Revised copy for review only. BAS 14 July 78

1061

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DOW 798843

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EVALUATION OF THE MUTAGENIC POTENTIAL OF 2,4,5-T AND TCDD IN RESPONSE TO A RPAR ON 2,4,5-T

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Under the ammended Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the basic environmental standard for major regulatory determinations is "unreasonable adverse effects on the environment". The term is defined in Section 2 (bb) of the act to mean "any unreasonable risk to man or the environment taking into account the economic, social, and environmental costs and benefits of the use of any pesticide". The Environmental Protection Agency's registration guidelines establish the data requirements for registration of a pesticide. Sections 162.84-1 to 162.84-5 of these guidelines establish the requirements for testing to establish the safety of compounds for mutagenic effects in order to register a compound. In making the determination that a rebuttable presumption has arisen, the registration regulations require the agency to take into account the type of effect, the statistical significance of the findings, and whether the tests were conducted in accordance with the material requirements for valid tests as recognized by experts in the field. Section 162.3 (y) of the registration regulations defines the term "mutagenic" as "the property of a substance or mixture of substances to induce changes in the genetic complement of either somatic or germinal tissue in subsequent generations". An important aspect of this definition is that the EPA considers the demonstration

DOW98845

of heritable change as fundamental to identifying a compound as mutagenic and of potential concern for the health of humans.

Multi-test evidence is necessary for assessing the mutagenic potential of a test substance since it is well recognized that it is unlikely that a single test system is capable of assessing all potential types of genetic change with sufficient sensitivity and, in addition, incorporate the toxicological concerns relevant to mammalian physiology and metabolism, as well.

Many new test systems have evolved in recent years or are in the process of being developed presently, especially in the area of in vitro short-term tests to evaluate mutagenic and carcinogenic potential. Nearly all of these tests suffer from lack of validation between laboratories and across different classes of chemicals. Until a satisfactory degree of reliability has been demonstrated for these tests, it does not seem appropriate to use the results of such tests for making critical regulatory decisions. For example, in regard to the microbial tests in particular, consideration must be given to the fact that it is impossible to simulate the route of exposure encountered by man in these tests, liver microsomes are not an accurate reflection of

DOW798846

the integrated metabolic processees of an intact animal or even of an intact liver, such test systems circumvent the natural ability of the intact animal to deal with foreign substances through disposal of the material or its metabolites, and the influence of dose level upon response is not interpretable. Therefore, the usefulness of these test systems even for the process of defining the needs for further testing has significant limitations.

DOW 79884?

The Environmental Protection Agency has issued a rebuttable presumption against registration (RPAR) of 2,4,5-T on the basis of two triggers, oncogenicity and teratogenicity/feto-toxicity (1). They concluded that there were insufficient data on the mutagenic potential of 2,4,5-T and TCDD, and that further testing was needed. Mutagenicity was not a trigger for the RPAR.

The results of the laboratory tests to assess the mutagenic potential of 2,4,5-T and TCDD are summarized in Tables 1 and 2, respectively. Our review of the literature revealed considerable additional information to that included in the RPAR Position Document 1.

TESTS IN NON-MAMMALIAN SYSTEMS

Negative results were reported by Anderson et al. (2) in spot tests with salmonella; ten different phenoxyalkanoic acids and five commercial formulations containing these compounds did not increase the frequency of back mutations. A group of 110 different herbicides was evaluated for their ability to induce point mutations in one or more of four different microbial systems using histidine-requiring mutants of Salmonella typhimurium. The amount of 2,4,5-T used in the test plates was not indicated.

Division of Pesticide Chemistry, 30 August ... entitled "Mutagenesis Induced in Mutant Strains of Salmonella typhimurium by Pesticides" by Ercegovich and Rashid (3). 2,4,5-T was found to be "doubtfully mutagenic only after activation". In the absence of enzyme activation, 2,4,5-T was not mutagenic in any of the five strains of organisms (TA-100, TA-1535, TA-98, TA-1537, or TA-1538). In the presence of enzyme activation, there was no increase in the number of revertants in the frameshift strains (TA-98, TA-1537, or TA-1538); the increase in the number of revertants in the base substitution strains (TA-100 or TA-1535) was less than two-fold. This increase of less than a two-fold magnitude was referred to by the authors as a doubtful mutagenic effect; increases less than two-fold are generally not considered to represent a positive response in this test.

Seibert and Lemperle (4) tested 32 herbicides for their ability to induce mitotic gene conversion in a diploid strain of the yeast, Saccharomyces cerevisiae. The culture medium contained 1000 ppm 2,4,5-T; no evidence of mutagenic activity was found for 2,4,5-T. Shirasu and co-workers (5) conducted tests with the REC-assay procedure using H17 REC^+ and M45 REC^- strains of Bacillus subtilis. The "dose" of 2,4,5-T was 20 µg/plate; no evidence of mutagenicity was found.

as an *in vitro* cytogenetics test using cultured human lymphocytes. In all cases, no evidence of mutagenicity was observed with 2,4,5-T. The methodology employed was not described in sufficient depth to critically review the procedures used. Another study in *Saccharomyces cerevisiae* RAD18 has recently been reported by Zetterberg (7). 2,4,5-T was found to be mutagenic at an acidic pH but not in solutions with neutral pH. 2,4,5-T concentrations in the range of 0.02-0.06 mg/ml were evaluated over a range of pH values. The authors concluded that while 2,4,5-T caused mutations in acidic conditions, it was unlikely that mutations would be induced at neutral pH, such as would be found in gametes. Cells would apparently not take up the dissociated form of 2,4,5-T.

Numerous mutagenicity studies have been conducted in the fruitfly, *Drosophila melanogaster*. In a study by Davring and Sunner (8) chromosomal defects in oocytes and nurse cells of Drosophila females were reported at very low concentrations of a commercial formulation of 2,4,5-T butoxyethyl ester. The significance of these observations

250 ppm in the medium provided for the flies; Magnusson gave 2,4,5-T at a concentration of 1000 ppm.

Several sex-linked recessive lethal studies have been reported in Drosophila. In a study reported by Mujumdar and co-workers (10), Drosophila were given 2,4,5-T at concentrations of 250 or 1000 ppm in their diet. A slight increase in sex-linked recessive lethals was found at the high concentration, 1000 ppm. In contrast to these positive findings, Vogel and Chandler (11) found no effect on the incidence of sex-linked recessive lethals in Drosophila given 2,4,5-T at concentrations of approximately 1000 and 2000 ppm in their diet. As already indicated above, Magnusson (9) found an increase in the incidence of direct recessive lethals in Drosophila given 1000 ppm 2,4,5-T in their diet. This was not accompanied by cytogenetic changes in the insects.

strain which was unstable for the white locus, which depends on introduction of foreign DNA in the white region; the system is based on forward mutations in the inserted DNA. There was no evidence of mutagenicity at 100 ppm of 2,4,5-T.

TESTS IN MAMMALIAN SYSTEMS

In addition to the above tests in non-mammalian systems, numerous tests have also been conducted in mammalian systems. Dominant lethal tests were performed in mice by Buselmaier (13) by injecting 100 mg 2,4,5-T/kg intraperitoneally. The males were subsequently mated during six weeks after treatment with untreated females in order to assess all stages of spermatogenesis. There was no indication of an increased pre- or postimplantation loss. Buselmaier (13) also tested for point mutations in the host-mediated assay using Salmonella typhimurium and Serratia marcescens. 2,4,5-T caused no effect at a dose level of 500 mg/kg. Thus, 2,4,5-T was found to be negative in both the dominant lethal test and the host-mediated assay.

erythrocytes from the mouse bone marrow. In another host-mediated assay by Styles (15), serum from rats treated with 2,4,5-T was used with *his* mutants of Salmonella typhimurium. No increase in the number of mutants was observed.

The results of several cytogenetic studies appear in the 2,4,5-T literature. In a study by Yefimenko (16) rats were administered 2,4,5-T at dose levels of 0.001-1 mg/kg. Chromatid aberrations and chromosome adhesions were reported after a dose of only 0.01 mg/kg of the butylester of 2,4,5-T to male rats. This interpretation is based on the information contained in an English abstract and a translation of the original article from the Russian literature. In a study translated from the Japanese literature (Fujita, 17), the effect of 2,4,5-T on cultured human lymphocytes is reported. The concentrations of 2,4,5-T ranged from 0.025-25 µg/ml. Chromosome and chromatid aberrations were reported, but as mentioned in the RPAR position document 1, it is not possible to distinguish whether this was a toxic effect or a potential genetic effect of 2,4,5-T.

An in vivo cytogenetics study was reported by Majumdar and Hall (18) in which gerbils were given 2,4,5-T at dose levels of 10-100 mg/kg/day. Aberrations of chromosomes and chromatids were observed which may have been related to toxic effects rather than mutagenic changes. The judgment of the authors in their classification of aberrations has been challenged (Ramel, 19) and leaves the value of the data reported in this paper highly questionable. Another in vivo cytogenetics study was reported by Davring and Hultgren (20) in which mice were dosed with a commercial 2,4,5-T ester formulation. Chromosomal aberrations were observed in the mouse bone marrow cells in this study but close examination of the data reveals that the effects may well be caused by the solvent and/or emulsifier used in the herbicide preparation and not by the 2,4,5-T itself. The same preparation was used in Davring's experiments in Drosophila (8).

In addition to these published reports on studies of the mutagenic potential of 2,4,5-T, several additional pieces of unpublished data are included. An abstract published by Babbitt and co-workers (21) is attached regarding studies of the effects of 2,4,5-T on two human cell lines - spleen fibroblasts and lymphocytes. No data were included in the published abstract.

potential, the results of such studies should not be [illegible] in the overall assessment of mutagenic potential. In the case of 2,4,5-T, a study was conducted at the Dow Chemical Company in which Sprague-Dawley rats were maintained on diets providing dose levels of 2,4,5-T of 3, 10, and 30 mg/kg/day for three successive generations (22). Effects were not seen in this reproduction study which were suggestive of mutagenic effects of 2,4,5-T. If 2,4,5-T were a potent mutagen which caused changes which were transmissable to subsequent generations, a reproduction study would reveal the effects of a potent mutagen. The results of this reproduction study, considered together with the rest of the data from all of the mutagenesis studies, indicate that 2,4,5-T is not a [redacted] mutagen. [redacted].

The results of the mutagenicity studies on TCDD are summarized in Table 2. Hussain and co-workers (23) reported that TCDD was mutagenic by causing intercalation in bacterial systems. An increased frequency of mutations was found in E. coli Sd-4. No effects were seen in Salmonella typhimurium TA-1530, but increased mutation frequencies were seen in strain TA-1532, a

University of California at Berkeley, to Dr. Philip Kearney, U.S.D.A., dated 11 March 1974 contains additional information on the effect of TCDD in the Salmonella tester strains developed by Ames (24). No evidence of mutagenic effects was observed in these tests.

Two different mammalian tests have indicated a lack of mutagenic potential for TCDD. Khera and Ruddick (25) reported the results of a dominant lethal study in Wistar rats that were dosed with 4 or 8 µg TCDD/kg/day for 7 consecutive days. The male rats were caged with untreated virgin females for 5 days during 7 sequential mating trials after dosage with TCDD. The dose levels administered were clearly toxic, since 11 of 20 and 2 of 20 male rats died after receiving 8 or 4 µg/kg/day, respectively. No dominant lethal effects were observed. An in vivo cytogenetics study in rats has been reported by Green and Moreland (26). In their study, rats were given 10 µg TCDD/kg orally five times in one week; additional groups of rats were given 5, 10, 15 or 20 µg TCDD/kg as a single dose intraperitoneally. Examination of the bone marrow of these rats revealed no increase in the incidence of chromosomal aberrations relative to control rats. Another unpublished study by Green is mentioned in a

TCDD/kg twice weekly for 13 weeks. An increase in the number of chromosome breaks occurred at dose levels of 2 and 4 µg/kg in male rats and at 4 µg/kg in female rats compared to the frequency of chromosome breaks at the lowest dose level. The results of this latter study are not available for review and inclusion in this response.

The results of the mutagenesis tests on TCDD indicate that under some test conditions, this chemical may be mutagenic in microbes. In whole animal mammalian tests, TCDD has not been found to be mutagenic in the reports of studies that have been available for review. The recent data of Sidney Green's representing rats which were dosed on a subchronic basis with TCDD will need to be examined before a more definitive answer can be given regarding the mutagenic potential of TCDD in mammalian systems.

Based upon the definition by EPA of the term "mutagenic" (the property of a substance or mixture of substances to induce changes in the genetic complement of either somatic or germinal tissue in subsequent generations), the results of studies of the mutagenic potential of 2,4,5-T do not indicate that this herbicide represents a mutagenic hazard.

demonstrated.

CONCLUSION

In conclusion, numerous studies have been conducted to evaluate the mutagenic potential of 2,4,5-T and TCDD in nonmammalian and mammalian test systems. None of the studies in microbial organisms indicate a significant mutagenic effect for 2,4,5-T. One study in yeast indicated mutagenic changes at acidic pH's but not at a neutral pH as found in mammalian tissues. Positive and negative results were seen in studies in Drosophila. Tests in whole-animal mammalian systems have either indicated negative results (dominant lethal test, host-mediated assay and micronucleus test), or uninterpretable results (cytogenetic studies). Thus, 2,4,5-T should not be considered a mutagen, and might be classified, at most, as a weak mutagen in some test systems.

Additional data from one laboratory which have not been published are reported to indicate a positive cytogenetic change in rats dosed subchronically with TCDD. The final evaluation of the mutagenic potential of TCDD should take these data into account when they become available.

Saccharomyces cerevisiae D_4	1000 ppm	-	32 Herbicides were tested	1	Not listed
Bacillus subtilis	20 µg/place	-	166 Pesticides were tested	5	Not listed
E. coli (5-MT resistance)	?	-			
E. coli (Gal R^3 strain)		-			
Serratia marcescens (a 21 and a 742 strains)	?	-	31 Pesticides were tested, very little description of methods	6	Not listed
E. coli (strep. resistance)	?	-			
Saccharomyces cerevisiae	?	-			
In Vitro Cytogenetics (human lymphocytes)	?	-			
Saccharomyces cerevisiae RAD 18	0.02-0.06 mg/ml	+	Mutations only at pH < 4.5; mutations unlikely at neutral pH, as in gametes	7	Not listed
Drosophila melanogaster (effect on oogenesis)	250 ppm in diet	+	50% Decrease in fertility at 250 ppm chromosome effects seen; unsubstantiated in other tests	3	172
Drosophila melanogaster (sex-linked recessive lethal)	250, 1000 ppm in diet	+	Slight increase in sex-linked recessive lethals at 1000 ppm	10	178
Drosophila melanogaster (sex-linked recessive lethal)	3.6, 7.2 mM (0.92, 1.84 mg/ml)	-	No effect on sex-linked recessive lethals	11	Not listed
Drosophila melanogaster (In vivo cytogenetics)	1000 ppm in diet	-	Same sample used by Davring and Sunner (1971)	9	Not listed
Drosophila melanogaster (direct recessive lethal)	1000 ppm in diet	+	-	9	Not listed
Drosophila melanogaster (somatic mutations)	25-200 ppm in diet	-	Tested in genetically stable and unstable strains	12	Not listed
Host-Mediated Assay and Dominant Lethal (mice)	100 mg/kg IP	-	-	13	Not listed
Micronucleus test (mouse)	100 mg/kg	-	-	14	Not listed
Host-Mediated Assay (salmonella typhimurium)	?	-	Used serum from rats dosed with 2,4,5-T (abstract)	15	Not listed
In Vivo Cytogenetics (rats)	0.001-1 mg/kg	+	Abstract and translation only	16	151
In Vitro Cytogenetics (human lymphocytes)	10^{-7} -10^{-4}M) (0.025-25 µg/ml)	+?	Not possible to tell if effect was mutagenic or toxic	17	149
In Vivo Cytogenetics (gerbils)	10-100 mg/kg/day	+ at 70 & 100 mg/kg	Questionable classification of aberrations	18	169
In Vivo Cytogenetics (mice)	Not stated	+	Effect may have been due to solvent or emulsifier	20	171

TABLE 2

Summary - Laboratory Tests For Mutagenicity of TCDD

Test System	Dose(s) Tested	Results	Comments	Reference Number	RPAR Reference Number
TCDD: Non-Mammalian Mutagenic Tests					
Ames Test and E. Coli	up to 4.0 µg/plate	+	-	23	24
Ames Test	Not stated	-	A memo to U.S.D.A.	24	Not listed
TCDD: Mammalian Mutagenic Tests					
Dominant Lethal (rats)	0, 4, 8 µg/kg	-	High dose level lethal to some of the males	25	6
In Vivo Cytogenetic (rats)	0, 5, 10, 15, 20 µg/kg	-	Abstract plus text of oral presentation	26	119

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DOW 798861

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798862

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17330

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798863

DOW 798864

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OW 798865

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1062
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RESPONSE BY THE DOW CHEMICAL COMPANY TO THE ENVIRONMENTAL PROTECTION AGENCY'S DECISION AND EMERGENCY ORDER SUSPENDING REGISTRATIONS FOR CERTAIN USES OF 2,4,5-TRICHLOROPHENOXY ACETIC ACID (2,4,5-T) AND 2-(2,4,5-TRICHLOROPHENOXY) PROPIONIC ACID (SILVEX) IN A NOTICE TO REGISTRANTS DATED FEBRUARY 28, 1979.

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DOW 798840

The Environmental Protection Agency (EPA) banned certain uses of 2,4,5-T and Silvex on February 28, 1979. This action was taken on the basis of reported miscarriages in certain areas of Oregon. The EPA concluded a causal relationship between the occurrence of these miscarriages and spraying of forests with 2,4,5-T. The Dow Chemical Company believes that such a conclusion is totally unjustifiable for numerous reasons, the most important of which are summarized as follows.

1) Inadequate data are reported to support EPA's conclusions. The critical data needed to substantiate their conclusion (and therefore their action) are neither in the report nor available from EPA.

2) The data are uninterpretable because of sampling problems. EPA did not even measure the spontaneous abortion rates. Significant differences in medical care between the three areas make it impossible to draw conclusions because comparable data were not collected in each area. In addition, the data were collected by two different teams of investigators, a practice which can easily bias results.

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3) EPA's evaluation of the data would more appropriately be called manipulation of numbers to support a preconceived bias. A more objective evaluation showed no cause and effect relationships.

4) EPA's conclusion is in marked contrast with findings in other human populations throughout the world that have been exposed to 2,4,5-T and TCDD. Exposure of people to sufficient TCDD to cause non-reproductive toxicity (chloracne) did not cause malformations, abortions, stillbirths, or chromosomal damage.

5) Measurements made on animals and humans exposed to 2,4,5-T indicate that the maximum amount of 2,4,5-T and TCDD which could possibly have been absorbed, if any, by the residents of Alsea, Oregon, would be far too low to be of any toxicological significance.

A more in-depth critique of EPA's report is included in the two attached documents entitled:

1. Critique of "Report of Assessment of a Field Investigation of Six-Year Spontaneous Abortion Rates in Three Oregon Areas in Relation to Forest 2,4,5-T Spray Practices".

2. Analysis of EPA Decision to Declare an Emergency Suspension of 2,4,5-T as Related to Studies in Animals.

DOW 798842

TABLE 4

Summary - Laboratory Tests For Mutagenicity of 2,4,5-T

Test System	Dose(s) Tested	Results	Positive Mutagen Employed	Comments	Reference
2,4,5-T: Non-Mammalian Mutagenic Tests					
Ames Test	Not stated	-	Yes	110 Herbicides were tested	Andersen, K. J. et al. 1972 J. Agr. Food Sci. 20, 649
Ames Test	4.9 µg/plate	+	Yes	Doubtfully mutagenic after activation	Ercegovich, C. D. and Rashid, K. A., 1977 (unpublished)
Drosophila	250 ppm	+	No	50% Decrease in fertility at 250 ppm chromosome effects seen	Davring and Summer, 1971 Hereditas. 68, 115
Drosophila	0, 250, 1000 ppm	+	Yes	Slight increase in sex-linked recessive lethals	Mujumdar, S. K. and Golia, J. K., 1974 Can. J. Genet. Cytol. 16, 465
2,4,5-T: Mammalian Mutagenic Tests					
Host-Mediated Assay and Dominant Lethal (mice)	500-1000 mg/kg IP	-	No	-	Buselmaier, Von W. et al. 1972 Biol. Zbl. 91, 311
In Vitro Cytogenetics (human lymphocytes)	10^{-7}-10^{-4}M	?+	No	Not possible to tell if effect was mutagenic or toxic	Fujita et al. 1975 J. Jap. Assoc. Rural Med. 24, 77
In Vivo Cytogenetics (gerbils)	10-100 mg/kg/day	+ at 70 & 100 mg/kg	No	-	Mujumdar, S. K. and Hall, R. C., 1973 J. Heredity 64, 213
In Vivo Cytogenetics (mice)	Not stated	+	?	-	Davring and Hultgren, 1977 Hereditas. 85, 123-134

DOW 798822

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DOW 798823

TABLE A-3 (Continued)

Drug	Safety Factor	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference Number
Chlordane	5	Rat	Dietary	1000 ppm	-	-	-	30
Diazinon	21	Rabbit	Oral	7 mg/kg	-	-	-	31
Phaltan	470	Rabbit	Oral	80 mg/kg	-	-	-	32
Imidan	20	Rabbit	Oral	35 mg/kg	-	-	-	32
Paraquat	10	Mice	Oral	20 mg/kg	-	-	-	33

*Based on the no-effect dose level in teratology studies.

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Drug
Maneb
Zineb
Carbaryl
Thiram
Ethylene Thiourea (ETU)
Malathion
Trinzine
Captan
Dinoseb
Mirex
Dimethoate
2,4-D

CIHS

TABLE A-3

Safety Factors For Selected Pesticides*

Safety Factor	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observati
1,852	Rat	Oral	0.5 g/kg	-	1 g/kg	Exencepially Phocomelia
392	Rat	Oral	1.0 g/kg	-	2 g/kg	Exencepially Phocomelia
47	G. Pig, Hamster	Oral	125 mg/kg	250 mg/kg	300 mg/kg	Cervical ve fused
543	Hamster	Oral	125 mg/kg	-	250 mg/kg	Skeletal d
Not Calculable	Rat	Oral	-	-	10 mg/kg	Hydrocepha neural tu
0.8	Rat	Oral	105 mg/kg	-	205 mg/kg	Hydronephr
Not Calculable	Rat	?	-	-	30 mg/kg	Cleft pala absent ma
223	Rabbit	Oral	38 mg/kg	75 mg/kg	-	Resorptio
4	Mice	Oral	20 mg/kg	32 mg/kg	-	Decreased length
Not Calculable	Rat	Dietary	-	5 ppm	-	Decreased size
Not Calculable	Mice	Water	-	60 ppm	-	Decreased survival
2.5	Rat	Oral	25 mg/kg	50 mg/kg	-	Subcutan minor sk effects

TABLE A-2

Safety Factors For Selected Industrial Chemicals*

Drug	No-Effect Dose TLV	TLV	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference Number
Vinyl Chloride	50	1 ppm	Mice	Inhal.	50 ppm	500 ppm	-	Skeletal variants	13
Acrylonitrile	2	20 ppm	Rats	Inhal.	40 ppm		80 ppm	Malf. - variety	14
Methyl Ethyl Ketone	<5	200 ppm	Rat	Inhal.	<1000	1000 ppm	-	Skeletal variants	15
Chloroform	<3	10 ppm	Rats	Inhal.	<30	30 ppm	-	Skeletal variants	16
1,1-Dichloromethane	19	200 ppm	Rats	Inhal.	3800	6000	-	Skeletal variants	15
Carbon Tetrachloride	<30	10 ppm	Rats	Inhal.	<300	300	-	Skeletal variants	15
Pentachlorophenol	5	0.5 mg/m^3	Rats	PO	5 mg/kg	15 mg/kg	-	Skeletal variants	17
Captan	38	5 mg/m^3	Rabbit	PO	37.5 mg/kg		75	Malformations	18
2,4,5-T	10	10 mg/m^3	Mice	Oral	20				22

*Based on the no-effect dose level in teratology studies.

DOW 798825

TABLE 5

Summary - Laboratory Tests For Mutagenicity of TCDD

Test System	Dose(s) Tested	Results	Positive Mutagen Employed	Comments
TCDD: Non-Mammalian Mutagenic Tests				
Ames Test and E. Coli	up to 4.0 µg/plate	+	No	-
Ames Test	Not stated	-	Yes	A memo to USDA
TCDD: Mammalian Mutagenic Tests				
Dominant Lethal (rats)	0, 4, 8 µg/kg	-	No	Highest dose level lethal to some of the males
In Vivo Cytogenetic (rats)	0, 5, 10, 15, 20 µg/kg	-	No	Abstract only
In Vivo Cytogenetics (rats)	0.001 - 1 mg/kg	+	No	Abstract and translation only

DOW 798826

Reference

Hussain, S. et al. 1972
Ambio. 1, 32-33

Ames, B. N., 1974
(unpublished)

Khera, K. S. and Ruddick, J. A., 1973
Advances in Chemistry 120, 70-84.

Green, S., 1975
Toxicol. Appl. Pharmacol. 33, 161

Yefimenko, L. P., 1974
Gig. Tr. Prof. Zabol. 18, 24-27.

DOW 798827

Dominant Lethal tests conducted at extremely high dose levels have shown negative results. This is a significant observation, especially when considered in conjunction with the results of other tests that have been conducted on these compounds.

also reported on a Dominant Lethal test with 2,4,5-T in NMRI mice at a dose level of 1,000 mg/kg administered intraperitoneally, with no indication of a dominant lethal effect.

DOW 7988828

One additional piece of unpublished data is attached, consisting of a communication from Bruce Ames, Professor of Biochemistry, University of California at Berkeley, to Dr. Philip Kearney, USDA, dated 11 March 1974. Dr. Ames has evaluated the mutagenicity of TCDD in his tester strains of Salmonella and has not found positive responses.

An abstract published by Babbitt et al. is also attached regarding work in progress on the effect of 2,4,5-T on two human cell lines, spleen fibroblasts and lymphocytes. No data are included in this abstract.

As indicated in the Document prepared by the Working Group on 2,4,5-T, there are significant limitations in the majority of the reports cited on mutagenicity studies on 2,4,5-T and TCDD. Considered collectively, the results of the tests do not indicate that either chemical is a potent mutagen. Generally, the results from in vivo mammalian tests which assess germ cell mutation are the most relevant for predicting risks to humans. With each of these materials, the

DOW 798829

The EPA has concluded that there are insufficient data on the mutagenic potential of 2,4,5-T and TCDD and that further testing is needed. The results of the laboratory tests to assess the mutagenic potential of 2,4,5-T and TCDD are summarized in Tables 4 and 5, respectively. Our review of the literature revealed very little additional information to that included in the RPAR Document No. 1 on 2,4,5-T. Two additional references on mutagenicity studies on 2,4,5-T are submitted to supplement the review by the EPA. A paper by Andersen et al. (1972) is included which was an evaluation of 110 herbicides for their ability to induce point mutations in four different microbial systems. None of the herbicides, including 2,4,5-T, caused point mutations in 8 strains of Salmonella typhimurium as well as the T4 bacteriophage and E. coli.

The second paper is by Buselmaier et al. (1972) who evaluated a number of pesticides, including 2,4,5-T, in the Host-Mediated assay and the Dominant Lethal test in mice. The Host-Mediated assay was conducted in NMRI mice using mutants of the bacteria Salmonella typhimurium and Serratia marcescens introduced into the mice by intraperitoneal injection. No mutagenesis was found at 2,4,5-T dose levels of 500 mg/kg, and doses of the n-butyl ester of 2,4,5-T of 1,000 mg/kg. These investigators

DOW 798800

RPAR OF 2,4,5-T: TERATOGENICITY/FETOTOXICITY AS A TRIGGER

Summary The Environmental Protection Agency has issued a rebuttable presumption against registration (RPAR) of 2,4,5-T on the basis of two triggers, oncogenicity and teratogenicity. Regarding the latter, the EPA has concluded that an ample margin of safety does not exist for the population at risk (women of childbearing age) for dermal and inhalation exposure and for cumulative oral, dermal, and inhalation exposure to both 2,4,5-T and TCDD. Dow contends that the risk is negligible to the developing embryo and fetus from the proper use of products containing 2,4,5-T. The teratogenic potential of 2,4,5-T and TCDD given to laboratory animals alone or in combination has been extensively investigated. In fact, few if any other pesticides, drugs, or industrial chemicals have been challenged to the extent that 2,4,5-T and TCDD have been studied. TCDD is teratogenic in mice but not rats. 2,4,5-T is teratogenic in mice but not rats, rabbits, hamsters, cattle, or primates at dose levels below one which produces maternal toxicity. Years of experience conducting teratology studies have taught us that mice are highly susceptible to any form of stress, including airplane travel, high temperatures, or high doses of materials as common as sugar, salt,

DOW 79S501

and vitamins. Positive results in mice but not in other species of laboratory animals is generally not considered sufficient evidence to identify a chemical as a significant teratologic risk for humans.

Studies in laboratory animals have shown that the presence of TCDD in 2,4,5-T products does not contribute to their teratogenic potential. In fact, TCDD must be present at concentrations many times greater than the present specifications will permit before the toxicity of TCDD becomes detectable. Thus, measures which protect against toxicity from 2,4,5-T are sufficient to protect against TCDD as well.

Whether or not there is an ample margin of safety for any chemical depends on many factors, including the toxicity of the chemical, the likelihood of over-exposure, and the effectiveness of control measures to prevent exposure. The EPA has used exaggerated figures for calculating the exposure level of applicators to 2,4,5-T products. This tends to decrease the apparent margin of safety. In the real world, the margin of safety for 2,4,5-T is at least as great as it is for other pesticides, drugs, or industrial chemicals which are not considered to pose a significant hazard to the population at risk.

DOW7983003

teratogenic effect is very often an acute exposure at a critical organogenetic period to an agent such as a chemical, infectious organism, or some form of energy. Chronic exposure is certainly not needed to cause a teratogenic effect. Teratogenicity is not dependent upon delayed toxic effects. The observation of the result of the insult might well be delayed on the basis of the duration of gestation, but the injury is coincident with the time of dosage. Therefore, I assume that this Toxicologic endpoint was classified as other chronic or delayed toxic effects simply as a matter of convenience rather than for a scientific reason.

On page 66 of the document, the statement is made that a rebuttable presumption shall arise if chronic chemical exposure in test animals produces injury for the reproductive system and/or the fetus. This is inconsistent with the widely accepted principle of teratogenesis which states that teratogenic effects are related in a very time specific manner to dosage with an agent which results in an adverse effect. This point is important because the margins of safety that are considered appropriate for oncogenic effects are based on the assumption that animals or humans will be exposed to the potential oncogen on a chronic basis. The margin of safety for teratogenic potential must take into account

DOW 198804

that this insult may be incurred on the basis of an acute exposure and chronic exposure is not necessary or may not be sufficient.

Throughout the document, the majority of references to studies in the literature ignore the fact that the chemicals were given only on certain days of gestation. The dose levels used in the studies are defined on a mg/kg basis; in teratologic studies, it is critical to indicate that these dose levels are on a mg/kg/day basis on specified days of gestation only. Dosage in the majority of the studies ranged from a single day to periods of time greater than the duration of major organogenesis. Since the days of major organogenesis vary between species, it is critical to define the number of days of gestation during which the administration of chemicals is conducted.

It is unfortunate that no attempt was made to standardize the terms used to describe the developmental variations observed in the reports. Such terms as "anomalies, malformations, fetotoxic, teratogenic, embryotoxic, deformities" were used in an apparent interchangable manner, usually quoting directly the phrases used by the authors in their reports. Admittedly, definitions vary between laboratories but perhaps the data could be made less inconsistent between laboratories if an effort

DOW 798805

was made to standardize terms in reviewing literature. Another short-coming of the review is that all data appeared to be given equal weight and validity in the eyes of the Working Group. No judgement was made on the quality of the studies, which in some cases varied considerably from what would be considered an appropriate experimental design. In cases where studies have significant limitations based on experimental design, these short-comings should have been pointed out and taken into consideration in interpreting the results of the study and accepting the interpretations of the authors. For example, one of the studies cited was written by Sokolik, I. (1973; RPAR reference no. 131). The value of the information presented in this report is questionable compared to many of the other reports for several reasons. There are insufficient data presented to allow the reader to draw conclusions independent from those stated by the author; the amount of control data included is negligible; the number of animals per group is not stated; the author concludes that the teratogenic action of the compound is due to the presence of "dioxide" (I assume he means dioxin) but he does not specify the level of dioxin in the sample of 2,4,5-T; no reference is made to the maternal toxicity which surely must have been existent at the dose levels utilized; there is no indication of the number of fetuses examined or the number of resorptions observed in the litters. In addition, the findings in this study are

DOW 79S806

inconsistent with those found with the trichlorophenoxyacetic acids in numerous other studies in rats. Because of all of these limitations, it is difficult to give the same credence to the results of this study as to the other studies which were conducted in a more appropriate manner.

Another study was cited in a manner which precluded comparison of the data to the results of other studies cited. In the paper by Courtney (1977; RPAR reference no. 134, correct reference is Arch. Environm. Contam. Toxicol. 6, 33-46, 1977), the dose levels were expressed on a millimolar basis, thus requiring conversion to a weight basis for comparison to all other data. The conversion is as follows: 1.00 mM/kg = 255.5 mg/kg, 0.80 mM/kg = 204.4 mg/kg, and 0.45 mM/kg = 115.0 mg/kg. The number of pregnant mice per experimental group in this study was very small. Half of the 2,4,5-T - groups consisted of only 5-6 litters; a significant proportion of the fetuses from these few litters was resorbed, leaving very few fetuses upon which to make a scientific judgement.

The Working Group was also fairly selective in citing conclusions by authors. For example, in the study reported by Collins and Williams (1971; RPAR reference no. 124), the conclusion of the authors is included, that 2,4,5-T is teratogenic in the hamster, but no mention is made of the

DOW 798807

author's conclusion that based on these data, there is no indication that 2,4,5-T represents a hazard to humans. Within this report, the authors state that "there is a wide margin of safety between the exposure levels of 2,4,5-T permitted by registered uses existing prior to April, 1970, on food crops and the levels producing teratologic changes in the hamsters used here. Since April, these uses have been cancelled so that the amount of 2,4,5-T now in use has still further decreased the possible hazard to humans". Such conclusions by authors should not have been ignored by the Working Group.

The results of reproduction and teratology studies should have been kept separate and distinct from each other. Since teratologic studies are acute in design and reproduction studies are designed to evaluate the effect of chronic exposure to chemicals, the findings of the two different kinds of studies cannot be equated. For example, on page 79 of the document, reference is made to "preliminary data from Dow (110) indicate that TCDD may have effects at 0.01 µg/kg in rats". This statement is in reference to the results of a reproduction study but is included in the middle of a paragraph describing the results of teratologic studies. Such statements should be amended to indicate that it is in reference to a three-generation reproduction study to avoid the possible confusion between the two types of studies.

DOW 738808

Overview on Teratogenicity of 2,4,5-T and TCDD:

The risk to the developing embryo and fetus of humans from the proper use of products containing 2,4,5-T is negligible. The teratogenic potential of 2,4,5-T and TCDD given to laboratory animals alone or in combination has been extensively investigated. In fact, few if any other pesticides, drugs, or industrial chemicals have been evaluated to the extent that 2,4,5-T and TCDD have been studied. A large number of teratology studies have been conducted using a variety of laboratory and non-laboratory animal species. Studies have been reported on mice, rats, hamsters, rabbits, sheep, Rhesus monkeys, and reindeer, using samples of 2,4,5-T with varying degrees of purity. The content of the contaminant TCDD ranged from <0.01 ppm to approximately 45 ppm. In certain studies, 2,4,5-T and TCDD were administered simultaneously, using dose levels of 50 to 100 mg/kg/day of 2,4,5-T in combination with 0.01 to 3 µg/kg/day of TCDD. Considered overall, the studies indicate that purified 2,4,5-T is teratogenic in mice; dose levels of 45 mg 2,4,5-T/kg/day or higher were generally associated with cleft palate and/or kidney malformations in mice. Studies conducted at the National Center for Toxicological Research (Highman et al. 1977, RPAR reference No. 45) indicate that the reported fetal "cystic kidneys" in offspring of mice given 2,4,5-T were due to a retardation

DOW 788809

sub-human primates at dose levels below one which produced maternal toxicity. Such a data set would generally not be considered sufficient evidence to identify a chemical as a significant teratologic risk for humans.

Regarding the effects of 2,4,5-T and TCDD in combination, studies in three different species of animals have indicated that a TCDD level in 2,4,5-T of 1 ppm or less does not enhance or potentiate the toxic effect of 2,4,5-T. In a teratology study conducted here at The Dow Chemical Company (unpublished data) the effect of 2,4,5-T alone was compared to the effect of 2,4,5-T administered in combination with TCDD to Sprague-Dawley rats. Purified 2,4,5-T was administered on days 6 through 15 of gestation at one dose level, 50 mg/kg/day. In addition, these rats were dosed with 0.01, 0.03, 0.06, 0.125, 0.5, or 1 µg TCDD/kg/day. Both materials were administered orally. Hemorrhage in the intestineal wall was observed at dose levels of 0.06 µg/kg/day TCDD and higher. Cleft palates were observed at 0.5 and 1 µg/kg/day given in combination with 50 mg/kg/day of 2,4,5-T. Therefore, at a dose level of 50 mg of 2,4,5-T/kg/day, 2,4,5-T would have to contain more than 1 ppm TCDD to cause intestinal hemorrhage and would require more than 10 ppm to cause a teratogenic effect, that is, cleft palate. A copy of this reported data is attached.

DOW 798810

in fetal renal development and down-growth of the renal papilla into the pelvis of the kidney. Thus, 2,4,5-T is not a renal teratogen in mice but simply retards the normal development of the fetal kidney. Cleft palate, then, is the one malformation in mice which is relatable to dosage with 2,4,5-T. Cleft palate is the most easily induced malformation in most strains of mice, being induced by most forms of stress including such things as airplane travel, high temperatures, or high dose levels of materials as common as sugar, salt, and vitamins (Golberg, 1971, Fd. Cosmet. Toxicol. 9, 65-80). 2,4,5-T was not found to be teratogenic in other species of mammals. The one study in rats in which malformations were found is a study which is difficult to interpret because of the lack of information included in the report (Sokolik, 1973, RPAR reference No. 131). Even at high dose levels, administration of 2,4,5-T to mammalian species other than the mouse was associated primarily with adverse effects on the development of the skeletal structures, changes which reflect retarded development rather than malformations and are very likely associated with the stress of being given large doses of 2,4,5-T rather than the direct action of the agent with the genetic material of the embryo or fetus. Collectively, 2,4,5-T has caused cleft palate in some strains of mice and is not teratogenic in six other species of animals, including

Courtney and Moore (1971; RPAR reference No. 128), conducted a study in which mice were dosed with 100 mg/kg/day of analytical grade 2,4,5-T plus 1 µg/kg/day of TCDD. The additional TCDD did not potentiate the effect of 2,4,5-T given alone; thus, a dose equivalent to 2,4,5-T containing 10 ppm TCDD was no more toxic to the developing embryo and fetus of mice than 2,4,5-T with less than 0.05 ppm of TCDD.

DOW 798811

In hamsters, Collins and Williams (1971; RPAR reference No. 124), studied the effect of 2,4,5-T containing various levels of TCDD. No fetal abnormalities were observed at dose levels of 2,4,5-T at or below 80 mg/kg/day. Among groups of hamsters dosed with 2,4,5-T containing 0.1, 0.5, 2.9 and 45 ppm of TCDD, only the highest TCDD level (45 ppm) clearly increased the toxicity of 2,4,5-T to hamster embryos and fetuses.

In another teratology study in mice, Neubert and Dillman (1972, RPAR reference No. 127) studied the teratogenic effects of 2,4,5-T in mice along with TCDD. A potentiation of the teratogenic effect of 2,4,5-T and TCDD (based on the incidence of cleft palate) was observed when teratogenic doses of one substance were combined with a threshold dose of the other. However, for such potentiation to occur, more than 1.5 ppm TCDD was required. The authors concluded that a TCDD content of less than 1 ppm did not cause such an effect.

DOW 798812

TCDD is teratogenic in mice but not in rats. As with 2,4,5-T, the manifestations in mice include abnormalities of the kidney and cleft palate. In rats, the primary effect observed was death of the embryos or fetuses rather than malformations. The no-effect dose levels of TCDD, as noted in the RPAR document No. 1, are such that TCDD does not contribute to the potential hazards of 2,4,5-T.

In summary, numerous studies in laboratory and non-laboratory animals have indicated that the risk is negligible to the developing embryo and fetus from the proper use of products containing 2,4,5-T. In addition, the presence of TCDD in 2,4,5-T does not contribute to the teratogenic potential of 2,4,5-T. TCDD must be present at concentrations many times greater than the present specifications will permit before the toxicity of TCDD becomes detectable.

Comments On The Margin Of Safety Of 2,4,5-T:

The margin of safety for any chemical depends on numerous factors, including the inherent toxicity of a chemical, the likelihood of over-exposure and the effectiveness of control measures to prevent exposure. The hazard associated with exposure to a chemical is a function of inherent toxicity and the amount of exposure. As already discussed in the preceeding section, 2,4,5-T and TCDD are not considered to be

potent teratogens across species. TCDD is embryotoxic and fetotoxic at very low dose levels in laboratory animals but the exposure of humans is such that the hazard is considered negligible.

DOW 798813

The EPA has estimated the amount of exposure of applicators using 2,4,5-T products. Their estimates of exposure are based on assumptions and exaggerated figures which are not supportable by actual observations on people spraying 2,4,5-T. Based on pharmacokinetic data in humans and the excretion of 2,4,5-T by spray applicators, an estimate of the actual dose of 2,4,5-T has been calculated and is presented elsewhere in this rebuttal. Based on those calculations, the lowest margin of safety under any condition of use of 2,4,5-T products is greater than 50. This is considerably greater than the margins of safety calculated by the EPA. Their use of exaggerated figures for calculating exposure tended to decrease the apparent margin of safety.

In reality, the margin of safety for 2,4,5-T is at least as great as it is for many other drugs, industrial chemicals, or pesticides which are not considered to pose a significant hazard to the population at risk. For example, the information summarized in Table 1 includes calculated safety factors for selected drugs. Many of the drugs, including ones which

DOW 798814

are routinely taken by pregnant women, have margins of safety somewhat lower than 50. For example, based on a teratology study, the margin of safety for aspirin is only 3; on the basis of results of a reproduction study, the margin of safety is less than 2. The margin of safety for meclizine, a drug commonly used to treat the symptoms of morning sickness during pregnancy, is only 12.

The safety factors for a number of chemicals commonly encountered in the industrial workplace are summarized in Table 2. Again, the margins of safety for this group of widely encountered industrial chemicals are all in the range of 50 or less. A Threshold Limit Value (TLV) has been established for each of these chemicals and defines what is accepted to be a safe level of exposure for a 40 hr work-week. The TLV for 2,4,5-T is 10 mg/m^3 of air; based on the tidal volume of humans doing light work the margin of safety is 10. Thus, the branches of government which regulate the exposure limits for chemicals in the workplace consider a margin of safety of 10 to be sufficient for 2,4,5-T.

The safety factors for a number of pesticides is summarized in Table 3. Pesticides were selected on the basis of the use of the compound and the availability of teratologic data. In searching for references on these chemicals, it was obvious that in no case was there as much teratologic data

Positive results in mice but not in other species of laboratory animals is generally not considered sufficient evidence to identify a chemical as a significant teratologic risk for humans.

DOW 798815

Studies in laboratory animals have shown that the presence of TCDD in 2,4,5-T does not contribute to the teratologic potential of 2,4,5-T. In fact, TCDD must be present at concentrations many times greater than present specifications will permit before the toxicity of TCDD becomes detectable. Thus, measures which protect against toxicity from 2,4,5-T are sufficient to protect against TCDD as well.

Whether or not there is an ample margin of safety for any chemical depends on numerous factors including the inherent toxicity of a chemical, the likelihood of over-exposure, and the effectiveness of control measures to prevent exposure. By using exaggerated figures for calculating the exposure level of applicators to 2,4,5-T products, the EPA has estimated margins of safety which are somewhat lower than the margins which actually exist under use conditions. Based on data collected on spray applicators, the margin of safety under real world conditions exceeded a factor of 50. This is at least as great as the margin of safety for many other drugs, industrial chemicals, or pesticides to which women are exposed during pregnancy which are not considered to pose a significant hazard to the population at risk. Thus, 2,4,5-T

DOW 798816

Conclusions:

The EPA has issued a rebuttable presumption against reregistration of 2,4,5-T on the basis of two triggers, oncogenicity and teratogenicity. Regarding the latter, the EPA has concluded that an ample margin of safety does not exist for the population at risk (women of childbearing age) for dermal and inhalation exposure and for cumulative oral, dermal, and inhalation exposure to both 2,4,5-T and TCDD.

The hazard associated with the use of any chemical is a function of the inherent toxicity of the chemical and the amount of exposure. In the case of 2,4,5-T, the risk to the developing embryo and fetus from the proper use of products containing 2,4,5-T is negligible. The teratogenic potential of 2,4,5-T and TCDD given to laboratory animals alone or in combination has been extensively investigated. In fact, few if any other pesticides, drugs, or industrial chemicals have been evaluated to the extent that 2,4,5-T and TCDD have been studied. TCDD is teratogenic in mice but not rats. 2,4,5-T is teratogenic in mice but not rats, rabbits, hamsters, cattle, or primates at dose levels below one that produces maternal toxicity. The evidence of teratogenicity in mice is limited to cleft palate in certain strains of mice, a finding which is shared by many other chemicals or other forms of stress including such things as airplane travel, high temperatures, or high doses of materials as common as sugar, salt, and vitamins.

OCW 798817

as for 2,4,5-T. In some cases, the published data on these chemicals is limited to studies in chick embryos. The safety factors were calculated using the same assumptions as were used by the EPA for 2,4,5-T regarding the amount of exposure and the absorption through skin. The directions on the labels of these pesticides were used to calculate the dose level to the user of the pesticide. Approximately half of the materials have a safety factor of less than 50. The lowest safety factor, less than 1, was found for malathion.

The information contained in Tables 1 through 3 indicate that margins of safety in the range of 5 to 50 are not uncommon for a large number of common chemicals to which women of childbearing age are routinely exposed. This includes widely used drugs, chemicals in the work environment, as well as pesticides. In the case of 2,4,5-T, the margin of safety under work conditions with no special precautions exceeds a factor of 50. This far exceeds the margin of safety for many chemicals to which the population at risk is commonly exposed. Thus, in addition to 2,4,5-T and TCDD not being potent teratogens, the margin of safety based on level of exposure also indicates that there is negligible risk to users of 2,4,5-T products.

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and TCDD are not considered to pose a significant risk to the developing embryo and fetus under conditions of use of 2,4,5-T products.

DOW 798138

DOW V88819

TABLE 1

Safety Factors For Selected Drugs

Drug	Safety Factor*
Dilantin (anticonvulsant)	20
Valium (tranquilizer)	170
Phenobarbital (anticonvulsant)	10
Meclizine (antihistamine)	12
Norethindrone (progestin)	50
Norethynodrel (progestin)	4
Vitamin A	40
Acetazolamide (diuretic)	7
Morphine (analgesic)	666
Aspirin: teratology -	3
reproduction -	<2
Acetaminophen (analgesic): reproduction -	<6

*The no-effect dose level in teratology studies divided by the therapeutic dose for humans. See Appendix Table A-1 for further data on the drugs listed.

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DOW 798820

TABLE 2

Safety Factors For Selected Industrial Chemicals

Drug	Safety Factor*	TLV
Vinyl Chloride	50	1 ppm
Acrylonitrile	2	20 ppm
Methyl Ethyl Ketone	<5	200 ppm
Chloroform	<3	10 ppm
1,1-Dichloroethane	19	200 ppm
Carbon Tetrachloride	<30	10 ppm
Pentachlorophenol	5	0.5 mg/m^3
Captan	38	5 mg/m^3
2,4,5-T	10	10 mg/m^3

*The no-effect dose level in teratology studies divided by the current TLV. See Appendix Table A-2 for further data on the chemicals listed.

DOW 198821

TABLE 3

Safety Factors For Selected Pesticides

Drug	Safety Factor*
Maneb	1,852
Zineb	392
Carbaryl	47
Thiram	543
Ethylene Thiourea (ETU)	Not Calculable**
Malathion	0.8
Triazine	Not Calculable**
Captan	223
Dinoseb	4
Mirex	Not Calculable**
Dimethoate	Not Calculable**
2,4-D	2.5
Chlordane	5
Diazinon	21
Phaltan	470
Imidan	20
Paraquat	10

*Based on the no-effect dose level in teratology studies.

**A no-effect dose level was not established. See Appendix Table A-3 for further data on the pesticides listed.

TABLE 4

Summary - Laboratory Tests For Mutagenicity of 2,4,5-T

Test System	Dose(s) Tested	Results	Positive Mutagen Employed	Comments	Reference
2,4,5-T: Non-Mammalian Mutagenic Tests					
Ames Test	Not stated	-	Yes	110 Herbicides were tested	Andersen, K. J. et al. 1972 J. Agr. Food Sci. 20, 649
Ames Test	4.9 µg/plate	+	Yes	Doubtfully mutagenic after activation	Ercegovich, C. D. and Rashid, K. A., 1977 (unpublished)
Drosophila	250 ppm	+	No	50% Decrease in fertility at 250 ppm chromosome effects seen	Davring and Summer, 1971 Hereditas. 68, 115
Drosophila	0, 250, 1000 ppm	+	Yes	Slight increase in sex-linked recessive lethals	Mujumdar, S. K. and Golia, J. K., 1974 Can. J. Genet. Cytol. 16, 465
2,4,5-T: Mammalian Mutagenic Tests					
Host-Mediated Assay and Dominant Lethal (mice)	500-1000 mg/kg IP	-	No	-	Buselmaier, Von W. et al. 1972 Biol. Zbl. 91, 311
In Vitro Cytogenetics (human lymphocytes)	10^{-7}-10^{-4}M	?+	No	Not possible to tell if effect was mutagenic or toxic	Fujita et al. 1975 J. Jap. Assoc. Rural Med. 24, 77
In Vivo Cytogenetics (gerbils)	10-100 mg/kg/day	+ at 70 & 100 mg/kg	No	-	Mujumdar, S. K. and Hall, R. C., 1973 J. Heredity 64, 213
In Vivo Cytogenetics (mice)	Not stated	+	?	-	Davring and Hultgren, 1977 Hereditas. 85, 123-134

DOW 798822

TABLE A-3 (Continued)

Drug	Safety Factor	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference Number
Chlordane	5	Rat	Dietary	1000 ppm	-	-	-	30
Diazinon	21	Rabbit	Oral	7 mg/kg	-	-	-	31
Phaltan	470	Rabbit	Oral	80 mg/kg	-	-	-	32
Imidan	20	Rabbit	Oral	35 mg/kg	-	-	-	32
Paraquat	10	Mice	Oral	20 mg/kg	-	-	-	33

*Based on the no-effect dose level in teratology studies.

DOW 798823

TABLE A-3

Safety Factors For Selected Pesticides*

Drug	Safety Factor	Species	Route	No-Effect Dose	Embryotoxic Effect
Maneb	1,852	Rat	Oral	0.5 g/kg	-
Zineb	392	Rat	Oral	1.0 g/kg	-
Carbaryl	47	G. Pig, Hamster	Oral	125 mg/kg	250 mg/kg
Thiram	543	Hamster	Oral	125 mg/kg	-
Ethylene Thiourea (ETU)	Not Calculable	Rat	Oral	-	-
Malathion	0.8	Rat	Oral	105 mg/kg	-
Triazine	Not Calculable	Rat	?	-	-
Captan	223	Rabbit	Oral	38 mg/kg	75 mg/kg
Dinoseb	4	Mice	Oral	20 mg/kg	32 mg/kg
Mirex	Not Calculable	Rat	Dietary	-	5 ppm
Dimethoate	Not Calculable	Mice	Water	-	60 ppm
2,4-D	2.5	Rat	Oral	25 mg/kg	50 mg/kg

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DOW 798824

Teratogenic Effect	Observations	Reference Number
1 g/kg	Exencephaly and Phocomelia	20
2 g/kg	Exencephaly and Phocomelia	20
300 mg/kg	Cervical vertebrae fused	21
250 mg/kg	Skeletal defects	21
10 mg/kg	Hydrocephalus, neural tube defects	22
205 mg/kg	Hydronephrosis	23
30 mg/kg	Cleft palate and absent mandible	24
-	Resorptions	25
-	Decreased fetal length	26
-	Decreased litter size	27
-	Decreased pup survival	28
-	Subcutaneous edema and minor skeletal effects	29

TABLE A-2

Safety Factors For Selected Industrial Chemicals*

Drug	No-Effect Dose TLV	TLV	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference Number
Vinyl Chloride	50	1 ppm	Mice	Inhal.	50 ppm	500 ppm	-	Skeletal variants	13
Acrylonitrile	2	20 ppm	Rats	Inhal.	40 ppm		80 ppm	Malf. - variety	14
Methyl Ethyl Ketone	<5	200 ppm	Rat	Inhal.	<1000	1000 ppm	-	Skeletal variants	15
Chloroform	<3	10 ppm	Rats	Inhal.	<30	30 ppm	-	Skeletal variants	16
1,1-Dichloroethane	19	200 ppm	Rats	Inhal.	3800	6000	-	Skeletal variants	15
Carbon Tetrachloride	<30	10 ppm	Rats	Inhal.	<300	300	-	Skeletal variants	15
Pentachlorophenol	5	0.5 mg/m^3	Rats	PO	5 mg/kg	15 mg/kg	-	Skeletal variants	17
Captan	38	5 mg/m^3	Rabbit	PO	37.5 mg/kg		75	Malformations	18
2,4,5-T	10	10 mg/m^3	Mice	Oral	20				22

*Based on the no-effect dose level in teratology studies.

DOW 798825

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TABLE 5

Summary - Laboratory Tests For Mutagenicity of TCDD

Test System	Dose(s) Tested	Results	Positive Mutagen Employed	Comments	Reference
TCDD: Non-Mammalian Mutagenic Tests					
Ames Test and E. Coli	up to 4.0 µg/plate	+	No	-	Hussain, S. et al. 1972 Ambio. 1, 32-33
Ames Test	Not stated	-	Yes	A memo to USDA	Ames, B. N., 1974 (unpublished)
TCDD: Mammalian Mutagenic Tests					
Dominant Lethal (rats)	0, 4, 8 µg/kg	-	No	Highest dose level lethal to some of the males	Khera, K. S. and Ruddick, J. A., 1973 Advances in Chemistry 120, 70-84.
In Vivo Cytogenetic (rats)	0, 5, 10, 15, 20 µg/kg	-	No	Abstract only	Green, S., 1975 Toxicol. Appl. Pharmacol. 33, 161
In Vivo Cytogenetics (rats)	0.001 - 1 mg/kg	+	No	Abstract and translation only	Yefimenko, L. P., 1974 Gig. Tr. Prof. Zabol. 18, 24-27.

DOW 798826

Dominant Lethal tests conducted at extremely high dose levels have shown negative results. This is a significant observation, especially when considered in conjunction with the results of other tests that have been conducted on these compounds.

DOW 798827

also reported on a Dominant Lethal test with 2,4,5-T in NMRI mice at a dose level of 1,000 mg/kg administered intraperitoneally, with no indication of a dominant lethal effect.

DOW 798828

One additional piece of unpublished data is attached, consisting of a communication from Bruce Ames, Professor of Biochemistry, University of California at Berkeley, to Dr. Philip Kearney, USDA, dated 11 March 1974. Dr. Ames has evaluated the mutagenicity of TCDD in his tester strains of Salmonella and has not found positive responses.

An abstract published by Babbitt et al. is also attached regarding work in progress on the effect of 2,4,5-T on two human cell lines, spleen fibroblasts and lymphocytes. No data are included in this abstract.

As indicated in the Document prepared by the Working Group on 2,4,5-T, there are significant limitations in the majority of the reports cited on mutagenicity studies on 2,4,5-T and TCDD. Considered collectively, the results of the tests do not indicate that either chemical is a potent mutagen. Generally, the results from in vivo mammalian tests which assess germ cell mutation are the most relevant for predicting risks to humans. With each of these materials, the

DOW 79882

RPAR OF 2,4,5-T: MUTAGENICITY

The EPA has concluded that there are insufficient data on the mutagenic potential of 2,4,5-T and TCDD and that further testing is needed. The results of the laboratory tests to assess the mutagenic potential of 2,4,5-T and TCDD are summarized in Tables 4 and 5, respectively. Our review of the literature revealed very little additional information to that included in the RPAR Document No. 1 on 2,4,5-T. Two additional references on mutagenicity studies on 2,4,5-T are submitted to supplement the review by the EPA. A paper by Andersen et al. (1972) is included which was an evaluation of 110 herbicides for their ability to induce point mutations in four different microbial systems. None of the herbicides, including 2,4,5-T, caused point mutations in 8 strains of Salmonella typhimurium as well as the T4 bacteriophage and E. coli.

The second paper is by Buselmaier et al. (1972) who evaluated a number of pesticides, including 2,4,5-T, in the Host-Mediated assay and the Dominant Lethal test in mice. The Host-Mediated assay was conducted in NMRI mice using mutants of the bacteria Salmonella typhimurium and Serratia marcescens introduced into the mice by intraperitoneal injection. No mutagenesis was found at 2,4,5-T dose levels of 500 mg/kg, and doses of the n-butyl ester of 2,4,5-T of 1,000 mg/kg. These investigators

TABLE VIb

Safety Factors For Selected Drugs[a]

Drug	No-Effect Dose / Therapeutic Dose	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference
Dilantin (anticonvulsant)	20	Mice	Oral	100 mg/kg	125 mg/kg	150 mg/kg	Cleft palate	Murray et al. Tox. Appl. Pharmacol. 1978, In Press
Valium (tranquilizer)	170	Mice	Oral	100 mg/kg	-	140 mg/kg	Cleft palate	Miller et al. TAP[b] 32, 53-61, 1973
Phenobarbital (anticonvulsant)	10	Rabbit	Oral	-	-	50 mg/kg	Skeletal defects	McColl et al. TAP 10, 244-252, 1967
Meclizine (antihistamine)	12	Rat	Oral	5 mg/kg	-	25 mg/kg	Cleft palate microstomia	King, C.T.G., Science 141, 353-355, 1963
Norethindrone (progestin)	50	Mice	Oral	0.5 mg/kg	-	1.0 mg/kg	-	Andrew et al. Teratology 5, 249, 1972
Norethinodrel (progestin)	4	Mice	Oral	0.15 mg/kg	-	0.6 mg/kg	Cranial retardation	Gidley et al. Teratology 3, 339-344, 1970
Vitamin A	40	Mice	Oral	-	-	400,000 IU/kg	Exencephaly Cleft palate Microphthalmia	Giroud et al. C. R. Soc. Biol. 156, 449-450, 1962
Acetazolamide (diuretic)	?	Rat	Dietary	40 mg/kg	-	150 mg/kg	Limb deformities	Layton et al. Science 149, 306-308, 1965
Morphine (analgesic)	666	Mice	SC	200 mg/kg	-	300 mg/kg	Exencephaly	Johanneson et al. Acta Pharm. Toxicol. 31, 305-313, 1972
Aspirin: teratology	3	Rat	Oral	200 mg/kg	-	225 mg/kg		Thompson et al. Report of Dow Chemical Company, (unpublished data)
reproduction	<2	Mice	Dietary	Effects at lowest dose, 130 mg/kg				Wright, H. N. TAP 11, 280-292, 1967
Acetaminophen (analgesic): reproduction	<6	Mice	Dietary	Effects at lowest dose, 130 mg/kg				Wright, H. N. TAP 11, 280-292, 1967

[a]Based on the no-effect dose level in teratology studies.

[b]TAP = Toxicology and Applied Pharmacology.

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TABLE A-1

Safety Factors For Selected Drugs*

Drug	No-Effect Dose / Therapeutic Dose	Species	Route	No-Effect Dose	Embryotoxic Effect	Teratogenic Effect	Observations	Reference Number
Dilantin (anticonvulsant)	20	Mice	Oral	100 mg/kg	125 mg/kg	150 mg/kg	Cleft palate	1
Valium (tranquilizer)	170	Mice	Oral	100 mg/kg	-	140 mg/kg	Cleft palate	2
Phenobarbital (anticonvulsant)	10	Rabbit	Oral	-	-	50 mg/kg	Skeletal defects	3
Meclizine (antihistamine)	12	Rat	Oral	5 mg/kg	-	25 mg/kg	Cleft palate microstomia	4
Norethindrone (progestin)	50	Mice	Oral	0.5 mg/kg	-	1.0 mg/kg	-	5
Norethynodrel (progestin)	4	Mice	Oral	0.15 mg/kg	-	0.6 mg/kg	Cranial retardation	6
Vitamin A	40	Mice	Oral	-	-	400,000 IU/kg	Exencephaly Cleft palate Microphthalmia	7
Acetazolamide (diuretic)	7	Rat	Dietary	40 mg/kg	-	150 mg/kg	Limb deformities	8
Morphine (analgesic)	666	Mice	SC	200 mg/kg	-	300 mg/kg	Exencephaly	9
Aspirin: teratology	3	Rat	Oral	200 mg/kg	-	225 mg/kg		10
reproduction	<2	Mice	Dietary	Effects at lowest dose, 130 mg/kg				11
Acetaminophen (analgesic): reproduction	<6	Mice	Dietary	Effects at lowest dose, 130 mg/kg				12

*Based on the no-effect dose level in teratology studies.

MOW 798831

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DOW 798832

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Murray, F. J. and Hammond, P. B. (1978) Teratogenicity CNS toxicity, and plasma levels of diphenylhydantoin in pregnant mice and rats. Toxicol. Appl. Pharmacol. (in press).

2. Diazepam: (valium)

Miller, R. P. and Becker, B. A. (1973) Teratogenicity of oral diazepam and diphenylhydantoin in mice. Toxicol. Appl. Pharmacol. 32, 53-61.

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5. Norethindrone:

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10. Aspirin:

Thompson, D. J., Strebing, R. J., Gerbig, C. G. and Molello, J. A. (1971) Teratogenicity study on aspirin in the Spraque-Dawley rat. Report of Dow Chemical Company. Unpublished data.

DOW 7988833

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DOW 798834

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DOW 798835

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Schwetz, B. A., Keeler, P. A., and Gehring, P. J. (1974) The effect of purified and commercial grade pentachlorophenol on rat embryonal fetal development. Toxicol. Appl. Pharmacol. 28, 151-161.

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19. 2,4,5-T:

Roll, R. (1971) Studies on the teratogenic effect of 2,4,5-T in mice. Fd. Cosmet. Toxicol. 9, 671-676.

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DOW 798836

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Robens, J. F. (1969) Teratogenic studies of carbaryl, diazinon, norea, disulfiram and thiram in small laboratory animals. Toxicol. Appl. Pharmacol. 15, 152-163.

22. Ethylenethiourea:

Khera, K. S. (1973) Teratogenic effects of ethylene-thiourea in rats and rabbits. Toxicol. Appl. Pharmacol. 25, 455-456.

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Kalow, W. and Marton, A. (1961) Second-generation toxicity of malathion in rats. Nature 192, 464-465.

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Kennedy, G. L., Fancher, D. E. and Calandra, J. C. (1968) An investigation of the teratogenic potential of captan, folpet and difolatan. Toxicol. Appl. Pharmacol. 13, 420-430.

DOW 798837

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Gibson, J. E. (1973) Teratology studies in mice with 2-sec-butyl-4,6-dinitrophenol (dinoseb). Fd. Cosmet. Toxicol. 11, 31-43.

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Budreau, C. H., and Singh, R. P. (1973) Teratogenicity and embryotoxicity of demeton and fenthion in CF1 mouse embryos. Toxicol. Appl. Pharmacol. 24, 324-332.

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Schwetz, B. A., Sparschu, G. L. and Gehring, P. J. (1971) The effect of 2,4-dichlorophenoxyacetic acid and esters of 2,4-D on rat embryonal, fetal and neonatal growth and development. Fd. Cosmet. Toxicol. 9, 801-817.

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Ingle, L. (1952) Chronic oral toxicity of chlordane to rats. Arch. Ind. Hyg. Occup. Med. 6, 357-367.

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DOW 798838

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Fabro, S., Smith, F. L. and Williams, R. T. (1966) Embryotoxic activity of some pesticides and drugs related to phthalimide. Fd. Cosmet. Toxicol. 3, 587-590.

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MN071036 1085

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ECON-O-GRAM WRITE-DO NOT TYPE DOW DATE 19 June 78

NAME DEPARTMENT BUILDING AND OR LOCATION

TO: L.L. Smith, 9008 P.J. Gebring, 1803
D.D. McCollister, 9008 R.J. Kociba, 1803
M.L. Leng, 9008 K.S. Rao, 1803

RPAR on 2,4,5-T — Teratology & Mutagenicity responses for your review and comments. The attachments are included only with the copy to Marguerite Leng.

Bern Schwetz G-0231

NAME DEPARTMENT—BUILDING AND OR LOCATION PHONE

FROM:

NO ENVELOPE REQUIRED
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USE REVERSE SIDE FOR REPLY

17385

1072

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

9008 Building
May 16, 1978

B. B. Holder
H&ER Medical
2030 Dow Center

cc: J. H. Davidson
M. J. Traynor, 2030 P. D. McCollister

SEVESO AND 2,4,5-T RPAR

Attached are the two references on the Seveso incident cited in the 2,4,5-T RPAR. No. 99 is a brief letter by C. D. Dewse in The Lancet, August 14, 1976 while No. 100 is a brief article by Hay in Nature, October 14, 1976.

In our rebuttal to the 2,4,5-T RPAR we will likely include an update of that situation, with additional references as appropriate. A brief summary from you and copies of additional documents would be of great help. Due to time constraints, I would need your comments by June 15.

Marguerite

Marguerite L. Leng
Government Registration
Health and Environmental Research

Attachment

abc

MN 053305

DOW 3847739

17386

LIFE IS FRAGILE · HANDLE WITH CARE

1077

M. L. Lentz

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

9008 Building
July 28, 1978

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*J. A. Gray, 2030
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*E. E. Kenaga, 9008
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*A. H. Morgan, 9008
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*B. A. Schwetz, 1803
M. J. Traynor, 2030
R. D. Vatne, 9008
*M. A. Weaver, 2040
C. S. Williams, 9008
M. E. Winquist, 2040
C. D. Woods/J. M. Capriccioso, 2030
Action File (2)

Should make this available in Organics & Pharm. Group meeting in Midland. Dave Schwetz, Clay Williams & Don Donaldson.

DOW 360376
MN 053305

2,4,5-TRICHLOROPHENOL RPAR/POSITION DOCUMENTS

Mary Reece, OSPR/EPA, informed me on Monday, July 24, 1978, that the 2,4,5-TCP RPAR had issued, and mailed an unofficial copy of the Notice (11 pages) and the Position Document (65 pages plus 98 references) to me which arrived on Thurs., July 27, 1978. The official RPAR Notice and Position Document was mailed (certified) to all registrants on July 25, 1978, and is scheduled to be published in the Federal Register on Aug. 2, 1978. We have 45 days (Sept. 18, 1978) to submit evidence in rebuttal of the presumption and may request an additional 60-day extension. The public will also be given an opportunity for comment and for submitting additional information relevant to the presumption. Registrants and other interested parties may also submit, for consideration, data on benefits which they believe would justify registration or continued registration.

Nov. 17

According to the Position Document, the "2,4,5-TCP and its salts have been registered for pesticidal use since 1948. Current EPA records indicate that 42 registrants have 94 Federally-registered products and one State-registered product containing 2,4,5-TCP or its salts. These products are usually formulated as wettable powders, emulsifiable concentrates, dry powders, liquids, or ball briquettes. Dow Chemical Company is the major manufacturer of technical grade 2,4,5-TCP (DOWICIDE® 2) and Na-2,4,5-TCP (DOWICIDE B). The 2,4,5-TCP and its salts are frequently mixed with other pesticides including pentachlorophenol, tetrachlorophenol, and sodium pentachlorophenate."

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"2,4,5-TCP is available in both a technical and analytical form. Technical grade 2,4,5-TCP (DOWICIDE 2) currently contains 95% active ingredient and 5% inert ingredients in which TCDD is present at a maximum of 0.099 ppm. Technical grade Na-2,4,5-TCP (DOWICIDE B) contains 85% Na-2,4,5-TCP and 15% inert ingredients in which TCDD is present at a maximum of 0.099 ppm (Dow Chemical Company Sales Specifications, 1976). The dioxin content in both of these products does not exceed the limit of 0.1 ppm recommended by the Advisory Committee to the EPA Administrator on May 7, 1971."

