been exposed to 2,4,5-T, because in the Bionetics study of rats *every* dosage level produced deformed fetuses. A "no-effect" level was never achieved.

DOW 1 129065

the thirty-second emergency-dumping procedure. Dr. Pfeiffer has recalled going along as an observer on a United States defoliation mission last March, over the Plain of Reeds area of Vietnam, near the Cambodian border, during which the technician at the spray controls was unable to get the apparatus to work, and thereupon dumped his whole load. "This rained down a dose of 2,4,5-T that must have been fantastically concentrated," Dr. Pfeiffer has said. "It was released on a very watery spot that looked like headwaters draining into the Mekong River, which hundreds of thousands of people use? In another instance, he has recalled, a pilot going over the area of the supposedly "friendly" Catholic refugee villages of Ho Nai, near Bien Hoa, had serious engine trouble and dumped his whole spray load of herbicide on or near the village. In such instance, the concentration of 2,4,5-T dumped upon an inhabited area in Vietnam probably averaged about a hundred and thirty times the concentration reccommended by 2,4,5-T manufacturers as both effective and safe for use in the United States.

Theoretically, the dangers inherent in the use of 2,4,5-T should have been removed by means of the steps promised in the White House announcement last October. A quick reading of the statement by Dr. DuBridge (who is also the executive secretary of the President's Environmental Quality Council) certainly seemed to convey the impression that from that day onward there would be a change in Department of Defense policy on the use of 2,4,5-T in Vietnam, just as there would be a change in the policies of the Departments of Agriculture and the Interior on the domestic use of 2,4,5-T. But did the White House mean what it certainly seemed to be saying about the future military use of 2,4,5-T in Vietnam? The White House statement was issued on October 29th. On October 30th, the Pentagon announced that no change would be made in the policy governing the military use of 2,4,5-T in South Vietnam, because—so the Washington *Post* reported on October 31st—"the Defense Department feels its present policy conforms to the new Presidential directive." The *Post* article went on:

"A Pentagon spokesman's explanation of the policy, read at a morning press briefing, differed markedly from the written version given reporters later.

"When the written statement was distributed, reporters were told not to use the spokesman's [previous] comment that the defoliant . . . is used against enemy 'training and regroupment centers.'

"The statement was expunged after a reporter asked how use against such centers conformed to the Defense Department's stated policy of prohibiting its use in 'populated areas.'"

But the statement wasn't so easily expunged. A short time later, it was made again, in essence, by Rear Admiral William E. Lemos, of the Policy Plans and National Security Council Affairs Office of the Department of Defense, in testimony before a subcommittee of the House Foreign Affairs Committee, the only difference being that the phrase "training and regroupment centers" became "enemy base camps." And in testifying that the military was mounting herbicidal operations on alleged enemy base camps Rear Admiral Lemos said:

"We know . . . that the enemy will move from areas that have been sprayed. Therefore, enemy base camps or unit headquarters are sprayed in order to make him move to avoid exposing himself to aerial observation."

If one adds to the words "enemy base camps" the expunged words "training and regroupment centers"—centers that are unlikely to operate without an accompanying civilian population—what the Defense Department seems actually to be indicating is that the "areas remote from the population" against which the United States is conducting military herbicidal operations are "remote from the population" at least in part *because* of these operations.

As for the Bionetics findings on the teratogenic effects of 2,4,5-T on experimental animals, the Department of Defense indicated that it put little stock in the dangers suggested by the report. A reporter for the *Yale Daily News* who telephoned the Pentagon during the first week in December to inquire about the Defense Department's attitude toward its use of 2,4,5-T in the light of the Bionetics report was assured that "there is no cause for alarm about defoliants." A week or so later, he received a letter from the Directorate for Defense Information at the Pentagon which described the Bionetics results as based on "evidence that 2,4,5-T, when fed in large amounts to highly imbred and susceptible mice and rats, gave a higher incidence of birth defects than was normal for these animals." After reading this letter, the *Yale Daily News*

DOW 1129067
16669

business of manufacturing 2,4,5-T. It appears that the presence of a dioxin contaminant in the process of manufacturing 2,4,5-T is a constant problem among all manufacturers. Three years ago, Dow was obliged to close down its 2,4,5-T plant in Midland, Michigan, for several months and partly rebuild it because of what Dow people variously described as "a problem" and "an accident." The problem—or accident—was that workers exposed to the dioxin contaminant during the process of manufacture came down with an acute skin irritation known as chlor-acne. The Dow people, who speak with considerable pride of their toxicological work ("We established our toxicology lab the year Ralph Nader was born," a Dow public-relations man said recently, showing, at any rate, that Dow is keenly aware of Nader and his career), say that the chloracne problem has long since been cleared up, and that the current level of the dioxin contaminant in Dow's 2,4,5-T is less than one part per million, as opposed to the dioxin level in the 2,4,5-T used in the Bionetics study, which is alleged to have been between fifteen and thirty parts per million. A scientist at the DuBridge office, which has become a coordinating agency for information having to do with the 2,4,5-T question, says that the 2,4,5-T used by Bionetics was "probably representative" of 2,4,5-T being used in this country—and presumably in Vietnam—at the time it was obtained but that considerably less of the contaminant is present in the 2,4,5-T now being produced. Evidently, the degree of dioxin contamination present in 2,4,5-T varies from manufacturer to manufacturer. What degree of contamination high or low, was present in the quantities of 2,4,5-T shipped to South Vietnam at various times this spokesman didn't seem to know.

The point about the dioxin contamination of 2,4,5-T is an extremely important one, because if the suspicions of the Dow people are correct and the cause of the fetus deformities cited in the Bionetics study is not the 2,4,5-T but the dioxin contaminant, then this contaminant may be among the most teratogenically powerful agents ever known. Dr. McLaughlin has calculated that if the dioxin present in the Bionetics 2,4,5-T was indeed responsible for the teratogenic effects on the experimental animals, it looks as though the contaminant would have to be at least ten thousand times more teratogenically active in rats than thalidomide was found to be in rabbits. Furthermore, it raises alarming questions about the prevalence of the dioxin material in our environment. It appears that under high heat the dioxin material can be produced in a whole class of chemical substances known as trichlorophenols and pentachlorophenols. These substances include components of certain fatty acids used in detergents and in animal feed.

As a consequence of studies that have been made of the deaths of millions of young chicks in this country after the chicks had eaten certain kinds of chicken feed, government scientists are now seriously speculating on the possibility that the deaths were at the end of a chain that began with the spraying of corn crops with 2,4,5-T. The hypothesis is that residues of dioxin present in the 2,4,5-T remained in the harvested corn and were concentrated into certain byproducts that were then sold to manufacturers of chicken feed, and that the dioxin became absorbed into the system of the young chicks. One particularly disquieting sign of the potential of the dioxin material is the fact that bioassays made on chick embryos in another study revealed that all the embryos were killed by one twenty-millionth of a gram of dioxin per egg.

Perhaps an even more disquieting speculation about the dioxin is that 2,4,5-T may not be the only material in which it appears. Among the compounds that several experienced biologists and toxicologists suspect might contain or produce dioxin are the trichlorophenols and pentachlorophenols, which are rather widely present in the environment in various forms. For example, a number of the trichlorophenols and pentachlorophenols are used as slime-killing agents in paper-pulp manufacture, and are present in a wide range of consumer products, including adhesives, water-based and oil-based paints, varnishes and lacquers, and paper and paper coatings. They are used to prevent slime in pasteurizers and fungus on vats in breweries and are also used in hair shampoo. Along with the 2,4,5-T used in the Bionetics study, one trichlorophenol and one pentachlorophenol were tested without teratogenic results. But Dr. McLaughlin points out that since there are many such compounds put out by various companies, these particular samples might turn out to be —by the reasoning of the allegation that the 2,4,5-T used by Bionetics was unusually dirty—unusually clean.

DOW-1 129069

16670

There have been a number of reports from Vietnam both of animal abortions and of malformed human babies that are thought to have resulted from spraying operations in which 2,4,5-T was used. But such scattered reports, however well founded, cannot really shed much more light on the situation. The fact is that even in this country, the best-fed, richest, and certainly most statistics-minded of all countries on earth, the standards for testing materials that are put into the environment, into drugs, and into the human diet are grossly inadequate. The screening system is so coarse that as a teratology panel of the Mrak Commission warned recently, in connection with thalidomide, "the teratogenicity of thalidomide might have been missed had it not produced malformations rarely encountered." In other words, had it not been for the fact that very unusual and particularly terrible malformations appeared in an obvious pattern—for example, similarly malformed babies in the same hospital at about the same time—pregnant women might still be using thalidomide, and lesser deformations would, so to speak, disappear into the general statistical background. As for more subtle effects, such as brain damage and damage to the central-nervous system, they would probably never show up as such at all. If such risks existed under orderly, normal medical conditions in a highly developed country, how is one ever to measure the harm that might be done to unborn children in rural Vietnam, in the midst of the malnutrition, the disease, the trauma, the poverty, and the general shambles of war?

DEPARTMENT OF AMPLIFICATION,
New York, March 5, 1970.

The EDITORS,
The New Yorker

DEAR SIRS: In an article that appeared in *The New Yorker* on February 7th, I wrote that Dr. Lee DuBridge, the President's science adviser, issued a statement last October at the White House saying that because a laboratory study had shown a "higher than expected number of deformities" in the fetuses of mice and rats exposed to the herbicide 2,4,5-T, agencies of the United States government would take action to restrict the use of that substance in this country and in Vietnam, where it was being used in extensive military defoliation operations. This action, Dr. DuBridge announced, would include the cancellation, by January 1st of this year, of Department of Agriculture permits for the use of 2,4,5-T on some American food crops unless the Food and Drug Administration had by then been able to determine a safe concentration of the herbicide in foods. Dr. DuBridge further announced that the Department of Defense would thenceforth "restrict the use of 2,4,5-T to areas remote from the population" in Vietnam. His statement added that these actions and others "will assure the safety of the public while further evidence [of the alleged harmful effects of 2,4,5-T] is being sought."

Four months have passed, and 2,4,5-T is still being used as widely as ever. The Department of Agriculture has yet to cancel its permits for the use of the herbicide on food crops in this country, and the Department of Defense is continuing to use it in populated areas of Vietnam. In the meantime, officials of the Dow Chemical Company, which is one of the largest producers of 2,4,5-T, have been maintaining that the samples of 2,4,5-T used in the study cited by Dr. DuBridge, which was done by the Bionetics Research Laboratories, of Bethesda, Maryland, were uncharacteristic of the 2,4,5-T currently being produced, because the material tested by Bionetics—which did not come from Dow—was contaminated to an unusual extent by a toxic substance identified as symmetrical 2,3,6,7-tetrachlorodibenzo-*p*-dioxin. This contaminant, usually called dioxin, was alleged by the Dow people to be present in the Bionetics samples at a concentration of approximately twenty-seven parts per million, and they claim that the 2,4,5-T that Dow is currently producing contains the dioxin contaminant in concentrations of less than one part per million. The Dow people maintain that their currently produced 2,4,5-T does not appear to have the effect of deforming rat fetuses. In January, a Dow official told the Department of Health, Education, and Welfare, "We strongly urge that action concerning the status of 2,4,5-T be held in abeyance until [Dow's] testing program is completed [in] April." The United States government's failure so far to place the promised restrictions on the use of 2,4,5-T in this country may in part be attributed to this plea.

45-362—70——9

NOV 1 1290 71

16671

Toxicology of Chlorinated Dibenzo-p-dioxins

by B.A. Schwetz,* J.M. Norris,* G.L. Sparschu,* V.K. Rowe,* P.J. Gehring,* J.L. Emerson,† and C.G. Gerbig †

DOW 331974
MM 022777

Severe toxicological responses have been associated with certain chlorodibenzodioxins. One of these responses is chloracne, a folliculosis first associated with skin contamination by chlorohydrocarbons in 1899 (*1*). Serious outbreaks of chloracne-like lesions associated with runaway reactions in the production of 2,4,5-trichlorophenol occurred in Germany in the early 1950's (*2*). 2,4,5-Trichlorophenol itself does not cause acne (*3*), but the contaminants which may be formed in the uncontrolled production of 2,4,5-trichlorophenol are extremely potent acnegens (*2*). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and tri- and tetrachlorodibenzofuran were isolated from the contaminants formed in 2,4,5-trichlorophenol production and were demonstrated to be strongly positive acnegens when applied to rabbit ears (*3*). By using the rabbit ear test, the acnegenic potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) was confirmed in 1962 (*4*). In addition, 2,3,7,8-TCDD is extremely toxic in the chick embryo assay (*5*) and is highly embryotoxic in rats (*6*). Another chlorodibenzodioxin, hexachlorodibenzo-*p*-dioxin (HCDD), is known to be positive for the chick edema factor, a condition characterized by hydropericardium, ascites, and anasarca (*5*, *7*).

*Chemical Biology Research, The Dow Chemical Co., Midland, Michigan 48640.

† Human Health Research and Development Center, The Dow Chemical Co., Zionsville, Indiana 46077.

Experimental

Materials

The chlorodibenzodioxin samples used in these studies are identified and described in Table 1. Studies were limited in some cases by availability of pure samples.

Acute Lethality

Samples of 2,7-dichlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, hexachlorodibenzo-*p*-dioxin, and octachlorodibenzo-*p*-dioxin were evaluated for acute oral lethality in several animals as summarized in Table 2.

Test materials were administered as suspensions in corn oil or as corn oil: acetone (9:1) solutions in single doses by gavage. The animals were deprived of feed for 16 hr before dosing. After dosing, they were observed for signs of toxicity including body weight changes for two to eight weeks.

Lethality of 2,3,7,8-TCDD via skin absorption was tested on rabbits of mixed sexes with doses of 31.6, 63, 126, 252, and 500 μg/kg body weight. The compound was applied as a 0.01% solution in acetone to the abdominal skin which had been shorn. After the acetone evaporated, the trunk of each rabbit was wrapped in cotton to prevent ingestion. The rabbits were housed in individual holding cages and were observed for signs of toxicity including body weight changes for three weeks.

Parenteral lethality was determined by injecting rabbits of mixed sexes intraperi-

CORRECTION: the term μg or mg/kg-day on pp 89-97 should read μg or mg/kg/day.

16672

toneally with 31.6, 63, 126, 252 and 500 μg/kg of 2,3,7,8-TCDD as a 0.01% corn oil suspension; control rabbits were injected with corn oil. The rabbits were housed in individual holding cages and were observed for signs of toxicity for four weeks. The LD_{50}'s were calculated by the Weil modification of the Thompson method (*8, 9*) or by the Litchfield and Wilcoxon method (*10*). The acute lethality studies were terminated when it was evident that the survivors were not showing signs of toxicity.

Eye Irritation

Rabbit eyes were examined prior to experiments and found to be free from defects or irritation. Approximately 2 mg of 2,7-DCDD, 2,3,7,8-TCDD, HCDD, or OCDD were instilled in the conjunctival sac of one eye; the contralateral eye served as a control. The eyes were examined at various times after treatment for conjunctival redness and chemosis, iritis, and corneal injury. Responses were categorized according to intensity.

Rabbit Ear Bioassay For Acnegenic Activity

Acnegenic activity of 2,7-DCDD, 1,2,3,4-TCDD, 2,3,7,8-TCDD, HCDD, and OCDD was tested by applying 0.1 ml of either a solvent solution or the supernatant of a solvent suspension of each compound to the inner surface of the rabbit's ears five days a week for four weeks. The ears were examined weekly for signs of chloracne, inflammation and hyperkeratosis. The responses were divided into five categories: (1) none, (2) very slight, (3) slight, (4) moderate, and (5) severe.

Responses in the first three categories include no response to mild irritation, increased ear thickness, slight enlargement of the follicular aperture, slight exfoliation and slight crust formation. These responses alone are not considered indicative of chloracnegenic activity. Categories 4 and 5 are indicative of acnegenic response and are characterized by comedo formation, increased ear thickness and hyperkeratosis.

Teratology

Pregnant adult Sprague-Dawley (Spartan strain) female rats weighing approximately 250 g were used to study teratogenicity of the chlorinated dibenzo-*p*-dioxins. The day sperm were first present in a vaginal smear was considered day zero of pregnancy. The animals were housed individually in wire-bottom cages in a room controlled for temperature, humidity, light cycle and noise. Commercial laboratory rat chow and water were provided with choice.

Corn oil: acetone (9:1) solutions with varying amounts of test material were given in 2.5 ml/kg dosages by gavage. Dosages were calculated using daily body weights. Rats were treated with 100 mg of 2,7-DCDD/kg-day, 0.1, 1.0, 10, or 100 μg HCDD/kg-day and 100 or 500 mg OCDD/kg-day on days 6 through 15 of gestation. Control rats received 2.5 ml/kg of corn oil: acetone (9:1) orally. All rats were observed daily throughout pregnancy and were weighed on days 6, 13, and 21 of gestation. Pregnant females were sacrificed by carbon dioxide anesthesia on day 21 of gestation; the uterine horns were exteriorized through a midline incision in the abdominal wall, and the number and position of live, dead, and resorbed fetuses were noted. After being weighed and sexed, the fetuses were examined for external anomalies; the crown-rump length was measured with a vernier caliper. Half of each litter was preserved in Bouin's solution and later examined for soft tissue anomalies (*11*); the other half was preserved in alcohol, cleared and stained with Alizarin Red-S, and examined for skeletal abnormalities (*12*).

A 2 × 2 contingency table was used to evaluate the frequency of anomalies and resorptions within the fetal population and between litters. Body weight and body measurements were statistically analyzed by an analysis of variance and Tukey's test (*13*). In all cases, the level of significance was $P<0.05$.

Chick Bioassay for Chick Edema Factor

The bioassay for chick edema factor was

UCM 54910

16673

Table 3. Lethality of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [a]

Species and sex	Sample [b]	Route of administration	Time of death, days postadministration	LD_{50}, mg/kg	Dose, mg/kg	Number deaths/number treated
Rat, male	c	Oral	9–27	0.022	0.008	0/5
					0.016	0/5
					0.032	10/10
					0.063	5/5
Rat, female	c	Oral	13–43	0.045 (0.030–0.066)		
Guinea pig, male	c	Oral	5–34	0.0006 (0.0004–0.0009)		
Guinea pig, male	d	Oral	9–42	0.0021 (0.0015–0.0030)		
Rabbit, mixed	c	Oral	6–39	0.115 (0.038–0.345)		
	c	Skin	12–22	0.275 (0.142–0.531)		
	c	Intraperitoneal	6–23	—	0.032	0/5
					0.063	2/5
					0.126	2/5
					0.252	2/5
					0.500	3/5
Dogs, male	c	Oral	9–15		0.30	0/2
					3.00	2/2
Dogs, female	c	Oral	—		0.03	0/2
					0.10	0/2

[a] Responses to individual doses are given in those cases in which an LD_{50} could not be calculated. The LD_{50} for oral administration to rabbits was calculated by using the method of Litchfield and Wilcoxon (*9*); the remaining values were calculated by using the Weil modification of the method of Thompson (*15, 16*).

[b] Letters refer to sample identification in Table 1.

ient pain and conjunctival inflammation, initially. Treatment with 2,3,7,8-TCDD was associated with delayed conjunctival chemosis 13-22 days later. By day 27, the chemosis had subsided, but the rim of the eyelid was thickened and encrusted. In rabbits treated with HCDD, the rim of the eyelid was encrusted 27 days after treatment. Neither corneal injury nor iritis was observed in any of the animals following instillation of the chlorodibenzodioxins in the conjunctival sac.

Acnegenic Response

Both 2,3,7,8-TCDD and HCDD produced acne in the rabbit ear bioassay as indicated by the formation of comedones. Solutions of 2,3,7,8-TCDD (sample c) in benzene ranging in concentration from 0.04 to 400 μg/ml produced a positive response with severity increasing with concentration. A negative response was obtained with a solution of 0.004 μg/ml. In contrast, a chloroform solution of 1,2,3,4-TCDD, 50 μg/ml, did not produce a positive response. With HCDD (samples a, b, c, and d), a response was produced by solutions of 10 to 50 μg/ml in chloroform and dimethoxyethane. Chloroform extracts from 10% suspensions of 2,7-DCDD or OCDD were negative, indicating that these have a low order or possibly no acnegenic activity.

Teratogenicity

The effects of chlorodibenzodioxins on maternal and fetal body measurements, incidence of fetal resorptions and anomalies are given in Tables 4 and 5.

2,7-DCDD. Rats treated with 100 mg/kg-day on days 6 through 15 of gestation gained slightly more weight during pregnancy than controls but showed no toxicity. There was no effect on fetal body measurements, or incidence of resorptions, or gross, soft tissue or skeletal anomalies.

HCDD. Administration of 0.1–100 μg HCDD/kg-day was associated with a dose-related decrease in maternal weight-gain

DOW 3119178

16674

Table 4. Effect of treatment with chlorinated dibenzo-*p*-dioxin on maternal and fetal body measurements and the incidence of fetal resorption.

Test compound (sample) [a]	No. of litters	Maternal weight gain, g [b]			Fetal body weight, g [c]	Fetal crown–rump [c] length, mm	Fetal resorptions, %	
		Days 6–13	Days 13–21	Days 6–21			Population [d]	Litter [e]
Control	30	36 ± 2	101 ± 6	137 ± 8	5.68 ± 0.05	44.5 ± 0.1	7 (22/337)	47 (14/30)
2,7-Dichlorodibenzo-*p*-dioxin (d)								
100.0 mg/kg-day	7	31 ± 1	122 ± 4	152 ± 5	5.80 ± 0.09	44.2 ± 0.2	6 (5/ 86)	57 (4/ 7)
Hexachlorodibenzo-*p*-dioxin (c)								
0.1 μg/kg-day	19	28 ± 2	102 ± 5	130 ± 5	5.73 ± 0.04	43.8 ± 0.1	5 (10/217)	47 (9/19)
1.0 μg/kg-day	19	27 ± 3	99 ± 5	126 ± 6	5.93 ± 0.16	45.7 ± 0.5	9 (20/218)	74 (14/19)
10.0 μg/kg-day	18	22 ± 3 [f]	97 ± 5	119 ± 6	5.12 ± 0.05 [f]	42.6 ± 0.2 [f]	25 [f] (57/229)	94 [f] (17/18)
100.0 μg/kg-day	19	6 ± 2 [f]	13 ± 7 [f]	19 ± 9 [f]	3.65 ± 0.28 [f]	35.2 ± 0.7 [f]	85 [f] (194/227)	100 [f] (19/19)
Octachlorodibenzo-*p*-dioxin (d)								
100.0 mg/kg-day	12	32 ± 2	100 ± 8	131 ± 7	5.73 ± 0.09	43.6 ± 0.4	8 (11/131)	42 (5/12)
500.0 mg/kg-day	17	35 ± 3	115 ± 4	150 ± 5	5.69 ± 0.05	44.5 ± 0.2	5 (9/199)	41 (7/17)

[a] Sample identified in Table 1; administered on days 6–15 of gestation as a corn oil: acetone (9:1) solution.
[b] Mean ± S.E. for various gestation times.
[c] Mean of litter means ± S.E.
[d] % (number resorptions/number implantations).
[e] % (number litters with at least one resorption/number litters).
[f] Significantly different from control by an analysis of variance and Tukey's test (measurements) or the 2 × 2 contingency table (resorptions), $P < 0.05$.