"2,4,5-TCP and its salts are used in the textile industry to preserve emulsions used in rayon spinning and silk yarns, in the adhesive industry to preserve polyvinyl acetate emulsions, in the leather industry as a hide preservative, and in the automotive industry to preserve rubber gaskets. The sodium salt is used as a preservative in adhesives derived from casein, as a constituent of metal cutting fluids and foundry core washes to prevent breakdown and spoilage, as a bactericide/fungicide in recirculating water in cooling towers, and as an algicide/slimicide in pulp/paper manufacturing industry.

There are some minor uses of 2,4,5-TCP and its salts in disinfectants which are of major importance relative to human exposure. These include use on swimming-pool-related surfaces; household sickroom equipment; food processing plants and equipment; food contact surfaces; hospital rooms; sickroom equipment; and bathrooms (including shower stalls, urinals, floors, and toilet bowls)."

DOW360377

The triggers for the RPAR are discussed in the Position Document and include:

1. Oncogenicity - based on the oncogenic effects of the contaminant, TCDD. The Working Group has concluded that there is evidence to indicate that TCDD can produce oncogenic effects in rats.

2. Fetotoxicity - based on the study by Neubert and Dillmann (1972) entitled, "Embryotoxic Effects in Mice Treated with 2,4,5-Trichlorophenoxyacetic Acid and 2,3,7,8-Tetrachlorodibenzo-p-Dioxin." The dermal and inhalation exposures for 2,4,5-TCP or its salts were compared to the no-adverse-effect level for fetotoxicity, 0.9 mg/kg body weight/day (Neubert and Dillmann, 1972). Since the difference between this no-adverse-effect level and the calculated exposure did not represent an adequate margin of safety, the Working Group recommended issuance of the RPAR based on fetotoxicity.

Other data have associated TCDD with mutagenic and teratogenic effects, and chemical carcinogenesis in test animals, and toxic effects on humans and other mammals; however, these data are not presently sufficient to support issuance of an RPAR. EPA solicits information regarding these or any additional adverse effects on man or the environment caused by TCP/TCDD.

The RPAR Notice states that it is "the first of several which will give public notification of the Agency's progress in reviewing this chemical." It emphasizes that "a notice of rebuttal presumption against registration and continued registration is not a notice of intent to cancel the registration of a pesticide, and may or may not lead to cancellation."

Copies of the official RPAR Notice are attached, and the Position Document (with the references) are attached for those whose names on the distribution list are preceded by an asterisk. I would appreciate it if these people would review the Document and comment on any errors or weak points as soon as possible, so said comments can be considered for our overall response. Persons designated to assist in preparing the rebuttal will be notified as soon as Mr. McCollister returns (Aug. 2). If I have inadvertently omitted anyone who should be advised of the subject notice and document, please let me know and I will rectify the situation. Also, if anyone else needs a copy of the Notice and/or the Document, please advise me.

Maggie

Margaret L. Wulf
Government Registration
6-6376

rdp

DOW 360378

4 Atrazine

DOW CHEMICAL U.S.A. 1064

MN 0615 93

1706 MIDLAND, MICHIGAN 48640

May 31, 1978

Date of meeting changed to June 7

TO: PROPONENTS OF PHENOXY HERBICIDES

SUBJECT: GROUP MEETING TO COORDINATE EFFORTS ON RPAR FOR 2,4,5-T

MEETING TIME AND PLACE: June 7, 1978 - 9:00 a.m.

Ramada O'Hare
6600 Mannheim Rd.
Des Plains, Illinois
Tel. (312) 827-5131

2,4,5-T is a useful and necessary tool for the following:

1. Right-of-way brush control
2. Rangeland brush control
3. Forestry
4. Weed control in rice

In order to better coordinate the activities of these interests, it was thought desirable to assemble a group of individuals that could mobilize the necessary effort to respond to the RPAR.

Reasons for the meeting:

1. Establish lines of communications among the key groups present.
2. Determine capabilities of each organization in regards to making key political and user contacts.
3. How to achieve maximum effect with the news media on forthcoming benefit statements from user groups.
4. Review of RPAR process and the necessary jobs that need to be done.
5. Review of legal actions in various states regarding 2,4,5-T.

Come prepared to participate by reporting what work your organization has underway and difficulties encountered to date.

We will see you June 7.

J. H. Davidson
Agricultural Products Department

nc

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FRIDAY, APRIL 21, 1978
PART II

MN0544479

DOW 169581

federal register

ENVIRONMENTAL PROTECTION AGENCY

PESTICIDE PROGRAMS

Rebuttable Presumption Against Registration and Continued Registration of Pesticide Products Containing 2, 4, 5–T

17391

[6560-01]

ENVIRONMENTAL PROTECTION AGENCY

[FRL 882-2; OPP-30000/26]

PESTICIDE PROGRAMS

Rebuttable Presumption Against Registration and Continued Registration of Pesticide Products Containing 2,4,5-T

AGENCY: Office of Pesticide Programs, Environmental Protection Agency (EPA).

ACTION: Notice of rebuttable presumption.

SUMMARY: 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) has been found to exceed certain risk criteria set forth in 40 CFR 162.11. This notice requests registrants and other interested persons to submit rebuttals and other information on the presumption and to submit any other data on the risks and benefits of this pesticide chemical. This notice is the first of several which will give public notification of the Agency's progress in reviewing this chemical.

DATE: Rebuttal evidence and other information must be received on or before June 5, 1978.

ADDRESS MATERIAL TO: Federal Register Section, Technical Services Division (WH-569), Office of Pesticide Programs, EPA, Room 401, East Tower, 401 M Street SW., Washington, D.C. 20460.

FOR FURTHER INFORMATION CONTACT:

Harvey Warnick, Office of Special Pesticide Reviews, Office of Pesticide Programs (WH-566), Room 447, East Tower, EPA, 202-755-5754.

SUPPLEMENTARY INFORMATION: The Deputy Assistant Administrator, Office of Pesticide Programs, EPA, has determined that a rebuttable presumption exists against registration and continued registration of all pesticide products containing 2,4,5-T.[1]

I. Regulatory Provisions

A. *General.* Title 40, § 162.11, of the Code of Federal Regulations for the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended (86 Stat. 973, 89 Stat. 751, 7 U.S.C. 136 et seq.), provides that a rebuttable presumption against registration shall arise if the Agency determines that a pesticide meets or exceeds any of the risk criteria relating to acute and chronic toxic effects set forth in § 162.11(a)(3). If it is determined that such a rebuttable presumption has arisen, the regulations require that the registrant be notified by certified mail and afforded an opportunity to submit evidence in rebuttal of the presumption. In addition, the Agency has determined that the public should also be given notice of the bases for the presumption to provide an opportunity for comment and to solicit additional information relevant to the presumption.

A notice of rebuttable presumption against registration is issued when the evidence related to risk meets the criteria set forth in § 162.11(a)(3). It is emphasized that a notice of rebuttable presumption against registration and continued registration of a pesticide is not a notice of intent to cancel the registration of a pesticide, and may or may not lead to cancellation. The notice of intent to cancel is issued only after the risks and benefits of a pesticide are carefully considered and it is determined that the pesticide may generally cause unreasonable adverse effects to the environment.

All registrants and applicants for registration are invited pursuant to 40 CFR 162.11(a)(4) to submit evidence in rebuttal of the presumptions listed in part II of this notice and, in the case of oncogenicity, to submit information which relates to the assessment of oncogenic risks as set forth in the Agency's Interim Procedures and Guidelines for Health Risk and Economic Impact Assessment of Suspected Carcinogens (May 25, 1976; 41 FR 21402). Registrants and other interested parties may submit for consideration data on benefits which they believe would justify registration or continued registration. In addition, any registrant may petition the Agency to voluntarily cancel a current registration pursuant to section 6(a)(1) of FIFRA.

B. *Rebuttal criteria.* Section 162.11(a)(4) provides that a registrant may rebut the presumption by sustaining the burden of proving:

(1) In the case of a pesticide presumed against pursuant to the acute toxicity or lack of emergency treatment criteria, "that when considered with the formulation, packaging, method of use, and proposed restrictions on the directions for use and widespread and commonly recognized practices of use, the anticipated exposure to an applicator or user and to local, regional, or national populations of nontarget organisms is not likely to result in any significant acute adverse effects" (40 CFR 162.11(a)(4)(i));

(2) In the case of a pesticide presumed against pursuant to the chronic toxicity criteria, "that when considered with proposed restrictions on use and widespread and commonly recognized practices of use, the pesticide will not concentrate, persist or accrue to levels in man or the environment likely to result in any significant chronic adverse effects" (40 CFR 162.11(a)(4)(ii)); or

(3) In either case, that "the determination by the Agency that the pesticide meets or exceeds any of the criteria for risk was in error" (40 CFR 162.11(a)(4)(iii)).

C. *Benefits information.* In addition to submitting evidence to rebut the presumption of risk, § 162.11(a)(5)(iii) provides that a registrant "may submit evidence as to whether the economic, social, and environmental benefits of the use of the pesticide subject to the presumption outweigh the risk of use." If the risk presumptions are not rebutted, the benefit evidence[2] submitted by the registrant, applicants, and other interested persons will be considered by the Administrator in determining the appropriate regulatory action. Specifically, § 162.11(a)(5)(iii) provides that if the benefits appear to outweigh the risks, the Administrator may issue a notice of intent to hold a hearing pursuant to section 6(b)(2) of FIFRA to determine whether the registration(s) should be cancelled or application(s) denied. Alternatively, if the "benefits do not appear to outweigh the risks, the Administrator shall issue a notice pursuant to section 3(c)(6) or section 6(b)(1) of the Act, as appropriate." Moreover, if at any time the Administrator determines that a pesticide poses an "imminent hazard" to humans or the environment, a notice of suspension may be issued pursuant to section 6(c) of the Act.

II. Presumptions

Registrations and applications for registration of pesticide products containing 2,4,5-T meet or exceed the 40 CFR 162.11(a)(3) risk criteria relating

[1] A position document, containing an appendix of references, background information, and other material pertinent to the issuance of this notice, has been prepared by the Agency Working Group on 2,4,5-T and is also published with this notice.

[2] Registrants or other interested persons who desire to submit benefit information should consider submitting information on the following subjects, along with any other relevant information they desire to submit:

1. Identification of the major uses of the pesticide, including estimated quantities used by crop or other application.
2. Identification of the minor uses of the pesticide, including estimated quantities used by category such as lawn and garden uses and household uses.
3. Identification of registered alternative products for the uses set forth in (1) and (2) above, including an estimate of their availability.
4. Determination of the change in costs to the user of providing equivalent pesticide treatment with any available substitute products.
5. Assessment of regulation impact upon user productivity (e.g., yield per acre and/or total output) from using available substitute pesticides or from using no other pesticides.
6. If the impacts upon either user costs or productivity are significant, a qualitative assessment of the regulation's impact on production of major agricultural commodities and retail food prices of such commodities.

DOW 16983

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to oncogenic effects and teratogenic and/or fetotoxic effects in mammalian test species. The Agency's basis for concluding that these risk criteria have been met or exceeded is set out in "2,4,5-T: Position Document 1," which follows. Copies of attachments to the Position Document which are not published with this notice are available for public inspection in the Office of Special Pesticide Reviews. Information protected from disclosure pursuant to FIFRA section 10 cannot be provided. Specific inquiries concerning the Position Document, as well as requests for access to these files, should be directed to Project Manager Harvey Warnick, Office of Special Pesticide Reviews (WH-566), EPA, Room 447, East Tower, 401 M Street SW., Washington, D.C. 20460, 202-755-5754.

A. *Oncogenicity.* 40 CFR 162.11(a)(3)(ii)(A) provides that a rebuttable presumption shall arise if a pesticide "(i)nduces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation or dermal exposure * * *." As a further clarification of the provision, the preamble to the Agency's Interim Procedures and Guidelines for Health Risk and Economic Impact Assessment of Suspected Carcinogens (May 25, 1976; 41 FR 21402) states that "a substance will be considered a presumptive cancer risk when it causes a statistically significant excess incidence of benign or malignant tumors in humans or animals."

On the basis of scientific studies and information summarized in the Position Document, the Agency has concluded that all registrations and applications for registration of pesticide products containing 2,4,5-T and/or its dioxin contaminant (TCDD) exceed this risk criterion, and that a rebuttable presumption against new or continued registration of such products has arisen.

B. *Other chronic or delayed toxic effects.* 40 CFR 162.11(a)(3)(ii)(B) provides that rebuttable presumption shall arise if a pesticide "(p)roduces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety * * *."

On the basis of scientific studies and information summarized in the Position Document, the Agency has concluded that all registrations and applications for registration of pesticide products containing 2,4,5-T and/or TCDD exceed this risk criterion for teratogenic and/or fetotoxic effects and that a rebuttable presumption against new or continued registration of such products has arisen.

III. Additional Grounds for Review

As discussed in detail in the attached Position Document, some data has associated 2,4,5-T and/or TCDD with mutagenic effects in test animals and TCDD with toxic effects in humans. The data and analyses available at this time with respect to these effects are not sufficient to warrant the issuance of a Rebuttable Presumption. The Agency specifically solicits further evidence bearing on these possible adverse effects. All comments and information received with respect to the potential adverse effects, including analysis thereof, may serve as a basis for a final decision on registering pesticides containing 2,4,5-T and/or TCDD.

IV. Registrations and Products Subject to the Notice

All registrants and applicants for registration listed below are being notified by certified mail of the rebuttable presumption existing against registration and continued registration of their products.

The registrants and applicants for registration shall have 45 days from the date this notice is sent or until June 5, 1978, to submit evidence in rebuttal of the presumption. However, the Administrator may, for good cause shown, grant an additional 60 days during which such evidence may be submitted. Notice of such an extension, if granted, will appear in the Federal Register.

A registrant or applicant for registration may, if it desires, assert a business confidentiality claim covering part or all of the informaion submitted in rebuttal. The registrant or applicant may assert the claim by placing on or attaching to the information a cover sheet, stamped or typed legend, or other suitable form of notice employing language such as "trade secret," "proprietary," or "company confidential." Allegedly confidential portions of otherwise nonconfidential documents should be clearly marked.

If a confidentiality claim is asserted, the information covered by the claim will be disclosed by EPA only to the extent and by means of the procedurec set forth in 40 CFR part 2, subpart B (41 FR 36906; September 1, 1976). If no confidentiality claim accompanies the information at the time it is received by EPA, EPA will place the information in the public comment file where it will be available for public inspection.

If a registrant or applicant does assert a confidentiality claim for some but not all, of the information submitted to EPA in rebuttal, the registrant or applicant should furnish two copies of the information to EPA. The first copy should contain all of the information submitted in rebuttal with information claimed as confidential clearly identified. The second copy should be identical to the first except that all information claimed as confidential should be deleted. The second copy will be placed in the public comment file. The first copy will be treated in accordance with the procedures set out above.

V. Duty to Submit Information on Adverse Effects

Registrants are required by law to submit to EPA any additional information regarding any adverse effects on man or the environment which comes to a registrant's attention at any time, pursuant to section 6(a)(2) of FIFRA and 40 CFR 162.8(d). If any registrant of 2,4,5-T products has any published or unpublished information, studies, reports, analyses, or reanalyses regarding any adverse effects in animal species or humans, residues, and claimed or verified accidents to humans, domestic animals, or wildlife, which have not been previously submitted to EPA, the material must be submitted immediately. When responding to this notice, each registrant shall submit a written certification to the Agency that all information regarding any adverse effects known to the registrant has been submitted. In addition, the registrants should notify EPA of any studies currently in progress, including the purpose of the study, the protocol, the approximate completion date, and a summary of all results observed to date.

VI. Public Comments and Inspection

During the time allowed for submission of rebuttal evidence, specific comments on the presumptions set forth in this notice and on the material contained in the Position Document are solicited from the public. In particular, any documented episodes of adverse effects to humans, domestic animals, or wildlife, and information as to any laboratory studies in progress or completed are requested to be submitted to EPA as soon as possible. Specifically, information on the fate and effects of 2,4,5-T, its impurities, metabolites, and degradation products on flora and fauna, particularly animals with metabolism similar to man, is solicited. Similarly, any studies or comments on the benefits from the use of 2,4,5-T are requested to be submitted. All comments and information received, as well as any other relevant information and analysis thereof, which come to the attention of the Agency may serve as a basis for final determination pursuant to § 162.11 (a)(5).

All comments and information should be sent to the Office of the Federal Register Section at the address given above, if possible in triplicate to facilitate the work of the

DOW 1695 83

Agency and others interested in inspecting them. The comments and information should bear the identifying notation "OPP-30000/26." Comments received after the specified time period will be considered only to the extent feasible, consistent with the time limits imposed by 40 CFR 162.11(a)(5)(ii).

All written comments and information filed pursuant to this notice will be available for public inspection in the Office of the Federal Register Section from 8:30 a.m. to 4 p.m. during normal working days. Interested persons are encouraged to take advantage of the opportunity to inspect Agency files during normal working hours since: (1) All of the information received may serve as a basis for final determination pursuant to § 162.11(a)(5), and (2) the Agency will not generally publish a summary of information received in the FEDERAL REGISTER at the close of the rebuttal period.

Your cooperation is solicited in identifying any errors or omissions which may have been made in the following computer listings. Corrections to the listings may not necessarily be published in the FEDERAL REGISTER, but rather handled by mail with affected parties. Omissions will be corrected by notice in the FEDERAL REGISTER.

Dated: April 11, 1978.

EDWIN L. JOHNSON,
Deputy Assistant Administrator for Pesticide Programs.

2,4,5-T: POSITION DOCUMENT 1

2,4,5-T WORKING GROUP, U.S. ENVIRONMENTAL PROTECTION AGENCY

I. Background

A. *Chemical/physical characteristics.* The herbicide commonly known as 2,4,5-T (chemical name, 2,4,5-Trichlorophenoxyacetic acid) has an empirical formula of $C_8H_5Cl_3O_3$. The pure acid form occurs as white crystals and has a molecular weight of 255.49. The melting point is 156.5°C. Its solubility in water is 278 parts per million (ppm) at 25°C; it is also soluble in acetone, ethanol, ether, and alkaline solutions (*1*). The esters of 2,4,5-T are formulated to be emulsifiable in water and soluble in most oils, while its amine salts are soluble in water but insoluble in petroleum oils (*2, 3*).

B. *Manufacturing process and contaminants.* 2,4,5-T is produced commercially by a process using 1,2,4,5-tetrachlorobenzene as the starting material which is reacted with methanol and sodium hydroxide under high temperature and high pressure to give the sodium salt of 2,4,5-trichlorophenol (2,4,5-TCP).[1]

This product is reacted with chloroacetic acid under mildly alkaline conditions. Sulfuric acid (H_2SO_4) is then added to the product of this step to produce 2,4,5-T. The acid form of 2,4,5-T can be readily reacted with a variety of alcohols to produce a large selection of esters and with amines to produce amine salts (*3*).

During the first step in the manufacturing process of 2,4,5-T, if temperature and pressure are not carefully controlled, highly toxic contaminants, polychlorinated dibenzo-p-dioxins, may be formed in large quantities. The particular dioxin formed is dependent on the chlorophenols present (*4*). The term dioxin does not apply to any one compound but to a group of related substances, which are distinguished by the number and orientation of chlorine atoms they contain. Dioxin toxicity also varies with the position and numbers of chlorines attached to the phenol rings.

In the 2,4,5-T manufacturing process an especially toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is formed when the reaction temperature is excessive (*8, 9, 10, 11, 12*), most commonly at temperatures above 160°C. Halogens at the 2, 3, and 7 positions are known to produce toxic dioxins (*13*). In the case of TCDD, the chlorine atoms are attached at the 2, 3, 7, and 8 positions which are considered the most toxic positions possible (*14*). The dioxin contaminant in 2,4,5-T is of particular concern because of its extremely high toxicity, and because of the apparent inability of manufacturers to produce 2,4,5-T without the contaminant, TCDD (*7*).[2]

TCDD occurs as a white crystalline solid. It is 99.5 percent decomposed at 800°C. TCDD has the following solubility in various solvents at 25°C (*7*).

Solvent	Solubility[1]
Acetone	0.012
Benzene	0.057
Dimethylsulfoxide	<00.01
Methanol	0.001
Water	[3]0.00000002

[1] Weight percent.
[3] (0.2ppb)

It has been recognized for quite some time that chlorinated dibenzo-p-dioxins occur as possible by products (contaminants) in the manufacturing of chlorinated phenols (*15*). The formation of TCDD during production of 2,4,5-TCP was demonstrated by Kimmig and Schulz (*16*). TCDD was obtained from the pyrolysing of 2,4,5-TCP by Higginbotham et al. (*11*). They noted that the specific dioxin formed depended on the chlorophenol pyrolysed. Kearney et al. (*17*), however, reported that TCDD is historically associated with any pesticide derived from 2,4,5-TCP. A number of researchers (*12, 18, 19, 20, 21*) have reported on the formation of TCDD by thermal decomposition of the sodium salt of 2,4,5-TCP under alkaline conditions during the manufacturing process.

Since 1950, most of the chemical industry has known that large quantities of TCDD may be formed as a byproduct of the 2,4,5-TCP manufacturing process if the procedures are not carefully controlled. At one time, 2,4,5-T was produced which contained

[1] 2,4,5-TCP is the subject of a separate Rebuttable Presumption Against Registration (RPAR) Position Document. It is discussed in this document because both it and its contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may be present in some commercial 2,4,5-T and in 2,4,5-T samples used in animal experiments.

[2] Since manufacturers are unable to produce 2,4,5-T without TCDD, all references to 2,4,5-T in this document refer to 2,4,5-T contaminated with some level of TCDD.

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17884

and to growth of sidecats grammma plants; all of which were factors contributing to rapid reduction of herbicide concentrations.

When considering the persistence of 2,4,5-T, the persistence of its manufacturing contaminant, TCDD, must also be considered. Helling et al. (*39*) found that TCDD was not photodecomposed on soil. TCDD was found to be immobile in Norfolk and Lakeland sandy loams, Hagerstown silty clay loam, Barnes Clay loam, and Celeryville muck, and was not leached further into soil by rainfall or irrigation. During surface erosion of soil, however, lateral transport of TCDD could occur. The persistence of TCDD in Lakeland loamy sand and Hagerstown silty clay loam at 1, 10, and 100 ppm was studied by Kearney et al. (*46*) for 360 days. After 1 year these researchers recovered 56 and 63 percent of the originally applied TCDD in Hagerstown and Lakeland soils, respectively. Helling et al. (*39*) observed that TCDD's persistence was predictable since it is insoluble in water.

(2) *Persistence: Water.* Current information indicates that, although some 2,4,5-T may enter streams flowing through or adjacent to areas being sprayed, residue levels in streams will be very low. Norris (*47*) reported the results of an intensive study of stream contamination from spray projects on range and forest lands in Oregon which showed that peak concentrations of phenoxy herbicides seldom exceeded 0.1 ppm and that herbicide residues persisted for only a few hours in nearly all streams. Norris (*47*) speculated, however, that application of herbicides to marshy areas may result in high-level, long persistence of chemical residues in nearby streams.

The Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency (*48*) stated that all available data indicated that the amount of 2,4,5-T entering water is small and does not persist long. It is adsorbed on clay or absorbed by biota within a matter of days.

Phenoxy chemicals entering water may be lost by volatilization, degradation, adsorption on sediment, adsorption by biota, and dilution as additional stream water passes through the site. Almost all authorities agree that there is adsorption on bottom sediment (*48, 49, 50*).

Kenaga (*51*) stated that esters of 2,4,5-T in most kinds of water, except highly acidic waters, are usually hydrolysed within a matter of days. When the 2-ethylhexyl ester of 2,4,5-T was applied to water in the laboratory at a concentration of 1 ppm for an hydrolysis study, 58 percent remained after 4 hours; 33 percent after 8 hours; and 12 percent after 16 hours.

Trichell et al. (*52*), studying the loss of herbicides in runoff water, found 2 μg/ml of 2,4,5-T in runoff water 24 hours after it was applied at 2.24 kg/ha, after which 1.3 cm of rainfall was simulated on sod-covered plots of 3 percent slope. Four months after application, concentrations of 2,4,5-T in runoff water had diminished to 0.04 μg/ml.

Edwards and Glass (*53*) monitored runoff and percolation of 2,4,5-T at Coshocton, Ohio, for 14 months following application of 11.2 kg/ha of 2,4,5-T and found that 5.8 g/ha, or over 0.05 percent of the herbicide, was lost from the treated area. Most of the 2,4,5-T was removed in runoff water during the first 4 months after application, and more than half of the loss occurred the first month after treatment.

Kearney et al. (*46*) concluded that contamination of underground water supplies with TCDD seemed very unlikely, since vertical movement of TCDD did not occur in a wide range of soil types. The fact that no leaching occurred, however, would not preclude runoff loss when soil erosion is significant (*39*).

(3) *Transport.* Isensee and Jones (*54*) measured uptake of TCDD from soil by two crop species. Oats (*Avena sativa*) and soybeans (*Glycine max*) were grown in Lakeland sandy loam soil treated with 0.06 ppm TCDD. The concentration of TCDD in soil was approximately 4,000 times greater than the amount that would be deposited in soil from an application of 2,4,5-T (with 1 ppm TCDD) at a rate of 2 pounds/acre in the top one-third inch of the soil surface. The tops of these plants were harvested at intervals to maturity. Mature oats and soybean tops contained less than 1 part per billion (ppb) TCDD. TCDD was detected (with a detection limit of 1 ppb) in mature oat grain, while no TCDD was found in the bean of soybeans. The authors concluded that soil uptake of TCDD by plants was highly unlikely, since little or no TCDD was taken up by oats or soybeans under the conditions of this experiment (*54*).

(4) *Bioaccumulation.* Woolson et al. (*55*) conducted a study to determine if TCDD residues could be detected in bald eagle (*Haliaectas leucocephalus*) tissue extracts, as a representative of the top of a food chain. Scientists at the Patuxent Wildlife Center (U.S. Department of the Interior, Laurel, Md.) collected, and furnished to these researchers, 19 bald eagle carcasses from Alaska, Maine, North Dakota, Wisconsin, Michigan, Minnesota, Arkansas, Illinois, Missouri, Maryland, Virginia, Iowa, New York, New Jersey, and Florida between 1966 and 1971. These States were selected as sampling sites in order to provide a widely dispersed sample population. The eagle tissues were prepared and extracted as described by Mulhearn et al. (*56*). Woolson et al. (*55*) detected no dioxin residues at a level of 0.06 ppm TCDD, the lower limit of detection for most pesticides in tissue samples run by the Patuxent Wildlife Research Center at that time. The authors stated that the nondetection of dioxin residues could imply that there was no dioxin buildup in the food chain; that the buildup was less than the [then] current detectable level of 0.05 ppm [50 ppb]; that the eagles examined were not contaminated, although other samples might be; or that other species could feed on a different food chain to accumulate dioxins.

Isensee and Jones (*57*) exposed several organisms in a model aquatic ecosystem to ^{14}C-labeled TCDD for up to 31 days to determine the distribution and bioaccumulation potential in the aquatic environment. Soil containing from 0.0001 to 7.45 ppm adsorbed ^{14}C-TCDD was placed in aquaria, containing eight snails (*Physa sp.*) a few strands of algae (*Oedogonium cardiacum*), and 10 ml of old aquarium water containing various diatoms, protozoa, and rotifers. Fifteen duckweed (*Lemna minor*) plants were also added to one aquarium. Samples of daphnids were taken for analysis at 30 days; and two mosquito fish (*Gambusia affinis*) were added to each tank. Three days later all of the organisms were removed for analysis, and two fingerling channel catfish (*Ictalurus punctatus*) were added to each tank and exposed for 6 days.

The authors stated that all organisms in both treatment and control tanks prospered during this exposure period, indicating that TCDD was not toxic at the concentrations used. TCDD accumulated in all organisms. At the highest TCDD concentration (7.45 ppm) algae accumulated 6,600±960 ppb TCDD; snails, 1,820±170 ppb; daphnids, 10,400±480 ppb; and Gambusia, 1,380±220 ppb. Catfish were not analyzed for TCDD residues. At the second highest TCDD concentration (3.17 ppm), however, catfish accumulated 730±130 ppb TCDD. The authors stated that accumulation in all of the test organisms from soil containing 0.1 ppb TCDD is important since this concentration approaches the concentration which would occur under normal field use of 2,4,5-T. The authors concluded that the data suggested that under certain circumstances (discharge of storm runoff from recently treated rangeland into a small pond), water-eroded surface soil or debris may contain enough TCDD for measurable residues (parts per thousand (ppt) quantities) to accumulate in fish or other aquatic organisms. However, the authors speculated that TCDD, originating from 2,4,5-T applications, discharged into large lakes, streams, or estuaries would probably become sufficiently diluted so that no measurable accumulation would occur.

As part of a broad study to determine whether 2,4,5-T use leads to TCDD accumulation in the environment, Shadoff et al. (*58*) collected samples of fish, mud, water, and human milk from areas in Texas and Arkansas. The Texas samples of water, mud, catfish, and walleyed pike were collected from the San Angelo Reservoir, an impoundment of the North Concho River. The authors stated that this watershed has large acreages that have been sprayed with 2,4,5-T at 0.5 pounds/acre (2,4,5-T acid equivalent) for brush control. These researchers also obtained six samples of human milk from mothers residing in general area of the San Angelo Reservoir. In addition, bass from a 12-acre pond in the heart of the Arkansas rice-growing area were collected. Water from this pond is used to flood rice fields treated with the equivalent of 1.25 pounds/acre of 2,4,5-T, acid, 4 to 8 weeks prior to flooding. The water is later drawn off the fields and pumped back into the pond for reuse. In addition, the pond is supplemented by water from wells and by water collected as runoff from surrounding rice fields during the rainy season. The authors stated that this cycle had been in use (including the proper use of 2,4,5-T) for 18 years up to the time of their study. The authors stated that no TCDD was detected in the tissues sampled, using a gas chromatography-mass spectrometry procedure with a detection limit which averaged less than 10 ppt. No evidence was found that TCDD is accumulating in the environment from the use of 2,4,5-T described in this study.

G. *Residues.*—(1) *Soil.* Woolson et al. (*55*) studied Lakeland sandy soil to determine if TCDD residues could be detected in soil receiving exceedingly large applications of 2,4-D and 2,4,5-T. The heaviest rate of 2,4,5-T application was 947 pounds-acre applied serially during 1963 through 1964, while the lightest rate was 100 pounds-acre applied serially during 1968 and 1969. During this period, it was not uncommon for commercial samples of 2,4,5-T to contain levels of 30 to 40 ppm TCDD.

The authors were able to detect small amounts of 2,4,5-T in the soil samples. They observed that the residue level decreased with time after application and stated that leaching and microbial decomposition could account for this decrease. Using a detection

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17885

limit of less than 1 ppb, the authors did not detect an TCDD at any depth in 36-inch core samples of the soil.

(2) *Water.* In October 1965, the U.S. Geological Survey initiated a limited program of pesticide monitoring on 11 waterways in the west United States (*59*). The streams, representing agricultural areas were the probability of observing pesticide residues would be greater, included the Missouri, Brazos, Yellowstone, Sacramento, Colorado, Arkansas, Yakima, Rio Grande, and Snake Rivers. Pesticides chosen for analysis included the insecticides aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane, and the herbicides 2,4,-D, 2,4,5-T, and silvex. The authors reported that no herbicide was found at any time at any station during the first year of the sampling program. The lower limit of sensitivity (detection) was 5 ppt.

Manigold and Schulze (*60*), reporting on the results of the U.S. Geological Survey stream monitoring program for the 2-year period October 1966 to September 1968, observed that beginning in August 1967 2,4-D, silvex, and 2,4,5-T had been detected frequently. 2,4,5-T was found in 28 of the 320 samples and ranged from 0.01 to 0.07 ppb. The authors stated that the established criteria permitted 100 μg/liter (ppb) for herbicides. These authors reported that the analytical procedures were changed from the preceding report to use Law's sample cleanup procedure, which permits routine detection of pesticides at 0.005 μg/liter in most waters.

Norris (*47*) observed that peak concentrations of phenoxy herbicides seldom exceeded 0.1 ppm in streams contaminated from spray projects on range and forest lands in Oregon.

Lawson (*61*) studied 2,4,5-T residues in storm runoff from three small watersheds in Arkansas. Two watersheds, one cleared and the other partially cut, were sprayed with the isooctyl ester of 2,4,5-T. A third watershed, adjacent to the two treated ones, was used as a control. Spraying was done in September 1971, June 1972, and July 1973, either to control woody sprouts and broadleaf vegetation or just to provide herbicide application for monitoring. The cleared watershed was treated with 4 pounds acid equivalent per acre and the partially cut site with 2 pounds/acre.

In water samples taken after the first runoff-producing storm in October 1971, Lawson (*61*) detected an average of 2.1 ppm 2,4,5-T from the cleared watershed and 1.0 ppm from the partially cut site. Maximum amounts detected were 2.2 and 1.3 ppm for the two areas. No 2,4,5-T was detected from the control site.

Only trace amounts (less than 0.2 ppm) were detected from each of the two treated sites after the next runoff-producing storms in November 1971. None was detected from the control.

In approximately 90 samples taken after storms during the period December 1971 through September 1973, no 2,4,5-T was detected by Lawson (*61*) in the runoff from the treated or control water sheds.

Since TCDD is immobile in soil (*39*) and soluble in water at only 0.2 ppb (*7*), the possibility of ground water contamination is virtually nonexistent (*46*). TCDD could be present in runoff when soil erosion is significant (*39*), and thus TCDD contamination of water bodies could occur.

A recent National Academy of Sciences report on drinking water stated that 2,4,5,-T and TCDD have never been detected in drinking water; the limit of detection was in the parts per trillion. However, the report did project the toxicity of 2,4,5,-T and TCDD, their acceptable daily intake, and suggested no-adverse-effect levels (*62*).

(3) *Air.* Prior to 1970, phenoxy herbicides were widely used for early postemergence control of weeds in wheat. Johnson (*63*) reported that air samples collected during spring and summer in the state of Washington where these crops are grown contained as much as 0.06 μg/m³ 2,4-D and 2,4,5-T. Assuming that a man inhales about 30 m³ of air per day, the authors estimated that exposure to 0.06 μg/m³ would amount to inhalation of 1.8 μg phenoxy herbicide/day or 0.025 μg/kg of body weight per day for a 70 kg man.

Ambient air monitoring for pesticides in predominantly agricultural areas of 28 states was conducted by the National Air Monitoring Program in calendear years (CY) 1970 through 1972 using ethylene glycol impinger type samples. Table 1 records the arithmetic mean of residues of 2,4,5-T detected in this program (*64*).

Table 1. Air Monitoring Data for 2,4,5-T in 28 State Monitoring Programs (1970 to 1972)

Name of State or City	2,4,5-T Ester Monitored For	ng/m³ CY 1970	ng/m³ CY 1971	ng/m³ CY 1972
Louisana	Isopropyl ester	-	ND	1.9
Montana		ND	ND	0.8
New Mexico		-	ND	1.0
Idaho		ND	ND	1.7
Illinois	BOEE	ND	3.6	ND
Oregon		ND	0.5	ND
Tennessee		1.1	ND	ND
Tennessee	Isooctyl ester	ND	2.7	ND
Oklahoma		ND	14.6	ND

ND = Not Detected.

(4) *Animals.* Phenoxy acetic acids are relatively strong acids, and animals rapidly excrete them unchanged in their urine (*35*). In their study of the fate of atrazine, kuron, silvex, and 2,4,5-T in the dairy cow, St. John et al. (*65*) found that dairy cows given 2,4,5-T and silvex in their feed at 5 ppm for four days, completely eliminated both 2,4,5-T and silvex as soluble salts in the urine two days after dosing stopped.

Zielinski and Fishbein (*66*) treated female C57BL/6 mice with a single subcutaneous injection of 100 mg/kg body weight of 2,4,5-T in dimethylsulfoxide solution. They sacrificed the animals at various intervals after injection and analyzed in toto for 2,4,5-T. The amounts recovered as percentage of the amount injected indicated decreasing levels at the following time intervals after dosing: at 0 hours, 77.1±5.0%; at 16 hours, 56.9±4.2%; and at 24 hours 23.7±3.6%.

In a preliminary report of a two-year chronic toxicity feeding study, Dow Chemical USA (*110*) reported the following residue data for rats fed indicated TCDD doses: 24,000 ppt in liver and 8,100 ppt in fat of females ingesting 2,200 ppt/day; 5,100 ppt in liver and 1,700 ppt in fat of females ingesting 220 ppt/day; and 540 ppt in liver and fat of females ingesting 22 ppt/day. The preliminary report gives no residue data for treated males, or for controls of either sex.

Piper et al. (*67*) studied the fate of 2,4,5-T following oral administration to rats and dogs. Four groups of three male and three female Sprague-Dawley rats (Spartan strain) and two male and two female adult beagle dogs were given single doses of ^{14}C-labeled 2,4,5-T by intubation at 5, 50, 100, and 200 mg/kg body weight in rats and 5 mg/kg body weight in dogs. The authors combined data obtained for males and females since the pharmacokinetics of 2,4,5-T were essentially the same in each sex. In this study, the clearance half-life for 5 mg/kg 2,4,5-T from dog plasma was 77.0 hours; in rats the half-life was 4.7 hours at 5 mg/kg and 4.2 hours at 50 mg/kg. At doses of 100 and 200 mg/kg body weight, the clearance half-life for rats increased to 19.4 and 25.2 hours, showing that the pharmacokinetics of 2,4,5-T varies with dose as well as with species. The authors suggested that the half-life values at 100 and 200 mg/kg body weight indicated that these doses may have exceeded the excretory capacity of the rats.

Zitko (*68*) assayed chlorinated dibenzodioxin residues in aquatic animals, but was unable to detect these compounds (detection limit: 0.04 μg/g [ppm] for TCDD) in any of several aquatic animals from Canadian locations. The author had selected species from high trophic levels of the aquatic food web to measure cumulative pesticide contamination. More recently, using improved analytical methods for detection of dioxin at ppt levels, Baughman and Meselson (*69*) found mean TCDD levels ranging from 18 ppt to 810 ppt in fish and crustaceans taken from Vietnamese rivers in August and September 1970. TCDD levels tended to be higher in fish from interior rivers than in those from seacoast locations. In comparison, Baughman and Meselson found less than 3 ppt TCDD in fish obtained in a market in Cape Cod, Massachusetts. In another study, Matsumura and Benesat (*70*) placed TCDD-coated sand directly in an aquarium containing brine shrimp, mosquito larvae, and fish (silverside). TCDD pickup was low in fish (2 ppb) and brine shrimp (157 ppb) under the experimental conditions. But mosquito larvae, which are bottom feeders, showed a surprisingly high rate of pickup (4,150 ppb). The authors concluded that TCDD was not likely to accumulate in as many biological systems as DDT because of TCDD's low solubility in water and lipids, as well as its low partition coefficient in lipids.

(5) *Plants.* Clark et al. (*36*) reported that, when herbicides are applied to rangeland, the levels of phenoxy herbicides available for ingestion by grazing livestock depend upon the nature and degree of cover, the rate and mode of application, time after application, and climate conditions. Studies by Morton et al. (*44*) showed that residues on grass immediately after application of 2,4,5-

DOW 659588

T are not likely to exceed 100 to 150 ppm for each pound of actual herbicide applied per acre.

Leng (37) stated that herbicide residues in or on plants declined rapidly, with a half-life of one to two weeks due to photodecomposition by sunlight, wash-off by rain, metabolism by plants, and dilution from growth of plants. 2,4,5-T was applied to grass in four states at an application rate of 4 pounds/gallon, 3 gallons/acre; initial residues immediately after treatment in California averaged 684 ppm (or 57 ppm/pound applied per acre); 1,668 ppm (or 139 ppm/pound) in Michigan; 1,464 ppm (or 122 ppm/pound) in North Carolina; and 1,332 ppm or (111 ppm/pound) in Texas. After two weeks, residues in the four locations averaged 26 to 34 ppm/pound per acre. After 16 weeks, all residues had declined to an average 3 ppm/pound applied per acre.

Bauer et al. (71) treated grass species indigenous to Victoria County, Tex., with 2 pounds/acre 2,4,5-T ester. One month after application the concentration averaged 4,060 ng/g (ppb) for 2,4,5-T acid and 2,890 ng/g (ppb) for 2,4,5-T ester. Six months after application the concentration averaged 60 and 170 ng/g (ppb) for 2,4,5-T acid and ester, repectively.

Getsendaner and Hummel (72)[1] described a 1969 study in which a 2,4,5-T propylene glycol butyl ether ester formulation was sprayed on Texas grass at an application rate equivalent to 12 pounds of 2,4,5-T per acre; this rate was 6 to 24 times the usual rate applied to grazing lands for brush control. At this time, manufacturing specifications for no detectable TCDD in 2,4,5-T used a method sensitive to 1ppm. The authors found that residues of TCDD decreased rapidly from about 500 ppt TCDD within one day of application, to about 35 ppt TCDD after four weeks, and about 15 ppt TCDD after 16 weeks. The TCDD decrease roughly paralleled the loss of 2,4,5-T from the same grass.

(6) *Humans.* Matsumura (73) studied 2,4,5-T in the blood and urine of human male volunteers who had ingested the chemical. After ingesting 150 mg (2.2 mg/kg), the plasma concentration of 2,4,5-T in one subject reached a peak of 21.1 μg/ml after four hours. A linear, semi-logarithmic concentration-time curve (a gradient of −0.065) four hours post-treatment indicated first order elimination and absorption kinetics.

In a second part of this study, Matsumura gave two male volunteers single oral doses of 100 mg 2,4,5-T. Urine samples were collected over 72 hours. About 45 percent of the orignial dose was found in urine collected during the first 24 hours after treatment; 60 percent had been recovered 36 hours after treatment; and after 72 hours, more than 80 percent of the original dose of 2,4,5-T had been recovered.

Gehring et al. (74) also studied the fate of 2,4,5-T following oral administration to man. Five male volunteers, ages 31 to 58 years, each ingested a single 5 mg/kg oral dose of analytical grade 2,4,5-T, with a purity greater than 99 percent and less than the detectable level (0.05 ppm) TCDD, directly or as a slurry in milk. Blood, urine and feces were collected at intervals for up to 96 hours after ingestion. Essentially all (88.5±5.11 percent) of the 2,4,5-T ingested by these subjects was excreted unchanged in the urine after 96 hours. The plasma 2,4,5-T concentration increased rapidly following ingestion and after 7 hours reached a peak of approximately 57 μg/ml, after which the plasma contained 65 percent of the 2,4,5-T in the body, of which 99 percent was bound reversibly to protein.

Kohli et al. (75) also studied absorption and excretion of 2,4,5-T in man. Eight male volunteers, age 25 to 35 years, received a single oral dose (2, 3, or 5 mg/kg) analytical grade 2,4,5-T with a purity greater than 99 percent. Urine was collected up to 96 hours, and blood samples were collected up to 168 hours. 2,4,5-T was detected in some two-hour urine samples, indicating rapid excretion of the compound. More than half of the 2,4,5-T was excreted in the urine in the first 48 hours, although small quantities were still being excreted at 96 hours.

2,4,5-T appeared in all plasma samples 1 hour after 2,4,5-T ingestion, indicating rapid absorption. Maximum concentration (approximately 25 μg/ml for the 5 mg/kg dose) was reached between 7 and 24 hours after ingestion and began to decline at a first-order rate after 32 hours.

These investigators concluded that 2,4,5-T was readily absorbed from the gastrointestinal tract, that it was eliminated unchanged in the urine, and that the half-life for plasma clearance was 18.8±3.1 hours. These authors pointed out that, in general, higher recoveries were reported by Gehring et al. (74) who used an electron capture detector, instead of the flame-ionization detector used in their study.

The National Human Monitoring Program for Pesticides, through its cooperative arrangement with the Health and Nutritional Examination Survey II (Hanes II project), is currently analyzing human urine samples for silvex, 2,4,5-T, and 2,4,5-TCP (66). The survey is scheduled for completion in 1979, but some extremely tentative results are available. No quantifiable 2,4,5-T residues have been detected in the first 400 samples; however, trace amounts (<10 ppb) have been found in a few samples.

Dougherty and Piotrowska (177) reported on screening of human urine for environmental contamination with toxic residues by negative chemical ionization mass spectrometry. The procedure is based on solvent extraction with minimal clean-up followed by examination with negative chemical ionization mass spectrometry for organochlorine residues and related compounds with masses greater than 130 daltons. Urine for the screening procedure was obtained from students at Florida State University (28 dorm residents; 21 football team members; and 11 swimming team members). The authors reported that the limited survey of human urines indicates contamination of the subjects with 2,4,5-T, pentachlorophenol, other polychlorophenoxy acids, and numerous unknown compounds. The authors indicated that 2,4,5-T was found in 36 percent (9/25) of the dorm residents; 24 percent (4/11) of the football team; and 9 percent (1/11) of the swimming team. The authors attempted to define the source of the contamination by applying the same screening procedure to environmental substrates and suggested the food chain (beef fat in the case of 2,4,5-T) as one significant source of the contamination.

(7) *Animal products.* Kocher et al. (76) surveyed beef fat from cattle grazing on land where 2,4,5-T had been applied to determine if TCDD was present in this tissue. None of the 2,4,5-T samples used were available for analysis for TCDD conten... The authors did not know whether the samples were produced before 1972 (when maximum allowable TCDD content was 1 ppm) or after 1972 (when maximum allowable TCDD content was 0.1 ppm). None of the 16 samples from Sugarland (Tex., Mo., and Okla.), showed TCDD residues when analyzed by a gas chromatography-mass spectrometry detection technique (detection limits: 3 to 6 ppt). Three of the eight samples from Mertson, Tex., where animals had grazed for 30 days in a fenced pasture sprayed in its entirety with 2,4,5-T, gave positive responses at the detection limit of 3 to 4 ppt TCDD.

In another surveillance study, Mahle et al. (77) analyzed milk from cows grazing on grass treated with 2,4,5-T in accord with normal agricultural practices. Twenty-five samples were collected from different farms in Oklahoma, Arkansas, and Missouri; these areas were selected as representative of those where 2,4,5-T is used to control broadleaf weeds and brush in pasture and rangeland. Milk purchased in Midland, Mich., an area where 2,4,5-T is not used, provided control samples. Based on gas chromatography-mass spectrometry data (detection limit: 1 ppt), the authors stated that control samples were indistinguishable from the samples from treated areas and concluded that TCDD was not present.

The residue levels reported in animal products in the studies cited below were obtained in laboratory feeding studies and not from animals grazing on pastures and rangelands treated at dosage rates recommended on registered product labels. Nevertheless, residues obtained in these feeding studies could occur in the environment and at these same levels since animals grazing on forage plants immediately after treatment at recommended rates of application could ingest 2,4,5-T in amounts similar to those fed in the studies.

Leng (37) found no residues greater than 0.05 ppm in milk or cream of cows ingesting 10 to 30 ppm 2,4,5-T. At 100 ppm 2,4,5-T in the diet, traces of 2,4,5-TCP (0.06 ppm) were found in milk and cream. When the diet contained high levels of 2,4,5-T, equivalent to 300 and 1,000 ppm in the total diet, residues of 2,4,5-T and 2,4,5-TCP ranged from 0.15 to 0.5 ppm in milk of individual cows.

Leng (37), reporting on residues in meat and meat byproducts, stated that calves slaughtered after ingesting 300 ppm 2,4,5-T in total diet contained average residues of 0.12 to 0.28 ppm in muscle, fat, and liver, and 3.3 ppm in kidney. Animals fed 900 to 2,000 ppm 2,4,5-T in the total diet and slaughtered without withdrawal had proportionally higher average residues in tissue. No residues were detected (detection limit: 0.05 ppm) in most tissues when animals were given untreated feed for one week after they had been on the highest levels (1,800 and 2,000 ppm) of 2,4,5-T for 4 weeks. Residues of 2,4,5-T declined rapidly in tissues as soon as animals started to eat untreated feed.

Clark and Palmer (78) found 0.08 ppm 2,4,5-T in omental fat of each of two sheep given four oral doses of either 0.15 or 0.75 mg/kg of the propylene glycol butyl ester of 2,4,5-T. They also found 368 ppm 2,4,5-T in kidneys of animals killed by 4 daily 250 mg/kg doses of a 2,4,5-T ester.

Clark et al. (36) found 2,4,5-T no higher than 0.05 ppm in muscle or fat of sheep held one week on untreated feed. Residues

[1] Studies submitted by registrants as part of petitions for residue tolerances are classified confidential, pending outcome of litigation in U.S. District Court.

of the metabolite 2,4,5-TCP were not detected in the fat of any of the animals. They also found that the 2,4,5-T level in liver and kidneys was less than 0.05 ppm after the animals were on untreated feed for 7 days.

Leng (*79*) found low levels of 2,4,5-T in muscle and fat of calves receiving 300 to 900 ppm 2,4,5-T in the diet and much higher residues in tissues of animals fed 1,800 ppm 2,4,5-T for 28 days. Calves fed 300 ppm 2,4,5-T showed 0.12 and 0.28 ppm 2,4,5-T in muscle and fat, respectively. Calves fed 900 ppm showed 0.24 and 0.38 ppm in muscle and fat, respectively. And at 1,800 ppm in the diet, calves showed 1.2 and 2.0 ppm in muscle and fat, respectively. In this same study, Leng found relatively low residues of 2,4,5-T in liver at feeding levels of 300 and 900 ppm (0.2 and 1.0 ppm, respectively) and sharply increased residues (7.9 ppm) at 1,800 ppm, indicating that the threshold level may have been exceeded at this higher dosage level. Residues of 2,4,5-T in kidney appeared to be proportional to the level in the diet.

Eighty-five samples of beef fat were analyzed for TCDD content under the auspices of the EPA Dioxin Implementation Plan (see section II). These beef fat samples included 18 samples from control areas and 67 samples from areas previously treated with 2,4,5-T. None of the 18 control samples had detectable amounts of TCDD at a detection limit of 10 ppt. Of the 67 samples from areas previously exposed to 2,4,5-T, one showed a positive TCDD level of 60 ppt; two appeared to have TCDD at 20 ppt; and five may have had TCDD levels which ranged from 5 to 10 ppt. The values for these five samples were at or below the limits of detection of 10 ppt. Forty-three beef liver samples were analyzed and showed no TCDD residues at a detection limit of 10 ppt.

(8) *Food.* Evidence that very little 2,4,5-T gets into food is seen in results of Market Basket Surveys conducted by the Food and Drug Administration (FDA). Of the 134 total diet samples involving 1,600 food composites (Market Basket Survey) analyzed from 1964 through April 1969, only three contained 2,4,5-T. Two were dairy products containing 8 to 13 percent fat with 0.006 and 0.19 ppm in the fat. A single meat, fish, and poultry composite from Boston consisting of 17 to 23 percent fat was found to contain 0.003 ppm 2,4,5-T on a fat basis (*81, 82, 83, 84*).

FDA Market Basket Survey samples from 1969 through July 1974 showed no 2,4,5-T residues (detection limit: 0.02 ppm) in 155 total diet samples involving 1,869 food composites (*85, 86, 87, 88, 89*).

(9) *Human exposure via industrial accidents.* There have been a number of industrial accidents during manufacture of chlorinated phenols that have resulted in human exposure to TCDD.

Whiteside (*90*) reported on a 1949 explosion at a chemical plant producing 2,4,5-T in Nitro, W. Va. The release of intermediate chemicals led to 228 cases of chloracne among exposed workers. Whiteside stated that symptoms of affected workers included skin eruptions, shortness of breath, intolerance to cold, palpable and tender liver, loss of sensation in extremities, damage to peripheral nerves, fatigue, nervousness, irritability, insomnia, loss of libido, and vertigo.

Goldmann (*91*) reported on a 1953 accident at a 2,4,5-TCP production plant in Germany. Temperature and pressure rose explosively in the autoclave, forming previously unknown, very toxic chlorinated hydrocarbons; 42 persons contracted serious cases of dermatitis, in which 14 persons suffered consequent damage to internal organs, and seven persons experienced disturbances of the nervous system. A similar accident occurred in Amsterdam in 1963 when an explosion in a 2,4,5-T factory resulted in 50 workers contracting chloracne (*90*).

In 1954, 31 workers in a Hamburg, Germany, chemical plant producing 2,4,5-T from technical 2,4,5-TCP contracted chloracne (*16, 16, 93*) and suffered the physical and psychological symptoms associated with it (*93*). Kimmig and Schuls (*16*) extensively investigated the workers' conditions and conducted experiments treating the skin of a rabbit's ear with chemicals to which workers had been exposed. These researchers tentatively identified the causative agent of the chloracne as TCDD. Bauer et al. (*15*) conclusively identified TCDD as the cause of chloracne.

In 1964 workers in a 2,4-D and 2,4,5-T plant in the United States developed chloracne (*93, 94*). Bleiberg et al. (*94*) found evidence of porphyria cutanea tarda (PCT) of varying degrees of severity in 11 out of 29 workers. PCT had never before been described as related to chloracne, nor had it been ascribed to industrial exposure in the United States. The authors stated that either the finished chemicals or some intermediate were responsible for both diseases.

The Fine Chemicals Unit of Coalite and Chemical Products Ltd. located at Bolsover, Derbyshire, in England had been producing 2,4,5-TCP for nearly 3 years without incident when an explosion occurred at midnight on April 23, 1968. As a result of this exothermal reaction, TCDD had accidentally been produced. Workers at this plant where accidentally exposed to TCDD, and 79 cases of chloracne were recorded, many of them severe (*9, 95*).

Beginning in May 1971 an accidental poisoning episode occurred in the United States that affected humans, horses, and other animals. The exposure was related to the spraying of waste oil, contaminated with TCDD, on riding arenas to control dust. Three days after spraying, sparrows and other birds were found dead on the arena floor. Of 85 horses exercised within the arena, 62 became ill, and 48 died. The first horse died on June 20, 1971. Horses continued to die as late as January 1974. Human illnesses were less severe, but did include one case of hemorrhagic cystitis in a 6-year-old girl who frequently played in the arena. Analysis showed the arena contained 31.8 to 33.0 μg/g TCDD (*96, 97, 162*).

Beale et al. (*98*) presented follow-up information on the 6-year-old girl involved in this accidental poisoning. These authors stated that the girl's symptoms resolved in three to four days and did not recur. Results of a repeat voiding cystogram three months later appeared normal. Cystoscopy at this time did, however, demonstrate numerous punctate haemorrhagic areas in the bladder, especially in the region of the trigone. Five years later, an investigation showed that this girl had grown normally; results of a physical examination, including a detailed neurological examination, were normal. Cystogram and liver-function tests were also normal, as was the urinary excretion of uroporphyrins, coproporphyrins, and thyroid function.

On July 10, 1976, an accident at the ICMESA chemical plant in the Seveso Region of Italy released 2 to 10 pounds of TCDD over a wide area (*96, 98, 100*). Hundreds of animals died, many area residents reported skin disorders, and an area of 110 hectares was evacuated (*101*). Reports of the immediate symptoms and indications of many long-term effects are just becoming available.

Seveso inhabitants initially experienced numerous, burnlike skin lesions which gradually receded; Whiteside (*90*) believed this type of lesion was probably due to direct contact with the sodium hydroxide and phenolic components of the fallout. Two and a half months after the explosion, however, children and young people in the zone most affected by the fallout developed symptoms of true chloracne, a sign of dioxin poisoning, on their faces, arms, and bodies. By November 1976, 28 people had developed confirmed cases of chloracne, and the number rose to 38 by December 1976; one year later, the number of confirmed cases of chloracne was 130.

A number of Seveso women were pregnant at the time of the accident. Whiteside (*90*) reported that the number of legal and illegal abortions performed after the accident probably totalled 90. Results of a survey by an epidemiological commission showed that 183 babies were delivered in the two months following the accident and that there were 51 spontaneous abortions as distinct from induced abortions (approximately double the rate of spontaneous abortions previously reported for the area). Whiteside (*90*) reported that eight cases of birth abnormalities have been noted to date among babies born to women in the Seveso area who were pregnant at the time of the explosion. Physicians in the Seveso area have had difficulty relating this directly to the explosion, however, since this incidence of birth abnormalities was not disproportionate to the usual incidence of abnormal births.

H. *Tolerances.* There are no tolerances established for 2,4,5-T in or on food crops. Likewise, no tolerances have been set specifically for TCDD in or on food crops. However, 40 CFR 180.202 does establish a tolerance of 0.05 ppm for hexachlorophene on cotton seed (a nonhuman dietary food item), with a stated limitation that the technical grade hexachlorophene used in the formulation shall not contain more than 0.1 ppm TCDD. The limitation does not constitute a tolerance (*102*).

I. *Pesticide episode reports system (PERS).* EPA's Pesticide Episode Response Branch of the Office of Pesticide Programs maintains a Pesticide Episode Reports System (PERS) which collects reports of pesticide exposure affecting humans, domestic animals, livestock, and wildlife (*103*). According to their records, there were 96 episodes from 1966 to April 1977 involving 2,4,5-T.

Many of these 96 episodes recorded effects in more than one area of the environment. Plant damage was reported 60 times, effects on humans 16 times, water contamination 14 times, effects on domestic animals and soil contamination 7 times each, general environmental contaminations 3 times, and fish kills and complaints against use of 2,4,5-T twice each.

There was substantial evidence in 13 of the 96 episodes linking 2,4,5-T to the episode's effects; there was circumstantial evidence in 20 of the episodes for involvement of 2,4,5-T; there was insufficient evidence in 62 of the episodes to prove or disprove involvement of 2,4,5-T; and one episode had no verification status listed.

Of the 13 episodes for which there was substantial evidence linking 2,4,5-T to the

17858

episode's effects; two involved humans (including one suicide); 2,4-D was also involved in both episodes. Three episodes involved plant damage from drift of herbicides; 2,4,5-T residues were found in plant samples in two episodes; 2,4-D was also involved in one of these episodes. Two episodes involved fish kills resulting from accidental spills into streams, with 2,4-D involved in both incidents; in one of these episodes, 6,000 fish (90 percent juvenile salmon) were killed; residues of both 2,4-D and 2,4,5-T were found in these fish. Two incidents involved soil contamination when two warehouses were destroyed by a tornado and fire; many other pesticides were involved in both instances. Two episodes involved domestic animals; in one, 24 cows died after herbicide application. Arsenic residues were found in two cows, and arsenic contamination of the herbicide mix was suspected. In the other instance, 8 cows drank water contaminated with 2,4,5-T; residue levels of 0.03 and 0.02 ppm were found in the milk five and eight days, respectively, after the incident. Two hundred and forty gallons of milk were dumped. One incident involved water contamination as a result of a warehouse fire; many other pesticides were also involved.

II. Regulatory History

2,4,5-T was developed during World War II and was first registered as a pesticide on March 2, 1948 (3). Since then, it has been the subject of several Federal regulatory actions.

On April 13, 1966, the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) published an announcement in the FEDERAL REGISTER abolishing the "No Residue and Zero Tolerance" concepts as scientifically untenable. Future registrations would be granted on the basis of either "Negligible Residue" or "Permissible Residue." Industry was given until December 31, 1967, to comply by obtaining tolerances for residues of 2,4,5-T in all treated food, feed products, and byproducts (in addition no registrations would be continued beyond December 31, 1970).

Following this action, a series of Pesticide Registration (PR) Notices were issued over several years, extending certain "no residue" and "zero tolerance" registrations beyond the December 31, 1967, deadline for obtaining residue tolerance. (These and all following PR Notices are cited in Reference 104.) Among uses of 2,4,5-T extented beyond the deadline were uses on pasture grasses and rangeland; on apples (McIntosh), blueberries (low bush), cereal grains (undesignated), rice, and sugarcane; and in lakes and ponds.

PR Notice 70-8 issued by the USDA on March 10, 1970, identified data needs for certain compounds. 2,4,5-T was identified as one of the compounds requiring further teratogenic studies.

PR Notice 70-11 published on April 20, 1970, suspended 2,4,5-T products bearing certain directions for use. The suspended uses were all uses in lakes, ponds, or on ditch banks; and liquid formulations for use around the home, recreation areas, and similar sites.

PR Notice 70-13 issued by the USDA on May 1, 1970, cancelled 2,4,5-T products bearing certain directions for use. The cancelled uses were all granular 2,4,5-T formulations for use around the home, recreational areas, and similar sites; and all 2,4,5-T uses on food crops intended for human consumption.

All registrants were advised of these actions, and two of the 2,4,5-T registrants, Dow Chemical and Hercules Incorporated, exercised their right under Section 4(c) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 135 et seq.) to petition for referral of the cancellation (rice use only) to an Advisory Committee.

As provided by Section 4(c) of FIFRA (1964 amendment), a nine-member Advisory Committee of scientists was appointed to consider all relevant facts, submit a report and recommendations regarding registration for certain uses of 2,4,5-T, and state the reasons or bases for these recommendations. Their report was submitted to the Administrator of the Environmental Protection Agency on May 7, 1971 (48). The Committee recommended that use of 2,4,5-T be permitted in forestry, range land, and rights-of-way providing that the limit of 0.1 ppm of contamination with TCDD be set for all future production of 2,4,5-T; that 2,4,5-T be applied no more than once a year at any one site; and that 2,4,5-T be applied with proper caution so that it will not contaminate other areas where it may come into contact with humans.

The Committee also recommended that this action be reviewed again when existing deficiencies in information about possible magnification of TCDD in the food chain have been rectified by specific research.

In the meantime, PR Notice 70-22, published by the USDA on September 28, 1970, addressed the presence of chlorodioxin contaminants in economic poisons. This notice stated that the USDA had determined that certain toxic chlorodioxins (such as TCDD) may be present as contaminants in the basic materials used in formulating 2,4,5-T and silvex. The notice also stated that the presence of such chlorodioxins constituted a possible hazard to man since they had been found to be extremely toxic to laboratory animals, and that appropriate regulatory action would be taken under provisions of FIFRA since products containing chlorodioxins are considered to be in violation of FIFRA.

Dow Chemical obtained an injunction against EPA in July 1972, enjoining further administrative action against 2,4,5-T. The United States Court of Appeals for the Eighth Circuit overturned the injunction in 1973, and administrative proceedings were allowed to go forward.

On July 20, 1973, a notice of intent to hold public hearings on *all* uses of 2,4,5-T was filed with the EPA Hearing Clerk under section 6(b)(2) of FIFRA, as amended 1972. All federally approved uses of 2,4,5-T were to be explored in a public hearing scheduled for April 1974, following completion of an intensive monitoring program for detecting dioxin in the ppt range (38 FR 19666, July 29, 1973).

On May 10, 1974, the information hearing was expanded to include all insecticides and herbicides having 2,4,5-TCP in their manufacturing process. These included silvex, erbon, and ronnel, as well as 2,4,5-T and 2,4,5-TCP, all of which may contain TCDD.

On June 24, 1974, EPA withdrew cancellation and information-gathering proceedings initiated against 2,4,5-T and related compounds because of its inability to monitor food for TCDD residues with the necessary analytical precision. Although the 2,4,5-T notice of hearing was withdrawn, the Agency stated that it "will continue its TCDD residue monitoring program and will take such further action as it deems appropriate once the results of the monitoring project are available" (39 FR 24050 June 28, 1974).

On July 25-26, 1974, the Agency held a Dioxin Planning Conference in Washington, D.C., primarily for those parties having an interest in the withdrawn 2,4,5-T/dioxin hearings, to address data analysis and retrieval (in the areas of analytical methodology, toxicology, and monitoring) with emphasis on analytical methodology for TCDD at the ppt level. As a result, the Agency established a Dioxin Implementation Plan (DIP) intended to identify a preferable analytical methodology to monitor human and environmental samples for TCDD.

On-going TCDD studies under the DIP include: An analytical method validation study to produce statistically defensible data; monitoring for residues in human milk in the Pacific northwest; additional beef fat residue studies; additional technical pesticide residue studies; and an environmental monitoring program for TCDD residues in soil, water, and biota.

III. Summary of Scientific Evidence Relating to Rebuttable Presumption

The following adverse effects of 2,4,5,-T and/or TCDD have been found to exceed the criteria for issuance of a rebuttable presumption as stated in § 162.11 of the Code of Federal Regulations (CFR 40). Because of industry's apparent inability to produce 2,4,5-T without TCDD contamination, none of the studies cited are for pure 2,4,5-T. The effects of TCDD must also be considered when assessing 2,4,5-T by the Agency's risk criteria.

A. *Oncogenic effects.* 40 CFR 162.11(a)(3)(ii)(A) provides that a rebuttable presumption shall arise "if a pesticide's ingredient(s) * * * (i)nduces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation or dermal exposure * * *." Section 162.3(bb) defines the term oncogenic as "the property of a substance or a mixture of substances to produce or induce benign or malignant tumor formation in living animals."

The studies summarized below indicate that 2,4,5-T containing less than .05 ppm TCDD and/or TCDD alone have oncogenic effects in two mouse strains and one rat strain. Since 2,4,5-T, as currently formulated, contains TCDD (at a maximum amount of 0.099 ppm), a rebuttable presumption against the registration of 2,4,5-T products has arisen because of the oncogenic effect of 2,4,5-T and its contaminant, TCDD.

(1) 2,4,5-T—(a) *Effects of dietary 2,4,5-T (<0.05 ppm TCDD) on rodents.* In their bioassay of 2,4,5-T for carcinogenicity in mice, Muranyi-Kovacs et al. (*105*) administered 2,4,5-T (containing <0.05 ppm TCDD)* to inbred C3Hf and XVII/G mice. The mice were given 100 mg/liter of 2,4,5-T in the drinking water for two months beginning at six weeks of age. During the succeeding 18 to 20 months, the mice were given 2,4,5-T mixed in the diet at a concentration of 30 ppm *ad libitum*.

In C3Hf mice, 48 percent of the treated females ($^{15}/_{31}$) and 55 percent of the treated males ($^{16}/_{29}$) developed tumors, compared with control values of 21 percent ($^{6}/_{28}$) and 49 percent ($^{21}/_{43}$), respectively (Table 2). The differences between the number of tumors observed and the number expected were sig-

*This TCDD level is less than the 0.1 ppm TCDD currently found in most commercial formulations (see Section I.B).

DOW 1695290

nificant for female mice at all sites ($p<0.03$) and for the combined sexes ($p<0.01$).[7] For non-incidental tumors, the differences were significant for each sex and the combination; no significant differences were found in incidental tumors.[8] No other strain-sex combination yielded statistically significant values (*106*). Rare types of tumors, not seen in the control animals, were observed in the treated C3Hf females.

A decrease in survival time for mice with tumors was noted in both male and female treated C3Hf mice when compared with controls. C3Hf treated male mice survived an average of 511 days compared with 630 days for control male mice. According to the evaluation by EPA's Carcinogen Assessment Group (CAG). (*106*), this difference was significant ($p<0.001$). Treated female C3Hf mice survived 620 days compared with 680 days for control females. Chemically induced oncogenic effects typically show long latency periods. The finding of reduced longevity among treated animals as compared with controls complicates the assessment of the potential oncogenic effects of 2,4,5-T.

In XVII/G mice, 84 percent of the treated females (16/19) and 75 percent of the treated males (15/20) developed tumors, compared with control values of 53 percent (21/40) and 78 percent (25/32), respectively.

An increase in survival time for mice with tumors over controls was noted among the XVII/G treated animals. There was an average survival time of 583 days for treated male mice compared with 521 days for control male mice. Treated females survived 641 days compared with 569 days for control females. According to CAG (*106*), the difference was significant ($p<0.01$) in females.

(b) *Effects of subcutaneous injection and oral administration of 2,4,5-T (30 ppm TCDD) on rodents.* Innes et al. (*107*) studied the tumorigenicity of 2,4,5-T, containing about 30 ppm TCDD, in two hybrid strains of mice, designated as "X" and "Y", after oral or subcutaneous administration of the maximum tolerated dose (table 3). The testing was performed at Bionetics Research Laboratores, under contract from the National Institutes of Health. Results of the studies were calculated comparing treated groups with matched and pooled controls.[9]

In the subcutaneous study, mice were given a single injection of 21.5 mg/kg of 2,4,5-T in a dimethyl sulfoxide (DMSO) solution at approximately 18 months of age. Seventeen percent (5/30) of the treated "Y" males developed pulmonary adenomas, compared with 1 percent (1/91) of the matched controls and 3 percent (5/188) of the pooled controls. This increased incidence of pulmonary adenomas was significant relative to both control groups ($p=0.024$ matched and $p=0.04$ pooled) (*106*).

In the oral study, 21.5 mg/kg of 2,4,5-T in gelatin was administered daily by stomach tube, beginning at 7 days of age. After weaning, 60 ppm of 2,4,5-T was mixed in the diet and provided ad libitum until the end of the study at approximately 18 months. Gross and histological examinations were made of all major organs and visible lesions; thyroid glands were not examined. According to CAG's evaluation (*106*), there were no significant differences between 2,4,5-T treated and control groups of mice with respect to tumors at specific sites or total number of tumor-bearing animals.

Table 2. Oncogenic Effects of 2,4,5-T on Mice[a/]

Strain	Sex	Dietary Level (ppm)[b/]	Mean Survival Time (days)	Mice with Leukemia and Lung and Liver Tumors No/Total No[c/]	%	Incidence of Tumors Total	Lung	Liver	Leukemia	Other
C3HF	M	0	630	21/43	49	22	2	19	—	1[d/]
		80	511[e/]	12/22	55	13	—	10	2	1[f/]
	F	0	680	9/44	21	9	5	3	1	—[g/]
		80	620	12/25	48	13	—	8	3	2[h/]
XVII/G	M	0	521	25/32	78	27	22	4	—	1[i/]
		80	583	15/20	75	16	14	—	1	1[j/]
	F	0	569	21/40	53	24	20	—	2	2[k/]
		80	641[l/]	16/19	84	16	15	—	1	—

a/ Data from Muranyi-Kovacs (105).
b/ Estimated daily oral dose = 12 mg/kg body weight.
c/ Effective number of mice are mice surviving longer than 300 days or developing a tumor before 300 days of age.
d/ Pleomorphic salivary gland tumor.
e/ Fibrosarcoma; one hyperplastic urinary bladder and one hyperplastic forestomach not included.
f/ One osteogenic sarcoma; two sarcomas; two cutaneous tumors; one cervical tumor.
g/ Forestomach tumor.
h/ Urinary bladder papilloma; two hyperplastic lesions of urinary bladder not included.
i/ Two hemangiomas.
j/ $p < 0.01$ compared with controls.
k/ $p < 0.001$ compared with controls.

Table 3. Tumors in Mice Ingesting 2,4,5-T

Strain	Sex	Dose (ppm)	Mice with Tumors No/Total No	%	Mice with Specific Tumors: Reticulum Cell Sarcoma	Tumor Type Pulmonary Adenoma & Carcinoma	Hepatoma
X	M	0	5/15	33	0	2	3
		(matched)					
		0	22/79	28	5	5	8
		(pooled)					
		60	6/18	33	1	1	4
	F	0	2/18	11	1	1	—
		(matched)					
		0	8/87	9	4	3	—
		(pooled)					
		60	1/81	6	—	1	—
Y	M	0	3/18	17	—	3	—
		(matched)					
		0	16/90	18	1	10	5
		(pooled)					
		60	3/18	17	2	—	1
	F	0	1/15	7	1	—	—
		(matched)					
		0	7/82	9	3	3	1
		(pooled)					
		60	2/18	11	1	—	—

[7] The investigators found no significant sex-related differences.

[8] Incidental tumors are tumors discovered at necropsy of an animal which died from some other cancer; nonincidental tumors are tumors diagnosed during life or which caused the death of the animal.

[9] Because this was a large scale screening study, several control groups were used. No significant differences were found among these groups.

(2) *TCDD.*—(a) *Oncogenic effects of low levels of TCDD on rodents.* Van Miller et al. (*109*) recently reported the results of a 2-year feeding study with male Sprague-Dawley rats. Ten groups of ten animals per group were fed ground chow containing 0, 1, 5, 50, or 500 ppt (=10^{-12} gram TCDD/gram food), and 1, 5, 50, 500, or 1,000 ppb (=10^{-9} gram TCDD/gram food) TCDD.

Food intake (10=±4g/day) was significantly lower in rats ingesting the three highest dose levels (50,500, or 1,000 ppb TCDD) than in controls (21±2 g/day), and none of the rats in these three groups gained weight after the start of the experimental diet. All rats receiving these three dose levels died between the second and fourth week of treatment.

On the other hand, food intake for rats on other dose levels was similar to controls (20±2 g/day). Weight gain was significantly less for rats given 5 ppb TCDD (391±54 g) as compared to controls (531±44 g). In these seven groups only one animal died before the 30th week, and that death occurred in the 500-ppt group at the 17th week. In the 5- and 1-ppb groups, all animals died by the 90th week of the experiment. Table 4 shows the mortality figures for all groups.

TABLE 4.—*Mortality in rats ingesting various levels of TCDD*

Dose	Week of 1st death	Number of rats dead at 95th week[a]
0[b]	68	6/10 (60%)
1 ppt[c]	86	2/10 (20%)
5 ppt[d]	33	4/10 (40%)
50 ppt[e]	69	4/10 (40%)
500 ppt[f]	17	5/10 (50%)
1 ppb[g]	31	10/10 (100%)
5 ppb[h]	31	10/10 (100%)
50 ppb[i]	3	10/10 (100%)
500 ppb[j]	2	10/10 (100%)
1,000 ppb[k]	2	10/10 (100%)

[a] Surviving animals sacrificed at 95 weeks.
[b] Control group. Diet contained no TCDD.
[c] Approximate weekly dose was 0.0003 μg/kg body weight.
[d] Approximate weekly dose was 0.001 μg/kg body weight.
[e] Approximate weekly dose was 0.01 μg/kg body weight.
[f] Approximate weekly dose was 0.1 μg/kg body weight.
[g] Approximate weekly dose was 0.4 μg/kg body weight.
[h] Approximate weekly dose was 2.0 μg/kg body weight.
[i] Approximate weekly dose was 24 μg/kg body weight.
[j] Approximate weekly dose was 240 μg/kg body weight.
[k] Approximate weekly dose was 500 μg/kg body weight.

Laparotomies were performed on all rats surviving through the 60th week, and all tumors observed were biopsied. Rats were maintained on these diets until the 78th week and were then placed on the control diet. Surviving animals were killed at 95 weeks. Complete necropsies were done at death or sacrifice, and tissue samples were microscopically examined. Special staining methods were used to "aid in the diagnosis of neoplasms."

Tumorigenic and toxic effects were observed in rats in the six lowest dose groups. The overall incidence of neoplasms in the six experimental groups was 38 percent (23/60), compared with 0 percent (0/10) in the control group. The difference is statistically significant (*106*). Neoplastic nodules and cholangiocarcinomas of the liver were observed in 40 percent (4/10) of the rats ingesting 5 ppb TCDD; two animals had both neoplastic nodules of the liver and cholangiocarcinomas. One rat (10 percent) in the 1 ppb group had hepatic carcinoma compared to none of the controls. Hepatic tumors were not found in other dose groups (table 5).

Table 5. Liver Tumors in Rats Ingesting TCDD[a]

Dose (ppb)	Rats With Neoplastic Nodules		Rats With Cholangio-carcinomas		Rats With Nodules plus Carcinomas	
	No.	%	No.	%	No.	%
0	0/10	0	0/10	0	0/10	0
1	0/10	0	1/10	10	1/10	10
5	4/10	40[b]	2/10	20[b]	4/10	40[b]

a/ Data from Van Miller (109).
b/ Two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

Tumors developed in 46 percent (23/50) of the rats ingesting 5, 50, or 500 ppt and 1 or 5 ppb TCDD, compared to none (0/10) in the control rats. Van Miller et al. noted that "nineteen (57 percent) (sic—Agency calculation is 54 percent (19/35)) of the animals that died in the six groups fed subacute levels of TCDD had neoplastic alternations." Carcinomas were observed in the ear duct, kidney, and liver. Three retriperitoneal histiocytomas were described as metastasizing to the "lungs, kidney, liver, and skeletal musculature." According to CAG's evaluation (*106*), statistically significant increases in tumors at all sites were found in rats fed 5, 500, 1,000, and 5,000 ppt as compared with control animals (p=0.05) (table 6). Three of the ten deaths which occurred in the 5-ppb dose group were attributed to aplastic anemia. One animal in the 500-ppt group had a severe liver infarction.

Dow Chemical U.S.A. (*110*) has provided EPA with a preliminary report of a study of TCDD's chronic toxic effects in Sprague-Dawley rats. Groups of 50 rats of each sex were fed 0.1, 0.01, or 0.001 μg TCDD/kg body weight daily for 2 years. To provide these dose levels, the concentrations or TCDD in the diet were approximately 2,200, 210, and 22 ppt. Eight-six animals of each sex were used as controls.

Table 6. Total Tumors in Rats Ingesting TCDD[a]

Dose[b]	Tumors			Rats With Tumors	
	Benign	Malignant	Total	No.	%
0	0	0	0	0/10	0%[c]
1 ppt	0	0	0	0/10	0%
5 ppt	1	5	6[d]	5/10	50%[a]
50 ppt	2	1	3[f]	3/10	30%
500 ppt	2	2	4[g]	4/10	40%[h]
1 ppb	0	5	5[i]	4/10	40%
5 ppb	8	2	10[j]	7/10	70%

a/ Data from Van Miller (109).
b/ Rats administered 50, 500, and 1,000 ppb were all dead within four weeks.
c/ Forty male rats used as controls for another study that were received at the same time and kept under identical conditions did not have neoplasms when killed at 18 months.
d/ One rat had ear duct carcinoma and lymphocytic leukemia. The following tumor types were each observed in one rat: adenocarcinomas (kidney), malignant histiocytoma (retroperitoneal), angiosarcoma (skin), and Leydig cell adenoma (testis).
e/ Three rats died with aplastic anemia.
f/ The following tumor types were each observed in one rat: fibrosarcoma (muscle), squamous cell tumor (skin), and astrocytoma (brain).
g/ The following tumor types were each observed in one rat: fibroma (striated muscle), carcinoma (skin), sclerosing seminoma (testis), and adenocarcinoma (kidney).
h/ One rat had a severe liver infarction.
i/ One rat had cholangiocarcinoma and malignant histiocytomas (retriperitoneal). The following tumor types were each observed in one rat: angiosarcoma (skin), glioblastoma (brain), and malignant histiocytoma (retroperitioneal).
j/ One rat had squamous cell tumor (lung) and neoplastic nodule (liver). Two rats had cholangiocarcinoma and neoplastic nodule (liver). Three rats had squamous cell tumors (lung). One rat had neoplastic nodule.

Dow 1695592

17401

Dow (*110*) reported "discernible increases" in the incidence of hepatocellular carcinomas of the liver and of squamous cell carcinomas of the lung, hard palate/nasal turbinates, and tongue in rats at 0.1 μg/kg. They also reported decreased incidences of pituitary, uterine, mammary gland, pancreatic, and adrenal gland tumors at this dose level. Dow also reported that this dose level produced increased mortality, decreased body weight gain, and changes in blood chemistry values which suggested severe toxicity. Hepatocellular nodules and alveolar hyperplasia were observed in the 0.01 μg/kg group. A squamous cell carcinoma of the hard palate was observed in one female receiving this dose; Dow considered this unrelated to TCDD treatment because a similar tumor occurred in "other concurrent studies." At 0.001 μ/kg there were no "discernible effects in male rats and an increased incidence of [reversible] swollen hepatocytes in female rats."

Dow's preliminary report does not include control data, quantitative data on tumor incidence, or statistical analyses. CAG has not evaluated this study. Table 7 describes the available tumor information. Dow has submitted the final report for this study, which CAG is currently reviewing.

Table 7. Tumors in Sprague-Dawley Rats Ingesting TCDD[a/]

Dose		
ug/kg/day	ppt	Tumors
0	0	----
0.001	22	----
0.01	210	Hepatocellular Nodules
		Squamous Cell Carcinoma[b/]
		Alveolar Hyperplasia
0.1	2,220	Hepatocellular Carcinoma[c/]
		Squamous Cell Carcinoma[d/]

a/ Data from Dow Chemical USA (110), a preliminary report.
b/ Hardpalate squamous cell carcinoma observed in only one female rat.
c/ Observed only in females.
d/ Squamous cell carcinoma observed in lungs, hardpalate/-nasal turbinate, or tongue.

(b) *Effects closely related to oncogenicity in test animals.* Many chemically nonreactive carcinogens are enzymatically converted to biologically active carcinogens. The enzyme aryl hydrocarbon hydroxylase (AHH) is strongly implicated in this process (*112*). For example, the incidence of bronchiogenic carcinomas in humans (*113*) and mouse sarcomas induced by 3-methyl-cholanthrene (*114*) have been related to the level of inducibility of AHH (*99*).

Kouri et al. (*114*) studied AHH induction in human lymphocyte cultures by TCDD. The authors stated, "TCDD itself is not a potent carcinogen in mice; however, the synergistic action of TCDD with 3-methylcholanthrene (MC) produces cancer in different strains of mice in direct proportion to the degree of elevation of the induced hydroxylase activity and associated cytochrome P_1-450 content." Their study showed a positive correlation between basal enzyme activity and enzyme levels maximally inducible by either TCDD or MC. They also found that TCDD is about 40 to 60 times more potent than MC as an inducer of hydroxylase activity in cultured human lymphocytes. These authors further suggested that, because of the relatively high levels of TCDD in certain parts of the world, TCDD may also present considerable long-term risk because of possible synergism in chemically initiated oncogenesis, in addition to short-term risks posed by its toxic and teratogenic properties.

The implication of TCDD in AHH inducibility has also been reported by Poland and Glover (*115, 116*) and Poland et al. (*117*). In their studies on chick embryo livers, Poland and Glover (*115*) found that all dioxins which are potent inducers have halogens at three of the four lateral ring positions and at least one nonhalogenated carbon atom. Poland and Glover (*116*) compared the potency of TCDD as an inducer of hepatic AHH with that of MC, the most commonly employed inducing agent. They stated that analysis of the data by a computer program for bioassay showed that TCDD was 28,840 times as potent as MC on a molar basis. (The 95 percent confidence interval of the potency ratio is 2.07 to $3.95\sqrt{10}$ [9]) The index of precision, [9], was 0.18. Poland et al. (*117*) suggested that a hepatic cytosol species which binds TCDD is the receptor for the induction of hepatic aryl hydrocarbon hydroxylase.

Allen et al. (*118*) conducted a study in which female rhesus monkeys were fed diets containing 500 ppt TCDD for nine months. Anemia, thrombocytopenia, and leukopenia were the most debilitating changes. The altered lymphopoiesis could be associated with immune suppresion. The authors reported widespread hypertrophy, hyperplasia, and metaplasia in the epithelium of monkeys exposed to TCDD, and related this to data showing increased tumor frequency in TCDD fed rats.

(3) *Preliminary epidemiological studies.* Two epidemiological studies lend support to a finding of increased tumorigenicity due to 2,4,5-T exposure. The English summary of a Swedish paper by Hardell (*108*) stated that "there were seven cases of malignant mesenchymal tumors in 87 persons [who had been] exposed to 2,4,5-T over a period of 10-20 years." In five of the cases, exposure had been direct and comparatively massive. The latent period of 10 to 20 years is in agreement with that assumed for chemical car-

DOW 169593

17402

cinogenesis. The statistical distribution of 7 of the 87 patients deviated from the national average with a dominance of tumors in males.

Tung (*128*) reported an elevated incidence of primary liver cancers among Vietnamese following the wide application of "Agent Orange" as a defoliant during the years 1961 to 1962. "Agent Orange" is composed of equal parts 2,4,5-T and 2,4-D (2,4-dichlorophenoxyacetic acid) and is contaminated with TCDD. During 1962 to 1968, 18 percent (791/7911) of all cancers were liver cancers, compared with 3 percent (159/5442) during 1955 to 1961. The latent period involved is shorter than that normally assumed for chemical carcinogenesis; the possibility of a shorter latent period for some chemicals, however, cannot be eliminated. Neither of these studies is sufficient to be the basis of any firm conclusions concerning a causal connection between 2,4,5-T and cancer. But in view of the results obtained in experimental animals, they warrant noting.

The Working Group concludes that there is sufficient evidence to indicate that 2,4,5-T, containing TCDD at levels as low as 0.05 ppm, and TCDD alone can produce oncogenic effects in mamalian species. Since 2,4,5-T, as currently formulated, contains TCDD (at a maximum amount of 0.099 ppm), a rebuttable presumption against registration of 2,4,5-T products has arisen because of the oncogenic effects of 2,4,5-T and TCDD.

B. *Other chronic or delayed toxic effects.* 40 CFR 162.11(a)(3)(ii)(B) provides that "a rebuttable presumption shall arise if a pesticide's ingredient(s) • • • (p)roduces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety." This section reflects concern that chronic exposure to chemicals may result in injury to the reproductive system and/or the fetus and provides that a rebuttable presumption shall arise if chronic chemical exposure in test animals produces such results.

The studies summarized below show that 2,4,5-T containing 0.5 ppm or less TCDD produces teratogenic and/or fetotoxic effects in mice at 30 mg/kg, in rats at 100 mg/kg, in hamsters at 40 mg/kg, and in birds at 1 mg/kg. Other studies show that pesticide-free TCDD is fetotoxic and/or teratogenic at doses as low as 0.125 µg TCDD/kg in rats and 0.1 µg TCDD/kg in mice. Specifically, these studies show that exposure to TCDD and/or 2,4,5-T containing TCDD during pregnancy is associated with statistically significant increases in the incidence of cleft palate, kidney anomalies, skeletal and intestinal tract anomalies, and embryonic resorption. (Maternal toxicity has also been observed in many of these studies, primarily in the form of reduced weight gain and increased liver-to-body weight ratio. Whenever it has appeared particularly relevant, details have been cited in the individual studies.)

The Working Group has concluded from these studies that 2,4,5-T containing TCDD, 2,4,5-T without detectable dioxin, and TCDD alone produce fetotoxic and teratogenic effects in mammals. The Working Group has also concluded that an ample margin of safety does not exist for the population at risk (women of child-bearing age) for dermal and inhalation exposure and for cumulative oral, dermal, and inhalation exposure to both 2,4,5-T and/or TCDD. For these reasons, the Working Group recommends issuance of a rebuttable presumption based on the fetotoxic and teratogenic effects of 2,4,5-T and/or TCDD.

(1) *Pesticide-free TCDD.* A Bionetics Research Institute study on 2,4,5-T provided the first indication that TCDD adversely affected mammalian development (*123*). In this study, detailed with later confirming studies in Section III.B.(*2*) below, 2,4,5-T significantly increased the frequency of cleft palate, kidney anomalies, and fetal mortality in the litters of treated dams. The 2,4,5-T used in this study contained approximately 30 ppm TCDD. Subsequent studies, detailed in this section, using pesticide-free TCDD have established that TCDD alone produces these effects, and that the TCDD contaminant may be the principal chemical determinant of the fetotoxic and teratogenic effects in mammals exposed to the pesticide 2,4,5-T.

(a) *Studies in which TCDD produced teratogenic and/or fetotoxic effects in mice.* Courtney and Moore (*128*) studied TCDD's embryotoxic and teratogenic effects in three mouse strains (Table 8). Test animals were administered 1 or 3 µg TCDD/kg body weight subcutaneously in solutions of 100 percent dimethylsulfoxide (DMSO) on days 6 to 15 of gestation. DMSO was administered as the control. TCDD produced cleft palates in all three strains. At 3 µg/kg, 30 percent (⅗) of the CD-1 litters had fetuses with cleft palates compared to 0 percent (%) of the controls; 71 percent (⅝) of the C57BL/6 litters had cleft palates at 3 µg/kg as compared to 0 percent (⅗) of the controls; and 22 percent (⅔) of the DBA/2 litters had cleft palates, as compared to 0 percent (⅗) of the controls. The authors also found a marked increase in the incidence of kidney anomalies in all strains. One especially sensitive strain, C57BL/6, developed kidney anomalies in 100 percent (⅙) of the litters as compared to 9 percent (⅗) in the controls. Maternal liver-to-body weight ratio was significantly increased in the inbred strains, C57BL/6 and DBA/2, but not in the randomly bred CD-1 mice. TCDD had no effect on fetal mortality, fetal weights, or maternal weights at the doses administered.

Table 8. Teratogenic Effects of TCDD in Mice and Rats[a/]

Strain	Dose (ug/kg)	Litters Affected/Live Litters: Cleft Palate #	%	Kidney Anomalies #	%	Average Fetuses Affected/Live Litters: Cleft Palate #	%	Kidney Anomalies #	%
Mouse									
CD-1	0(DMSO)	0/9	0	3/9	33	0/9	0	1/9	11
	1	1/9	11	5/9	56	2/9	22	4.6/9	51
	3	3/10	30	10/10	100	1/10	10	6.5/10	65
DBA/2	0(DMSO)	0/23	0	3/23	13	0/23	0	1/23	4
	3	2/29	22	8/9	89	1/9	11	1.8/9	20
C57BL/6	0(DMSO)	0/23	0	2/23	9	0/23	0	1/23	4
	3	5/7	71	7/7	100	2.6/7	37	3/7	43
Rat									
CD	0(DMSO)	0/9	0	0/9	0	0/9	0	0/9	0
	0.5	0/6	0	4/6	67	0/6	0	1.8/6	30

a/ Data from Courtney and Moore (128).

In another study in which six dioxins were administered subcutaneously and orally to CD-1 mice, Courtney (*133*) found TCDD to be the most fetotoxic and teratogenic of the dioxin compounds, by either route of exposure at all dose levels tested (Table 9). On days 7 to 16 of gestation, TCDD was administered orally at 25 to 400 µg/kg body weight and subcutaneously at 25 to 200 µg/kg.

Mortality per litter increased with the dose and reached 97 percent (oral) and 76 percent (subcutaneous) in the litters administered TCDD, as compared with a mortality of 6 and 14 percent in the oral and subcutaneous control groups, respectively. The most common anomalies observed were cleft palates and malformed kidneys. All of the fetuses in the 200 and 400 µg/kg (oral) and 200 µg/kg (subcutaneous) groups exhibited cleft palates as compared to 0 percent of the controls. Of the fetuses in the 200 µg/kg (oral) group, 100 percent had kidney malformations as compared to 1 percent of the controls. Other anomalies observed were hydrocephalus, open eye, and club foot. Edema and petechiae were also observed in fetuses administered the high doses.

Table 9. Fetotoxic and Teratogenic Effects of TCDD in CD-1 Mice[a/]

Dose (ug/kg per day)	Route of Administration	% Average Fetal Mortality/Litter	Average # Abnormal Fetuses per Litter	Abnormal Anomalies/Total Fetuses: Cleft Palate %	Kidney Anomalies %	Club Foot %
25	Oral	6	4.6	3	34	3
50	Oral	13	8.1	19	72	7
100	Oral	14	8.3	66	71	13
200	Oral	87	1.5	100	100	14
400	Oral	97	0.4	100	50	50
25	Subcutaneous	36	6.7	82	53	11
50	Subcutaneous	56	5.0	79	58	17
100	Subcutaneous	72	3.5	89	95	0
200	Subcutaneous	76	3.1	100	38	18
5% emisele corn oil (0.1 ml)	Oral	6	0.8	0	1	4
DMSO[b/]	Subcutaneous	14	0.2	0	0	1

a/ Data from Courtney (133).
b/ DMSO = dimethylsulfoxide.

DOW 1695 94

Moore et al. (*174*) also found that TCDD caused fetotoxic and teratogenic responses in C57BL/6 mice at 1 μg/kg administered on days 10 through 13 of gestation. Compared with 0 percent incidence (0/27) in the control litters, 94 percent (15/16) of the treated litters exhibited kidney anomalies, and 19 percent (3/16) had cleft palates. At 3 μg/kg, the incidence of these anomalies was 100 percent (14/14) and 86 percent (13/14), respectively.

Neubert and Dillman (*127*) tested the embryotoxic and teratogenic effects of TCDD in NMRI mice (Table 10). In one test, pregnant mice were given varying doses of TCDD (0.3 to 9 μg/kg) by intubation on days 6 to 15 of gestation. At 9 μg/kg, 100 percent (%) of the viable litters had resorptions; 67 percent (%) of all litters had total resorptions. Oil control values were 32 and 0 percent for litters with resorptions and litters with total resorptions, respectively. Cleft palate was observed in all of the litters and 82 percent of the fetuses at 9 μg/kg; comparable oil control values were 6 and 0.7 percent, respectively, statistically significant ($p<0.01$) proportions of the fetuses evidenced cleft palate at 3, 4.5, and 9 μg/kg (3, 13, and 82 percent, respectively) when compared with the oil control.

Table 10. Embryotoxic and Teratogenic Effects of TCDD on NMRI Mice[a/]

Dose[b/] (ug/kg)	Litters Affected/Viable Litters: Resorptions #	Resorptions %	Cleft Palate #	Cleft Palate %
0	23/95	24	6/95	6
oil	21/65	32	4/65	6
0.3	7/13	54	0/13	0
3.0	16/24	67	7/24	29
4.5	5/12	42	6/12	50
9.0	3/3	100	3/3	100
9.0	3/6	50	5/6	83

a/ Data from Neubert and Dillman (127).
b/ All doses administered on days 6 to 15, except second 9.0 ug/kg dose which was administered on days 9 to 13.

In this study, a single oral dose of 45 μg/kg TCDD on day 6 produced resorption in 100 percent of the viable litters; 23 μg/kg on day 10 led to 50 percent resorptions. Seventy-one percent of the viable litters had embryos with cleft palate when 45 μg/kg was given as a single dose on day 11. Control values were 24 percent for litters with resorption and 6 percent for litters with cleft palates.

Smith et al. (*135*) administered 0.001, 0.01, 0.1, 1.0, and 3.0 μg TCDD/kg body weight per day to CF-1 mice by gavage from days 6 through 15 of gestation (Table 11). The percentage of resorptions per implantation was significantly higher in treated mice than in the controls only in the 1.0 μg/kg group. Cleft palate occurred in 71 percent of the litters treated at 3.0 μg/kg and in 21 percent of the litters treated at 1.0 μg/kg; bilateral dilated renal pelvises occurred in 28 percent of the litters treated at 3.0 μg/kg, and in 5 percent of the litters treated at 1.0 μg/kg. No significant increase in either cleft palate or dilated renal pelvis was observed at 0.1, 0.01, or 0.001 μg/kg. None (0/34) of the control litters had cleft palate or abnormal kidneys.

Table 11. Fetotoxic and Teratogenic Effects of TCDD in CF-1 Mice[a/]

Dose (ug/kg)	Incidence of Cleft Palate in Litters per Live Litters #	%	Litters With Resorbed Fetuses per Live Litters #	%	Litters With Dilated Renal Pelvis per Live Litters #	%
0	0/34	0	25/34	74	0/34	0
0.001	2/41	5	30/41	73	0/41	0
0.01	0/19	0	17/19	89	0/19	0
0.1	1/17	6	16/17	94	0/17	0
1.0	4/19	21[b/]	18/19	95	1/19	5
3.0	10/14	71[b/]	11/14	78	4/14	28[b/]

a/ Data from Smith et al. (135).
b/ Statistically different from controls by the Fishers exact probability test ($p < 0.05$).

Neubert et al. (*175*) estimated the ED-50 for cleft palate in fetuses to be 40 μgTCDD/kg per day (Table 12). The no-effect-level during days 6 to 15 of gestation was estimated to be 2 μg/kg per day for NMRI mice. No pronounced fetal mortality was observed when 3 μgTCDD/kg body weight was administered on days 6 to 15 of pregnancy.

DOW 169595

Table 12. Occurrence of Cleft Palate in Offspring of Mice Fed TCDD[a/]

Strain	Dose (ug/kg)	% Cleft Palates per Total Fetuses Examined	Affected Litters/Total Litters #	%
CD-1	0	<0.3	0/29	0
	3	3	3/10	30
[illegible]	0	<1	0/23	0
	3	8	2/9	22
[illegible]	0	0.7	10/160	6
	3	3	7/24	29
C57B1	0	<1	0/23	0
	3	22	5/7	71

a/ Data from Neubert et al. (175).

(b) *Studies in which TCDD produced teratogenic and or fetotoxic effects in rats.* Sparschu et al. (*129*) administered TCDD to Sprague-Dawley rats by gavage at 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg per day on days 6 through 15 of gestation (Table 13). Intestinal hemorrhages were observed in 14 percent (18/127) of the fetuses at 0.125 μg/kg; 36 percent (36/99) at 0.5 μg/kg; and 57 percent (4/7) at 2.0 μg/kg; none (0/246) of the control fetuses had intestinal hemorrhages. At 8.0 μg/kg per day, all fetuses (100 percent) were resorbed as compared to 20 percent (63/308) in the controls. Fetal weights were depressed at 0.125, 0.5, and 2 μg/kg. This effect was statistically significant ($p<0.05$) in all groups except females at 0.5 μg/kg. No adverse effects were noted in the fetuses whose mothers were fed 0.03 μg/kg. The authors concluded that TCDD induced a high level of maternal and fetal toxicity and that 0.03 μg/kg per day was the no-effect-level for fetal and embryotoxic effects in rats.

Table 13. Intestinal Hemorrhages in Offspring of Sprague-Dawley Rats Fed TCDD[a/]

Dose (ug/kg per day)	Fetuses Affected/-Fetuses Examined #	%	Litters Affected/-Litters Examined #	%
0 (control)	0/246	0	0/24	0
0.03	0/115	0	0/10	0
0.125	18/127	14	7/10	70
0.5	36/99	36	10/12	83
2.0	4/7	57	2/4	50
8.0	---	---	---	---

a/ Data from Sparschu et al. (129).

Khera and Ruddick (*6*) studied the perinatal effects of TCDD in Wistar rats. In one test, rats were orally administered 0.125, 0.25, 0.5, and 1.0 μg TCDD/kg per day on days 6 through 15 of gestation (Table 14). Visceral lesions were observed at 0.25 μg/kg and above; slight decreases in fetal weight were also seen. Postnatal effects of prenatal exposure to TCDD were studied by allowing offspring of treated dams to be reared by untreated dams until weaning. Reduced survival, body weight gain, and reproductive ability in the progeny were observed after maternal treatment with 0.5 and 1.0 μg/kg. No fetotoxic effects were observed at 0.125 μg/kg.

In a second experiment, rats were treated orally with 1, 2, 4, 8, and 16 μg TCDD/kg body weight per day on days 6 through 15 of gestation. TCDD treatment reduced fetal weight, and the number of live fetuses per litter, and produced visceral lesions in 50

DOW 1695596

percent (%) of the 1.0 μg/kg fetuses and 43 percent (%) of the 2.0 mg/kg fetuses, as compared to none (%) in the controls. The incidence of skeletal anomalies was comparable to that in the controls at all dose levels. Doses of 1 μg/kg or more produced maternal toxicity; 4 μg/kg or more produced 100 percent embryomortality. The authors concluded that oral treatment of pregnant Wistar rats with 0.25 μg (or more)/kg per day on days 6 to 15 of gestation adversely effected rat development.

Table 14. Teratogenic Effects of TCDD in Wistar Rats[a/]

Dose (ug/kg)	Avg. # Live Fetuses/Litter	Avg. Fetal Weight (grams)	Fetuses with Skeletal Anomalies/-Total # Examined #	%	Fetuses with Microscopic Visceral Lesions/Total # Examined #	%
Test 1						
Untreated control	10.7	4.82	5/107	5	0/13	0
Treated control	11.0	4.51	21/116	18	0/11	0
0.125	10.6	4.64	3/121	2	0/38	0
0.25	10.9	4.79	6/109	6	1/33	3
0.5	10.5	4.46	10/105	10	3/31	10
1.0	9.3	4.10	6/81	7	3/10	30
Test 2						
Untreated control	11.5	4.68	8/116	7	0/10	0
Treated control	9.8	4.77	9/89	10	0/10	0
1.0	6.5	4.17	7/80	9	3/6	50
2.0	6.0	3.31	7/57	12	3/7	43
4.0	0					
8.0	-					
16.0	0					

a/ Data from Khera and Ruddick (6); treated controls given anisole-corn oil.

Courtney and Moore (*128*) administered TCDD to CD rats subcutaneously in solutions of 100 percent DMSO on days 6 through 15 of gestation (Table 8). DMSO was administered as the control. Kidney anomalies were found in four of the six litters (67 percent) whose dams were administered 0.5 μg/kg as compared to 0 percent (0/9) in the controls. TCDD did not affect fetal mortality, fetal weight, or cleft palates in the fetuses.

Dow Chemical USA (*110*) conducted a three-generation reproductive study on Sprague-Dawley rats continuously fed the equivalent of 0.001, 0.01, or 0.1 μg TCDD/kg per day. A preliminary report cites reduced fertility and litter survival in f_1 rats as the reasons for discontinuing the 0.1 μg/kg dose level; significantly reduced fertility was also observed at 0.01 μg/kg. "Clearly evident" indications of toxicity at 0.01 μg/kg among f_2 and f_3 litters included smaller litter size at birth, plus decreased survival and growth of neonates. Dilated renal pelvis was observed in each of the three f_1 rats at 0.1 μg/kg which survived to adulthood. Increased fequency of this anomaly was also seen among weanlings at lower doses; however a dose-related or generational correlation could not be made. In summary, Dow concluded that "the reproductive capacity of rats ingesting TCDD was clearly affected at dose levels of 0.01 and 0.1 μg/kg per day, but not at 0.001 μg/kg per day, through three successive generations." The preliminary report did not include the numerical data necessary for Agency evaluation. Analysis will continue as these become available.

Adverse reproductive effects due to TCDD

DOW 169597

17408

have also been observed in hamsters and chickens. Gastrointestinal hemorrhage was noted in hamster fetuses after administration of TCDD at 0.5 μg/kg per day on days 6 to 10 of gestation (*48; 63*). Buu Hoi et al. (*121*) established that 0.02 μg/kg TCDD caused teratogenic effects in chick embryos. Bowes et al. (*137*) and Verrett (*136*) confirmed these results. They found abnormalities in the beaks, eyes, and feet of chick embryos after TCDD exposure.

(c) *Summary.* Studies have established that TCDD is fetotoxic and teratogenic at doses as low as 0.125 μg/kg in rats (*129*) and at 0.3 μg/kg in mice (*127*); preliminary data from Dow (*110*) indicates that TCDD may have effects at 0.01 μg/kg in rats. Cleft palate and kidney anomalies have been observed in rats, mice, and hamsters. No fetotoxic or teratogenic effects have been observed at doses of 0.03 μg/kg in rats (*129*) and 0.1 μg/kg in mice (*135*). Table 15 lists the no-effect-levels in rats and mice for teratogenicity from TCDD.

Table 15. No-Effect-Levels for Teratogenesis from TCDD

Species	Route of Administration	No-Effect-Level μg/kg per day	Reference
Rat	Subcutaneous	<0.5	Courtney and Moore (128)
	Oral	0.125	Khera and Ruddick (6)
	Oral	0.03	Sparschu et al. (129)
Mouse	Subcutaneous	<1.0	Courtney and Moore (128)
	Oral	<0.3	Neubert and Dillman (127)
	Oral	0.1	Smith et al. (135)

(2) 2,4,5-T (*TCDD contamination ranging from undetectable to 30 ppm*)—(a) *Teratogenic and fetotoxic effects in rodents.* Courtney et al. (*123*) developed the first evidence that a 2,4,5-T pesticide product was teratogenic and fetotoxic (Table 16).[10] The 2,4,5-T used in this study contained approximately 30 ppm TCDD. The pesticide was administered daily either orally or subcutaneously on days 6 to 14 of gestation in C57BL/6 mice, days 6 to 15 in AKR mice, and days 10 to 15 in Sprague-Dawley rats. Subcutaneous administration of 113 mg/kg body weight resulted in significant increases in the incidence of cleft palate and cystic kidneys[11] in the embryos of both strains of mice, and fetal mortality in the C57BL/6 mice. Oral administration of the same dose caused increased incidence of cleft palate and fetal mortality in both strains and cystic kidneys in C57BL/6 mice. Courtney et al. also reported increases in liver-to-body weight ratios in fetal mice.

These investigators also found that 4.6, 10, or 46.4 mg/kg 2,4,5-T given orally to Sprague-Dawley rats produced kidney

[10]Results of this study were published by the Department of Health, Education, and Welfare (*121*) and by Clegg (*122*).

[11]In a recent report on studies measuring renal alkaline phosphatase in fetal mice, Highman et al. (*45*) attributed the increased incidence of "cystic kidneys" in the offspring of 2,4,5-T treated animals to retarded development, rather than true teratogenesis. Reduction in fetal weight and increased incidence of cleft palate were also observed among the fetuses of treated dams.

Table 16. Teratogenic Evaluation of 2,4,5-T[a] in Mice[b]

Mouse Strain	Route of Administration	Dose (mg/kg)	# Litters	Per Litter: Avg. # Live Fetuses	Per Litter: Abnormal Fetuses %	Per Litter: % Fetuses with Cleft Palate	Per Litter: % Fetuses with Cystic Kidney	Fetal Mortality %	Abnormal Litters %
C57BL/6[c]									
Nontreated	——	——	72	5.8	11	<1	1	26	38
Control	Subcutaneous	d/	106	5.5	12	<1	2	29	42
Control	Stomach tube	d/	32	7.1	14	0	1	15	41
Treated	Subcutaneous	21.5[e]	6	7.7	12	0	0	3	50
Treated	Subcutaneous	113.0[e]	18	4.4	57[f]	22[f]	41[f]	42	86[f]
Treated	Stomach tube	46.4[f]	6	8.5	37[g]	2	33[f]	8	100[g]
Treated	Stomach tube	113.0[f]	12	4.8	70[f]	23[f]	48[f]	47[f]	100[f]
C57BL/6[d]									
Nontreated	——	——	8	5.1	31	0	7	36	71
Control	Subcutaneous	d/	10	6.1	8	0	0	23	30
Treated	Subcutaneous	113.0[e]	10	7.7	77[f]	29[f]	60[f]	11	100[f]
AKR[d]									
Nontreated	——	——	58	7.1	5	<1	<1	16	19
Control	Subcutaneous	d/	72	6.9	4	<1	<1	15	24
Control	Stomach tube	d/	12	8.8	0	0	0	9	0
Treated	Subcutaneous	113.0[e]	14	6.9	29[f]	28[f]	1	23	71[f]
Treated	Stomach tube	113.0[f]	7	5.3	55[f]	55[f]	0	32[g]	100[f]

a/ Contained approximately 30 ppm TCDD.
b/ Data from Courtney et al. (123).
c/ Treated from day 6 through 14 of pregnancy. Killed on day 18 of gestation.
d/ Treated from day 9 through 17 of pregnancy. Killed on day 18 of gestation.
e/ Treated from day 6 through 15 of pregnancy. Killed on day 19 of gestation.
f/ Dose, 100 ul DMSO per mouse.
g/ Dose, 100 ul honey solution (honey to water, 1:1) per mouse.
h/ Administered as a solution of 2,4,5-T in 100% DMSO in a volume of 100 ul per mouse.
i/ 2,4,5-T was suspended in a honey solution (honey to water, 1:1) in a volume of 100 ul per mouse.
j/ p = 0.01.
k/ p = 0.05.

DOW 1695598

17407

anomalies and other embryotoxic effects at all levels (Table 17). The occurrence of hemorrhagic gastrointestinal tracts in rat fetuses was also reported.

Roll (*125*) found embryotoxic and teratogenic effects in NMRI mice after prenatal exposure to 2,4,5-T containing 0.05±0.02 ppm dioxin (Table 18). 2,4,5-T at 20 to 130 mg/kg body weight was administered orally to the dams on each of days 6 to 15 of gestation. At 90 or 130 mg/kg, the percentage of resorptions and/or dead fetuses was markedly increased relative to the controls; however, maternal toxic effects were also observed at these dose levels.[12] Statistically significant, dose-related reductions in fetal weight were observed at 20 mg/kg and above.

Cleft palate increased among fetuses exposed to 35 mg/kg or more and was significant when compared with control values. Skeletal retardation effects, manifested as insufficient ossification, were also observed. The teratogenic no-effect level in mice for this 2,4,5-T was considered to be 20 mg/kg. Later studies with a specially prepared sample of 2,4,5-T with no detectable amounts of dioxin (detection limit: <0.02 ppm) confirmed these results in mice (*125, 126*). By contrast, daily oral administration of 25 to 150 mg/kg of either the dioxin-free or commercial grade 2,4,5-T (<0.1 ppm dioxin) did not produce teratogenic effects in FW 49 rats (*236*).

Neubert and Dillman (*127*) also studied the effects of 2,4,5-T in NMRI mice, using three samples containing either (A) less than 0.02 ppm dioxin, (B) 0.05±0.02 ppm dioxin (provided by Dr. Roll), or (C) an unknown amount of dioxin (Table 19). Their results confirmed those obtained by Roll (*125*). 2,4,5-T was administered to the dams orally in rape-seed oil on each of days 6 through 15 of gestation at 8 to 120 mg/kg body weight.

The average number of resorptions was significantly higher than the oil control at 60, 90, and 120 mg/kg of sample (A), and 90 mg/kg of samples (B) and (C). Total resorption of one litter was observed in four of the groups (30, 45, 60, and 90 mg/kg) treated with sample (A) and in three of the litters treated with 90 mg/kg of sample (B); none was seen in the controls. Fetal weight was significantly depressed in all treated groups compared with the oil control.

The percentage of fetuses with cleft palate was significantly higher than the control group in all 2,4,5-T groups treated with 45 mg/kg or more. In the group treated with 120 mg/kg 2,4,5-T containing <0.02 ppm dioxin, 54% (⅞) of the litters and 11% (¹⁵⁄₁₄₈) of the fetuses exhibited cleft palate compared with oil control values of 6% (⅙) and 0.7% (⅞₀₀), respectively.

These investigators also tested the butyl ester of 2,4,5-T and found similar effects. In experiments combining 2,4,5-T and TCDD, potentiation of teratogenic effects was observed. Sixty mg/kg of 2,4,5-T (sample A) combined with 0.3 ug/kg TCDD increased cleft palate frequency among fetuses from 5 to 14%. In this study no cleft palates were observed among fetuses treated only with 0.3 ug/kg TCDD.

Table 17. Teratogenic Evaluation of 2,4,5-T[a] in Rats[b]

Test Animal	Route of Administration	Dose (mg/kg)	# Litters	Per Litter: Avg. # Live Fetuses	Per Litter: Abnormal Fetuses %	Per Litter: % Fetuses with: Enlarged Renal Pelvis	Per Litter: % Fetuses with: Cystic Kidney	Fetal Mortality %	Abnormal Litters %
Rats[c]									
Nontreated	----	---	7	9.9	9	9	0	11	43
Control	Stomach tube	d/	14	8.7	12	12	<1	1	57
Treated	Stomach tube	4.6[e]	8	8.2	36[e/]	18	21	12	88
Treated	Stomach tube	10.0[e]	7	7.1	48[e/]	17	30[e/]	28[e/]	86
Treated	Stomach tube	46.4[e]	6	2.7	60[h/]	27	33[h/]	59[e/]	67

a/ Contained approximately 30 ppm TCDD.
b/ Data from Courtney et al. (123).
c/ Treated from day 10 through 15 of pregnancy. Killed on day 20 of gestation.
d/ Dose, 200 ul honey solution (honey to water, 1:1) per rat.
e/ 2,4,5-T was suspended in a honey solution (honey to water, 1:1) in a volume of 200 ul per rat.
f/ p = 0.01.
g/ p = 0.05.
h/ The sample size was possibly too small to show a significant difference.

Table 18. Embryotoxic Effects of 2,4,5-T in NMRI Mice[a]

Dose mg/kg	Implantations per Pregnancy	Resorptions and/or Dead Fetuses No./Total No.	Resorptions and/or Dead Fetuses %	Fetal weight (grams)	Cleft Palate No./Viable No.	Cleft Palate %
0	10.1	19/332	5.7	1.23	6/313	1.9
20	9.8	30/344	8.7	1.09	6/314	1.9
35	9.5	22/248	8.9	1.06	14/226	6.2
60	9.9	15/208	7.2	1.05	19/193	9.8
90	9.8	35/293	11.9	0.86	39/258	15.1
130	9.6	191/316	60.4	0.73	61/125	48.8

a/ Data from Roll (125).

[12] Although the LD-50 for female NMRI mice had been previously determined to be 778 mg/kg, an increased maternal mortality rate was seen at 130 mg/kg and weight gain was depressed at doses above 60 mg/kg (*125*).

DOW 169599

17408

Table 19. Embryotoxic Effects of 2,4,5-T[a]

Treatment	Dioxin Content (ppm)	Dose (mg/kg)	Resorption (RES)			Fetal Weight (grams)	Cleft Palate (CP)	
			% Litters with RES	% RES/Implantation Sites	RES/Single Litter w/RES (N)		% Litters with CP	% Fetuses with CP
None	—	—	24	4	0.6	1.26[b]	6	0.6
Oil control	—	0.4 ml	32	4	0.5	1.30	6	0.7
2,4,5-T (A)	<0.02	8.0	35	3	0.4	1.27[b]	<7	<1
		15.0	38	5	0.5	1.15[b]	8	1
		30.0	56	7	0.8	1.09[b]	11	1
		45.0	55	6	0.6	0.98[b]	16	3[b]
		60.0	63	11	1.2[b]	1.01[b]	20	5[b]
		90.0	53	8	1.7[b]	1.02[b]	35	8[b]
		120.0	54	10	1.3[b]	0.95[b]	54	11[b]
2,4,5-T (B)	0.05	30.0	44	6	0.6	1.11[b]	22	2
		60.0	57	7	0.4	1.11[b]	71	9[b]
		90.0	71	8	1.0[b]	0.99[b]	86	23[b]
2,4,5-T (C)	unknown	90.0	71	13	1.4[b]	1.00[b]	72	26[b]

a/ Data from Neubert and Dillman (127); 2,4,5-T sample (b) received from Moll (125).
b/ $p \leq 0.01$.

Bage et al. (*132*) injected NMRI mice subcutaneously with 50 and 110 mg/kg 2,4,5-T (<1.0 ppm dioxin) on each of days 6 through 14 of gestation. At 110 mg/kg, 2,4,5-T was teratogenic, causing fetal death, cleft palate, and other anomalies.

Courtney and Moore (*128*) studied the effects of 2,4,5-T in CD-1 random-bred mice, two strains of inbred mice, DBA/2J and C57BL/6J, and CD rats (Table 20). 2,4,5-T containing 0.5 ppm (technical) or 0.05 ppm (analytical) TCDD was administered subcutaneously to mice at 50 to 150 mg/kg in DMSO and orally to rats at 10 to 80 mg/kg in sucrose on each of days 6 to 15 of gestation. At 100 mg/kg or more, both 2,4,5-T samples produced significant reductions, which appeared to be dose related, in fetal weight in all strains of mice; rats were not affected. 2,4,5-T was fetocidal at two doses, but the investigators considered this effect to be due to maternal toxicity.

Both 2,4,5-T samples produced cleft palate in mice. For CD-1 dams treated with 100 mg/kg of either 2,4,5-T sample, 40% of the litters and two fetuses per affected litter evidenced cleft palate compared with 0% in the control (Expt. 3). No cleft palates were

Table 20. Embryotoxic Effects of Analytical and Technical 2,4,5-T[a]

Species	Compound	Dose (mg/kg)	% Fetal Mortality per Litter	Fetal Weight (grams)	Cleft Palate (CP)		Kidney Anomalies	
					% Litters Affected	# CP per Affected Litter	% Litters Affected	# Affected Fetuses per Affected Litter
CD-1 Mouse								
Expt. 1	DMSO	—	6.6	1.35	0	0	0	0
	2,4,5-T (Tech.)	50	6.6	1.26	0	0	0	0
	2,4,5-T (Tech.)	100	7.5	1.00	33	3.0	0	0
	2,4,5-T (Tech.)	150[b]	51.7	0.91	100	5.3	0	0
Expt. 2	DMSO	—	8.8	1.02	0	0	33	1.0
	2,4,5-T (Analy.)	100	9.6	0.73[d]	89	4.4	78	1.7
Expt. 3	DMSO	—	8.4	1.09	0	0	63	2.0
	2,4,5-T (Tech.)	100	10.7	0.85[d]	40	2.0	80	2.4
	2,4,5-T (Analy.)	100	11.6	0.86[d]	40	2.0	100	4.2
	2,4,5-T (Analy.)	125	12.9	0.71[d]	78	5.4	67	4.3
DBA/2 Mouse	DMSO	—	26.1	0.85	0	0	13	1.0
	2,4,5-T (Tech.)	100	27.0	0.67[d]	27	1.0	9	1.0
C57Bl/6 Mouse	DMSO	—	10.8	0.99	0	0	9	1.0
	2,4,5-T (Tech.)	100	15.9	0.75[d]	40	1.2	0	0
CD Rat	Sucrose	—	3.4	2.48	0	0	0	0
	2,4,5-T (Tech.)	10	1.8	2.40	0	0	20	1.0
	2,4,5-T (Tech.)	21.5	1.4	2.54	0	0	38	1.3
	2,4,5-T (Tech.)	46.4	3.8	2.20	0	0	14	2.0
	2,4,5-T (Tech.)	30.0[c]	52.7	2.30	0	0	50	4.0

a/ Data from Courtney and Moore (128).
b/ Investigators thought this data to be close to a maternal toxic dose.
c/ Maternal LD-40.
d/ $p < 0.05$.

DOW 169600

17409

observed among the rat fetuses. To verify this observation, a second group of rats was given two 150 mg/kg doses of technical 2,4,5-T subcutaneously at the time of palate closure (days 13 to 14). Again, no cleft palates were observed; however, there was a significant increase in fetal mortality among treated animals (14%) when compared with the controls (0%).

Fetuses of CD-1 mice treated with analytical 2,4,5-T also showed increased incidences of kidney anomalies; the response to technical 2,4,5-T was not as great. At 100 mg/kg, 100% of the litters and 4.2 fetuses per affected litter of dams treated with analytical 2,4,5-T displayed kidney anomalies, compared with 80% and 2.4 for technical 2,4,5-T and 63% and 2.0 for controls (Expt. 3). The effect in inbred strains of mice was comparable with control values. In rats, technical 2,4,5-T at all dose levels produced higher incidences of litters affected and numbers of fetuses per litter affected than seen in the control animals. The maximum effects on kidney anomalies in rats were 50% of the litters and 4.0 fetuses per litter at 80 mg/kg, compared with 0% in the control litters.

In another study using CD-1 mice, Courtney (*134*) administered 0.45 to 1.0 mM/kg body weight per day of 2,4,5-T (0.05 ppm dioxin) either orally or subcutaneously during various segments of the gestation period (Table 21).[12] Cleft palate was seen in all groups treated with 2,4,5-T; there were no instances of this anomaly within the control groups. At 0.8 mM/kg, 48% of total fetuses and 37% of the litters evidenced this malformation. Statistically significant ($p \leq 0.05$) increases in the percentage of fetuses dead and/or resorbed were observed at the highest doses. All dose levels had adverse effects on fetal weight. The author noted that by slightly altering experimental conditions, the cleft palate effect and the effects on fetal mortality and fetal weight could be produced independently.

[12] Maternal toxicity was also observed, evidenced by reductions in maternal weight gain and increased liver-to-body weight ratios (*134*).

Table 21. Embryotoxic Effects of 2,4,5-T in CD-1 Mice[a]

Vehicle	Dose (mM/kg)	Days Dosed	Viable Normal Fetuses #/total #	Viable Normal Fetuses %	% Fetal Mortality	Fetal Weight (grams)	Cleft Palate (avg. %) Fetuses	Cleft Palate (avg. %) Litters
oil:Ac[b]	—	10-15	75/80	94	6	0.95	—	—
	—	11-13	99/112	88	11	0.94	—	—
	—	12-15	108/126	86	13	1.01	—	—
	0.45	10-15	86/107	80	17	0.89	7	6
	0.80	11-13	88/122	72	14	0.87	16	14
	0.80	11-14	21/59	36	29[e]	0.87	48	37
	1.00[d]	12-15	75/82	91	8	0.96	1	1
DMSO[c]	—	12-15	152/171	89	12	1.03	—	—
	1.00	12-15	11/68	16	72[f]	0.70	48	67

a/ Data from Courtney (134).
b/ Corn oil:Acetone (9:1)—oral.
c/ Dimethylsulfoxide — subcutaneous.
d/ This concentration exceeded the solubility characteristics of the vehicle. Doubling the volume of vehicle resulted in effects more consistent with those found at lower doses.
e/ $p \leq 0.05$.
f/ $p \leq 0.001$.

Table 22. Effects of 2,4,5-T on Wistar Rat Fetuses[a]

Compund	Dose (mg/kg)	# of Litters	Avg. # per Litter Viable Fetuses	Avg. # per Litter Dead Fetuses	Fetal Weight (grams)	Avg. % Malformed Fetuses per Litter[b]
T-1	Treated Control	14	11.1	0.6	4.65	15
	50	7	12.9	1.3	4.84	24
	100	9	11.3	1.9	4.60	29
T-2	Treated Control	10	9.2	0.6	5.34	10
	25	13	10.5	0.8	5.06	15
	50	12	11.7	0.5	5.15	9
	100	9	8.6	2.4	4.57	32
	Treated Control	10	12.6	0.7	4.67	26
	25	11	12.7	0.5	5.15	10
	50	14	11.5	1.4	4.91	28
	100	10	11.0	0.6	4.35	36
	150	5	11.6	2.2	3.98	56
T-3	Treated Control	10	11.8	0.7	5.31	17
	25	14	12.2	0.9	5.00	11
	50	2	11.0	0.5	4.75	56
	100	12	12.6	0.9	5.00	37
	150	1	11.0	1.0	3.00	91
T-4[c]	50	8	11.3	1.1	4.94	14

a/ Data from Khera and McKinley (130).
b/ One or more skeletal malformation (viable fetuses).
c/ No treated control given.

DOW169601

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Khera and McKinley (*130*) studied the prenatal and postnatal effects of 2,4,5-T in Wistar rats, using four samples containing no TCDD (detection limit: 0.8 mg/kg) (Table 22). Twenty-five to one hundred fifty mg/kg body weight per day were administered to the dams, orally in gelatin or corn oil, on days 6 to 15 of gestation. At 25 and 50 mg/kg, the differences between experimental and control values were minimal. However, at 100 and 150 mg/kg, there were significant ($p < 0.05$) effects on fetal weight, number of dead fetuses, and percentage of malformed fetuses per litter.[14] The larger proportion of malformed fetuses in the treated groups resulted from either an increased incidence of skeletal anomalies also seen in the controls or a low incidence of abnormalities not observed in the controls. The former category included wavy ribs, retarded ossification, extra ribs, and a variety of sternal defects; the latter included fused ribs, small-sized distorted scapula, malformed humerus shaft, and bent radius or ulna. Abnormal kidneys were observed in 7 to 45% of the examinined fetuses treated with sample T-1, compared with a control value of 20 to 35%.

In the postnasal portion of the study, after normal delivery, survival rate, sex ratio, and pup weight on days 1 and 21 were compared. Although treated pups surviving from day 2 to 21 were slightly smaller at some dose levels, there were no significant differences from controls for any variable. In some experiments, litters were standardized at 8 pups on day 2, and the remaining littermates examined for defects. The increased incidences of malformations among treated groups were comparable to those found in the prenatal study. Assuming the same incidence for pups not examined, the investigators concluded that there were no real differences in survival rates among control and treated groups. The butyl ester of 2,4,5-T produced similar toxic effects.

Sokolik (*131*) orally administered 100 and 400 mg/kg and 50 and 200 mg/kg of 2,4,5-T and its butyl ester to rats of the Rappolovo line on each of days 1 to 14 or 1 to 16 of pregnancy. At 100 mg/kg, 2,4,5-T produced embryos with a combination of deformities including absence of lower jaw, abnormal hind limbs, and exophthalmos. At 400 mg/kg, the embryos of treated rats evidenced cleft palate, hydrocephalus, hydronephrosis, and abnormalities of the upper limbs which included tridactyly, webbed toes, and abnormal shortness.

The butyl ester of 2,4,5-T was more toxic than the parent compound, causing more than 30 percent embryonic mortality at 200 mg/kg. The lower dose, 50 mg/kg, also caused high mortality among the embryos. Cleft palate, hydronephrosis, hydrocephalus, and extensive gastrointestinal hemorrhages were also observed within the treated groups. From these results, the author concluded that 2,4,5-T and its derivatives have a high potential for teratogenic activity.

Collins and Williams (*124*) tested seven samples of 2,4,5-T from different sources for embryotoxic effects in golden Syrian hamsters (*Mesocricetus auratus*) (Table 23). The dioxin contents ranged from not detectable (detection limit <0.1 ppm) to 45 ppm. Daily oral doses of 20 to 100 mg/kg body weight were administered in acetone:corn oil:carboxymethyl cellulose (1:5.8:10) on days 6 to 10 of gestation. 2,4,5-T with no detectable dioxin significantly ($p < 0.05$) reduced fetal weight and fetal viability per litter at all levels tested.

Total fetal mortality was greatly increased at all levels when compared with controls and was dose-dependent, as was the effect on fetal viability. The increased incidence of gastrointestinal hemorrhage also appeared to be dose related. At 100 mg/kg, "pure" 2,4,5-T caused increased incidences of malformations and reductions in the number of live fetuses per litter. One "pure" sample, F, at 100 mg/kg significantly reduced fetal weight from 1.8 to 1.6 grams, reduced fetal viability from 96.7 to 71.4 percent, and increased abnormalities from 3.5 to 40 percent. The anomalies associated with 2,4,5-T containing no dioxin were exencephaly, eye abnormalities, delayed head ossification, and hind limb deformities.

Increasing the level of dioxin contamination increased fetal mortality and the incidence of abnormalities per litter; fetal viability was reduced. A clear correlation was found between the level of dioxin and abnormalities per litter. Although the incidence of hemorrhages also increased, no relationship between it and dioxin level could be found. Bulging eyes (absence of eyelid) and delayed ossification were the most common anomalies seen among fetuses exposed to dioxin-contaminated 2,4,5-T; exencephaly, edema, cleft palate, ectopic heart, and fused ribs were also observed.

Emerson et al. (*141*) found no adverse effects of commercial 2,4,5-T, containing 0.5 ppm TCDD, on fetal development in Sprague-Dawley derived rats and New Zealand white rabbits. Daily oral doses of 2,4,5-T in gelatin were administered to the rats at 1 to 24 mg/kg on days 6 to 15 of gestation; to the rabbits at 10 to 40 mg/kg on days 6 to 18 of gestation. The investigators found no maternal or embryonic toxic effects in either species, nor was 2,4,5-T considered teratogenic under the conditions of these experiments. The most frequently observed abnormalities were accessory ribs, hydronephrosis, and retardation in the development of the sternebrae. With the exception of partially ossified sternebrae in both species and bilateral accessory ribs in the rabbit, the incidence of these anomalies was greater in the control animals than in the examined treated groups.

Sparschu et al. (*140*) orally administered 2,4,5-T, containing 0.5 ppm TCDD, to rats in daily doses of 50 and 100 mg/kg on days 6 to 15 and 6 to 10 of gestation, respectively. Results are given in Table 24. At 50 mg/kg, there were no significant maternal or embryonic toxic effects attributable to 2,4,5-T except for an increased incidence of delayed skull ossification, and a single fetus with in-

[14] Statistical significance was determined using the average value per dose level. Data from T-4 were not used in this analysis.

Table 23.—Embryotoxic Effects of 2,4,5-T in Hamsters[a]

Compound	Dioxin Content (ppm)	Dose (mg/kg)	Fetuses: % Total Mortality	Fetuses: Avg # Live per Litter	Fetuses: Avg Weight[b] (grams)	% Fetal Viability per Litter	% Abnormalities per Live Litter	% Hemorrhages per Total Live Fetuses
Control	—	—	3.8	11.0	1.8	96.7	3.5	0.32
A	45	20	32.3	7.3	1.7	68.1[d]	25.0[d]	28.4
		40	74.3	3.7	1.7	25.8[d]	33.3[d]	75.7
		80	94.4	0.8	1.6	5.3[d]	100.0[d]	42.9
		100	100.0	0	—	0[d]	—	—
B	2.9	40	7.2	9.1	1.7	93.1	0	0
		80	9.8	10.4	1.7	90.1[d]	12.5	2.4
		100	11.4	12.8	1.7	88.9[d]	50.0[d]	13.9
C	0.5	20	8.5	12.6	1.7[d]	90.8[d]	0	8.6
		40	4.0	13.4	1.6[d]	95.9	11.1	2.5
		80	43.6	6.6	1.6[d]	58.3[d]	40.0[d]	12.5
		100	57.2	5.1	1.5[d]	30.2[d]	30.0[d]	7.6
D	0.1	40	2.4	11.4	1.8	97.8	0	0
		80	33.3	7.8	1.7	68.3[d]	0	2.1
		100	57.1	6.0	1.6[d]	57.2[d]	0	5.6
E	ND[c]	40	16.7	11.2	1.5[d]	88.2[d]	0	1.5
		80	25.9	8.7	1.5[d]	69.1[d]	0	4.2
		100	58.3	6.3	1.5[d]	53.1[d]	16.3[d]	0
F	ND	100	31.1	7.3	1.6[d]	71.5[d]	40.0[d]	6.8
G	ND	100	30.0	8.3	1.6[d]	68.3[d]	0	16.7

[a] Data from Collins and Williams (124).
[b] Apparently normal weights for samples A and B attributed to edema.
[c] Not detected.
[d] $p < 0.05$.

17411

Table 24. Effects of 2,4,5-T on Fetal Development of Rats[a/]

Parameter	Dose (mg/kg per day) 0	50	100
# Viable fetuses			
Total	252	203	13[b/]
Mean per litter	11	11	---
% Resorptions			
Litters	68	61	100
Total fetuses	6.7	12.1	75[c/]
Fetal weight (grams)			
Male	4.41	4.38	3.57[c/]
Female	4.17	4.15	3.52[c/]
Sex Ratio (M:F)	53:47	44:56	23:77
Abnormalities (% fetuses examined)			
Poorly ossified sternebrae			
Fifth	15.2	22.1	57.1[c/]
Second and fifth	3.0	4.2	14.3
Multiple	8.3	12.6	14.3
Malaligned sternebrae	0.8	2.1	28.6[c/]
Delayed ossification			
Interparietal	3.8	16.8[c/]	28.6[c/]
Parietals	3.0	16.8[c/]	57.1[c/]
Frontals	0.8	7.4[c/]	14.3

a/ Data from Sparschu et al. (140).
b/ All viable fetuses from one litter.
c/ $p < 0.05$

testinal hemorrhage. At 100 mg/kg, 2,4,5-T was toxic to both dams and fetuses.[15]

Resorptions were observed in all litters; 75 percent were totally resorbed. Fetal weight was significantly ($p < 0.001$) reduced in both sexes and the sex ratio was shifted in favor of females. Abnormalities observed which had significantly ($p < 0.05$) higher incidences than in the controls were poorly ossified and malaligned sternebrae and delayed skull ossification. The investigators concluded that the delayed ossification observed in this study was a reversible manifestation, rather than a true teratogenic effect.

(b) *Adverse Reproductive Effects in Other Mammalian Test Systems.* Adverse reproductive effects of 2,4,5-T exposure have been observed in other mammalian test systems. Lloyd et al. (*173*) reported on *in vivo* enzymatic studies showing reduced uptake and metabolism of testosterone by the prostate gland in male mice treated orally with doses of 2,4,5-T (6.25, 12.5, or 25 mg/kg, ten times daily).

Yefimenko (*151*) reported on the effects of acute and chronic exposure to the butyl ester of 2,4,5-T on gonadal and somatic tissue in an *in vivo* cytogenetic study in male albino rats. Chronic effects on the gonads were observed after exposure to 0.1 ug/kg for two and one half months. Adverse effects (seen at seven months, when the experiment was terminated), which were considered persistent effects, included testicular atrophy, decreased sperm count, desquamated tubules, and aberrant cells in the germinal epithelium. Chromosomal aberrations were also observed during the chronic phase of the experiment. EPA evaluation of this study found inadequacies in the methodology which would prevent the drawing of firm conclusions from this data (*106*).

Recent studies in rats by Sjoden and Soderberg [cited in (*25*)] appear to show that prenatal exposure to 2,4,5-T leads to behavioral abnormalities and changes in thyroid activity and brain seritonin levels in the progeny. Single oral doses of 100 mg/kg were administered to the dams on days 7, 8, or 9 of pregnancy.

(c) *Adverse Effects in Avian Species.* Embryotoxic effects in avian species due to 2,4,5-T exposure have been reported. Verrett (*136*) studied the effects of 2,4,5-T, containing either 27 or 0.5 ppm TCDD, on chicken eggs. The 2,4,5-T was injected through the air cell of the eggs, either preincubation or on the fourth day of incubation. The sample containing 27 ppm TCDD was found to be more lethal (LD-50=25 ug/egg) than the less contaminated sample (LD-50=100 ug/egg). Both samples produced teratogenic effects, including chick edema, eye defects, beak defects (primarily cleft palate), and short, twisted feet resulting from tendon slippage. Teratogenic effects were observed at doses as low as 1 ppm (50 ug/egg) with the sample containing 0.5 ppm TCDD and as low as 0.125 ppm (6.25 ug/egg) with the sample containing 27 ppm TCDD.

Lutz and Lutz-Ostertag (*135*) studied the action of 2,4,5-T, in aqueous solution at a concentration of 2 to 10 g/liter, on the embryonic development of quail (*Coturnix coturnix japonica*), chicken (*Gallus gallus*), pheasant (*Phasianus colchicus*), and two partridge species (*Alectoris rufa* and *Perdrix perdrix*). The 2,4,5-T was administered by dipping, spraying, and organo-typic cultures. Abnormal genital tracts were observed in all species, indicating abnormal sexual differentiation. Further, morphological changes in the testes often gave the appearance of true testicular atrophy. In another study, 2,4,5-T affected fertility in birds of both sexes (*139*).

(d) *Studies in Avian Species in Which Adverse Effects Were Not Observed.* Using 2,4,5-T contaminated with less than 0.1 ppm dioxin, Strange and Kerr (*142*) found no abnormal development in chicken embryos. Doses of 12.5, 25, 50, 75, 100, and 125 mg/kg were injected into eggs on days 0 and 5 of incubation; observations were made 48 hours later. At this developmental stage, kidneys were not sufficiently developed to detect the tubule lesions reported by Bjorklund and Erne (*143*).

(e) *Summary.* Studies have established that 2,4,5-T is fetotoxic and teratogenic at doses as low as 35mg/kg (0.05 ± 0.02 ppm TCDD) in mice (*125*); 4.6 mg/kg (approximately 30 ppm TCDD) in rats (*123*); and 20 mg/kg (0.5 ppm TCDD) in hamsters (*124*). Cleft palate and kidney anomalies have been observed in mice, rats, and hamsters. No fetotoxic or teratogenic effects (no-effect levels) have been observed at doses of 20 mg/kg (0.05 ± 0.02 ppm TCDD) in mice (*125*) and 25 to 150 mg/kg (0.05 ± 0.02 ppm TCDD) in rats (*125*).

(3) *Exposure Analysis.* In order to determine whether a rebuttable presumption should be issued based on reproductive and fetotoxic effects, pursuant to § 162.11(a)(3)(ii)(B), the Working Group must determine whether or not an ample margin of safety exists between the levels of 2,4,5-T and/or TCDD which produce reproductive and fetotoxic effects, and the level(s) to which humans can reasonably be anticipated to be exposed.

The cancellation of uses of 2,4,5-T on food crops intended for human consumption and for use around the home, recreation sites, aquatic areas, and ditch banks in 1970 was thought to have eliminated the potential exposure to that portion of the population at risk (women of child bearing age).

Social changes over the last few years, however, have given women the opportunity for employment in areas that once were considered open only to men. Since women of child-bearing age are now employed in occupations such as pesticide applicators, operators of highway construction and maintenance equipment, foresters, and chemical formulators, they have become part of the

[15] The high rate of maternal mortality caused dosing to be stopped on day 10, instead of day 15. Significant reductions in weight gain were also observed.

population at risk with potential exposure to 2,4,5-T and/or TCDD.

In order to determine whether an ample margin of safety exists, the Working Group must first determine how much 2,4,5-T a woman could be exposed to through oral, dermal, or inhalation exposure. For each of these analysis, the Working Group assumes a woman to weigh 60 kg. The following calculations are based on an exposure analyses for 2,4,5-T and TCDD performed by EPA's Criteria and Evaluation Division [CED] (*166*).

(a) *Oral Exposure:* For purposes of this analysis, the Working Group considered currently registered uses where the possibility of oral exposure to 2,4,5-T and/or TCDD existed. Treatment of range and pasture land could result in oral exposure through ingestion of meat and milk from animals grazing on the treated area. Since actual data on residues of 2,4,5-T in animals grazing on treated rangeland is unavailable, for purposes of the 2,4,5-T oral exposure analysis, the Working Group used residue information obtained in a feeding study (*37*) in which cattle were fed considerably higher amounts of 2,4,5-T than they would normally be exposed to in grazing on treated land. The following calculations are based on the average quantities of food eaten per day (1.5 kg), as reported by Lehman (*144, 165*).