16675

DOW 331980

Table 6. Results of chick edema bioassay: body weight, food consumption, and pericardial fluid volume calculations of chicks treated with chlorodioxins.

Treatment (sample)[a]	n	Body weight, g[b] Day 0	Body weight, g[b] Day 21	Food consumption, g[c]	Pericardial fluid volume ml ± S.E.	Pericardial fluid volume Mean log (100 × ml)	Calculated t value	Positive for chick edema factor based on Calculations	Positive for chick edema factor based on Gross lesions
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (d)[d]									
0 μg/kg	10	46 ± 1	199 ± 5	17.4	0.16 ± 0.02	1.1717	—	—	—
0.01 μg/kg	10	44 ± 1	196 ± 7	16.7	0.14 ± 0.02	1.1181	−0.74	No	No
0.10 μg/kg	10	45 ± 1	203 ± 7	17.2	0.19 ± 0.01	1.2688	+1.69	No	No
1.0 μg/kg[e]	2	42 ± 1	196 ± 24	33.7	2.34 ± 0.08	2.3680	+21.7	No	Yes
10.0 μg/kg[f]	9	42 ± 1	No survivors	11.2	1.29 ± 0.62	1.5661	+1.47	No	Yes
Hexachlorodibenzo-*p*-dioxin (c)[d]									
0 μg/kg	9	38 ± 1	194 ± 5	17.4	0.15 ± 0.01	1.1771	—	—	—
0.1 μg/kg	10	42 ± 1	197 ± 4	17.8	0.11 ± 0.02	0.9978	−1.93	No	No
1.0 μg/kg	10	43 ± 1	196 ± 6	18.2	0.09 ± 0.01	0.9387	−4.57	No	No
10.0 μg/kg[g]	9	38 ± 1	187 ± 7	16.7	0.81 ± 0.01	1.7294	+3.82	No	Yes
100.0 μg/kg[h]	10	36 ± 1	No survivors	13.3	0.62 ± 0.24	1.5650	+2.72	No	Yes
Octachlorodibenzo-*p*-dioxin (d)[i]			(Day 20)						
0% of diet	12	45 ± 1	141 ± 8	13.3	0.08 ± 0.01	0.8053	—	—	—
0.1% of diet	11	45 ± 1	124 ± 5	9.5	0.06 ± 0.01	0.7889	−0.21	No	No
0.5% of diet	11	43 ± 1	196 ± 10	10.9	0.09 ± 0.01	0.9002	+0.91	No	No

[a] Sample identified in Table 1.
[b] Mean ± S.E.
[c] Grams/chick/day.
[d] Administered orally as a corn oil: acetone solution.
[e] Animals died on days 9, 11, 11, 14, 15, 17, 18, and 19 of treatment.
[f] Animals died on days 3, 4, 4, 4, 5, 8, 8, 9, 12 and 15 of treatment.
[g] One animal died on day 19 of treatment.
[h] Animals died on days 5, 5, 6, 7, 8, 10, 11, 11, 15 and 17 of treatment.
[i] Fed in the diet (0.1% ≅ 100 mg/kg, 0.5% ≅ 500 mg/kg).

16676

DOW 331982

Microscopic examination of this organ revealed a highly variable pattern and degree of hepatic necrosis with various degrees of degeneration and regeneration of the hepatocytes, depending upon the post-treatment interval. Necrosis was observed both in the centrilobular and periportal areas. The degree of necrosis of the liver was not sufficient to conclude that it was responsible for death. Hepatic lesions were observed in rats, mice, rabbits, and dogs. In addition to hepatic involvement, other changes observed sporadically include fat necrosis, periarteritis, serous atrophy of fat, and ascites.

Discussion and Summary

The studies reported here confirmed the high toxicity of 2,3,7,8-TCDD. In addition, some perspective of the relative toxicities of 2,7-DCDD, HCDD, and OCDD has been obtained. 2,7-DCDD and OCDD failed to cause death in female rats given oral doses of 1 g/kg; even larger doses were given to mice without causing death. Limited data suggest that oral doses of approximately 100 mg/kg of HCDD are needed to cause death in male rats. In the teratology study, no deaths occurred following administration of 100 μg/kg of HCDD to female rats for 10 consecutive days.

2,3,7,8-TCDD is much more toxic than the other chlorodibenzodioxins studied; the LD_{50} ranged from 0.6 μg/kg in male guinea pigs to 115 μg/kg in rabbits. Dogs appear to be less sensitive than rabbits. Others have reported 100% mortality in rabbits treated with 10 μg/kg (*15*) and chick embryos treated with 0.05 μg/egg (*5*).

Death following treatment with a lethal dose of 2,3,7,8-TCDD is often delayed for several weeks. Among the animals which died following treatment, approximately half the deaths occurred between 13 and 18 days after treatment, with one animal dying as late as 43 days after a single oral dose. In mice and rabbits, there is a marked individual difference in susceptibility to this compound which makes it difficult to conduct acute lethality studies.

If the results of the rabbit eye irritation test can be extrapolated to man, accidental contact of these chlorodibenzodioxins with the eyes should not present a serious threat to vision. However, repeated contact with the skin of small amounts of either 2,3,7,8-TCDD or HCDD may be expected to produce chloracne. Sensitivity to 2,3,7,8-TCDD was recognized by industry years ago, and precautions have been taken to minimize its occurrence and prevent contamination of worker's skin. HCDD is apparently a less potent acnegen than 2,3,7,8-TCDD.

As previously reported, 2,3,7,8-TCDD is highly embryotoxic (*6*). The no-effect level for embryotoxicity was 0.03 μg/kg-day of 2,3,7,8-TCDD. In contrast to the high embryotoxicity of the symmetrical 2,3,7,8-TCDD, 1,2,3,4-TCDD was not embryotoxic at doses as high as 800 μg/kg-day (*16*).

By previously described definitions of teratogenicity and embryotoxicity (*17*), HCDD is teratogenic in the rat at a 100 μg/kg-day dose level, given orally on days 6 through 15 of gestation. Treatment of pregnant rats with HCDD caused embryotoxicity evidenced by a dose-related decrease in fetal body weight and crown–rump length and an increase in the incidence of fetal resorptions (Table 4). Likewise, the incidence of certain soft tissue and skeletal anomalies increased in a manner related to the dose level of HCDD (Table 5). A 0.1 μg/kg-day dosage of HCDD had no effect on embryonal or fetal development.

OCDD caused embryotoxicity but was not teratogenic at 500 mg/kg-day. OCDD and 2,7-DCDD caused neither teratogenicity nor embryotoxicity at 100 mg/kg-day. Khera and Ruddick (*16*) reported that the administration of 2 mg 2,7-DCDD/kg-day was associated with microscopic myocardial and pericardial lesions in rat fetuses. However, examination of sections of myocardium and pericardium from fetuses of dams treated with 100 mg doses in this study revealed no morphological differences from controls.

Both 2,3,7,8-TCDD and HCDD give positive results in chick edema bioassays (Table 6). This HCDD result is consistent with a previous report that the HCDD isolated

DOW331984

16677

16. Khera, K. S., and Ruddick, J. A. Polychlorodibenzo-*p*-dioxins: Perinatal effects and dominant lethal test in Wistar rats. Advan. Chem. Ser. 121, R. F. Gould, Ed., American Chemical Society, Washington, D.C., in press.

17. Schwetz, B. A., Sparschu, G. L., and Gehring, P. J. The effect of 2,4-L and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. Food Cosmet. Toxicol. 9: 801 (1971).

DOW 331986

16678

DOW 3318799
MM 022799

Formation of Dibenzodioxins and Other Condensation Products from Chlorinated Phenols and Derivatives

by H.G. Langer,* T.P. Brady,* and P.R. Briggs*

Introduction

Chlorodioxins can be formed in a two-step condensation reaction from *ortho*-substituted chlorophenoxy radicals or anions (*1–3*). The first route is of significance only where strongly oxidizing conditions exist such as a reaction of chlorine with pentachlorophenol at elevated temperature. For the second route we have investigated the condensation of alkali metal salts of chlorinated phenols which occurs spontaneously when these metal salts are heated to temperatures above 300°C. Since this reaction is strongly exothermic, it proceeds to completion in a very narrow temperature range once initiated.

For bimolecular reactions involving *ortho* chlorines of both phenate molecules, dioxins would be formed according to the scheme shown in eq. (1) as one of the possible reaction pathways. If these phenates contain halogen substituents in *meta* or *para* positions, these also are involved in the condensation reactions and the product distribution depends on a variety of factors such as (1) the total number of halogen substituents which determines the ease of removal of halogen; (2) the arrangement of the molecules within a crystal if this reaction takes place in the solid state, which in turn is influenced by the metal cation involved; (3) steric effects which may facilitate the attack by the second molecule at a particular carbon or may prevent a nucleophilic attack at a particular site; (4) electronic effects which in this case allows halogen atoms in *meta* positions to be removed more readily than those in the other ring positions (*4*).

*Eastern Research Laboratory, Dow Chemical USA, Wayland, Massachusetts 01778.

Experimental

Thermal reactions were carried out in bulk as described below or in a differential thermal analysis cell using a DuPont 900 Differential Thermal Analyzer.

Residues and sublimates were analyzed by mass spectrometry on an A.E.I. MS-12 instrument coupled to a D.E.C-PDP-12 computer using a program called MASH (*5*).

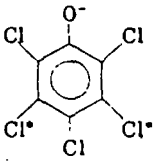
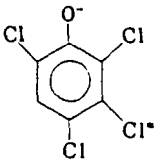
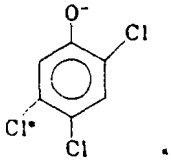
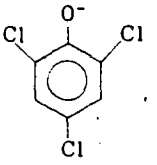
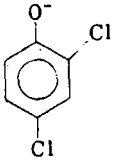
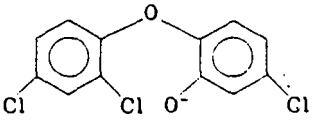
Results

The results of our extensive studies are summarized in Table 1, which contains all the pertinent information on this complicated reaction system and with this the answers to many questions regarding dioxin formation.

From electronic considerations Cl atoms marked with an asterisk (*) and located in *meta* positions are more likely to be removed than any of the others (*4*). The first two reactions occur in the solid state where the molecules—anions as well as alkali metal cations—are locked into positions which may have an important effect on the reaction

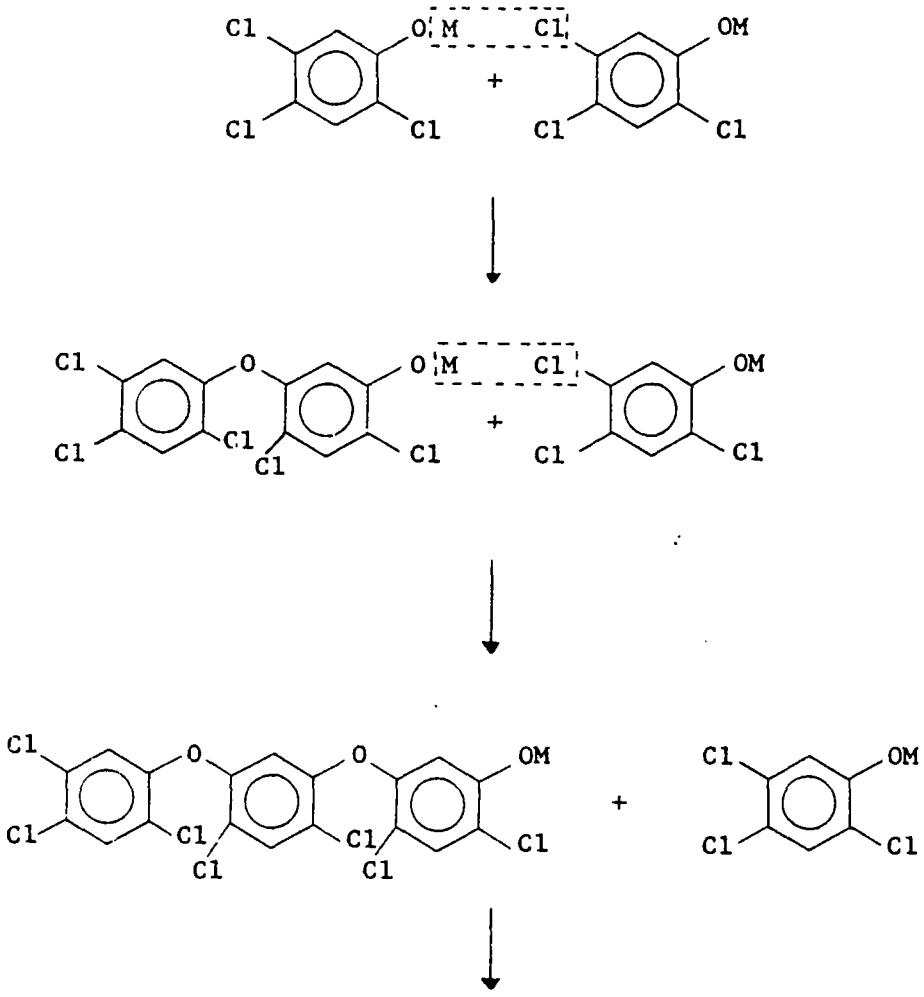
16679

Table 1. Pyrolysis products from Na chlorophenates by mass spectrometry.

Compound	m/e	Relative intensity, %	Species		Approx. yield, %	
Sodium pentachlorophenate (solid)	456	100	Dioxin	(8 Cl)	80	
	684	2	Trimer	(12 Cl)		
	912	0.3	Tetramer	(16 Cl)		
Sodium 2,3,4,6-tetrachlorophenate (solid)	388	100	Dioxin	(6 Cl)	30	
	582	3	Trimer	(9 Cl)		
Sodium 2,4,5-trichlorophenate (at mp)	320	100	Dioxin	(4 Cl)	<1	
	35–356	30	Dimers	(5 Cl)		
	480	4	Trimers	(6 Cl)		
Sodium 2,4,6-trichlorophenate (at mp)	320	100	Dioxin	(4 Cl)	up to 15	
	480	33	Trimer	(6 Cl)		
	640	50	Tetramer	(8 Cl)		
	800	30	Pentamer	(10 Cl)		
Sodium 2,4-dichlorophenate (at mp)	252	100	Dioxin	(2 Cl)	<3	
	378	10	Trimer	(3 Cl)		
	504	1	Tetramer	(4 Cl)		
Sodium Irgasan (molten)	252	100	Dioxin	(2 Cl)	7	
	378	10	Trimer	(3 Cl)		
	414	20	Trimer	(4 Cl)		
	504	16	Tetramer	(4 Cl)		
	540	12	Tetramer	(5 Cl)		

DOW-31898

16680



etc. (2)

DOW 331900

REFERENCES

1. Kulka, M. Octahalogenodibenzo-*p*-dioxins. Can. J. Chem. 39: 1937 (1961).
2. Tomita, M., Ueda, S. I., and Narisada, M. Dibenzo-*p*-dioxin derivatives. XXVII. Synthesis of polyhalodibenzo-*p*-dioxin. Yakugaku Zasshi 79: 186 (1959).
3. Pohland, A. E., and Yang, G. C. Preparation and characterization of chlorinated dibenzo-*p*-dioxins. J. Agr. Food Chem. 20: 1093 (1972).
4. Weingarten, H. Ullman condensation. J. Org. Chem. 29: 977 (1964).
5. Briggs, P., Dix, D. T., Glover, D., and Kleinman, R. A multiinstrument data acquisition system for use with mass spectrometry. Am. Lab. 4: 57 (Sept. 1972).
6. Buu-Hoi, N. P., et al., Preparation, proprietés et identification de la "dioxine" (tetrachloro-2,3,7,8-dibenzo-*p*-dioxine) dans les pyrolysats de defoliants a base d'acide trichloro-2,4,5-phenoxy-acetique, et de ses esters et des vegetaux contaminnés. C. R. Acad. Sci. (Paris) Ser. D 273: 708 (1971).
7. Boer, F. P., et al., The crystal and molecular structure of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin. Acta Cryst. B 28: 1023 (1972).

16681

TESTIMONY OF A. T. TALCOTT

SHOECRAFT ET AL. VS. DOW

DOW 1469660

I am A. T. Talcott, administrator of Regulations and Labeling in the Quality Assurance Department of The Dow Chemical Company. I have a Bachelor of Science degree in chemistry from Alma College, Alma, Michigan, and have done graduate work at Michigan State University but have no advanced degree. I joined The Dow Chemical Company, Midland, Michigan, in 1957 as a chemist in their analytical laboratories and moved through a series of technical promotions in the analytical chemical field. In 1965 I moved to the Labeling/ Product Registration section of the Legal Department, becoming supervisor of that section in 1975. In 1976 I became manager of Product Safety Compliance in Dow's Quality Assurance Department, which included the functions of labeling, material safety data sheets, and product registration. In 1979 I assumed my present position where I am responsible for labeling policies on Dow products.

I am a member of the American Conference on Chemical Labeling, the Labeling Standard Revision Committee of the Chemical Manufacturers Association, a member or chairman of several committees dealing with regulations and labeling within the Chemical Specialties Manufacturers Association, a former member of the Labeling and Precautionary Information Committee of the Chemical Manufacturers Association, and the Labeling Committee of the National Paint and Coatings Association. I was also one of two industry representatives participating with EPA to put together their National Symposium on Pesticide Labeling in 1974.

I will testify on the history and procedures used in the development of labels by The Dow Chemical Company and to the registration requirements for labeling used by the Pesticide Registration Division of EPA which was part of the USDA in 1968 prior to the formation of EPA. I will also testify to

-- health professionals, technical specialists, and legal and regulatory counsel in areas pertinent to such a product.

The labeling used on KURON weed and brush killer in the time period 1967 through 1969 was in great part specified in government regulations for pesticides which were called economic poisons under the statute in effect at that time. These regulations required the product to bear a label containing the name of the product, the name and address of the manufacturer or registrant, the net contents, an ingredient statement which was prescribed in a special manner, a warning or caution statement as appropriate to regulations prescribing same, and directions for use--which if complied with, would be adequate for the protection of the public. These elements of a label were further prescribed in a certain manner by the regulations and interpretations promulgated by the Pesticide Registration Division and required a preclearance or acceptance by that Division before they could be used on the package of the pesticide. This labeling was also submitted to the state of Arizona and other states for review as part of the state registration of the product required prior to sale.

The product name KURON, the Company name, and certain other elements such as the registration number required to appear on the label are for the most part self-explanatory.

For precautionary labeling purposes, pesticides were placed in one of four different categories based on certain toxicity data that would be ~~in~~ derived from animal tests provided to the Pesticide Registration Division by the manufacturer. These were ~~called~~ identified as categories one, two, three, and four, with category one being materials that were highly toxic and requiring the word "poison" and the skull and crossbones to be used on

*Trademark of The Dow Chemical Company

fold decreases in toxicity from the lower-numbered category.

During this time precautionary statements used on pesticides were set forth in a document called "Interpretation 18, Revision 2" published in the Federal Register of March 9, 1962 as an interpretation of 7 CFR, Part 362. There were two ways in which this interpretation was used. One related to signal words and statements of hazard that were used on pesticides containing specifically-listed active ingredients and the other part set forth general procedures based on the toxicity categories previously mentioned which was applicable to KURON weed and brush killer.

Applying the principles stated in Interpretation 18, one comes up with the precautionary labeling which was accepted by the government as adequate to protect the public and used by Dow on its product KURON weed and brush killer.

Quoting from section 362.116(b)(2)(III), "Labels of products in the third category should carry the word "caution" in statements indicating the means of avoiding the principal hazards of use. Use of the skull and cross bones, the word "poison," and antidote statements are not necessary for these products."

Another part of the label which may need more explanation is the ingredient statement. Ingredient statements are required to be made on all pesticide products and must be stated in certain ways. The constituents of a product must be characterized as either active or inert ingredients. The active ingredients must be described by their chemical name.

The two ways in which ingredient statements can be listed were specified in section 362.7 of the regulations. These were to list each active ingredient and its percentage and the total of the inert ingredients or to list the total of the active ingredients and each individual active ingredient in descending order of percentage without the actual percentage of each ingredient plus the

this product. Such a listing would not have been acceptable from the government's standpoint since this is not an active pesticidal constituent; additionally, the initials TCDD do not represent a common chemical name acceptable to the federal government so such listing would again be prohibited. The other reason for not listing TCDD as an ingredient is that the limits of analytical detection at the time of production of the KURON in question were such that TCDD was not found in this product.

There are other sections of the label that are, of course, pertinent to the allegations made concerning the product KURON weed and brush killer. Among these are the instructions under a section titled "Warning" that "applications by airplane, ground rigs, and hand dispensers should be carried out only when there is no hazard from drift." Other instructions which are meaningful to a user of such products are the instructions that "coarse sprays are less likely to drift" as well as the instructions on application rates and times for specific applications of the product.

The procedures followed for labeling pesticidal products are an application of the principles that are included for labeling of chemicals set forth in the American National Standard for Precautionary Labeling ANSI Z129.1. This is a consensus standard which was not published until 1976, however, does have the benefit of review of such organizations as the American Society of Agricultural Engineers, the National Safety Council, the American Society of Safety Engineers, the Society of Toxicology, AFL-CIO, the American Conference of Governmental

Industrial Hygienists, and numerous other government, industry, and independent organizations with an interest in conveying precautionary information.

These principles prescribe a signal word, statement of hazard, precautionary measures, and as appropriate, instructions in case of contact or exposure as procedures for providing precautionary labeling. The labeling used by Dow on KURON weed and brush killer demonstrates these principles.