Table 25. 2,4,5-T Oral Exposure Analysis

	Whole Milk	Meat (Beef)
No-adverse-effect level for teratogenicity in mice	20 mg/kg	20 mg/kg
Average level of 2,4,5-T identified	0.103 ppm[a/]	0.2 ppm[a/]
% of food item in total human diet	19.6%	4.6%
Average amount of food eaten per day	1.5 kg	1.5 kg
Exposure to 2,4,5-T per day	0.0005 mg/kg	0.0002 mg/kg

a/ Animals were fed at 300 ppm 2,4,5-T in the diet for 2 to 3 weeks. This is a worst case assumption for cows grazing on freshly-treated pasture without a withdrawal period; all milk and meat was obtained from such cows. Meat (beef) includes muscle, fat, and liver tissues which constitute the major portion of edible meat.

To find the average daily intake of a single food item, multiply the average daily food intake by the percent of that item in the total diet: For milk, 1.5 kg×19.6%=0.294 kg; and for meat (beef), 1.5 kg×4.6%=0.069 kg.

The quantity of 2,4,5-T in the average daily diet equals the average daily intake of each food item multiplied by the level of 2,4,5-T in the food item: For milk, 0.294 kg×0.103 ppm=0.03 mg; and for meat (beef), 0.069 kg×0.2 ppm=0.014 mg.

The theoretical exposure of an average woman equals the amount of 2,4,5-T in the daily diet divided by the weight of the average woman: For milk, 0.03 mg/60 kg=0.0005 mg/kg; and for meat (beef), 0.014 mg/60 kg=0.0002 mg/kg; total exposure from milk and beef products could be 0.0007 mg/kg per day.

Existing data on TCDD residues in animals grazing on treated rangeland are too meager to use for an analysis of TCDD exposure to humans through ingestion of meat or milk from animals so exposed.

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and the calculated oral exposure level for 2,4,5-T (0.0007 mg/kg per day) does constitute an ample margin of safety. Since this risk criterion for other chronic adverse effects has not been met or exceeded, a rebuttable presumption does not arise.

(b) *Dermal Exposure.* In order to conduct these analyses, the Working Group must determine the amount of 2,4,5-T and/or TCDD which would come in contact with the skin and the amount that would be absorbed.

(1) *Spray Applicator: Back-pack Sprayer.* For purposes of this analysis, the Working Group assumes the applicator to be a 60-kg woman of child-bearing age, and the site of application either a right-of-way or spot treatment of pasture or rangeland. The equipment is a back-pack sprayer (*166*). The following calculations of exposure are based on dilution for spraying of three pints of formulated product per 32 pints of water. Typical 2,4,5-T formulations, based on inspection of a large number of registered labels (*166*), range from 4 to 6 pounds active ingredient (acid equivalent) per gallon. The product used in this exposure analysis has an assumed concentration of 4 pounds 2,4,5-T per gallon. Label recommendations vary from a recommended dilution of 0.094 to 4 pounds acid equivalent per 32 pints of water. A dilution rate of 1.6 pounds per 32 pints has been selected as representative of a typically-used spray mixture.

Wolfe et al. (*166*) studied dermal exposure to fenthion during hand back-pack spraying for mosquitoes for ten situations. Exposure ranged from 0.1 to 6.2 mg/hr, with a mean value of 3.6 mg/hr (6 ml/hr). Method of application was a hand pressure sprayer using a 0.06 percent spray. Workers wore short-sleeved, open-necked shirts with no gloves or hat. Based on Wolfe's data, CED (*166*) calculated a dermal exposure of approximately 0.177 pints per day. CED (*166*) also determined that approximately 10 percent of the 2,4,5-T and TCDD coming in contact with the skin of the applicators would be absorbed even after washing, based on absorption studies with other pesticides (*145, 146, 165*).

Table 26. Back-pack Sprayer Dermal Exposure Data

	2,4,5-T	TCDD
Use Dilution rate	3 pints (1.6 pounds 2,4,5-T) per 32 pints water	3 pints (0.00000016 pounds TCDD) per 32 pints water
Amount of diluted material gotten on skin daily	0.18 pint	0.18 pint
% Diluted material absorbed	10%	10%
Exposure level	409 mg	0.0409 ug
Dose level	6.8 mg/kg	0.0007 ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

DOW169604

The following calculations (see Table 27 for mathematics) will give the daily dermal exposure for both 2,4,5-T and TCDD: (1) Convert the dilution rate to grams; (2) multiply this figure by 1,000 (for 2,4,5-T) to convert to milligrams and by 1,000,000 (for TCDD) to convert to micrograms; (3) multiply this figure by the daily dermal dose of diluted material; (4) multiply this figure by the percent absorbed; and (5) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (6.8 mg/kg), as well as the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 μg/kg) and this calculated exposure level for TCDD (0.0007 μg/kg), do not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T and/or TCDD pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

(ii) *Spray Applicator: Tractor-mounted, Low-boom Spray Equipment.* For the purpose of this analysis, the Working Group assumes the applicator to be a 60-kg female of childbearing age clearing brush on either rangeland or rights-of-way. The same product cited above (2,4,5-T at 4 pounds/gal) is being used, and the dilution rate is 1.6 pounds of formulation to 32 pints of water (equal to 4 pounds of 2,4,5-T per 10 gallons of water). Based on exposure studies using similar equipment but a different herbicide (*147*), the Working Group determined that, during an eight-hour working day, the applicator would get 0.048 pints of diluted material on her skin. The Working Group determined that 10 percent of the pesticide on the skin would be absorbed (*145, 146, 163*).

The following calculations (see Table 29 for mathematics) will give the daily dermal exposure for both 2,4,5-T and TCDD: (1) Convert the dilution rate to grams; (2) multiply this figure by 1,000 (for 2,4,5-T) to convert to milligrams and by 1,000,000 (for TCDD) to convert to micrograms; (3) multiply this figure by the daily dermal dose of diluted material; (4) multiply this figure by the percent absorbed; and (5) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

Table 27

2,4,5-T	TCDD
1) 1.6 pounds/32 pt X 454 g/pound = 22.70 g/pt;	1) 0.00000016 pounds/32 pt X 454 g/pound = 0.00000227 g/pt;
2) 22.70 g/pt X 1,000 mg/g = 22,700 mg/pt;	2) 0.00000227 g/pt X 1,000,000 ug/g = 2.27 ug/pt;
3) 22,700 mg/pt X 0.18 pt = 4,086 mg;	3) 2.27 ug/pt X 0.18 pt = 0.41 ug;
4) 4,086 mg X 10% = 408.6 mg	4) 0.41 ug X 10% = 0.041 ug;
5) 408.6 mg / 60 kg = 6.8 mg/kg per day	5) 0.041 ug / 60 kg = 0.0007 ug/kg per day

Table 28. Dermal Exposure Data (Tractor Mounted Equipment)

	2,4,5-T	TCDD
Use Dilution rate	3 pints (1.6 pounds 2,4,5-T) per 32 pints water	3 pints (0.00000016 pounds TCDD) per 32 pints water
Amount of diluted material gotten on skin daily	0.048 pint	0.048 pint
% Diluted material absorbed	10%	10%
Exposure level	109 mg	0.0109 ug
Dose level	1.8 mg/kg	0.00018 ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

Table 29

2,4,5-T	TCDD
1) 1.6 pounds/32 pt X 454 g/pound = 22.70 g/pt;	1) 0.00000016 pounds/32 pt X 454 g/pound = 0.00000227 g/pt;
2) 22.70 g/pt X 1,000 mg/g = 22,700 mg/pt;	2) 0.00000227 g/pt X 1,000,000 ug/g = 2.27 ug/pt;
3) 22,700 mg/pt X 0.048 pt = 1,089.6 mg;	3) 2.27 ug/pt X 0.048 pt = 0.109 ug;
4) 1,089.6 mg X 10% = 108.96 mg;	4) 0.109 ug X 10% = 0.011 ug;
5) 108.96 mg / 60 kg = 1.8 mg/kg per day	5) 0.011 ug / 60 kg = 0.00018 ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (1.8 mg/kg), as well as the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03μg/kg) and this calculated exposure level for TCDD (0.00018 μg/kg), do not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T and/or TCDD pursuant to 40 CFR 162.11(a)(3)(ii)(B).

(iii) *Aerial Application: Exposed Population Directly Beneath Spray Plane.* Caplan et al. (*167*), working with aerially applied malathion in oil sprays applied at 0.46 pounds per 0.76 gallons water/acre, determined a dermal exposure to persons directly beneath the spray plane for bare skin (head, neck, shoulders, forearms, hands, and thighs) of 3.556 mg/day. With these data, an equivalent dermal exposure for 2,4,5-T and TCDD, aerially applied at 4 pounds acid equivalent 2,4,5-T per 10 gallons water/acre, can be determined.

Table 30. Dermal Exposure Data (Aerial Application)

Dermal exposure to aerially applied malathion	3.556 mg/0.46 pounds malathion per acre	
	2,4,5-T	TCDD
Use Dilution rate	4 pounds 2,4,5-T per 10 gallons of water/acre	0.0000004 pounds TCDD per 10 gallons of water per acre
% Diluted material absorbed	10%	10%
Exposure level	3.1 mg	0.0003 ug
Dose level	0.051 mg/kg	5×10^{-6} ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

The following calculations (see Table 31 for mathematics) will give the daily dermal exposure for both 2,4,5-T and TCDD: (1) Divide the dermal exposure to malathion by the malathion application rate and multiply by the application rate of 2,4,5-T and TCDD to obtain the dermal exposure; for TCDD, multiply this figure by 1,000 to convert to micrograms; (2) multiply this figure by the percent absorbed; and (3) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

Table 31

2,4,5-T	TCDD
1) 3.556 mg/0.46 pounds X 4 pounds = 31 mg;	1) 3.556 mg/0.46 pounds X 0.0000004 pounds = 0.000003 mg X 1,000 = 0.003 ug;
2) 31 mg X 10% = 3.1 mg;	2) 0.003 ug X 10% = 0.0003 ug;
3) 3.1 mg/ 60 kg = 0.051 mg/kg per day	3) 0.0003 ug / 60 kg = 5×10^{-6} ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 μg/kg) and this calculated dermal exposure level for TCDD (5×10^{-6} μg/kg) does constitute an ample margin of safety. The Working Group also considers, however, that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (0.051 mg/kg) does not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR 162.11(a)(3)(ii)(B).

(c) *Inhalation Exposure: Aerial Application.* There are no studies available on inhalation exposure of 2,4,5-T. There are, however, several studies on inhalation exposure to malathion (167, 168) which CED used as a model for this 2,4,5-T exposure analysis (*164*). Caplan et al. (*167*) determined an air concentration, for unprotected persons directly beneath the spray plane during application and for two hours afterward, of 0.067 mg malathion/m^3 from aerial application of 0.46 pounds AI/gallon per acre. The collection period spanned the course of the actual application time plus two hours thereafter. The authors considered the sampling technique to be equivalent to average inspira-

DOW 169606

tion through the nostrils. This inhalation exposure (amount available for inhalation) was 12 percent of the applied malathion. Caplan et al. further reported that the average median diameter (=volume median diameter, or vmd [16]) was 100 microns. Based on work by Akesson and Yates (*168*), CED (*164*) estimated that the size of the malathion droplets which could be inhaled was under 60 microns. Since 2,4,5-T is typically applied as a medium or coarse spray, while malathion is applied as a fine spray, the percent of 2,4,5-T droplets small enough to be inhaled (under 60 microns) would be less than the percent of malathion droplets small enough to be inhaled. According to Akesson and Yates (*168*), 2 percent of 2,4,5-T spray droplets would be available for inhalation (or ¼ the amount of malathion droplets available for inhalation), on a "worst case" basis.

The following calculations (see Table 33 for mathematics) will give the daily inhalation exposure for both 2,4,5-T and TCDD: (1) Multiply the air concentration of malathion by the amount of 2,4,5-T and TCDD applied, then multiply this figure by ¼ for the inhalation exposure to 2,4,5-T and TCDD; for TCDD, multiply this figure by 1,000 to convert to micrograms; (2) multiply this figure by the breathing rate; (3) multiply this figure by eight [8] to get the 8-hour exposure total; and (4) divide this figure by the weight of the applicator for the inhalation exposure to 2,4,5-T or TCDD per 8-hours exposure.

The Working Group considers that the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 μg/kg) and this calculated dermal exposure level for TCDD (2×10^{-4} μg/kg) does constitute an ample margin of safety. The Working Group also considers, however, that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (0.026 mg/kg [17]) does not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR 162.11(a)(3)(ii)(B).

(d) *Cumulative Exposure.* The Working Group has also considered the possibility of a single individual being exposed through two or more of the above routes. The results (derived from Tables 27, 29, and 31) are shown in Table 34. The Working Group also notes that possible cumulative exposure to several dioxin-containing pesticides could increase the total body burden and increase total risk from dioxin exposure.

The Working Group considers that the differences between the no-adverse-effect level of TCDD for teratogenic effects (0.03 μg/kg) and the calculated cumulative exposure levels for TCDD in Situations 2 and 3 (see Table 34) do constitute an ample margin of safety. The Working Group also considers, however, that the differences between the no-adverse-effect levels of 2,4,5-T and TCDD for teratogenic effects (20 mg/kg and 0.03 μg/kg, respectively) and the calculated cumulative exposure levels for 2,4,5-T in Situations 1, 2, and 3 and TCDD in Situation 1 (see Table 34) do not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR 162.11(a)(3)(ii)(B).

[16] The vmd is that droplet size which divides the total volume of drops in half, i.e., 50 percent of the volume is in drops above the vmd size and 50 percent below it.

[17] Johnson (63) (see Section I.G.(3)), in a review article, calculated a daily inhalation exposure to phenoxy herbicides of 0.025 μg/kg for a 70-kg adult. The calculations were based on actual air monitoring data of air samples collected in two wheat-growing areas in the state of Washington during spring and summer and analyzed for phenoxy herbicides. The author did not specify how soon after application the samples were taken.

Table 32. Inhalation Exposure Data (Aerial Application)

Air concentration of aerially applied malathion	0.067 mg/m^3 with application rate of 0.46 pounds malathion per gallon per acre	
	2,4,5-T	TCDD
Use Dilution rate	4 pounds 2,4,5-T per 10 gallons of water/acre	0.0000004 pounds TCDD per 10 gallons of water per acre
Lung Absorption Rate	100%	100%
Breathing Rate	1.8 m^3/hr	1.8 m^3/hr
Exposure level	0.34 mg per 2 hr	0.000032 ug per 2 hr
Dose level	0.023 mg/kg per 8 hr	2 X 10^{-6} ug/kg per 8 hr
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

Table 33.

2,4,5-T	TCDD
1) 0.067 mg/cu m per 0.46 pounds X 4 pounds = 0.58 mg/cu m X 1/6 = 0.097 mg/cu m;	1) 0.067 mg/cu m per 0.46 pounds X 0.0000004 pounds = 0.000000058 mg/cu m X 1/6 = 0.000000009 mg/cu m X 1,000 = 0.000009 ug/cu m;
2) 0.097 mg/cu m X 1.8 cu m/-hr = 0.17 mg/hr;	2) 0.000009 ug/cu m X 1.8 cu m/hr = 0.000016 ug/hr;
3) 0.17 mg/hr X 8 = 1.36 mg;	3) 0.000016 ug/hr X 8 = 0.000128 ug;
4) 1.36 mg / 60 kg = 0.026 mg/kg exposure per day	4) 0.000128 / 60 kg = 2×10^{-6} ug/kg per day

17416

Table 34. Cumulative Exposure to 2,4,5-T and TCDD

Situation #1: 2,4,5-T	Situation #1: TCDD
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 6.8 mg/kg	Dermal- 0.0007 ug/kg
Inhal.- 0.2 mg/kg[a/]	Inhal.- negligible[a/]
Cum. = 7.0 mg/kg	Cum. = 0.0007 ug/kg
Situation #2: 2,4,5-T	**Situation #2: TCDD**
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 1.8 mg/kg	Dermal- 0.00018 ug/kg
Inhal.- 0.05[a/]	Inhal.- negligible[a/]
Cum. = 1.85 mg/kg	Cum. = 0.00018 ug/kg
Situation #3: 2,4,5-T	**Situation #3: TCDD**
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 0.051 mg/kg	Dermal- 5×10^{-6} ug/kg
Inhal.- 0.026 mg/kg	Inhal.- 2×10^{-6} ug/kg
Cum. = 0.0777 mg/kg	Cum. = 7×10^{-6} ug/kg

a/ Calculations were made on a worst-case basis as 3% of dermal exposure based on Wolfe (179) who states, "over 97% of the pesticide to which the body is subjected during most exposure situations, and especially to applicators of liquid sprays, is deposited on the skin." TCDD inhalation exposure values were negligible: Situation #1, 21×10^{-6} ug/kg; Situation #2, 54×10^{-7} ug/kg.

IV. STUDIES RELATING TO POSSIBLE ADVERSE EFFECTS

This section addresses other types of adverse effects of 2, 4, 5-T for which the Working Group has determined that insufficient evidence exists to initiate a rebuttable presumption. The Agency solicits comments from registrants and other interested parties on the evidence listed below, and requests submission of any additional studies or relevant information on 2, 4, 5-T and/or TCDD relative to these potential adverse effects.

A. *Mutagenicity.* Section 162.11(A)(3)(ii)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) induce mutagenic effects, as determined by multitest evidence.

(1) *2, 4, 5-T*—(a) *Positive Study.* Majumdar and Golia (*178*) fed male *Drosophila melanogaster* either 250 or 1,000 ppm dioxin free 2, 4, 5-T (obtained from Eastman Kodak) for 15 days. They were then mated to sets of virgin females to generate three 4-day broods of offspring. F_1 flies were allowed to mate, and F_2 flies were scored for X-linked recessive lethals. No differences among broods were noted, and data from all broods were pooled. The percent lethals in controls, 250 and 1,000 ppm groups were dose-related and were 0.05, 0.026, and 0.66 percent, respectively. The control vs. 1,000 ppm lethal rates were significantly different from one another ($p < 0.01$). Ethyl methane sulfonate (250 ppm) was included as a positive control; it yielded 13.70 percent lethals. The total number of flies in each experimental group was no larger than 2,000.

(b) *Negative Studies.* The mutagenicity of 2, 4, 5-T was evaluated by Ercegovich et al. (*148*), employing the procedure of Ames, using five strains of *Salmonella typhimurium* without activation. They concluded that 2, 4, 5-T is not mutagenic.

Fujita et al. (*149*) reported chromosomal abnormalities in *in vitro* cytogenetic studies of human lymphocytes exposed to 10^{-7} to 10^{-4} M of 2, 4, 5-T, which contained 0.09 ppm TCDD. Breaks, deletions, and rings were observed. Chromatid breaks increased with increasing concentrations of 2, 4, 5-T. It was not possible to distinguish whether this was a toxic effect or a potential genetic effect (*150*).

Majumdar and Hall (*169*) reported on the cytogenetic effects of 2,4,5-T[16] on in vivo bone-marrow cells of Mongolian gerbils. The animals were injected with total amounts of 2,4,5-T at the rate of 50, 150, 250, 350, or 500 mg/kg body weight over the 5-day period of the study. Increasing numbers of chromatic gaps, breaks, and fragments were observed at 250, 350, and 500 mg/kg doses. No exchange figures or isochromosome gaps or breaks were observed. This is not a definitive experiment for indicating the potential of 2,4,5-T for causing heritable chromosome damage (*170*). Toxicity effects of the chemical could give similar results (*170*).

Davring and Hultgren (*171*) reported on an *in vivo* study on the cytogenetic effects on bone-marrow cells of *Mus musculus* (male mice) induced by a Swedish commercial 2,4,5-T ester formulation[16] and its components. The study showed that 2,4,5-T commercial products can affect chromosomal and reproductive mechanisms. Two different strains of mice were used with similar results for both. These results correlated with effects seen in Drosophila. The authors stated that chromatid inter- or intraexchanges were never observed. This study was not carried out sufficiently for the demonstration of chromosomal effects such as rearrangements in future generations of somatic cells (*171*).

Davring and Sunner (*172*) demonstrated cytogenetic effects of a Swedish commercial 2,4,5-T formulation[16] on oogenesis and early embryogenesis in *Drosophila melanogaster*. A 50 percent decrease in fertility for the flies was determined to be 250 ppm. This level is 40 to 60 times less than field use concentration levels. Reproductive and chromosomal effects were observed.

[16]The 2,4,5-T used in this study was purchased from Eastman Kodak Co., Rochester, N.Y., and contained no measurable amount of TCDD. The authors do not indicate the limit of sensitivity.

[16]The concentration of TCDD was guaranteed to be less than 0.1 ppm in the product.

[16]TCDD concentration was less than 0.1 ppm in this formulation which was tested at practical field use concentrations or lower.

DOW 699608

17417

(2) *TCDD*—(a) *Positive Studies.* Hussain et al. (*24*) evaluated the mutagenic activity of TCDD (99 percent pure) on three different microbial test systems. In the first study, TCDD significantly increased the incidence of reverse mutations in *Escherchia coli* Sd-4 administered 2 μg/ml TCDD from streptomycin dependence to streptomycin independence. This was the only dose at which mutations were clearly observed. No details of the experimental protocol were given, and statistical methods were apparently not employed in assessing the data.

The second test by Hussain et al. (*24*) studied reverse mutation from histidine dependence to histidine independence in *Salmonella typhimurium* (Strains TA 1532 and TA 1530). TCDD was positive in TA 1532 but negative in TA 1530. This indicated that TCDD acts as a frameshift mutagen. ICR-170 was used as a positive control in the test with TA 1532. No positive or negative controls were tested in TA 1530.

In the third test Hussain et al. (*24*) observed slight prophage induction in *E. coli* K-39. However, data from this test were difficult to evaluate because the solvent used, dimethyl sulfoxide, causes cellular effects.

A preliminary report on the chromosomal analysis of hospital patients exposed to TCDD in the accident at the Seveso, Italy, factory was presented at the Department of Health, Education, and Welfare meeting on October 12, 1976 (*152*). An increased number of chromosomal lesions (gaps, chromatidic and chromosomal breaks, and rearrangements) were observed in somatic cells of the 2- to 28-year-old males and females tested. Cytogenetic studies of tissues from therapeutic abortions performed on women who were exposed to TCDD during the accident indicated that there was chromosomal damage to cells in maternal peripheral blood, and placental and fetal tissues. These preliminary results were based on a small number of samples, and no specific data are available at this time (*150*).

(b) *Negative Studies.* Khera and Ruddick (*6*) conducted dominant lethal tests in which male Wistar rats received TCDD at dosages of 4 and 8 μg/kg per day. The studies indicated that no dominant lethal mutations arose during the 35 days after treatment. The period examined corresponded to postmeiotic stages of spermatogenesis.

A cytogenetic screening study of the effects of TCDD on bone marrow cells of male Osborne-Mendel rats was performed by the Food and Drug Administration (*119*). Two separate experiments were performed. The first was a multiple dose test in which 10 μg TCDD/kg per day was administered by intubation for 5 consecutive days. In the second test, single doses of 5, 10, and 15 μg TCDD/kg were administered intraperitoneally and 20 μg/kg (the highest dose) was administered orally. There was no evidence from these studies to indicate that TCDD produced cytogenetic damage in the bone marrow of these male rats. Toxicity, which was indicated by a slight weight loss, was noted in rats that received a single dose of 15 or 20 μg/kg (the highest dose levels).

Green (*119*) conducted a short-term investigation of several dioxins, using male Osborne-Mendel rats, to determine what potential these substances had to produce cytogenetic damage in rat bone-marrow. In one study all of the dioxins were tested by being intubated in the rats for five consecutive days at 10 μg/kg per day. A second study involved TCDD alone administered orally at 20 μg/kg and intraperitoneally at 5, 10, and 15 μg/kg. The author found no evidence that any of the substances tested produced cytogenetic damage in the bone marrow of male rats.

In conclusion, although Hussain et al. (*24*) have demonstrated that TCDD does appear to act as a point (gene) mutagen, the evidence is weak for heritable genetic effects since the level of mutagenic testing is meager and there were some major deficiences in some tests. However, the study by Hussain et al. does not fulfill the criterion of multitest evidence as prescribed in 40 CFR 162.11. Although TCDD does appear to have the potential to act as a chromosomal mutagen from the *in vivo* cytogenetic studies (*152*), specific data are not yet available from the Seveso accident.

(3) *Chromosomal Damage.* The Working Group also wishes to call attention to three studies (previously discussed in Sections III.B.(2)(b), IV.(1), and IV.(2)(b)), which indicate that 2,4,5-T and/or TCDD may cause chromosomal damage. Fujita et al. (*149*) reported chromosomal abnormalities in *in vitro* tests on human lymphocytes exposed to 2,4,5-T; abnormalities included breaks, deletions, and rings. Yefimenko (*167*) reported damage to bone-marrow cell chromosomes (including breaks, true aberrations, or rearrangements) in *in vivo* tests on rat gonadal and somatic tissue exposed to butyl ester 2,4,5-T. The preliminary HEW report (*152*) on the Seveso incident indicated an increased number of chromosomal lesions (gaps, chromatic breaks, and rearrangements) in somatic cells of 2- to 28-year-old humans exposed to TCDD.

The Working Group concludes that there is a data gap on mutagenic effects and that further evidence and testing is needed on the mutagenicity of 2,4,5-T and TCDD. The Working Group would like to evaluate more detailed and specific information as it becomes available from the Seveso accident. Relevant information or studies on the mutagenetic effects of 2,4,5-T and/or TCDD should be submitted to the Agency, and the option for re-evaluating their mutagenic properties must be left open should more conclusive evidence become available.

B. *Toxicity to Humans: TCDD*—(1) *Chloracne.* A number of researchers have reported illness ascribed to TCDD (*90, 93, 95, 153*). Most of these toxic effects have occurred in chemical plant workers after accidental exposure to the dioxin. While a number of ill effects have been reported, the most widely known is chloracne.

Chloracne is a severe skin disease resulting from exposure to highly chlorinated dibenzo dioxins. It is a disease of the follicular and sebaceous glands. Its symptoms and signs include skins lesions, follicular hyperkeratosis, and the formation of large sebaceous cysts, inflammed tubercles, and pustules. In addition to these symptoms, chloracne is often accompanied by a brownish keratinization of the skin, cystitis, pyelonephritis, depression, hirsutism, fatigue, neurological disturbances, raised cholesterol levels, liver damage, and psychological manifestations (*10; 15, 16, 154, 155, 156, 157*). Several researchers have observed that chloracne is not only irritating and persistent but also very difficult to cure. It is one of the most frequently contracted forms of occupational dermatitis, occurring primarily in chemical plant employees engaged in the production of 2,4,5-T and 2,4,5-TCP (*16, 95, 155, 156, 158*).

The first report on a toxic material being the causative agent for an occupational skin disease appears to have been by Dr. Karl Herxheimer in 1899. Dr. Herxheimer diagnosed the cause of dermatological problems in a German factory worker as exposure to chlorine ions in the production of caustic potash (*159*). It is from this early diagnosis that we get the name chloracne. During the early 1950's there were a series of industrial accidents in Germany resulting in an outbreak of chloracne in the employees of chemical plants manufacturing 2,4,5-T and 2,4,5-TCP. The symptoms of the employees of one of these factories in Hamburg, Germany, were extensively investigated by Kimming and Schulz (*16*). These researchers, using the rabbit ear test, proved that the cause of the chloracne was a contaminant found in crude 2,4,5-TCP and not the formulated 2,4,5-TCP. Later on, Bauer et al. (*15*) conclusively identified TCDD as the causative agent of chloracne.

(2) *Porphyria cutanea tarda and δ-Aminolevulinic Acid Synthetase.* Porphyria cutanea tarda (PCT), a form of hepatic porphyria, is another disease caused by exposure to TCDD and often accompanies chloracne. PCT occurs primarily in industrial workers associated with the manufacture of 2,4,5-T (*93, 94, 160*).

The symptoms of porphyria cutanea tarda, a defect in hepatic metabolism of porphyrins, are fragility of the skin, photosensitivity of the skin, hyperpigmentation, over-production of porphyrins, hirsutism, and neurological and intestinal disorders (*94, 160*). It is also characterized biochemically by an increase in the activity of the mitochondrial enzyme δ-aminolevulinic acid (ALA) synthetase, which is the first and rate-limiting enzyme in heme biosynthesis (*160*). TCDD was thought to be a potent inducer of ALA activity in chick embryo liver (*115*). Goldstein et al. (*161*) reported that TCDD was found to induce ALA synthetase and hepatic prophyria in mice. These researchers stated that at that time [1973] TCDD was the most potent porphyrogenic chemical known. Poland and Kende 1976 (*4*) found that the duration of ALA induction from TCDD exposure is prolonged, most likely due to the long biological half-life of TCDD. These researchers also found that ALA synthetase inducers have halogen atoms occupying at least three of the four lateral ring positions (positions 2, 3, 7, and 8), and that there is at least one free, non-halogenated ring position. TCDD fulfills all of these requirements.

2,4,5-T: Position Document 1

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1743

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DOW 169612

17421

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DOW 169613

17422

**** PRODUCT SEARCH LISTING **** PAGE 1

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT *NAME AND ADDRESS*

* 000004 *ORTHO CHEMICAL CO., INC.*
2 HUNZ AVE.
TIMBVILLE NY 13495

PRODUCT NAME

0001 *BUNKER KILLBRUSH*

REGISTRANT *NAME AND ADDRESS*

* 000100 *BRULIN & COMPANY INC*
PO BOX 27??
INDIANAPOLIS IN 46??0

PRODUCT NAME

0000 *LIVERSDALE CONCENTRATE*

REGISTRANT *NAME AND ADDRESS*

* 000148 *THOMPSON-HAYWARD CHEMICAL COMPANY*
BOX 2383
KANSAS CITY KS 66110

PRODUCT NAME

0211 *DE-ESTER DED-WEED LV-3S*

0212 *DED-WEED LV-6 BRUSH KIL LOW VOLATILE ESTER*

0272 *DE-ESTER DED-WEED AMINE-T AMINE SALT*

0311 *DED-WEED LV-6 BRUSH KIL*

0420 *2,4,5-T ISOOCTYL ESTER TECHNICAL*

**** PRODUCT SEARCH LISTING **** PAGE 2

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

CONTINUED REGISTRANT 000148

0193 *DED-WEED AMINE-T AMINE SALT*

0200 *DED-WEED 88-6 BRUSH KIL*

0206 *DED-WEED LV 2-T BRUSH KIL*

REGISTRANT *NAME AND ADDRESS*

* 000186 *ABATCH CHEMICAL DIVISION ENTRADA IND INC*
P O BOX 8219
SALT LAKE CITY UT 84108

PRODUCT NAME

0227 *BASCO 2,4,5-T LOW VOLATILE ESTER 4LB. E.C.*

0231 *BASCO 2,4,5-T BUTYL ESTER 4# E.C.(2,4,5-T EMUL.CONC.)*

0261 *BASCO BRUSH KILLER LOW VOLATILE 2,4-D 2,4,5-T EMUL. C.*

REGISTRANT *NAME AND ADDRESS*

* 000179 *BOLLE M M COMPANY*
BOX 588
ALEXANDRIA VA 22306

PRODUCT NAME

0064 *BU-HID 4, D. BRUSH KILLER BKE-4*

0065 *BU-HID 4, D. BRUSH KILLER BKE-6*

0067 *BU-HID 4,D. BRUSH KILLER BKE- 2#2*

**** PRODUCT SEARCH LISTING **** PAGE 3

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT *NAME AND ADDRESS*

* 000228 *TOBACCO STATES CHEMICAL COMPANY*
BOX 479
LEXINGTON NY 40501

PRODUCT NAME

0142 *TOBACCO STATES BRAND ESTER BRUSH KILLER 2#2*

0155 *TOBACCO STATES BRAND LOW-VOL BRUSH KILLER 2#2*

REGISTRANT *NAME AND ADDRESS*

* 000228 *RIVERDALE CHEMICAL COMPANY*
420 E 17TH ST
CHICAGO HEIB IL 60411

PRODUCT NAME

0015 *RIVERDALE BRUSH KILLER 1 CONTAINS LOW VOLATILE 2,4,5-T & 2,4,D*

0016 *RIVERDALE WEEDESTROY 2,4,5-T*

0027 *2,4,5-T LOW VOLATILE ESTER*

0028 *RIVERDALE BRUSH KILLER 4 ESTER*

0045 *RIVERDALE BRUSH KILLER 2*

0075 *RIVERDALE FORMULA 6 BRUSH KILLER*

0077 *RIVERDALE 2,4,5-T AMINE BRUSH KILLER*

0078 *WEEDESTROY 2,4,5-T ESTER 4*

0091 *2,4,5-T LV6*

0119 *RIVERDALE BUTOXY 2,4,5-T LOW VOLATILE ESTER*

0120 *RIVERDALE BUTOXY BRUSH KILLER 2 LOW VOLATILE ESTER*

**** PRODUCT SEARCH LISTING **** PAGE 4

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT *NAME AND ADDRESS*

* 000239 *CHEVRON CHEMICAL COMPANY*
ORTHO DIVISION 940 HENSLEY WAY
RICHMOND CA 94801

PRODUCT NAME

0125 *ORTHO LV BRUSH KILLER Type*

REGISTRANT *NAME AND ADDRESS*

* 000264 *AMCHEM PRODUCT INC*
BROOKSIDE AVE
AMBLER PA 19002

PRODUCT NAME

0000 *WEEDONE 2,4,5-T*

0010 *WEEDONE BK 12*

0019 *WEEDONE KR 66*

0021 *WEEDONE I.B.E.*

0053 *WEEDAR AMINE BK*

00C2 *WEEDAR 2,4,5-T*

0071 *2,4,5-T LOW VOLATILE ESTER BRUSH KILLER*

0073 *2,4-D-2,4,5-T LOW VOLATILE ESTER BRUSH KILLER*

0080 *AMCHEM TRIMURAL*

0080 *AMINE 2,4,5-T FOR RICE*

0089 *WEEDONE 2,4,5-T SPECIAL AIR SPRAY FORMULA*

0183 *AMCHEM DIMURAL*

DOW 169614

17423

**** PRODUCT SEARCH LISTING **** PAGE 5

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000200

**000121* AMCHEM ENVERT-8T
**000123* AMCHEM ENVERT-8
**000126* AMCHEM TRIBASIL SUPER + 6
**000130* BINAMUL SUPER 6
**000161* AMCHEM EMULSAMINE 2,4,5-T
**000163* EMULSAMINE BR WOODY PLANT HERBICIDE
**002000* ASPLUNDH 1650-6 BRUSHKILLER
**002040* EMULSAYER! 10V
**002090* EMULSAYER! 20S
**002340* TECHNICAL 2, 4, 5-T ESTER (FOR USE IN THE MANUFACTURE OF HERBICIDES)
**002600* WEEDONE ISK DOOR INHIBITED
**002690* ENVERT-8T
**002820* AMCHEM BR WOODY PLANT HERBICIDE
**002830* AMCHEM BR WOODY PLANT HERBICIDE
**002860* AMCHEM 2,4,5-T WOODY PLANT HERBICIDE
**002880* AMCHEM 2,4,5-T WOODY PLANT HERBICIDE
**002870* WEEDONE 2,4,5WT WOODY PLANT HERBICIDE DOOR INHIBITED
**002900* WEEDONE 2,4,5-T SPECIAL AIR SPRAY FORMULA

****

REGISTRANT *NAME AND ADDRESS*

* 000334 NYBAN CORPORATION
919 N 18TH ST
CHICAGO IL 60607

********** PRODUCT NAME **********

**** PRODUCT SEARCH LISTING **** PAGE 6

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000334

**000253* GCC 649
**000308* GCC-645

****

REGISTRANT *NAME AND ADDRESS*

* 000350 MOTT MANUFACTURING COMPANY INC
PLEASANT VALLEY NY 12569

********** PRODUCT NAME **********

**000000* MOTT'S POISON IVY KILLER

****

REGISTRANT *NAME AND ADDRESS*

* 000359 RHODIA INC, AGRICULTURAL DIVISION
P.O. BOX 125
MONMOUTH JUNCTION, NJ 08852

********** PRODUCT NAME **********

**000178* RHODIA 2,4,5-T LOW VOLATILE ESTER 4L
**000179* RHODIA LOW VOLATILE BRUSH KILLER NO. 2
**000412* RHODIA LOW VOLATILE BRUSH KILLER NO.3
**000413* RHODIA 2,4,5-T LOW VOLATILE ESTER 4L
**000540* UTILITY BRUSH KILLER NO. 4
**000540* CHIPMAN AMINE BRUSH KILLER
**000570* CHIPMAN MIXTURE SPECIAL MIXTURE NO. 1
**000600* RHODIA 2,4,5-T 1OCTYL ESTER (TECHNICAL)

**** PRODUCT SEARCH LISTING **** PAGE 7

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000350

**000682* CHIPMAN 2,4-D AND 2,4,5-T ESTER MIX TECHNICAL
**000685* CHIPMAN D AND T MIX NO. 8
**000812* T & C LAWN WEED KILLER CONCENTRATE
**000815* SHIFT MIX NO. 1
**000816* SHIFT MIX NO. 2
**000845* VISKO-RHAP LOW VOLATILE ESTER 1U-1T FOR AIR APPLICATION
**000846* VISKO-RHAP LOW VOLATILE ESTER 1U-1T FOR GROUND APPLICATION
**000849* VISKO-RHAP WEL-SOLUBLE AMINE A-3T
**000851* VISKO-RHAP LOW VOLATILE ESTER 2T FOR GROUND APPLICATION
**000852* VISKO-RHAP LOW VOLATILE ESTER 2T FOR AIR APPLICATION
**000853* VISKO-RHAP 4 LOW VOLATILE 2 T
**000855* VISKO-RHAP LOW VOLATILE ESTER 3T

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REGISTRANT *NAME AND ADDRESS*

* 000007 IMPERIAL INC
BOX 828
CHEMANOUAH IA 51001

********** PRODUCT NAME **********

**001345* IMPERIAL LOW-VOL SUPER BRUSH KILLER
**001346* IMPERIAL LOW-VOL 2,4,5-T
**001365* IMPERIAL NO.4 LOW VOLATILE 2,4,5-T BRUSH AND WEED KILLER
**001366* IMPERIAL NO. 2 + 2 LOW VOLATILE BRUSH AND WEED KILLER

DOW 169615

**** PRODUCT SEARCH LISTING **** PAGE 8

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

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REGISTRANT *NAME AND ADDRESS*

* 000013 GANFELD & BRUNER CHEMICAL COMPANY
1406-08 ST LOUIS AVE
KANSAS CITY, MO 64101

********** PRODUCT NAME **********

**000025* PIONEER BRAND BRUSH KILLER NO. 23 BUTYL ESTER

****

REGISTRANT *NAME AND ADDRESS*

* 000049 TECHNE CORPORATION
C/O REGULATORY AFFAIRS DEPT. FARMLAND IND., INC.
P. O. BOX 7305
KANSAS CITY, MO 64116

********** PRODUCT NAME **********

**000140* BUTOXYL ESTER
**000020* BROM-KILL CONCENTRATE (ESTER)
**000050* BROM-KILL 4LB. BUTYL ESTER CONCENTRATE
**000120* LOW VOL 4 LB AMINE KILL
**000127* NO. 4 LOW VOLATILE 2,4,5-T
**000317* 2,4,5,-T 4LB BUTYL ESTER

****

REGISTRANT *NAME AND ADDRESS*

* 000044 UAP CHEMICAL U S A
PO BOX 1700
GREENVILLE SC 29602

********** PRODUCT NAME **********

17424

DOW 1696L6

**** PRODUCT SEARCH LISTING **** PAGE 9

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000404

000199	VERN 245
000272	TIPPON 1& BRUSH AND WEED KILLER
000273	TIPPON 2-8
000289	VERTON CE WEED AND BRUSH KILLER
000340	VERTON 7 HERBICIDE
000351	BRUSH KILLER 4T
000352	BRUSH KILLER 2-4
000364	DOW TURFLON 155 MIXTURE BRUSH KILLER
000407	TURFLON 2P5 MIXTURE HERBICIDE
000422	VERTON 4T
000491	2,4,5-T BUTOXY ETHANOL ESTERS
000492	2,4,5-T BUTOXY PROPYL ESTERS
000494	2,4,5-T BUTYL ESTERS
000495	2,4,5-T ISOOCTYL ESTERS
000497	DOW BRUSH KILLER & HERBICIDE
000498	DOW BRUSH KILLER IV HERBICIDE

REGISTRANT	NAME AND ADDRESS
000476	STAUFFER CHEMICAL COMPANY LABELING & REGISTRATION 1200 SOUTH 47TH ST RICHMOND, CA 94804

PRODUCT NAME

**000000	2,4,5-T TRIETHYLAMINE 4#S
**000230	2,4-D+2,4,5-T AMINE 2-2 #S

**** PRODUCT SEARCH LISTING **** PAGE 10

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000476

000401	2,4-D+2,4,5-T ISOOCTYL ESTER 2-2E LOW VOLATILE BRUSH KILLER
000498	2,4,5-T ISOOCTYL ESTER 4-E LOW VOLATILE BRUSH KILLER
000431	2,4,5-T BUTYL ESTER 4-E
000436	2,4-D 2,4,5-T BUTYL ESTERS 1.33-.66-E
000439	STAUFFER 2,4-D 2,4,5-T BUTYL ESTERS 2-2-4 EMULSIFIABLE LIQUID

REGISTRANT	NAME AND ADDRESS
000524	MONSANTO COMPANY AGRICULTURAL PRODUCTS 800 N. LINDBERGH BLVD. ST. LOUIS, MO 63166

PRODUCT NAME

**000047	2,4,5-T ISOPROPYL ESTER, TECHNICAL GRADE
**000070	TECHNICAL GRADE 2,4,5-T BUTYL ESTER
**000075	MONSANTO 2,4-D + 2,4,5-T BUTYL ESTER BRUSH KILLER
**000081	2,4,5-T LOW VOLATILE ESTER BRUSH KILLER
**000095	D-61 MONSANTO 2,4,5-T ISOOCTYL ESTER
**000097	MONSANTO 2,4,5-T ESTER BRUSH KILLER
**000098	2,4-D +2,4,5-T AMINE BRUSH KILLER
**000108	MONSANTO 2,4,5-T AMINE BRUSH KILLER
**000110	BRUSH-O-CIDE BRUSH KILLER
**000111	BRUSH BLITZ
**000149	2,4-D/2,4,5-T MIXED ESTERS N-BUTYL AND ISOBUTYL
**000150	2,4-D/2,4,5-T BUTYL ESTERS
**002540	2,4-D/2,4,5-T LOW VOLATILE ESTER BRUSH KILLER (CODE36)

**** PRODUCT SEARCH LISTING **** PAGE 11

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 000524

000258	2,4,5-T ISOBUTYL ESTER TECHNICAL GRADE

REGISTRANT	NAME AND ADDRESS
000951	BAIRD & MCGUIRE INC SOUTH ST HOLBROOK MA 02343

PRODUCT NAME

**000111	4DT AMINE BRUSH KILLER
**000116	4T AMINE BRUSH KILLER
**000157	4T-ESTER BRUSH KILLER
**000158	4DT-ESTER BRUSH KILLER
**000183	BRUSH KILLER (LOW VOLATILE EMULSIFIABLE CONCENTRATE) LV 3D/6T
**000184	BRUSH KILLER (LOW VOLATILE EMULSIFIABLE CONCENTRATE) LV-6T

REGISTRANT	NAME AND ADDRESS
000556	AGRICO INC BOX 456 SPANG PLAZA MO 58201

PRODUCT NAME

**000556	AGRICO BRUSH KILLER

**** PRODUCT SEARCH LISTING **** PAGE 12

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
000572	HICKLAND CHEMICAL CO. INC. P.O. BOX 809 CALDWELL, NJ 07006

PRODUCT NAME

**000035	HICKLAND BRUSH KILLER (ESTER FORM) LOW VOLATILE

REGISTRANT	NAME AND ADDRESS
000577	SHERWIN-WILLIAMS COMPANY 101 PROSPECT AVE NW CLEVELAND OH 44101

PRODUCT NAME

**000124	ACME POISON IVY KILLER

REGISTRANT	NAME AND ADDRESS
000632	MUTUAL DEALERS WHOLESALE INC 2361 HAMPDEN AVE ST. PAUL MN 55114

PRODUCT NAME

**000822	SUNTHRONE L V BRUSH KILLER

REGISTRANT NAME AND ADDRESS

* 000044 IMPERIAL CHEMICALS CORP
4529 E SIGURD ST
PITTSBURG CA 94564

PRODUCT NAME

000200 TECHNICAL 2-ETHYLHEXYL ESTER OF 2,4,5-T

000203 TECHNICAL N-BUTYL ESTER OF 2,4,5-T

REGISTRANT NAME AND ADDRESS

* 000477 DIAMOND SHAMROCK CHEM. AGRICULTURAL CHEM. DIVISION
A UNIT OF DIAMOND SHAMROCK CORP 1100 SUPERIOR AVE
CLEVELAND OH 44114

PRODUCT NAME

000095 LINE KIDEN 2D BRUSH KILLER

000097 LINE KIDEN 4T BRUSH KILLER

000102 LINE KIDEN LV 30/3T BRUSH KILLER

000103 BRUSH KILLER DIAMOND CANE LV 30/3T-4B

000106 DIAMOND CANE CONCENTRATE LV-4T-4B

000131 LINE KIDEN AMINE 4T BRUSH KILLER

000136 FENCE KIDEN 4T BRUSH KILLER

000161 LINE KIDEN LV-2T BRUSH KILLER

000172 LINE KIDEN INVERT I CONCENTRATE

000174 LINE KIDEN INVERT D/T CONCENTRATE

000195 DACAMINE 4T BRUSH KILLER

**CONTINUE REGISTRANT 000477

000199 DACAMINE 2D/2I

000238 THALLOAT 2D/2I DIAMINE SALT OF 2,4-D & 2,4,5-T

000267 TECHNICAL 2-ETHYLHEXYL-T (2,4,5-T)

000268 TECHNICAL ISOBUTYL-T (2,4,5-T)

000269 TECHNICAL BUTYL-T (2,4,5-T)

000271 TECHNICAL ISOOCTYL-T (2,4,5-T)

000273 THALLOAT 4T CONTAINS 4.8 POUNDS 2,4,5-T ACID EQUIVALENT PER GALLON

000297 AMINE 2D/2I

003010 LO-VOL 2D/2I

003011 LO-VOL-4I

003012 LO-VOL 4T

003240 DIAMOND SHAMROCK BRUSH KILLER LO-VOL 2D/2I

003245 DIAMOND SHAMROCK BRUSH KILLER 8P LO-VOL 4T

REGISTRANT NAME AND ADDRESS

* 000740 WA OIL CO
P.O. BOX 910
COLUMBIA MO 65204

PRODUCT NAME

000050 W.F.A. LO-V 2,4,5-T

000051 W.F.A. 2,4,5-T BUTYL ESTER

000052 W.F.A. LO-V SUPER BRUSH KILL

000053 W.F.A. SUPER BRUSH KILL (BUTYL ESTER)

000188 W.F.A. AERIAL ESTER NO. 44

REGISTRANT NAME AND ADDRESS

* 000002 LILLY CHAS M COMPANY MILLER RD DIV
7737 N.E. KILLINGSWORTH
PORTLAND OR 97218

PRODUCT NAME

000128 MILLER'S 2,4,5-T AMINE 4

000181 MILLER'S LV BRUSH KILLER

000184 MILLER LV 2,4,5-T ESTER

000220 MILLER'S BLACKBERRY & BRUSH KILLER

000329 MILLER'S LV 2,4,5-T ESTER 6A

000528 MILLER'S LV BRUSHKILLER D

000529 MILLER'S LV 2,4,5-T ESTER FOUR 6P

000530 MILLER'S BRUSH KILLER

REGISTRANT NAME AND ADDRESS

* 000870 VELSICOL CHEMICAL CORP
341 EAST OHIO STREET
CHICAGO IL 60611

PRODUCT NAME

000104 BANVEL SIV OIL SOLUBLE INDUSTRIAL HERBICIDE

000170 BANVEL - 71V

000174 BANVEL - 32V WATER SOL IND HERBICIDE FOR BRUSH & BROADLEAF WE

000180 BANVEL SIV OIL SOL INDUS.-COMM. FOR BRUSH AND BROADLEAF WEED CONTRO

000210 VELSICOL VEGAIRUL 4 2D-4I HERBICIDE FOR BRUSH CONTROL

**CONTINUE REGISTRANT 000870

000218 VELSICOL VEGAIRUL LV-2D-2I HERBICIDE FOR BRUSH CONTROL

000219 VELSICOL VEGAIRUL BE-2D-2I HERBICIDE FOR BRUSH CONTROL

000220 VELSICOL VEGAIRUL BE-4T HERBICIDE FOR BRUSH CONTROL

000222 VELSICOL VEGAIRUL 8-4D HERBICIDE FOR BRUSH CONTROL

000228 VELSICOL VEGAIRUL LV-4P HERBICIDE FOR BRUSH CONTROL

000229 VELSICOL VEGAIRUL LV-4T HERBICIDE FOR BRUSH CONTROL

000230 VELSICOL VEGAIRUL 8-4I HERBICIDE FOR BRUSH CONTROL

000231 VELSICOL VEGAIRUL 4-4I HERBICIDE FOR BRUSH CONTROL

REGISTRANT NAME AND ADDRESS

* 000912 FARMERS UNION CENTRAL EXCHANGE INC
POST OFFICE BOX "G"
ST PAUL MN 55165

PRODUCT NAME

000050 CO-OP BRUSH KILLER LV 262 BRUSH AND WEED KILLER

REGISTRANT NAME AND ADDRESS

* 001169 AMCO OIL CO.
200 E. RANDOLPH DR.
CHICAGO IL 60601

PRODUCT NAME

000018 AMCO BRUSH KILLER

000020 AMCO 2, 4, 5-T LV ESTER FOR BRUSH CONTROL

COW 169617

DOW 1699618

**** PRODUCT SEARCH LISTING **** PAGE 17

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 001140

000079 AMOCO 2,4,5-T AMINE

REGISTRANT	*NAME AND ADDRESS*
* 001140	HIGHLAND CORP INC 2021 EAST HENNEPIN AVE MINNEAPOLIS MN 55413

********** PRODUCT NAME **********

000617 HIDLAND 50-50 *** LOW VOLATILE BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 001200	U.S. EAGLE, INC. P.O. BOX 50051 DALLAS, TX 75250

********** PRODUCT NAME **********

000020 EAGLE BRUSH KILL 2,4,5-T SOLUTION

REGISTRANT	*NAME AND ADDRESS*
* 001222	INDIANA FARM BUREAU COOP ASSN INC 47 SO PENNSYLVANIA ST INDIANAPOLIS IN 46204

********** PRODUCT NAME **********

000036 LOW CONCENTRATED 2,4,5-T

000000 COOP LOW CHAIN LOW VOLATILE FOUR POUND BRUSH KILLER

**** PRODUCT SEARCH LISTING **** PAGE 18

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 001258	KLIN CHEMICALS KLIN CORPORATION 120 LONG RIDGE ROAD STAMFORD, CT 06904

********** PRODUCT NAME **********

001058 LV ESTER 2T BRUSH KILLER

001078 LV ESTER 4T BRUSH KILLER

002368 NATRIBON BUTYL ESTER 2T BRUSH KILLER

002618 NATRIBON BUTYL ESTER 7T BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 001269	DEWITT CHEMICAL COMPANY BOX 343 ATLANTA GA 30301

********** PRODUCT NAME **********

000313 DE WITT 8-77 WEED KILLER

001068 DEWITT NO. 17 BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 001279	ZEP MANUF CORP BOX 2015 ATLANTA GA 30301

********** PRODUCT NAME **********

000055 ZEP WEED KILLER

**** PRODUCT SEARCH LISTING **** PAGE 19

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

**CONTINUE REGISTRANT 001270

001280 ZEP 8-76 BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 001301	LAND O'LAKES C/O IMPERIAL INC. PO BOX 429 SHENANDOAH IA 51601

********** PRODUCT NAME **********

000020 FELCO SUPER BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 001360	UNIVERSAL COOPERATIVES INC PO BOX 656 ALLIANCE OH 44601

********** PRODUCT NAME **********

000020 BRUSH KILLER

000056 UNICO LOW BRUSH KILLER CONTAINS 2,4-D AND 2,4,5-T LOW VOLATILE ES

000057 UNICO 2,4,5-T LOW ESTER BRUSH KILLER

000556 UNICO LV T BRUSH KILLER I-T

**** PRODUCT SEARCH LISTING **** PAGE 20

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 001605	STATE CHEMICAL MFG CO INC 3140 HAMILTON AVE 44114

********** PRODUCT NAME **********

000001 FORMULA 600 BRUSH-KIL SELECTIVE WEED AND BRUSH KILLER

000008 FORMULA 200 BRUSH KIL SELECTIVE WEED & BRUSH KILLER

000078 FORMULA 220-4 BRUSH-KIL

REGISTRANT	*NAME AND ADDRESS*
* 001769	NATIONAL CHEMSEARCH DIV USACHEM INC 2727 CHEMSEARCH BLVD IRVING TX 75060

********** PRODUCT NAME **********

001686 CHEMSEARCH CHEMSTEEN 145

REGISTRANT	*NAME AND ADDRESS*
* 001772	MULDER CORP INC 1421 FIFTH AVE HUNTINGTON WV 25702

********** PRODUCT NAME **********

000072 CHEMFORM BRAND BUTOXY BRUSH KILLER

17427

**** PRODUCT SEARCH LISTING **** PAGE 21

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
001026	DAVY BRAND MFG COMPANY 3111 3D AVE ST LOUIS MO 63110

PRODUCT NAME

000010 BRN-15

REGISTRANT	NAME AND ADDRESS
001969	FARNAM CHEMICAL BOX 160 GRAND LEDGE MI 48837

PRODUCT NAME

000071 PUISON IVY & BRUSH KILLER 40

REGISTRANT	NAME AND ADDRESS
001990	FARMLAND IND., INC. C/O REGULATORY AFFAIRS DEPT. P. O. BOX 7305 KANSAS CITY, MO 64116

PRODUCT NAME

000106 CHIP WEED-OUT (2,4,5-T LOW VOLATILE ESTER & POWDR)

000131 CHIP WEED-OUT BRUSH KILLER LOW VOLATILE

000160 WEED-OUT 2,4,5-T BUTYL ESTER

000227 CHIP WEED OUT (BRUSH KILLER BUTYL ESTER)

**** PRODUCT SEARCH LISTING **** PAGE 22

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
002126	GRACE W R & COMPANY AGH CHEM C H FIDWELL PO BOX 277 100 N MAIN ST MEMPHIS TN 38101

PRODUCT NAME

000137 MACU LOW VOLATILE 20 - 27

000705 MACU 2,4,5-T LOW VOLA BRUSH KILLER-4T CONT 4 LBS 2,4,5-T GAL

REGISTRANT	NAME AND ADDRESS
002155	SCHMIDT,INC. 1429 FAIRMONT AVE N W ATLANTA GA 30318

PRODUCT NAME

000845 FORMULA 400 BRUSH KILLER

REGISTRANT	NAME AND ADDRESS
002169	PATTERSON CHEMICAL COMPANY INC 1400 UNION AVE KANSAS CITY, MO 64101

PRODUCT NAME

000649 PATTERSON'S BRUSH KILLER NO. 200

**** PRODUCT SEARCH LISTING **** PAGE 23

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
002217	PBI-GORDON CORPORATION 300 NO 3RD ST KANSAS CITY KS 66118

PRODUCT NAME

000070 LV BRUSH KILLER

000078 LOW VOLATILE NO. 400 2,4,5-T ESTER WEED KILLER

000090 SUPER BRUSH KILLER NO. 400 BUTYL

000100 BUTYL ESTER 4#E 2,4,5-T BRUSH KILLER

000556 2,4,5-T AMINE

REGISTRANT	NAME AND ADDRESS
002362	AFRO-MIXABLE CHEMICAL CORP AGR PRO & LABELING AERO-MIXER CENTER OKLAHOMA CITY OK 73102

PRODUCT NAME

000838 POISON IVY KILLER

REGISTRANT	NAME AND ADDRESS
002393	MEMPHIS AGRI. CHEMICAL CO. P.O. BOX 7532 MADISON WI 53707

PRODUCT NAME

000380 LOW VOLATILE 2-4 BRUSH KILLER

**** PRODUCT SEARCH LISTING **** PAGE 24

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
002518	SANTELLI P A TREE EXPERT COMPANY 2770 SUMMER ST STAMFORD CT 06905

PRODUCT NAME

000080 BAKHI-VERT COMPUMENT A.

REGISTRANT	NAME AND ADDRESS
002737	PUEBLO CHEMICAL & SUPPLY COMPANY DRS 1270 - 2200 N. ST. JOHN KANSAS CITY, MO 64116

PRODUCT NAME

000812 PUEBLO 4 LB. BRUSH KILLER

000831 PUEBLO BRUSH KILLER LOVOL 20/27

REGISTRANT	NAME AND ADDRESS
002736	STULL CHEM COMPANY 1800 PAULSON DRIVE SAN ANTONIO, TX 78219

PRODUCT NAME

000049 INSTEMUL DTA 60

DOW 169619

17428

DOW169620

**** PRODUCT SEARCH LISTING **** PAGE 26

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 001035	JEFUNU CHEMICALS PO BUX 80288 ATLANTA GA 30341

PRODUCT NAME

**000390 UEFUNU 66-64 LOW-VOLATILE HERBICIDE FOR CONTROLLING WOODY PLANTS

REGISTRANT	*NAME AND ADDRESS*
* 001770	ECONOMY SPRAY COMPANY BOX 217 MONTPELIER, IA 52758

PRODUCT NAME

**000130 NO. 2 & 2 BRUSH KILLER

**000480 ECONOMY 2,4,5-T BUTYL ESTER 4E

REGISTRANT	*NAME AND ADDRESS*
* 001805	SMITH-DOUGLASS DIV. BORDEN CHEMICAL, BORDEN INC. 5100 VIRGINIA BEACH BLVD. NORFOLK, VA 23501

PRODUCT NAME

**001240 SMITH-DOUGLASS HANDLE BRUSH KILLER

**001280 SMITH-DOUGLASS 2,4,5-T LOW VOLATILE ESTER BRUSH KILLER

**** PRODUCT SEARCH LISTING **** PAGE 27

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 004031	GUUU-LIFE CHEMICALS INC BOX 687 EFFINGHAM IL 62401

PRODUCT NAME

**000860 GUUU-LIFE 2-2 LOW VOLATILE BRUSH KILLER ISOCTYLESTER 2,4-D+2,4,5-T

**000870 GUUU-LIFE 4 Lb. 2,4,5-T LOW VOLATILE BRUSH KILLER

**000890 GUUU-LIFE 4 Lb. 2,4,5-T BRUSH KILLER BUTYL

REGISTRANT	*NAME AND ADDRESS*
* 005905	HELENA CHEMICAL CO ATTN: [illegible], 5100 POPLAR AVE, SUITE 2900 MEMPHIS TN 38137

PRODUCT NAME

**000740 HELENA 20-20 BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 006290	COMET MANUFACTURING COMPANY 1381 DALTON AVENUE N E ATLANTA GA 30306

PRODUCT NAME

**000810 COM-U-BRUSH LV 20-10 LOW VOLATILE ISOOCTYL ESTER OF 2,4,5-T AND 2,4-D

**000819 COM-D-BRUSH ISOCTYL BRN KLR SLV LOW VOL ISOOL ESTR 2,4,5-TE,2,4-D

**** PRODUCT SEARCH LISTING **** PAGE 28

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 004715	COLUMBIA INTERNATIONAL CORP 2400 HAROLD N.E. ALBUQUERQUE, N.M. 87110

PRODUCT NAME

**001730 BEST 4 SERVIS BRAND LO-VOL BRUSH KILLER

**001740 BEST 4 SERVIS BRAND LO-VOL 2,4,5-T BRUSH KILLER

**001820 BEST 4 SERVIS BRAND BUTYL ESTER 4WD 2,4,5-T BRUSH KILLER

**002000 BUTYL BRUSH KILLER 3-5

REGISTRANT	*NAME AND ADDRESS*
* 004820	ABCO INC 210 INDUSTRY BLVD SOUTH HUNTINGTON, PA 15642

PRODUCT NAME

**000370 B. 4. - DT 135

**000630 ABCO 4EB-65 BRUSH KILLER

REGISTRANT	*NAME AND ADDRESS*
* 000017	REUEL LITTLE TREE INJECTION COMPANY BOX 288 RADILL OK 73446

PRODUCT NAME

**000020 REUEL LITTLE TREE INJECTION FLUID FORMULA NO. 2

**** PRODUCT SEARCH LISTING **** PAGE 29

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 006720	SOUTHERN HILL CREEK PRODUCTS COMPANY INC BOX 1090 TAMPA FL 33601

PRODUCT NAME

**000120 SHCP SPECIAL ESTER LV 0 & 7

REGISTRANT	*NAME AND ADDRESS*
* 000702	STERN CHEM CORP BOX 3078 MONROE LA 71204

PRODUCT NAME

**000810 REDUEA 130 LOW VOLATILE ESTER WEED GRASS AND BRUSH KILLER

**000821 REDUEA-65 LOW VOLATILE ESTER WEED GRASS & BRUSH KILLER

**000320 2,4,5-T TREE KILLER

REGISTRANT	*NAME AND ADDRESS*
* 000900	DILL J J COMPANY BOX 788 KALAMAZOO MI 49005

PRODUCT NAME

**000730 DILL 2,4,5-T LOW VOLATILE ESTER 4L BRUSH AND WEED KILLER

17429

CONTINUED REGISTRANT 000043

000450 HARCO LU-VOL 20/2T

000510 HARCO LU-VOL 4T

REGISTRANT	NAME AND ADDRESS
000340	PRECISION LAMINATORIES INC PO BOX 127 WAUKEGAN IL 60084

PRODUCT NAME

000050 LIQUI-DATE BRUSH KILLER (ESTER FORM)

REGISTRANT	NAME AND ADDRESS
000450	HARCO CHEMICALS INC POST OFFICE BOX 7A041

PRODUCT NAME

000010 FORMULATION NO. 100 BRUSH & WEED KILLER

REGISTRANT	NAME AND ADDRESS
000770	RIVERSIDE CHEMICAL COMPANY P.O. BOX 171109 995 RIDGE LAKE BLVD MEMPHIS TN 38117

PRODUCT NAME

000122 RIVERSIDE HERBICIDE LOW VOLATILE 4T

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
010305	CHEVRON INTERNATIONAL CORPORATION 7625 OLD BRAMPTON AVE CLEVELAND OH 44127

PRODUCT NAME

000002 OLD GARDNER HERB'S FREU

REGISTRANT	NAME AND ADDRESS
011515	AMC CHEMICAL CORPORATION 17000 W. EIGHT MILE ROAD SOUTHFIELD, MI 48075

PRODUCT NAME

000040 TRIM ESTERS KILLS WOODY PLANTS LOW VOLATILITY

REGISTRANT	NAME AND ADDRESS
033330	FORMULA UNLIMITED P.O. BOX 7340 MEMORIAL AVE STATION LONGVIEW TX 75601

PRODUCT NAME

000001 BIRCH RANGELAND BRUSH SPRAY ULV 40-4T

000005 FORMULA UNLIMITED INDUSTRIAL BRUSH SPRAY ULV 30-3T

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	NAME AND ADDRESS
007273	DRUSH CHEMICAL INCORPORATED 4999 NORTH MAIN STREET ROCKFORD IL 61101

PRODUCT NAME

- 000020 BRUSH OFF 6-1 AMINE BRUSH KILLER
- 000060 BRUSH-OFF B & I BRUSH KILLER
- 000077 BRUSH-OFF 2-4-D BRUSH KILLER
- 000085 WEED AND BRUSH-OFF AMINE FORMULA 900
- 000086 BRUSH-OFF LOW VOLATILE BRUSH & WEED KILLER
- 000091 BRUSH-OFF 2-D AMINE BRUSH KILLER

REGISTRANT	NAME AND ADDRESS
000125	MILLER PHARM & SONS INC [illegible] W EMERALD AVE CHICAGO IL 60647

PRODUCT NAME

- 000125 BRUSH & WEED KILLER TO CONTROL UNWANTED WEEDS & WOOD PLANTS

REGISTRANT	NAME AND ADDRESS
000825	NAMCO INC 10500 SATURN BLVD. SUITE 101 [illegible], NV 89043

PRODUCT NAME

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

CONTINUE REGISTRANT 000779

- 000123 RIVERSIDE HERBICIDE LOW VOLATILE 2-4-5T
- 000126 RIVERSIDE HERBICIDE 4-6# 2,4,5-T

REGISTRANT	NAME AND ADDRESS
010668	ATHEA LABORATORIES INC 4100 N FIRST ST MILWAUKEE WI 53212

PRODUCT NAME

- 000000 LVOE KILLS BRUSH,LEAF WEEDS AND WOODY PLANTS LOW VOLATILE SELECTIVE
- 000000 WEED & BRUSH KILLER NO. 7
- 000019 2,4,5-T LOW VOLATILE ESTER BRUSH AND WEED KILLER

REGISTRANT	NAME AND ADDRESS
010278	HUNTER DIV. OF USA CHEMICAL INC. P.O. BOX2263 IRVING TX 75222

PRODUCT NAME

- 000000 HUNTER HAR WEED KNOC

DOW 1699621

DOW169622

**** PRODUCT SEARCH LISTING **** PAGE 33

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

REGISTRANT	*NAME AND ADDRESS*
* 039128	ENVIRONMENTAL PROCESS RESEARCH RIVER OAKS BANK TOWER 200 AIRLY SUITE 610 HOUSTON, TX 77019

PRODUCT NAME
**000001* BEST * SERVIS BRAND BUTYL BRUSH KILLER 4-4
**000002* BEST * SERVIS BRAND BRUSH & RANGE CLEAR 3-3
**000003* BEST * SERVIS BRAND BRUSH & RANGE CLEAR 4-4

REGISTRANT	*NAME AND ADDRESS*
* 039511	VENTAC, INC. STE. 1200 CLARK TOWER 5100 POPLAR AVE. MEMPHIS, TN 38137

PRODUCT NAME
**000018* BRUSH-RHAP AMINE A-2D-2T HERBICIDE
**000017* BRUSH-RHAP A-4T HERBICIDE 2,4,5-T AMINE.
**000018* BRUSH-RHAP J A-4T HERBICIDE 2,4,5-T
**000019* BRUSH-RHAP BUTYL ESTER A-4T HERBICIDE,2,4,5-T
**000021* BRUSH-RHAP BUTYL-2D-2T HERBICIDE
**000022* BRUSH-RHAP LV-4T HERBICIDE 2,4,5-T
**000023* BRUSH-RHAP LV-5T HERBICIDE 2,4,5-T
**000024* BRUSH-RHAP LV-4T HERBICIDE
**000025* BRUSH-RHAP LV DEF-5T HERBICIDE
**000026* BRUSH-RHAP LV DEF-4T HERBICIDE

**** PRODUCT SEARCH LISTING **** PAGE 34

10/03/77 FEDERALLY REGISTERED PRODUCTS CONTAINING 2,4,5-T ESTERS/SALTS

*CONTINUE REGISTRANT 039511	
	**000027* BRUSH-RHAP LV DEF-4T HERBICIDE
	**000028* BRUSH-RHAP LV DEF 3D-3T HERBICIDE
	**000029* BRUSH-RHAP LV DEF-2D-2T HERBICIDE
	**000030* BRUSH-RHAP LV 3D-3T HERBICIDE
	**000032* BRUSH-RHAP LV-2D-2T HERBICIDE
	**000033* BRUSH-RHAP JL+ 2D-2T HERBICIDE
	**000034* BRUSH-RHAP JL+-4T HERBICIDE
	**000035* BRUSH-RHAP JL+-4T HERBICIDE
	**000036* BRUSH-RHAP JL+ 3D-3T HERBICIDE
	**000050* AMINE DA-1.5D-1.5T HERBICIDE
	**000051* TRIAMSAMINE DA-3T HERBICIDE 2,4,5-T OIL SOLUBLE AMINE
	**000053* 2,4,5-T BUTYL ESTER
	**000054* 2,4,5-T LOW VOLATILE ESTER
	**000055* 2,4,5-T LOW VOLATILE DEF-ESTER

**** PRODUCT SEARCH LISTING **** PAGE 1

10/03/77 APPLICANTS FOR REGISTRATION OF PRODUCTS CONTAINING 2,4,5-T ESTERS/SALT

REGISTRANT	*NAME AND ADDRESS*
* 000140	THOMPSON-HAYWARD CHEMICAL COMPANY BOX 2383 KANSAS CITY KS 66110

PRODUCT NAME
**101180* OED-WEED LV-4
**101181* OED-WEED LV-5S
**101182* OED-WEED LV-6

REGISTRANT	*NAME AND ADDRESS*
* 000264	AMCHEM PRODUCT INC BRUNSWICK AVE AMBLER PA 19002

PRODUCT NAME
**101178* ENVERT DT
**101179* ENVERT DT

REGISTRANT	*NAME AND ADDRESS*
* 000464	DOW CHEMICAL U S A PO BOX 1706 MIDLAND MI 48640

PRODUCT NAME
**068119* MESQUITE 7 HERBICIDE
**068120* TURFLON SPECIAL LV 2&5 HERBICIDE

**** PRODUCT SEARCH LISTING **** PAGE 2

10/03/77 APPLICANTS FOR REGISTRATION OF PRODUCTS CONTAINING 2,4,5-T ESTERS/SALT

*CONTINUE REGISTRANT 000464	
	**068121* TURFLON 225E HERBICIDE
	**068122* TURFLON 225 MIXTURE HERBICIDE

REGISTRANT	*NAME AND ADDRESS*
* 000557	SWIFT AGRICULTURAL CHEMICAL CORP. 111 WEST JACKSON BOULEVARD CHICAGO, IL 60604

PRODUCT NAME
**004082* SWIFTS GOLD BEAN BRUSH KILL 22
**004083* SWIFTS GOLD BEAN BRUSH KILL 55

REGISTRANT	*NAME AND ADDRESS*
* 000676	VELSICOL CHEMICAL CORP 341 EAST OHIO STREET CHICAGO IL 60611

PRODUCT NAME
**079179* BANVEL 2+2 HERBICIDE FOR PASTURE,RANGELAND GRASSES AND NONCROPLAN
**079180* BANVEL 2+4 HERBICIDE
**079181* BANVL 2+2 HERBICIDE

17431

REGISTRANT NAME AND ADDRESS

* 003296 PENN STAPPEL CO.
P.O. BOX 3280
SAN ANTONIO TX 78205

PRODUCT NAME

003296 STAPPEL'S 2,4,5-T

010172 STAPPEL'S BRUSH KILLER NO.3

REGISTRANT NAME AND ADDRESS

* 003579 DRAKEN CHEMICAL CORPORATION
P O BOX 48
HONOLULU, HI 96810

PRODUCT NAME

032790 ULTRAMAR LV-6A

REGISTRANT NAME AND ADDRESS

* 010627 INDUSTRIAL PAYMENTS
PO BOX 312
SAN MARCOS TX 78666

PRODUCT NAME

042420 IND-BUR 140

REGISTRANT NAME AND ADDRESS

* 011668 HUNCH INC
11837 SAN JERONIMO ROAD
SAN ATUNIO TX 78217

PRODUCT NAME

018171 BUNCHUN BRUSH CONTROL MIXTURE T

REGISTRANT NAME AND ADDRESS

* 025203 AMERICAN NATION CORP
P.O. BOX 666
LONGMONT, CO 80501

PRODUCT NAME

039598 QUICK KILL 44# WEED KILLER LIQUID

REGISTRANT NAME AND ADDRESS

* 037367 UNI-CHEM CORP. OF FLORIDA
POA 6136 - 2601 N.W. 55TH CT.
FT. LAUDERDALE, FL 33310

PRODUCT NAME

031264 UNI CHEM BRUSH AND WEED KILLER

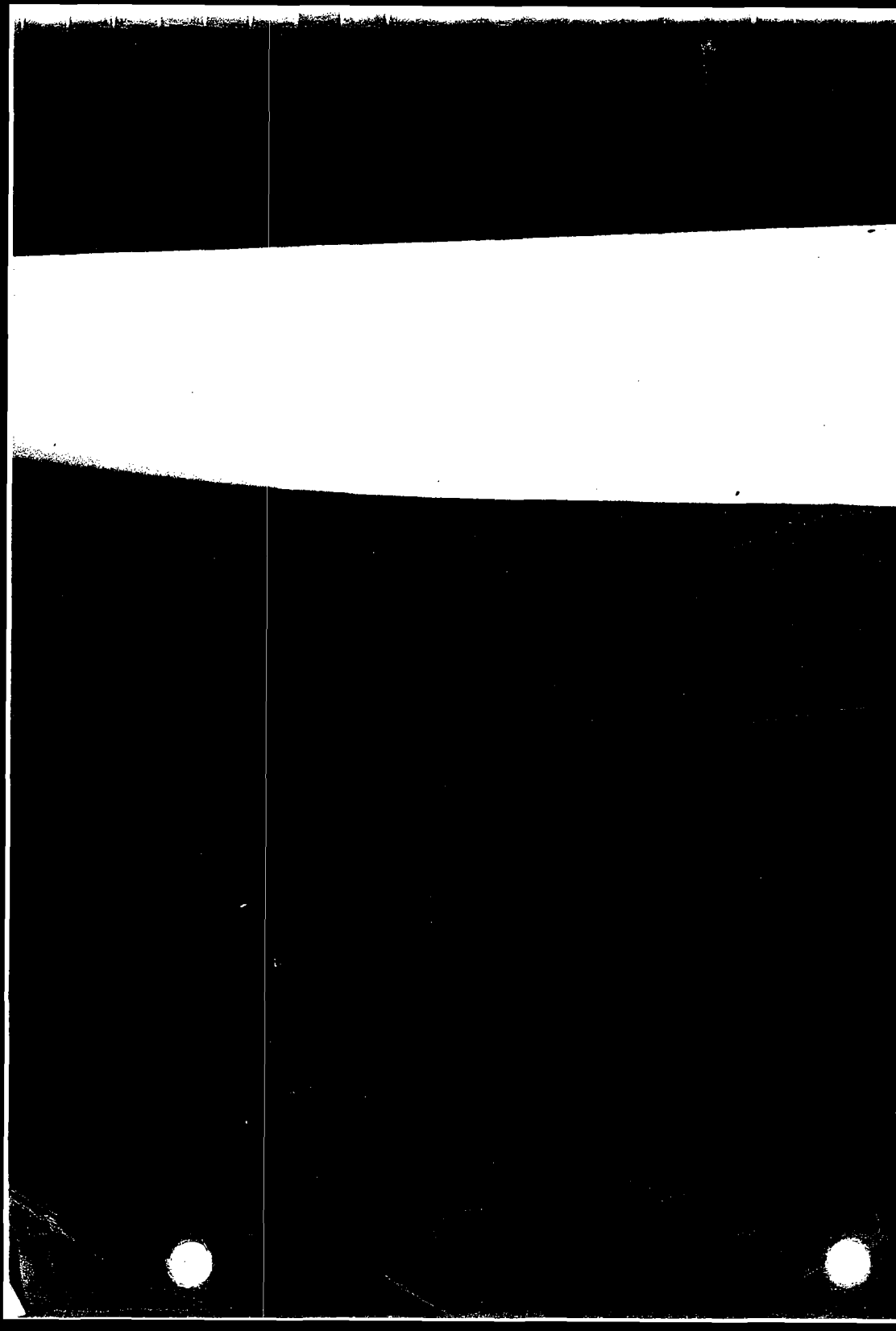
DOW 169623

[illegible]	[illegible] NAME AND ADDRESS [illegible]	
•	067351	COLUMBIA CHEMICAL & FERT. CO. INC. ROUTE 1, BOX 2548 LONDAUNE, CO 40581

[illegible] PRODUCT NAME [illegible]

[illegible] COLUMBIA'S DMA SPOT 60430 KILLER

[FR Doc. 78-10340 Filed 4-20-78; 8:45 am]



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12693

DOW215964630

(RPZHAW) (1980)
TRANS. MID. NO. 12,693
NTC. NO.
WARSAW. PANSTWOWY ZAKLAD HIGIENY. ROCZNIKI. WARSAW.
V.31(6)611-14 (1980)
Absorption of 2,4-dichlorophenoxyacetic acid throush the skin.
AUTHOR: Senczuk, Witold

TRANSLATOR: Lilienheim
DATE: November 1981

REQUESTED BY: L. Griswold, 1803
TRANSLATION NO.: 81-11-7

17434

Trans. 81-11-7
Record 19011
MID. 12,603
CODEN RPZHAW

12693

Witold Senczuk, Halina Pogorzelska, Monika Debska

ABSORPTION OF 2.4-DICHLORPHENOXYACETIC ACID THROUGH THE SKIN

Texicology Department of the Institute of Bicanalysis and medium analysis of the Medical Academy of Poznan
Director: Prof. Dr. W. Senczuk

Percutaneous absorption of 2.4-dichlorphenoxyacetic acid by indirect method was investigated, determining the amount of 2.4-D in the urine of rats.