The procedures used within Dow to develop labeling in 1967 involved the initial labeling being developed from a use standpoint by a technical specialist from our Agricultural Products Department along with the individual responsible for submitting registration applications to the federal government, and precautionary information coming from a labeling specialist like myself. This draft copy then would be typed and sent for review by the numerous specialists within the Company with input on the health and safety aspects coming from toxicologists, industrial hygienists, medical doctors, individuals from our quality function in connection with our manufacturing people providing input on the composition of the product, trademark and patent comments coming from our attorneys, and the original three persons reviewing these inputs and those of our marketing personnel. Once these inputs had been composited, the labeling was sent to the federal government for their acceptance or suggestions for change if they did not accept it.

Upon return of a copy of the accepted labeling, the labeling specialist would initiate the actions responsible for getting the labels or the preprinted container copy to our production people for application on containers of the final product.

It is important to remember when developing or reviewing labeling for a product that the labeling must be based on the product as a whole; consequently, the labeling must be viewed in its entirety, not just in parts--since it is the whole product that is used, not a part.

I have compared the labeling used by other pesticide producers of similar products and find the labeling used, which also was required to be submitted to the Pesticide Registration Division before being used, to be essentially the same where applicable as that used on the product KURON weed and brush killer.

Based on my education, training, and experience, it is my opinion that the label used on the product KURON weed and brush killer in the time period 1967 through 1969 met or exceeded all regulations and standards of care pertaining to such products; and further the directions and precautions if followed were adequate to prevent accident or injury with the handling and application of the product.

DOW 1469665

2702

Industrial Vegetation Management

DOW
758726
MN073280

1698

R. S. Wallace
Research Herbicide Technical Specialist
Dow Chemical U.S.A.

DOW 758727

The current status of phenoxy herbicides

BACKGROUND

In January of 1971 a review of "The Status of 2,4-D, 2,4,5-T, Silvex and MCPA Herbicides" was reported in DOWN TO EARTH* (Vol. 26, No. 4). It now seems desirable and beneficial to repeat pertinent parts of that paper as well as to include additional data and information generated since then.

This new review concerns itself with 2,4-D (2,4,-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), silvex [2-(2,4,5-trichlorophenoxy) propionic acid] and MCPA (2-methyl-4-chlorophenoxy acetic acid). While these herbicides appear to be quite similar in structure, each compound exhibits unique characteristics with respect to selectivity for broadleaf weed and/or brush control. The formulation of these compounds into specific esters or amines further contributes to species selectivity as well as to particular techniques for spray preparation and application.

The phenoxy herbicides have been utilized commercially for twenty years or more without harm to humans or wildlife and were registered on the basis of safety tests considered adequate by USDA at the time.

In 1966 the USDA and FDA, acting on a study conducted by the National Academy of Sciences, declared that the concepts of "no-residue" and "zero tolerance" registration of pesticides on food crops were no longer tenable and residue tolerances would have to be established to enable continued registration under these concepts. In order to accomplish this, additional toxicity and analytical studies, updated to meet current requirements, have been required by USDA and FDA. Pesticide regulation has now been transferred to the Environmental Protection Agency (EPA). Scientific studies subsequent to April 1966, when the USDA abolished the no residue status, have shown the phenoxy herbicides to represent no hazard to man and his environment when properly applied in accordance with label recommendations. The issue of 2,4,5-T on food crops is still undergoing review by EPA, primarily at the insistence of environmental groups and as an outgrowth of its previous usage in Viet Nam. It is extremely important to recognize that *industrial, forestry or rangeland usage of 2,4,5-T are not affected by the current controversy*. The 2,4,5-T issue has become one of principle rather than monetary value to the manufacturers and it has been in this context that Dow has continually pursued the scientific route to resolve the potential hazard of 2,4,5-T to man and his environment.

Chronology of Events

Development of the phenoxy herbicides in the mid-forties resulted in three major herbicides for right-of-way, forestry and other industrial markets. These products (2,4-D, 2,4,5-T and silvex) are not protected by patents. Seven commercial companies were manufacturing one or more of these three products and or MCPA at the time when USDA announced abolishment of the no residue status on food and feed crops on April 13, 1966. These were Diamond, Dow, Hercules, Monsanto, Rhodia, Thompson and Thompson Hayward. They got together to form the Industry Task Force on Herbicide Tolerances (ITFHT). The history of these events since...

16689

Task Force modifies petitions by requesting 300 ppm residue tolerance for each phenoxy herbicide in/on range or pasture grasses

[illegible] 7, 1971 Judgment entered by U S Circuit Court of Appeals, District of Columbia. Re: Petition for review of order by Secretary of Agriculture.

Harrison Wellford, et al (petitioners)

vs.

Wm. William D. Ruckelshaus

Petitioners asked for "Suspension" (which would represent total ban on use) of 2,4,5-T registrations for:

1. All granular 2,4,5-T formulations for use around the home, recreation areas and similar sites.
2. All 2,4,5-T uses on food crops intended for human consumption.

The case is remanded to EPA administrator "for further consideration and for a decision supported by a reasoned analysis of the relevant factors."

January 18, 1971 Ruckelshaus announces 60 day review beginning January 18. All interested groups and individuals are invited to submit in writing any additional data or arguments that bear on whether 2,4,5-T is or is not an imminent hazard to the public.

February 1, 1971 Scientific Advisory Committee holds first meeting on cancellation of 2,4,5-T for crop use as appealed by Dow and Hercules (May 28, 1970).

May 7, 1971 2,4,5-T Advisory Committee report submitted to EPA administrator, but not released at this time.

July 23, 1971 2,4,5-T Advisory Committee report released by EPA. Committee recommends, by a 8:1 vote, restoring registration of 2,4,5-T to status existing prior to April 1970 with following conditions:

(1) 0.1 ppm tolerance of 2,4,5-T on edible food crops

(2) limit of 0.5 ppm TCDD* on existing 2,4,5-T inventories and 0.1 ppm on future 2,4,5-T production with certified analysis being furnished by manufacturer to EPA

(3) formulations around home bear warning of possible danger to pregnant women and animals so as to reduce exposure to an absolute minimum

(4) conduct specific research on TCDD for potential soil accumulation and food chain magnification

(5) additional post registration monitoring for adverse affects

August 6, 1971 EPA issues a purported "Determination and Order" on 2,4,5-T which continues the cancellation for use on food crops additionally. [illegible] hearing to afford registrants and the public the opportunity to present the pertinent economic and other data relating to the balance that the law requires to be struck between benefit and risk." Hearing to be held in the fall but no specific date set.

October 1, 1971 The Dow Chemical Company objects to EPA "order" of August 6 and requests EPA administrator to write a proper determination order with findings of fact based upon all data before him including the report of the 2,4,5-T Advisory Committee given to EPA on May 7.

November 4, 1971 EPA states the August 6 order should remain in effect in spite of Dow's objections to the order, thereby continuing the cancellation of 2,4,5-T on rice. Ruckelshaus states that the hearing should proceed "forthwith"

October-November 1971 Task Force submits amendments to petitions containing additional data on residues in meat and meat by-products of cattle and sheep for each phenoxy compound and its corresponding phenol as requested by EPA July 12, 1971. Receipt acknowledged by EPA and review of petitions begins anew.

December 6, 1971 As of this time, no date for the public hearing on 2,4,5-T has been set. No word has been received relative to the tolerances being requested for the phenoxies on food crops including grass, meat and milk from use on pasture and rangeland.

Registration Status

Tolerances have been established for several formulations of 2,4-D in apples, barley, grapefruit, lemons, oats, oranges, pears, rye and wheat. A tolerance has also been established for 2,4-D in asparagus from use of only the sodium salt.

Data originally submitted with the petitions in December, 1967 is expected to be sufficient for 2,4-D in blueberries, cranberries, and grapes and for silvex in apples and plums. Data on residues in grass includes that from previous work and from residue samples analyzed by Dow in 1970.

Additional work supported by the Task Force provided necessary residue data on certain crops as shown in Table 1. For virtually all of this work, new analytical methods had to be developed to permit analyses down to 0.1 ppm phenoxy acid in crops and to 0.05 ppm acid or corresponding phenol in the animal tissues and milk.

Residue analyses indicate 0.1 ppm for all phenoxy acids in most food crops at time of harvest. Negligible residue tolerances [illegible] 0.1-0.2 ppm were proposed for each phenoxy compound in all food crops [illegible] 2,4-D [illegible] permit

*TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin, a highly toxic impurity that may be present in trace amounts in 2,4,5-T.

DOW-SR-00728

same level established in April 1967 for small grains.

Feeding studies in dairy cows were conducted for each phenoxy compound at levels of 30, 100 and 300 ppm for two weeks and at 1000 ppm for three weeks in the total diet. Milk was collected and analyzed for residues. The data indicate that grazing restrictions for dairy cattle are needed only for 2,4,5-T, and that two weeks should suffice. Current restrictions imposed by EPA in April 1971 require no grazing by dairy cattle for six weeks after treatment with 2,4,5-T. No restriction should be necessary for dairy cattle grazing on areas treated with 2,4-D, silvex or MCPA.

The phenoxies were also fed to beef cattle and sheep at levels up to 2000 ppm in the total diet for four weeks. Animals were then slaughtered immediately or one week after withdrawal from a diet containing 2000 ppm herbicide. Based upon the 300 ppm tolerance requested in/on grass from application of phenoxies up to 2 pounds per acre, no grazing restriction should be necessary for 2,4-D or MCPA. The current limitation for 2,4,5-T approved by EPA should suffice for both silvex and 2,4,5-T. This states: "Do not slaughter meat animals grazing on treated area within two weeks after application." (Figure 1 shows typical residue on/in grass from 2,4,5-T applications. This dissipation curve is similar for 2,4-D and Silvex with variation attributable more to location where applied than to the specific phenoxy herbicide applied.)

Toxicology

Negligible residue tolerances can be obtained based on information from 90 day feeding studies in two species of mammals. However, tolerances at higher (permissible) residue levels require two-year feeding studies in rats and dogs, plus fertility and reproduction studies in rats. At the time the phenoxy herbicides were developed, these long term feeding studies were not necessary since these compounds were registered on a "no-residue" basis.

FDA has conducted two-year feeding studies and reproduction and fertility studies with 2,4-D. Dow has conducted two-year feeding studies with silvex but not reproduction or fertility studies. Ninety day feeding studies have been run on rats and dogs for 2,4-D, 2,4,5-T, silvex and MCPA. The no-ill effect levels are shown in Table 2.

Based on single oral doses in rats, 2,4-D, 2,4,5-T, silvex and MCPA are classed as "slightly toxic" with LD_{50} values ranging from 300 to 700 mg/kg body weight.

2,3,7,8 Tetrachlorodibenzo p Dioxin

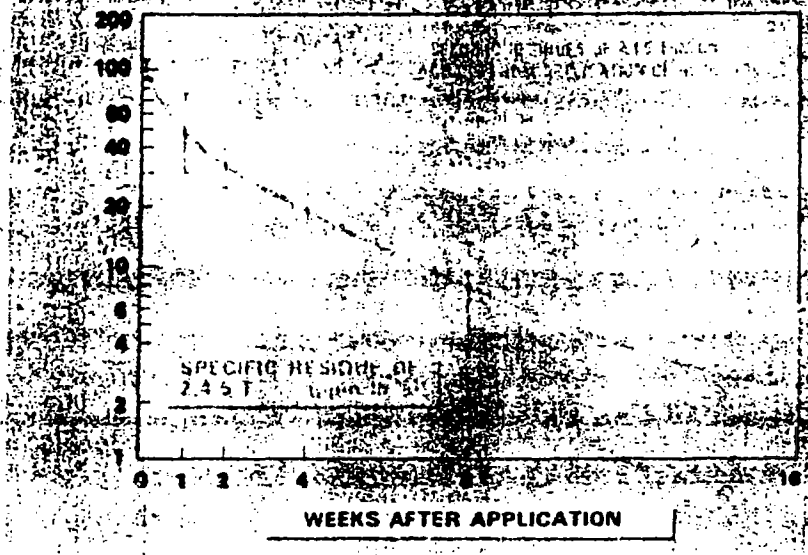
The word teratology has recently become much more familiar. It was [illegible] when studies by Bionetics Research Laboratory implied that 2,4,5-T at very high dosage levels was teratogenic, producing malformed fetuses in mice and rats. Subsequent studies showed that a toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was responsible for some of the findings attributed to 2,4,5-T. The sample of 2,4,5-T employed in the Bionetics [illegible] contained ± 27 ppm TCDD.

Additional studies have shown that oral administration of 2,4,5-T containing 0.1 ppm TCDD produced no teratogenic effects in rats at doses as high as 50 mg/kg/day and in rabbits at doses as high as 40 mg/kg/day. The obvious concern is to produce 2,4,5-T without the contaminant.

TCDD can be formed in the manufacture of 2,4,5-trichlorophenol, the precursor to 2,4,5-T. The conditions required for its formation are high temperatures in the presence of base, conditions which can occur in the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenol.

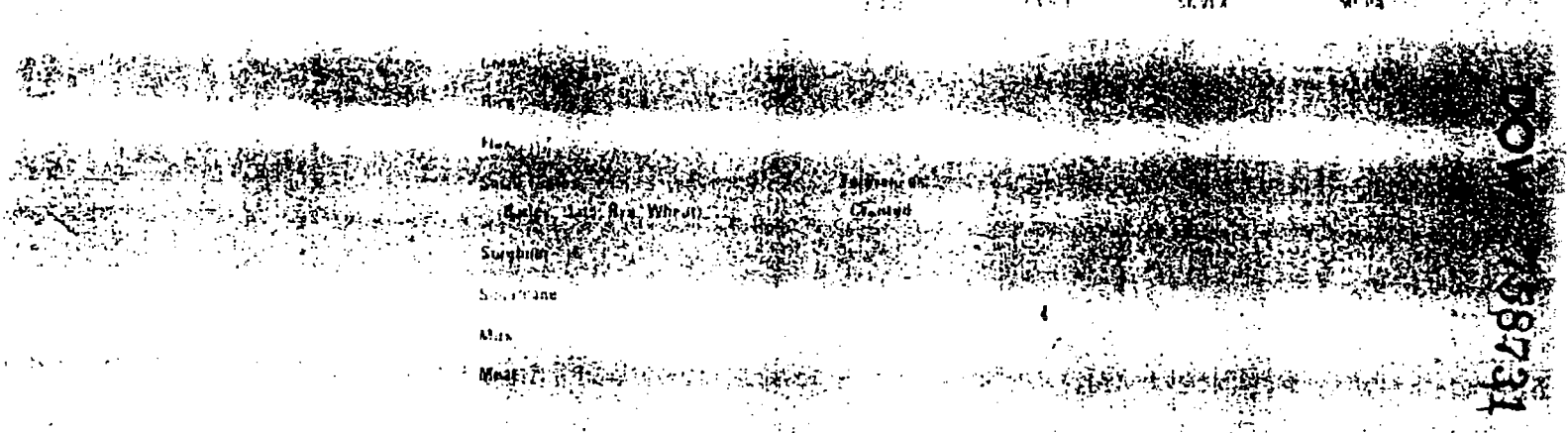
No detectable dioxins have been found in 2,4-D. This is due to the fact that the precursor 2,4-dichlorophenol is made by direct chlorination of phenol and not by alkaline hydrolysis of 1,2,4-trichlorobenzene.

FIGURE 1.



16691

TABLE 1 Crops and tissues sampled for residue analysis ( ) under the ITFPHT program



To date, analytical methods have been developed and validated to a sensitivity of 0.1 ppm for TCDD in 2,4,5-T acid. With proper manufacturing controls, 2,4,5-T can be produced with no detectable TCDD as measured by these analytical methods.

Considerable data on TCDD have been generated during the past two years. Early studies on dioxin were reported by J. E. Johnson, The Dow Chemical Company, at the American Institute of Biological Sciences meeting in Bloomington, Indiana, August 26, 1970 on "The Public Health Implications of Widespread Use of the Phenoxy Herbicides and Picloram." This paper was published in the September 1, 1971, issue of BioScience (Vol. 21, No. 17) and concludes with the statement that "Impurities can be an important factor – particularly the chlorodibenzo-p-dioxins – but these can be controlled by proper manufacturing techniques." Photodecomposition of chlorinated dibenzo-p-dioxins was shown by D. G. Crosby et al (Science, Vol. 173, pp. 748-749, August 20, 1971). Work on dioxins was culminated in a full day symposium in sessions of the Pesticide Chemistry Division at the American Chemical Society meeting held in Washington D.C. September 13-17, 1971. The USDA Agricultural Research Report of October 1971 on "A Close Look At TCDD" contained the following statements with respect to this compound:

"The Agricultural Research Service studies on TCDD in the chlorinated compounds with particular emphasis on 2,4,5-T were started in February 1970 at Beltsville, Md. by a research team led by biochemist Philip C. Kearney.

"Our research," said Dr. Kearney, "shows that TCDD is immobile in soils, relatively persistent but not readily taken up by the plants nor translocated to other plant parts, and it can be washed off plants."

Dr. Kearney also said, "We are certain that TCDD is not produced biosynthetically from 2,4,5-T in soils, nor in any other manner except in the manufacturing process. Therefore, current regulations and careful quality control during manufacturing can assure that the environment is protected from all effects of TCDD-contaminated 2,4,5-T [illegible]."

THE FUTURE

Recent developments indicate that the cloud surrounding the 2,4,5-T controversy is clearing. Trade memorandum R-14 October 15, 1971 from the Canada Department of Agriculture stated the following:

"Trade memorandum T-53 of May 11, 1970 and T-53 amendments of June 12 and August 24, 1970 define the current registration status of 2,4,5-T products. Since this memorandum was issued, a great deal of research has been carried out on the teratogenicity of 2,4,5-T and of its contaminant, 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD). It is recognized that "pure" 2,4,5-T may have some teratogenic properties in some test animals at high dosages and that TCDD is an extremely potent teratogen at extremely low dosages. However, it has now been concluded that [illegible] sufficiently low level of TCDD contamination, 2,4,5-T represents no undue risk to humans, animals or the environment when used as outlined in memorandum T-53."

Fenoprop is recognized as being subject to the same TCDD contamination as 2,4,5-T because of a similar manufacturing process. However, [illegible] that [illegible]

16692

DOW 7587732

ties. The standards of acceptance for technical fenoprop, in terms of TCDD content, will be the same as for technical 2,4,5-T.

It is now proposed that for registration purpose all 2,4,5-T and fenoprop products to be sold in Canada must have a declared and guaranteed TCDD content in the technical material of less than 0.5 ppm. Also, the source (i.e. the manufacturer and place) of technical 2,4,5-T and fenoprop must be identified. The quality control measures used are also to be described. Finally, the analytical method used to determine the TCDD content must be fully described and shown to be sensitive to a level of 0.5 ppm TCDD. 2,4,5-T and fenoprop products are not to be registered for sale for 1972 unless these conditions are met. These criteria will in future be a part of the protocol for registration and re-registration of 2,4,5-T and fenoprop products.

Adherence to these standards will justify deletion of the following warnings from 2,4,5-T product labels:

(1) 'Exposure of pregnant women by any route of contact to 2,4,5-T may be harmful to the offspring.'

(2) 'Direct exposure of some women to this product may cause a health hazard.'"

The council of the Society of Toxicology in a letter to the editor of Science (Vol. 174, November 5, 1971) commented as follows on 2,4,5-T:

"The 2,4,5-T controversy involves a fundamental issue in safety evaluation. The issue is simply whether or not demonstration of a teratogenic effect in some species of animal, at a dosage level far in excess of any possible human exposure, constitutes scientific grounds for banning the chemical. A small minority of toxicologists believe in the affirmative, but the overwhelming majority do not. The EPA scientific advisory committee on 2,4,5-T, which included several toxicologists and teratologists, specifically endorsed the majority opinion. To accuse the scientific advisory committee of "bias" for defending a commonly held scientific principle is unwarranted. To select a committee of ten experts who might share the opposite "bias" would be extremely difficult if not impossible."

The USDA has issued two reports dealing with the benefits of phenoxy herbicides:

Restricting the Use of Phenoxy Herbicides—Costs to Farmers.

Agricultural Economic Report No. 194

Restricting the Use of 2,4,5-T: Costs to Domestic Users

Agricultural Economic Report No. 199

The USDA states that prohibiting the use of phenoxy herbicides "would cost the U. S. farmers an additional $290 million to maintain current agricultural production. In addition, farmers and their families would have to work 20 million more hours to control the weeds without these herbicides. For this extra labor, the farmers would obtain no additional income."

Over a million dollars has been expended over the past two years to prove the safety of phenoxy herbicides to man and his environment. From a scientific base the phenoxy herbicides can contribute economically, efficiently, and safely in the future for the control of broadleaved weeds and brush on food crops, pasture, rangeland, and noncropland areas as they have for over 20 years.

16693

cc: A. M. Griswold, Executive Res.
J. C. VanderWeele, Penta Office
Rowe/Hoyle, Biochem. Res. Lab.
W. A. Kelching, Dowicides
Knapp/Kleine, Zurich
J. R. Myers
FBS File

2712

DOW CHEMICAL INTERNATIONAL LIMITED

CABLE: DOWINTAL

1714 EASTMAN ROAD
MIDLAND, MICHIGAN U.S.A.

February 22, 1957

DOW 1716970

C. H. Boehringer Sohn
22b Ingelheim am Rhein
Germany

Reference: Dr. Wey/B

Gentlemen:

Subject: Preparation of Trichlorophenol to Avoid Chloracne

Thanks very much for your letter of February 11 and the attached description of the work you have done on the preparation of Trichlorophenoxyacetic Acid to avoid the formation of Chloracne exciters and consequent dermatitis to your workmen. This work is of very great interest to us and we appreciate your thoughtfulness in remembering our earlier correspondence on this matter.

Yours very truly,

O.K.
F.B.S.

Frank B. Smith
Technical Director

FBS/wmc

cc: Otto Krahn

NOTE: Early in 1955 subject company communicated with L. Givaudan & Cie. S. A. in Geneva concerning skin irritation from 2,4,5-Trichlorophenol which was referred to us by the Givaudan Corp., Delawanna, New Jersey. On January 27, 1955 we wrote to Boehringer enclosing a data sheet describing the hazards due to toxicity and precautions for safe handling and use of 2,4,5-Trichlorophenol and answered seven specific questions regarding experience in our own plant.

F.B.S.