DOM21564464

The production of pesticides and their application give rise to wide possibilities of poisoning with these compounds (1,2,3,5), and this prompted the determination of the degree of percutaneous absorption of one of most frequently used protective agents, namely 2.4-dichlorphenoxyacetic acid (2.4-D).

Experimental Part

Material and Method

The tests were carried out on 28 rats of the Wistar species, weighing each 200 g ± 10g. The animals were divided into seven groups of four rats each group.

Commercial preparation "Pielik"(2.4-dichlorphenoxyacetic acid: translator) containing about 85% of sodium salt of 2.4-dichlorphenoxyacetic acid was used for the tests. The preparation was used in the form of aqueous solutions of different 2.4-D concentrations.

Results of Determinations

1. Determination of 2.4-D acid in the urine depending on the concentration of the tested compound in the solution

The tests were carried out with the use of aqueous solutions of "Pielik" containing 0.5, 1, 2, 4 and 5% of 2.4-D acid. After six hours there was determined the amount of 2.4-D acid excreted with urine. The results are shown in table 1 and in Fig. 1.

2. Determination of 2.4-D acid in urine depending on the period of exposure

The influence of the exposure period (2, 4 and 6 hours) on the excretion of 2.4-D with urine was determined by using aqueous solution of "Pielik" containing 4% of the tested acid. The results of the determination of 2.4-D acid in the collected portions of urine are shown in table II. Fig. 2 graphically

illustrates the results

Table 1. Results of the determination of 2.4-D acid in urine depending on the concentration of the compound in the solution

Solution %	Animal No	Amount of 2.4-D acid in urine (mg) 1st day (24 hours)	2nd day	3rd day	jointing after three days
0.5	1	0,9	1,4	1,2	3,5
	2	1,0	1,5	1,0	3,5
	3	1,0	2,8	1,0	4,6
	4	1,0	1,6	1,1	3,6
average	Srednia	0,97	1,77	1,07	3,81
1,0	1	3,4	2,4	1,0	6,8
	2	1,0	1,6	1,2	3,8
	3	7,6	2,2	0,0	9,8
	4	2,5	1,0	0,0	3,5
average	Srednia	3,62	1,80	0,55	5,97
2,0	1	1,2	5,1	1,8	8,1
	2	2,0	8,7	1,7	12,4
	3	1,6	4,8	2,6	9,0
	4	2,5	1,8	1,1	5,4
average	Srednia	1,82	5,10	1,80	8,72
4,0	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
average	Srednia	4,45	4,00	3,80	12,25
5,0	1	5,5	4,7	4,9	15,1
	2	2,7	2,6	2,1	7,4
	3	3,6	5,8	6,8	16,2
	4	4,6	6,8	7,2	18,6
average	Srednia	4,10	4,97	5,25	14,32

Table II. Results of the determination of 2.4-D acid in urine depending on the exposure period

Period of Exposure	Animal No	Amount of 2.4-D acid in urine (mg) 1st day (24 hours)	2nd day	3rd day	jointing after three days
2	1	1,6	0,8	0,9	3,3
	2	4,7	3,8	1,7	10,2
	3	2,5	1,7	0,8	5,0
	4	3,0	1,6	0,8	5,4
	Srednia	2,95	1,97	1,05	5,97
average					
4	1	1,8	1,3	1,4	7,5
	2	1,4	3,3	1,5	8,2
	3	1,9	2,5	2,2	6,6
	4	3,2	10,2	4,0	17,7
	Srednia	2,07	5,15	2,27	9,50
average					
6	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
average	Srednia	4,45	4,00	3,80	12,25

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Discussion of the Results

It has been determined that the amount of the substance excreted with urine is proportional to the 2.4-D concentration in the tested solutions(Fig.1). As the 2.4-D concentration increased, the amount of this compound excreted with urine, as compared with the preceding group, i.e. of lower 2.4-D concentration of 2.4-D increased by about 50%. This related to 0.5 to 4% concentrations, but after exposure to 5% concentration, in comparison with the group of animals exposed to 4% solution, 17% less 2.4-D acid were excreted with urine.

Also the period of exposure influenced the amount of 2.4-D acid absorbed by skin. This relation was proportional for 2, 4 and 6 hour exposure (Fig. 2). This can provide a basis for further research in determining the biochemical absorption index.

Figure1. Excretion with urine of 2.4-D acid depending on the concentration of the compound in the solution

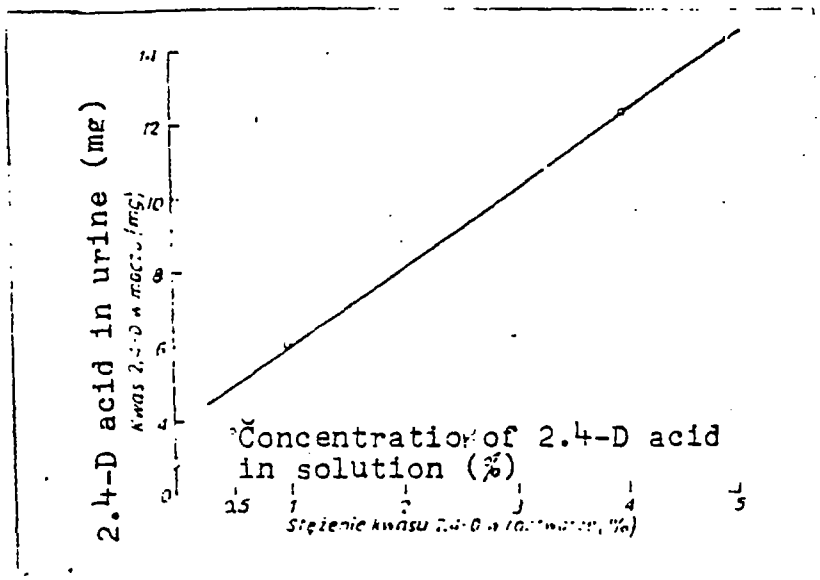
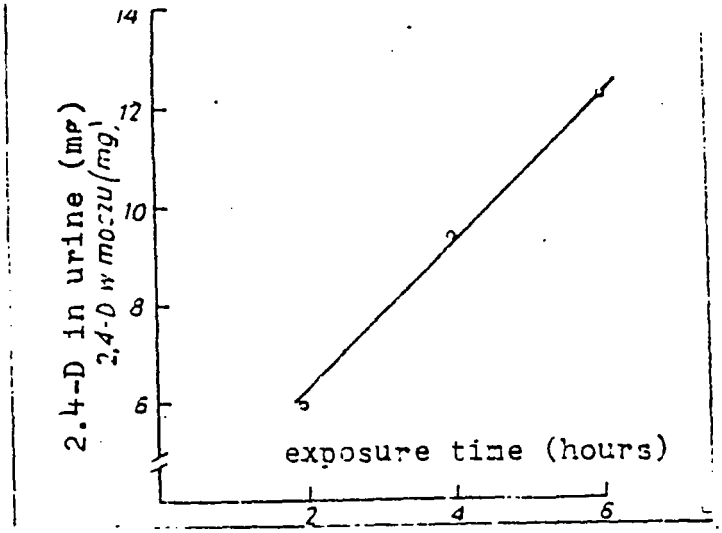


Figure 2. Excretion with urine of 2.4-D acid depending on the period of exposure.



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A Russian summary corresponds to the following English summary.

W. Seńczuk, H. Pogorzelska, M. Dębska

ABSORPTION OF 2,4-DICHLORPHENOXYACETIC ACID THROUGH THE SKIN

Summary

The degree of percutaneous absorption of one of the most frequently used herbicides -- 2,4-dichlorphenoxyacetic acid — was investigated. The investigations were carried out with different concentrations of this acid in aqueous solutions of „Pielika" and in relation to the duration of exposure. *Wistar* rats were used in these experiments.

The dermal exposure was done by the tail method. The urinary excretion of the acid was determined by gas chromatography after previous extraction of the acid from urine and its estrification.

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1. *Bobiagin D. A., Syrkin A. B., Łupinosow J. W., Zajcewa L. A.:* Diejstwije na organizm czełowieka i żiwotnych gierbicidow-proizwodnych chlorfienoksiuksusnoj kisloty. Farmakol. i Toksikol., 1969, 32, 747. — 2. *Jirasek L., Kalensky J., Kubec K.,* Acne chlorina a porphyria cutanea tarda pri vyrobe herbicid, Cs. dermatol., 1973, 48, 306. — 3. *Radionow A. D., Chumachenko A. N., Kurilenko I. I.:* Sanitary — toxicologic features of a herbicide of sodium, ammonium and dimethylammonium salts of 2,4-dichlorophenoxyacetic acid, Gig. Sanit., 1967, 32, 100. — 4. *Seńczuk W., Pogorzelska H.:* Budowa chemiczna a toksykodynamiczne właściwości pochodnych kwasów fenoksykarboksylowych. Część II. Metody oznaczania pochodnych kwasu fenoksyoctowego i fenoksypropionowego w moczu i krwi. Roczniki PZH. Praca oddana do druku. — 5. *Zankiewicz W., Rutkowska N.:* Zatrucie Pielikiem, Medycyna Pracy, 1967, 18, 446.

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1. Influence on the organism of humans and animals of herbicides on the basis of chlorphenoxyacetic acid, Pharmacology and Toxicology, 1969, 32, 747.
2. Percutaneous absorption in the production of herbicides
3. see above in English
4. Chemical structure and toxidynamic properties of derivatives of phenoxycaboxylic acids. Part II . Method for determination of derivatives of phenoxyacetic and phenoxypropionic acid in urine and blood. PZH Annuals. Paper remitted to printing.
5. Poisoning with 'Pielik', Occupational Medicine

July 17, 1980

60-780 Poznan, Grunwaldzka street 6

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Roczn. PZH, 1980 T. XXXI, Nr 6

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WITOLD SENCZUK, HALINA POGORZELSKA, MONIKA DĘBSKA

WCHŁANIANIE KWASU 2,4-DWUCHLOROFENOKSYOCTOWEGO PRZEZ SKÓRĘ

Zakład Toksykologii Instytutu Bioanalizy i Badania Środowiska
Akademii Medycznej w Poznaniu
Kierownik: prof. dr hab. *W. Seńczuk*

Zbadano wchłanianie przez skórę kwasu 2,4-dwuchlorofenoksyoctowego metodą pośrednią oznaczając ilość 2,4-D w moczu szczurów.

Produkcja pestycydów i ich stosowanie stwarza duże możliwości zatrucia tymi związkami [1, 2, 3, 5], stąd celowym było określenie stopnia wchłaniania przez skórę, jednego z częściej używanych środków ochrony roślin, a mianowicie kwasu 2,4-dwuchlorofenoksyoctowego (2,4-D).

CZĘŚĆ DOŚWIADCZALNA

Materiał i metodyka

Badania wykonano na 28 szczurach, szczepu *Wistar*, o masie ciała 200 g ± 10 g. Zwierzęta podzielono na 7 grup po 4 szczury.

Do badań użyto preparatu handlowego „Pielik" zawierającego około 85% soli sodowej kwasu 2,4-dwuchlorofenoksyoctowego. Preparat używano w postaci roztworów wodnych o różnych stężeniach 2,4-D.

Ogony zwierząt po dokładnym umyciu i obliczeniu powierzchni, zanurzano w probówkach zawierających około 20 cm³ badanego roztworu o temp. 37- 40 °C. Po określonym czasie ekspozycji (p. niżej) zwierzęta przenoszono do klatek metabolicznych typu „Simax", po czym przez 72 godziny zbierano dobowe porcje moczu. Dwa razy dziennie podawano zwierzętom sondą do żołądka po 5 cm³ wody. Zawartość kwasu 2,4-D w moczu określano metodą chromatografii gazowej, po uprzednim wyekstrahowaniu badanego związku z moczu i jego estryfikacji [4].

WYNIKI OZNACZEŃ

1. Oznaczanie kwasu 2,4-D w moczu w zależności od stężenia badanego związku w roztworze

Badania wykonano przy użyciu wodnych roztworów „Pielika" zawierających 0,5, 1, 2, 4 i 5% kwasu 2,4-D. Po 6 godzinach określano ilość wydalonego z moczem kwasu 2,4-D. Wyniki przedstawiono w tabeli I oraz na rycinie 1.

2. Oznaczanie kwasu 2,4-D w moczu w zależności od czasu ekspozycji

Wpływ czasu ekspozycji (2, 4 i 6 godzin) na wydalanie z moczem 2,4-D określono stosując wodny roztwór „Pielika" zawierający 4% badanego

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Tabela I. Wyniki oznaczania kwasu 2,4-D w moczu w zależności od stężenia związku w roztworze

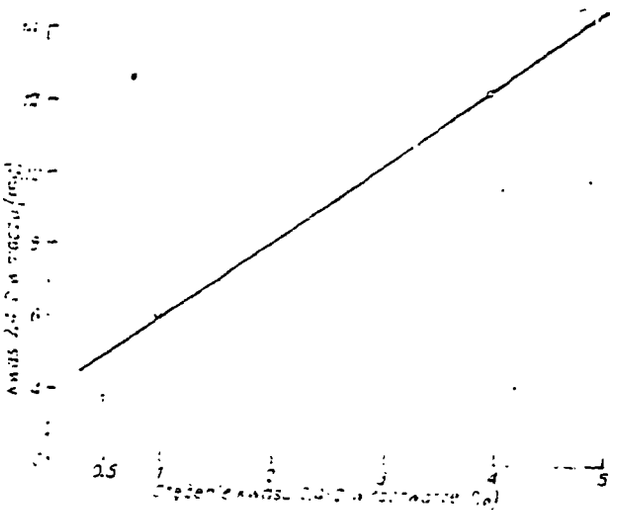
Roztwór %	Zwierzę Nr	Ilość kwasu 2,4-D w moczu (mg) I doba	II doba	III doba	Łącznie po trzech dobach
0,5	1	0,9	1,4	1,2	3,5
	2	1,0	1,5	1,0	3,5
	3	1,0	2,8	1,0	4,8
	4	1,0	1,6	1,1	3,6
	Średnia	0,97	1,77	1,07	3,81
1,0	1	3,4	2,4	1,0	6,8
	2	1,0	1,6	1,2	3,8
	3	7,6	2,2	0,0	9,8
	4	2,5	1,0	0,0	3,5
	Średnia	3,62	1,80	0,55	5,97
2,0	1	1,2	5,1	1,8	8,1
	2	2,0	8,7	1,7	12,4
	3	1,6	4,8	2,6	9,0
	4	2,5	1,8	1,1	5,4
	Średnia	1,82	5,10	1,80	8,72
4,0	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
	Średnia	4,45	4,00	3,80	12,25
5,0	1	5,5	4,7	4,9	15,1
	2	2,7	2,6	2,1	7,4
	3	3,6	5,8	6,8	16,2
	4	4,6	6,8	7,2	18,6
	Średnia	4,10	4,97	5,25	14,32

kwasu. Wyniki oznaczania kwasu 2,4-D w zebranych porcjach moczu przedstawiono w tabeli II. Ilustracją graficzną wyników jest rycina 2.

OMÓWIENIE WYNIKÓW

Stwierdzono, że ilość substancji wydalonej z moczem jest proporcjonalna do stężenia 2,4-D w badanych roztworach (ryc. 1). W miarę wzrostu stężenia 2,4-D ilość tego związku wydalonego z moczem, w porównaniu z grupą poprzednią, tzn. o niższym stężeniu 2,4-D wzrastała o ok. 50%. Dotyczyło to stężeń od 0,5 do 4%; natomiast po ekspozycji na roztwór 5%, w porównaniu z grupą zwierząt eksponowanych na roztwór 4%, z moczem wydaliło się o ok. 17% mniej kwasu 2,4-D.

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Ryc. 1. Wydalanie z moczem kwasu 2,4-D w zależności od stężenia związku w roztworze.

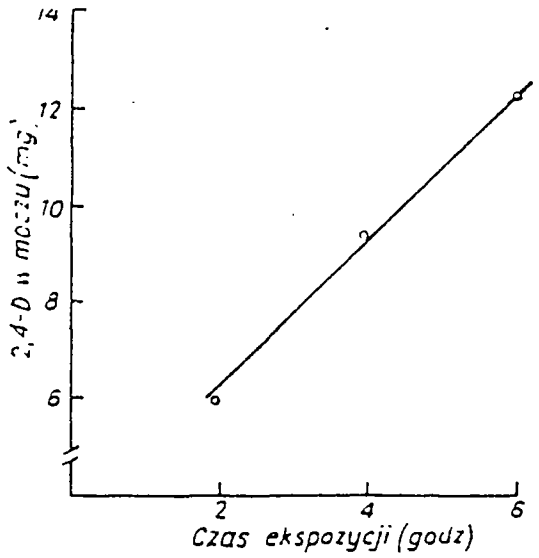
Czas ekspozycji wpływał również na ilość kwasu 2,4-D wchłoniętą przez skórę. Zależność ta była proporcjonalna dla 2, 4 i 6 godzinnej ekspozycji (ryc. 2). Może to stanowić podstawę do dalszych badań nad opracowaniem biochemicznego wskaźnika wchłaniania.

Tabela II. Wyniki oznaczania kwasu 2,4-D w moczu w zależności od czasu ekspozycji

Czas ekspozycji (h)	Zwierzę Nr	Ilość kwasu 2,4-D w moczu (mg)			
		I doba	II doba	III doba	Łącznie za trzech dobach
2	1	1,6	0,8	0,9	3,3
	2	4,7	3,8	1,7	10,2
	3	2,5	1,7	0,8	5,0
	4	3,0	1,6	0,8	5,4
	średnia	2,95	1,97	1,05	5,97
4	1	1,8	1,3	1,4	7,5
	2	1,4	3,3	1,5	8,2
	3	1,9	2,5	2,2	6,6
	4	3,2	10,5	4,0	17,7
	średnia	2,07	5,15	2,27	9,40
6	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
	średnia	4,15	4,00	3,80	12,25

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Ryc. 2. Wydalanie z moczem kwasu 2,4-D w zależności od czasu ekspozycji.

B. Seńczuk, H. Pogorzelska, M. Dębska

РЕЗОРБЦИЯ ЧЕРЕЗ КОЖУ 2,4-ДИХЛОРФЕНОКСИУКСУСНОЙ КИСЛОТЫ

Резюме

Определялась степень резорбции через кожу одного из наиболее часто применяемых гербицидов, 2,4-дихлорфеноксиуксусной кислоты (2,4-D).

Исследования проводились в зависимости от концентрации этой кислоты в водном растворе препарата „Пелик" а также от времени экспозиции хвоста применяемых животных (крыс линии Вистар) на действие 2,4-D.

Количество выделяемого с мочой 2,4-D определяли методом газовой хроматографии после его экстракции из мочи и этерификации.

W. Seńczuk, H. Pogorzelska, M. Dębska

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PIŚMIENNICTWO

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or as a conjugate within 96 to 144 hours, when each was administered orally at 5 mg per kg body weight. In rats and dogs, 2,4,5-T administered by intubation resulted in rapid unchanged 2,4,5-T urinary excretion by rats, but much slower excretion in dogs with some metabolism occurring. Excretion occurred by first order processes with half elimination values ($E_{1/2}$) for man, rats, and dogs of 20, 24, and 87 h, respectively. In man, Sauerhoff et al. (2) found $E_{1/2}$ values of 18 h for 2,4-D. Kohli et al.(5,6) and Sauerhoff et al. (2) found $E_{1/2}$ plasma values of 35, 19 and 12 h for 2,4-D, 2,4,5-T, 2,4-D respectively. Sauerhoff et al. (7) observed that urinary excretion was even more rapid ($E_{1/2}$ = 11 h) when 2,4,5-T was administered intravenously to rats. Distribution and elimination of 2,4,5-T was markedly altered when larger doses were administered.

Exposure studies by Lavy et al. (1) on forest applicators showed a 5-day mean excretion of 0.033 mg/kg body weight from single day exposures. A considerable range of 2,4,5-T was measured in the urine for different crew members, with a mixer excreting the highest level (0.096 mg/kg) and a flagman the lowest level (0.001 mg/kg) per exposure. In a subsequent 2,4-D study on forestry workers, T. L. Lavy, J. D. Walstad, R. R. Flynn, and J. D. Mattice (1981, unpub), found mean values of less than 0.02, 0.07, 0.006, 0.013, and 0.001 mg/kg body weight, respectively, for batchmen, pilots, mechanics, supervisors, and observers.

The objective of the current study was to obtain measurements of 2,4-D levels in urine from workers involved in the application of the herbicide under normal use conditions. Specifically, 2,4-D levels were measured in urine samples collected prior to, during, and after actual spray operations from workers involved in ground and aerial applications of amine and ester formulations of 2,4-D to wheat. The resultant data were used to calculate exposure doses and urinary excretion relationships.

Materials and Methods

Protocol. Volunteers for the 2,4-D exposure study consisted of 26 workers involved in the ground application in Eastern North Dakota and 17 workers involved in the aerial application in Eastern Washington during the spring of 1980. No suggestion or attempt was made to alter work habits or clothing worn by the workers. Instructions were given for the taking, handling, and storing urine samples and recording dates of 2,4-D application and urine collection, equipment used, hours and acres sprayed, formulation, wind condition, and job. A summary of the experimental conditions and parameters is given in Table I. Prior to the experiments most participants completed an information sheet which listed age, sex, weight, job, years of experience with 2,4-D, and applicator equipment type. During the experiments most participants listed type of clothing and weather conditions.

Sampling. Individual workers from aeria vided 24-hour urine samples on approximate all continuous 2,4-D application. This represente ered a typical situation during the height of to wheat in Eastern Washington. Individual ae applications provided six consecutive day 24-h for a one-week period following a single 2,4-D participant in both groups were requested to single voided urine sample prior to experiment

Table 1. Summary of Conditions and Parameters Agricultural Applicators.

Item	Application Method and St	
	Aerial (Washington)	
Equipment	4 Thrush Commanders	2
	4 Grumman Ag-Cat	1
	4 Pipers	0
	1 Snow	0
	1 Cessna	0
Occupation	14 Pilots	3
	5 Mixer/loaders	4
Clothing	Variable	
Rates lb/A	--	0.
kg/ha	--	0.
Formulation	--	24
Sex	18 Males, 1 Female	25
	Geometric mean / Range	Geometr
Age	33 / 21-57	
Work experience	7 / 1-20	
Sprayed acres	9000 / 2500-30,000	
ha	3650 / 1000-16,200	
Hours per day	10 / 2-16	
Days per year	45 / 14-60	

[a]Pilots, 4.2 h and mixer/loaders, 5.6 h.

Sample handling. All urine samples were st each subject until collected and transported to on receipt of the frozen urine samples, they wer (in the case of the North Dakota workers they we the Plant Metabolism Laboratory, Fargo, North Da prior to shipping to Beltsville, Maryland), assi returned to a refrigerator (5°C) pending analysi

Analysis. The Washington tank urine samp at the ARS Yakima Agricultural Research Laborato Dakota samples at the ARS Pesticide Degradation Beltsville, Maryland. The method used by both l similar procedure based on a method developed at and Sell, unpub.). Several randomly selected ur

a conjugate within 96 to [illegible] hours, when each was administered orally at 5 mg per kg body weight. In rats and dogs, 2,4-T administered by intubation resulted in rapid unchanged 2,4-T urinary excretion by rats, but much slower excretion in dogs with some metabolism occurring. Excretion occurred by first order processes with half elimination values ($E_{1/2}$) for man, rats, and dogs of 23, 14, and 87 h, respectively. In man, Sauerhoff et al. (2) found $E_{1/2}$ values of 18 h for 2,4-D. Kohli et al. (5,6) and Sauerhoff et al. (2) found $E_{1/2}$ plasma values of 33, 19 and 12 h for 2,4-D, 2,4,5-T, and 2,4-D respectively. Sauerhoff et al. (7) observed that urinary excretion was even more rapid ($E_{1/2}$ = 11 h) when 2,4,5-T was administered intravenously to rats. Distribution and elimination of 2,4,5-T was markedly altered when larger doses were administered.

Exposure studies by Lavy et al. (3) on forest applicators found a 6-day mean excretion of [illegible]33 mg/kg body weight from three day exposures. A considerable range of 2,4,5-T was measured in the urine for different crew members, with a mixer excreting the highest level (0.096 mg/kg) and a flagman the lowest level (<1 mg/kg) per exposure. In a subsequent 2,4-D study on forestry workers, T. L. Lavy, J. D. Walstad, R. R. Flynn, and J. D. Mattice (1980, unpub), found mean values of less than <0.02, 0.02, 0.003, and 0.001 mg/kg body weight, respectively, for flagmen, pilots, mechanics, supervisors, and observers.

The objective of the current study was to obtain measurements of 2,4-D levels in urine from workers involved in the application of the herbicide under normal use conditions. Specifically, 2,4-D levels were measured in urine samples collected prior to, during, and after actual spray operations from workers involved in ground and aerial applications of amine and ester formulations of 2,4-D to wheat. The resultant data were used to calculate exposure doses and urinary excretion relationships.

Materials and Methods

Protocol. Volunteers for the 2,4-D exposure study consisted of 20 workers involved in the ground application in Eastern North Dakota and 17 workers involved in the aerial application in Eastern Washington during the spring of 1980. No suggestion or attempt was made to alter work habits or clothing worn by the workers. Instructions were given for the taking, handling, and storing of urine samples and recording dates of 2,4-D application and urine collection, equipment used, hours and acres sprayed, formulation, wind condition, and job. A summary of the experimental conditions and parameters is given in Table I. Prior to the experiments most participants completed an information sheet which listed age, sex, weight, job, years of experience with 2,4-D, and motor equipment type. During the experiments most participants recorded type of clothing and weather conditions.

Sampling. Individual workers from aerial applications provided 24-hour urine samples on approximate alternate days for the continuous 2,4-D application. This represented what may be considered a typical situation during the height of 2,4-D applications to wheat in Eastern Washington. Individual workers from ground applications provided six consecutive day 24-hour urine samples for a one-week period following a single 2,4-D application. Each participant in both groups were requested to provide a one-time single voided urine sample prior to experimental initiation.

Table I. Summary of Conditions and Parameters of 2,4-D Exposure to Agricultural Applicators.

Item	Application Method and State			
	Aerial (Washington)		Ground (North Dakota)	
Equipment	4 Thrush Commanders		3 Pull Type	
	1 Grumman Ag-Cats		21 Self-propelled	
	4 Pipers		10 Cab	
	1 Snow		16 No cab	
	1 Cessna			
Occupation	14 Pilots		9 Sprayers	
	5 Mixer/loaders		16 Mixer/loaders	
Clothing	Various		Various	
Rates lb/A	--		0.25 - 3.50	
kg/ha	--		0.28 - 3.36	
Formulation	--		24 Amine, 4 ester	
Sex	18 Males, 1 Female		25 Males, 1 Female	
	Geometric mean	Range	Geometric mean	Range
Age	33	21-64	39	18-63
Work experience	7	1-20	10	1-35
Sprayed acres	9000	2500-40,300	300	12-1200
ha	3650	1000-16,200	200	10-1200
Hours per day	5[a]	3-16	--	--
Days per year	45	14-60	2	1-14

[a] Pilots, 4.2 h and mixer/loaders, 5.6 h.

Sample Handling. All urine samples were stored frozen by each subject until collected and transported to a laboratory. Upon receipt of the frozen urine samples, they were weighed, thawed (in the case of the North Dakota workers they were subsampled at the Plant Metabolism Laboratory, Fargo, North Dakota, and refrozen prior to shipping to Beltsville, Maryland), assigned a code, and returned to a refrigerator (5°C) pending analysis.

Analysis. The Washington State urine samples were analyzed at the ARS Yakima Agricultural Research Laboratory and the North Dakota samples at the ARS Pesticide Degradation Laboratory at Beltsville, Maryland. The method used by both laboratories was a similar procedure based on a method developed at Yakima (Maitlen and Sell, unpub.). Several randomly selected urine samples were

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exchanged between the two laboratories for comparative analysis. (Deviations from this method used at Beltsville are shown in the following parenthesis.) Briefly the method involved placing 25 mL urine (25 mL), 2 mL 37% KOH (5 mL 1 N KOH), 5 mL methanol, and glass beads (sand) in a boiling flask and refluxing the contents for 1 h. After cooling, the contents were transferred to a 250 mL separatory funnel, the flask rinsed with 25 mL water (50 mL w. NaCl) and 25 mL dichloromethane (DCM) added. The funnel contents were shaken, allowed to separate, and the bottom DCM layer discarded. The upper layer was acidified with 2 mL H_3PO_4 (10 mL 20% H_3PO_4) and 20 mL DCM (15 mL) was added to the funnel. The funnel contents were shaken and allowed to separate. The bottom DCM layer was collected in a boiling flask. Two additional extractions with DCM were collected and the funnel contents discarded.

The DCM solution in the boiling flask was transferred to a separatory funnel the boiling flask was rinsed with 60 mL 3% K_2CO_3 (twice with 25 mL 3.6% $KHCO_3$), and the rinses transferred to the funnel. The funnel contents were shaken vigorously for 1 min and allowed to separate and the bottom layer was discarded. Three mL H_3PO_4 (15 mL 20% H_3PO_4) was carefully added to the separatory funnel and gently swirled until effervescence diminished. The acidified solutions were extracted three times with 20 mL DCM (15 mL) and each DCM extract dried through anhydrous Na_2SO_4 in a 125 mL Erlemeyer flask (100 mL beaker). The DCM solution was carefully evaporated to dryness.

Samples were methylated by one of two procedures. Ten milliliters methanol and 1 mL of 14% BF_3 in methanol were added to the residue in the flask and the contents boiled on a hot plate for 2.5 min. Excess methanol was evaporated with dry air. About 0.5 mL of a brown oily substance remained. Two mL of 95% hexane plus 5% toluene were added to the oil residue, mixed and placed into the refrigerator until gas-liquid chromatographic (GLC) analysis. An alternate methylation procedure involved transferring the residue to a 15 mL pointed tube with four 0.5 mL increments of BF_3-[illegible] in methanol (1:2 v/v). The pointed tube was stoppered and placed in an 80°C tube heater. After 1 h the tube was removed and allowed to cool. Five milliliters hexane and 2 mL saturated NaCl were added to the tube and the contents mixed thoroughly on a vortex mixer. Four mL of the hexane layer were pipeted from the tube and placed into a vial. The contents of the vial were evaporated just to dryness with dry N_2 at room temperature. Four milliliters of hexane were added to the vial and the contents shaken. Aliquots from the vial were taken for GLC assay.

GLC conditions. Dilutions for GLC assays were made when necessary. The GLC parameters were: ^{63}Ni electron-capture detector; glass columns were filled with 3% OV 101 on Gas Chrom Q (4 mm i.d. by 1.2 m), 11% (QF-1 + OV-17) on Gas Chrom Q (4 mm i.d. by 1.8 m), and 5% OV 101 on Chromsorb W (4 mm i.d. by 1.8 m) all

dryness with dry [illegible]

80/100 mesh; 3/95 [illegible] at 200 and 180° column temperatures represents the absolute values whereas the Beltsville laboratory plus a value 2.5 times the recov[illegible] samples.

Results and Discussion

Recoveries of [illegible] are given in Table II and compar[illegible] laboratory exchanged samples was used as an internal standard. The Beltsville laboratory found

Table II. Recoveries [illegible] water) and 2,4,5-T [illegible]

Added ng	Recovered %
58	73 ± 14
120	75 ± 8
240	83 ± 6
480	72 ± 3
960	84 ± 3
2,400	83 ± 5
4,800	87 ± 4
Average	80 ± 7

[illegible]Standard error of mean [illegible]

Table III. Comparative [illegible] between the Yakima and Be[illegible]

Sample number	Laboratory: Yakima	Beltsville
	North Dakota Samples	
1	ND[a]	ND
2	0.17	0.16
3	0.01	0.003
4	ND	ND
5	0.61	0.65
6	0.24	0.29
7	0.18	0.10
8	ND	ND
9	0.03	0.01
10	0.44	0.38
11	2.63	2.94
12	0.31	.033

[a]None detected.

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... partitioning ...
... methanol and ... are shown in the
... synthesis.) Briefly the ... involved placing
... (25 mL), 2 mL 37% KOH (... KOH), 5 mL methanol,
... (sand) in a boiling ... and refluxing the con-
... Using caution, the ... were transferred to a
... funnel, the flask ... with 25 mL water (50 mL
... and 25 mL dichloromethane ... added. The funnel con-
... tion, ... vented to remove ... The bottom DCM layer
... upper layer was ... with 2 mL H_3PO_4 to
... and ... to the funnel.
... were ... and ... separated. The
... the ... flask. Two additional
... 0% were collected ... funnel contents

... solution in the boiling ... was transferred to a
... the boiling flask ... with 0.5 mL
... with 10 mL 5% KHCO3, ... rinses transferred
... the funnel contents ... vigorously for 1
... and the ... layer was discarded.
... 1% ... was ... added to the ...
... and ... solution. ... fluorescence diminished.
... were ... with 2 mL DCM
... drops ... to
... solution was
evaporated to dryness.

... were methylated by one of ... procedures. Ten milli-
... and 1 mL of 14% BF_3 in ... were added to the
... the flask and the contents ... on a hot plate for
... with dry air. About ...
... substance ... 95% hexane plus
... were added to the ... and placed into
... until ... analysis. ...
... procedure ... the resi-
... tube with four ... increments of BF_3-
... (44:2 V/v). The ... was stoppered
... in ... water. ... the tube was removed
to cool. Five milliliters hexane and 2 mL saturated
... to the tube and the contents mixed thoroughly on a
... Four mL of the hexane layer were pipeted from the
... into a vial. The contents of the vial were evapo-
... dryness with dry ... at ... temperature. Four
... of hexane were added to ... and the contents
... from the vial were ... GLC assay.

... Conditions. ... for GLC assays were made with
the GLC parameters were: ... electron-capture detec-
... were filled with 1.5% ... on Gas Chrom Q (4 mm
... m), II (OF-1 + OV-17) on ... Chrom Q (4 mm i.d. by
... OV 101 on Chromosorb W (... m, i.d. by 1.8 m) all

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70/[illegible] mesh; 5/95 SE$_4$ Ar gas at 90, 30, or 60 mL/min and 160, 200 and 180° column temperatures, respectively. The data reported represents the absolute values measured by the Yakima Laboratory, whereas the Beltsville laboratory subtracted from all field samples a value 2.5 times the recorder peak height of control urine samples.

Results and Discussion

Recoveries of 2,4-D (80%) and 2,4,5-T (92%) added to urine are given in Table II and comparative 2,4-D analysis in several laboratory exchanged samples is given in Table III. The 2,4,5-T was used as an internal standard at the Beltsville laboratory. The Beltsville laboratory found higher 2,4-D amounts in many urine

Table II. Recoveries of 2,4-D (propylene glycol butyl ether ester) and 2,4,5-T (isooctyl ester) Added to 25 mL urine.

2,4-D		2,4,5-T	
Added ng	Recovered %	Added ng	Recovered %
48	73 ± 14[a]	27	98 ± 10
120	75 ± 9	68	68 ± 7
240	83 ± 6	135	96 ± 4
480	72 ± 5	270	93 ± 6
960	84 ± 3	540	100 ± 4
2,400	83 ± 5	1,350	95 ± 4
4,800	87 ± 4	2,700	97 ± 6
Average	80 ± 7		92 ± 6

[a]Standard error of mean from 4 to 12 trials.

Table III. Comparative 2,4-D (ppm) Analysis of Urine Samples between the Yakima and Beltsville Laboratories.

Sample number	Laboratory		Sample number	Laboratory	
	Yakima	Beltsville		Yakima	Beltsville
	North Dakota Samples			Washington Samples	
1	ND[a]	ND	13	0.04	0.05
2	0.17	0.16	14	0.06	0.05
3	0.01	0.003	15	0.19	0.25
4	ND	ND	16	0.32	0.51
5	0.61	0.66	17	1.50	2.00
6	0.24	0.29	18	1.05	1.43
7	0.18	0.10	19	1.21	2.02
8	ND	ND	20	1.08	1.17
9	0.03	0.01	21	1.25	1.53
10	0.44	0.38	22	1.88	2.24
11	2.61	2.94	23	0.83	0.93
12	0.31	.033	24	0.20	0.23

[a]None detected.

samples, especially the high 2,4-D urine content obtained in samples. The procedure measures conjugated 2,4-D, also (4).

Aerial application workers. The total amount of 2,4-D applied to wheat on a weight basis, exposure period, and mean daily excretion rate expressed on a body weight basis for the aerial application workers is shown in Table IV. A preexperimental one-time void sample was collected from most participants. These one-time samples contained from 0.1 to 1.6 ppm 2,4-D, with an average of 1.4 ppm. The mean daily excretion rate for all workers was 0.012 mg/kg body weight. From an occupational standpoint, the per day excretion rate was substantially higher for the mixer/loader (mean 0.02 mg/kg body weight) as compared to pilots (mean 0.006 mg/kg), and probably associated with their handling the 2,4-D concentrate. The low number of participants reporting exposure information relative to 2,4-D amount handled and hours of exposure negated (Table I) any valid statistical analysis of the data when worker number 1 was not included.

From the information provided by the participants, we were unable to find any relationship between clothing worn and 2,4-D excretion levels. The mixer/loader excretion 2,4-D levels (Table IV) correspond to the 0.02 mg/kg 2,4-D excreted by batchmen of T. L. Lavy, J. D. Walstad, R. R. Flynn, and J. D. Mattice, (1980, unpub), but considerably lower (0.06 mg/kg) than the 2,4,5-T mixers of Lavy's study and the 0.077 mg/kg in pilots to a low of 0.01 mg/kg in supervisors of forestry workers in Smith's et al. (8) study. Likewise, the wheat 2,4-D pilots excreted 0.006 mg/kg compared to the 0.02 mg/kg forestry pilots of Lavy et al. (unpub).

Ground application workers. Urinary excretion samples from ground applicators and mixer/loaders from North Dakota were taken on 6 consecutive days after a single 2,4-D exposure. Most participants provided one preexperimental urine sample, which contained from nondetectable quantities to 0.4 ppm 2,4-D.

The total amount of 2,4-D applied, hours of exposure, and daily and total excretion on a body weight basis is shown in Table V. Three parameters-occupation, amount mixed or applied, and exposure period were correlated with the amount of 2,4-D excreted in urine. When the occupational series is compared to the total amount (mg/kg) of 2,4-D excreted, three subgroups are identifiable, (a) applicators, 0.005; (b) mixer/loaders, 0.007; and (c) mixer/loader/applicators, 0.018; i.e., the excretion levels increased a<b<c. This reflects, in part the longer average exposure time (3.5 h) and the handling of 2,4-D concentrate for the mixer/loader/applicator group when compared to the mixer/loader (2.4 h) and applicator (3.5 h) groups (Figure 1). For example, the calculated 2,4-D excretion rate for the mixer/loaders would be 0.002 mg/kg/hour of 2,4-D exposure.

Total 2,4-D excretion was also correlated with the amount of 2,4-D mixed or applied (Figure 2). Assuming a one day exposure to

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Table IV. 1980 2,4-D 12/Day Intermittent
to Aerial Applicators in Wa

Worker	Job	12-Day Application 2,4-D A.I. Amount	
		lb	kg
1	M/L a/	500	225
2	M/L	1,200	1,300
3	M/L	1,360	730
4	M/L	---	---
5	M/L	---	---
6	M/L	---	---
7	M/L/P	360	190
8	P	1,050	475
9	P	1,120	510
10	P	950	430
11	P	---	---
12	P	100	115
13	P	830	375
14	P	---	---
15	P	750	335
16	P	---	---
17	P	---	---
Mean	overall	995	456
Mean	M/L b/	1,290	687
Mean	P	800	360
Ratio	M/L÷P	1.6	1.9

a/ Mixer/Loader/Pilot.
b/ For 80 kg person.
c/ Including No. 7.

ally the high 2,4-D urine conte[illegible] Washington Nam-
edure measures conjugated 2,4-D, also (4).

ication workers. The total amount of 2,4-D applied
eight basis, exposure period, and mean daily excre-
ssed on a body weight basis for the aerial appli-
is shown in Table IV. A preexperimental one-time
collected from most participants. These one-time
ed from 0.1 to 1.6 ppm 2,4-D, [illegible] average of
ean daily excretion rate for all workers was 0.012
ht. From an occupational standpoint, the per day
was substantially higher for the mixer/loader (mean
weight) as compared to pilots (mean 0.006 mg/kg),
sociated with their handling the 2,4-D concentrate.
of participants reporting exposure information
-D amount handled and hours of exposure negated
alid statistical analysis of the data when worker
t included.
nformation provided by the participants, we were
ny relationship between clothing worn and 2,4-D
s. The mixer/loader excretion 2,4-D levels (Table
to the 0.02 mg/kg 2,4-D excreted by hatchmen at T.
Halstad, R. R. Flynn, and J. D. Tarrice, (1980),
siderably lower (0.006 mg/kg) than the 2,4,5-T
study and the 0.007 mg/kg in pilots to a low of
pervisors of forestry workers in Smith's et al.
wise, the wheat 2,4-D pilots excreted 0.006 mg/kg
0.02 mg/kg forestry pilots of Lavy et al. (unpub).

ication workers. Urinary excretion samples from
rs and mixer/loaders from North Dakota were taken
days after a single 2,4-D exposure. Most parti-
one preexperimental urine sample, which contained
le quantities to 0.4 ppm 2,4-D.
mount of 2,4-D applied, hours of exposure, and
excretion on a body weight basis is shown in Table
ters-occupation, amount mixed or applied, and ex-
re correlated with the amount of 2,4-D excreted in
occupational series is compared to the total
f 2,4-D excreted, three subgroups are identifi-
ators, (a) 0.005; (b) mixer/loaders, 0.007; and (c)
licators, 0.018; i.e., the excretion levels in-
This reflects, in part the longer average exposure
he handling of 2,4-D concentrate for the mixer/
r group when compared to the mixer/loader (2.4 h)
3.5 h) groups (Figure 1). For example, the ex-
cretion rate for the mixer/loaders would be 0.002
4-D exposure.
excretion was also correlated with the amount of
pplied (Figure 2). Assuming a one day exposure to

Table IV. 1980 2,4-D 12-Day Intermittent to Continuous Exposure to Aerial Applicators in Washington.

Worker	Job	12-Day application 2,4-D a.i. amount		Time	Mean daily excretion of 2,4-D
		lb	kg	hour	[illegible] mg/kg x 10 [illegible]
1	M/L[a]	500	225	6	6.5 [b]
2	M/L	2,200	1,000	24	54.5 [b]
3	M/L	1,600	730	28	4.9
4	M/L	---	---	--	3.8
5	M/L	---	---	--	34.0
6	M/L	---	---	--	18.5
7	M/L/P	860	390	22	10.0
8	P	1,050	475	8	3.3
9	P	1,120	510	14	9.7
10	P	950	430	20	[illegible]
11	P	---	---	18	3.3 [b]
12	P	100	415	4	1.4
13	P	830	375	23	1.3
14	P	---	---	15	0.6 [b]
15	P	740	335	19	10.1
16	P	---	---	--	2.5
17	P	---	---	--	20.2
Mean	overall	995	450	16	11.6
Mean	M/L[c]	1,290	585	20	19.5
Mean	P	800	360	14	5.0
Ratio	M/L÷P	1.6	1.6	1.4	3.3

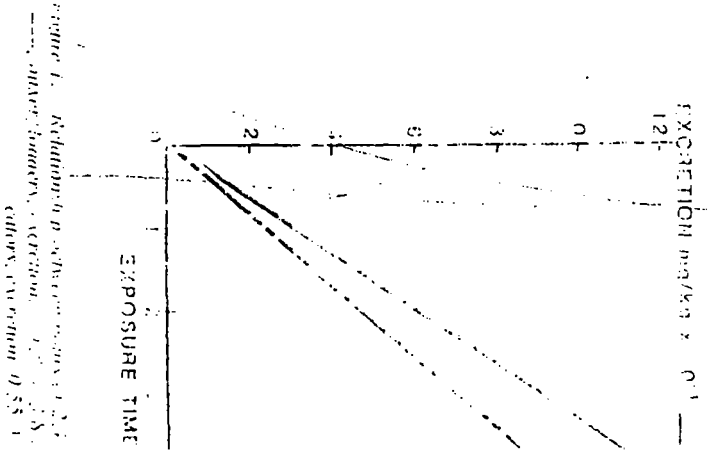
a/ Mixer/Loader/Pilot.
b/ For 80 kg person.
c/ Including No. 7.

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Table V. 1980 2,4-D 1-Day Exposure to Ground Applicators in North Dakota.

Worker	Job	1-Day application 2,4-D a.i. amount lb	kg	Time hour	Daily excretion of 2,4-D, mg/kg × 10^{-3} 0 [a/]	1	2	3	4	5	6	Total exposure mg/kg X 10^{-3}
1	A [b/]	23	10	1	ND [c/]	0.05	0.10	0.10	ND	ND	0.05	0.30
2	A	--	--	1.5	0.09	ND	ND	ND	ND	ND	ND	0.09
3	A	40	18	3	ND	0.07	0.07	0.07	ND	ND	ND	0.21
4	A	100	45	2	ND	ND	ND	ND	ND	ND	ND	0.0
5	A	120	54	7	ND	0.48	0.19	0.07	0.14	0.34	0.34	1.56
6	A	100	45	5	ND	1.03	2.12	1.71	0.68	0.27	0.14	5.95
7	A	--	--	3	ND	10.41	5.21	1.30	0.55	0.41	ND	17.9
8	A	43	20	3	ND	0.06	0.35	0.52	0.29	0.06	0.17	1.45
9	A	23	10	2	0.16	4.00	19.4	24.0	15.2	6.37	6.89	76.0
10	A	107	49	6	0.07	4.13	4.48	4.73	1.46	1.27	1.00	17.0
11	M/L	20	9	1	ND	0.93	0.83	0.58	0.07	0.07	0.14	2.54
12	M/L	23	10	1	ND	0.88	0.13	0.44	ND	0.20	ND	1.65
13	M/L	23	10	1	ND	0.33	1.13	1.13	0.47	0.33	0.20	3.59
14	M/L	50	23	3	ND	1.74	1.67	1.52	0.53	0.98	0.68	7.12
15	M/L	35	16	3.5	ND	3.96	3.19	4.22	1.17	0.91	0.97	16.4
16	M/L	58	26	4	0.13	0.75	0.78	2.16	5.64	4.26	0.54	14.3
17	M/L	42	19	3	ND	0.28	0.84	0.62	0.34	ND	ND	2.08
18	M/L/A	--	--	15	0.21	3.36	8.71	8.43	5.79	5.57	2.64	34.7
19	M/L/A	112	51	11	ND	5.60	1.60	2.33	2.00	0.27	1.20	13.0
20	M/L/A	200	91	3	0.38	1.29	2.80	6.38	10.4	2.56	20.5	44.2
21	M/L/A	14	6	3	ND	2.36	4.79	6.07	5.64	1.26	0.93	21.0
22	M/L/A	--	--	9.5	ND	1.35	4.30	4.16	4.78	2.92	2.53	20.1
23	M/L/A	30	14	3	0.12	1.07	0.73	0.33	1.13	0.93	--	4.31
24	M/L/A	40	18	6	0.35	2.07	3.50	2.93	1.86	2.79	1.07	15.2
25	M/L/A	83	38	6	ND	0.91	1.04	0.73	0.37	0.37	0.18	3.66
26	M/L/A	162	46	10	0.27	3.74	5.79	5.68	5.84	1.37	1.89	23.6
Mean	overall	63	(29)	4.7	0.09	1.96	2.60	3.08	2.47	1.36	0.90 [d/]	12.7 [d/]
Mean	A	70	(32)	3.4	0.03	2.02	3.18	3.25	1.64	0.97	0.86	12.0
Mean [e/]	A	76	(34)	3.5	0.02	1.80	1.18	0.94	0.34	0.26	0.19	4.93
Mean	M/L	39	(18)	3.4	0.02	1.27	1.51	1.17	0.96	0.36	0.36	6.8
Mean	M/L/A	83	(38)	7.3	0.21	2.42	3.37	4.11	4.02	2.22	1.49 [d/]	17.8 [d/]

a/ Day 2,4-D was applied. b/ Mixer/Loader/Applicator. c/ None detected. d/ Without worker 20. e/ Without worker 9.



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			hour	a/	1	2	3	4	5	6	mg/kg X 10^{-3}
1	A	23	1	ND	0.05	0.10	0.10	ND	ND	0.05	0.30
2	A	--	1.5	0.09	ND	ND	ND	ND	ND	ND	0.09
3	A	40	3	ND	0.07	0.07	0.07	ND	ND	ND	0.21
4	A	100	2	ND	ND	ND	ND	ND	ND	ND	0.0
5	A	120	7	ND	0.48	0.19	0.07	0.14	0.34	0.34	1.56
6	A	100	5	ND	1.03	2.12	1.71	0.68	0.27	0.14	5.95
7	A	--	3	ND	10.41	5.21	1.30	0.55	0.41	ND	17.9
8	A	43	3	ND	0.06	0.35	0.52	0.28	0.06	0.17	1.45
9	A	23	2	0.16	4.00	19.4	24.0	15.2	6.37	6.89	76.0
10	A	107	6	0.07	4.13	4.40	4.73	1.40	1.27	1.00	17.9
11	A/L	20	1	ND	0.93	3.83	0.50	0.07	0.07	0.14	2.54
12	A/L	23	1	ND	0.80	0.13	0.44	ND	0.20	ND	1.65
13	A/L	50	3.5	ND	0.33	1.13	1.13	0.47	0.33	0.20	3.59
14	A/L	35	4	ND	1.74	1.52	1.52	0.53	0.98	0.68	7.12
15	A/L	58	3	ND	3.96	3.40	4.22	1.17	0.91	0.97	16.4
16	A/L	42	3	ND	0.78	0.78	2.16	5.64	4.26	0.54	14.1
17	A/L	--	3	ND	0.26	0.91	0.63	0.34	0.41	ND	2.08
18	A/L/A	--	15	0.31	0.36	8.71	8.43	4.73	2.37	2.61	34.7
19	A/L/A	112	11	0.30	5.60	1.64	2.33	2.04	0.27	1.20	13.8
20	A/L/A	200	6	0.30	1.29	2.80	6.36	10.4	10.27	2.50	44.2
21	A/L/A	14	3.5	ND	2.36	4.79	6.07	5.64	1.26	0.93	21.0
22	A/L/A	--	3.5	ND	1.35	1.38	4.16	4.70	2.92	2.53	20.1
23	A/L/A	30	3	0.12	1.07	0.73	0.33	1.13	0.93	--	4.31
24	A/L/A	40	3	0.33	2.07	3.50	2.81	1.86	2.79	1.07	15.2
25	A/L/A	83	3	ND	0.91	1.04	0.71	0.37	0.37	0.18	3.66
26	A/L/A	102	10	0.27	3.74	2.79	5.68	5.83	3.37	1.89	23.6
Mean	overall	63	4.7	0.09	1.96	3.80	3.08	2.47	1.36	0.90d/	12.7d/
Mean e/	A	70	3.4	0.03	2.02	3.18	3.25	1.83	0.87	0.86	12.0
Mean	A/L	39	3.5	0.02	1.80	1.18	0.94	0.34	0.26	0.19	4.93
Mean	A/L/A	83	7.9	0.21	1.27	1.51	1.51	1.17	0.96	0.36	6.84/
					2.42	3.37	4.11	4.02	2.22	1.49d/	17.8d/

a/ Day 2,4-D was applied. b/ Mixer/Loader/Applicator. c/ None detected. d/ Without worker 10.
e/ Without worker 9.

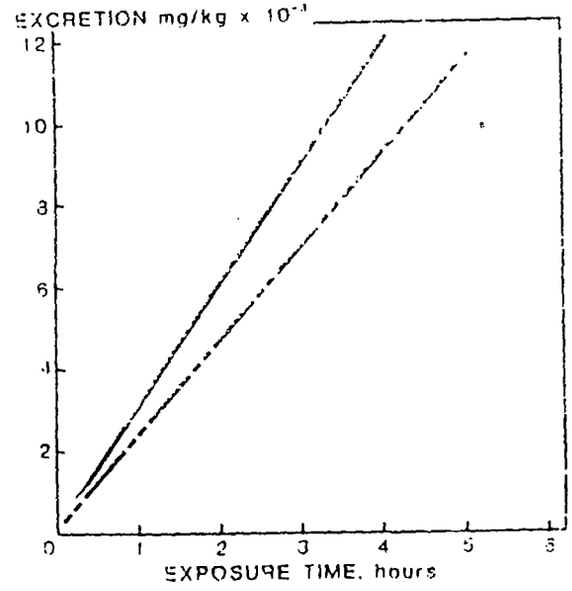


Figure 1. Relationship between hours on 2,4-D exposure and excretion rate. Key: — · — · mixer/loaders, excretion: 1.8 +- 3.8 h (r: 0.75[a]*); - - - -, ground applicators, excretion: 0.55 +- 2.2 h (r: 0.68*[a]*)*

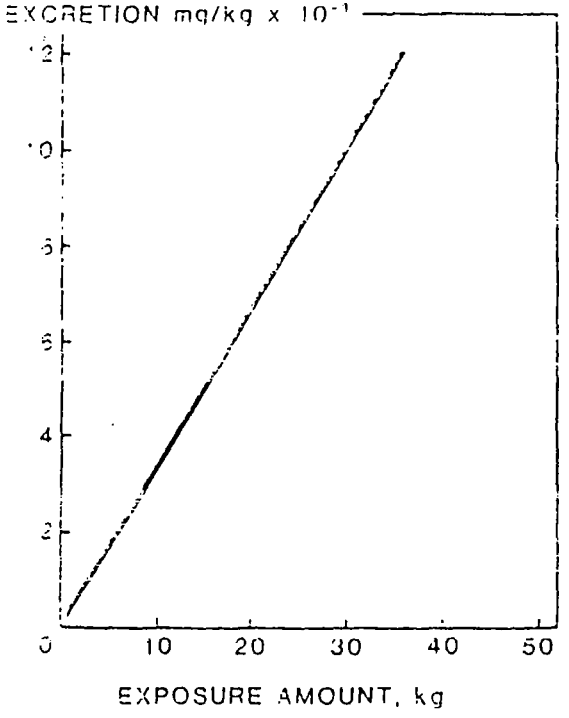


*Figure 2. Relationship between amount of 2,4-D applied and excretion rate (excretion = 1.15 ± 0.31 mixed or applied (r: 0.61**).*

45 kg (100 lb) of 2,4-D, the total amount exc
would be 0.015 mg/kg body weight. It should
the amount of 2,4-D mixed or applied or the e
not be the most important relationship to stu
The care or carelessness with which the worker
and the type of protective clothing worn are
ingful relationship. In this study, because t
worn was not specified in the protocol, the re
worn was too variable to make any statistical
amounts of 2,4-D excreted.

One-half of all ground applicators exceed
imum daily excretions of 0.0003 mg/kg (Table VI
ably more indicative of the exposure level for
rather than the 0.003 mg/kg maximum mean level
ground workers.

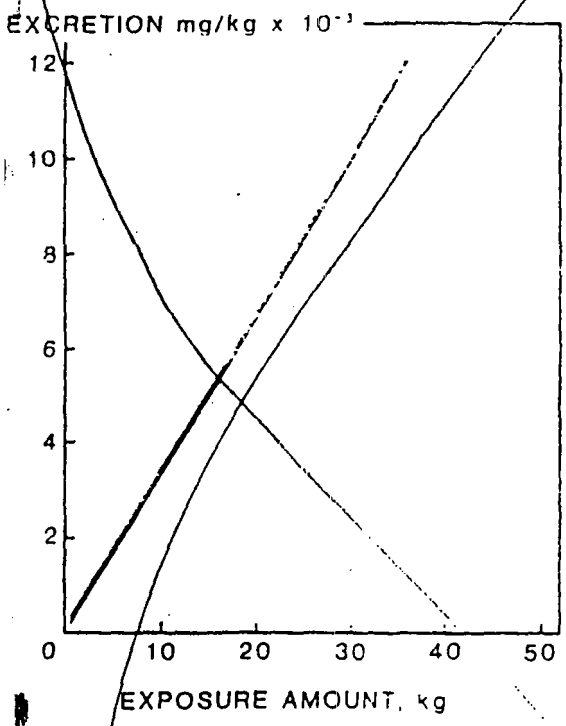
Table VI. Individual Frequencies for Maximum
Excretions of North Dakota Ground Ap

2,4-D Excretion mg × 10^{-1}/kg body weight	
0 – 1.9	1
[illegible]	
[illegible]	
[illegible]	
8 – 9.9	
10 – 11.9	
24 – 25.9	

Comparisons with other studies. The uptake of 2,4-D by aerial and ground workers was not weight, or type of clothing. However, 2,4- associated with job for both aerial and ground ors of 2,4-D applied and with hours of exposure for Presumably this reflects the amount of 2,4-D ex by absorption through the skin, inhalation, and gestion. Gehring et al.(4) Kohli et al. (6), (7) observed that all ingested or bodily absor by man was absorbed into the plasma and excrete ther unchanged or as a conjugate. In the case of al. (5), indicated 2,4-D would follow a similar they recovered 77% of the ingested 2,4-D and 5 after 96 h.

Gehring et al. (4), and Kohli et al. (5,6), al. (2) found urinary excretion and plasma elim $E_{1/2}$ times of 23 and 19 h, respectively, for 2, 18 h, respectively, for 2,4-D. The $E_{1/2}$ time mixer/loaders, and mixer/loader/applicators, r 35, 39, and 48 h. An $E_{1/2}$ time for forest 2,4- calculated from Lavy's et al. (1) data was fou longer $E_{1/2}$ times associated with the 2,4-D (t

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Relationship between amount of 2,4-D applied and excretion rate (excretion = .15 + 0.31 mixed + applied; r = 0.61").

45 kg (100 lb) of 2,4-D, the total amount excreted over 6 days would be 0.015 mg/kg body weight. It should be pointed out that the amount of 2,4-D mixed or applied or the exposure period may not be the most important relationship to urine excretion levels. The care or carelessness with which the worker performs his duty and the type of protective clothing worn may provide a more meaningful relationship. In this study, because the type of clothing worn was not specified in the protocol, the resultant clothing worn was too variable to make any statistical correlation with amounts of 2,4-D excreted.

One-half of all ground applicators exposed to 2,4-D had maximum daily excretions of <0.002 mg/kg (Table VI), which is probably more indicative of the exposure level for an average worker rather than the >0.003 mg/kg maximum mean given in Table V for all ground workers.

Table VI. Individual Frequencies for Maximum 2, 4-D Daily Urinary Excretions of North Dakota Ground Applications.

2,4-D Excretion mg x 10^{-3}/kg body weight	Frequency
0 - 1.9	13
2 - 3.9	3
4 - 5.9	3
6 - 7.9	1
8 - 9.9	1
10 - 11.9	2
24 - 25.9	1

Comparisons with other studies. The urinary excretion level of 2,4-D by aerial and ground workers was not associated with age, weight, or type of clothing. However, 2,4-D excretion was associated with job for both aerial and ground workers and with amount of 2,4-D aplied and with hours of exposure for ground workers. Presumably this reflects the amount of 2,4-D taken into the body by absorption through the skin, inhalation, and possibly some ingestion. Gehring et al.(4) Kohli et al. (6), and Sauerhoff et al. (7) observed that all ingested or bodily absorbed 2,4,5-T or 2,4-D by man was absorbed into the plasma and excreted in the urine either unchanged or as a conjugate in the case of 2,4-D. Kohli et al. (5), indicated 2,4-D would follow a similar pattern because they recovered 77% of the ingested 2,4-D and found no metbolites after 96 h.

Gehring et al. (4), and Kohli et al. (5,6), and Sauerhoff et al. (2) found urinary excretion and plasma clearances for man with $E_{1/2}$ times of 23 and 19 h, respectively, for 2,4,5-T, and 33 and 18 h, respectively, for 2,4-D. The $E_{1/2}$ times for applicators, mixer/loaders, and mixer/loader/applicators, respectively, were 35, 39, and 48 h. An $E_{1/2}$ time for forest 2,4,5-T applicators, calculated from Lavy's et al. (1) data was found to be 52 h. The longer $E_{1/2}$ times associated with the 2,4-D (this study) and

2,4,5-T (2) workers compared to 2,4-D or 2,4,5-T ingestion studies (2,4,5,6) probably reflects the slower rate of plasma absorption of the herbicides, compared to ingestion. The data suggests the workers did a poor job of skin protection and subsequent washing.