DS

16695

2718

DOW 1 627630

WN027035

STATEMENT OF

LEWIS A. SHADOFF

ON

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

IN

THE ENVIRONMENT

8/4/76

16699

DOW 1 627631

My name is Lewis Shadoff. My residence address is 3031 N. 11 Mile Road, Coleman, Michigan. I am an Analytical Specialist of the Analytical Laboratires of the Dow Chemical Company's Midland Division. I graduated from the Polytechnic Institute of Brooklyn in 1961 with the B.S. degree in chemistry. I then attended Kansas State University and was awarded a Ph.D. in chemistry in 1966 having done my doctoral research in mass spectrometry. Since then, I have been employed by Dow Chemical working in mass spectrometry. In my present capacity, I have responsibility for the operation and maintenance of three gas chromatograph-mass spectrometers including a high resolution unit.

ANALYTICAL INVESTIGATION OF TCDD

I have been working on the analysis of TCDD since 1970 determining the purity of synthesized material (1) and the TCDD content of 2,4,5-T ester products.

Since April, 1973, I have been involved in the development of gas chromatography-mass spectrometry methods for the determination of TCDD at the picogram level (corresponding to the part per trillion level in biological samples after sample preparation).

Gas chromatography is an analytical technique used to separate a mixture into its component parts. The separated components may then be analyzed by a mass spectrometer which yields information about the atomic composition of these components. This combination has been used as an extremely sensitive and specific detection method for TCDD.

The concept of parts per trillion is difficult to envision. If it were salt we were analyzing, it would correspond to the

ability to detect one grain of salt in an olympic size swimming pool of fresh water by analyzing one shot-glass full of water.

We have searched for TCDD in the following samples by this technique:

DOW 1 627632

ENVIRONMENTAL SAMPLES ANALYZED FOR THE PRESENCE OF TCDD

SAMPLE	SOURCE	NUMBER SAMPLES	TCDD CONTENT*	LIMIT OF DETECTION
Human Milk (ref. 2)	Texas	6	N.D.	Ave. 3 ppt, range 1-6
	Michigan	1	N.D.	
Fish (2)	Texas	20	N.D.	Ave. 7 range 2-18 ppt
	Arkansas	20	N.D.	
Mud(2)	Arkansas	2	N.D.	4 and 6 ppt
	Texas	2	N.D.	3 ppt
Water(2)	Arkansas	2	N.D.	Ave. 0.2 ppt
	Texas	2	N.D.	0.1 ppt
Cow's Milk(3)	Oklahoma Missouri Arkansas	25	N.D.	0.5 or 1 ppt
Beef Fat(4)	Texas Oklahoma Missouri	24	21 spls N.D. 3 spls 3-4 ppt	Ave. 4, range 2-7 ppt
Rice(5)		19	N.D.	Ave. 4, range 2-7 ppt
	(12 of them commercial)			
Shrimp	Japan	1	N.D.	1 ppt
	Vietnam	3	N.D.	

*N.D. = not detected at the detection limits listed.

The significance of low part per trillion TCDD content is not presently known. It is under study at the EPA, Dow Chemical, and IITRI.

The methodology and some of the results have been reported at scientific meetings (2). Publication of the work is now pending in journals whose articles are reviewed by scientific peers.

The cow's milk was collected from cow's grazing on grass known to have been treated with 2,4,5-T.

16701

The fish, human milk, mud, and water were taken from environments where 2,4,5-T had been used on rangeland and rice fields. Those samples from Texas were from the San Angelo, Texas Reservoir which is an impoundment of the North Concho River and has its water shed large acreages that have been sprayed with 2,4,5-T herbicides for the control of mesquite (0.5 lbs/acre 2,4,5-T acid equivalent) and brush (3-4 lbs/acre 2,4,5-T acid equivalent). The samples from Arkansas were taken from a 125-acre pond in the heart of the Arkansas rice growing area near Grady, Arkansas. This water is used to flood the rice fields which have been sprayed with the equivalent of 1.25 lb/acre of 2,4,5-T acid four to eight weeks previously. Later, when the water is drawn off the fields it it pumped back into the pond for reuse. Water in the pond also collects run-off from surrounding rice fields during the rainy season and is supplemented by wells. This cycle has been in use (including the proper use of 2,4,5-T herbicide) for 18 years up to the time of this study. Thus, these should represent likely locations for the accumulation of TCDD if it occurs.

Twelve of the rice samples were collected from grocery stores throughout the United States. The rest were taken from experimental plots.

The shrimp was supplied by the Kien Hoa Shrimp Company.

REFERENCES

1. W. W. Muelder and L. A. Shadoff, Adv. in Chem. Ser. 120, (1973).

2. R. A. Hummel and L. A. Shadoff, 170th National Meeting of the American Chemical Society, Chicago, Illinois (1975).

DOW 1 627633

3. Mahle, N. H., Higgins, H. S., and Gertzendaner, M. E., "Search For The Presence of 2,3,7,8-Tetrachloro-p-dioxin in Bovine Milk," submitted to Bull, Environ. Cont. Toxic. for publication.

4. Kocher, C. W., Mahle, N. H., Hummel, R. A., Shadoff, L., and Getzendaner, M. E., "Search For The Presence of 2,3, 7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Beef Fat," submitted to Bull, Environ. Cont. and Toxic. for publication.

5. Jensen, D., Hummel, R. A., Madrid, Ester, and Turley, June, "Search For 2,3,7,8-Tetrachloro-p-dioxin in Rice," to be published.

MRM 1 627634

2030

Acta vet. scand. 1966, 7, 240-252.

DOW 2583313
MN 022772

From the National Veterinary Institute, Stockholm, Sweden.

DISTRIBUTION AND ELIMINATION OF CHLORINATED PHENOXYACETIC ACIDS IN ANIMALS*)

By
Kurt Erne

The distribution, elimination and metabolism in plant tissues of chlorinated phenoxyaliphatic acids have been the subject of numerous investigations (see e.g. *Audus* 1964). The fate of phenoxy acids in the animal organism, however, has not been so thoroughly investigated.

According to early studies unsubstituted phenoxyacetic acid is excreted unchanged in urine by man, dogs, rats and rabbits (*Williams* 1959). The o- and *p*-chloroderivatives also were shown to be excreted renally, without conjugation, in the rabbit (*Leuck & Lewis* 1947). On feeding ω-phenoxybutyric and -caproic acids to rabbits, some phenoxyacetic acid was detected in the urine, along with the unchanged acids, indicating partial β-oxidation.

Recently a group of workers at Cornell University, N.Y., in a series of feeding experiments with cattle and using a gas chromatographic method of analysis, found that chlorinated phenoxyacetic acids (2,4-D, MCPA and 2,4,5-T) were completely eliminated with the urine in intact form, as was a chlorinated 2-phenoxypropionic acid (2,4,5-TP) (*Gutenmann et al.* 1963 a, b; *Lisk et al.* 1963; *Bache et al.* 1964 a, b; *St. John et al.* 1964). Chlorinated 4-phenoxybutyric acids (2,4-DB and MCPB) were largely degraded in the rumen of cattle, but small amounts of the β-oxidation products, the corresponding phenoxyacetic acids, were also detected in the urine. A 2,4,5-TP ester was largely eliminated in urine as the parent acid. None of the phenoxy herbicides given

*) Supported by a grant from "Albert Hjärnefonden".

16704

DOW 2583141

to cattle could be detected in the milk. *Clark et al.* (1961), applying a tracer technique, reported the rapid renal elimination of orally administered 2,4-dichlorophenoxyacetic acid in a sheep. 96 % of the activity was excreted with the urine in 72 hours, and blood levels dropped to below 0.05 p.p.m. in 21 hours.

Experiments with other animal species do not seem to have been reported.

The present paper presents results from studies of the distribution and elimination of some chlorophenoxyacetic acids in rats, pigs, calves and chickens. The experiments were performed in connection with toxicity studies which will be reported separately (*Björklund & Erne* 1966).

EXPERIMENTAL

Materials

2,4-D amine. Aqueous solution containing 20 mg of 2,4-D per ml, as the triethanolamine salt, prepared by diluting a commercial formulation.

2,4-D K-Na salt. Aqueous solution containing 20 mg of 2,4-D per ml, prepared by dissolving the pure acid (m.p. 138—142°C) in a slight excess of aqueous KOH-NaOH (1:1) and adjusting pH to about 7.

2,4,5-T amine. Aqueous solution containing 20 mg of 2,4,5-T per ml, prepared by dissolving the pure acid (m.p. 155—157°C) in a slight excess of aqueous triethanolamine and adjusting pH to about 7.

2,4-D ester. Emulsion containing 20 mg of 2,4-D per ml, as the butyl ester, prepared by homogenizing a commercial formulation, in a petroleum solvent, with water.

Animals

Data given for weight and age refer to conditions at start of experiment.

Albino rats, Anticimex strain, 180—220 g, both sexes.

Pigs, Swedish "Lantras" breed, 20—25 kg, 10—12 weeks, castrated males, and females.

Calves, SRB breed, 55—65 kg, 6—8 weeks, both sexes.

Chicks, broiler, one week, male.

Chickens, White Leghorn breed, 0.8—1.0 kg, 8—10 weeks, both sexes, and New Hampshire breed, 2.2 - 2.9 kg, adult hens.

Methods

Administration. Before dosing animals were starved overnight. Doses were given orally, in short term experiments by stomach tube, in long term experiments in feed or drinking water.

Sampling. At selected time intervals before and after dosing, blood samples were withdrawn in heparinized tubes, in rats at

16705

DOW 258315

exsanguination from a neck artery, in pigs from vena cava cranialis, in calves from a jugular vein and in chickens from a wing-vein. Plasma was separated by centrifugation.

A number of the experimental animals were sacrificed at selected time intervals, by exsanguination after anesthetizing with either chloroform (rats and poultry) or nembutal sodium (pigs). Tissues were examined for gross pathological and histological changes (*Björklund & Erne* 1966) and samples collected for analysis. Samples were stored at —20°C until analyzed.

Analytical method. The chlorinated phenoxyacetic acids were isolated from body fluids and tissues by solvent extraction, separated from extractives by thin-layer chromatography and quantitatively determined photometrically according to a technique described previously (*Erne* 1966 a). When required, the procedure was scaled down to handle samples as small as 1 g. The method gives the total concentration of chlorophenoxyacetic acids. The limit of determination of the method is 0.1—0.2 µg of 2,4-D per g of sample, with the reduced sample amounts, however, about 0.5—1 µg/g.

RESULTS

A. *Plasma levels*

1. *Single dose*

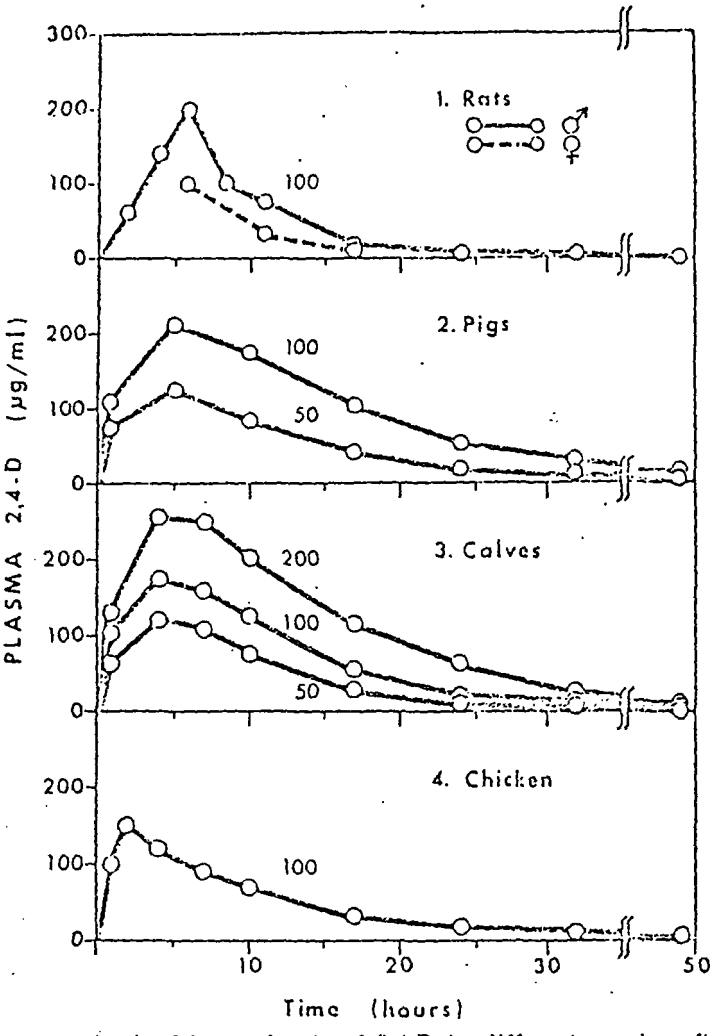
a. *2,4-D amine.* Groups of rats (male and female), pigs, calves and chickens (Leghorn and New Hampshire) were given 2,4-D amine salt, in the form of a technical formulation, as single oral doses equivalent to 50—200 mg of 2,4-D per kg body weight. On each dosage level 25 male and 10 female rats, 5—10 pigs and chickens and 2 calves were used. Blood samples were taken (in rats after killing) at 2—3-hour intervals during the first 12 hours and thereafter less frequently, and plasma analyzed for 2,4-D. Plasma concentration-time curves are shown in Figs. 1—4. (In addition, a number of the animals were killed at certain intervals in order to study tissue distribution, see section C, below.)

The peak plasma concentration of 2,4-D was usually attained within 2 hours after dosing in chickens and within 4—7 hours in the mammalian species. In the species examined a dose of 100 mg/kg gave a peak level of about 100—200 µg/ml. The plasma levels for female rats were lower than those for the males. The half-life values of 2,4-D in plasma, calculated from the curves after plotting on a semi-logarithmic scale, are given in Table 1.

b. *2,4-D K-Na salt.* The experiments were repeated with the potassium-sodium salt of 2,4-D. Ten male rats and 2 calves were given single doses of 100 mg/kg.

16708

DOW 258316



Figures 1—4. Plasma levels of 2,4-D in different species after a *single* oral dose of *2,4-D amine* (50, 100 or 200 mg 2,4-D/kg). The curves for pigs, calves and chickens were obtained with 3—5 animals on each dosage level and the curves for rats with 25 males and 10 females, 2—4 animals on each point of the curve.

The curves obtained and the half-life values found (Table 1) were similar to those obtained with 2,4-D amine.

c. *2,4,5-T amine*. Ten male rats and 2 pigs were given single oral doses of a 2,4,5-T amine salt (100 mg/kg). The plasma concentration-time curves, as well as the plasma half life values found, were similar to those obtained with the 2,4-D salts.

W 258317

d. *2,4-D ester.* A technical formulation containing the butyl ester of 2,4-D was given as single oral doses of 100 mg/kg to 14 male rats, 4 pigs and 2 calves. Plasma samples were analyzed for total 2,4-D as in the preceding experiments. Typical curves are shown in Fig. 5. Curves for pigs were intermediate between those for rats and calves. The plasma half-life values are given in Table 1.

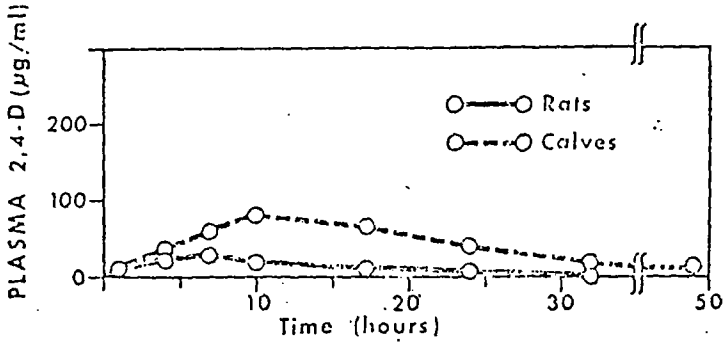


Figure 5. Plasma levels of 2,4-D in rats and calves after a *single* oral dose of *2,4-D butyl ester* (100 mg 2,4-D/kg). The curves were obtained with 14 rats (2 on each point of the curve) and 2 calves.

Table 1. Plasma half-life values, in different species, of phenoxyacetic derivatives given as a single oral dose of 100 mg/kg.

Compound	Species	Plasma half-life hours
2,4-D amine salt	Rat, male	2.9 ± 0.1
„ „ „	Rat, female	3.3 ± 0.5
„ „ „	Pig	12 ± 2
„ „ „	Calf	7.5 ± 0.8
„ „ „	Chicken	7.7 ± 0.7
„ K-Na „	Rat, male	3.5 ± 0.5
„ „ „	Calf	8 ± 0.6
„ ester	Rat, male	6 ± 1
„ „	Pig	10 ± 0.8
„ „	Calf	10 ± 1
2,4,5-T amine salt	Rat, male	3 ± 0.6

2. *Repeated administration*

2,4-D amine and 2,4-D ester were given as repeated oral doses (50 mg 2,4-D/kg/day) to two groups of 5 and 1 pigs, respectively. In both groups the plasma level of 2,4-D was seen to decline and the urinary excretion to increase as exemplified in

16705

pigs no. 8 A and 15 A developed signs of poisoning. Values in brackets denote the corresponding urinary concentrations of 2,4-D.

Number of doses given	Plasma 2,4-D, µg/ml (at 24 hrs. after last dose)			
	Amine		Ester	
	Pig no. 4 A	Pig no. 8 A	Pig no. 9 A	Pig no. 15 A
3	35 (200)	230	190	155
4	—	315	240	—
7	—	335	24	295
8	8	520 (90)	25	—
23	6 (550)	—	7 (250)	—

Table 2 by pigs no. 4 A and 9 A. One animal in each group, however, did not tolerate the repeated administration; the plasma levels continued rising during the experimental period, and signs of poisoning gradually developed (pigs no. 8 A and 15 A, Table 2).

In an experiment with two hens, 2,4-D amine (300 mg/kg/day) was given orally for 12 and 24 days, respectively, and during the experimental period the plasma level (at 3 hours after dosing) dropped from 150 to 20 µg/ml, on the average.

B. *Blood cells*

In *in vitro* experiments 2,4-D was added, at two concentration levels, to horse blood and the samples agitated at room temperature for 5 hours and 24 hours, respectively. Then the blood cells were separated by centrifuging for 30 minutes at 2500 r.p.m., washed by suspending in two volumes of either fresh plasma or physiological saline for 5 minutes and centrifuging for another 5 minutes and then haemolyzed by diluting with two volumes of water. For comparison some samples were washed in physiological saline three times. The 2,4-D levels found in plasma and blood cells are presented in Table 3.

A small proportion of the 2,4-D, about 10 % of the plasma level, was found in blood cells washed once. The proportion did not change on prolonged contact between plasma and cells. Washing with plasma removed more of the 2,4-D from the blood cells than did washing with saline. Repeated washing with saline removed 2,4-D almost completely from the cells.

8318

16709

DOW255319

Table 3. *In vitro* distribution of 2,4-D between horse plasma and blood cells at two concentration levels. Before analysis the blood cells were washed either once (5 min.) with 2 volumes of fresh plasma (A) or of physiological saline (B) or three times (15 min. each time) with 2 volumes of physiological saline (C). Values given are means of results from duplicate experiments.

Time of contact hours	2,4-D, μg/ml							
	10 μg 2,4-D added per ml whole blood				100 μg 2,4-D added per ml whole blood			
	Plasma	Blood cells			Plasma	Blood cells		
		A	B	C		A	B	C
5	13	1.5	2.1	<0.1	135	11	17	<0.1
24	12	1.5	2.0		122	10	18	

The results reported in Table 4 were obtained under physiological conditions with rats receiving single oral doses of 2,4-D amine and 2,4-D ester.

Table 4. *In vivo* distribution of 2,4-D between rat plasma and blood cells after a single oral dose of either 2,4-D amine or 2,4-D butyl ester (100 mg 2,4-D/kg in each case). Before analysis the blood cells were washed for 5 min. with 2 volumes of physiological saline. Values given are the means of results from two animals.

Time after dosing hours	2,4-D, μg/ml			
	Amine		Ester	
	Plasma	Blood cells	Plasma	Blood cells
5	170	20	25	3
24	2.5	0.8	8.5	1.2

C. *Tissues*

Groups of rats (male and female), pigs and chickens (Leghorn and New Hampshire) were given *single* oral doses of 2,4-D amine (100- 200 mg 2,4-D/kg), and at different time intervals 3 -4 animals from each group were sacrificed and tissues and body fluids analyzed for 2,4-D. The experiments were performed in connection with the plasma level studies described above. The results are summarized in Tables 5 - 7.

The effect on tissue distribution of *repeated administration* of 2,4-D was studied in feeding experiments with rats (male and

16710

DOW 258320

Table 5. Tissue levels of 2,4-D in male and female *rats* at different intervals after a *single* oral dose of 2,4-D amine (100 mg 2,4-D/kg). Each value is the mean of results from 3–4 animals, with the range given in brackets.

Tissue	2,4-D, μg/g (fresh weight)			
	6 hours		24 hours	
	Males	Females	Males	Females
Plasma	150 (130—171)	70 (55—87)	2 (1.2— 3.0)	1.5 (0.8— 2.1)
Liver	90 (65—120)	35 (23—42)	5 (3 — 9)	3 (2.9— 5.2)
Kidney	250 (182—289)	145 (90—185)	27 (19 —40)	15 (10 —23)
Lung	140 (104—176)	60 (15—75)	8 (6 —11)	6 (4 — 8)
Spleen	80[a])	—	6	—
Skeletal muscle	23 (12— 29)	14 (8 — 21)	2 (1.3— 3.1)	0.6 (0.2— 1.3)
Feces	70 (55— 90)	—	18 (11 —27)	—

[a]) One animal.

Table 6. Tissue levels of 2,4-D in *pigs* at different intervals after a *single* oral dose of 2,4-D amine (100 mg 2,4-D/kg). Each value is the mean of results from 3–4 animals, with the range given in brackets.