In the Gehring et al. (4) study, the one-time ingestion was 5 mg 2,4,5-T/kg body weight. The per day excretion was 1.9, 1.5, 0.71, and 0.33 mg/kg for days 1 to 4, respectively. The Kohli et al. (5,6), studies for 2,4-D and 2,4,5-T showed the same order of magnitude for 5 mg/kg dosages, but proportionately less for 1 and 0.1 mg/kg dosages. In this experiment, the per day excretion for the other ground workers (Table V) on days 1 to 5, respectively, were 1.2, 1.4, 1.5, 1.4, and 1.1 mg x 10^{-3}/kg, about one-thousandth as much as the former.

Gehring et al. (4) also calculated the concentration of 2,4,5-T in plasma from repeated doses. They found that a maximum concentration was reached after 3 days when the rate of 2,4,5-T clearance from plasma equaled the amount ingested. The analogy can be applied to the urinary excretion amounts, also, because plasma clearance rate was exactly the same as the urinary excretion rate. Therefore, it would appear that the aerial workers (Table IV, Figure 3) had essentially reached the maximum 2,4-D excretion plateau.

Concluding Remarks. Based on the results presented here, some estimate of 2,4-D exposure can be calculated. For example, the ground application workers exposure to 2,4-D can be expressed as percent daily excretion (Figure 4). By knowing the total urinary excretion for any given day after exposure, the total exposure level can be calculated. In another example, the all-jobs per day urinary excretion of the aerial workers (Table IV) was 0.012 mg/kg. A 35 or 40 year-old 80 kg worker with 30 years experience of 30 days exposure per year could have absorbed and excreted as much as [illegible] 2,4-D in his lifetime, which is about double the single injestion dosages reported by Gehring et al. (4), Kohli et al. (5,6), and Sauerhoff et al. (2) without any known ill effects.

Aknowledgements

The authors thank the 43 volunteer 2,4-D applicators who participated in this study; North Dakota State University Extension personnel and the Washington State Chapter of National Agricultural Aviation Association who contacted the participants; W. C. Aller, Yakima Laboratory for collection of urine samples from Eastern Washington and assistance in the analysis of these samples; and D. S. Frear, Plant Metabolism Laboratory, USDA, SEA-AR, Fargo, North Dakota.

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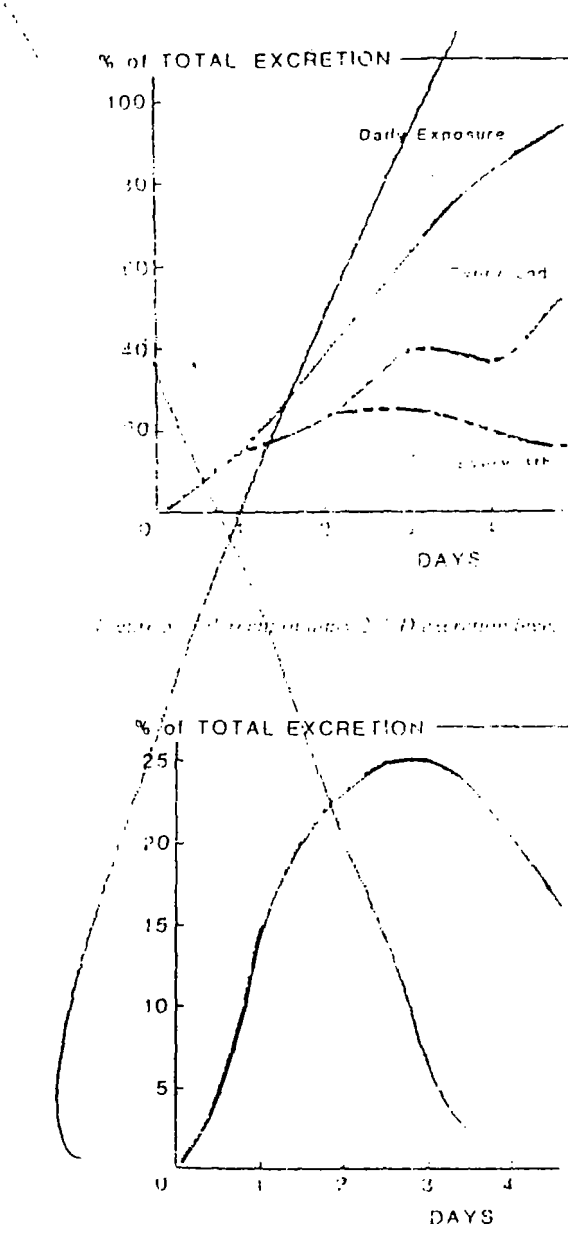


Figure 4. Percent of daily 2,4-D excretion from [illegible] days (%: 0.04 + 15 d − 11 d² + 0.12 d³ (r: 0.99

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1) workers compared to 2,4-D or 2,4,5-T ingestion studies probably reflects the slower rate of plasma absorption bicides, compared to ingestion. The data suggests the d a poor job of skin protection and subsequent washing. he Gehring et al. (4) study, the one-time ingestion was 5 t/kg body weight. The per day excretion was 1.0, 1.4, 0.33 mg/kg for days 1 to 4, respectively. The Kohli et studies for 2,4-D and 2,4,5-T showed the same order of for 5 mg/kg dosages, but proportionately less for 2 and osages. In this experiment, the per day excretion for /loader ground workers (Table 4) on days 1 to 5, respec- ere 1.2, 1.4, 1.5, 1.4, and 1.1 x 10^{-3} /kg, about one- h as much as the former.

ing et al. (4) also calculated the concentration of n plasma from repeated doses. They found that a maximum tion was reached after 3 days when the rate of 2,4,5-T from plasma equaled the amount ingested. The analogy plied to the urinary excretion amounts, also, because earance rate was exactly the same as the urinary excre- . Therefore, it would appear that the aerial workers , Figure 3) had essentially reached the maximum 2,4-D plateau.

Remarks. Based on the results presented here, some es- 2,4-D exposure can be calculated. For example, the lication workers exposure to 2,4-D can be expressed as ily excretion (Figure 4). By knowing the total urinary for any given day after exposure, the total exposure be calculated. In another example, the all-jobs per day cretion of the aerial workers (Table IV) was 0.012 50 to 60 year-old 80 kg worker with 30 years experience exposure per year could have absorbed and excreted as 9 g 2,4-D in his lifetime, which is about double the estion dosages reported by Gehring et al. (4), Kohli et , and Sauerhoff et al. (2) without any known ill

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thors thank the 43 volunteer 2,4-D applicators who par- n this study; North Dakota State University Extension nd the Washington State Chapter of National Agricultur- Association who contacted the participants; W. C. ma Laboratory for collection of urine samples from hington and assistance in the analysis of these sam- . S. Frear, Plant Metabolism Laboratory, USDA, SEA-AR, h Dakota.

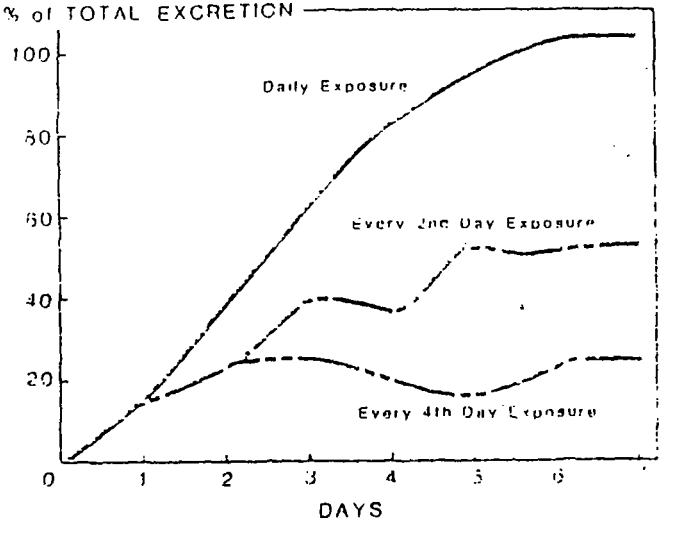


Figure 3. Percent of daily 2,4-D excretion levels from repeated exposure.

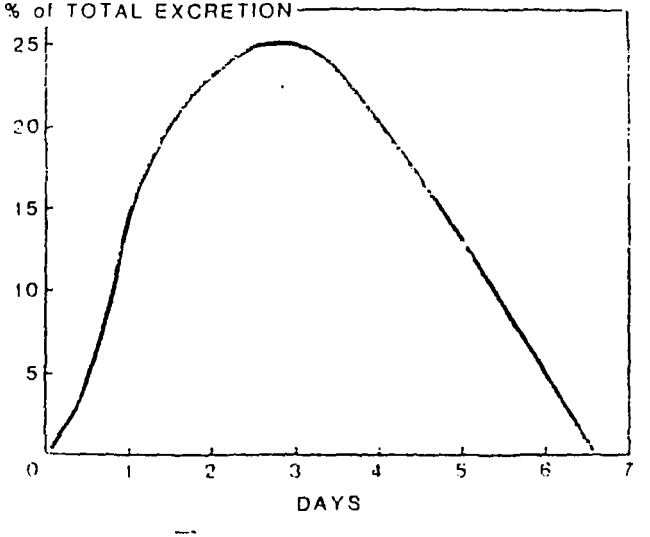


Figure 4. Percent of daily 2,4-D excretion from a single exposure for the first 6 days (% = 0.04 + 15 d − 1.1 d² + 0.12 d³ (r = 0.99) based on ground applications)

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Review of Studies with 2,4,5-Trichlorophenoxyacetic Including Applicators Under I

M. L. FENG, J. C. RAMSEY, and W. H. BRAU
The Dow Chemical Company, Health and Environm
L. L. LAY
University of Arkansas, Altheimer Laboratory, Fayet

decades (Table I). In April 19[illegible] 1979 [illegible] a temporary suspen[illegible] richlo-ef-ay and pastures (2). [illegible] not affected. Hearing began in the [illegible] on potential risk [illegible]

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The Lancet · Saturday 26 June 1982

No. 8287 VOL. I FOR 1982

HERBICIDES, OCCUPATION, AND CANCER

SIR,—In their review of the phenoxy herbicide/cancer controversy Dr Coggon and Professor Acheson (May 8, p. 1057) ask for more information. I have updated an occupational mortality study[1] which sheds further light on the matter.

There is considerable exposure in Washington State to phenoxy herbicides and chlorophenols in agriculture and forestry and the wood products industry. 2,4-D is used extensively in wheat farming, roadside brush and weed control, management of unwanted forest growth, and in pulp mills and sawmills for control of bluestaining fungi.

Table I shows mortality due to soft tissue sarcomas, Hodgkin's disease, and the non-Hodgkin's lymphomas for men in occupations which have exposure to phenoxy herbicides and chlorophenols.

Only farmers NOS (not otherwise specified) have a significant excess of soft tissue sarcoma, but the total proportional mortality rate (PMR) for these occupations is not significantly increased. Similarly, papermakers have a significant excess of Hodgkin's disease while the PMR for Hodgkin's disease in these occupations is only 102.

Table II shows the 15 occupations (of 218) with the highest PMRs for soft tissue sarcomas. None of them have an obvious exposure to the phenoxy herbicides or chlorophenols. The dry cleaners, printers, and mechanics have solvent exposure.

The mortality patterns seen here do not offer convincing support for the phenoxy herbicide and chlorophenol link to soft tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphomas.

Supported in part by National Institute for Occupational Safety and Health contract no 210-80-0088.

Epidemiology Section,
Washington State Department of
Social and Health Services,
Olympia, Washington 98504, U.S.A.

SAMUEL MILHAM, JR

1. Milham S. Occupational mortality in Washington State, 1950–1979. HEW publication (NIOSH) (in press).

TABLE I—OBSERVED/EXPECTED DEATHS (AND PMRs) FOR SELECTED CANCERS* IN OCCUPATIONS WITH EXPOSURE TO PHENOXY HERBICIDES AND CHLOROPHENOLS: WASHINGTON STATE WHITE MALE DEATHS, AGE 20+, 1950–79

Occupation	Soft tissue sarcomas	Hodgkin's disease	Lympho-sarcoma, reticulo-sarcoma	Other lymphomas
Foresters	..	..	1/1 (126)	..
Farmers (NOS)	25/16 (157)†	18/36 (107)	72/71 (101)	41/40 (103)
Orchardists	3/2 (145)	9/5 (196)	12/9 (136)	3/5 (56)
Nurserymen	..	..	1/1 (71)	..
Ranchers	1/2 (47)	2/5 (38)	9/10 (91)	6/6 (102)
Wheat farmers	1/2 (40)	5/6 (80)	13/11 (121)	9/6 (148)
Orchard labourers	1/1 (99)	2/3 (67)	2/4 (46)	0/2 (0)
Landscapers, gardeners	1/2 (56)	4/4 (89)	4/8 (52)	5/4 (116)
Paper/pulp millworkers	3/3 (91)	17/9 (194)†	17/13 (122)	7/8 (92)
Sawmill workers	10/10 (98)	23/27 (88)	42/45 (94)	23/24 (95)
Tree farmers		3/3 (118)	3/4 (72)	3/2 (154)
Railroad labourers	4/3 (120)	5/9 (58)	12/15 (83)	12/7 (169)
Total	49/41 (120)	109/107 (102)	188/192 (98)	109/104 (105)

†$p<0.05$.
*ICD nos are respectively: 197; 201; 200·0–200·1; 202–202·7.
Expected deaths based on age-at-death and year-of-death specific proportionate mortality (rounded to nearest whole numbers). PMR = Obs/Exp × 100.

TABLE II—OCCUPATIONS WITH HIGHEST PMRs FOR SOFT TISSUE SARCOMAS (7TH ICD 197): WASHINGTON STATE WHITE MALE DEATHS, AGE 20+, 1950–79

	Observed	Expected	PMR
Marine engineers	3	<0·5	>600
Butchers	5	1	446
Laundry, dry cleaners	3	1	>300
Plumbers, pipefitters	10	3	300
Teachers	7	3	272
Students	4	2	250
Pressmen, printing	3	1	209
Construction labourers	8	4	195
Car salesmen	3	2	184
Bricklayers	2	1	179
Navy officers, enlisted men	4	2	177
Aeronautical engineers	2	1	176
Bus drivers	2	1	175
Aluminium workers	2	1	158
Mechanics (NOS)	6	4	156

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Bull. Environm. Contam. Toxicol. 29, 586–591 (1982)

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Measurement of 2,4-Dichlorophenoxyacetic Acid (2,4-D) After Occupational Exposure

P. K. Taskar, Y. T. Das, J. R. Trout, S. K. Chattopadhyay, and H. D. Brown

New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, NJ 08903

CUM71504

The phenoxy herbicides have been used to control broad-leafed weeds in crops, water sources, forests, pastures, rangelands, gardens, lawns, and urban and industrial sites. Because of the efficiency, economy and safety, the phenoxy herbicides, especially 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D), besides other herbicides, remain essential for agricultural and other uses (DIAZ-COLON & BOVEY 1976). Use of 2,4,5-T and 2,4-D on a large scale to defoliate vegetation has resulted in controversy about the fate of the chemicals. Many of the hazardous properties ascribed to 2,4,5-T are to a lesser extent shared by 2,4-D. As a result, a number of papers dealing with the fate in the environment, toxicological effects on living organisms (DIAZ-COLON & BOVEY 1976; SAUERHOFF et al. 1977; PIPER et al. 1973) have been published. COOPER (1974) compared 2,4,5-T and 2,4-D. He has reported that commercial samples of 2,4-D in mice were teratogenic and embryotoxic. Increasing controversy about their safe use has raised doubt about their utility in achieving vegetation management objectives.

A Dow Chemical report on Forestry Applicators exposure to 2,4-D has recently been published (LEVY 1980). Nonetheless, in total, only meager information regarding human (e.g., gardeners, small farmers, etc.) exposure to 2,4-D is available. This study reports an investigation conducted under conditions characteristic of actual use pattern to determine the quantity of body surface contact of applicator with the commercial 2,4-D formulation and the quantity of the chemical measurable in serum and urine.

MATERIALS AND METHODS

Eleven healthy young male (ages 19 to 31 years) human volunteers were selected and were examined by the University physician prior to the work. Each subject was examined for blood pressure, pulse rate and body temperature on the day of work, both before and after the work period, and similar examinations were made every morning for the next three days. Sterile gauze pads (size 0.67 sq. ft.) were attached on the back and chest of each volunteer. A paper cap (area 0.44 sq. ft.) was used to cover the head.

The study was carried out either on the campus of Rutgers University or near the campus in New Brunswick, New Jersey during the period from May 1981 to August 1981. The location was covered with

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: Acid (2,4-D)

and H. D. Brown

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weeds and grass. Each area was roughly between one-half to one acre.

Weather conditions during this study were moderate, temperature ranging between 65° and 78° with humidity from 59 to 89%. Wind was fairly calm throughout this study except on one occasion, when it gusted to 10 miles per hour.

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The spraying material consisted of DMA-4 concentrate (obtained as a gift from Dow Chemical, U.S.A.) which contained 3.8 lb acid equivalent/gal 2,4-D. The herbicide was mixed with tap water prior to use and the resulting mixture was applied at a rate of approximately 1 gal/30 min by a hand sprayer held at hip level. A DuPont air sampler was attached to the applicator and was kept running during the spraying operation. The areas were covered by spraying six gallons in about three hours as per label instruction.

To ascertain body surface exposure, gauze pads and caps were collected immediately after the work and washed in methanol. Air filter absorbents were also washed in methanol. Residue separated by methanol was evaporated to dryness and then treated with 15% BF_3 in methanol. Finally, it was extracted with n-hexane (YIP 1975).

Blood samples were drawn before the work and at the end of the day's work. Three more blood samples were drawn on the following three mornings. Overnight pooled urine samples were collected before the work and the day's collections were made during and/or after the treatment. Then the pooled urine samples were collected twice a day for the next 4 to 7 days. Blood sera and urine samples were treated, extracted and analyzed by the method of SAUERHOFF et al. (1977).

Vegetation samples and soil samples from the treated area were collected before and immediately after the treatment and then once every 24 hours for the next three days. Samples were treated and analyzed by the methods of DAVIDONIS et al. (1980) and OLSON et al. (1978).

2,4-D residue extracted in n-hexane was analyzed on a gas chromatograph (Tracor Model No. 560, equipped with electron capture detector and a six foot glass column packed with 3% OV-101 on 80/100 supelcoport).

Gas chromatographic conditions used were as follows:

Detector, Electron capture Ni 63 temperature:	350°C
Gas: Methane: Argon	(5:95)
Flow rate through the column	25 ml/min
Sample volume injected	1 µl
Column material	3% OV-101 on 80/100 Supelcoport
Length of the column	6 ft
I.D. of the column	2 mm
Column temperature	187°C
Injection port temperature	200°C

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Clinical determinations on blood serum and urine were made through the automated procedures of a commercial diagnostic laboratory.

RESULTS AND DISCUSSION

Distribution of 2,4-D on human volunteers (applicators) body surface is presented in Table 1.

Table 1. Distribution of 2,4-D Residue on Body Surface

Applicator No.	Head µg/sq. ft.	Chest µg/sq. ft.	Head µg/sq. ft.
1	8.55	7.81	5.10
2	14.06	11.68	10.60
3	7.06	3.37	3.77
4	222.20	171.50	99.63
5	34.22	94.53	41.16
6	141.80	111.50	74.23
7	76.16	68.39	46.64
8	16.99	13.56	11.86
9	19.12	14.58	10.29
10	18.55	31.46	4.46
11	17.80	14.95	18.63

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At the end of a day's work, the applicators had an average 2,4-D residue of 52.41 µg/sq. ft. on the head, 49.39 µg/sq. ft. on the ches and 29.67 µg/sq. ft. on the back.

2,4-D residue data on vegetation and soil surface are summarized in Tables 2 and 3, respectively.

Table 2. 2,4-D Residue on Vegetation (µg/g)

Treated Area	0	X	X+1	X+2	X+3
1	0.59	28.85	16.15	16.67	20.16
2	14.74	29.60	22.43	4.32	2.13
3	00.00	25.35	7.70	1.92	3.44

Table 3. Amount of 2,4-D on Soil Surface (µg/g)

Treated Area	0	X	X+1	X+2	X+3
1	1.24	3.70	1.90	2.80	7.30
2	3.58	4.21	0.72	1.59	7.25
3	0.00	0.13	0.15	0.27	0.80

0 = pretreatment; X = posttreatment; X+1 = 1 day after treatment; X+2 = 2 days after treatment; X+3 = 3 days after treatment.

The average value of 2,4-D residue on vegetation in the treated area was 27.93 µg/g of plant tissue at the end of the working day (i.e., first day). The residue decreased to 8.57 µg/g in plant tissue on

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ead sq. ft.
5.10
0.60
3.77
9.63
1.16
4.23
6.64
1.86
0.29
4.46
8.63

ad an average 2,4-D
sq. ft. on the chest

face are summarized

g/g)

X+3
20.16
2.13
3.44

(µg/g)

X+3
7.30
7.25
0.80

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orking day (i.e.,
plant tissue on

the fourth day. On the other hand, the average value of soil residue was 2.68 µg/g soil at the end of the first day which, however, increased to 5.12 µg/g on the fourth day.

Analysis of 2,4-D retained by the air monitor filter revealed detectable residue. The amount of air drawn through the filter was calculated to be 56.6 to 84.96 liters, and the measured 2,4-D residue was 43.1 to 60.1 parts per trillion.

Level of 2,4-D residue plus intrinsic phenolics in serum and urine was measured. The tables below present values calculated by substracting the measured base (intrinsic phenolics) from each subsequent measurement.

Table 4. Amount of 2,4-D Measured as Phenolics in Serum

Applicator No.	2,4-D Residue (ng/ml) X	X+1	X+2	X+3
1	--	714.3	198.7	0
2	60.4	116.1	196.1	48.3
3	128.4	38.0	—	396.1
4	285.4	--	—	—
5	482.0	440.6	113.6	188.8
6	--	654.6	--	466.3
7	179.9	74.8	150.2	134.1
8	15.6	52.2	40.1	79.9
9	21.2	12.8	14.0	--
10	--	74.3	39.8	17.3
11	--	55.6	27.9	114.8

X = posttreatment; X+1 = 1 day after treatment; X+2 = 2 days after treatment; X+3 = 3 days after treatment.

Table 5. 2,4-D Measured as Phenolics Excreted in the Urine

Applicator No.	1	12	24	36	48	60	72	84
1	3.39	--	--	2.67	1.97	--	17.47	25.26
2	3.95	--	0.63	2.78	2.53	1.24	3.22	--
3	1.40	7.15	6.02	--	--	—	7.94	1.22
4	7.33	13.27	--	1.45	22.44	16.76	4.98	--
5	1.00	1.47	1.28	0.99	2.30	3.00	1.95	7.07
6	--	5.67	—	13.44	3.92	--	--	3.21
7	0.90	5.34	--	--	—	--	--	--
8	0.11	0.57	2.77	1.34	3.46	2.84	3.08	5.30
9	0.75	—	0.85	2.46	8.28	6.57	1.46	4.09
10	1.94	1.73	1.63	2.83	2.21	2.73	8.48	4.35

1 = posttreatment

The average base value for phenolics in serum was 70.7 ng/ml immediately before exposure. Immediately after the day's work, the value

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went up to 106.63 ng/ml and again to 203.03 ng/ml on the second day. The value, however, came down to 70.95 ng/ml on the third day and again went up to 130.51 ng/ml on the fourth day. Average phenolic compounds ranged from 3.81 µg (zero level exposure) to 5.05 µg on the fifth day in 12 hours of pooled urine.

The effect of 2,4-D exposure on red blood cell (RBC), white blood cell (WBC), hemoglobin concentration (HG), hematocrat (HCT), platelet and many other blood parameters were studied. No appreciable changes were noticed. However, some changes were observed in clinical analysis made on blood serum (19 parameters were studied) and urine.

The clinical residue and weather data were critically scrutinized to both linear models and regression models. The primary emphasis of the analysis was to identify the parameters that recorded any significant change. This was accomplished by subtracting the baseline value from the observed value and then dividing it by the baseline. Analysis of the resulting values revealed highly significant (α=0.01) changes in the serum creatinine and total protein levels, and significant (α=0.05) change in the urine specific gravity. It was also seen that the total protein and BUN/creatinine ratio were positively correlated with the residue on the chest of the volunteers and with their height. When the peak residue was related with the external factors, it was seen that the serum residue was positively correlated with the 2,4-D concentration in the air and the amount of chemical that had landed on the head and back. Likewise, the urine residue was positively correlated with the air volume and the height of the volunteers. Precipitation (rainfall-high humidity) was negatively correlated with the serum residue, presumably due to decreased chances of vaporization and subsequent inhalation.

Results of this study indicate that there was a considerable amount of 2,4-D in the air, on the vegetation and soil surface of the treated areas. An appreciable quantity of 2,4-D landed on the body surface and possibly entered into the applicator's body through inhalation and/or through body surface (dermal) absorption. Lengthy retention times have been observed in some subjects (Table 5). These findings are consonant with the report of LEVY et al. (1980) for foresters. Clinical physiology data otained here are consistent with long retention times for this chemical. We surmise, but have no specific evidence, that the 2,4-D molecule or some portion (phenolic) binds to serum protein and is elminated as a function of the turnover of the protein molecules. Other than the clinical (chemical) changes noted above, we have seen no pronounced adverse effect of 2,4-D exposure.

ACKNOWLEDGEMENT

New Jersey Agricultural Experiment Station Publication No. D-04502-1-82, supported by State funds and by Grant No. USDA-TPSU-RU-0-191 of the U.S. Department of Agriculture/Pennsylvania State University.

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Accepted August 29, 1982

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K-2372-(100)
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JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH



Volume 10
Numbers 4-5
October-November 1982

JTEHD6 10(4-5) 521-857 (1982)
ISSN: 0098-4108

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K-12372-(100)
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ALLERGIC HYPERSENSITIVITY TO THE HERBICIDE 2,4-D IN BALB/c MICE

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Following several reports of human allergic hypersensitivity, the ubility of 2,4-D (2,4-dichlorophenoxyacetic acid) to elicit 2,4-D-specific IgE antibodies and delayed-type hypersensitivity in BALB/c mice was studied. 2,4-D-specific IgE antibodies were detected in mouse sera following the second intraperitoneal immunization with 1, 10, or 100 μg 2,4-D-keyhold limpet hemocyanin conjugate with aluminum hydroxide adjuvant. Specific IgE was determined with the rat passive cutaneous anaphylaxis test using a conjugate of 2,4-D with bovine serum albumin for challenge. The highest antibody titers and a measurable response in all mice were seen in the group that received 1 μg of 2,4-D conjugate. Dinitrophenyl-specific IgE was measured at all intervals examined in mice immunized with a dinitrophenyl-keyhole limpet hemocyanin conjugate. 2,4-D applied epicutaneously on 2 d or over 4 wk failed to elicit delayed-type hypersensitivity as measured by change in ear thickness, incorporation of 5-[^{125}I]iodo-2'-deoxyuridine, or histology following challenge on the ear. No 2,4-D-specific IgE antibodies were detected in serum during the 4-wk sensitization period. Similar treatment with a known sensitizer, dinitrofluorobenzene, produced delayed hypersensitivity. Following 4 wk of dosing, low titers of dinitrophenyl-specific IgE antibodies were elicited.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) in its ester and sodium, amine, and lithium salt formulations is the most commonly used phenoxy herbicide, with, for example, 18.3 million kg used in the United States in 1974 by agriculture, government, and industry (Keil et al., 1977). Human health hazards associated with the use of 2,4-D have been relatively insignificant (Bovey and Young, 1980). However, 2,4-D has been implicated in occasional reports of hypersensitivity reactions in humans.

Balo-Bango et al. (1973) described a case involving severe bullous dermatitis in a man following exposure to a cornfield sprayed with 2,4-D. In another report, a female orchard worker in contact with the herbicide

This research was supported in part by Cooperative Agreement CR-807295 from the Environmental Protection Agency, a National Institute of Environmental and Health Sciences training grant in toxicology (ES-07097), and the Utah Agricultural Experiment Station, Project 603.

We thank Rita M. Nelson for her valuable technical assistance.

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Journal of Toxicology and Environmental Health, 10:729–741, 1982
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0098-4108/82/090729-13$2.25

17465

5-[^{125}I]Iodo-2'-deoxyuridine ([^{125}I]-UdR), activity 5.3 Ci/mg, was purchased from Amersham, Arlington Heights, Ill.

Keyhole limpet hemocyanin (KLH) was obtained from Pacific Bio-Marine Supply Co., Venice, Calif., and purified according to Garvey et al. (1977). The final purified KLH was dissolved in phosphate buffered saline (0.15 *M* phosphate, 0.15 *M* NaCl, pH 7.2) and sterilized by filtration. The protein concentration was calculated from Kjeldahl nitrogen determination performed in duplicate based on a nitrogen content of 15.4% (Fairless et al., 1967).

Aluminum hydroxide adjuvant was prepared according to the method of Levine and Vaz (1970).

Animals

BALB/c female mice, 9 wk old, and Sprague-Dawley rats, 400–680 g, were obtained from Simonsen Laboratories, Inc., Gilroy, Calif.

Preparation of Conjugates

2,4-D-KLH EEDQ was used to couple 2,4-D and KLH using a method modified from that of Fatori and Hunter (1980). The reaction was performed in an ice bath and the pH was monitored continually. 2,4-D (0.48 g) was dissolved in 2 ml of acetone and water (2:1) at pH 4.0. EEDQ (0.108 g) in 0.7 ml acetone was added to the 2,4-D and allowed to react for 10 min. The pH was maintained at 4.0 with 2 *N* HCl. The 2,4-D and EEDQ mixture was added drop-by-drop to a stirred solution of KLH (0.168 g in 1.5 ml of phosphate buffered saline (0.15 *M* phosphate, 0.15 *M* NaCl, pH 7.2) over 3 min. The pH was raised to 8.0 with 0.5 *N* NaOH and maintained for 2 h. The preparation was extensively dialyzed against phosphate buffered saline (PBS, 0.01 *M* phosphate, 0.15 *M* NaCl, pH 7.2), sterilized by filtration, and analyzed.

To determine conjugated 2,4-D, the protein complex was acid hydrolyzed, extracted with benzene, and the extract taken to dryness and redissolved in methanol. The sample was analyzed for 2,4-D using a Varian Model 5000 high-performance liquid chromatograph using 35% acetonitrile and 65% water (pH 3.0) at a flow rate of 2.0 ml per min through a reversed-phase C-18 column. Absorbance at 285 nm was recorded. All analyses were performed in duplicate. Protein concentration was estimated by Kjeldahl nitrogen determinations.

2,4-D-BSA The conjugation of 2,4-D and BSA was performed using the coupling agent EDC at room temperature. 2,4-D (0.221 g) was dissolved in 2 ml dioxane, and the 2,4-D solution was rapidly added to a solution of EDC (0.192 g dissolved in 2 ml water) with the pH maintained at 4.75 with 0.5 *M* NaOH. This mixture was slowly added to a solution of BSA (0.168 g in 10 ml water) at pH 6.0 with rapid stirring. The time from the addition of the water to the EDC to the completion of the addition to the protein solution was found to be critical and was kept at

17466

of approximately 2 cm^2. 2,4-D was administered at 11.8 and 118.4 mg/ml and DNFB at 5 mg/ml in acetone and ethyl alcohol (4:1). The doses of 2,4-D were equivalent to 2 and 20 times the dose of DNFB on a molar basis. Control mice were treated similarly with the solvent alone.

Six days after the second sensitization (d 8), the mice were challenged on the right ear with 20 μl of solvent and the left ear with 20 μl of 2,4-D (11.9 mg/ml) or DNFB (2 mg/ml).

Following challenge, DTH was evaluated by measurements of ear thickness, ear histology, and the radioisotopic method of Vadas et al. (1975). At 10 h after challenge, each mouse received 2 μCi of [^{125}I]-UdR in 0.1 ml of sterile saline intravenously. Ear thickness was measured 18 h later, with a Mitutoyo engineer's micrometer, in units of 10^{-2} mm. The pinnae were cut off at the hair line, placed in buffered formalin in individual tubes, and counted on a Beckman gamma counter. The ratios of the thicknesses of the left ear to the right ear and of the counts per 5 min of the left ear to the right ear were calculated. For histology, the ears were cut and stained with hematoxylin and eosin and also toluidine blue for mast cells and basophils.

Other groups of 10 mice received sensitizing applications of the test materials or solvent on the abdomen on d 1, 2, 8, 15, 22, and 29. On d 8, 15, 22, and 29, a blood sample was taken from each mouse as described previously. The sera were tested for 2,4-D- and DNP-specific IgE antibodies using the PCA test. Ten 2,4-D- or DNFB-treated mice and 6 solvent-treated mice were challenged on the ears 6 d after the final sensitization (d 35). Ear thickness was measured 24 h after challenge.

The means of the test and appropriate control groups were compared using the *t*-test for equal and unequal variances, as determined by *F* ratios (Brownless, 1960).

RESULTS

The degrees of conjugation of the 2,4-D and DNP haptens with KLH and BSA are presented in Table 1. The KLH preparations did not achieve as high a degree of conjugation as the BSA preparations, but the conjugates were considered adequate for sensitization.

2,4-D-KLH injected intraperitoneally elicited IgE antibodies specific for 2,4-D following the second immunization, as demonstrated by positive PCA reactions using 2,4-D-BSA as the challenge antigen (Fig. 1). The specificity of the response for 2,4-D was ensured by the use of the BSA conjugate rather than the KLH conjugate for challenge. The highest titers were produced 7 d after secondary immunization in the mice that were sensitized with 1 μg of 2,4-D-KLH. All the mice in that group produced detectable 2,4-D-specific IgE. Fewer mice responded to the 10 μg and 100 μg doses of 2,4-D-KLH. The geometric mean of the titers for the group receiving 10 μg was lower than for the 1 μg group. The single responding

17467

study investigated the sensitizing potential of 2,4-D in the BALB/c mouse when applied epicutaneously and when coupled to a protein to simulate an allergic conjugate that might form upon coupling to body proteins in the exposed human. IgE antibodies specific for 2,4-D were elicited by sensitization with 2,4-D-KLH. Epicutaneous applications of 2,4-D did not produce either DTH or detectable circulating specific IgE.

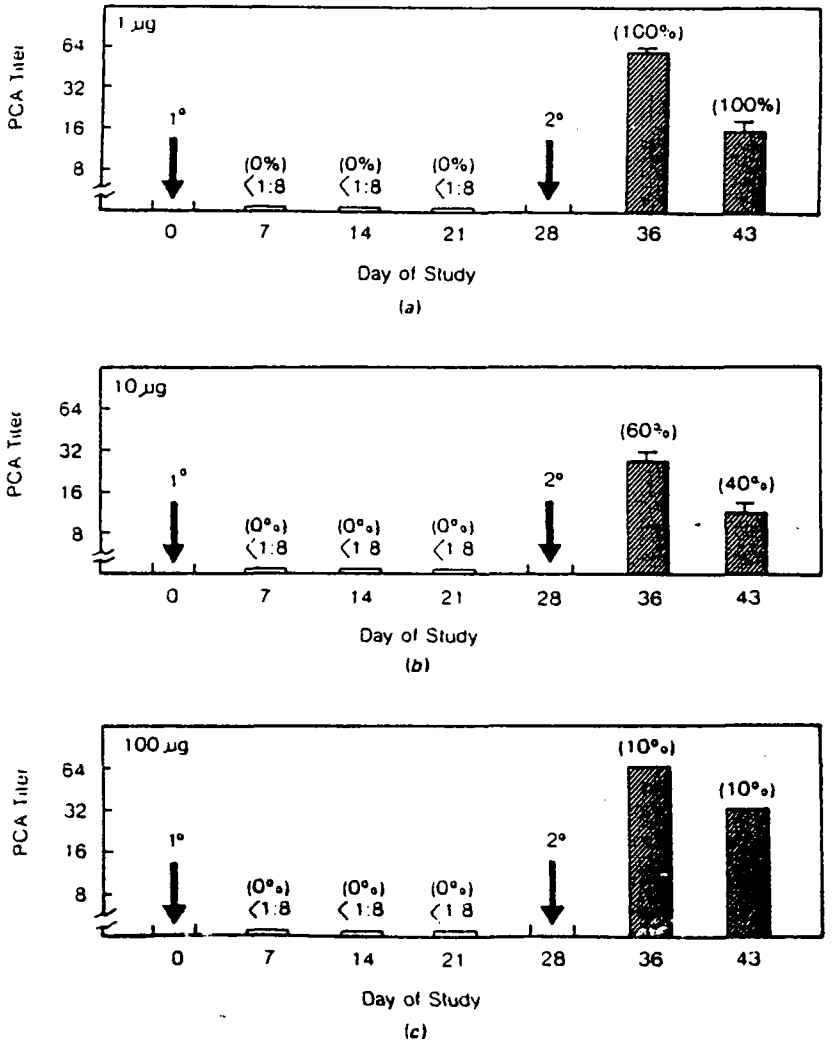


FIGURE 1. IgE antibody response specific for 2,4-D in female BALB/mice. Doses of 2,4-D-KLH administered intraperitoneally at primary (1°) and secondary (2°) immunizations were (*a*) 1 μg, (*b*) 10 μg, and (*c*) 100 μg per mouse. The bars represent the geometric mean ± the standard error of the PCA titer for mice producing detectable circulating IgE antibodies. The percentage of mice that produced detectable antibodies is shown at the top of each bar.

17468

The lowest dose of conjugate, 1 μg, elicited the highest IgE titers with the highest proportion of mice responding as compared to the 10- and 100-μg doses. This pattern of response was reported first by Levine and Vaz (1970), who noted that high doses of benzylpenicilloyl and DNP conjugates elicited little or no IgE antibody production while eliciting good levels of IgG antibody. Tamura and Ishizaka (1978) showed that immunization with relatively large doses of ovalbumin resulted in the generation of antigen-specific suppressor T-lymphocytes that were not demonstrated in mice that were immunized with small doses. These researchers concluded that the IgE response in relation to dose depended on the elicitation of suppressor T-lymphocytes and not solely on the activity of helper T-lymphocytes or B memory cells. Other studies in mice and rats indicated that the size of the primary dose of antigen is important in determining the primary response as well as the secondary response (Hamaoka et al., 1974; Jarrett, 1978; Lee and Sehon, 1978). In this study, the 10- and 100-μg doses seemed unable to elicit a primary IgE response but did generate a dose-related decrease of the secondary response both in terms of a decrease in the percentage of mice with

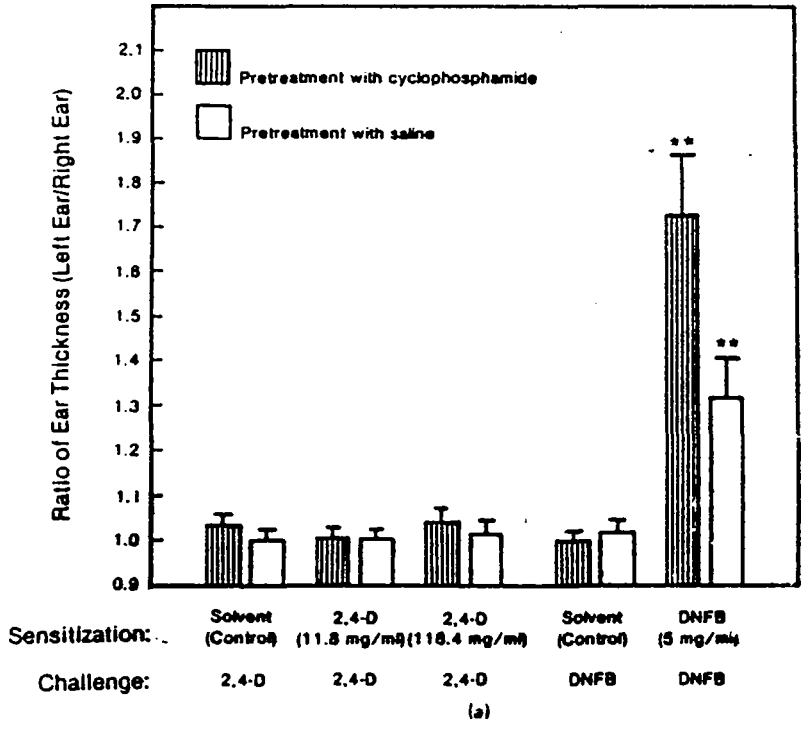


FIGURE 3. Delayed-type hypersensitivity following epicutaneous sensitization on two consecutive days. (*a*) Ear thickness.

17469

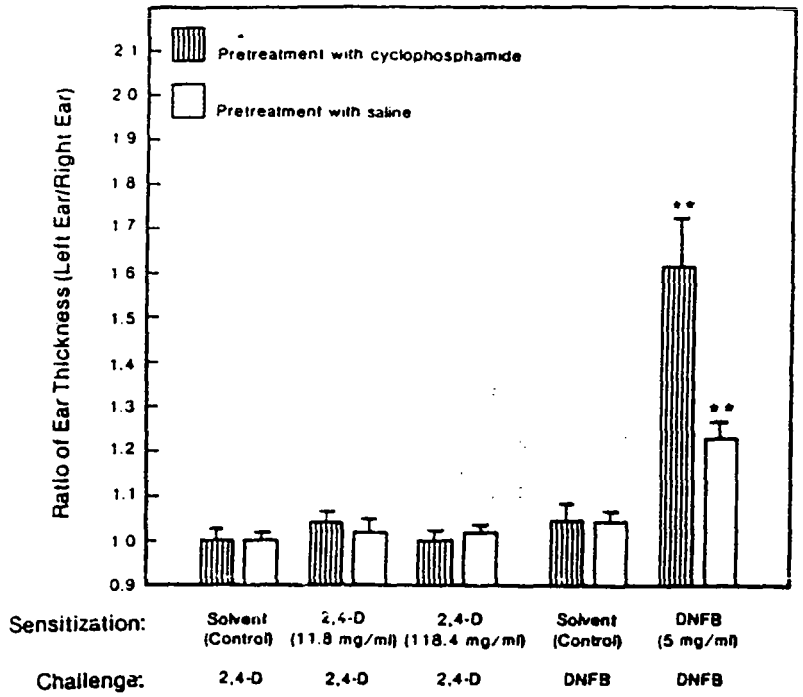


FIGURE 4. Delayed-type hypersensitivity following multiple epicutaneous sensitizations over 4 wk. The left ear was challenged with test compound, and the right ear with solvent. The bars represent the mean from 10 2,4-D- or DNFB-treated or 6 solvent-treated female BALB/c mice + standard error. The asterisks indicate that the mean is significantly greater than the comparable control mean ($p < 0.01$) according to the *t*-test for unequal variances.

to dinitrochlorobenzene reported by Chase (1947). Cyclophosphamide pretreatment appeared to increase the number of mice producing detectable IgE toward DNP but not the titers.

Based on these experiments, it appears that 2,4-D carboxylate-coupled to protein is capable of eliciting a specific IgE response, albeit a relatively low response compared to that of a strong sensitizer like a DNP conjugate. Although 2,4-D acid administered epicutaneously did not cause delayed-type hypersensitivity, other formulations of 2,4-D such as esters, amines, and other salts need to be tested to determine their sensitizing potential.

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Received February 10, 1982
Accepted April 3, 1982

DUM21566420

J. Agric. Food Chem. 1982, 30, 375–381

(2,4-Dichlorophenoxy)acetic Acid Exposure Received by Aerial Application Crews during Forest Spray Operations

Terry L. Lavy,* John D. Walstad, Rinda R. Flynn, and John D. Mattice

505

In three helicopter spray operations, forest workers were monitored for exposure to and internal dose of (2,4-dichlorophenoxy)acetic acid (2,4-D). Levels of 2,4-D were measured in air near the breathing zone, on denim patches to estimate dermal exposure, and in the urine excreted for 2 days before and 5 days after the spraying to determine internal dose. Each crew made two applications about 1 week apart to compare exposure from crew members wearing customary clothing and following normal precautions (T-1) with that of the same crew members wearing protective apparel and following special hygienic practices (T-2). External exposure was low with the highest level at 0.0911 mg/kg of body weight for a batchman in T-1. The total internal dose determined by urine analyses ranged from nondetectable to 0.0557 (in T-1) or 0.0237 (in T-2) mg/kg of body weight. Those crewmen working most closely with the spray concentrate or handling spray equipment (pilots, mechanics, and batchman–loaders) showed the highest doses. Protective clothing and good hygienic practices limited exposure. On the basis of analyses of toxic levels of 2,4-D in laboratory animals, human exposure levels in these tests were well below that which might endanger health.

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Considerable research has shown the effectiveness, economic importance, toxicological effects, and environmental behavior of herbicides. However, only a limited number of studies have measured amounts and disposition of herbicide actually absorbed by applicators (Sauerhoff et al., 1977; Kolmodin-Hedman et al., 1979). Such knowledge is important for the safety of those whose use herbicides and for the understanding of those who regulate their use [e.g., U.S. Environmental Protection Agency (1978)]. This kind of information was recently acquired for the herbicide (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) (Lavy, 1978; Lavy et al., 1980a,b; Ramsey et al., 1978). The purpose of this study was to provide similar information for (2,4-dichlorophenoxy)acetic acid (2,4-D).

The rapid degradation of 2,4-D makes it one of the least persistent herbicides in the environment (Norris, 1966, 1970; Lavy et al., 1973; WSSA Herbicide Handbook Committee, 1979). Furthermore, the long history of 2,4-D use with no reports of ill effects to applicators suggested that harmful levels were probably not being absorbed. Nevertheless, scientific data were needed to document the extent of exposure occurring to workers under actual field conditions. The results of this study, when used in conjunction with toxicological data, provide a meaningful evaluation of applicator safety during use of 2,4-D under various conditions.

Techniques have been developed in recent years to measure external exposure to pesticides (Durham and Wolfe, 1962) and internal phenoxy herbicide dose as measured in the urine excreted (Sauerhoff et al., 1977; Lavy et al., 1980a). The 2,4,5-T present in the urine has been shown to be a reliable indicator of the dose received during application of 2,4,5-T spray (Lavy, 1978; Gehring et al., 1973). Since 2,4-D is quite similar structurally, and since it is metabolized in a similar fashion (Sauerhoff et al., 1977), the same technique has been used to ascertain the dose of 2,4-D absorbed by workers during application (Kolmodin-Hedman et al., 1979).

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The objectives of this study were (1) to measure both external exposure and internal dose received by helicopter crews spraying 2,4-D in the forest, (2) to determine whether protective clothing significantly altered these measurements, and (3) to evaluate the relationship between exposure or dose and the worker's duties.

MATERIALS AND METHODS

Measurements Taken. Tests were designed to determine the quantity of 2,4-D in the ambient air of the worker's breathing zone, the quantity of 2,4-D that came into contact with denim patches attached to the worker's clothing, and the quantity and rate of 2,4-D that was subsequently excreted in the urine of each worker.

Air Samples. Battery-powered air sampling monitors were attached to all workers involved in the spraying program to provide an estimate of respiratory exposure to 2,4-D ester. The monitors contained amberlite XAD-2 resin, which is effective in trapping airborne vapors and spray droplets of 2,4-D esters (Johnson et al., 1977). After the air monitors were exposed during the treatment period, the tubes containing the resin were removed and placed in small, airtight containers until analysis.

For these analyses, the total volume of air inhaled was estimated by multiplying the rate (1740 L/h) at which a person doing this kind of work breathes (Durham and Wolfe, 1962) by the total time of the exposure period (148 min). The total volume of air inhaled multiplied by the concentration of 2,4-D (micrograms per liter) from the resin of the air monitors gives the total amount of 2,4-D inhaled. This amount is expressed on a basis of micrograms per kilogram of body weight.

Patches. Before the spray operation, denim patches were attached to crew members' clothing near bare skin areas. For estimation of exposure to the wrist area, a 2.5 × 15 cm strip was stapled to each shirt cuff. So that exposure to the bare skin of the head and neck could be estimated, one 2.5 × 40 cm strip was stapled to the back of the collar of the shirt, extending to the points of the collar on each side, and another 2.5 × 40 cm strip was similarly attached to the hat band, extending from the front and around both sides toward the back. The patches were attached to and removed from workers by persons not exposed to the spray material and were then shipped to the analytical laboratory in individual glass containers

Reprinted from JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 1982, 30, 375.
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17472

Table I. Summary of Batching, Loading, and Spraying Information

	T-1			T-2		
	crew 1	crew 2	crew 3	crew 1	crew 2	crew 3
batching operation						
time required (approx.), min	15	5	60[a]	6	5	18
volume mixed, gal	1000	1000	1000	1000	1000	1000
loading operation						
number of loads	13	14	17	13	14	17
volume/load (approx.), gal	80	70	60	80	70	60
time required/load (approx.), min	0.5	1	1	0.5	1	1
total time loading, min	9	14	18	7	14	24
spraying operation						
time required/load (approx.), min	3	2	3.5	2.5	2	2.5
total time flying,[b] min	43	29	60	34	27	45

[a] The transfer pump failed to operate, so the batching operation was conducted manually by using pails. [b] The actual "trigger time" for spraying was about half of the flying times.

immediately after the spray operation.

In addition to height and weight measurements, a photograph was taken of each worker in spray attire to determine the area of bare skin exposed, based on values provided by Durham and Wolfe (1962). The area of bare skin, in conjunction with the amount of 2,4-D deposited on the denim patches (known area), was used to estimate dermal exposure.

Urine Collection. The pharmacokinetic behavior of 2,4-D and 2,4,5-T in humans has been established for a 6-day period (Sauerhoff et al., 1977; Gehring et al., 1973). The total collection period in this study covered 2 days before the spraying occurred, the spray day, and at least 5 days after each spray application. Polyethylene-lined containers (2.5-L capacity) were used by each crew member to collect all urine excreted over consecutive 12-h intervals throughout the test. The urine samples collected prior to spraying were used to detect background levels, if any, of 2,4-D present in the urine and to check for interferences that might confound analysis. Workers were instructed to avoid contaminating the urine. The urine was kept in a cool location before being transported at 3–4-day intervals to the laboratory for analysis.

Crew Description. Three different helicopter crews (designated crews 1, 2, and 3) participated in the study. Each crew was comprised of a helicopter pilot, mechanic, batchman–loader, supervisor, and two observers. Observers were included in the study to provide an estimate of the 2,4-D dose received by individuals who were not directly involved in the spray application but were 25–175 yards from the helicopter loading zone at the time the spraying was done.

Test Description. Each crew performed two applications, which were approximately 1 week apart. In the first treatment (T-1), crew members made use of conventional spray techniques and performed their normal duties using ordinary precautions (Lavy, 1980). They followed label instructions and other legal regulations but received no additional guidance from research personnel.

A second treatment (T-2), in which additional precautions were used, was conducted following the conclusion of the T-1 phase of the study. During T-2 the workers wore special protective clothing consisting of disposable Tyvek coveralls. (Tyvek is the trade name for Du Pont's spun-bonded olefin fabric, consisting of a lightweight paper covered with a durable plastic film.) In addition, pilots wore normal flying gloves and headgear, mechanics, batchman–loaders, and supervisors wore chemically impervious gloves and boots, clean hats, and goggles, and observers wore cotton or rubber gloves, clean hats, and goggles. During T-2 it was permissible for research personnel to suggest to crew members how to minimize exposure. For example, crew members were instructed to wash their hands before rest stops and meals and also to take showers and change into clean clothing soon after the spray operation.

Both treatments were designed with the intent that the only exposure to phenoxy herbicides would occur on the actual spray day of T-1 and T-2. All selected crew mem-

Table II. Recovery Data for Air, Patch, and Urine Analyses

analyses of fortified samples	no. of replicates	2,4-D acid equiv added[a]	av % recovery ± SD
air[b]		μg	
laboratory	4	0.068	87 ± 19
	4	0.36	81 ± 0.8
	4	0.72	78 ± 3
	4	3.5	80 ± 6
field	4	18.0	115 ± 13
	3	83.0	71 ± 2
patches[c]		mg	
laboratory	4	0.0037	156 ± 17
	4	0.0074	122 ± 4
	4	0.015	95 ± 12
	4	0.030	101 ± 9
field	8	0.038	118 ± 9
	8	0.075	104 ± 13
	8	0.375	105 ± 9
	8	0.750	112 ± 13
urine[d]		ppm	
laboratory	16	0.0386	98 ± 54
	20	0.565	74 ± 7
	12	2.300	73 ± 7
field	6	0.0328	98 ± 48
	12	0.0645	86 ± 26
	12	0.246	70 ± 11
	12	0.842	73 ± 10

[a] The solution used to fortify samples was a dilution of Esteron 99 Concentrate in hexane. [b] The limit of detection ($S/N = 2$) for air monitors was established as 0.05 μg. Only one air monitor collected from the workers indicated the presence of 2,4-D, and it was within the range of 0.068–0.36 μg shown above. The average recovery value, which includes trapping efficiency, for fortified standards in this range was 84% [(87 + 81)/2]. Thus, an 84% recovery figure was used to correct the value from the air monitor worn by worker 33 as shown in Table III. [c] The limit of detection ($S/N = 2$) was established as 0.0037 mg. Most of the 2,4-D values for patch samples worn by workers fell within the range shown above where 100% recovery (within experimental error) was obtained. Thus, there was no need to correct the values for patch samples collected from workers for percent recovery. [d] The limit of detection ($S/N = 2$) was established as 0.04 ppm. Urine samples shown in Table V collected from workers during the study were corrected for percent recovery by using a sliding scale as constructed from the above recovery data.

17473

Table III. Estimation of Respiratory Exposure to 2,4-D As Determined from Analyses of Air Monitors Worn by Workers[a]

worker		T-1 (ordinary precautions)				T-2 (special precautions)			
no.	wt, kg	2,4-D on resin,[b] μg	air vol sampled, L	concn, μg/L	estimated respiratory exposure/ body wt, μg/kg	2,4-D on resin,[b] μg	air vol sampled, L	concn, μg/L	estimated respiratory exposure/ body wt, μg/kg
11	89.0	nd	16.6	nd	nd	nd	7.59	nd	nd
12	74.9	nd	19.9	nd	nd	nd	11.00	nd	nd
13	79.5	nd	24.0	nd	nd	nd	23.34	nd	nd
14	97.6	nd	17.9	nd	nd	nd	20.52	nd	nd
15	72.6	nd	15.4	nd	nd	nd	15.19	nd	nd
16	72.6	nd	14.1	nd	nd	nd	10.70	nd	nd
21	68.6	nd	18.2	nd	nd	nd	14.41	nd	nd
22	95.3	c	c	c	c	nd	18.50	nd	nd
23	90.8	nd	20.0	nd	nd	nd	19.45	nd	nd
24	102.2	nd	18.6	nd	nd	nd	20.75	nd	nd
25	84.0	nd	24.1	nd	nd	nd	9.76	nd	nd
26	72.6	nd	17.2	nd	nd	nd	12.17	nd	nd
31	88.5	nd	30.8	nd	nd	nd	8.27	nd	nd
32	89.9	nd	23.8	nd	nd	nd	17.03	nd	nd
33	79.5	0.13	23.1	0.0056	0.30[d]	c	c	c	c
34	90.8	c	c	c	c	nd	18.94	nd	nd
35	79.5	nd	32.1	nd	nd	nd	14.60	nd	nd
36	74.9	nd	21.9	nd	nd	nd	15.31	nd	nd

[c] The limit of detection ($S/N = 2$) was 0.05 μg. Values which were below the limit of detection are represented by nd (not detected). [b] All values have been corrected for percent recovery (84%) according to data presented in Table II. [c] Pump failure precluded meaningful analyses of these samples. [d] Respiratory exposure per body weight for worker 33 was determined as follows: (0.13 μg of 2,4-D/23.1 L of air × 1740 L of air/ha × 148 min × 1 h/60 min)/79.5 kg of body weight = 0.30 μg of 2,4-D/kg of body weight.

bers claimed to have had no known exposure to 2,4-D for at least 2 weeks prior to the study. As a precautionary measure, however, urine samples were collected for the 2 days preceding T-1. Seven days elapsed between the spray days in T-1 and T-2 for crew 1, and 6 days elapsed between the 2 spray days for crews 2 and 3.

Treatment Description. Personnel engaged in supervising and applying the herbicide treatment were trained, licensed, and certified by the appropriate state and federal agencies. The spray applications complied with state and federal regulations and were representative of routine aerial treatments in forestry.

The application areas, typical of the Pacific Northwest, were located near Raymond, WA, Cottage Grove, OR, and Gardiner, OR, for crews 1, 2, and 3, respectively. At each geographic location, two sites were selected within 10 miles of each other for the respective T-1 and T-2 applications. Each site consisted of an approximate 100-acre tract within a larger forested area. These tracts contained Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] in need of release from competing vegetation.

Treatments were applied by helicopter at each location in late February and early March of 1980. The formulation used was Esteron 99 Concentrate, which contains 4 lb of acid equivalent (a.e.) of 2,4-D/gal as the propylene glycol butyl ether ester. The herbicide was mixed with diesel oil and water at the rate of 0.5:0.5:9 gal, and the resulting mixture was applied at the rate of 10 gal/acre, which provided a 2,4-D application rate of approximately 2 lb of a.e./acre. Therefore, each 100-acre tract received about 1000 gal of this mixture.

Treatments were applied in the early morning hours. Temperatures ranged from 4 to 13 °C, and relative humidities were usually in excess of 80%. Winds were generally calm, with occasional gusts up to 5 mph. Weather conditions were typical for the season and were well within the regulatory restrictions for spraying.

Treatment Logistics. Table I displays the information collected during the treatment phase of the study. With one exception, batching of the 1000 gal of spray mixture took from 5 to 18 min, depending on the pumping system employed. The exception occurred during T-1 when crew 3 required nearly 1 h to transfer the concentrate manually from the drum to the batch truck via 2-gal pails because the pumping system failed to operate.

Loading the spray mixture from the batch truck into the helicopter tanks took 30–60 s/load. The volume per load varied from 60 to 80 gal, depending on payload capacity of the helicopter. The spraying operation took 2–4 min/load, depending on ferry distance. Actual "trigger time" was 1–2 min/load. Total time required for loading and spraying the 1000 gal ranged from 41 to 78 min/treatment. These spray periods were comparable to those in routine forestry operations (American Paper Institute/National Forest Products Association, 1978).

Chemical Recovery Data. Laboratory procedures used to analyze air monitors, patches, and urine were similar to those used by Johnson et al. (1977) or Lavy et al. (1980a). To ensure that no 2,4-D was broken down prior to analysis and to establish the percent recovery for the analytical techniques, we prepared a series of samples fortified with known 2,4-D amounts for each sample type (air; patches; urine) in the field shortly after collection. These were compared to samples fortified similarly at the laboratory just prior to analysis. Limits of detection were established by a signal-to-noise ratio of two ($S/N = 2$).

Recovery data are shown in Table II. Reported values for experimental samples shown in Tables III–V have been corrected for percent recovery as discussed in Table II. In all cases, the 2,4-D values are reported as the acid form. Phenoxy esters are converted in the body to the acid form, which is then excreted in urine (Gehring et al., 1975).

The Amberlite XAD-2 resin from the air monitors and the denim patches used to intercept 2,4-D spray mist were analyzed by gas chromatographic techniques (Lavy et al., 1980a,b; Johnson et al., 1977). The limits of detection were established as 0.05 μg for the air monitors and 0.0037 mg for the patches.

17474

Urine samples used to measure the dose of 2,4-D received by crew members were analyzed by a modification of a gas chromatographic technique used by Lavy et al. (1980a). When the methyl ester of 2,4-D was prepared, it was found to elute at a retention time similar to those of interfering peaks from the urine. The method was modified by preparing the butyl ester of 2,4-D with BF_3–butanol. The limit of detection was established as 0.04 mg/kg in the urine. Two different columns were used routinely in the ^{63}Ni gas chromatographs to confirm results obtained from air, patch, and urine sample analyses.

RESULTS AND DISCUSSION

Air Samples. Thirty-two of the 33 air monitors which remained functional during the test did not contain any 2,4-D at the limit of detection of 0.05 μg (Table III). The only air monitor containing a detectable amount of 2,4-D was worn during T-1 by the batchman–loader (worker 33), one of the crew members who manually transferred the 2,4-D concentrate from the drum to the batch truck due to a malfunction of the normal pumping system. Subsequent conversations with this worker revealed that odors associated with the concentrate were discernible during the transfer process. Therefore, it is possible that his activity may have accounted for the 2,4-D detected by that particular monitor. The amount detected during the test was equivalent to 0.30 μg of 2,4-D/kg of body weight.

Patch Samples. The amount of 2,4-D deposited on the denim patches attached to each crew member's clothing is shown in Table IV. Levels detected were generally very low, and in many instances no detectable exposure occurred. The exposure that did occur appeared directly related to workers' duties. Batchman–loaders and mechanics usually showed some degree of dermal exposure; observers showed almost none. Observers in crews 2 and 3 were stationed outside the spray areas during the 2,4-D application. However, worker 15, an observer in crew 1, was inadvertently in the spray area during T-2. Table IV shows that his patches received slight amounts of 2,4-D.

Since all patches were placed on the outside of the clothing in both T-1 and T-2, no major differences in patch exposure between the two tests would have been expected. An exception occurred in T-1 for crew 3 (particularly the mechanic, worker 32, and the batchman–loader, worker 33), who manually transferred the concentrate from the drum to the batch truck when the transfer pump did not work. This operation increased the opportunities for exposure to the concentrate, and visual observations of these workers' clothing revealed that certain portions did indeed become contaminated during the transfer process. The transfer pump worked properly for this crew during T-2. This fact probably accounts for the reduced amounts of 2,4-D detected on the patches of the mechanic and batchman–loader in T-2 (Table IV).

Five of the six patches on the headbands of batchman–loaders contained higher amounts of 2,4-D than their other patches (Table IV). These workers attached the quick-couple batch truck hose to the helicopter during reloading. The wind generated by the whirling helicopter blades often prompted the batchman–loaders to hold their hats in place. Although these individuals often wore gloves (always in T-2), the gloves were sometimes contaminated with 2,4-D which could have been easily transferred to the patch on the headbands as they held onto their hats.

Urine Samples. Whether intake of 2,4-D was through dermal, respiratory, or other exposure, the actual dose can be determined by analyzing for 2,4-D in the urine excreted. The total quantity of 2,4-D excreted during T-1 and T-2 (spray day plus 5 days thereafter) was determined by

Table IV. Estimation of Dermal Exposure to 2,4-D As Determined from Analysis of Patches[a]

worker no.	worker wt, kg	T-1 dermal exposure area,[b] cm^2	T-2 dermal exposure area,[b] cm^2	T-1 (ordinary precautions) amount of 2,4-D on patches, mg: right wrist (37.5 cm^2)	left wrist (37.5 cm^2)	neck (100 cm^2)	head (120 cm^2)	estimated dermal exposure/body wt,[c] mg/kg	T-2 (special precautions) amount of 2,4-D on patches, mg: right wrist (37.5 cm^2)	left wrist (37.5 cm^2)	neck (100 cm^2)	head (120 cm^2)	estimated dermal exposure/body wt, mg/kg
11	90	650	650	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	76	650	430	0.007	0.007	0.019	0.046	0.0023	0.024	nd	nd	nd	0.0005
13	80	580	580	0.009	nd	0.029	0.056	0.0023	0.008	nd	0.010	0.047	0.00016
14	98	1620	540	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	73	1580	540	nd	nd	nd	nd	nd	0.018	0.012	0.035	0.114	0.0045
16	73	760	640	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
21	69	760	760	nd	0.019	0.005	0.004	0.0010	nd	nd	nd	nd	nd
22	96	1470	430	0.048	0.011	nd	0.053	0.0059	0.047	0.596	0.032	0.383	0.0162
23	91	800	540	0.013	0.047	0.015	2.974	0.0911	0.177	0.263	0.008	0.686	0.0216

DOW2156365

17475

24	102	800	540	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
25	84	1580	540	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
26	73	760	540	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
31	89	800	800	0.012	nd	nd	0.011	0.0007	0.004	nd	nd	nd	0.0001
32	90	1580	540	0.962	0.055	0.008	0.010	0.0617	0.014	0.007	nd	0.008	0.0006
33	80	800	540	0.356	0.375	0.010	0.458	0.0409	0.048	0.348	0.005	0.042	0.0102
34	91	1580	540	nd	0.008	nd	nd	0.0005	nd	0.134	nd	nd	0.0027
35	80	1620	540	nd	nd	nd	nd	nd	0.170	nd	nd	nd	0.0039
36	75	1580	540	nd	nd	nd	0.007	0.0005	nd	nd	nd	nd	nd

[a] The limit of detection ($S/N = 2$) was 0.0037 mg. Where a value of nd (not detected) was obtained, a zero was used for averaging purposes. [b] The dermal exposure area was calculated after viewing photographs of each worker, reviewing treatment notes, and using values obtained by Durham and Wolfe (1962). [c] The estimated dermal exposure per body weight (milligrams per kilogram) represents the amount of 2,4-D that may be deposited on a worker's skin in relation to his size and apparel, as calculated from [2,4-D on four patches (mg) × dermal exposure area (cm^2)]/[total patch area (295 cm^2) × body weight (kg)].

Table V. Daily Quantities of 2,4-D Excreted in the Urine and Estimation of Total Dose Received by Each Worker[a]

worker			2,4-D excreted daily (T-1), mg								T-1[b]		2,4-D excreted daily (T-2), mg								T-2[d]	
no.	duty	wt, kg	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	total 2,4-D, mg	2,4-D/ body wt, mg/kg	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	total 2,4-D, mg	2,4-D/ body wt, mg/kg
11	pilot	89.0	nd[c]	nd	nd	nd	0.113	nd	0.048	nd	0.161	0.00179	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	mechanic	74.9	nd	nd	0.025	nd	nd	0.008	nd	nd	0.033	0.00044	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	batchman	79.5	nd	0.022	0.019	0.120	0.032	nd	nd	nd	0.171	0.00215	nd	nd	0.016	nd	nd	0.026	nd	nd	0.042	0.00053
14	supervisor	97.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.037	nd	nd	0.029	0.037	0.00038
15	observer	72.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.041	nd	nd	nd	nd	nd	nd	0.041	0.00056
16	observer	72.6	nd	nd	0.040	nd	nd	nd	nd	nd	0.040	0.00055	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	mean (crew 1)										0.068	0.00082									0.20	0.00024
21	pilot	68.6	0.372	0.445	0.519	0.536	1.156	0.760	0.402	0.446	3.819	0.0557	0.193	0.525	0.389	0.119	0.060	0.339			1.625	0.0237
22	mechanic	95.3	nd	0.189	0.057	0.090	0.074	nd	nd	nd	0.221	0.00232	0.034	0.191	0.109	0.043	0.080	0.035			0.492	0.00516
23	batchman	90.8	0.243	0.253	0.347	0.165	0.554	0.186	0.160	0.306	1.718	0.0189	0.284	0.527	0.354	0.330	0.121	0.163			1.779	0.0196
24	supervisor	102.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			nd	nd
25	observer	84.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			nd	nd
26	observer	72.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			nd	nd
	mean (crew 2)										0.960	0.0128									0.649	0.0081
31	pilot	88.5	nd	nd	0.046	nd	0.063	nd	nd	0.073	0.182	0.00206	0.103	0.042	0.025	nd	nd				0.170	0.00192
32	mechanic	89.9	nd	0.073	0.228	0.434	0.180	0.133	0.148	0.098	1.221	0.0136	0.026	nd	0.083	nd	0.070	0.170			0.349	0.00388
33	batchman	79.5	0.168	0.238	0.542	0.773	0.657	0.337	0.340	0.345	2.994	0.0377	0.261	0.283	0.240	0.306	0.314	0.337			1.741	0.0219
34	supervisor	90.8	nd	nd	0.140	0.162	0.165	0.093	nd	0.078	0.628	0.00692	nd	nd	nd	nd	nd	nd			nd	nd
35	observer	79.5	nd	nd	nd	0.088	nd	nd	nd	nd	0.088	0.0011	nd	nd	nd	nd	nd	nd			nd	nd
36	observer	74.9	nd	nd	0.030	nd	nd	0.064	nd	nd	0.094	0.0013	nd	nd	nd	nd	nd	nd			nd	nd
	mean (crew 3)										0.868	0.0104									0.377	0.0046

[a] Crew 1 = workers 11-16; crew 2 = workers 21-26; crew 3 = workers 31-36. [b] Treatment 1 (T-1) = ordinary precautions. Totals include values from spray day (day 3) plus the next 5 days. [c] nd = not detected; a zero was used for averaging purposes. [d] In treatment 2 (T-2) special precautions were observed. Totals include values from spray day (day 10 for crew 1; day 9 for crews 2 and 3) plus the next 5 days.

DOW215636

Table VI. Comparisons of Respiratory Exposure, Dermal Exposure, and Total Dose of 2,4-D Received during Treatment 1 (T-1) and Treatment 2 (T-2) for Similar-Duty Workers

	estimated respiratory exposure, μg/kg		estimated dermal exposure, mg/kg		dose, mg/kg[a]	
workers	T-1	T-2	T-1	T-2	T-1	T-2
pilots						
11	nd	nd	nd	nd	0.00179	nd
21	nd	nd	0.0010	nd	0.0557	0.0237
31	nd	nd	0.0007	0.0001	0.00206	0.00192
mean[b]	nd	nd	0.0005	nd	0.0198	0.00854
mechanics						
12	nd	nd	0.0023	0.0005	0.00044	nd
22	nd	nd	0.0059	0.0162	0.00232	0.00516
32	nd	nd	0.0617	0.0006	0.0136	0.00388
mean[b]	nd	nd	0.0233	0.0058	0.00545	0.00301
batchmen						
13	nd	nd	0.0023	0.006	0.00215	0.00053
23	nd	nd	0.0911	0.0216	0.0189	0.0196
33	0.30	nd	0.0409	0.0102	0.0377	0.0219
mean[b]	0.10	nd	0.0448	0.0111	0.0196	0.0140
supervisors						
14	nd	nd	nd	nd	nd	0.00038
24	nd	nd	nd	0.0004	nd	nd
34	nd	nd	0.0005	0.0027	0.00692	nd
mean[b]	nd	nd	0.0002	0.0010	0.00231	0.00013
observers						
15	nd	nd	nd	0.0045	nd	0.0056
16	nd	nd	nd	nd	0.00055	nd
25	nd	nd	nd	nd	nd	nd
26	nd	nd	nd	nd	nd	nd
35	nd	nd	nd	0.0039	0.0011	nd
36	nd	nd	0.0005	nd	0.0013	nd
mean[b]	nd	nd	0.0001	0.0014	0.00049	0.00009

[a] Values include 2,4-D excreted on the spray day plus 5 days following. [b] Where a value of nd (not detected) was obtained, a zero was used for averaging purposes.

multiplying the 2,4-D concentration detected in each sample by the volume of urine in that sample and then summing the values to give a total amount of 2,4-D for each treatment period. When divided by the body weight of the individual involved, the result was an estimate of the 2,4-D dose received over each treatment period. Pharmacokinetic analysis indicated that greater than 95% of the absorbed dose of 2,4-D was excreted in the urine by the end of each treatment period (Ramsey et al., 1980).

Less than 30% of the 524 urine samples analyzed contained levels of 2,4-D above the 0.04-ppm detection limit (Table V). Table VI reveals that most of the positive samples were from the crew members most closely involved with the actual spraying—batchman-loaders, pilots, and mechanics. Thus, there was a relationship between worker duties and levels of 2,4-D detected in the urine. Except for one pilot (worker 21), who had assisted in cleaning spray nozzles, batchman–loaders and mechanics showed the highest levels of 2,4-D in the urine, while observers received the lowest levels. Urine samples from observers standing near the heliport rarely contained any 2,4-D and then in only negligible amounts approaching the limit of detection. The only supervisor excreting 2,4-D (worker 34) was probably exposed when he helped manually transfer the chemical during the T-1 application. Similar exposure did not occur during T-2, and no 2,4-D was detected in his urine in T-2 (Table V).

If one assumes a no-observable-effect level of 24 mg/kg of body weight, as determined from toxicology tests with laboratory animals, then safety factors for the categories of workers involved in this test are substantial (Hall, 1980). At least a thousand times the actual dose received would be necessary to correspond to the toxic levels found to show an effect in the laboratory tests.

Members of crew 1 generally received smaller doses of 2,4-D than did members of the other two crews (Table VI). On the basis of milligrams of 2,4-D per kilogram of body weight over a 6-day period, 2,4-D excretions in urine from crew 1 members ranged from not detectable to 0.00215, while members for crew 2 and crew 3 showed maximum doses of 0.0557 and 0.0377, respectively, for T-1 (Table V). Analysis of variance showed that the amounts of 2,4-D excreted in urine following T-1 were significantly greater ($p \leq 0.014$) than those following T-2. However, when crew 3 was eliminated from the comparisons (because of the different procedure used to transfer the concentrate from the drum to the batch truck), the level of difference between T-1 and T-2 decreased ($p \leq 0.15$).

The differences between T-1 and T-2 might have been greater if all the 2,4-D absorbed during T-1 had been excreted before T-2 began. Nevertheless, if 2,4-D in this study was excreted in a manner similar to the data presented by Sauerhoff et al. (1977), then over 95% of the 2,4-D dose from T-1 would have been excreted in the week prior to initiating T-2.

Five workers in crews 2 and 3, with duties as pilot, mechanics, and batchman–loaders, came into T-1 with low levels of 2,4-D in their urine (Table VI). These findings were confirmed by using two gas chromatographic columns and two different methylating agents. The presence of 2,4-D in the prespray samples was surprising in view of the facts that (1) the worker information sheets filled out by the workers indicated that none of them recalled applying phenoxy herbicides for at least 2 weeks before the study was conducted and (2) human excretion rates for 2,4-D and 2,4,5-T indicated that over 95% of the material absorbed before that time should have been excreted in the 2-week period. Conversations held with these five workers after the study, revealed that they cleaned, assembled, and tested their spray equipment before the study was conducted. Exposure to 2,4-D may have occurred during these activities. Although certain members of crew 1 also per-

DOW 2156367

formed these tasks, they apparently followed more hygienic practices, since they exhibited little, if any, prespray dose of 2,4-D.

CONCLUSION

This study found that some crew members involved in the aerial application of 2,4-D for forestry purposes absorbed low levels of 2,4-D, but the doses as indicated by urine analyses were several orders of magnitude below the no-observable-effect level determined in toxicology studies. These results are in agreement with those of Nash et al. (1981). The doses were comparable to those found in an earlier test involving aerial application of 2,4,5-T but were substantially lower than those found for ground application of that herbicide (Lavy et al., 1980a).

Nevertheless, this study established that, if desired, exposure to 2,4-D can be reduced by wearing standard protective apparel and following good hygienic practices. This is particularly true for mechanics, batchman–loaders, and pilots, since they are closely involved with the mixing and application of the herbicide. For example, wearing chemically impervious rubber gloves and using modern siphon pumps and drip-free coupling systems on transfer hoses virtually eliminated dermal exposure (the primary route of entry).

Supervisors and observers rarely exhibited any tangible exposure to 2,4-D, even when in close proximity to the spray operation. Those who handled equipment and received detectable doses of 2,4-D did not show levels which would appear to constitute health hazards (Hall, 1980).

ACKNOWLEDGMENT

We are indebted to Dr. Logan Norris, Pacific Northwest Forest and Range Experiment Station of the U.S. Department of Agriculture Forest Service at Corvallis, OR, for the patch design and choice of patch location on workers. Also, we appreciate the assistance of M. Davis in preparing this manuscript.

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Received for review March 10, 1981. Revised manuscript received October 21, 1981. Accepted October 21, 1981. This research was supported by the National Forest Products Association. Published with permission of the Director of Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville. The use of trade names does not imply endorsement of this product by the authors or organizations supporting this research.

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K-2372-(100)
1993

Arch. Environ. Contam. Toxicol. 12, 71–76 (1983)

Archives of Environmental Contamination and Toxicology

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1DOM215963356

Laundering Procedures for Removal of 2,4-Dichlorophenoxyacetic Acid Ester and Amine Herbicides from Contaminated Fabrics[1]

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Abstract. Denim fabrics were contaminated with 2,4-dichlorophenoxyacetic acid (2,4-D) ester and 2,4-D amine formulations and laundered. The variables examined were pesticide formulations, temperatures, pre-rinse conditions, detergents, ammonia additive, and number of washings. A clean fabric, designated the "transfer fabric", was laundered with the contaminated denim to assess amounts of herbicide transferred during laundering. Solubility of the formulation was a major factor in ease of removal and transfer during laundering. The amine is water-soluble and was readily removed during the laundry process. Generally, 60°C water temperature and heavy-duty liquid detergent without ammonia additive provided better pesticide removal than other tested conditions. Pre-rinse was not a contributing factor in herbicide removal, but resulted in significantly less amounts of 2,4-D transferred to concomitant laundry. A second washing cycle removed significantly greater amounts of the herbicide than a single washing.

Emphasis on reducing accidental exposure to pesticides is paramount as pesticide use has increased dramatically in the last four decades (Deichmann 1972). Concern has increased for the safe handling and usage, environmental contamination, and effects of pesticides on non-target species.

The three natural routes for a compound to enter the human body are oral, respiratory, and dermal. Wolfe *et al.* (1967) showed that the major route of pesticide exposure and absorption was through the skin and not the respiratory system. This was explained by the fact that only small particles enter the lungs, but larger, more numerous particles settle on surfaces such as skin, clothing, and soil. Concern for dermal pesticide exposure is highest among agricultural workers, farmers, aerial sprayers, and formulators because of the duration of the application season. Since the skin is the main site of pesticide exposure and route of absorption, attention has focused on clothing items which cover and protect skin surfaces. There is evidence that such clothing can be contaminated by pesticide (Finley *et al.* 1969, 1977, and 1979). Metcalfe (1972) found biologically active methyl parathion (*O,O*-dimethyl *O-p*-nitrophenyl phosphorothioate) (MeP) residue in fabrics after three washings. She also recorded MeP transfer from contaminated fabrics to clean fabrics during concomitant laundering. Recent work by Easley *et al.* (1981) indicated varied success in the removal of MeP from fabrics by laundering. MeP emulsifiable concentrate formulation was more difficult to remove from cotton and cotton/polyester denim fabrics than encapsulated or wettable powder formulations. Fiber content was not a contributing factor in the removal of MeP regardless of formulation. Of the laundering procedures investigated, pre-rinsing followed by a wash and two rinses resulted in overall increased levels of pesticide removal. Laundry additives of ammonia or bleach were relatively ineffective in assisting the detergent in removal of MeP. Additionally, Laughlin *et al.* (1981) found that washing equipment retained and provided for possible transference to subsequent laundry.

The majority of pesticide laundering studies have investigated insecticides; however, the largest por-

[1] Published as paper number 6784, Journal Series, Nebraska Agricultural Experiment Station. Research was conducted under Project Nebraska 94-010 and North Central Region Pesticide Impact Assessment Program Project No. 144

0090-4341/83/0012-0071 $01.20
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tion of pesticide active ingredient (a.i.) applied to crops and/or pastureland in the U.S. are herbicides (EPA 1980, USDA 1981). Certain herbicides, including 2,4-D, are under scrutiny by the U.S. Environmental Protection Agency (EPA) because of potential adverse health effects. Approximately 1,500 products containing 2,4-D are registered with the EPA, and over 60 million pounds a.i. were used in 1980. This herbicide is used in the control of broadleaf weeds in small grains, field crops, rangeland, and turf.

Interest concerning the impact of 2,4-D on human health surfaced following the EPA's emergency suspension of 2,4,5-T [(2,4,5-trichlorophenoxy) acetic acid] and Silvex [2-(2,4,5-trichlorophenoxy) propionic acid] on February 28, 1979. This interest was due to the chemical similarities of 2,4-D and 2,4,5-T and the concern that 2,4-D could be contaminated with dioxin [2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD)] which had been linked to fetotoxic and carcinogenic effects. EPA initiated a review to determine if 2,4-D should be investigated under the Rebuttable Presumption Against Registration (RPAR) process. EPA concluded that scientific toxicology studies on 2,4-D did not indicate that continued use of the herbicide posed significant health hazards; however, additional studies were suggested.

The purpose of the present study was to investigate laundry procedures required to remove 2,4-D from contaminated fabrics and evaluate the potential for dermal exposure following laundering.

Materials and Methods

Fabrics: Two types of fabrics were used: 1) denim fabrics represented pesticide-contaminated clothing laundered in a home laundry situation; and 2) transfer fabrics represented textile items laundered in the same load with contaminated fabrics. An 80% cotton-20% polyester blend (2/1 twill denim greige goods) was chosen for the contaminated fabric. A 50% cotton-50% polyester double knit, similar in weight to an infant's T-shirt (5.7 g/m^2), was the selected transfer fabric. The transfer fabric was laundered concomitantly with a contaminated denim fabric.

Chemicals: Either 2,4-D ester or 2,4-D amine solution was used to contaminate denim swatches. A 1.25% solution, commonly used for agricultural application, was prepared from field formulations [amine formulation: alkanol amine salts, 2,4-D acid equivalent 36.5% (1.7 kg/3.79 L); ester formulation: propylene glycol butyl ether ester, 2,4-D acid equivalent 44.9% (1.8 kg/3.79 L)].

Fabric Contamination: Denim swatches, 8 × 8 cm, were contaminated by pipetting two ml of 2,4-D formulation onto the fabric. A magnetic stirrer provided uniform agitation of the 1.25% solution during the contamination process. Swatches were suspended from a drying rack while pipetting was performed, and remained suspended to air dry.

Laundering Conditions

A contaminated denim swatch was individually laundered along with an uncontaminated transfer swatch in 150 ml detergent solution calculated proportionally to a standard 45 L washing machine volume. Laundering of each swatch was done in stainless steel canisters of an Atlas Launder-Ometer (Model B5). A water bath maintained the water temperature for a 12 min detergent wash and two rinse cycles of five and three min, respectively.

Temperature of Laundering: Fabrics were laundered in either a 60°C (hot) water wash and 49°C (warm) water rinses or a 30°C (cold) water wash and 30°C water rinses. Although previous studies for laundering contaminated clothing (Easley *et al.* 1982) had recommended 60°C or above wash temperature, current energy costs have caused lower temperatures for family laundering (Loveday 1979).

Pre-Rinse: Fabrics were subjected to either a "pre-rinse" or "no pre-rinse" treatment. The pre-rinse was the first step in a four-step process in which each contaminated swatch was pre-rinsed in 150 ml distilled water for two min prior to laundering. A 49°C water pre-rinse was selected for the 60°C water wash; a 30°C pre-rinse was used with the 30°C water wash. Contaminated fabrics which were not pre-rinsed were laundered in the standard three-step process of a wash and two rinses. Transfer fabrics were not subjected to the pre-rinse cycle; these swatches were introduced at the washing cycle.

Detergent: Since previous work had identified heavy duty liquid (HDL) detergents and high phosphate detergents as effective for methyl parathion removal (Easley *et al.* 1982), these two detergents were used. The non-phosphate HDL was Dynamo-[a], a nonionic liquid detergent manufactured by the Colgate-Palmolive Co. The 12% phosphate detergent was American Association of Textile Chemists and Colorists (AATCC) Standard Detergent 124, widely used in textiles research. Detergent solutions were proportionally prepared using 0.2 ml HDL detergent/150 ml water [0.13% solution (manufacturer's recommendation)] and 0.3 g AATCC detergent/150 ml water [0.2% solution (AATCC standard method)].

Laundry Additive: Ammonia (NH_4) additives are used in the home laundering process to assist detergents in cleaning. Commercial pesticide applicators use ammonia-water solutions to decontaminate spray equipment. Therefore, contaminated fabrics were laundered with sudsy household ammonia as a laundry additive or without ammonia in the washing solution. The ammonia (3.5%–4% ammonia concentration) was added on a basis of 0.4 ml/150 ml water, equivalent to 250 ml ammonia additive per 45 L washing machine volume.

Repeated Washing: The effect of repeated washings on pesticide removal had received limited investigation (Metcalfe 1972). Contaminated fabrics were laundered through one complete cycle or two complete laundry cycles in this investigation. Using a 2 × 2 × 2 × 2 × 2 × 2 block design (pesticide formulation,

[a] The use of tradenames does not signify product endorsement

17480

temperature, pre-rinse, detergent, ammonia additive, repeated washing), the contaminated denim fabrics were laundered along with uncontaminated transfer fabrics. Unlaundered contaminated fabrics (controls) were analyzed as an indicator of initial contamination and as a baseline (0%) for calculating percent pesticide removed and percent transferred. All variables tested in this study were replicated three times.

Extraction Procedures

Procedure development indicated analysis was more effective in the 2,4-D methyl ester state. Therefore, techniques were developed to convert 2,4-D ester and amine to 2,4-D methyl ester.

2,4-D Ester: 2,4-D ester is essentially insolubie in water, but soluble in most organic solvents. Experimentation with appropriate solvents for extraction was undertaken during procedure development. Maximum recovery was obtained with acetone. Fabric swatches were extracted twice with two-150 ml aliquots of acetone in a two hr mechanical shaking process, as described by Easley *et al.* (1981). Aliquots of 2,4-D ester extract were saponified by adding 2 ml of a solution made from 6.4 g potassium hydroxide (KOH) in 10 ml H_2O plus 90 ml ethylene glycol (EG) (Aly and Faust 1963). The resultant solution was evaporated to 2 ml under an N_2 gas stream which removed the acetone. An additional 8 ml KOH-EG were added. The solution was vortex stirred and heated in a 70°C water bath for 10 min, poured into a 60 ml separatory funnel, and acidified with 4 ml 6 *N* sulfuric acid (H_2SO_4). Salt formed during neutralization and was redissolved by adding 25 ml distilled water. Two 8-ml aliquots of chloroform ($CHCl_3$) were used to extract the 2,4-D acid. The combined $CHCl_3$ extracts were evaporated to approximately 0.10 ml with a N_2 stream evaporator, after which 2 ml boron trifluoride (BF_3) in methanol were added to achieve esterification of the 2,4-D acid. The solution was heated to 70°C for 10 min and quenched in an ice bath. Five ml of a saturated salt solution and 10 ml iso-octane were added and vigorously vortexed for three min to effect partitioning of 2,4-D methyl ester into iso-octane. After phase separation, a 2-ml aliquot of the iso-octane layer was pipetted into a vial for gas chromatographic analysis.

The 2,4-D ester transfer fabric extractions were prepared for analysis in the same manner, with the exception that the entire acetone extract was concentrated by vacuum rotary evaporation to approximately five ml prior to saponification with KOH-EG.

2,4-D Amine: 2,4-D amine is extremely soluble in water (300 g/100 g H_2O) and detectability of residues after laundering needed to be thoroughly explored. Amine-contaminated laundered denim swatches were extracted in two-150 ml aliquots of 0.15 *N* sodium hydroxide solution during two hr of mechanical shaking. The entire extract was poured into a 500 ml separatory funnel and acidified with 10 ml 6 *N* H_2SO_4 (Bucholtz 1981). The 2,4-D acid was extracted with a 35 ml aliquot of $CHCl_3$. A severe emulsion problem during phase separation was reduced by the addition of three ml absolute ethanol. Following two min vigorous shaking, the phases were allowed to separate. The chloroform phase and emulsion were drained into a 125 ml separatory funnel. A second chloroform extraction was made and combined with the first using the same procedures. Following phase separation in the 125 ml separatory funnel, the chloroform extract was drained into a 250 ml round bottom flask. Remaining water and emulsion in the 125 ml separatory funnel were re-extracted with a 10-ml aliquot of $CHCl_3$, following separation, $CHCl_3$ was added to the combined extracts in the round bottom flask. Contents in the flask were concentrated to five ml by rotary vacuum evaporation. The chloroform extract was transferred to a test tube and further evaporated to 0.10 ml using a N_2 stream. Following addition of BF_3-methanol, the procedures were the same as described for 2,4-D ester extraction. The 2,4-D amine transfer fabric extractions did not result in an emulsion formation following the addition of $CHCl_3$; therefore, only two 35 ml chloroform extractions were performed.

Gas Chromatography Procedures

A Hewlett-Packard gas chromatograph (Model 5840A) with a flame ionization detector was used for analysis. The separation column was 1.83 m × 3 mm I.D. packed with 3% OV-25 on 100–120 mesh Chromosorb W HP. The identical column temperature was 185°C, and temperature was programmed to increase at a rate of 5°C/min to 189°C. Temperature of the inlet was 195°C and the detector was 300°C. Nitrogen carrier gas flow was 1,290 ml/hr, air was 13,200 ml/hr, and hydrogen was 1,320 ml/hr. External standard quantification was performed using a standard solution of 2,4-D methyl ester (Dow Chemical Co., 99.98% purity) with recalibration every fifth sample. Peak area measurement was electronically calculated by a dedicated microprocessor. Two injections were made for each sample and averaged per replication. The amount of 2,4-D extracted from unlaundered controls and laundered swatches was expressed in nanograms (ng)/cm².

Statistical Analysis

Differences in the amount of 2,4-D ester or amine (ng cm²) between controls and laundered swatches were expressed in percentages of 2,4-D removed and transferred, and subjected to arc sin transformation. A factorial analysis of variance and a Duncan's multiple range test were used for data analysis. Indication of significance was at the $p \leq .05$ level.

Results

Due to the complexity of the research design, the findings are discussed separately for 2,4-D ester and 2,4-D amine formulations. The treatments of pre-rinse, water temperature, detergent, ammonia additive, and repeated washing are presented for each formulation. Furthermore, the amount of herbicide transferred during laundering is discussed for each formulation.

2,4-D Ester Formulation

The mean percents of 2,4-D herbicide removed from contaminated fabrics ranged from 28.86% to 44.99%. Amounts transferred ranged from 1.05% to 2.05% of the initial contamination (Table 1). The

17481

Table 1. Mean percent 2,4-D ester removed by laundering from contaminated denim fabric and transferred to concomitantly laundered fabric

Treatments		Mean % removed		Mean % transferred[1]	
Pre-rinse					
Pre-rinse-wash		36.52[a,2]	±12.19[3]	1.48[a]	±0.78
Wash (only)		34.50[a]	±14.99	1.71[b]	±0.80
Temperature					
60°C wash/49°C rinse		44.99[a]	±11.88	2.14[a]	±0.75
30°C wash/30°C rinse		26.03[b]	± 7.40	1.05[b]	±0.34
Detergent					
HDL		39.80[a]	±13.67	1.61[a]	±0.85
AATCC 124		31.22[b]	±12.32	1.58[a]	±0.75
Repeated wash/additive					
Detergent	1 wash	32.08[a]	±10.56	1.89[a]	±0.89
Detergent + NH_3	1 wash	28.86[a]	±13.35	2.05[a]	±0.82
Detergent	2 washes	40.59[b]	±13.09	1.28[b]	±0.48
Detergent + NH_3	2 washes	40.51[b]	±13.53	1.16[b]	±0.52

[1] Mean % transferred compares the amount (ng/cm²) of 2,4-D ester on control with amount (ng/cm²) in transfer fabric
[2] Mean followed by the same letter within treatments are *not* significantly different at $p \leq .05$ level
[3] Standard deviation

low residue removal is attributable to the insolubility of 2,4-D ester in water, yet this insoluble formulation apparently was carried to and retained in the transfer fabric even though the transfer fabric was taken through a complete laundry cycle.

Pre-rinse: No significant difference was found between pre-rinsed samples and non-pre-rinsed samples, although removal was slightly greater for 2,4-D ester contaminated denim fabrics which had been pre-rinsed. For the transfer fabrics, pre-rinse plus laundry resulted in significantly less 2,4-D transferred to concomitantly laundered fabric than those that were not pre-rinsed.

Temperature: A significant difference was found between the hot (60°C wash/49°C rinse) and the cold (30°C wash/30°C rinse) laundry procedures for both the contaminated denim and transfer fabrics. The higher water temperatures (Table 1) were more effective in removing the ester formulation from the fabric, but also resulted in increased transfer of the pesticide to concomitantly laundered fabric. This may be due to increased solubility of the ester formulation at higher temperatures.

Detergent: The use of HDL detergent resulted in significantly better removal than that of AATCC Standard Detergent 124 for the contaminated denim, regardless of temperature or laundry procedure. However, the amount of 2,4-D ester in the transfer fabric was not significantly different when a comparison was made between the two detergents. This indicated that the amount of 2,4-D transferred was similar regardless of detergent type.

Ammonia Additive: For both the contaminated denim and transfer samples, detergent washing alone was generally more efficient than detergent plus ammonia additive in removing residues from the fabrics. The use of ammonia at the concentration tested appeared to inhibit removal of 2,4-D ester.

Repeated Washing: Repeated washing was significantly better than single washing in the removal of 2,4-D ester from the initially contaminated denim fabric. Repeated washing also resulted in less 2,4-D ester being sorbed by the transfer fabric.

2,4-D Amine Formulation

Laundering procedures removed 99.17% to 99.65% of 2,4-D amine from the contaminated denim fabric (Table 2). These levels of removal were due in part to the extreme solubility of the amine formulation in water solutions. Differences found in the removal of the 2,4-D amine formulation, though statistically significant, were not meaningful in a practical sense because of the nearly complete elimination by the laundering procedures tested and low toxicity of this herbicide to humans. Minute amounts (0.02% to 0.26%) of 2,4-D amine were retained by the transfer fabric after concomitant laundering.

17482

...2,4-D amine removed by laundering from contaminated denim fabrics and transferred to concomitantly laundered

	Mean % removed		Mean % transferred[1]	
	99.53[a2]	±0.49[1]	0.08[a]	±0.11
	99.37[a]	±0.75	0.21[b]	±0.15
	99.54[a]	±0.55	0.17[a]	±0.16
	99.36[a]	±0.71	0.12[a]	±0.16
	99.65[a]	±0.29	0.18[a]	±0.14
	99.24[b]	±0.81	0.11[a]	±0.17
...wash	99.39[ab]	±0.38	0.26[a]	±0.11
	99.17[a]	±0.49	0.25[a]	±0.19
...washes	99.63[b]	±0.42	0.02[b]	±0.14
	99.61[b]	±0.37	0.04[b]	±0.08

...mpares the amount (ng/cm²) of 2,4-D amine on control with amount (ng/cm²) in transfer fabric
...same letter within treatments are *not* significantly different at $p \leq .05$ level

...No significant difference in herbicide ...ved between the four-step washing ...included a pre-rinse cycle and the ...treatment. However, pre-...reduced the amount of 2,4-D ...ferred as compared to the three-step ...

...significant differences were not ...en the hot (60°C wash/49°C rinse) and ...rinse) laundering proce-... the transfer fabrics. ...water-soluble, it apparently ...d from the fabric regardless ...

...ificant difference between HDL ...°C Standard Detergent 124 ...denim contaminated fabrics. ...fabrics. The detergents were ...statistically compared. These ...sons were similar to those of the ...ation (Table 1).

...Findings from the 2,4-D amine ...ine transfer fabrics indicated that ...e may have inhibited residue re-...). Detergent alone was generally ...ns than detergent plus ammonia ad-...h the difference was not significant.

...shing: A second washing significantly ...moval of 2,4-D amine from the con-...nim fabric and decreased amounts of residue transferred to concomitantly laundered fabrics.

Conclusions

General conclusions and recommendations resulting from this study include: 1) 2,4-D formulations which are water-soluble are more effectively removed through the laundering process than insoluble formulations; 2) 60°C wash/49°C rinse water temperatures result in better residue removal than 30°C temperatures for the ester formulation; 3) heavy duty liquid detergent provides for greater removal of 2,4-D during laundry than does a high phosphate detergent; 4) 2,4-D can be transferred from contaminated clothing to textile items laundered in the same wash load; therefore, it is imperative that contaminated clothing be laundered separately; 5) pre-rinsing is helpful when laundering contaminated fabrics, and does result in significantly less 2,4-D being transferred to other fabrics in the same wash; 6) ammonia as a laundry additive offered no advantage in the removal of 2,4-D residues during laundering; and 7) two launderings of contaminated fabrics result in greater removal than one laundering; however, the effects of more than two washings needs further investigation.

Differences in the amount of 2,4-D herbicide removed from contaminated denim fabrics and transferred to other fabrics during concomitant laundering is dependent upon pesticide formulation. Solubility of the formulation was a major factor in ease of removal through laundering. Because of the rela-

tively low toxicity of this herbicide, transfer in laundering is not a major concern. It could be anticipated that exposure due to prolonged dermal contact could be minimized through proper laundering of clothing contaminated with 2,4-D.

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Manuscript received March 15, 1982; accepted June 28, 1982.

17284

K-2312-
1983

The Science of the Total Environment, 31 (1983) 203—218
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

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CARCINOGENICITY AND TOXICITY OF 2,4-DICHLOROPHENOXY-ACETIC ACID

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DOW CHEMICAL
JAN 1 8 1984
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(Received February 9th, 1983; accepted May 4th, 1983)

D00M21563326

ABSTRACT

2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

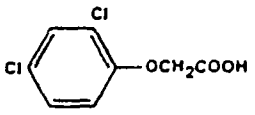
INTRODUCTION

The auxinlike, plant growth-promoting chemical, 2,4-dichlorophenoxy-acetic acid (2,4-D), has been used for may years as a plant herbicide [1]. The acid, its salts, and its esters are used to kill dicotyledonous weeds and certain other plant species. The chemical is readily absorbed from the digestive tract of animals and excreted by the kidney. Acute and subacute toxicity tests have shown that 2,4-D can cause poisoning in animals.

2,4-D is widely used as a herbicide to control certain types of vegetation. It is sprayed from airplanes on many millions of acres to kill shrub and broad-leaved plant life on specialized farms and range and pasture lands used for grazing. 2,4-D is used to kill vegetation adjacent to railroads, highways and other roads, pipe lines, power lines, and in and around lakes, ponds and irrigation ditches. It is used in aerial spraying operations of forest lands to kill broad-leaved vegetation and encourage soft wooded trees such as spruce and pine. It is present in preparations sold at retail for home gardeners and is widely used on home lawns and grass covered areas used for recreational purposes. 2,4-D is also used on cereal crops such as rice, on sugar cane and to control ripening of bananas and citrus fruits, to delay preharvest dropping of some fruits, and for the control of rot when lemons are stored. 2,4-D

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17485



2, 4-D

was extensively sprayed together with 2,4,5-trichlorophenoxyacetic acid on millions of acres as a defoliant in Vietnam.

With the widespread use of this herbicide, possible toxic effects are important. 2,4-D finds its way to humans through direct contact either occupationally or by spraying humans purposely or accidentally. Drift after spraying can contaminate gardens, plant nurseries, water and other areas. Residues may inadvertently be encountered in food, vegetables, meat and water.

The production and use of 2,4-D can only be estimated because the data often is considered as trade secret by the manufacturers. The latest data available are for the year 1975. An estimated 27 million kg were used in the U.S.A. The Federal Republic of Germany and the U.K. are the major producing countries of western Europe where annual production was estimated to be 3—30 million kg. In eastern Europe it was estimated to be less than 10 million kg. In Japan production amounted to 511 thousand kg.

This review includes, to the best of our knowledge, every study on the carcinogenicity of 2,4-D, or its esters, and a metabolite, 2,4-dichlorophenol in animals. The raw data and histological sections were reviewed for the FDA 2,4-D Rat Study and FDA 2,4-D Dog Study [1—3] and the results are based on this examination. The Innes et al. Mouse Studies for 2,4-D, or its esters [4], the Archipov and Kozlova 2,4-D Mouse and Rat Studies [5], and the Boutwell and Bosch 2,4-Dichlorophenol Mouse Study [6] are also included.

Statistical tests of significance were p (probability) values obtained with Fisher's exact test and tests for positive linear trend and departure from linear trend.

FDA 2,4-D RAT STUDY

Osborne-Mendel rats, three weeks of age, ingested either 0, 5, 25, 125, 625, or 1250 ppm of 2,4-D in the diet for 104 weeks [1, 3]. There were 25 males and 25 females at each dose. The rats were individually caged and were weighed weekly. Hematologic studies were done periodically.

Survivors were killed after 104 weeks, and organ weights were taken for heart, liver, spleen, kidneys, and testes. Organs were also weighed from moribund rats killed after 52 weeks; however, no organ weights were included from rats that died.

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TABLE 1
NUMBER OF MALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA RAT STUDY)

Dose (ppm)	Carcinomas	p	Sarcomas	p	Both[a]	p
0	0/25 (0%)		1/25 (4%)		1/25 (4%)	
5	2/25 (8%)		2/25 (8%)		3/25 (12%)	
25	6/25 (24%)	0.011	4/25 (16%)		9/25 (36%)	0.0053
125	6/25 (24%)	0.011	6/25 (24%)	0.049	9/25 (36%)	0.0053
625	5/24 (21%)	0.022	4/24 (17%)		6/24 (25%)	0.043
1250	3/23 (13%)		7/23 (30%)	0.018	9/23 (39%)	0.0033
5—1250	22/122 (18%)	0.012	23/122 (19%)	0.052	36/122 (30%)	0.0038
				0.021[b]		0.039[b]
		0.057[c]				0.034[c]

[a] Some rats had both carcinoma and sarcoma and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

Detailed histologic examination was made of tissues from six male rats and six female rats from the 1250 ppm 2,4-D groups and the control groups. Liver, kidney, spleen, and ovary or testes, as well as tumors and other gross lesions, were sectioned histologically from the rats on all other doses of 2,4,-D.

At the end of 104 weeks the following rats were alive: 12 males, 6 females (0 ppm); 8 males, 4 females (5 ppm); 9 males, 9 females (25 ppm); 9 males, 8 females (125 ppm); 10 males, 9 females (625 ppm); and 5 males, 11 females (1250 ppm).

Body weights and the organ—body weight ratios for liver, kidney, heart and testes were reported as showing no differences among the various groups. These results have little meaning, however, since the rats that died with the most chance of being abnormal were not included. The spleen—body weight ratio was slightly elevated in rats given 625 ppm or 125 ppm 2,4-D ($p < 0.05$).

Terminally, the red blood cells of rats given 5 ppm, 625 ppm, or 1250 ppm 2,4-D showed macrocytosis, slight polychromasia, and slight to moderate hypochromasia. These changes were not present, or were of a minor degree in control rats.

Malignant neoplasms at all sites in male rats

There were increased incidences of malignant neoplasms in male rats given all doses of 2,4-D (Table 1). Nine of 25 rats (36%) given 25 or 125 ppm ($p = 0.0053$) and 9 of 23 (39%) given 1250 ppm of 2,4-D ($p = 0.0033$) developed carcinomas and sarcomas. The incidences of these neoplasms were dose related ($p = 0.039$). For some unexplained reason, the rats receiving 625 ppm, 6 of 24 (25%), had fewer malignant neoplasms than male

17487

TABLE 2
NUMBER OF MALE RATS INGESTING 2,4-D WITH LYMPHOSARCOMAS AND NEUROSARCOMAS (FDA RAT STUDY)

Dose (ppm)	Lymphosarcoma	p	Subcutaneous neurosarcomas
0	0/25 (0%)		0/25 (0%)
5	2/25 (8%)		0/25 (0%)
25	4/25 (16%)	0.055	0/25 (0%)
125	5/25 (20%)	0.025	1/25 (4%)
625	3/24 (13%)		1/24 (4%)
1250	6/23 (26%)	0.0082	1/23 (4%)
5—1250	20/122 (16%)	0.018 0.034[a]	3/122 (2%)

[a] Test for positive trend.

rats in other groups ($p = 0.043$). Thirty-six of 122 (30%) 2,4-D-treated male rats had carcinomas and sarcomas ($p = 0.0038$).

Sarcomas in male rats

The sarcomas in 2,4-D-treated male rats were mainly lymphosarcomas, however, there were a small number of subcutaneous neurosarcomas (Table 2). Sarcomas were present in 4 of 25 rats (16%) ingesting 25 ppm, 6 of 25 rats (24%) ingesting 125 ppm ($p = 0.049$), and 7 of 23 (30%) receiving 1,250 ppm of 2,4-D ($p = 0.018$) (Table 1). Twenty-three of 122 (19%) male rats given 2,4-D had sarcomas ($p = 0.052$).

Carcinomas in male rats

Carcinomas in male rats treated with 2,4-D were seen in the endocrine system. Six of 25 male rats (24%) ingesting 25 or 125 ppm ($p = 0.011$) and 5 of 24 rats (21%) given 625 ppm ($p = 0.022$) of 2,4-D developed carcinomas (Table 1). Twenty-two of 122 (18%) male rats given 2,4-D had carcinomas ($p = 0.012$).

Malignant neoplasms at all sites in female rats

Eight of 20 (40%) female rats given 5 ppm ($p = 0.019$), 11 of 22 (50%) given 25 ppm ($p = 0.058$), 13 of 23 (57%) on 125 ppm ($p = 0.022$), 18 of 24 (75%) receiving 625 ppm ($p = 0.00047$), and 17 of 25 (68%) female rats ingesting 1,250 ppm ($p = 0.0022$) of 2,4-D developed malignant neoplasms at all sites (Table 3). Altogether, malignant neoplasms were observed in 67 of 114 female rats (59%) ingesting 2,4-D ($p = 0.0018$).

Lesions of the lymphoreticular system in female rats

The highest incidence of lymphosarcomas was 12 of 24 (50%) female rats given 625 ppm of 2,4-D ($p = 0.00007$) (Table 4). Female rats in the other

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TABLE 3
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA RAT STUDY)

Dose (ppm)	Carcinomas	p	Sarcomas	p	Both[a]	p
0	4/22 (18%)		1/22 (5%)		5/22 (23%)	
5	8/20 (40%)		5/20 (25%)		8/20 (40%)	
25	7/22 (32%)		8/22 (36%)	0.011	11/22 (50%)	0.058
125	10/23 (43%)	0.065	8/23 (35%)	0.013	13/23 (57%)	0.022
625	14/24 (58%)	0.0059	12/24 (50%)	0.00007	18/24 (75%)	0.00047
1250	13/25 (52%)	0.017	10/25 (40%)	0.0044	17/25 (68%)	0.0022
5—1250	52/114 (46%)	0.013	43/114 (36%)	0.0017	67/114 (59%)	0.0018
		0.017[b]		0.024[b]		0.0022[b]
				0.089[c]		0.090[c]

[a] Some rats had both carcinomas and sarcomas and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

2,4-D treatment groups had 24—27% lymphosarcomas ($p = 0.011$—0.018). Thirty-one percent of all females ($p = 0.00073$) developed lymphosarcomas. The findings are even more significant if hyperplasia of the lymphoreticular system is considered along with lymphosarcomas, i.e., 39% ($p = 0.00005$) of female rats ingesting 2,4-D. Female rats also had sarcomas of the uterus.

Carcinomas at all sites in female rats

Carcinomas at all sites were seen in 43% (10 of 23) ($p = 0.065$) of female rats on 125 ppm, 58% (14 of 24) ($p = 0.0059$) given 625 ppm, 52% (13 of 25) ($p = 0.017$) receiving 1250 ppm and 46% (52/114) ($p = 0.013$) of female rats given 2,4-D (Table 3). Carcinomas were found in the reproductive system, particularly the mammary gland, and occasionally in endocrine organs.

Neoplasms of the mammary gland in female rats

The incidence of mammary gland tumors was higher in treated female rats, particularly on gross examination, which is generally quite reliable. The number of rats with such tumors was less in all but one group after histologic examination (Table 5).

On gross examination, 18 of 21 female rats (86%) ingesting 25 ppm ($p = 0.0064$), 20 of 24 rats (83%) ingesting 625 ppm ($p = 0.0080$) and 16 of 23 rats (70%) given 1250 ppm of 2,4-D had neoplasms of the mammary gland, compared to 10 of 22 (45%) control female rats. The findings are quite different for the numbers of rats with histological sections examined by pathologists at FDA and sections available for this examination.

17489

TABLE 4
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH LESIONS OF THE LYMPHORETICULAR SYSTEM (FDA RAT STUDY)

Dose (ppm)	Hyperplasia	*p*	Lymphosarcoma	*p*	Hyperplasia and lymphosarcoma	*p*
0	0/22 (0%)		0/22 (0%)		0/22 (0%)	
5	0/22 (0%)		5/20 (25%)	0.018	5/20 (25%)	0.018
25	2/22 (9%)		6/22 (27%)	0.011	8/22 (36%)	0.0018
125	2/23 (9%)		6/23 (26%	0.012	8/23 (35%)	0.0023
625	4/24 (17%)	0.065	12/24 (50%)	0.00007	16/24 (67%)	< 0.00001
1250	2/25 (8%)		6/25 (24%)	0.016	8/25 (32%)	0.0034
5—1250	10/114 (9%)		35/114 (31%)	0.00073	45/114 (39%)	0.00005
						0.039[a]
						0.0004[b]

[a] Test for positive trend.
[b] Departure from linear trend.

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TABLE 5

NUMBER OF FEMALE RATS INGESTING 2,4-D WITH NEOPLASMS OF THE MAMMARY GLAND AND HISTOLOGIC EXAMINATION[a] (FDA RAT STUDY)

Dose (ppm)	Gross[b]	*p*	Histologic[c]	*p*	Histologic[d]	*p*
0	10/22 (45%)		10/22 (45%)		8/22 (36%)	
5	8/20 (40%)		7/20 (35%)		7/20 (35%)	
25	18/21 (86%)	0.0064	11/21 (52%)		11/21 (50%)	
125	8/23 (35%)		10/23 (43%)		9/23 (39%)	
625	20/24 (83%)	0.0080	14/24 (58%)		15/24 (63%)	0.070
1250	16/23 (70%)	0.091	15/23 (65%)		11/23 (45%)	
5—1250	70/111 (63%)		57/111 (52%)		53/111 (47%)	
		0.0233[e]		0.0334[e]		
		0.0008[f]				

[a] Corrected for survival time, i.e., time of appearance of first neoplasms of the mammary gland.
[b] Gross necropsy at FDA.
[c] Histological examination by FDA.
[d] Histological examination by Reuber.
[e] Test for positive trend.
[f] Departure from trend.

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Comments

It is worth noting that histological examination of tissues from this study was inadequate. Microscopic neoplasms would have been overlooked at the 5 to 625 ppm doses of 2,4-D (only gross neoplasms were sectioned histologically) and also even at the highest dose because only six rats of each sex were examined in detail. Also histological sections were not available for 19 mammary gland neoplasms described at the time of necropsy, and no explanation was given for this discrepancy.

Despite the shortcomings of this study, it must be considered as an acceptable study.

The largest numbers of rats alive were in the 25 to 1250 ppm doses; and the smallest number of rats alive was in the lowest dose, 5 ppm. These results suggest that rats in at least some of the 25 to 1250 ppm groups were not ingesting their diets because of toxicity. The differences in the incidences of neoplasms, therefore, would not be great.

Tumors were analyzed, on the basis of 25 rats per group, and the following conclusions were made by the authors: "There is a statistically significant ($p \leq 0.05$) linear relationship between the proportion of female rats with tumors and the level of the log dose, but not the arithmetic dose, indicating that there is a tendency for the proportion of female rats with tumors to increase with the log dosage. There is also a statistically significant ($p \leq 0.05$) linear relationship between the proportion of male rats with malignant tumors and the level of arithmetic and log dose. A comparison was made of the control group with each treatment group. Statistically significant ($p \leq 0.05$) differences were found only between the control group and the 1250 ppm dose level with respect to male rats with malignant tumors."

The authors also believed that the raw data and the pathologic interpretation did not support the statistical analyses and that "a carcinogenic effect of 2,4-D has not been shown". They also stated that "additional support for this interpretation has been given by the long-term study in mice".

Summary

Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats.

Conclusions

2,4-D is carcinogenic for male and female rats.

FDA 2,4-D DOG STUDY

Beagle dogs, 6 to 8 months old (3 males and 3 females per group) ingested 0, 10, 50, 100, or 500 ppm 2,4-D in the diet for 104 weeks [1, 2]. Organ weights were taken for brain, heart, liver, kidneys, spleen, thyroid, adrenals, and testes. Tissues from all dogs were studied grossly and microscopically.

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Many of the dogs ingesting 2,4-D lost weight. There were scattered lesions such as atrophy of the testes and prostate, interstitial nephritis, hemangioma of the adrenal, atrophic or cystic pituitary, atrophy of the thyroid, and hypoplasia of the bone marrow. Most of the lesions were seen in the endocrine organs. Control dogs generally did not have lesions.

Comments

Previous experience at FDA has shown that long-term chronic dog studies should be carried out for six years or longer in order for neoplasms to develop. Since they occurred predominantly in 2,4-D treated dogs, lesions may have progressed to neoplasms had the dogs been treated for a longer period of time.

Summary

A two-year feeding study in dogs cannot be considered a carcinogenicity study.

INNES et al. 2,4-D, OR ITS ESTERS, ORAL MOUSE STUDIES

1. 2,4-D

The maximum tolerated dose of 2,4-D was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" mice [4, 7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. 46.4 mg/kg was given in 0.5% gelatin daily by stomach tube beginning at 7 days of age. After the mice were weaned at 28 days of age, 149 ppm of 2,4-D was mixed directly in the diet and provided ad libitum. "Strain B" male and female mice were also given 100 mg/kg, followed by 323 ppm. Treatment was continued approximately 18 months.

Postmortem included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. Thyroid glands were not examined.

There was no increase in neoplasms in the 2,4-D-treated mice.

2. 2,4-D isopropyl ester

"Strain A" and "strain B" male and female mice were given 46.6 mg/kg of 2,4-dichlorophenoxyacetic acid, isopropyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 111 ppm in the diet for approximately 18 months.

There were neoplasms in the lungs of 4 of 18 "strain A" male mice (22%) ingesting 2,4-D isopropyl ester compared to 2 of 17 matched controls (12%) and 5 of 79 pooled male control mice (6%) ($p = 0.0584$) (Table 6).

In this study, "strain A" male mice receiving 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

17493

TABLE 6
NUMBER OF MALE AND FEMALE MICE INGESTING 2,4-D ISOPROPYL ESTER WITH NEOPLASMS OF THE LUNG (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female
"A" Matched	0	2/17 (12%)		1/18 (6%)
"A" Pooled	0	5/79 (6%)		3/87 (3%)
"A"	111	4/18 (22%)	0.0584	1/18 (6%)
"B" Matched	0	2/18 (11%)		0/17 (0%)
"B" Pooled	0	9/90 (10%)		3/82 (4%)
"B"	111	2/18 (11%)		0/17 (0%)

3. 2,4-D butyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, butyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 149 ppm in the diet for approximately 18 months.

Three of 18 "strain A" female mice (17%) and 4 of 87 pooled controls (5%) had reticulum cell sarcomas ($p = 0.0955$) (Table 7).

In this study, there was an increased incidence of reticulum cell sarcoma in 2,4-D butyl ester-treated "strain A" female mice.

4. 2,4-D isooctyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, isooctyl ester in 0.5% gelatin by stomach tube. After 28 days they ingested 130 ppm in the diet for approximately 18 months.

Neoplasms in treated mice were found mainly in the liver and lung. One "strain A" treated male mouse had an "angioma" of the liver and another an "angioma" of the spleen.

In this study, neoplasms of the liver were slightly increased in 2,4-D

TABLE 7
NUMBERS OF MALE AND FEMALE MICE INGESTING 2,4-D BUTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female	p
"A" Matched	0	1/17 (6%)		2/18 (11%)	
"A" Pooled	0	5/79 (6%)		4/87 (5%)	
"A"	149	0/18 (0%)		3/18 (17%)	0.0955
"B" Matched	0	1/18 (6%)		2/17 (12%)	
"B" Pooled	0	1/90 (1%)		3/82 (4%)	
"B"	149	2/18 (11%)	0.0714	0/16 (0%)	

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isooctyl ester-treated "strain A" male mice and tumors of the lung in "strain A" female mice. Two male mice had rare tumors; "angiomas" of the liver and spleen.

INNES et al. 2,4-D, OR ITS ESTERS, SUBCUTANEOUS MOUSE STUDY

A single subcutaneous injection of 2,4-D, or its esters, was given in the nape of the neck to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" at approximately the 28th day of age [7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. They were killed after approximately 18 months.

Mice received the following doses: 215 mg/kg or 464 mg/kg of 2,4-D in DMSO; 100 mg/kg of 2,4-D isopropyl ester, 25.5 mg/kg of 2,4-D butyl ester, or 21.5 mg/kg of 2,4-D isooctyl ester in corn oil.

Necropsy included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities. Histological examination was done on all major organs and of all grossly visible lesions.

Reticulum cell sarcomas in "strain B" female mice

Five of 17 "strain B" female mice (29%) given 2,4-D isooctyl ester had reticulum cell sarcomas, compared to 5 of 157 control female mice (3%) ($p = 0.0009$) (Table 8).

Summary

2,4-D isooctyl ester was carcinogenic for the lymphoreticular system (reticulum cell sarcomas) in "strain B" female mice.

ARCHIPOV AND KOZLOVA MOUSE AND RAT STUDIES

Three groups, 100 each, of CBA x C57/BL hybrid mice were used [5]. Two groups of mice received one drop of a 0.5% solution of 3-methylcholanthrene

TABLE 8

NUMBER OF "STRAIN B" MALE AND FEMALE MICE GIVEN A SINGLE SUBCUTANEOUS INJECTION OF 2,4-D ISOOCTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Dose (mg/kg)	Males	Females	p
0	0/161 (0%)	5/157 (3%)	
1	0/18 (0%)	5/17 (29%)	0.0009

in benzene on the skin for 3 weeks. Later the skin of one group of mice was painted with a 10% solution of the amine salt of 2,4-D in acetone. The skin of the third group of mice was painted with a 10% solution of the herbicide. Mice were observed for 20 months.

Neoplasms of the skin

Papillomas developed on the skin of 17.7% of the mice treated with 3-methylcholanthrene followed by 2,4-D, and none for the mice receiving 3-methylcholanthrene only or 2,4-D only.

Comments

Groups of rats also ingested 1/10th the LD_{50} of the amine salt of 2,4-D [5]. The dose was not given.

Summary

2,4-D is a promoter of neoplasms of the skin in mice.

BOUTWELL AND BOSCH 2,4-DICHLOROPHENOL MOUSE SKIN STUDY

Female Sutter mice, 2—3 months of age, were used [6]. Mice were painted on the skin with a single initiating dose of 0.3% dimethylbenzanthracene in acetone followed by 20% 2,4-dichlorophenol in benzene, benzene only, or no treatment. 2,4-Dichlorophenol was applied twice weekly. One group of mice was observed for 15 weeks and a second for 24 weeks.

Neoplasms of the skin

Mice treated with both chemicals developed an increased incidence of neoplasms of the skin. Thirteen of 27 treated mice (48%) ($p = 0.00607$) had papillomas and 3 of 27 treated mice (11%) had carcinomas of the skin, compared to 1 of 15 untreated mice (7%) with papillomas and 0 of 15 untreated mice (0%) after 15 weeks. Papillomas of the skin were observed in 12 of 16 treated mice (75%) ($p = 0.00004$) and carcinomas of the skin in 10 of 16 treated mice (62%) ($p < 0.00001$); whereas, papillomas were seen in 3 of 27 mice (11%) and carcinomas in 0 of 27 mice (0%) receiving benzene only after 24 weeks.

Conclusion

2,4-Dichlorophenol promoted the incidence of neoplasms of the skin in mice when administered after a single initiating dose of dimethylbenzanthracene.

DISCUSSION

2,4-D was found to be carcinogenic for rats in the original publication by

17496

Hansen et al. [1]. It was also concluded that "2,4-dichlorophenoxyacetic acid is carcinogenic in rats" in a preliminary review of the raw data, but not the histological sections, requested by Senator Edward M. Kennedy's Subcommittee on Administrative Practice and Procedure of the Committee on the Judiciary, United States Senate [8]. The examination and diagnoses of the histological sections, which was done for this review further strengthened the findings and conclusion that 2,4-D is carcinogenic in rats.

Experience has shown that it is necessary to review the raw data and histological sections of old chronic toxicity and carcinogenicity studies in animals [9, 10, 11]. The data from studies often is not adequately analyzed and the conclusions are suspect. It also is not unusual for results of such studies, which are statistically significant, to be ignored because they are described as not biologically significant. The pathology report for the FDA 2,4-D study in rats was completed in 1964; however, the results were not published until 1971 [1, 3].

2,4-D is mutagenic and teratogenic as well as carcinogenic [12]. 2,4-D and its derivatives are teratogenic in mice, rats and hamsters. 2,4-D induces skeletal malformations, cleft palats, eye malformations, subcutaneous edema and hemorrhages in the snout, abdominal cavity, liver and soft tissues in rats when administered during early pregnancy [7, 13—17]. 2,4-D is embryotoxic [18]. 2,4-D penetrates the placenta and the fetuses [19]. 2,4-D caused point mutations in animal cells without liver activation, damages DNA in a manner similar to ionizing radiation and stimulates mitosis [20—22]. Endothel-treated fruit flies developed an increase of recessive lethal mutations [23].

2,4-Dichlorophenol is a major product of the breakdown of 2,4-D by microorganisms [24]. The compound is also teratogenic in rats and carcinogenic in mice. [6, 14].

2,4-D is rapidly absorbed from the human gastrointestinal tract and distributed widely throughout the body in animals and in human beings [25—28]. It is fat soluble and rapidly absorbed through the skin and lungs [29]. Acute effects of 2,4-D poisoning in humans include headache, weakness, dizziness, nausea and vomiting, sore throat, irritation of nasal mucosa, impared sense of taste and smell, substernal chest pain and loss of consciousness [30]. Peripheral neuropathy, which begins with tingling and numbness in the extremeties, has been reported in humans hours or days after exposure to 2,4-D on the skin. The symptoms in some people increased through several weeks until pain, paresthesia, and paralysis were severe. Disability was protracted and recovery was incomplete even after a lapse of years [31, 32]. These symptoms resemble those of multiple sclerosis, and this has been confirmed by the finding of plaques of acute demyelination in all parts of the brain of a man who died after ingestion of 2,4-D [33].

The formation and occurrence of tumors in man and other mammals, such as rats and mice, is quite similar [11]. In tests undertaken to date, it has been demonstrated that virtually every chemical which has been found to be carcinogenic in man is also carcinogenic in one or more

mammalian test animals. It has been shown that if these compounds will produce tumors in one species, they will very likely produce tumors in more than one; thus adding weight to any finding of carcinogenicity in tests using any mammalian species.

Sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that a finding of carcinogenicity in one mammalian species should be deemed to have relevance for other mammalian species — including man. This threat may not be manifested for up to 30 or 40 years, given the long latent period of many known human carcinogens. Given the state of carcinogenicity testing and knowledge, there can be only one safe tolerance for a carcinogen — an absolute zero tolerance — and the only way a substance shown to be carcinogenic in test animals will not be a threat to human health is simply to prevent that substance from entering the environment. To the extent that it does enter man's environment, it will constitute a very real threat to his health.

Recent reports have described an increase of malignant neoplasms in human beings exposed to phenoxyacetic acids. Humans exposed to phenoxyacetic acids or chlorophenols showed an increased risk for the development of soft-tissue sarcomas [34, 35]. Results also have indicated that humans in contact with phenoxy acids may also develop malignant lymphomas [36].

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ACKNOWLEDGEMENT

Statistical analyses were done by C. A. Riggs, Information Systems Department, Frederick Cancer Research Center. English translations were kindly supplied by Ruth M. Shearer, Issaquah Health Research Institute, Issaquah, Washington.

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Arch. Environ. Contam. Toxicol. 14, 427–435 (1985)

Archives of Environmental Contamination and Toxicology

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518
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Forestry Workers Involved in Aerial Application of 2,4-Dichlorophenoxyacetic Acid (2,4-D): Exposure and Urinary Excretion

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Abstract. Urinalysis was conducted on six volunteer workers involved in mixing and loading 2,4-D ester solutions into aircraft and in guiding the spray aircraft in two conifer release programs during 1981 and 1982. Exposures were reduced by wearing a full line of protective clothing. Two females and one male were involved in mixing the spray for 109 aircraft loads over an 11-day period in 1981. During the 1981 operation, the highest daily excretion of 2,4-D in the urine was 0.30, 0.94 and 9.59 μg/kg body weight for the three workers. In 1982, three male workers were involved, one diluting the concentrated solution and loading the aircraft, and two marking the swaths for aerial application over an 18 day period. The highest daily excretion of 2,4-D in the urine was 7.73, 8.37, 22.2 μg/kg body weight for the three workers. One of the authors, acting as a bystander, was directly sprayed in the 1982 season and 0.44% was absorbed based on urine analysis. The highest daily excretion of 2,4-D in his urine was 4.75 μg/kg body weight. For all seven people, the calculated exposure was less than the no-effect level of 10 mg/kg of body weight/day by a large margin of safety. The presence of 2,4-D in urine samples in the pre-spray period and its slow disappearance during the post spray period prompted further investigation. Swabs of internal surfaces of living quarters revealed deposits of 2,4-D from 0.7 to 288 μg/0.1m^2 and on spray equipment from 0.7 to 184 μg/0.1m^2.

Studies by Franklin *et al.* (1982), Kolmodin-Hedman and Erne (1980), Lavy *et al.* (1980, 1982), Libich *et al.* (1984), Nash *et al.* (1982), Poland *et al.* (1971), and Sauerhoff *et al.* (1977) have shown that chlorophenoxy herbicides (2,4-D and 2,4,5-T) were absorbed into the body by either the dermal or inhalation routes and almost totally eliminated in the urine as the unchanged parent material. Thus, urinary concentrations of phenoxy compounds can be considered as a quantitative indicator of exposure.

While studying forestry workers applying 2,4,5-T, Lavy *et al.* (1980) found that inhalation of the herbicide accounted for only a very small fraction of that entering the body, while dermal absorption was a thousand times greater. In the study of Libich *et al.* (1984) the ratio of dermal to inhalation absorption was between 11 and 50:1 depending on the degree to which the equipment delivered a mist or coarse spray droplet.

This two-year study was designed to measure direct and indirect exposure of seven workers; (a) four employed in mixing and loading aircraft with 2,4-D, (b) two balloon men directing aerial application and (c) one bystander. For an assessment of the source of indirect exposure, internal surfaces of living quarters and vehicles were swabbed for contamination.

Materials and Methods

Field Operation

In 1981 and 1982 field operations were monitored involving the spraying of 2,4-D ester to suppress competing plants and to promote regenerating conifer forest. These operations were performed by two paper companies located in Kapuskasing and Iroquois Falls, in northeastern Ontario, Canada. The formulations applied were 2,4-D iso-octyl esters (Esteron LV-600 and Pfizer 2,4-D Ester LV600) with a concentration of 600 g/L acid equivalent (ae) and dispensed from 205 L metal drums.

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During the mixing procedure, the contents of each drum were pumped directly into a mixing tank together with water as the diluent. During loading, the herbicide solution was pumped into tanks in the aircraft employing a high capacity pump. To minimize spillage during the loading operations, a dry-break connection was fitted between the end of the hose and the inlet port of the aircraft tank in 1981 and a quarter turn ball valve was installed at the end of the hose in 1982.

Urine samples were collected in 1981 from a crew of three persons employed in mixing and loading 2,4-D into fixed wing aircraft (Grumman Ag-CAT) in order to investigate exposure. Their ages and weights appear in Table 1. Workers F1, F2, and M1 used newly purchased mixing and loading equipment. Two workers (F1 & M1) were employed in mixing and loading spray solutions while a third (F2) was involved in recording, supervising, and assisting in mixing and loading. As added duties, workers F1 and M1 rinsed empty drums, hosed down spray equipment and the loading site, cleaned spray nozzles on aircraft, and repaired leaks in the loading and mixing system. Urine samples were collected in 1982 from a spraycrew using equipment that had been used for several years. The crew included one mixer and loader (M2) and two balloon men (M3 & M4). Their ages and weights appear in Table 1. Worker M2 was employed for duties similar to those of workers F1 and M1 in 1981. Workers M3 and M4 were balloon men marking the swaths and giving the helicopters (Bell Long Ranger) ground guidance on each flight pass during the spray operation. Worker M3 occasionally assisted M2 in mixing the concentrate before it was loaded into the helicopter.

Worker Protection

While mixing, loading and cleaning-up in 1981, the three workers wore at all times protective clothing including a disposable suit made of "Tyvec" (a liquid repelling material not completely impervious to organics or solutions of low surface tension, but which allowed the exchange of air) as well as neoprene gloves, rubber boots, hard hats, face shields, and respirators (half face type). Over the 11-day period, each employee was issued with 4 or 5 new disposable suits, and 2 or 3 new sets of cartridges for their respirators. Showers were taken within one hour after each working period (Table 1). In 1982, worker M2 wore the following protective gear: hard hat with flip-up visor, respirator (MSA Comfo II, half face type #460968) with filter, (GMP Pesticide Combination Filter, #448847), "non-breathing" Stanley Kem Wet® Wear jacket and pants (July 9 and 10), Stanley Kem "non-breathing" coveralls (July 11–July 27), neoprene gloves, and rubber boots. The contents of the cartridge filter between July 6–28 worn by worker M2 were analyzed for residues. Workers M3 and M4 wore the following protective clothing: a hard hat, "non-breathing" Stanley Kem coveralls and rubber boots. When worker M3 assisted M2 in mixing 2,4-D he wore the same apparel as worker M2. All three workers changed coveralls after every 4 to 5 spray operations, *i.e.*, every 2 to 3 days. A shower was taken by all three workers within one hour after each spray session.

Bystander

The US Environmental Protection Agency (EPA) has required the US Forest Service to prepare a worst case analysis for forestry use of herbicides. Our study was designed to determine the worst case exposure for a bystander (*i.e.*, being accidently directly sprayed).

At a forest site near Kirkland Lake, Ontario a bystander (B1-Table 1) wearing shorts, a T-shirt and sneakers, with bare arms, legs and head was sprayed by a Bell G-2 helicopter flying 90 km/hr at a height of 11 m above the ground. The helicopter had a 9.8 m rotor and spray boom of the same length mounted in front of the skids. Flat fan nozzles (28#6520) oriented back 45° below horizontal were fitted on the boom and a pressure of 195 kPa was used. To assist in determining the amount of 2,4-D actually deposited on the bystander (B1), two subjects (B2 and B3) were positioned one on either side of B1. Each wore two disposable "Tyvec" suits (one on top of the other to avoid possible contamination from work clothes). The suits consisted of hoods, smocks and pants giving full body coverage. The helicopter sprayed three parallel adjacent swaths, each 20 m wide, and the three subjects were located in the center of the second pass. The site chosen for the test was a clearing of 20 m diameter on a road. Application was made between 2010 and 2017 hr on 17 August, 1982 (18°C, 52% relative humidity, and no wind). The 2,4-D was applied at a rate of 1.62 kg ae in a total spray volume of 55 L/ha.

The outer disposable suits of B2 and B3 were cut off and divided into four portions: hoods, arms, torso, and legs. Each portion was placed in 500 ml of methanol and transported to the laboratory for extraction and analysis. Bystander B1 removed his clothes and showered three hr after the event.

Deposit surface areas were computed for the three bystanders. Since the clothing worn by B1 was skin tight, it was assumed that the surface area presented for deposit was equal to his body surface area. The latter was determined by constructing skin tight garments which were then cut up and measured. Since the clothing (protective suits) worn by B2 and B3 were very baggy the total surface area of these garments did not represent the surface area presented for deposit. The deposit surfaces were computed by measuring the surface areas of the various parts of clothing on life size front view photographs of B2 and B3. It was assumed that the surfaces of arms, legs and torso were cylindrical so that three dimensional surface areas could be calculated by multiplying the cross-sectional areas by pi. Cross-sectional areas were also measured on a life size photograph of B1. The ratio of measured three dimensional areas to measured cross sectional areas for B1 were: arms—3.12; torso—3.33; legs—3.37. Thus the method for estimating the surface areas of B2 and B3 available for deposit is reasonable although confidence limits can not be placed on the calculated areas. B2 and B3 were assumed to have the same size heads as B1 but the surface area for deposit was reduced by the area covered by goggles and respirator.

Urine Sampling

All urine excreted by the six workers and the bystander was collected, beginning several days prior to commencement of spraying and continuing until a number of days after the end of the spraying period. In all cases, there was no guarantee that there was no exposure to 2,4-D during the pre-spray period. All direct contact with 2,4-D was avoided during the post spray period. For each 24-hr period, the total urine per person was collected in a 3L plastic jug which was refrigerated until shipped to the laboratory for analysis.

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Table 1. Information on six workers and a bystander involved in aircraft application of 2,4-D in conifer release programs during two successive years

Item	1981 Workers[a]			1982 Workers[a]			1982 Bystander
	F1 (M + L)	M1 (M + L)	F2 (S)	M2 (M + L)	M3 (M + B)	M4 (B)	B1
Age (years)	21	20	22	25	23	32	38
Body Weight (kg)	51	70	57	80	82	75	84
Duration of direct exposure (hr)	65	65	65	50	51	39	
Urine: Specific gravity							
Mean	1.013	1.016	1.015	1.018	1.013	1.013	1.012
SD	0.005	0.003	0.005	0.008	0.005	0.009	0.003
Number of showers (during spray period)	22	11	15	18	18	15	

[a] M + L—mixer and loader, S—supervisor, M + B—mixer and balloon man, B—balloon man, F—female, M—male

Surface Swabs

Following the 1982 and 1983 spray seasons, selected surface areas of helicopters, vehicles, work place, and living and eating quarters were swabbed with cotton balls wetted with methanol. A total of 87 areas each 0.1 m^2 were swabbed at two sites in northeastern Ontario where aerial forestry spray programs using 2,4-D were conducted. A clean pair of disposable plastic gloves were used for the collection of each sample. Cotton balls wetted with 2 mL of methanol were used to swab the surfaces in two directions. The areas were then swabbed a second time using dry cotton balls. All swabs from each site were placed in vials, sealed and retained for analysis. With handles, knobs and latches, where the areas were less than 0.1 m^2, a correction factor was used to give comparable areas of 0.1 m^2.

Analytical Procedure

On receipt of each 24-hr urine sample, the volume was measured and the specific gravity determined. Single 50 mL samples of urine or swabs were hydrolyzed with 250 mL of 0.1 N NaOH and washed twice with 25 mL of 20% dichloromethane in *n*-hexane. The aqueous phase was acidified with 2.5 mL of 1:1 H_2SO_4 and extracted with 50 and 30 mL of ethyl ether. The ether extracts were combined and dried with Na_2SO_4 and evaporated to dryness. The herbicide residues were esterified with boron trifluoride in methanol (14% BF_3) at 80°C for 30 min. The methyl esters of the herbicide were extracted with benzene and water was added to the mixture to remove the excess boron trifluoride. The benzene extracts were dried with anhydrous Na_2SO_4 and the total volume adjusted to 5 mL by evaporation.

A Tracor 550 gas chromatograph equipped with an electron capture (^{63}Ni) detector and an electron capture linearizer was used to quantitate herbicide residues. The column was packed with 5% Dexsil® 300 GC on Chromosorb W and the column temperature was 160°C. For confirmation purposes, a gas-liquid chromatograph fitted with an electroconductivity detection system set in the chlorine mode was used. Urine spiking tests showed that 2,4-D recoveries varied from 85% to 90%.

Results

Details on age and weight of workers and the bystander and periods of exposure appear in Table 1 together with mean volumes and specific gravity of each person's urine.

1981 Operation

Over the 11 day period in August 1981, the three employees were involved in filling 109 aircraft hoppers with a 6.5% water emulsion of 2,4-D iso-octyl ester (Table 2). Workers handled 7995 L of 2,4-D concentrate (600 g ae/L) and loaded a total of 73,895 L of the 6.5% spray mixture at the rate of 678 L per load. The area sprayed was 2,195 ha. The total direct exposure time for all three workers over the 11 day period was 65 hr (Table 1). All three workers were exposed to 2,4-D as evidenced by the presence of the chemical in urine (Table 2).

The amounts and concentrations of 2,4-D voided in urine per 24-hr period for the three workers are given in Table 2. A slow build-up in the concentrations of 2,4-D in the urine of worker M1 was observed. At no time did his urine exceed a concentration of 45 μg/L 2,4-D, a maximum excretion rate of 0.94 μg 2,4-D/kg body weight per day (Tables 2 and 3). The mixer and loader (F1) had a much greater concentration of 2,4-D in her urine (rising in two peaks—323 and 326 μg/L) with a maximum level of 9.59 μg 2,4-D/kg body weight/day (Tables 2 and 3). The supervisor (F2) had the lowest 2,4-D concentrations in the urine (16 μg/L, and excreted a maximum of 0.30 μg/kg body weight/day (Tables 2 and 3). Attempts to calculate first order equations for these workers in the post-spray period were foiled by fluctuating 2,4-D excretion rates. This was explained by the fact that 2,4-D was absorbed at the work site both before the operation commenced and after the operation was completed (Table 2). Later swabs revealed that considerable contamination of internal surfaces of vehicles and living quarters

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Table 2. Daily excretion of 2,4-D by the three workers who mixed 7,995 L of 2,4-D concentrate (4.797 kg of 2,4-D acid equivalent) and loaded 73.895 L of herbicide mixture into aircraft (each load 678 L) in an 11-day period in 1981

Period	Date August noon-noon	Aircraft Loads[a]	Worker F1 (M + L) 2,4-D (μg/L)	Worker F1 (M + L) 2,4-D (μg)	Worker M1 (M + L) 2,4-D (μg/L)	Worker M1 (M + L) 2,4-D (μg)	Worker F2 (S) 2,4-D (μg/L)	Worker F2 (S) 2,4-D (μg)
Pre-spray	2[b]	0	<0.5[c]	0.3	<0.5[c]	0.6	<0.5[c]	0.3
	2–3	0	<0.5[c]	0.6	<0.5[c]	0.9	<0.5[c]	0.3
Spray Period	3–4	11	6.	11.	4.	5.8	0.5	0.4
	4–5	15	10.	18.	5.	9.3	4.	3.8
	5–6	18	14.	34.	11.	15.	9.	9.5
	6–7	15	44.	75.[d]	15.	12.	14.	15.
	7–8	13	43.	69.	18.	36.	5.	13.
	8–9	10	152.	167.	16.	30.	14.	17.
	9–10	4	323.	339.	22.	34.	16.	17.
	10–11	5	227.	245.	27.	26.	6	8.4
	11–12	3	61.	98.	15.	17.	14.	17.
	12–13	4	191.	248.	20.	15.	3.	5.1
	13–14	11	285.	413.	24.	18.	4.	4.8
Post Spray	14–15	0	326.	489.	45.	66.	7.	14.
	15–16		163.	179.	16.	14.	5.	7.
	16–17		43.	43.	17.	15.	0.5	0.6
	20–21		24.	17.	—	—	—	—
	21–22		—	—	—	—	0.5	0.6
	22–23		—	—	23.	16.	—	—

[a] Mixing was done during each time-period in which loading took place
[b] Midnight to noon—12 hr
[c] Limit of detection was 0.5 μg/L
[d] Gloves torn—direct contact with 2,4-D

occurred, that could account for these levels (Table 4).