Tissue	2,4-D, μg/g (fresh weight)			
	6 hours	24 hours	48 hours	72 hours
Plasma	210 (185 —230)	55 (43 — 64)	10 (5 — 16)	3 (1.5— 4.5)
Liver	115 (89 —125)	27 (18 — 35)	6 (3 — 9)	4 (2.5— 7)
Kidney	190 (165 —225)	36 (23 — 48)	10 (3 — 18)	5 (2 — 8)
Lung	90 (74 —107)	20 (14 — 32)	4 (3 — 6)	3 (2 — 4)
Spleen	65 (51 — 78)	17 (9 — 26)	3 (2 — 4)	—
Heart muscle	45 (38 — 53)	—	3[a])	—
Skeletal muscle	21 (15 — 28)	3 (2.5— 4.5)	2 (1.6— 2.7)	1.6 (1.0— 2.2)
Brain	12 (8 — 18)	3 (2 — 3.5)	1.5 (1.2— 1.8)	—
Fat	3 (1.2— 3.3)	2 (1.0— 2.1)	—	—
Skin	45 (30 — 55)	—	—	—
Bile	26 (17 — 36)	55 (40 — 71)	30 (15 — 38)	3 (1.5— 4.5)
Urine	85 (55 —125)	180 (110 —300)	95 (60 —135)	25 (10 —50)
Feces	150 (75 —205)	50 (40 — 65)	15 (10 — 25)	—
Thyroid	55 (42 — 63)	—	—	—
Adrenal	95 (80 —105)	—	—	—
Ovary	105[a])	—	—	—
Pancreas	60 (50 — 71)	—	—	—
Salivary gland	60 (48 — 69)		—	—
Lymph gland (mesenteric)	75 (61 — 83)		8[a])	—

[a]) One animal.

16711

DOW 2583321

Table 7. Tissue levels of 2,4-D in *chickens* at different intervals after a *single* oral dose of 2,4-D amine (200 mg 2,4-D/kg). Each value is the mean of results from 3 – 4 animals, with the range given in brackets.

Tissue	2,4-D, *pg/g* (fresh weight)		
	6 hours	24 hours	48 hours
Plasma	100 (72 —131)	15 (10 — 20)	5 (2 —7)
Liver	80 (50 —105)	25 (20 — 32)	3 (2 —4)
Kidney	120 (80 —170)	80 (53 —120)	7 (4 —8)
Lung	60 (40 — 82)	40 (28 — 49)	4 (2 —5)
Spleen	80 (50 — 90)	20 (10 — 28)	—
Skeletal muscle	3.5 (1.5— 4.2)	1.5 (0.8— 1.9)	1.2 (0.5—1.6)
Brain	1.5 (1.2— 2.1)	—	—
Fat	1.1 (0.9— 1.5)	—	—
Egg, yolk	< 0.1	0.2 (0.1— 0.3)	0.2 (0.1—0.4)
„ white	< 0.1	< 0.1	< 0.1

Table 8. Tissue levels of 2,4-D in rats, pigs, chicks and chickens after *repeated* oral administration for 2 months. Pigs were given 500 p.p.m. of 2,4-D amine in standard feed twice daily and the other species 1000 p.p.m. of 2,4-D amine in their drinking water. The pigs were killed 24 hours after the last meal; for the other species the interval between last dose and killing was irregular. Each value given is the mean of results from 3 – 4 animals, with the range given in brackets.

Tissue	2,4-D, *pg/g* (fresh weight)				
	Rats		Pigs	Chicks	Chickens
	Males	Females			
Plasma	10 (4 —20)	5 (2— 16)	22 (8 — 35)	12 (3 —25)	10 (3 —31)
Liver	25 (10 —39)	3 (3 —18)	6 (3 —14)	8 (5 —16)	15 (5 —20)
Kidney	45 (22 —60)	15 (8— 26)	12 (10 —16)	30 (7 —71)	20 (8 —56)
Lung	30 (19 —42)	6 (3— 11)	4 (2 — 6)	15 (6 —40)	12 (5 —48)
Skeletal muscle	7 (3 —10)	3 (2— 5)	2 (1.5— 3)	1.3 (0.3— 1.9)	3 (2 — 3.5)
Skin	4[a])	—	3[a])	—	—
Brain	—	—	2 (1.4— 2.6)	—	1.8[a])
Fat	1.5 (0.5— 2)	—	1.3 (0.6 — 2.2)	—	1.0 (0.5 — 1.3)
Egg, yolk	—	—	—	—	1.5 (0.5 — 2.1)
„ white	—	—	—	—	0.2 (0.1 — 0.4)

[a]) One animal.

16712

DOW 2583222

female), pigs, chicks (broiler) and chickens (Leghorn). The pigs were fed a standard diet, with 500 p.p.m. of 2,4-D amine added, twice a day, and the other animals were given 2,4-D amine, at a level of 1000 p.p.m., in their drinking water. Animals were sacrificed after feeding periods of 2 months to 2 years.

The major clinical signs, observed in all groups were anorexia and a reduced weight gain. Further clinical and morphological observations of the experimental animals will be reported elsewhere (*Björklund & Erne* 1966). Table 8 indicates the tissue levels of 2,4-D found after a feeding period of at least 2 months.

Table 9. Tissue levels of 2,4-D in pigs showing signs of poisoning on repeated oral administration of 2,4-D amine.

Tissue	2,4-D, μg/g (fresh weight)		
	Pig no. 3 A [a]	Pig no. 8 A [b]	Pig no. 12 A [c]
Plasma	300	580	530
Liver	230	300	225
Kidney	260	370	185
Lung	220	340	190
Spleen	130	225	150
Skeletal muscle	105	—	120
Brain	40	—	65
Urine	100	90	—

[a]) 10 daily doses of 50 mg/kg. Killed 48 hours after last dose.
[b]) 8 „ „ „ „ „ „ 96 „ „ „ „
[c]) 3 „ „ „ 100 „ „ 6 „ „ „ „

In another experiment, repeated oral doses of 2,4-D amine (50 and 100 mg 2,4-D/kg/day) were given by stomach tube to 5 and 2 pigs, respectively. Two animals of the first and one of the second group developed signs of poisoning (vomiting, locomotory disturbances, depression) and were killed. On analysis of tissues from these animals, the results given in Table 9 were obtained.

Further, four pigs were given repeated oral doses of 2,4-D *ester* (50 mg 2,4-D/kg/day) for up to one month. In three of these animals no untoward effects were seen, and in their tissues only low levels of 2,4-D were found (Table 10). (Intact ester could not be detected in tissues from any of the animals). One animal, however, exhibited signs of poisoning and was killed after 10 days (pig no. 15 A, Table 2). On analysis comparatively

16713

DOW258323

Table 10. Tissue levels of 2,4-D in pigs after repeated oral administration of 2,4-D *butyl ester* (equivalent to 50 mg 2,4-D/kg/day) for 1 month. The animals were killed 24 hours after last dose. Each value given is the mean of results from 3 animals, with the range given in brackets.

Tissue	2,4-D, *μg/g* (fresh weight)
Plasma	7 (5 —12)
Liver	10 (5 —17)
Kidney	17 (12 —25)
Lung	4 (2 — 7)
Skeletal muscle	1.5 (0.5 — 3)
Fat	0.5 (0.2 — 1.1)

high tissue levels of 2,4-D were found (230, 190, 205 and 80 μg/g in liver, kidney, lung and skeletal muscle, respectively.

Finally, with the main purpose of studying the possible effects of phenoxy acids on reproduction, a pregnant sow was given 2,4-D amine (500 p.p.m.) in the feed during the entire pregnancy (see *Björklund & Erne*). Of fifteen piglets delivered on parturition one was born dead and nine died within 24 hours. On analysis of tissues from the dead piglets considerable amounts of 2,4-D were found; for liver, kidneys and lungs the mean values, and the range, were 35 (14—77), 27 (13—58) and 30 (13—64) μg/g, respectively. The placenta contained 46 μg/g of 2,4-D and the plasma of the sow at parturition 28 μg/ml.

DISCUSSION

Absorption. 2,4-D, when administered orally as a technical formulation of an amine salt, was readily absorbed in all the animal species studied, as indicated by the peak plasma concentrations after a single dose being attained in a few hours (about 2 hours for chickens and 4—7 hours for rats, pigs and calves, Figs. 1—4). Similar results were obtained with an alkali salt of pure 2,4-D, thus indicating the absorption rate of 2,4-D to be essentially unaffected by the amine or other ingredient of the technical formulation. 2,4,5-T amine behaved similarly.

Oral administration of 2,4-D ester, on the other hand, resulted in low plasma and tissue levels of 2,4-D as compared with the soluble salts, probably as the result of incomplete absorption (Fig. 5, Table 10). Considering the poor solubility in water of the ester, a low absorption rate is also to be expected. Not at any

16714

DOW 2583224

time after dosing could intact ester be detected in plasma or urine of rats, pigs and calves. Only the acid was detectable, indicating the ester to undergo hydrolysis during absorption (*Erne* 1966 b).

Distribution. The absorbed phenoxy acids were distributed rapidly throughout the body (Tables 5—7). In all species the highest tissue levels were found in the excretory organs, liver and kidneys, and in the lungs and spleen. The levels in liver, kidney and lung at 6 hours approached, and at later times often exceeded, the plasma level. This was particularly true for rats and hens. A comparison between species may be based on the kidney levels. If expressed relative to the liver levels, these values at 6 hours and 24 hours, respectively, were 1.6 and 1.3 for pigs, 2.8 and 5.4 for male rats, 4.1 and 5.0 for female rats and 1.5 and 3.2 for chickens. Thus, rats exhibited the highest values throughout, and in both rats and chickens the values increased during the elimination phase. The difference in 6-hour values for male and female rats probably is not significant. Likewise, there seems to be no sex-difference in distribution pattern in rats, although the tissue levels found in the females consistently were inferior to those found in the males.

From the more detailed study performed with pigs (Table 6) is seen that comparatively high levels can be attained also in endocrine and other secretory organs. Concentrations of 2,4-D comparable to that in liver, or about 50 % of the plasma level, were found in ovaries and adrenals. In skin and heart muscle the levels were approximately 25 % and in skeletal muscle about 10 %, relative to plasma.

The brain level was usually low, about 5 % relative to plasma, although after toxic doses higher concentrations could be attained (see Table 9). In adipose tissue only traces of 2,4-D were found, or 1—2 % relative to plasma. The low brain level is consistent with current concepts of drug transfer across body membranes. For most drugs and foreign organic compounds the transfer can be explained in terms of a simple diffusion, of the unionized compound, across a lipoid membrane, the rate-determining factors being the ionization of the compound and the lipid-solubility of the unionized form (see for instance *Binns* 1961). Now, phenoxyacetic acids, as being relatively strong acids (pK_a values about 3), will be largely ionized and therefore not readily diffusible at plasma pH. Accordingly, they would not be expected to traverse the blood-brain barrier, as well as the placental barrier, readily.

16715

DOW258325

Against this background, the results obtained with the pregnant sow, suggesting a ready placental transfer of 2,4-D, seem interesting. The results may be attributable to different factors, such as change of placental permeability during pregnancy, lowering of placental pH as compared to plasma, with a consequent lowering of the degree of ionization of the phenoxy acid, and extraplacental transfer. However, the present limited evidence does not permit an assessment of the relative importance of the various factors.

The distribution pattern of 2,4-D, administered as the ester, did not differ appreciably from that observed with 2,4-D amine, although tissue levels were consistently lower.

The apparent distribution volume of the compounds under study can be estimated by plotting the dose-elimination curves (Figs. 1—4) on a semi-logarithmic scale (log. conc. v. time) and extrapolating the terminal, linear part of the plot back to zero time. By relating the dose given to the "zero time plasma levels" thus obtained, apparent distribution volumes of 25—50 % of the body weight were found for the salts of 2,4-D and 2,4,5-T, when administered orally as a single dose. These values are intermediate between the extracellular body volume (about 20 % of the body weight) and total body water (about 70 % of the body weight), thus suggesting some penetration of the phenoxy acids into the cells. Direct evidence for the permeability of blood cells to phenoxy acids was obtained in the experiments summarized in Tables 3 and 4. In the blood cells 2,4-D regularly could be detected in concentrations ranging between 10 and 20 % of the corresponding plasma levels. Moreover, the 2,4-D content of the cells could be easily removed by repeated washing, which indicates a ready passage of phenoxy acids across the erythrocyte wall. The distribution equilibrium between plasma and blood cells is obviously displaced towards plasma, a fact which is also reflected in plasma being superior to saline in washing efficiency. One reason for the equilibrium being displaced could conceivably be a partial binding of the phenoxy acids to plasma proteins, thus reducing the free (diffusible) fraction of phenoxy acids in plasma. Indications of such a protein binding have been obtained (*Erne* 1966 b).

Elimination. Elimination of the phenoxy acids from plasma, after being administered as amine or alkali salts, was rapid in all species studied. The semi-logarithmic dose-elimination curves

16716

DOW 258326

were linear in their terminal courses, indicating a first order elimination rate. The plasma half-life values calculated from these curves were about 3 hours for rats, 8 hours for calves and chickens and 12 hours for pigs (Table 1). No sex difference in respect to elimination rate was observed in rats, although the plasma levels were lower in the females than in the males.

The elimination rate from plasma of 2,4-D, given as the ester, was slightly reduced as compared with the soluble salts.

The rate of elimination of 2,4-D from tissues was lower than that from plasma (Tables 5—7). From the tabulated data, half-life values of 2,4-D ranging between 5 and 10 hours can be calculated for rats and between 10 and 30 hours for the other species. Generally, in 72 hours or less, tissue levels had dropped to a few μg/g. No retention of phenoxy acid was noted in the tissues examined. The results are in agreement with those obtained in sheep using C^{14}-labelled 2,4-D (*Clark et al.* 1964). The distribution pattern of 2,4-D on repeated administration was essentially similar to that obtained after a single dose, although individual variations were considerable, presumably owing to variations in feeding habit of the animals and in time between last water or food intake and time of killing (Tables 8 and 10). The results seem to support the view that halogenated phenoxyacetic acids are not likely to accumulate in tissues of clinically healthy animals.

Excretion. Judging from the analytical results the major excretory route is via the kidneys, at least in the mammalian species studied. Histopathological examination suggests that this is true for chickens as well (*Björklund & Erne* 1966). Only low levels of 2,4-D were found in feces from rats and pigs and in bile from pigs. That phenoxyacetic acids are predominantly excreted renally in cattle and sheep, was reported by *Lisk et al.* (1963), *Bache et al.* (1964 a), *St. John et al.* (1961) and *Clark et al.*

Interestingly, hens were found to be able to excrete part of ingested 2,4-D with the eggs (Table 6), a finding not previously reported. Most of the 2,4-D content of the egg was found in the yolk.

Adaptation. On prolonged administration of 2,4-D, given as amine or as ester in doses below the acutely toxic level, some experimental animals developed signs of an adaptation. In pigs, plasma levels of 2,4-D usually started to decline after about a

16717

week, the urinary excretion concurrently increasing (Table 2, pigs no. 4 A and 9 A). A similar response was seen in chickens.

It should be remarked, however, that pigs seem to be less tolerant to prolonged exposure to phenoxy herbicides than are the other species studied. In several instances, 2,4-D given as daily doses of 50 mg/kg/day eventually produced signs of poisoning in pigs with rising plasma and tissue levels of 2,4-D (*Björklund & Erne*).

DOW 2583277

REFERENCES

Audus, L. J.: The Physiology and Biochemistry of Herbicides. Academic Press, London & New York 1964.

Bache, C. A., D. J. Lisk, D. G. Wagner & R. G. Warner: Elimination of 2-methyl-4-chlorophenoxyacetic acid and 4-(2-methyl-4-chlorophenoxy)butyric acid in the urine from cows. J. Dairy Sci. 1964 a, *47*, 93—95.

Bache, C. A., D. D. Hardee, R. F. Holland & D. J. Lisk: Absence of phenoxyacid herbicide residues in the milk of dairy cows at high feeding levels. J. Dairy Sci. 1964 b, *47*, 298—299.

Binns, T. B., ed.: Absorption and Distribution of Drugs. Livingstone, Edinburgh & London 1964.

Björklund, N.-E. & K. Erne: Toxicological studies of phenoxyacetic herbicides. Acta vet. scand. 1966, 7. In press.

Clark, D. E., J. E. Young, R. L. Younger, L. M. Hunt & J. K. McLaran: The fate of 2,4-dichlorophenoxyacetic acid in sheep. J. Agric. Food Chem. 1964, *12*, 43—45.

Erne, K.: Determination of phenoxyacetic herbicide residues in biological materials. Acta vet. scand. 1966 a, 7, 77—96.

Erne, K.: Studies on the animal metabolism of phenoxyacetic herbicides. Acta vet. scand. 1966 b, 7. In press.

Gutenmann, W. H., D. D. Hardee, R. F. Holland & D. J. Lisk: Disappearance of 4-(2,4-dichlorophenoxy)butyric acid herbicide in the dairy cow. J. Dairy Sci. 1963 a, *46*, 991—992.

Gutenmann, W. H., D. D. Hardee, R. F. Holland & D. J. Lisk: Residue studies with 2,4-dichlorophenoxyacetic acid herbicide in the dairy cow and in a natural and artificial rumen. J. Dairy Sci. 1963 b, *46*, 1287—1288.

Levey, S. & H. B. Lewis: The metabolism of phenoxyacetic acid, its homologues, and some monochlorophenoxyacetic acids. New examples of β-oxidation. J. biol. Chem. 1917, *168*, 213—221.

Lisk, D. J., W. H. Gutenmann, C. A. Bache, R. G. Warner & D. G. Wagner: Elimination of 2,4-D in the urine of steers fed 4-(2,4-DB) or 2,4-D. J. Dairy Sci. 1965, *46*, 1435—1437.

16719

St. John, L. E., D. G. Wagner & D. J. Lisk: Fate of atrazine, kuron, silvex and 2,4,5-T in the dairy cow. J. Dairy Sci. 1964, 47, 1267–1270.

Williams, R. T.: Detoxication Mechanisms, 2nd Ed., Chapman & Hall, London 1959, p. 330.

DOW258328

SUMMARY

The distribution and elimination of two phenoxyacetic acids, 2,4-D and 2,4,5-T, were studied with a chemical method in rats, pigs, calves and chickens.

When administered orally as amine or alkali salts, the compounds were readily absorbed and distributed over the organism in all species studied. The absorption of 2,4-D in the form of an ester was incomplete, however, the ensuing plasma and tissue levels of 2,4-D being only low. (Intact ester could not be detected in plasma).

The highest tissue levels of 2,4-D and 2,4,5-T were found in liver, kidney, lung and spleen, the levels sometimes exceeding the plasma level. In blood cells 10—20 % of the plasma level was found. Penetration of 2,4-D into adipose tissue and into the central nervous system was restricted, whereas a ready placental transfer was demonstrated in swine. The distribution pattern did not show any significant species or — in rats — sex differences.

Elimination of the compounds was rapid, the plasma half-life being about 3 hours in rats, about 8 hours in calves and chickens and about 12 hours in pigs. The tissue half-life values ranged between 5 and 30 hours, the lower values being found in rats. No retention in tissues was noted, nor was accumulation seen on repeated administration.

In pigs and chickens an increased elimination rate was observed after repeated administration.

The major excretory route seemed to be via the kidneys in all species studied. Hens excreted small amounts of 2,4-D with the eggs.

ZUSAMMENFASSUNG

Verteilung und Elimination von chlorierten Phenoxyessigsäuren im Tierorganismus.

Die Verteilung und Elimination von zwei chlorierten Phenoxyessigsäuren, 2,4-D und 2,4,5-T, wurden an Ratten, Schweinen, Kälbern und Hühnern untersucht.

Nach oraler Eingabe, in Form der Amin- oder Alkalisalze, wurden die Verbindungen bei allen untersuchten Tierarten schnell resorbiert, und über den Organismus verteilt.

Orale Zufuhr von 2,4-D in Form eines Esters ergab jedoch nur niedrige Plasma- und Gewebsgehalte von 2,4-D, anscheinend infolge geringer Resorptionsgeschwindigkeit.

Nach einziger oraler Zufuhr von Phenoxyessigsäureaminsalzen wurden die höchsten Gewebskonzentrationen, bisweilen den Plasmaspiegel übersteigend, in Leber, Niere, Lunge und Milz gefunden. In

16719

den roten Blutkörperchen wurden 10—20 % des Plasmagehalts nachgewiesen. 2,4-D gelangte nur in niedriger Konzentration ins Fettgewebe und ins Zentralnervensystem. Dagegen wurde ein leichtes Durchdringen der Placentarbarriere beim Schwein nachgewiesen. Im Verteilungsmuster waren keine geschlechtlichen (bei Ratten) und nur mässige tierartsgebundene Variationen wahrzunehmen.

Die Verbindungen wurden schnell eliminiert; die Halbwertzeit in Plasma, nach einziger oraler Eingabe, betrug ca. 3 Stunden bei Ratten, ca. 8 Stunden bei Kälbern und Hühnern und ca. 12 Stunden bei Schweinen. Die Halbwertzeiten in den Geweben waren bei Ratten 5—10 Stunden und bei den anderen Tierarten 10—30 Stunden. Keine Gewebsretention von Phenoxysäuren wurde festgestellt, ebenso keine Akkumulation nach wiederholter Zufuhr.

Bei Schweinen und Hühnern wurde in einigen Fällen nach wiederholter Zufuhr eine gesteigerte Ausscheidung von Phenoxysäuren nachgewiesen.

Die Phenoxysäuren wurden bei allen untersuchten Arten hauptsächlich durch die Nieren ausgeschieden. Bei Hennen wurde eine Ausscheidung von kleinen Mengen 2,4-D mit den Eiern nachgewiesen.

SAMMANFATTNING

Distribution och eliminering av klorerade fenoxiättiksyror hos djur.

Två fenoxiättiksyrederivats, 2,4-D och 2,4,5-T, distribution och eliminering hos råttor, grisar, kalvar och höns har undersökts.

Tillförda oralt som amin- eller alkalisalter resorberades föreningarna lätt och fördelades snabbt över organismen hos alla undersökta djurarter.

Efter tillförsel av 2,4-D ester påvisades endast låga plasmahalter, synbarligen beroende på ofullständig resorption.

Lever, njure, lunga och mjälte visade de högsta vävnadshalterna, vilka ibland översteg plasmanivån. I erytrocyter återfanns 10—20 % av plasmahalten. Fettväv och centrala nervsystemet penetrerades endast i ringa grad av 2,4-D, medan placentabarriären till synes obehindrat passerades hos svin. Distributionsmönstret visade ej något signifikant art- eller, för råttor, könsberoende. Honråttor visade dock något lägre plasma- och vävnadshalter än hanråttor.

Föreningarna eliminerades snabbt; halveringstiden i plasma var c:a 3 timmar för råttor av båda könen, c:a 8 timmar för kalvar och höns och c:a 12 timmar för grisar. Halveringstiden i vävnaderna var av storleksordningen 5—30 timmar; råttorna hade de lägre värdena. Ingen retention i vävnaderna observerades och ej heller någon ackumulation efter upprepad tillförsel.

Hos grisar och höns iakttogs en uppträdad utsöndring efter upprepad tillförsel av 2,4-D.

Utsöndringen skedde huvudsakligen genom njurarna hos undersökta arter. Höns utsöndrade små mängder 2,4-D med ägget.

(Received March 22, 1966).

DOW 258329

16720

D. Toxic Effects of Herbicides and Conclusions

As mentioned in the introduction of this chapter, we are listing here a variety of information which could not properly be listed in the other sections. The items specifically included here are the following: (1) the mutagenic activity of herbicides on plants, bacteriophage, and bacteria, (2) the effect of herbicides on the embryonic development of beetle, (3) the concentration of herbicides in the tissues of fish, (4) the importance of selecting a formulation which will minimize the hazards of herbicides in aquatic environments, (5) an estimation of the hazard involved in the spraying of a man with herbicides,and (6) some general conclusions about the toxicity hazards relative to the use of herbicides.

Mutagenic Effects

Some of the persons who are critical of the use of herbicides feel that we are creating a monster which will eventually destroy us. One area of expressed concern deals with the possibility that herbicides will produce heritable changes in the genetic material of plants. The resulting plants, they fear, will no longer meet our need for the production of foods or they may be insidious weeds which do not respond to either chemical or biological control measures. Some studies have been conducted to determine the likelihood that herbicides may produce heritable changes in plants.

Doxey and Rhodes (1949) conducted a study of a plant-growth-regulator type of herbicide known as 4-chloro-2-methylphenoxyacetic acid (MCPA). In this study they exposed onion root tips to MCPA and observed the effects of this treatment on cell division and on the chromosomes. Chromosome "stickiness" and breakage were observed and the effects were judged to be similar in many respects to changes in mitosis produced by X-irradiation. In a series of three papers, Unrau and Larter (1952) and Unrau (1953 and 1954) discuss their findings on the effect of 2,4-D on the genetics of wheat and barley. The seed grain fields were sprayed with 8 to 16 oz of 2,4-D (EE) per acre 30 days prior to cytological examination. The chromosomes were studied in detail in those cells that were dividing during the process of pollen formation. Regardless of the date of application or the concentration of treatment, the herbicide caused "highly significant" abnormalities of chromosome behavior and the changes were visible as much as 30 days after treatment. Unrau warns that indiscriminate use of herbicides could have grave and far-reaching consequences especially in relation to the prime seed stocks of seed growers. There is reason to suspect that the herbicide interferes with self-pollination and this could explain the increase in the number of off-types or crosses obtained in Unrau's experiments.

DOW 120471

16721

Muhling et al. (1960) has studied the cytological effects of 2,4-dichlorophenol, 2,4-dichlorophenoxyethanol, 2,4-D, and other herbicides on pea seedlings (Pisum sativum). 2,4-D was clearly shown to be a prophase poison and to produce spindle inhibition resulting in chromosome configurations very similar to those produced by colchicine.

Dr. Kenneth J. Andersen (1967), Senior Research Microbiologist at Battelle Memorial Institute, Columbus Laboratories, is engaged in a research program for the United States Department of Agriculture to determine the possible mutagenic and genetic effects of the herbicides. As a part of this study, he grew histidine-requiring mutants of Salmonella typhimurium on a histidine-deficient medium to screen 120 of the 130 herbicides listed in the journal, Weeds. These included, 2,4-D, 2,4,5-T, picloram and cacodylic acid and the other herbicides which are currently important for noncropland vegetation control (see Table II-2). Since the S. typhimurium used is histidine-dependent it does not grow very well on the deficient medium unless there is a mutation which eliminates the histidine requirement. In his experiments he found that diethyl sulfate, beta-propiolactone, acridine mustard (ICR-91) and N-methyl-N'-nitro-N-nitrosoguanidine all produced the mutations required for the growth of the organism. On the other hand, none of the herbicides caused this mutation.

A second approach to the problem involved the use of T_4 bacteriophage to detect chemically induced mutations of the rII type. The number of mutant plaques, which characteristically were larger, with a clear center and a sharp edge, were compared to the normal T_4 plaques which were small, with a clear center surrounded by a halo. In addition to this characteristic plaque morphology, rII mutants form plaques on Escherichia coli but not E. coli KB while wild type T_4 bacteriophage forms plaques on both of the E. coli strains. In this evaluation system noninhibiting levels of the compounds (approximately 20 µg-1 mg/20 ml) were employed. The rate of spontaneous mutation (no test chemical present) by T_4 bacteriophage to the rII-type mutant, was about 0.11%. In the presence of the known mutagen, 5-bromouracil, the mutation frequency increased to about 2.5%. In the test system it was concluded that none of the herbicides were mutagenic.

Dr. Andersen has also performed experiments using a 2-aminopurine-induced rII mutant of T_4 bacteriophage to detect revisions from the mutant to the wild type which might be caused by the herbicides. Herbicides, in this detection, were again observed to be nonmutagenic.

McGahen and Hoffman (1966) have also investigated the possible mutagenic activity of herbicides. In their program they employed a screening technique similar to that used by Andersen (described above). In their study

DOW 120472

16722

they used both the 3- and also the 6-alkyl bromouracil herbicides (including bromacil) on a bacteriophage and concluded that it was not mutagenic by this test.

Dr. Verne Comstock (1967), U. S. Department of Agriculture and Agronomy Department at the University of Minnesota, has conducted a seven-year study on the effect of herbicide applications on the growth, yield and quality of flaxseed. During the years of 1956 to 1963 paired lines of four varieties of flaxseed were grown on plots which had received no herbicide or had received 15 to 20 oz/acre of MCPA per year (treatment was omitted in 1959). The performance of unsprayed subpopulations were compared with that of the subpopulations which had been sprayed in the F_2, F_3, and F_4 generations. "It can be concluded that the application of high rates of MCPA to successive generations did not cause a significant shift in these flax populations to more resistant biotypes."

Insect Development

Several investigators have considered the possibility that pesticides may under certain situations cause a disturbance in insect population which will result in a stimulation of a secondary pest. The reason for the increase in aphids in the grain fields in which 2,4-D amine has been used for weed control has now been investigated by Adams (1960). In her study three species of coccinellid beetles (Coccinella transversoguttata Fald., Hippodamia tredecimpunctata (L.), and Coccinella perplexa Muls.) were grown in the laboratory and sprayed with 2,4-D at various stages of their larval development (1 to 12 days). It was observed that spraying on the 12th day of larval development produced the highest mortality (70%) and about a 75% increase in time to maturity. Since this bettle is an important factor in the control of aphid populations on grain, weed control with herbicides should be timed so as to minimize the damage to the useful beetle.

DOW 120473

Concentration in Food Chains

One of the big concerns of the ecologists is this, are the herbicides concentrated at some point in the food chain so that some life form at or near the top of the food pyramid will receive a toxic dose? Fortunately there are few life forms which concentrate herbicides at all and in most species this concentration is a reversible process so that the concentration level remains small. Table VI-10 shows the amount of herbicide present in the tissues of fish after exposure to the concentrations shown. For comparison it may be noted that under these same conditions heptachlor accumulates in the bluegill

16723

DOW 120474

TABLE VI-10

AMOUNTS OF SOME HERBICIDES MEASURED AS RESIDUES IN ANIMALS AFTER 30-DAY EXPOSURES IN PONDS

Species	Pesticide	Locality	Amount of Pesticide in Water (ppm)	Amount of Residue in Animal (ppm)
Bluegill	2,4-D PGBE in oil	Tishomingo, Okla.	10	None detected
Redear Sunfish	Silvex,* PGBE in oil	Tishomingo, Okla.	1	0.5
Rainbow Trout	Paraquat emulsion	Denver, Colo.	1	0.11
Green Sunfish	Paraquat emulsion	Denver, Colo.	1	0.05
Bluegill	Paraquat emulsion	Denver, Colo.	1	1.21
Channel Catfish	Paraquat emulsion	Denver, Colo.	1	0.37
Bluegill	Sodium arsenite	LaCrosse, Wis.	0.23	0.40
Bluegill	Diquat	LaCrosse, Wis.	1	0.09

* Fenoprop.

Source: Cope, O.B., Contamination of the Freshwater Ecosystem by Pesticides, J. Appl. Ecology, 3 (Suppl.) 33-44 (1966).

16724

to the point where the tissue level of heptachlor is 300 times as great as the heptachlor level in water in which the fish is swimming (Cope, 1966).

Selection of Herbicides

At the present time there is no magic formula which will automatically select the herbicide which will control the vegetation best and provide the minimum damage to fish and wildlife. Instead herbicides must be carefully selected on the basis of the weed that needs to be controlled, the location of that weed, and the possible fish and wildlife which may be affected by its use. That selection of the proper formulation for the job is exceedingly important in the control of aquatic weeds without unnecessary toxic effects on the fresh-water ecosystem. In Table VI-11, the acute toxicities of a series of herbicide formulations to lake Emerald Shiners (Notropis atherinoides) are presented for three different exposure periods. The importance of the formulation employed is borne out by the observation that both the most toxic and the least toxic formulations are formulations of endothall and one is about 6,000 times as toxic as the other.

Spraying of Herbicides on Man

From an examination of unpublished results from the Patuxent Fish and Wildlife Center at Laurel, Maryland (Robert G. Heath, 1967), we conclude that dieldrin is at least 30 times more toxic than amitrole, atrazine, 2,4-D(BEE*), 2,4-D(DMS*), dalapon, diquat, diuron, monuron, picloram, simazine, 2,4,5-T(BEE) and 2,4,5-T acid to the two-week old young of mallards, ring-necked pheasants, bobwhite and coturnix.

One further comparison of the toxicity of herbicides and pesticides: the Federal Aviation Agency is much concerned about the toxicity of pesticides and their effect on the ability of the pilot to fly his plane safely. Dr. Paul Smith, Head of the Pharmacology Section at the Aeromedical Laboratories in Oklahoma City, said that the pilots spray insecticides during the early part of the year and then spray defoliants and herbicides in the summer for brush control. He said that the pilots themselves are always glad when they are through spraying the insecticides. He added that the flagman on the ground during the aerial spraying operation probably receives a higher dose of herbicides than the pilots. So far as he is aware there has not been a serious case of poisoning or toxicity as a result of the spraying of herbicides.

* BEE = Butoxyethyl ester; DMS = Dimethylamine salt.

DOW
120475
16725

TABLE VI-11

COMPARISON OF THE ACUTE TOXICITIES OF COMMERCIAL HERBICIDES TO LAKE EMERALD SHINERS (NOTROPIS ATHERINOIDES) IN MEDIUM HARD WATER. MEAN TEMPERATURE 69° - 72°F

Product	Mfr.	Liq. Gr.	Active Ingredient	TL_m, ppm active 4 hr	24 hr	48 hr	96 hr
Aquathol-Silvex	Pennsalt	L.	Endothall + Silvex	> 1,000	780	612	510
Weed Rhap 20	Reasor-Hill	Gr.	2,4-D, ethyl-hexyl ester	> 1,000	620	620	510
Amitrol T	Amchem	L.	Aminotriazole + am. thio-cyanate	910	455	455	420
Kurosal G	Dow	Gr.	Silvex, potas-sium salt	1,100	540	450	370
Crop Rider	Diam. Alk.	Gr.	2,4-D, ethyl-hexyl ester	500	280	280	280
Kurosal SL	Dow	L.	Silvex, potas-sium salt	509	420	310	270
Reglone (DB)	Chipman	L.	Diquat, di-bromide form	> 180	> 180	86.2	25.8
Reglone (DC)	Chipman	L.	Diquat, di-chloride form	> 180	15.5	11.7	9.1
Atlas A	Chipman	L.	Sodium arsenite	> 32	13.5	8.1	8.1 (As)
Esteron 99	Dow	Gr.	2,4-D, 2,4,5-T butyl esters	> 10	4.3	4.3	4.3
Garlon	Dow	L.	Dalapon + Silvex (butyl)	> 10	4.2	4.2	4.2
Urab	Allied	L.	3-phenyl-1, 1-dimethylurea trichloroacetate	5.6	4.7	4.3	3.8
Kuron	Dow	L.	Silvex, butyl ester	> 10	4.0	2.4	2.0
Hydrothol	Pennsalt	L.	Endothall, coco-amine salt	0.29	0.12	0.10	0.08

Source: Y.H. Swabey and C.F. Schenk, Proc. Third Annual Aquatic Weed Control Soc. 1963, pp. 20-28.

DOW 120476

16726

Item 3141

cc: John Gleason

14.036

DOW 11221.6

What Are Pesticides Doing To Human Beings?

REVISED and UPDATED
AFTER TWENTY YEARS

GRANVILLE F. KNIGHT, M.D., F.A.C.A.
President
PRICE-POTTENGER NUTRITION FOUNDATION

Orginally Published In MODERN NUTRITION MAGAZINE
April, September and December, 1952
Copyright © 1972 by Author

16727

What Are Pesticides Doing to Human Beings?

By Granville F. Knight, M.D., F.A.C.A.
Santa Monica, California 90403

"Nothing!"—say the publicity agents of the insecticide manufacturers. "Hysterical alarmists" is the quaint description applied to some of those who even suggest that the public is being harmed by their use. Groups urging that the newer pesticides not be released commercially until proven harmless to humans by exhaustive testing, have been accused of obstructing research and scientific progress!



"Nothing to worry about!"—repeat most of our entomologists. "Pesticides are poisonous. Yes. But with proper precautions those handling them are safe. And with the establishment of tolerances for residues on food the public is protected. Moreover, farmers can not bring to harvest the large crops needed throughout the world without the newer pesticides. Better the risk of some toxic residues on our food than widespread crop failure from insect attack." These conflicting statements remind one of the cheery whistle adopted by a small boy when passing a cemetery at night.

"Danger!" Widespread illness from the use of DDT on crops and in homes. This is the belief of a few keen observers in the medical profession. A handful of agriculturalists, entomologists and others agree. There is no question at all in the minds of those who have personally experienced DDT poisoning.

How may we account for such marked divergence of opinion? It is not difficult. The manufacturing companies are concerned with a booming business. The entomologists' responsibility lies mainly in protecting our crops from insect pests. Both groups are loath to accept evidence which might interfere with the production and use of the new insecticides. Knowing that some individuals exposed to large amounts of DDT remain in apparent good health, they refuse to believe that small, or even large, residues on food might be harmful to the general public.

Physicians, on the other hand, are primarily concerned with the health of the people and are quick to sense the threat of new poisons added to the diet. This is particularly true in view of the fact that DDT is very stable and may be concentrated in body fat up to at least 30 times the level in the food consumed. Unfortunately, scientific proof of toxicity to humans from low levels of intake is difficult.

The potential hazard to health is tremendous. It must be recognized by all parties concerned and controlled until *the facts are established beyond all doubt!*

The Delaney Committee

In spite of the assurance that there is nothing to fear, the public has become alarmed over the widespread use of poisonous sprays and dusts on our crops. And rightly so.

This alarm was communicated to the Congress which appointed a Select Committee to Investigate the Use of Chemicals in Food Products. The chairman is John J. Delaney, M.C. In addition to pesticides, this Committee is investigating the use of chemicals in other fields, such as drugs, cosmetics, food preservatives, and fertilizers.

For the past two years the Committee has been hearing testimony from prominent scientists, entomologists, nutritionists, food growers, manufacturers, physicians and others interested in the problem.

In the field of pesticides, testimony was overwhelmingly against the idea that the public health was being endangered by their use. However, a few physicians presented convincing clinical and laboratory evidence of widespread illness from contact with DDT on foods or in sprays.

Louis Bromfield was not only convinced of the hazard but from his own experience told the Committee, that with correct farming methods, few insecticides were necessary or desirable.

Recently L. G. Cox, director of technical projects for the Beech-Nut packing company stated that his firm had spent $660,000 in the past six years in an attempt to keep the newer pesticides out of baby foods and peanut butter. He also stated that a fund of $119,000 had been raised by the manufacturing chemists "to counteract unfavorable publicity." Mr. Cox related that in a number of instances his company had been forced to reject large orders of fruits and vegetables because of contamination with DDT, Chlordane and Benzene Hexachloride.

In a preliminary report to Congress in January, 1951, the Committee recognized

Reprinted from The Modern Nutrition Magazine April, September and December, 1952

11221

16728

the existence of a definite hazard to public health and continued hearings. These are still taking place. As a result of the evidence it is to be hoped that remedial legislation will be recommended to the Congress.

The purpose of this article is to give the members of the Academy the latest information on this highly controversial and extremely important subject.

Extent of the Problem

For the past thirty years or more there has been a gradual increase in the amount of poisonous chemicals used on crops for the control of insects and fungous diseases. During the same interval a corresponding decrease in the protein content of our plants and the nitrogenous content of our soil has been noted.

Since the introduction of DDT and similar compounds in 1946 the tonnage of the new insecticides in use has tremendously increased, while that of the older chemicals has dropped. The marked toxicity of these new compounds for most insects and animals, together with their unusual stability, has exchanged new problems for old.

It is no exaggeration to say that at the present time, with the exception of fish, it is a rare article of food that has not been dusted or sprayed with one or several poisons before reaching the consumer. Never before has the long-suffering public been made a guinea pig for the mass testing of so many exceedingly powerful chemicals.

Food Contamination

Theoretically crops are treated long enough before harvest so that weathering has reduced the residue below toxic levels. Practically this safeguard is of little comfort for the following reasons:

(1) Enforcement of regulations is not feasible except in the case of large farms. It is physically and financially impossible to police all the small farmers. How often has ignorant enthusiasm led to the application of many times the recommended amounts? Who knows? And how many small producers, if faced with the destruction of a crop by insect pests shortly before harvest, would have the fortitude to refrain from spraying at that time? And why should they hesitate? The trade journals tell them any danger to the consumer has been greatly exaggerated.

(2) When airplanes are used for dusting fields, drift to adjacent areas is frequent. This has been reported as leading to innocent, but heavy contamination of some crops.

(3) While our state and federal inspectors are conscientious and hard-working, they are limited in number. One wonders how much food grossly contaminated with poisons reaches the consumer. We know that some does. Spot check tests can discover only a portion of it.

(4) Our knowledge of the long range toxic effects of most of the new compounds is woefully inadequate. Therefore how can tolerance limits be arbitrarily set? Particularly when no tests have yet been perfected for even detecting the most recent ones. Logic dictates that until all the facts are known any residue on food is too much.

Other Contacts

As though poisonous residues on food were not enough, the public is exposed to powerful chemicals in other ways.

For some unknown reason—and in spite of evidence to the contrary—we have been sold the idea that DDT and chlordane are harmful for all practical purposes only to insects. The main basis for this impression seems to be these facts: (1) A comparatively few, healthy young men, when exposed to considerably larger doses of DDT than would be encountered in ordinary usage, showed no ill effects. (2) Animals exposed to DDT aerosols came through unharmed except for local irritation. No allowance has been made for the fact that many humans are malnourished, allergic and chronically ill; nor for the fact that the young are particularly susceptible to toxins of all kinds.

As a result of this propaganda, few households are without DDT aerosols or sprays for fly control. And where ants are a problem chlordane (even more toxic than DDT) is used for their destruction. Both of these substances may be encountered in hotels and restaurants. Naturally, contamination of food and utensils is to be feared.

Most commercial tree sprayers use the chlorinated hydrocarbons. In addition to this source of exposure, pleasure drivers on the highways in agricultural areas may be dusted by airplanes.

Is the Public Protected?

At the present time the Food and Drug Administration offers some protection, but only has jurisdiction over interstate commerce. The great weakness of the agency is this—while it has authority to set tolerances for pesticide residues on or in plant and animal foods, under existing law it can not prevent the use of a chemical before a tolerance has been set and the relative safety of the chemical determined.

Most of the state laws are similar. Therefore the state agencies labor under similar handicaps.

Potent new compounds are being synthesized at an amazing rate. Rough lethal doses for animal life can be determined in a few months by acute toxicity studies in the rat and larger animals. However, to learn the cumulative effects of repeated

11221B

16729

contact with small amounts of a chemical requires careful observation over several generations. This may take two or three years. In addition to being time-consuming, it is expensive.

In the meantime the partially tested pesticide may be manufactured, advertised, sold, and widely used! Is this protection?

It is only fair to state that some manufacturers insist on thorough investigation before releasing a new product. Others are not so careful, partly because they lack the funds and facilities for such work, and partly because the law does not demand it.

Under the present laws any company wishing to use a new chemical in or on food is not required to first consult with the Food and Drug Administration relative to merits or potential harmfulness. This agency may confiscate or condemn products only if they contain chemicals proven to be deleterious. The inadequacy of this law is obvious, but some illustrations are illuminating.

Agene was used for artificially aging flour over a period of thirty years before it was discovered that dogs fed with bread made from such flour developed "canine hysteria" or "running fits." The use of this substance has now been abandoned by the baking fraternity in favor of a less ? ? ? harmful substance. Denaturation of the proteins and damage to vitamins undoubtedly remains. Incidentally, the United States Supreme Court in 1919 declared bleached flour to be unfit for human consumption. If this judgment is still on the books, why has it never been enforced?

When the second of the "sulfa" drugs, sulfanilamide, was introduced some years ago, in addition to tablets, a liquid preparation was sought. The drug was relatively insoluble in the ordinary solvents, but finally a chemical was found that would dissolve an adequate dose. This was one of the group now used as an anti-freeze in car radiators. Without adequate animal tests the product was marketed. It was condemned only after a number of deaths due to the solvent occurred.

Other examples could be cited. How long must we wait before adequate pretesting may be counted on to prevent tragedies of this sort?

The Older Insecticides

Let us now consider the more important chemicals used for the destruction of pests. First of all we shall discuss those in use for many years—adequate enough to bring tremendous crops to maturity during the recent world conflict.

With the exception of selenium and arsenic, these chemicals do not transmigrate from the non-edible portions of a plant to those parts used for food. Nor do they penetrate to any extent into the fruit or leaf. Therefore, most of the residues may be removed by scrubbing with soap and water and/or by dipping for one minute in dilute hydrochloric acid.

The chronic toxicity of arsenic is well known. In small doses it may have a tonic effect. "Arsenic eaters" in the Tyrol feed it to their horses to increase their stamina and eat it themselves for the same reason. The custom is not highly recommended. As the chemical is usually metabolized and excreted in from one to six weeks, high chronic doses are necessary to produce damage.

However, such high contamination may occasionally be found. Livestock grazing in orchards which have been heavily treated with arsenic compounds may show serious symptoms from such fodder. Some years ago the American Medical Association condemned apples from one county in the State of Washington. These orchards had been so heavily treated with arsenic that dangerous amounts of this chemical had been taken up by the roots of the trees and translocated to the fruit.

Arsenic is often applied to fruits in the form of lead arsenate. The limit for the compound on apples was set in 1940 at 3.6 ppm (parts per million). The lead component is more dangerous in small amounts than the arsenic. Lead is stored in the bones at all dietary levels and may cause kidney damage. A high level of calcium in the diet tends to be protective.

Mercury is highly toxic and residual compounds are dangerous. As little as 0.5 ppm may induce storage in the kidney with resultant harm to that important organ. Mercury vapor used to preserve grain in storage has not been shown to contaminate such grain.

Selenium in the soil is absorbed by the roots and transmigrates to all parts of plants. The same process occurs following the application of sprays. This substance may not therefore be used on food crops. It causes abdominal pains, anemia, weight loss and liver damage. Cattle, cropping plants in seleniferous areas such as parts of Wyoming, develop the "blind staggers." This is followed by paralysis and death. Selenium sprays are used only on ornamental flowers.

Fluorine compounds are less toxic than lead arsenate. Their toxicity lies in their ability to precipitate calcium in the body. "Cryolite" is used as an apple spray. When so used the shipper is required to wash off the residue. Drinking water containing from 0.1 to 1 ppm has some effect in lowering the incidence of dental caries in young children. Even these concentrations may produce mottled enamel and interfere with bone metabolism.

112219

16730

112220

Nicotine is one of our most powerful, rapidly-acting poisons. One grain is the minimum lethal dose for man and even one-fifteenth of that may cause severe symptoms. It is a nerve poison, causing stimulation which is rapidly followed by depression. The alkaloid is volatile and therefore presents no chronic toxicity. Some of its salts may provide a residue hazard. For all practical purposes it is no problem except to the applicator.

The pyrethrins. These were originally obtained from the petals of a member of the chrysanthemum family grown in the Far East. Synthetic analogues are now available and have similar properties. These compounds are rapidly detoxified in animal tissues and the lethal dose is very high. Therefore, they are comparatively safe. However, they may produce severe asthma or other allergic reactions in those sensitive to ragweed pollen.

Rotenone from derris root is most poisonous to fish and not very toxic to man.

The thiocynates of which lethane is an example are absorbed through the skin. They release hydrogen cyanide, a powerful enzyme poison, in the body. Caution should be exercised if they are used as garden sprays. The solutions formulated for the control of flies are of a low order of toxicity for man. Nevertheless, inhalation should be avoided.

Since the introduction of the newer pesticides the use of the chemicals just described has rapidly decreased. The peculiar properties of the new compounds, together with their application to almost all foods, has created a potential hazard to health of unprecedented proportions.

Part II

For practical purposes, the newer pesticides may be divided into two main groups—the *chlorinated hydrocarbons* and the *organic phosphates*. While differing in mode of action and other properties, both types of compounds in sufficient quantity are lethal to all animal life and most insects.

The Chlorinated Hydrocarbons

In 1939 Swiss chemists discovered the insecticidal properties of DDT or dichlorodiphenyltrichloroethane. Soon afterwards BHC (hexachlorocyclohexane), commonly known as benzene hexachloride, was contributed by France and the United Kingdom.

Since that time American ingenuity has developed other compounds of similar type, namely toxaphene, TDE, chlordan, methoxychlor, lindane, aldrin and dieldrin. New compounds are constantly being synthesized.

Although still undergoing investigation, and with some reluctance on the part of



responsible authorities, DDT was released for commercial use in 1945. Farmers, entomologists and manufacturers had heard of the wonderful new insecticide and clamored for supplies. The pressure was great. Early results justified all expectations.

During the latter part of World War II, DDT had received extensive field trials in the protection of our armed forces from typhus and malaria. This was a calculated risk and there is no doubt that the destruction of lice and mosquitoes radically reduced the mortality from these dangerous diseases.

In Southern Europe our troops were dusted with DDT powder for the elimination of body lice. The same treatment vigorously applied to the population of Naples undoubtedly halted an epidemic of typhus fever.

In the tropics whole islands were dusted by airplanes. The unsuspecting mosquitos and flies were decimated. Other insects, bees and birds shared the same fate to a lesser extent.

An epidemic of gastroenteritis and so-called virus hepatitis affected our troops in Manila at the time this city was given the same treatment. The cause was never definitely determined but was attributed to unwashed vegetables acquired from the natives.

In the year 1950 it is estimated that one billion pounds of agricultural chemicals were manufactured. This gives an idea of the problem before us.

Physical and Chemical Properties

DDT is the least water soluble organic compound known to science. This fact helps to explain why chemists assumed —incorrectly as events have proved—that dairy cattle might be safely sprayed or dusted with that compound. It is however, quite soluble in many fats. Its usefulness as a pesticide and its danger, is to a considerable extent dependent upon this property.

DDT is exceedingly stable. It is not normally decomposed by sunlight nor by cooking temperatures. The leaching effect of rain is very slight. Residues on citrus fruits and foliage in California were found to be reduced by only 50% in 40 days. Ten per cent still remained after 90 days. In soil the compound is much more stable. One test plot still shows

16731

heavy contamination six years after one application to the soil.

BHC is a mixture of optical isomers with a musty smell. Its application has been found to produce an off-flavor in potatoes, walnuts and other crops. The Beech-Nut Packing Company has had to reject shipments of peanuts and vegetables purchased for its line of baby foods for this reason. With the exception of its gamma isomer, lindane, the compound is fairly stable. Lindane is the most active fraction and can now be purchased in a relatively pure form.

Chlordan has properties intermediate between DDT and BHC, but on the whole is more toxic. Aldrin and dieldrin are chlorinated naphthalenes of similar nature—dieldrin being the most toxic and residually effective of the present insecticides.

Toxaphene is a chlorinated camphene of marked stability. Methoxychlor shares with lindane the fact that it is relatively unstable and therefore presents less residual hazard. However, one investigator has stated, that with repeated exposure, the toxicity of lindane may increase as much as 100 times! This merits confirmation.

Toxicology

The chlorinated hydrocarbons are *nerve poisons* and their action is associated primarily with their solubility in tissue fats or lipoids. The cuticle or chitinous covering of most insects is water-repellent and seems to protect them from dessication or drowning. The outer layer of this cuticle is of a waxy nature and has a definite affinity for DDT. This fortuitous property renders most insets as vulnerable to contact with DDT as though no cuticle were present. And the marked stability of this chemical presents prolonged opportunity for such contact. Flies readily absorb the compound through their feet.

DDT has a similar action on the nervous system of insects and animals. Overstimulation of the sensory and motor nerves by lethal doses leads to tremors, convulsions and spastic paralysis. Death from exhaustion follows.

Toxicity for Man and Animals

The acute toxic oral dose for animals is about 250 mg/kg. For a 150 lb. man this would be about ½ ounce. The toxicity is enhanced when DDT is dissolved in dietary fat or in oils.

Suspensions and powders applied to the skin are believed to be non-toxic, but symptoms in man have occurred following contact with oily solutions.

The ordinary household aerosol bomb used for flies and mosquitos is thought to be harmless. Animal toxicity only appears after exposure to a concentration 4000 times that found in this contraption. However, Biskind has reported severe reactions to these sprays, probably as a result of hypersensitivity. Recent experiments suggest that repeated exposure may lead to respiratory infection in animals.

Because DDT is poorly metabolized and excreted by animals and is fat soluble, it is stored in the body fat. It is therefore a *cumulative poison*. The amount ingested in food may be concentrated as much as 30 times in fatty tissue. When the diet is contamintaed, DDT is excreted in comparatively large quantities in bovine and human milk, being found primarily in the cream. Thus, cows fed through the winter on hay containing 84-184 ppm of DDT, secreted enough of this chemical in their milk to cause loss of weight in steers drinking it. Their own suckling calves accumulated DDT to the extent of 825 ppm in their body fat. When rats were fed for several years on a diet containing only 1 ppm, they stored up to 30 ppm in their adipose tissue.

Chickens consuming contaminated rations magnify the DDT intake and excrete it in the yolks of their eggs.

It is conceivable that a rapid loss of weight in animals or humans storing large amounts of DDT in their body fat could release enough in the blood stream to cause symptoms of acute poisoning. Clinically this seems to occur.

At this point it should be noted that fat is a living tissue which is continuously being broken down and rebuilt. Moreover, all types of body cells are surrounded by semipermeable membranes containing lipoids, which can theoretically hold DDT. This chemical is known to inhibit one of the oxidative enzymes, cytochrome oxidase and may interfere with the action of others. Therefore this type of storage in body fat is not like keeping butter in a refrigerator. Toxic effects on each body cell even with high dilution are not too remote a possibility.

Pathology

Whereas rats do not show obvious signs or symptoms of poisoning on a ration containing less than 100 ppm of DDT, the inclusion of as little as 5 ppm in the diet over an extended period of time produces minimal but definite liver damage. This is reversible, if all DDT is removed from the diet. Liver necrosis may be preceded or accompanied by fatty degeneration or enlargement. Fatty changes are resistant to treatment with choline. While early stages of damage may be repaired, A. W. A. Brown states, "—but if further chronic doses prevent its regeneration, the detoxication mechanism of the liver for DDT is impaired."

The damage resulting from *severe poisoning* with the chlorinated carbons varies somewhat with the animal and the compound. However, few organs escape. Pathological changes reported include edema

112221

16732



of the brain and spinal cord, slight kidney damage, necrosis of the muscles, stomach and gall bladder, hemorrhages in the heart and gastrointestinal tract and degeneration of the endocrine glands. TDE has a unique tendency to cause atrophy of the adrenal gland.

Symptomatology

Acute DDT poisoning produces, in laboratory animals, a period of nervousness and hyperexcitability, with excessive blinking, cold skin, ruffled fur, lack of appetite and muscular weakness followed by the onset of fine tremors due to muscle fibrillation, particularly in the heart muscle, hind legs and back. Advanced poisoning leads to rapid weight loss, clonic convulsions, paralysis, and death. In addition to these, cattle and sheep develop staring eyes, diarrhea and staggering gait. Symptoms of mild chronic poisoning may include only nervousness and failure to gain weight normally.

Through 1951 a total of only 14 human fatalities due to DDT were reported. Most of these were the result of swallowing, accidentally or with suicidal intent, relatively large amounts of DDT dissolved in oil. A few severe reactions probably of allergic nature have been described. These included two deaths. Six cases of hemorrhagic purpura and one of agranulocytosis were recently reported.

Symptoms resulting from the accidental ingestion of *undissolved* DDT include nausea, vomiting, diarrhea, nervousness, sore throat, joint pains, muscular twitching, impairment of sight and insomnia. Recovery often takes place in a few days, but in one case took as long as twelve months.

Biskind from his observations has added the following: overwhelming fatigue and muscular weakness, peripheral neuritis, shooting pains, areas of skin hyperesthesia, sense of a lump in the throat, severe emotional depression and vague fear, and decreased vibratory sense.

It will be noted that many of these symptoms are typical of the so-called virus infections that have been widespread in the past five or six years.

Biskind has called attention to the fact that the symptoms described during an epidemic of "Virus X" in California several years ago corresponded almost 100% with those tabulated by him in cases of DDT toxicity. As a result of these observations some writers have quoted him as saying that the "Virus X" epidemic was the result of DDT poisoning. Biskind is emphatic in remarking that he "did not, and could not from the evidence at hand, make such a statement." While a virus believed to be responsible for this epidemic has been isolated, similarity of symptoms is conducive to much thought.

Evidence of Danger

At present the harmful effects of the chlorinated hydrocarbon pesticides on Mr. and Mrs. John Q. Public are debatable. The great majority of entomologists believe that residues on crops, in milk and eggs and meat are low enough to be harmless to humans, and that any possible toxicity is overbalanced by the necessity of these chemicals for the production of adequate food supplies. Their conclusions are open to question and I shall cite some evidence to the contrary.

Biskind, as one of the original physicians suspecting toxic effects from the new insecticides, has reported numbers of cases illustrating definite reactions to DDT, chlordan and BHC suggesting both allergic and direct toxic reactions from these compounds. While some afflictions were short-lived, others persisted for many weeks or months.

Pottenger noted that patients who reported gastrointestinal upsets, followed one or two weeks later by respiratory symptoms, often showed mild jaundice and evidence of liver damage. Fat biopsies analysed for DDT varied in content from 0 to 33 ppm. He felt the exhaustion and other associated symptoms could be attributed to exposure to DDT in food or household sprays. There was no question in his mind but that exposure to DDT could cause serious illness including one type of pneumonia.

Pottenger strongly recommends that any foods sprayed in the process of growing, preparation for market, or the final handling, should carry labels to that effect. Let the public know what it is buying.

Krohn and Pottenger in a medical paper awaiting publication, and included in their testimony before the Select Committee of the House of Representatives to Investigate the Use of Chemicals in Foods and Cosmetics, conclude as follows:

(1) The widespread use of chlorinated hydrocarbon insecticides has produced many cases of toxicity.

(2) These insecticides accumulate in fat and principally damage fat containing organs, as the liver and nervous tissue.

(3) In the presence of characteristic signs, symptoms and blood studies, detection of chlorinated hydrocarbon insecticides in a biopsy of the patient's fat confirms the diagnosis of insecticide poisoning.

(4) Chronic toxicity from these insecti-

112222

16733

cides has been observed more frequently than acute toxicity.

The writer has observed a small number of cases with negative fat biopsies but evidence of liver damage, that gave a significant history of exposure. He has also seen several cases of indisputable allergic reactions to these substances.

In other words, the presence of DDT in body fat proves that absorption of this chemical has taken place but does not necessarily indicate actual poisoning from that substance.

However the presence of liver damage favors such a supposition—particularly if more than 5 ppm of DDT is present in adipose tissue.

Contrariwise, an allergic or hypersensitive individual may theoretically exhibit violent reactions to minute amounts of DDT without absorbing measurable amounts. The only criterion necessary for diagnosis is the reproduction of symptoms upon repeated contact.

Sources of Exposure

There is adequate evidence to prove that many foods raised commercially contain at least traces of DDT or similar compounds. These include staple vegetables and fruits.

For all practical purposes DDT does not penetrate through the skin of fruits. However, it becomes incorporated in the skin or rind and can not be removed therefrom by any washing method known. In spite of the loss of nutrients present in this layer the only safe method of avoiding possible high contamination is to peel all fruit.

Beef, chicken, lamb and pork may contain relatively large amounts of DDT if the fodder of these animals was contaminated with the substance. The use of corn husks, pea vines and other heavily tainted sources of livestock feed renders this possibility a real one. Fatty portions of meat contain the largest quantities.

As has been mentioned before, milk, cream, butter and eggs may be serious offenders if contact with sprays or DDT containing food is adequate. Nor is margarine innocent. The vegetable fats in this product may be contaminated as a result of spraying or dusting.

It took many months before it was realized that dairy cattle sprayed for fly control with DDT or BHC in low dilution would excrete these substances in their milk for ten days or more. This even occurred when barns were fogged or whitewashed with DDT in spite of the fact that food bins were covered and the cattle removed. At the present time these chemicals are not recommended for use in dairies or on beef cattle. Instead, lindane and methoxychlor are suggested as substitutes.

Ironically enough, flies in most areas are becoming resistant to DDT. And what is more—once resistant to this chemical they rapidly seem to acquire immunity to others. Authorities therefore, are once again stressing the old methods of control —cleanliness and eradication of breeding places. And so we come full circle once more.

While translocation of DDT from the ground or foliage to the edible portion of plants seldom occurs, enough BHC and lindane may be so transported to give an off-flavor to potatoes and other vegetables. Therefore the use of these chemicals is decreasing.

Exposure to DDT in the home is all too common. Aerosol bombs for control of flies and fleas should be considered unsafe—as should chlordan powder or sprays for the destruction of ants and other pests. A case of poisoning from the latter compound was recently reported in the J.A.M.A.

The increasing use of lindane, DDT and similar compounds in restaurants and other public places by vaporization has been mentioned in a previous report and is attended by definite hazards.

Hypersusceptible individuals may incur damage from contact with outdoor sprays used domestically or commercially. They should be avoided.

Tentative Conclusions

The purpose of this article is not to create alarm but to alert thoughtful persons to the hazards inherent in the use of DDT and allied substances.

At the present time food production in the United States would be disrupted in a few weeks if the newer pesticides were discarded. This would be disastrous. However, the evidence herein presented points out inherent dangers which were not forseen, and if suspected were — and still are — ignored by many of those in responsible positions. More evidence of human toxicity is needed.

Your House Select Committee is doing a fine piece of investigation and deserves encouragement.

PART III

The Organic Phosphates

These compounds were developed in Germany during the past war for use as insecticides. Rumor has it that they may be the so-called "nerve gases" whose potentiality for harm brought bad dreams to our intelligence staff at that time. Be that as it may, these products are so toxic that they are limited to applications in the field by trained operators. Their use on livestock and pets is not recommended. (Department of Understatement.)

Physical and Chemical Properties

The organic phosphorus compounds are among the most toxic chemicals used for

112223

16734

pest control. Exposure to relatively small amounts may result in severe illness or death.

TEPP (or tetraethylpyrophosphate) is the most potent of the group from the acute standpoint. It is several times more toxic than nicotine. HETP (hetraethyltetraphosphate) is on a par with nicotine and owes most of its destructive potential to the percentage of TEPP present in the mixture. Both these compounds are rapidly hydrolysed and therefore dangerous for only a few days after application. They are also rapidly detoxified in the body and therefore not stored in the tissues.

Parathion is an ester of thiophosphoric acid and contains a sulfur radical. It is more slowly decomposed and as a result presents more of a hazard. It is also fat soluble to some extent.

Toxicology

There is little difference in susceptibility among various animal species to the toxic effects of these compounds. Several hundred human cases of poisoning have been reported with a number of fatalities—most of them due to carelessness in handling the products.

The organic phosphates are toxic by absorption through the skin, by inhalation and by ingestion. As little as one drop of concentrated solution in the eye has caused death. Whereas TEPP and HETP have failed to show cumulative effects, animal experiments have shown that repeated contact with small amounts of parathion may produce poisoning.

It has been estimated that 25 mg. of TEPP by mouth will produce moderately severe symptoms in man. 100 mg. orally would probably be fatal as would 500 mg. applied to the skin.

The alkyl phosphates inhibit the enzyme cholinesterase. This enzyme destroys acetylcholine, one of the chemicals liberated at nerve junctions and endings to transmit the nerve impulse. Thus a lack of the enzyme results in a marked prolongation of each nerve impulse affecting primarily the parasympathetic part of the nervous system. Nicotine-like effects are also produced.

On acute exposure symptoms develop rapidly once the cholinesterase level has been sufficiently lowered. They include loss of appetite, nausea, vomiting, abdominal cramps, excessive sweating, pupillary constriction, diarrhea, respiratory difficulty, headache, dizziness, muscle twitching and weakness. Severe cases develop coma, convulsions and death.

Repeated exposure to small doses may result in illness of severe degree. The enzyme system recovers fairly rapidly after one episode, but may take weeks after numerous subclinical contacts. Testing a blood sample for cholinesterase levels may help in diagnosis. Sedatives and the intravenous administration of atropine in doses of 1 to 2 mg. as often as every hour may be life saving in severe cases of poisoning. Death may occur within six to ten hours of the development of symptoms. Recovery from acute attacks is usually complete. Adequate protective clothing and special masks must be used by those handling these chemicals.

TEPP and HETP present no problem. Fruit pickers have become ill after entering an orchard treated with parathion several days after its application. Food crops are not supposed to be sprayed within thirty days of harvest. A safe residue on any one item of diet is considered to be 2 ppm of parathion. In the case of citrus fruit the peel will absorb and retain higher residues than are allowable. This is also true of other fruits. As regards apples and pears, if parathion is applied—"strictly in accordance with the recommendations of the Bureau of Entomology & Plant Quarantine of the U. S. Dept. of Agriculture with particular reference to the time between the last spraying and the harvesting of the fruit, normal weathering should result in parathion residue no greater than a fraction of a part per million."

Nevertheless toxic parathion residues have been reported by Pottenger in bread and canned fruit. Again the human equation enters the picture.

Susceptibility to the organic phosphates is variable as one would expect. In cases of suspected poisoning a physician should always be called. Blood tests may uncover chronic cases.

Absorption and Translocation

TEPP is rapidly absorbed into plant tissues as shown by growth inhibition or stimulation and other metabolic disturbances. The rapid breakdown of these compounds limits the importance of their absorption to the effect on plants.

Parathion has been reported to kill a variety of insects on plants when applied only to the soil. Biochemical studies and bioassay have shown that absorption and translocation does take place in insecticidal amounts. The relative stability of this chemical together with its toxicity makes these findings of great importance to animals and humans. Chemically altered parathion has been found in some citrus fruits. The toxicity has not yet been reported.

Systemic Insecticides

These are chemicals absorbed and translocated by actively growing plants in sufficient amounts to kill insects feeding at a site distant from the original application. The principal compounds of this group are "OMPA" (octamethylpyrophos-

112224

16735

phoramide), "Systox" (diethyl ethylmercaptoethylthiophosphate), triphosphoric acid penta dimethylamide and the bis fluorophosphine oxides.

After absorption the chemicals may be changed to less or more toxic compounds. Injury to the plant is possible. Translocation to edible portions may make them toxic or produce a tainted flavor. At the present time the use of these systemic pesticides on food crops is not permitted. They are still in the experimental stage and should always remain so as far as food for man or animal is concerned.

It should be stated once more that in addition to the systemics just described, evidence of absorption and translocation exists for arsenical compounds, parathion, benzene hexachloride, DDT, toxaphene, chlordane, dieldrin, selenium compounds, fluoroacetates, and fluorohydrins. The last four groups have been considered too toxic for use on food crops, although dieldrin has recently been released for use on peaches in California. Benzene hexachloride (as reported in Modern Nutrition for October) may no longer be applied to vegetables or fruits in this state. However, where it has been used in the past the soil may remain contaminated to a decreasing extent for five years or more.

It has taken a long time for the authorities to take such a step and now treated ground will not be free from traces of the chemical for eight or ten years more. In the meantime the population has been exposed to the effects of BHC for a period of four or five years. This is but one example of the price the public is paying for the use of new chemicals in and on our food without adequate investigation of possible harmful effects from these poisons.

Practical Aspects

By this time those who have followed these articles should be considerably alarmed over the number of poisons human beings in this atomic age must encounter. And rightly so! It is a deadly and grim situation. Naturally the question now arises—so what? Where do we go from here? What can I do to protect my family, myself and our wonderful Nation from what seems to be serious harm?

Unfortunately there is no pat answer. However, I am going to discuss steps which seem logical at the present time.

Legislative Action

While your government representatives in local, state and federal capacities realize there is a problem and wish to protect the public, they are under pressure from pesticide manufacturers and distributors whose chief interest naturally is in sales. These groups refuse to believe that exposure to small amounts of insecticides constitutes a great threat to public health.

Entomologists being primarily responsible for the production of large crops to feed a hungry world are loth to accept proof of chronic toxicity—and such proof in humans is difficult to obtain. (There is plenty of evidence of such potential hazard from animal experiments.) Meanwhile Departments of Agriculture are busy conducting investigations along lines of toxicity for crops, animals and man.

You are the guinea pigs and must write your state and federal representatives asking them to take action. You must also contact your local departments of health along the same lines. The other side of the question must be heard from.

Personal Precautions

In an attempt to avoid contaminated foods one must not go off the deep end and develop malnutrition in the process. Many foods from large truck farms where spraying directions are carefully followed and supervised contain no detectable residues. However, a certain number of crops, because of ignorance, willful neglect of advice, drift from nearby fields or other factors show slight or moderate and occasionally, heavy contamination. Surveys in the Los Angeles area in 1950 showed detectable residues of DDT in more than half the samples examined. About four percent were heavy. Arsenic contamination was negligible. This risk is unpredictable and at the present time cannot be prevented. The following suggestions may be helpful:

1. Whenever possible, purchase foods that have been grown without the use of toxic insecticides. At present the supply of such foods is markedly limited. Encourage the development of organic farms in your area. Grow your own vegetables.

2. Do not use sprays or aerosols in your house that contain DDT, Chlordane, lindane, BHC or methoxychlor. Make sure that commercial exterminating companies do not use these chemicals in your house or on your trees and shrubs. Do not eat in restaurants using vaporizers for fly control.

3. Peel all fruits and vegetables that lend themselves to such treatment. Scrape the outside layer from celery. Washing will not remove residues. Purchase eggs from local ranches that are not using DDT, BHC or chlordane in hen houses. (Methoxychlor and lindane are not cumulative and do not ordinarily build up in chicken tissues, hence in eggs.)

Milk, cream and butter are much safer than formerly. Most dairies now use methoxychlor and lindane for fly control and very little is excreted in the milk. Check with your local dairy to find out what chemicals they use. Shift to another dairy if yours is still using DDT, BHC or chlordane or dieldrin by the "strip" method.

112225

16736

Most vegetables growing in the ground such as potatoes, carrots and beets are comparatively safe. Contamination by BHC will produce an oil flavor and thus give warning. Potato skins may be eaten.

Fruits and vegetables that have at intervals shown contamination with DDT in the Los Angeles area are celery, cabbage, cauliflower, greens (spinach, parsley, endive, turnip, etc.) and lettuce. Apples and tomatoes are included, but quite safe when peeled. In case of stomach upsets it would be wise to avoid these vegetables for a few weeks.

Dried fruits may be higher in DDT than fresh fruit and they cannot be peeled. Some olives and grapes are suspect. Prunes show very little residue as a result of the alkali treatment given them.

Moderate amounts of salads greens are fairly safe and desirable food stuffs. Home sprouted soy beans, mung beans, alfalfa and the like from untreated seeds provide an excellent source of uncontaminated and nutritious salads.

Lean meats are satisfactory sources of protein and sea foods are naturally free of any residues.

4. Attention to other factors affecting the general health is important. Adequate rest, avoidance of worry and tension, moderation in the use of coffee, alcohol and tobacco, sufficient exercise and a diet low in refined carbohydrates need emphasis. Vitamin supplements such as yeast or vegex, dessicated liver, rice bran concentrates, fresh wheat germ, cod liver oil or its concentrates and kelp for trace minerals are helpful. More potent preparations are often indicated but should preferably be taken only under medical supervision.

Allergic states and chronic infection of any sort interfere with adequate metabolism and should be corrected if possible. Naturally other ailments should receive the necessary care.

5. Repeated attacks consisting of upper respiratory infections and/or gastrointestinal symptoms together with loss of weight and marked fatigue suggest pesticide toxicity or allergy. In any case of this sort see your physician. Other causes may be operative. If he finds nothing wrong suggest the possibility of insecticide poisoning and ask for a blood count and liver function tests. Do not try to diagnose and treat yourself. You are almost bound to be mistaken and to do more harm than good.

In definite cases of DDT poisoning a strict diet together with other nutritional therapy will be necessary. Recovery is often discouragingly slow but to be expected once the cause has been recognized.

Summary and Conclusions

The various types of chemicals used for the control of insect pests have been described together with their potential toxicity for man and animals. The fact that some of the chlorinated hydrocarbons, even when absorbed in trace amounts, may be concentrated at least thirty fold in the body fat of animals and human beings, renders even slight contamination of our foods a public health problem, of first importance. Such contamination is present and in susceptible individuals is producing illness. This fact must be faced and steps taken to protect the public interest. The Delaney Congressional Committee has been investigating the problem and will recommend legislation if this seems needed. The Committee needs the support of all.

Suggestions have been made by means of which the public may reduce the intake of insecticides to a minimum. It is impossible to avoid all contact.

If the use of insecticides were suddenly stopped crop failure and hunger would be widespread. Perhaps the solution may lie in the following steps:

(1) Fairly rapid abandonment of the more toxic pesticides in favor of less harmful ones.

(2) The development of widespread pest control by natural enemies. Excellent research on this subject is being carried on at the Riverside Experimental Station.

(3) As Pottenger has suggested, foods that have been sprayed or dusted should be so labelled including the insecticides used. The public has the right to know what it is buying.

(4) Testing of food crops before marketing. While costly it might be cheaper in the end, to test for BHC, DDT, arsenic, lead and parathion, where any or all of these have been used on food crops before the crop is sent to market. To allow for differences in concentration and other factors, a sample from each acre might be indicated. Thus heavily contaminated crops would be discovered before reaching the consumer.

(5) The passage of State or National laws condemning the manufacture of aerosols, sprays or vaporizers containing DDT, chlordane ,lindane or methoxychlor for use in homes or public places. These chemicals should be used only outdoors and then with extreme caution.

(6) The gradual spread of organic farming methods including the use of city wastes for fertilizer purposes. The work of E. Pfeiffer along the lines of special cultures to produce a rapid breakdown of organic material shows much promise. In spite of a great deal of skepticism, an increasing number of farmers and soil experts believe that healthy plants are more

112226

16737

resistant to insect pests and that healthy plants can only grow in rich soil. It is probable that the future of America and, indeed of the world, depends upon the rebuilding of our impoverished soil through the addition of organic matter, trace minerals and judiciously used commercial fertilizer—not upon the development of newer and more powerful insecticides.

2901 Wilshire Blvd., Suite 345

References

BISKIND, M. S.: Statement before the Select Committee to Investigate The Use of Chemicals in Food Products (Dec. 12) 1950.

BISKIND, M. S. and Irving Bieber: Am. Jl. Psychotherapy, III, No. 2 (April) 1949.

BROMFIELD, Louis: Statement before the Select Committee to Investigate the Use of Chemicals in Food Products (May) 1951.

BROWN, A. W. A.: Insect Control by Chemicals. 770 p., New York: John Wiley & Sons, Inc., 1951.

CASIDA, J. E. and T. C. ALLEN: Absorption and Translocation of insecticides by plants. Agricult. Chem., Vol. 7, No. 6 (June) 1952.

COUNCIL on PHARMACY AND CHEMISTRY: J.A.M.A., 146:367 (May 24) 1951.

COUNCIL on PHARMACY AND CHEMISTRY: Pharmacology and toxicology of certain phosphorus insecticides. J.A.M.A., 144:104-108 (Sept. 9) 1950.

DENDY, John: Statement before the Select Committee to Investigate the Use of Chemicals in Food Products (May) 1951.

EDITORIAL DEPT. J.A.M.A.: Insecticide storage in adipose tissue, 145: 735-736 (Mar. 10) 1951.

HOSKINS, W. M.: Deposit and residue of recent insecticides resulting from various control practices in California. Journ. Economic Entomol., 42:966, 1950.

KNIGHT, G. F.: Testimony before the House Select Committee to Investigate the Use of Chemicals in Foods and Cosmetics. Part 2, 1951.

KROHN, Bernard: Statement before the House Select Committee to Investigate the Use of Chemicals in Food Products. Part 2, 1951.

LANG, E. P., F. M. KUNZE and C. S. PRICKETT: Occurrence of DDT in human fat and milk. A.M.A. Archives Ind. Hyg. & Occ. Med., 3:245-246 (Mar.) 1951.

LEHMAN, A. J.: Pharmacological consideration of insecticides. J.A.M.A., 144:104-108 (Sept.) 1950.

LEHMAN, A. J.: Bulletin of the Association of Food and Drug Officials of the United States. Vol. XIV, No. 3 (July) 1950.

LEHMAN, A. J.: The Major toxic actions of insecticides. Bulletin N. Y. Acad. Med., 25:382-387 (June) 1949.

LEHMAN, A. J.: Some toxicological reasons why certain chemicals may or may not be permitted as food additives. Bull. Assn. Food and Drug Officials of U. S. Vol. 14, No. 3 (July) 1950.

LEMMON, Allen B., Chief, Bureau of Chem., Annual report for the calendar year 1950. The Bulletin, Dept. Agricult., State of California, Vol. 34, No. 4, 1950.

METCALF, R. L.: The Mode of Action of Organic Insecticides. Nat. Research Council, Wash., D. C., 1948.

POTTENGER, F.M. Jr., Testimony before the House Select Committee to Investigate the Use of Chemicals in Foods and Cosmetics, Part 2, 1951.

ROHWER, S. A. and H. L. Haller: Pharmacology and toxicity of certain phosphorus insecticides. J.A.M.A., 144:104-108 (Sept. 9) 1950.

Select Committee to Investigate the Use of Chemicals in Food Products: Report No. 3254, 81st Congress, 2nd Session, House of Representatives, Union Calendar No. 1139 (Jan. 3) 1951.

SIMMONS, S. W.: Health hazards of Economic poisons and related substances. Am. J. Trop. Med., Vol. 31, No. 4 (July) 1951.

ADDENDUM

In reprinting this article, which first appeared 20 years ago, it seems logical to briefly review what has happened since that time.

Chemical contamination of our environment has become obvious. DDT and other chlorinated hydrocarbon pesticides have unexpectedly been spread by wind currents thoughout the world: They have penetrated our food chains and are now found in high concentrations in some fish and seals, both in the Arctic and Antarctic. Many of our rivers are sewers and some of our lakes are dying as a result of changes caused by chemical effluents. Our coastal waters are so badly contaminated that in some areas many fish have been found with cancerous growths. As a result of DDT, the brown pelican may soon be extinct and other birds are seriously threatened.

Human beings carry in their fatty tissues about 12 parts per million of DDT or its degradation products. Many individuals have measurable residues of other chlorinated hydrocarbons, including the phenoxy herbicides and the polychlorinated biphenyls (PCBs). Degenerative diseases, including cancer, are on the increase.

Studies by the Bionetics Laboratories in 1965 — but not released until 1969 — showed that the phenoxy herbicides, 2,4–D, 2,4,5–T, 2,4–DP and Silvex not only produced birth defects but cancer in experimental mice. So did DDT and related chlorinated hydrocarbons, as well as other herbicides such as the Triazines, Paraquat and Zectran. *It is of considerable concern that DDT and 2,4-D have been used on our food crops since about 1945* and that 2,4-D, 2,4,5–T, Silvex and other herbicides have been available for use on lawns also during this period. Since 2,4,5–T and Silvex are contaminated by the 2,3,7,8 tetrachlorodibenzo-para-dioxins, *which, in animal experiments, have proven to be up to one million times more active than thalidomide in producing deformed young, contamination by these herbicides may be much more serious than suspected.* Incidentally, the toxic effects of the tetrachlorodibenzo-p-dioxins were extensively documented by M. Jacqueline Verrett, Ph.D., Division of Toxicology, F.D.A.,

112224

16738

in April of 1970. Obviously, the results of animal experiments cannot be necessarily applied to human beings. Nevertheless, the results are of great importance since minute amounts of carcinogenic chemicals may, at a much later date, produce cancer in susceptible individuals or animals. According to the Delaney Amendment to the Food, Drug and Cosmetic Act of 1945, any chemicals which produce cancer in animals must be banned from foods or cosmetics.

Phenoxy herbicides ,even though in trace amounts, now contaminate about thirty percent of our water supplies and a recent report indicates that the ubiquitous PCBs have been detected in fifteen to twenty percent of our drinking water. Such contamination may occur from local applications, effluents of various types, drift from aerial spraying, transport by winds from other parts of the country, or by as yet unknown means.

"Agent Orange," a mixture of equal parts of 2,4–D and 2,4,5–T, was used for many years as a defoliant in South Vietnam. This chemical weapon may have irreversibly destroyed the balance of life in the estuaries, as well as ruining as much as thirty percent of their hardwood forests. Not many individuals are aware of the fact that "Agent Orange," contaminated by the fantastically potent tetrachlorodibenzo-p-dioxins, was used for many years in our own forests before it was sprayed as a defoliant in Vietnam. Since it was banned as too dangerous in Vietnam in 1970, why has its use not been forbidden anywhere in the United States?

Mr. Ruckleshaus, Director of the Environmental Protection Agency, is attempting to limit the use of dangerous chemicals, such as DDT, 2,4,5–T, Silvex and similar chlorinated hydrocarbons, in order to protect our environment. He needs support. Powerful interests are opposing this move. Proof of definite damage is now at hand through the repeated pesticide spraying of Globe, Arizona, and the resulting extensive research of one of the victims — Billee Shoecraft — whose book "Sue the Bastards!" provides a cogent and moving account of her experiences. In the Globe area, plants and crops were destroyed; domestic and wild animals died or disappeared ;human beings and animals became ill; birth deformities were noted in animals, as well as human beings. Reproductive difficulties affected women and some of them long past the menopause — much to their consternation — bled from the uterus. Recurrent attacks of "virus" were frequent.

In 1971, the Bureau of Reclamation used Silvex on the Rio Grande River near San Acacia, about sixty miles south of Albuquerque, in an attempt to clear salt-bush from the river banks. The Bureau felt that this was using too much water. Without prior warning, helicopters sprayed the area, contaminating in the process members of two families and two herds of cattle. Both the human beings and the cattle became ill and many of the latter died. The remainder of the herds, even though they had been removed from the area and were eating voraciously, lost weight and had to be sold at a tremendous loss. Post-mortem examinations indicated damage to the liver and intestines, as well as to other internal organs. Thus, we know that Silvex can not only cause serious illness in cattle, but death as well, if the exposure is great enough. Fortunately, the human beings recovered.

2,4–D is contaminated by some of the dioxins and other noxious compounds. A recent study, reported to the French Academy of Sciences, involving the use of pheasant eggs injected with 2,4–D, revealed startling findings: Hatchability was reduced by about 70 percent and numerous embryos were deformed.

Most alarming was the finding that in fifty percent of the embryos, "the genital tract of the male embryo resembled that of a normal female." The authors state, "It is important to note that even the vapors of 2, 4-D have an analogous action . . . When non-treated eggs were put in treated nests, the embryos show the same sexual anomalies." Conclusion: "It is a question of feminization or physiological castration." (Are human beings exempt from this influence?)

Disturbing reports of cancer developing in Swedish workers using 2,4–D and 2,4,5–T along railroad rights-of-way and of deformed babies being born to two women in Australia exposed to 2,4,5–T, have apparently had no influence on those who are using these compounds in our forests and on our ranges.

Californians should know that for the past twenty years, 2,4–D, 2,4,5–T, 2,4–DB and/or Silvex have been, and still are being used in various combinations in our national forests up and down the coast, in order to control weed growth along firebreaks and power lines, as well as to destroy brush in planned brush conversion areas. Whether spraying is done by helicopter or ground crews, drift is inevitable and this may account for the fact that many Californians are showing the presence of these herbicides in their blood. Whereas herbicides are being used along state highways, the application of 2,4,5–T and Silvex have not been authorized by the State of California for three years. Compounds being used include Simazine and smaller amounts of Atrazine and Paraquat. It is, therefore, probable that most of the phenoxy herbicide contamination is the result of air-borne residues. This needs more study.

The extremely high incidence of virus pneumonitis in experimental animals exposed to pesticides during the Bionetics Studies for teratogenicity and carcinogenicity is highly significant.

In the past few months, an increasing number of illnesses seem to be associated with herbicide spraying in the Los Angeles area. These so-called "virus infections" tend to recur in waves and are extremely debilitating. Half a dozen patients have said they

112228

16739

felt they were dying. These occurrences are extremely disturbing.

Some specimens of beef contain residues of the synthetic estrogen, diethylstilbestrol, which has cancer-inducing properties, even in minute amounts. Meat, and particularly liver from these creatures, may also contain residues of the phenoxy herbicides and other chlorinated pesticides. Chickens supplied with food containing stilbestrol, of course, may also exhibit residues in their meat and livers. Just recently, the use of stilbestrol in animal feeds has been banned. However, pellets may still be implanted subcutaneously in animals, and whereas the residues will probably be less, there is no proof that some will not remain.

Lindane vaporizers are being phased out but are still being advertised and sold. "No Pest" strips may be just as dangerous as the vaporizers. (Two monkeys who chewed on their flea collars containing dichlorvos, the active ingredient of many "strips," were reported as suffering from repeated attacks of virus pneumonia together with other complications. One of them died and the other recovered after a prolonged illness.)

Hexachlorophene sponge baths for infants were recently found to cause serious poisoning and a number of deaths. A three percent solution of hexachlorophene had been used and it is now known that the scrotal area absorbs practically 100 percent of most compounds applied to it. Hexachlorophene has also been shown to contain other toxic contaminants, such as the dibenzodioxins. Hexachlorophene has now been banned for use in drugs and cosmetics, except on prescription. This is another example of long delay in recognizing toxic effects from the chlorinated hydrocarbons.

Last year, liquid formulations of 2,4,5–T were banned, together with any applications near water supplies, campgrounds, etc. On June 14, 1972, with a few exceptions, the use of DDT in the United States was ordered discontinued by the Environmental Protection Agency as of December 31, 1972. This is as it should be. If it sticks!

I suspect that recurrent, so-called "virus infections" which have appeared with increasing frequency over the years and which produce symptoms such as nausea, vomiting, diarrhea, prostration, severe headache, body pains, depression, sweats, low grade fever, sinus congestion, sore throat, laryngitis, hoarseness, feeling of constriction under the breastbone, "virus pneumonia," and severe pains in various parts of the body may all be due to contact with pesticides in our air, food and water, either acting as virus per se, or lowering resistance to viruses. (Morton Biskind, M.D., Westport, Connecticut, speculated on this fact 20 years ago.)

The suggestions in my original article are still valid. However, since the subject of mercury has surfaced, swordfish were found to be higher in mercury content than other fish. They were, therefore, banned temporarily and are now carefully screened so that swordfish appearing on the shelves is fairly safe if one does not eat it more than once weekly. Examination of museum specimens of various types of fish has shown the presence of mercury in those which were twenty, thirty or forty years old. This indicates that the importance of mercury has not yet been completely evaluated.

Lead contaminates the air of our cities, as do fluorides, nitrous oxides, etc. A good intake of Vitamin C helps to reduce lead toxicity. Vitamins A and E, together with C, help to minimize smog damage to the lungs. Liver, yeast and B-complex in addition to these vitamins and a good diet high in raw, uncontaminated foods grown in fertile soil, constitute logical nutritional insurance against infections and the development of degenerative diseases including cancer.

Obviously, resistance to toxic chemicals is related to active enzyme chemistry which, in turn, depends upon good nutrition and avoidance, insofar as that is possible, of all chemical compounds which are enzyme poisons.

Fluorides are now one of the most serious contaminants of our ecosystem. They are producing allergic and toxic reactions. Therefore, the endless drive by the U.S. Public Health Service and the American Dental Association — backed by our tax dollars — to promote artificial fluoridation of water supplies, seems not only stupid and foolhardy but dictatorial.

Unfortunately, other toxic herbicides, aside from the phenoxy herbicides are still being used for weed control on vacant lots and along highways and freeways in Los Angeles County, as well as in agricultural areas. These include Diquat, Paraquat, Atrazine and Simazine. (Paraquat possesses the unique quality of causing lung damage when it is inhaled; swallowed, or applied to the skin in sufficient quantity.)

Research funds are urgently needed to monitor foods, water, plants, and people for the presence of organic phosphates, herbicides and other chlorinated pesticides as well as lead, arsenic and mercury. Twenty thousand dollars is needed for an adequate investigation. Any donation will help to keep the work moving until larger gifts are received. Checks made payable to the Price-Pottenger Nutrition Foundation, labeled for "research purposes and forwarded to me, will be most welcome. Such donations are deductible for income tax purposes and will be acknowledged. We need your help!

Once again, I would suggest that you look for sources of uncontaminated foods; grow vegetables in your own backyard; make your opinions felt by writing your legislators. If our civilization is to survive, the use of potent and persistent pesticides must be reduced to an absolute minimum; mineral and organic matter returned to our soils and food grown for "quality" rather than "quantity," as recommended many years ago by W. A. Albrecht, Ph.D., Chairman, Dept. of Soils at the University of Missouri.

112229

Your future and that of your family depend upon your actions. Whereas individual activity is important, group action is more effective. Southern Californians should contact and join an American Nutrition Society chapter. There are chapters in Pasadena, San Fernando Valley, Los Angeles, Pomona and South Bay at Palos Verdes.

Whereas special situations may require the use of pesticides and the judicious use of artificial fertilizers, the widespread, indiscriminate use of these substances must be controlled if our food supply and health are to be preserved.

"Verbum sapienti satis est."

REFERENCES

HARMER, R. M.: *Unfit for Human Consumption*, 374 pp., New Jersey: Prentice-Hall, Inc., 1971, $6.95

HUNTER, B. T.: *Consumer Beware*, 440 pp., New York: Simon and Shuster, 1971, $8.95

MILLER, M. W. & G. G. Berg: *Chemical Fallout*, 301 pp., Springfield: Chas. C. Thomas, 1969, $12.85

SHOECRAFT, B.: *"Sue the Bastards!"* 460 pp., Phoenix: Franklin Press, 2436 E. Indian School Rd., 1971, $4.45.

DOW 112230