1982 Operation

In 1982, the cumulative exposure time was 50, 51 and 39 hr for the three workers during an 18 day spray period extending from 9 to 27 July (Tables 1 and 5). During this period, worker M2 loaded aircraft 135 times and mixed 45,877 L of 10.0–20.0% 2,4-D solution (Table 5). A total of 9,430 L of isooctyl ester (600 g/L a.e.) were mixed with water and 4,010 ha were sprayed. On average, worker M2 excreted 0.80 L of urine and a maximum excretion rate of 22.2 μg 2,4-D/kg body weight/day (Table 3 and 6). During the spray period, worker M2 was over-exposed to 2,4-D on the first day when a hose broke, and also on succeeding days when nozzles on the aircraft became clogged and required cleaning.

Worker M2 wore his respirator with the same cartridges during work duties between 6 and 28 July. Analyses revealed that 356 μg of 2,4-D had been absorbed during that period (Table 6). During the same period he excreted 12,659 μg of 2,4-D (Table 3). These data indicate that if no mask had been worn his urine output would only have been increased by 2.8%, suggesting that 97.2% of the exposure was from dermal contact.

Worker M3 assisted worker M2 from 9 to 16 July in mixing 2,4-D and in cleaning up the work site. On 9 and 15 July, spills occurred that increased his exposure. During the 18-day period worker M3, acting as a balloon man, guided 538 aerial application passes to conifer stands. (Table 5). The pilot of the aircraft shut off 2,4-D release before passing the balloon man, so as not to spray the worker; however, on 13 of the 538 passes (2.4%) the balloon man was aware of being exposed to spray drift. Worker M3 excreted an average 1.2 L urine/day with a maximum excretion rate of 8.37 μg 2,4-D/kg body weight/day (Table 3).

Worker M4 was employed solely in acting as a balloon man. In the 18 day operating period, he marked 498 aircraft passes (swaths) and reported that in only 4 cases (0.8%) did 2,4-D visibly drift over his position (Table 5). His urine output averaged 1.0 L per day and his maximum daily excretion rate was 7.73 μg 2,4-D/kg body weight.

Analysis of urine for the pre-spray period of all three workers indicated that 2,4-D was being absorbed on the work site, although no direct handling of 2,4-D was in progress (Table 6). This can be ex-

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Table 3. Rates and amounts of urine and 2,4-D excreted by the six workers in the pre-spray, spray, and post-spray periods 1981 and 1982

Worker	Parameter	Pre-spray period	Spray period	Post spray period	Total period
F1, M1, F2[a]	Period (d)	1.5	11	7, 9, 8	19.5, 21, 5, 20.5
F1	Total Urine (L)	1.75	16.81	7.53	26.09
	Mean 2,4-D (μg/L)	<0.5	123	94	103
	Total 2,4-D (μg)	0.9	1717	818	2536
	Mean 2,4-D (μg/kg bw/d)[b]	0.012	3.06	2.29	2.55
	Highest 2,4-D (μg/kg bw/d)		8.10	9.59	9.59
M1	Total Urine (L)	2.91	14.50	8.91	26.32
	Mean 2,4-D (μg/L)	<0.5	16.1	22.3	17.6
	Total 2,4-D (μg)	1.5	218	189	409
	Mean 2,4-D (μg/kg bw/d)	0.014	0.28	0.30	0.27
	Highest 2,4-D (μg/kg bw/d)		0.51	0.94	0.94
F2	Total Urine (L)	1.11	14.38	11.10	26.59
	Mean 2,4-D (μg/L)	<0.5	8.1	1.9	5.1
	Total 2,4-D (μg)	0.6	111	24.6	136
	Mean 2,4-D (μg/kg bw/d)	0.007	0.18	0.05	0.12
	Highest 2,4-D (μg/kg bw/d)		0.30	0.25	0.30
M2, M3, M4[a]	Period (d)	3	18	13, 10, 8	34, 31, 29
M2	Total Urine (L)	2.3	15.3	9.5	27.1
	Mean 2,4-D (μg/L)	69	837	423	611
	Total 2,4-D (μg)	156	12659	3617	16432
	Mean 2,4-D (μg/kg bw/d)	0.65	8.79	3.48	6.04
	Highest 2,4-D (μg/kg bw/d)		22.2	9.83	22.2
M3	Total Urine (L)	3.5	22.0	10.9	36.4
	Mean 2,4-D (μg/L)	7.6	261	159	203
	Total 2,4-D (μg)	26.5	5760	1543	7330
	Mean 2,4-D (μg/kg bw/d)	0.11	3.90	1.88	2.88
	Highest 2,4-D (μg/kg bw/d)		8.37	3.50	8.37
M4	Total Urine (L)	2.9	17.5	8.4	28.8
	Mean 2,4-D (μg/L)	4.7	243	109	181
	Total 2,4-D (μg)	13.6	4289	987	5290
	Mean 2,4-D (μg/kg bw/d)	0.060	3.18	1.65	2.43
	Highest 2,4-D (μg/kg bw/d)		7.73	7.31	7.73

[a] Post spray periods were 7d (F1), 8d (F2), 9d (M1), 13d (M2), 10d (M3) and 8d (M4)
[b] μg/kg body weight/d

plained from the results of the analysis of swabs taken from living quarters and equipment (Table 4). Urine analyses conducted 8 to 13 days after the end of the spray period revealed fluctuating 2,4-D excretion rates that failed to follow a first order disappearance equation and can be explained by the fact that all three workers continued to handle contaminated spray equipment and lived in contaminated quarters. The duties of M4 following the spray period brought him into less contact with contaminated equipment and this is reflected in lower post-spray excretion rates.

Bystander

The urine output of the bystander (B1) and his excretion of 2,4-D appears in Table 7. From 9 to 11, 16 to 17 and 23 to 24 August, the subject (B1) was in the vicinity of spray crews or spray equipment, and this is reflected in the pre-spray and post spray residues in the urine. On 9 to 11 and 16 to 17 August there was obviously unintentional contact with 2,4-D although the subject avoided direct contact with the spray crew (Table 4). Following the spray event the residue in the urine rose to 181 μg/L and seven days later declined to <0.1 μg/L (Table 7).

The areas of the various parts of clothing of B2 and B3 available for deposit together with the actual 2,4-D deposits are given in Table 8. The average deposit per unit area for each body part was used to compute that the total deposit on B1 was 164 mg and the deposit on exposed skin was 15.8 mg (Table 9). The intended aerial application rate was 1.62 kg ae/ha or 162 mg/m^2. As the surface area of B1 was 2.16 m^2 and as the spray would deposit principally on one side of the body (with the spray cloud moving horizontally in the same direction as the aircraft), there is reasonable agreement between the calculated and expected deposit. Assuming that the exposed bystander (B1) excreted all the 2,4-D which he absorbed, then the total 2,4-D absorbed

DOW21562287

17505

Table 4. Residues of 2,4-D in swabs taken from trucks, helicopters, offices and living quarters associated with two forestry spray programs

Residue of 2,4-D in μg/0.1 m² surface					
Living & eating quarters (after 1982 season)[a]		Living & eating quarters (after 1983 season)[a]		Supervisor's office (after 1983 season)	
Sink	6.4	Floor	4.8 ± .3	Chair arms	15
Counter top	3.9	Counter top	0.74	Desk—right	12
Door knobs	18 ± 25	Door knobs	9.8 ± 7.1	Desk—left	1.8
Refrigerator door handle	288	Refrigerator door handle	69		
Table tops	1.04 ± 0.73	Table tops	0.89 ± 0.53		
		Freezer door handle	12		
Sample numbers	13		15		3

	Seven trucks used 1982 and 1983	One truck not used on site		Two helicopters 1983	Control Swabs
Seats	0.8 ± 0.9	0.7	Seats	2.2 ± 3.1	<0.1
Steering wheel	34 ± 30	2.5	Controls	72 ± 12	
Door handles	45 ± 68	13	Door latches	6.7 ± 7.1	
Brake pedal	184	—	Pilot's helmet	12	
Tools	100	—	Passenger headsets	7.3 ± 5.4	
Sample numbers	34	3		14	5

[a] Nine months after 1982 season, immediately following 1983 season

Table 5. Work performance by three workers in 1982

Date	Worker M2 (M + L)		Worker M3 (M + B)				Worker M4 (B)		
July–Aug. noon–noon	Mixed (L)	Aero[a] loads	Mixed (L)	Swaths[b] (no.)	Rate (kg ae/ha)	Accid. sprayed	Swaths[b] (no.)	Rate (kg ae/ha)	Accid. sprayed
9–10	7328	3[c]		72	1.1	2	70	2.2	1
10–11	2452	0	2452	0	—	—	—	—	—
11–12	—	1	—	4	2.2	0	—	—	—
12–13	6461	19	6461	46	2.2	0	72	2.2	0
13–14	2797	22	—	89	2.2	5	8	2.2	0
14–15	—	6	—	25	2.2	0	79	2.2	1
15–16	6151	9	3677[d]	30	1.1	0	48	1.7	0
19–20	3664	13	—	52	1.1	—	96	1.7	1
20–21	2474	3	—	6	1.1	0	—	—	—
21–22	—	0	—	—	—	—	9	1.7	0
22–23	1203	15	—	40	1.1	6	16	1.7	0
23–24	6083	12	—	45	1.1	0	35	1.1	1
24–25	—	0	—	4	1.1	0	—	—	—
25–26	7264	22	—	73	1.1	0	30	1.1	0
26–27	—	10	—	52	1.7	0	35	1.7	0
Total	45877[e]	135	12590	538	—	13	498	—	4

[a] Each aircraft load 340 L
[b] Each swath 40 m wide
[c] Hose punctured while loading
[d] Spilled 2,4-D on leg
[e] Contained 9,430 L of 2,4-D concentrate.

was 716 μg (Table 7). This means that B1 absorbed only 0.44% of the 164 mg of 2,4-D deposited on his clothing and skin.

Assuming that little 2,4-D was absorbed through the head, which was covered with hair (beard and mustache as well as scalp hair), nor through the torso which was clothed, then most of the absorption would have had to occur through the exposed skin of legs and arms. The 716 μg of 2,4-D absorbed by B1 represents 4.5% of the 2,4-D

DOW2156288

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Table 6. Daily excretion of urine and 2,4-D by the three workers loading aircraft and directing spraying in 1982

Period	Date July–Aug.	Worker M2 (M + L)[a] 2,4-D (μg/L)	Worker M2 (M + L)[a] 2,4-D (μg)	Worker M3 (M + B) 2,4-D (μg/L)	Worker M3 (M + B) 2,4-D (μg)	Worker M4 (B) 2,4-D (μg/L)	Worker M4 (B) 2,4-D (μg)
Pre-spray	6–7	105	74	10.1	12	4.3	4.3
	7–8	70	56	4.1	4.5	4.1	3.7
	8–9	33	26	8.6	10	5.6	5.6
Spray	9–10	55	44	10.8	13	23	23
	10–11	1289	902	191	210	137	123
	11–12	963	770	181	199	146	146
	12–13	651	521	163	196	211	211
	13–14	1151	921	526	631	255	281
	14–15	845	761	202	222	214	214
	15–16	2153	1722	542	596	286	286
	16–17	2219	1775	556	667	417	375
	17–18	569	398	237	284	192	192
	18–19	363	290	75	90	89	98
	19–20	190	171	105	126	84	84
	20–21	231	208	141	169	401	401
	21–22	945	1134	276	331	368	368
	22–23	375	450	344	482	527	580
	23–24	659	527	429	686	507	456
	24–25	1821	1639	255	77	191	172
	25–26	434	304	193	386	113	68
	26–27	152	122	263	395	211	211
Post-spray	27–28	984	787	288	230	274	548
	28–29	586	293	105	74	237	119
	29–30	943	377	287	287	24	19
	30–31	625	500	261	261	136	82
	31–1	1090	654	347	278	40	40
	1–2	318	286	63	107	73	73
	2–3	88	70	57	91	47	47
	3–4	89	53	75	98	39	59
	4–5	170	204	45	32	—	—
	5–6	242	97	65	85	—	—
	6–7	136	95	—	—	—	—
	7–8	164	131	—	—	—	—
	8–9	70	70	—	—	—	—

[a] The charcoal and paper air filter taken from the respirator of M2 yielded 356 μg 2,4-D

calculated to have deposited on bare skin of arms and legs. This is a maximum value, since some 2,4-D was probably absorbed through the thin, tight fitting clothing worn by B1. The excretion of 716 μg of 2,4-D represents 8.5 μg 2,4-D body weight for the single exposure to subject B1.

Following exposure, the highest excretion of 2,4-D occurred on day 1, *i.e.*, 4.75 μg/kg body weight (Table 7). For the following eight days, the 2,4-D excretion declined with a half-residue decline of 16 hr and followed a first-order logarithmic equation where $\log Y = 2.73 - 0.44X$ ($r^2 = 0.94$ and $F = 86.9$) (y = excretion of 2,4-D in μg and x = time days).

Surface Swabs 1982 and 1983

In spite of having stayed away from the actual spray operation, the bystander lived and ate in the same quarters as the spray crew and excreted 2,4-D in his urine in the pre-spray period (Table 7). The three workers from the 1982 spray operations also excreted 2,4-D in pre- and post-spray periods when they were not handling 2,4-D. These unexplained residues prompted the authors to conduct a swabbing survey of vehicles, helicopters, and living quarters for 2,4-D residues. Results of 87 swabs presented in Table 4 indicate contamination of all internal surfaces of living and eating quarters, and internal surfaces of vehicles and helicopters used on the site.

For those residing in the field living quarters (mobile trailers located in remote area) in 1981 and 1982, water supplies for washing were limited and hence washing of sinks, counter tops, and refrigerators was also limited. Residues on swabs taken in 1983 were considerably lower than those col-

DOW2156289

17507

DOW2156290

Table 7. Urine excretion of bystander (B1) and amounts of 2,4-D voided following a single overspray at a rate of 162 mg 2,4-D aerm^{-2} on 17 August 1982 (Subject 38 yr old, weighed 84 kg and 196 cm tall)

Date[a] Aug./82	Urine (L)	2,4-D µg/L	2,4-D µg
15–16	1.3	10.7	13.9
16–17[b]	1.9	64.7	123
17–18	1.9	129	245
18–19	2.2	181	399[c]
19–20	1.6	16.0	25.6
20–21	1.4	16.6	23.2
21–22	2.0	5.6	11.2
22–23	2.1	3.3	6.93
23–24[b]	1.6	0.3	0.48
24–25	1.8	0.3	0.54
25–26	1.7	<0.1	0.17
26–27	1.7	<0.1	0.17
27–28	1.5	1.0	1.50
28–29	1.8	1.0	1.80
29–30	1.3	<0.1	0.13
30–31	1.8	<0.1	0.18
Means & Totals		µg/kg bw/d	µg
Pre-spray[b] (15–17)	3.2	0.82	137
Post spray (17–31)	24.4	0.61	716

[a] Sampling period commenced 0630 each day
[b] Around spray equipment
[c] 399 µg/d = 4.75 µg/kg bw/d

lected after the 1982 spray season due to improved water supplies provided for washing. The highest residues were observed on the handles and sides of refrigerators. Tables and sinks also had residues of 2,4-D.

The office of the supervisor revealed greater residues on the right hand side of this desk (12 µg/0.1 m^2) as compared to the left hand side (1.8 µg/0.1 m^2); the supervisor being righthanded. In comparing the trucks used for spraying, residues were observed on brake pedals, tools, door handles, and steering wheels. A newly purchased truck used on the site but not for spraying, also contained residues of 2,4-D on the same parts as on used trucks, albeit the residues were lower. This contamination of internal surfaces of vehicles, and of living quarters could explain why 2,4-D appeared in urine of workers in both the pre- and post-spray periods.

Discussion

Workers involved in mixing and loading aircraft, supervising the work and acting as balloon men absorbed low levels of 2,4-D in spite of the fact that precautions were taken to greatly reduce contact with the chemical. The 1981 crew (F1, F2 and M1) had lower urine levels of 2,4-D than the 1982 crew (M2, M3 and M4). This difference may be a consequence of the fact that the 1981 crew was inexperienced and hence possibly more cautious or it may be a reflection of the fact that the 1981 spray mix was less concentrated (6.5% vs 10–20%). Based on observation by the authors of spray operations, the following duties were considered to be in order of increasing direct exposure to workers:

1. Supervising and helping in the mixing and loading of aircraft.
2. Mixing concentrate and diluent
3. Loading aircraft or acting as balloon man
4. Cleaning up work site and washing drums
5. Spills or leaks during duties listed in 2 and 3, or overspraying of worker performing duties under 3

Based on the duties performed in 1981, F1 was the most exposed worker while F2 was the least exposed. This is borne out in the excretion of 2,4-D by these two individuals. Although worker M1 apparently had similar exposure to F1 his excretion of 2,4-D was closer to F2. In 1982, the potential exposure of workers M2, M3 and M4 were considered to be in descending order and this is borne out in the 2,4-D excreted by M2 which was almost double that of M3 and M4 being lower than M3.

Nash *et al.* (1982) reported that the mean daily urinary excretion of 2,4-D for mixer loaders involved in aerial applications was 20 µg/kg body weight with intermittent exposure. Our mixers and loaders averaged between 0.18 and 8.79 µg/kg body weight/d.

Based on an evaluation of published information on non-acute toxicity in mammalian species, a dose of 10 mg 2,4-D/kg body weight/day would appear to be near the no-observed effect level (NOEL) in laboratory animals (Hansen *et al.* 1971; Drill and Hiratzka 1953). Thus, for the bystander in this worst case situation (being directly sprayed) there was a 1176-fold safety factor. If an 18-hr half time for excretion of 2,4-D is assumed, the highest single day excretion by a worker (22.2 µg/kg body weight/day) can be extrapolated back to a maximum absorbed dose of 60 µg/kg body weight/day. Using the NOEL of 10 mg/kg body weight/day there was a 166-fold safety factor. Lavy *et al.* (1982) reported that the absorbed dose determined by urine analysis ranged from "not detected" to 55.7 µg/kg body weight in one subject and 23.7 µg/kg in a second subject.

Previous studies by Gehring and Betso (1978); Kohli *et al.* (1974); Sauerhoff *et al.* (1977); using human volunteers, showed that 2,4-D was absorbed from the gastro-intestinal tract into the blood plasma by a first-order rate process with a half life of approximately 12 hr. The herbicide was elimi-

17569

DOW2156291

Table 8. Surface areas of clothing worm by two subjects accompanying the bystander B1 and deposits of 2,4-D on the clothing

Part of clothing	Bystander B2			Bystander B3			Average
	Surface area	2,4-D deposits		Surface area	2,4-D deposits		2,4-D deposit
	m^2	mg	mg/m^2	m^2	mg	mg/m^2	mg/m^2
Hood	0.170	2.08	12.3	0.170	18.5	109	60.7
Arms	0.429	9.04	21.1	0.453	10.0	22.1	21.6
Torso	0.999	104	104	0.684	134	196	150
Legs	0.873	15.5	17.8	0.862	20.0	23.2	20.5
Total	2.47	131	52.9	2.17	183	84.3	68.6

Table 9. Surface areas of the parts of bystander B1's body and estimated 2,4-D deposits on each part

Part of body	Surface-area		2,4-D deposits	
	Total	Bare skin	Total	Bare skin
	m^2	m^2	mg	mg
Head	0.206		12.5	
Arms	0.399	0.251	8.61	5.41
Torso	0.859		129	
Legs	0.695	0.510	14.2	10.4
Total	2.16	0.761	164	15.8

nated essentially unchanged into the urine, also by an apparent first order rate process with 18–24 hr half life. (Gehring and Betso 1978; Libich *et al.* 1984; and Sauerhoff *et al.* 1977). Lavy (1980) reported that 2,4,5-T which is similar to 2,4-D can be absorbed through the skin and eliminated in the urine at a first order rate with a half life of 18 and 23 hr, respectively. In the model developed by Libich *et al.* (1984), the percutaneous absorption rate through the skin for 2,4-D was assumed to proceed with a half life of 12 hr, which was similar to that of absorption through the gastro-intestinal tract and was in the same ratio as the absorption and elimination rates for 2,4,5-T. In our study, the decline of 2,4-D in the urine of the bystander following the spray event had first order kinetics with a half time of 16 hr. With all six workers, indirect exposure to 2,4-D occurred after the spray operation through contact with contaminated equipment, vehicles and other objects at the camp site and, as a result, residues in their urine fluctuated from day to day and half life declines in 2,4-D excretion could not be calculated.

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Sauerhoff MW, Braun WH, Blau GE, Gehring PJ (1977) The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. Toxicology 8:3–11

Manuscript received July 16, 1984 and in revised form October 26, 1984.

17509

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521

HEALTH ASPECTS OF PESTICIDES

abstract bulletin

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June 1972 - Vol. 5, No. 6

Abstracts 72-1125-1353

Quarterly Index Issue

17510

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72-1163 Ganstad, E. O. (Planning Div., Civil Works Directorate, Office of the Chief of Engineers, Washington, DC) **Herbicidal control of aquatic plants.** *J. San. Eng. Div.* [illegible] *Soc. Civ. Eng.* [illegible] 1972. 19 references [illegible]

Removal of excessive aquatic plant growth required for optimal water resource utilization was performed by mechanical means at first but is now generally accomplished with the aid of chemicals, especially 2,4-D. The use of herbicides in accordance with recommendations for this purpose will generally have little or no deleterious influence on water quality, crop plant growth or wildlife. The 2,4-D residue levels for various application rates, depths and ambient temperatures indicate a [illegible] drop within 1 month. Herbicides registered for use on aquatic sites are reviewed. The regulations governing the use of these chemicals, however, leave something to be desired in the specification of use limitations, since rarely is a body of water used for a single purpose, and water frequently moves from one type of area to another before its herbicide content is entirely dissipated.

72-1164 Gillette, R. H. (Author address not given) **Oceans as alphabet soup: focus on DDT and PCB's.** *Sci. News 102* [illegible] 1972.

Recent studies on the occurrence of DDT and PCBs (polychlorinated biphenyls) in marine organisms have provided a wealth of facts, but the significance of the findings is difficult to determine. Results of studies performed by investigators from the Woods Hole Oceanographic Institution and presented at the Nobel Symposium in August of 1972 indicate that ingestion of DDT and PCBs by multicelled organisms which feed at the sea surface at night but migrate to considerable depths during the day may accelerate transport of organochlorine compounds from the biologically active upper ocean layers to the "abyssal sink." This removal process may explain why large-scale extinctions of life forms have not occurred, although environmental DDT concentrations are theoretically high enough to have caused them. The absence of the anticipated organochlorine concentration gradients in relation to land sources suggests that the atmospheric mode of organochlorine distribution is more important and the waterborne route less so on a worldwide scale, than previously assumed. An unexpectedly broad range of variability in DDT and PCB concentration up food chains was also encountered, possibly due to the significance of [illegible] food chains [illegible] pathways in aquatic organisms. Sediment [illegible] on the [illegible] expedition will be analyzed [illegible] organochlorine removal from the water column [illegible]

72-1165 Moriarty, F. (Monks Wood Experimental Station, Huntingdon, England) **Pollutants and food chains.** [illegible] 1972.

[illegible] possibility [illegible] concentration is reached [illegible] that [illegible] may absorb much more pollutant from the water [illegible] food [illegible] that [illegible]

72-1166 [illegible] **Thermal behavior of allethrin at 150 C in atmospheric environment.** [illegible]

[illegible] the atmosphere by heating [illegible] analyzed. Authentic [illegible] of possible decomposition products formed by isomerization, polymerization and hydrolysis of the starting material were synthesized and compared by GLC with the [illegible] constituents detected in the experiment. Under the vaporization conditions [illegible] of the allethrin vaporized without undergoing changes [illegible] decomposed [illegible] allethrin [illegible] has not been [illegible]

72-1167 [illegible] R. A. [illegible] (Dept. of [illegible] University of California, Davis, CA [illegible]) **Kelthane residues on Gravenstein apples and pomace: application and removal.** [illegible] 1972. [illegible]

Gravenstein apples [illegible] Kelthane [illegible] and its principal decomposition product [illegible] procedure [illegible] Kelthane [illegible] weight [illegible]. After [illegible] and [illegible] the peels were found to contain [illegible] the total residue [illegible] and the [illegible] probably due to contamination [illegible] washing with water [illegible] to the [illegible]

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526

activities and the cytoplasmic bromosulfophthalein (BSP)-glutathione conjugating enzyme system. The biphenyl nucleus played little role since biphenyl caused no induction of hepatic drug-metabolizing enzymes. Monochlorobiphenyl (4-Cl) was without influence; of the di- and tetra- chlorobiphenyls, substitution of chloro groups at the 3 and 4 positions resulted in enhanced induction of microsomal monooxygenases. The more highly chlorinated hexa- and octa- chlorobiphenyls caused an enhanced induction of monooxygenases, though differing little from one another. Nitro-reductase and carboxylesterase activities were not significantly affected by any of the agents whereas all agents, including biphenyl, caused a marked induction of the BSP-conjugating system.

528

6. ***The Fate of 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) Following Oral Administration to Man.*** P. J. Gehring, C. G. Kramer, B. A. Schwetz, J. O. Rost, V. K. Rowe, and J. S. Zimmer, The Dow Chemical Company, Midland, Michigan.

Five human male volunteers ingested a single dose of 5 mg kg 2,4,5-T without presenting detectable clinical effects. Concentrations of 2,4,5-T in plasma and its excretion were measured at intervals after ingestion. The clearances from the plasma, as well as the body, occurred via apparent first order rate processes with half-lives of 23.1 and 29.7 hr respectively. Essentially all of the 2,4,5-T was absorbed into the body and excreted unchanged via the urine. In the body, 65% of the 2,4,5-T resided in the plasma where 95 % was bound reversibly to protein. The volume of distribution was 0.097 liter kg. Utilizing the kinetic constants from the single dose experiment, the expected concentrations of 2,4,5-T in the plasma of individuals receiving repeated daily doses of 2,4,5-T were calculated. From these calculations, it was determined that the plasma concentrations would essentially reach a plateau value after 3 days. If the daily dose ingested in mg kg is I_m, the concentrations in the plasma after attaining plateau would range from $12.7 I_m$ to $22.5 I_m$ μg ml. This range would converge to approximately $17 I_m$ ug ml as the daily dose I_m is distributed throughout the day.

527

7. *Effects of 2,4,5-T and other organochlorides on the metabolism of [1,2-^{3}H]testosterone by male organs of reproduction.* J. W. Lloyd, J. A. Thomas, M. G. Mawhinney, and C. S. Durairaj, Department of Pharmacology and Urology, West Virginia University Medical Center, Morgantown, West Virginia.

Male mice treated with varying doses (6.25, 12.5 or 25 mg kg daily × 10, po) of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) led to a significant reduction in the ability of the prostate gland to assimilate radioactive testosterone (^{3}H-T). Regardless of the dosage, the average reduction in the uptake of ^{3}H-T by the prostate gland was about one-third. This treatment period did not cause any significant changes in the relative amounts of ^{3}H-T metabolites in the prostate, but the absolute amounts of at least three radiosteroids were lowered by this herbicide. The highest dose regimen of 2,4,5-T (viz. 25 mg kg daily × 10) caused a 33% reduction in the levels of dihydrotestosterone-^{3}H, a 37% reduction in androstanediol-^{3}H and a 14% reduction in prostatic androstenedione-^{3}H. Acute dose regimens failed to alter the assimilation of labeled testosterone by the mouse prostate gland. The 10-day dose regimen of 2,4,5-T had no significant effect upon the bioconversion of ^{3}H-T to its radiosteroid metabolites in homogenates of liver. Treatment with 2,4,5-T did not alter either the gravimetric responses of the male sex accessory organs nor did it affect prostate gland fructose levels. Although sex accessory organ [illegible]cAMP and ATP levels were unaffected by the 10-day administration of 2,4,5-T, preliminary data indicate that either dieldrin or DDT can alter these biochemical parameters. (Supported in part by a grant from the Environmental Protection Agency FPA 801630-02.)

8. *The Renal Transport of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) in vitro.* W. O. Berndt and T. Kostrubsky, Department of Pharmacology and Toxicology and Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire. (R. P. Smith).

The herbicides, 2,4-D and 2,4,5-T, are both weak organic acids and might be expected to be transported actively by renal tissue. This proposition has been examined using the tissue slice



17512

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technic, whereas tubule uptake is equated to renal tubular secretion. Both rat and rabbit renal cortical slices were prepared by the free-hand procedure. All incubations were performed at 25 C with 100% oxygen unless otherwise stated. The time-course of accumulation by rabbit tissue was characterized by a rapid initial phase and a slower secondary phase of uptake, although no steady state was reached after 4-5 hr of incubation. Usually 2,4,5-T was accumulated to a greater extent than 2,4-D. For example, at 3 hr the tissue slice:medium distribution ratio (S/M) for 2,4,5-T was 46 and for 2,4-D, 29. The uptake of both compounds was stimulated by acetate, pyruvate, and lactate, and inhibited by succinate. The metabolic inhibitors, 2,4-dinitrophenol and iodoacetamide, decreased uptake by 90%, as did nitrogen. The competitive inhibitor of renal organic acid transport, probenecid, reduced the S/M by 80%. Similar transport characteristics were noted for rat renal cortical slices. In addition a significant potassium requirement was noted for the uptake of both acids. These data are interpreted to mean that both herbicides are secreted actively by the mammalian kidney. Such a process may account for the relatively rapid plasma clearance reported in some *in vivo* studies. (Supported by PHS grant AM 13020).

9. *Non-teratogenicity of 2,4,5-Trichlorophenoxyacetic Acid in Monkeys (Macaca mulatta).* W. H. Doucette, J. Courtois, and L. Gerard, Albany Medical College, Albany, New York.

Reports that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) affects embryonic development in various species, mainly rodents, have left unresolved the question of potential hazard to man resulting from exposure to 2,4,5-T containing a minimal amount of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). An answer to this problem was sought by studying the action of 2,4,5-T in the pregnant nonhuman primate *M. mulatta*. Mature female rhesus monkeys were mated with males of proven fertility. Forty pregnant females were assigned to either the control group, or one of the dose-level groups: 0.05, 1.0, and 10.0 mg/kg. Technical Grade 2,4,5-T containing less than 0.05 ppm TCDD was administered in a No. 5 gelatin capsule by stomach tube daily from day 22 through day 38 of pregnancy. Of the 10 control monkeys, 9 delivered live offspring. (The spontaneous abortion rate among untreated females in our colony is approximately 12%.) Of the 10 monkeys in the 0.05 mg/kg group, all gave birth to normal babies. Seven of the monkeys receiving 1.0 mg/kg doses delivered live offspring; there was 1 stillborn fetus, and 1 abortion, and 1 female has yet to deliver. Six of the monkeys receiving 10.0 mg/kg doses had live offspring; there were 2 abortions, and 2 females have not delivered. Hematology, clinical chemistry, and urinalysis data obtained on all females prior to and immediately following the administration of the 2,4,5-T and at 2-month intervals until parturition indicated no toxicity to the female. Detailed examination of the live infants and the stillborn fetus, including skeletal X-ray studies, revealed no gross developmental abnormality in any of the groups. Under the conditions of this experiment there is no evidence that 2,4,5-T is teratogenic in the rhesus monkey, nor that it interferes in any way with normal development of the young. (Supported by Research Grant 2P01-ES00226-06 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant No. 5T01-ES00103-06.)

10. *The Significance of an Adaptation Phenomenon in Toxicity Studies.* T. Balazs, Food and Drug Administration, Washington, D.C.

Long term [illegible] studies in animals may overlook what happens in the very early stages of the treatment. [illegible] the repeated administration of various chemicals, certain degenerative tissue changes occur rather early; but under continued dosage healing occurs and apparent recovery [illegible] the toxic effect develops. W. de B. McNider (1928) and others described this [illegible] for renal and hepatic tissues; others described it for the gastric and alveolar epithelium and for the myocardium. This type of tissue resistance has several common features: (1) It develops only after an induced lesion. (2) It is not specific to the inducing agent, but extends to agents causing similar lesions. (3) It lasts during the period of dosing as well as for varying times thereafter. (4) Its duration after the interruption of the dosing appears to be a function of the turnover rate of the affected morphological or biochemical

17513

537

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[1975]

1

AU - Enikeeva NA

AU - Elina VA

AU - Dumkina GZ

AU - Murtazina LF

AA - USSR

TI - Industrial hygiene and health status of workers producing the herbicide amine salt of 2,4-D

SI - CA/086/160445C

SO - v sb., Aktual'n. Vopr. Gigieny Truda, Prof. Patol. i Tiksikol. v Neft., Neftekhim. i Khim. Prom-sti; 1975,45-50

LA - Russ

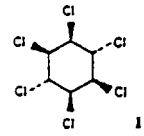
AB - CBAC COPYRIGHT: CHEM ABS Title only translated. Dichlorophenoxyacetic acid amine health hazard Herbicide manuf health hazard

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of plasma and red cell *cholinesterase* [9001-08-5] than did a nonindustrial population. The workers' subjective health complaints were correlated with lower enzyme levels and with pesticide exposure, and can not simply be ascribed to the anxiety engendered by their exposure.

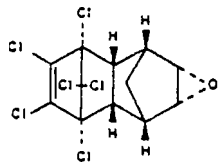
68995b **Measurements of multipolar anaphases production by γ-hexachlorocyclohexane in onion root tip cells.** Hervas, J. P.; Gimenez-Martin, G. (Dep. Citol., Inst. Biol. Celular, Madrid, Spain). *Cytobiologie* 1974, 9(2), 233-9 (Eng).



I

γ-Hexachlorocyclohexane (I) [58-89-9], which blocks cellular mitosis, induced the formation of multipolar anaphases in onion root tips. The frequency of this abnormal anaphase segregation was related to the I-exposure time of dividing cells. I may initiate the multipolar effect by acting on the mitotic spindle.

68996c **Toxicity of dieldrin (HEOD) to domestic fowl.** Brown, Vernon K. H.; Robinson, John; Thorpe, Ernest; Barrett, John W. (Tunstall Lab., Shell Res. Ltd., Sittingbourne/Kent, Engl.). *Pestic. Sci.* 1974, 5(5), 567-86 (Eng). Domestic fowl



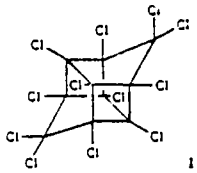
I

were fed control diet or diets contg. 10 or 20 μg/g *dieldrin* (I) [60-57-1]. Eggs and samples of blood were collected at intervals and the concn. of I was detd. The concns. of I in body tissues were also detd. after 7 and 13 months. Some of the birds died, but the health of the remainder appeared to be unaffected. The hen of the 20 ppm group showed an increased egg prodn., but chick mortality in this group was higher than in the other two groups. The dynamics of I in domestic fowl were qual. similar to that in other vertebrates and were consistent with the mamillary type compartmental model.

68997d **Demeton-O-methyl and dimethoate absorption by apple tree leaves.** Nowakowski, Zenon (Inst. Sadow. Skierniewice, Skierniewice, Pol.). *Postepy Nauk Roln.* 1974, 21(3), 25-30 (Pol). Only 11-27.6% of the labeled *dimethoate* [60-51-5] sprayed on 3-year-old apple trees at 100 ml of a 1% soln./tree was absorbed by the leaves within the first 3 days after application. Absorption of *demeton-O-methyl* [867-27-6] was much faster, 25, 40, and ~50% of the insecticide having penetrated the leaves 15 min, 4 hr, and 8 hr after application, resp.

68998e **Effect of DDVP on the cellular cycle in HEB and FL cell cultures.** Piekarski, Lech; Tokarz, Andrzej; Kugaczewska, Maria; Karlinski, Stefan (Zakl. Bromatol., Akad. Med., Warsaw, Pol.). *Bromatol. Chem. Toksykol.* 1974, 7(3), 341-5 (Pol). *DDVP* [62-73-7] decreased the mitotic index and increased the no. of cultured human amniotic cells (FL cells) in S phase, but did not have a similar effect on cultures of bovine embryonic liver cells (HEB cells).

68999f **Effects of mirex on litter organisms and leaf decomposition in a mixed hardwood forest in Athens, Georgia.** Lee, Beatrice Joy (Inst. Ecol., Univ. Georgia, Athens, Ga.). *J. Environ. Qual.* 1974, 3(4), 305-11 (Eng).

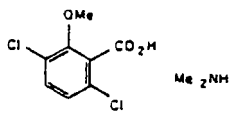


I

Application of *Mirex* (I) [2385-85-5] in corncob grit bait or in spray form to oak and dogwood leaf litter in mesh bags accelerated litter decompn. Ants were removed from bait and concd. spray plots up to 79 days, but had returned by the 334th day. Little difference was obsd. in macroarthropod populations except centipedes whose nos. appeared to decrease on the bait and concd. spray plots. Microarthropod populations showed significant differences with treatment, at different times during the expt., but no overall pattern could be detected that might explain the leaf decompn. acceleration noted with all treatments. Since microarthropods, macroarthropods and microflora are known to be involved in the decompn., and since neither arthropods could account for the change in decompn., the most probably affected by I would be the bacteria and fungi. These organisms were not monitored. I may accelerate decompn. through its ability to induce mixed-function oxidase in the microorganisms. Dogwood leaves were more affected than oak leaves.

69000k **Hygienic evaluation of the aftereffect of a complex of pesticides used in agriculture.** Dmitrieva, O. V. (Ryazan. Med. Inst. im. Pavlova, Ryazan, USSR). *Gig. Sanit.* 1974, (9), 107-9 (Russ). Decreases in the level of sulfhydryl groups and activity of *lactate dehydrogenase* [9001-60-9] in the blood serum were detected in human subjects, professionally exposed for 6 years to fungicides and pesticides, used in horticulture. Oral administration of *granosan* [107-27-7] at 0.05 LD_{50} to rats for 15 days before they were given *anthio* [2540-82-1] at a similar dose, increased the toxicity of the latter. Decreases in the activity of *cholinesterase* [9001-08-5] in the blood, esp. in the brain were obsd.

69001m **Effect of Banvel-D on several characteristics of the nitrogen metabolism of pea plants.** Ladonin, V. F.; Samoilov, L. N.; Posmitnaya, L. V.; Gruzdev, L. G.; Dyukina, N. N. (Vses. Nauchno-Issled Inst. Udobr. Agropochvoved., Moscow, USSR). *Agrokhimiya* 1974, (9), 120-7 (Russ).

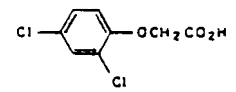


I

When sprayed in 0.02% solns. on pea plants at the 5-6 leaf pair stage, *Banvel D* (I) [2300-66-5] decreased the level of protein in the leaves and stems and changed the ratios between particular free amino acids. No changes were obsd. in the amino acid compn. in protein hydrolyzates.

K-2372

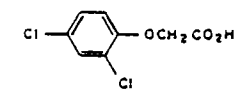
69002n **Cancerogenic properties of the herbicide ammonium 2,4-D amine salt.** Arkhipov, G. N.; Kozlova, I. N. (Lab. Kantserogenov, Inst. Pitan., Moscow, USSR). *Vopr. Pitan.* 1974, (5), 83-4 (Russ). *2,4-D amine salt* (I amine salt)



I

[2307-55-3] given to 165 rats in the diet at 1/10th the LD_{50} dose produced mammary gland fibroadenoma in 1 rat after 27 months and in another one after 31 months and produced hemangioma in 1 rat after 31 months. When administered orally or applied cutaneously at 1/10th the LD_{50} to CBA X C57/BL mice for 33 months, no tumors developed. However, in these mice this herbicide had cocarcinogenic effects with 3-methylcholanthrene when both were applied to the same area of skin.

69003p **Effect of 2,4-D on the RNase activity of tissues.** Eidel'nant, N. M.; Usmanova, R. F. (Sredneaziat. Nauchno-Issled. Inst. Fitopatol., Tashkent, USSR). *Agrokhimiya* 1974, (9), 128-30 (Russ). When sprayed at 100 μg/specimen on cotton



I

plants at budding, *2,4-D* (I) [94-75-7] increased the activity of *RNase* [9001-99-4] in the leaves and apex. The increase in enzyme activity was not accompanied by a decrease in the level of RNA.

69004q **Action of polychloropinene on the biligenic function of the liver.** Glukhova, L. G. (Ternopol. Med. Inst., Ternopol, USSR). *Farmakol. Toksikol. (Moscow)* 1974, 37(6), 699-701 (Russ). Polychloropinene (I) given to rats in large doses (1/2 LD_{50}), inhibited the intensity of bile secretion, and the excretion of *cholesterol* [57-88-5] with the bile. Also, I disrupted the bile's stabilizing properties.

69005r **Histochemical changes of oxidative-reductive enzymes in internal organs of a rat during multiple treatment with TMTD.** Agaeva, T. M.; Ismailov, O. B. (Azerb. Gos. Med. Inst. im. Narimova, Baku, USSR). *Sovrem. Probl. Biokhim. Dykhaniya Klin., Mater. Vses. Konf., 2nd* 1971 (Pub. 1972). 1, 250-1 (Russ). Edited by Usol'tseva, V. A. Ivanov. Gos. Med. Inst.: Ivanovo, USSR. When administered orally at 37 mg/kg/day to rats for 7 days, *TMTD* [137-26-8] inhibited the activities of *NAD diaphorase* [9079-67-8], *NADP diaphorase* [9001-68-7], and *succinic dehydrogenase* [9002-02-2]

DOW215618Z

46 AU - Konstantinova TK ; Shevelo FS
TI - Mutagenic activity of the combined effect of the herbicides 2,4-D and 2,4,5-T
SI - CA/099/017746Q
SO - Gig. Sanit.; ISS 4, 1983,21-2
LA - RUS
AB - CBAC COPYRIGHT: CHEM ABS The combination of 2,4-D (I) [94-75-7] and 2,4,5-T (II) [93-76-5] (given via the stomach as the Bu esters) was mutagenic in rats in the dominant lethal assay. II was considerably more mutagenic than I. The more mutagenic effect of I and II together, at a smaller dose, exerted a general toxic effects, suggesting selective action of these herbicides.

DOM2156181

20 AU - Konstantinova TK ; Shevelo FS
AD - Sarat. Med. Inst., Saratov
TI - Cytogenetic activity of the combined effect of the herbicides 2,4-D and 2,4,5-T
SI - CA/101/224518S
SO - Gig. Sanit.; ISS 9, 1984,28-9
LA - RUS
AB - CBAC COPYRIGHT: CHEM ABS A combined exposure of 2,4-D [94-75-7] and 2,4,5-T [93-76-5], at doses close to threshold or at levels detected in the environment, showed cytogenetic effects. A combination of doses (0.05 mg/kg) caused a greater cytogenetic effect in the rat bone marrow cells than that exerted by individual herbicides. This reflects a potentiating effect of the herbicides. The cytogenetic effect of the combined herbicides at small doses was selective and specific. The highest mutagenic effect in the bone marrow cells was from the

17517

DOM2159155

13 AU - BISHUN NP ; WILLIAMS DC ; MILLS J ; LLOYD N ; RAVEN RW ; PARKE DV
TI - Cromosome damage induced by chemicals.
SI - HEEP/74/04127
SO - CHEM-BIOL INTERACTIONS; 6 (6). 1973 375-392
AB - HEEP COPYRIGHT: BIOL ABS. During the past half century there was a substantial increase in the number and extent of usage of drugs, food additives, pesticides and other environmental chemicals. As a result mutation-induced susceptibility to disease, once largely self-eradicating, is now conserved and even propagated. In addition, over the same time period, there were added to human internal and external environments, numerous synthetic chemicals, the cytogenetic properties of some of which were reviewed, and to which man's metabolism has not necessarily been conditioned and adapted, and which may have the potential to augment the number of mutations of each succeeding generation. Far more must be learned about what humans are doing to themselves and their planet, and the fetuses of unborn children, and to the genetic heritage of these children, by the permissive use of the chemical wonders. It would be impracticable that one should wait for time to reveal harmful mutations before the chemical mutagens are researched and eliminated. Chromosomal studies and evaluation of mutagenic potential of all new and and established drugs, food additives and environmental chemicals should be integral aspects of the current practices of safety evaluation of these materials, and the same stringent principles should be applied to the numerous chemicals met with as solvents and intermediates in industrial processes. Persons exposed to high risk, such as those working in chemical industries, or patients on continous chemotherapy, should be offered routine chromosomal monitoring, and the appearance of adverse effects of abnormalities notified and correlated.

DOM21561150

8 AU - BALO-BANGA MJ ; ANTONI F ; ALTMANN H ; VINCZE I ; KOCSIS F
TI - EFFECT OF 2,4-D ON SEMICONSERVATIVE- AND DNA-REPAIR SYNTHESIS (CASE REPORT: BULLOUS DERMATITIS AFTER EXPOSURE TO 2,4-D)
SI - EMIC/73/014461
SO - BER OESTERR STUDIENGES ATOMENERG; NO.2218:1-7,1973
LA - ENG
AB - EMIC/ORNL SEE: CA 81-10307

17519

DOW215611 49

76 AU - Anon
AO - JRB Associates, Inc., McLean, VA.
TI - Review of Literature on Herbicides, Including Phenoxy Herbicides and Associated Dioxins: Volume I. Analysis of Literature
SI - NTIS/PB82-147414
SA - Veterans Administration, Washington, DC. Dept. of Medicine and Surgery.
SO - Govt Reports Announcements & Index (GRA&I), Issue 08, 1982
OD - NTIS/PB82-147414, 338p
PR - NTIS Prices: PC A15/MF A01
AB - TD3: Public Law 96-151 enacted December 20, 1979, mandated the Veterans Administration to conduct '... a comprehensive review and scientific analysis' of the worldwide literature on Agent Orange and other phenoxy herbicides. The need to conduct such a review was in response to an increasing awareness among veterans, the Congress and the public of the potential long-term health consequences of exposure to these herbicides and the contaminant dioxin as used during the Vietnam Conflict. Volume I presents a detailed scientific assessment of the literature concerning U.S. military personnel exposures to herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The following topics are covered: environmental fate and monitoring; metabolism, enzyme induction and mechanism of action; human exposure to TCDD from industrial and military uses; toxicity and mortality; mutagenicity and carcinogenicity. Each chapter concludes with references. Final rept.

John C. Ramsey 12 Oct. 1981

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LABORATORY REPORT CODE: HET K-002372-(27)

DATE ISSUED: 14 October 1980

DEPARTMENT: TOXICOLOGY RESEARCH LABORATORY

LAB. NO.: 1,7,9 PROBLEM NO.: 0,0,0,0,7,0,8

TITLE: Dose Levels of 2,4-D in Forest Workers

19 PAGES IN FULL REPORT

CRI NUMBER

AUTHOR(S): J.C. Ramsey, F.A. Smith, T.L. Lavy[1], C.N. Park, and W.H. Braun

AUTHOR(S) SIGNATURE(S): J.C. Ramsey 10/15/80 F.A. Smith JCR 10/15/80 T.L. Lavy JCR 10/15/80 C.N. Park JCR 10/15/80 W.H. Braun JCR 10/15/80

REVIEWER'S SIGNATURE

This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The dose level of 2,4-D absorbed by forest workers during the helicopter application of 2,4-D sprays has been determined. Three crews, each comprised of 6 workers, were engaged in 2 applications of ESTERON* 99*C herbicide. During the first application the workers used normal clothing and work practices. During the second application they were instructed to use more stringent protective measures to minimize exposure. Measurements of airborne 2,4-D during the spray applications revealed that the respiratory route contributes an insignificant proportion of the total potential for exposure. Total daily urine samples were collected from each worker on the spray application days and for 5 days thereafter. Analysis of total urinary 2,4-D provided reliable estimates of the total amount of 2,4-D absorbed. Fourteen of the 36 exposures did not reveal detectable quantities of urinary 2,4-D. Of the 22 exposures with detectable quantities of urinary 2,4-D, the maximum dose level was only 0.0557 mg/kg and the lowest was 0.00038 mg/kg. Those workers remote from the operations (supervisors and observers) received even lower dose levels than did those in closer contact with the sprays (pilots, mechanics, and batchmen). The low dose levels observed following spray operations using customary precautions (first application) were reduced slightly following spray operations using more stringent precautions (second application). Computer simulation of predicted body burden of 2,4-D upon repeated daily exposures demonstrated a maximum accumulation of only seven-tenths of the daily dose. In conclusion, the very low exposure potential demonstrated by this study, coupled with the low toxicity of 2,4-D, supports the continued registration and use of 2,4-D herbicides.

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[1]Department of Agronomy, University of Arkansas, Fayetteville.

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DOSE LEVELS OF 2,4-D IN FOREST WORKERS

J. C. Ramsey, F. A. Smith, T. L. Lavy[1],
C. N. Park, and W. H. Braun

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1803 Building
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Midland, MI 48640

14 October 1980

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DOSE LEVELS OF 2,4-D IN FOREST WORKERS

J. C. Ramsey, F. A. Smith, T. L. Lavy[1],
C. N. Park, and W. H. Braun

ABSTRACT

The dose level of 2,4-D absorbed by forest workers during the helicopter application of 2,4-D sprays has been determined. Three crews, each comprised of 6 workers, were engaged in 2 applications of ESTERON* 99*C herbicide. During the first application the workers used normal clothing and work practices. During the second application they were instructed to use more stringent protective measures to minimize exposure. Measurements of airborne 2,4-D during the spray applications revealed that the respiratory route contributes an insignificant proportion of the total potential for exposure. Total daily urine samples were collected from each worker on the spray application days and for 5 days thereafter. Analysis of total urinary 2,4-D provided reliable estimates of the total amount of 2,4-D absorbed. Fourteen of the 36 exposures did not reveal detectable quantities of urinary 2,4-D. Of the 22 exposures with detectable quantities of urinary 2,4-D, the maximum dose level was only 0.0557 mg/kg and the lowest was 0.00038

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DOW215572

mg/kg. Those workers remote from the operations (supervisors and observers) received even lower dose levels than did those in closer contact with the sprays (pilots, mechanics and batchmen). The low dose levels observed following spray operations using customary precautions (first application) were reduced following spray operations using more stringent precautions (second application). Computer simulation of predicted body burden of 2,4-D upon repeated daily exposures demonstrated a maximum accumulation of only seven- tenths of the daily dose. In conclusion, the very low exposure potential demonstrated by this study, coupled with the low toxicity of 2,4-D, supports the continued registration and use of 2,4-D herbicides.

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INTRODUCTION

A reliable assessment of the potential risk to workers during pesticide applications in the field is dependent on accurate knowledge of the quantity of the pesticide absorbed into the body. This report concerns measurements of the amount of 2,4-D absorbed by applicators of 2,4-D formulations in the forest.

EXPERIMENTAL

In an experiment conducted by T. L. Lavy (1) eighteen different workers were each engaged in 2 separate forest applications of 2,4-D formulations (ESTERON 99C applied by helicopter at a rate of approximately 2 lb. acid equivalents per acre). In the first application (T1) all personnel followed normal work practices, followed label directions, and operated in compliance with regulatory requirements. During the second application (T2), in addition to the foregoing procedures, the workers all wore protective clothing (disposable coveralls, chemically impervious gloves and boots, and clean hats), were instructed to wash before rest stops and meals, and were instructed in further good hygiene practices by research personnel in the field.[1]

[1]Deviations from prescribed operating conditions are noted in the report by Lavy (1).

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DOW 2155726

near the batch trucks at the heliports revealed detectable levels of 2,4-D esters. Only 2 of the 5 air monitors placed directly in the spray areas revealed detectable concentrations of 2,4-D esters (equivalent to 0.033 and 0.167 µg 2,4-D per liter of air). Further corroboration of the negligible exposure hazard to phenoxy herbicides via the airborne route is given by similar results following both aerial and ground application of 2,4,5-T sprays in the forest (8).

Patch Sample 2,4-D. Widely varying quantities of 2,4-D were detected on the various patch samples. But the poor correlation between the estimated quantity of 2,4-D absorbed based on these data, and the amount excreted in the urine (see following section) shows that this indirect method of estimating the absorbed dose has little validity. In a similar study with 2,4,5-T spray applications in the forest, patch samples were shown to be inadequate indicators of the amount of 2,4,5-T actually absorbed (8).

Urinary 2,4-D. The analysis of total urinary 2,4-D (on a daily basis) on the application day and for 5 days thereafter showed detectable quantities of 2,4-D for 22 of the 36 exposures comprising the study. The total quantity of urinary 2,4-D excreted on the application day and for 5 days thereafter

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DOW 2155728

in the urine as 2,4-D and 2,4-D conjugates by a first order process with an average half-life of approximately 11 hours.[1]

The foregoing data demonstrate that urine is indeed the major excretory route for 2,4-D in both rats and humans, and that urinary excretion is an efficient process that can be described by first order kinetics at non-saturating dose levels (i.e., below approximately 50 mg 2,4-D per kg (2)). Therefore, the measurement of total urinary 2,4-D and its conjugates during the spray application day and for 5 days thereafter (as reported in Table 1 and Reference 1) is considered to be a realistic approximation of the total quantity of 2,4-D absorbed.

Further support for the reliability of urinary 2,4-D measurements in estimating the absorbed dose is derived from comparison with another closely related phenoxyacetic acid herbicide, 2,4,5-T. This compound is also excreted almost exclusively in the urine of rats, dogs and humans (5,6,7) by a first order process that is apparently independent of the route of exposure. And the PGBE esters of 2,4,5-T (10), like the PGBE

[1]Sauerhoff et al. (4) calculated the half-life for urinary excretion of unconjugated 2,4-D as 17.7 hours. However, the inclusion of urinary conjugates of 2,4-D with these data (subjects no. 1, 2 and 3, reference 4) indicate an average half-life of approximately 11 hours for the excretion of both conjugated and unconjugated 2,4-D.

that the half-life values for the dermal absorption of the PGBE esters of 2,4-D and 2,4,5-T in rats are similar, approximately 20 hours and 24 hours, respectively (3,10).

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In order to calculate the pharmacokinetic fate of dermally absorbed 2,4-D in humans, numerical values for the rate constants of absorption and elimination are required. Based on the considerations above, the dermal absorption rate constant of 2,4-D esters in humans was assigned the same value as that for 2,4,5-T esters (half-life = 18 hours) (9). The urinary excretion rate constant of 2,4-D and conjugates was assigned the value previously determined in a human study (half-life = 11 hours) (4). These values then allowed the calculation of the amount of dermally absorbed 2,4-D that is excreted in the urine at any time following exposure (9). This calculation reveals that approximately 99% of a dermally absorbed dose of 2,4-D esters would be excreted in the urine as 2,4-D and conjugates during the exposure day and for 5 days thereafter.

These values for the rate constants were also used to obtain a computer simulation of the body concentration of 2,4-D that would result from repeated daily dermal exposures to 2,4-D esters. The results of this prediction are shown in Figure 1 in which the body burden of 2,4-D is given in units

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usual (T1) versus more stringent (T2) application procedures, possible differences between application crews, and different job descriptions within all 3 crews.

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Even though the dose levels received by the workers operating under usual conditions (T1) were very low, there was nevertheless a slight but statistically significant reduction ($p<0.05$, two-tailed Wilcoxon matched-pairs signed-rank test) in the dose levels received by these same workers when utilizing protective clothing (T2). Of the fifteen exposures in which urinary 2,4-D was detected in one or the other treatment (i.e., either T1 or T2), 11 exhibited a decrease in dose level during the second exposure (T2) whereas 4 showed an increase in urinary 2,4-D.

Among the 3 different crews, there was no statistically significant difference in dose levels when compared by a nonparametric analysis of variance. However a nonparametric t-test revealed that the workers of crew 1 received a significantly lower dose than did the workers of crew 3 ($p<0.05$).

As would be expected, those workers in closer contact with the sprays (pilots, mechanics, and batchmen) received a significantly greater dose level than did the workers more

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DOSE LEVELS OF 2,4-D IN FOREST WORKERS

J. C. Ramsey, F. A. Smith, T. L. Lavy, C. N. Park, and W. H. Braun

HET K-002372-(27) Problem #: 179-0000708

Date: 14 October 1980

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TABLE 1

ABSORBED DOSE OF 2,4-D IN FOREST WORKERS
ESTIMATED FROM URINARY EXCRETION OF 2,4-D*

		mg 2,4-D/kg Body Weight**	
		Ordinary Precautions	Special Precautions
CREW 1 -	Pilot	0.00179	nd
	Mechanic	0.00044	nd
	Batchman	0.00215	0.00053
	Supervisor	nd	0.00038
	Observer	nd	0.00056
	Observer	0.00055	nd
CREW 2 -	Pilot	0.0557	0.0237
	Mechanic	0.00232	0.00516
	Batchman	0.0189	0.0196
	Supervisor	nd	nd
	Observer	nd	nd
	Observer	nd	nd
CREW 3 -	Pilot	0.00206	0.00192
	Mechanic	0.0136	0.00388
	Batchman	0.0377	0.0219
	Supervisor	0.00692	nd
	Observer	0.0011	nd
	Observer	0.0013	nd

*From T. L. Lavy (1), Table 21, p. 48.

**nd = not detectable at a detection limit of 0.04 ppm 2,4-D in urine.

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733

BIOCHEMICAL RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY

Subject TOXICOLOGY AND HYGIENE:
2,4-DICHLOROPHENOXY ACETIC ACID

File T23.14-11-19
Chg. 1219
Rec'd
Fin'd 6-9-48
Work By E.M.Adams

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Rept. By E.M.Adams 6-22-48

DOW 21554 84

UNIT INDEX

A summary of available information.

INDEX HEADINGS

Acetic acid, 2,4-dichlorophenoxy -

SUMMARY OF HAZARD

The toxicity is such as to present but minor hazards of systemic effects. Skin irritation is possible from heavy prolonged contact with strong solutions or the solid acid. There is no hazard of absorption through the skin.

SUMMARY OF TOXICITY

The systemic toxicity was moderately high as indicated by single and repeated oral feedings to rats.

For single doses:

Largest dose survived by all rats	= 0.3 g./kg.
Smallest dose to which all rats succumbed	= 1.0 g./kg.

For repeated (20) doses; 0.3 g./kg. rapidly produced serious injury and death, 0.1 g./kg. had but very slight deleterious effect and 0.03 g./kg. had none.

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Examination of the affected animals revealed minor effects in liver and kidneys, the major action being local irritation in the stomach and gut.

When incorporated into the diet of rats, there was no adverse effect at concentrations which the rats would eat, 0.03% and less. At concentrations of 0.1% and more, the rats refused to eat.

The dichlorophenoxyacetic acid had but very slight effect when tested in solution upon the skin of animals.

There was no evidence of absorption through the skin.

In making and processing 2,4-dichlorophenoxyacetic acid there appears to be no serious hazard of systemic effects. Atmospheric contamination by dust offers the only concern and this would have to be quite severe to be excessive. Any such excessive exposure would probably be first indicated by symptoms referable to irritation in the upper respiratory passages or by gastrointestinal disturbances. By comparison with other common substances, it offers very little hazard of serious organic injury, even from repeated exposures.

Animal results indicate little irritating action upon the skin. There is the possibility that prolonged heavy exposures to solid or to strong solutions could cause a dermatitis. This applies to both the free acid and the sodium salt.

In the use of 2,4-dichlorophenoxyacetic acid, the solution concentrations are sufficiently low and the total amounts involved sufficiently small that there appears to be no hazard to the applicator or to farm animals.

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ontario hydro

Safety Services Department

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A STUDY TO ASSESS THE OCCUPATIONAL EXPOSURE TO 2,4-D HERBICIDES

Date: January 1981

Report: SSD-81-1

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H+S

Health & Safety Division

DOM2155451

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17538

TABLE OF CONTENTS

1.0 OBJECTIVES

2.0 SCOPE

3.0 INTRODUCTION

4.0 METHODS OF STUDY

4.1 Study Development

4.1.1 Breathing Air Sampling
4.1.2 Biological Monitoring (Urine)
4.1.3 The Urinary Model

4.2 Sampling Strategy

4.2.1 Air Sampling
4.2.2 Urine Sampling

5.0 RESULTS

6.0 DISCUSSION

6.1 Exposure vs Spray Operation
6.2 Major Exposure Pathways
6.3 Significance of Air Exposure
6.4 Significance of Urine Concentrations
6.5 Tordon vs 2,4-D/2,4-DP Exposures
6.6 Weekly Urinary 2,4-D Variations
6.7 Comparison with 1979 Urinary Levels

7.0 OBSERVATIONS AND CONCLUSIONS

8.0 RECOMMENDATIONS

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1.0 OBJECTIVES

The objectives of the 2,4-D study were

1.1 To evaluate the exposure hazard to 2,4-D at selected work sites.

1.2 To evaluate improvement due to changes of work practices and personal hygiene.

1.3 To assess the need for further monitoring in the future.

2.0 SCOPE

This study consisted of taking breathing air and urine samples during work with Tordon 101 and 2,4-D/2,4-DP at five selected work locations. Results of the tests are compared with existing guidelines for assessing the significance of exposures and with the type of work operations being performed.

3.0 INTRODUCTION

The term "2,4-D" refers to the chlorinated phenoxy herbicide 2,4-dichlorophenoxy acetic acid and its 35 derivative salt and ester forms. 2,4-D is used to control a variety of broadleaf plants in home lawns, agriculture and in rights-of-way for power lines.

To reduce drift and evaporation of 2,4-D most commercial products are produced as high molecular weight esters or as amine salts. These are diluted with water and applied as an aerosol spray.

2,4-DP refers to 2 (2,4 dichlorophenoxy)-propionic acid and its derivative salt and ester forms. Its herbicide properties are similar to 2,4-D and is often mixed with 2,4-D in a 1:1 ratio.

Tordon 101 is a trade name for a product made by Dow Chemical Co. containing 2,4-D and picloram in a 4:1 ratio.

Herbicides such as 2,4-D are toxic to plants because they interfere with chemically similar plant hormones known as auxins, which control plant growth. Human and animal metabolic process is different from plants and adsorbed 2,4-D is quickly eliminated from the body via the kidney with no metabolic transformation. This difference in metabolic processes makes 2,4-D highly toxic to plants but of low toxicity to man.

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Public concern about the potential adverse health effects of 2,4-D has intensified since the emergency suspension of 2,4,5-T (2,4,5-Trichlorophenoxy acetic acid) in 1979. This concern stems primarily from the chemical similarity of 2,4-D and 2,4,5-T as chlorinated phenoxy herbicides and the question of 2,4-D being contaminated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a manufacturing contaminant in 2,4,5-T. Evidence now seems to indicate that the harmful effects of 2,4,5-T were caused by the TCDD contaminant.

TCDD is not theoretically expected to be found in 2,4-D because the manufacturing processes and starting chemicals from which 2,4-D and 2,4,5-T are made are not the same. Other much less toxic dioxins are theoretically possible in 2,4-D and they have just recently been detected in trace amounts.

2,4-D has been in continuous use for more than 30 years and now is the most widely used pesticide. More than 70 million pounds of the active ingredient are distributed annually. The scientific evidence available at this time does not indicate the potential human expoure at current guidelines is sufficient to result in human health effects.

The objective of this study was to systematically evaluate the exposure hazard of herbicide applicators working for Ontario Hydro. Due to weather conditions, height of brush and terrain in which spray operations are conducted these herbicide applicators are exposed to 2,4-D in varying degrees by breathing the aerosols or through skin absorption by touching wetted brush, hose or spray equipment. This report evaluates such exposures and assesses their significance from the occupational health viewpoint.

4.0 METHODS OF STUDY

To determine the exposure level of workers using 2,4-D herbicide two methods of measurement were used; namely

(a) personal breathing air sampling to determine average daily exposure level

(b) biological monitoring (urine) to detect absorption during the work day.

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4.1 Study Development

4.1.1 Breathing Air Sampling

It was decided to use personal sampling pumps to sample the breathing air of each worker as he performed the herbicide spray operation. However, a suitable adsorbing medium had to be found for the 2,4-D. The use of midget impingers (with ethylene glycol) was not suitable for 2,4-D in a field study because of the rapid evaporation of the adsorbing medium over the sampling period and the possibilities of spillage and breakage of the impingers.

Solid sorbents would be ideal for such field applications. It was decided to use the standard NIOSH fluorisil sorbent tubes. Methods for desorbing or extracting the 2,4-D from the fluorisil were then developed by Chemical Research.

A series of spiking tests of 2,4-D on Fluorisil tubes gave recoveries of 86 to 99% with an average recovery of 92 ± 5%. After extraction the 2,4-D is transformed to the propyl ester and analyzed by gas chromatography.

The loading on the sorbent tubes could not be estimated with any accuracy so during the first week of the study half and full day samples were taken in parallel. Each sample had back up tubes to determine breakthrough or transport of 2,4-D on the solid sorbent. Aft analysis of this first week of sampling it was found a single sorbent tube was sufficient to measure the full day exposure of the workers.

4.1.2 Biological Monitoring (Urine)

Several studies have shown that the phenoxy herbicides such as 2,4-D and 2,4,5-T are absorbed by the body and almost 100% of the absorbed dose is eliminated unchanged via the urine. Thus the urinary concentration is a valuable indicator of the total body burden of 2,4-D i.e. that which adsorbed by inhalation, through ingestion and also absorbed through the skin.

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In a previous study of forestry workers using 2,4,5-T (1) it was shown that inhalation accounted for a very small fraction of the 2,4,5-T entering the body. The major fraction of the 2,4,5-T was absorbed through the skin. The quantity of 2,4-D absorbed may be quite different from the quantity to which workers are exposed under field conditions. During the handling and mixing of the concentrated chemical a very small skin exposure may be the equivalent of a full day of field exposure. Therefore, a proper assessment of the potential hazard to workers during herbicide applications is dependent on reliable estimates of the total quantity of herbicide absorbed under all conditions.

Since 2,4-D is essentially unchanged by the body the urinary concentrations can be used as a guide to the minimum estimate of the 2,4-D absorbed by the body.

4.1.3 The Urinary Model

Previous studies with 2,4-D ingested by human volunteers at a dose of 5 mg/kg of body weight showed that the 2,4-D was absorbed into blood plasma by a first order rate process with a half life of 11.7 hours and then eliminated essentially unchanged by an apparent first order rate process with a half life of 17.7 hours in the urine (2,3). No data exists for absorption rates of 2,4-D through the skin.

The herbicide 2,4,5-T which is similar to 2,4-D absorbs through the skin in an apparent first order rate process with a half life of 18 hours and is eliminated in the urine in a consecutive first order rate with a half life of 23 hours (4). For the purposes of this model the absorption rate through skin for 2,4-D is assumed to proceed with a half life of 12 hours which is similar to that of absorption through gastrointestinal tract and in the same ratio as the absorption and elimination rates for 2,4,5-T. These considerations provide the basis for the model below.

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$$D(t) \xrightarrow{ka} \boxed{B(t)} \xrightarrow{ke} E(t)$$

where

D(t) = amount of 2,4-D remaining on the skin to be absorbed at time t

B(t) = amount of 2,4-D in the body at time t

E(t) = amount of 2,4-D excreted at time t

ka and ke are first order rate constants for absorption and excretion respectively.

The differential equations and initial conditions describing this model can be expressed as follows:

	Initial Conditions
$\frac{d\,D(t)}{dt} = -ka\,D(t)$	$D(o) = Do$
$\frac{d\,B(t)}{dt} = ka\,D(t) - ke\,B(t)$	$B(o) = 0$
$\frac{d\,E(t)}{dt} = ke\,B(t)$	$E(o) = 0$

Calculating ka and ke from the previous studies,

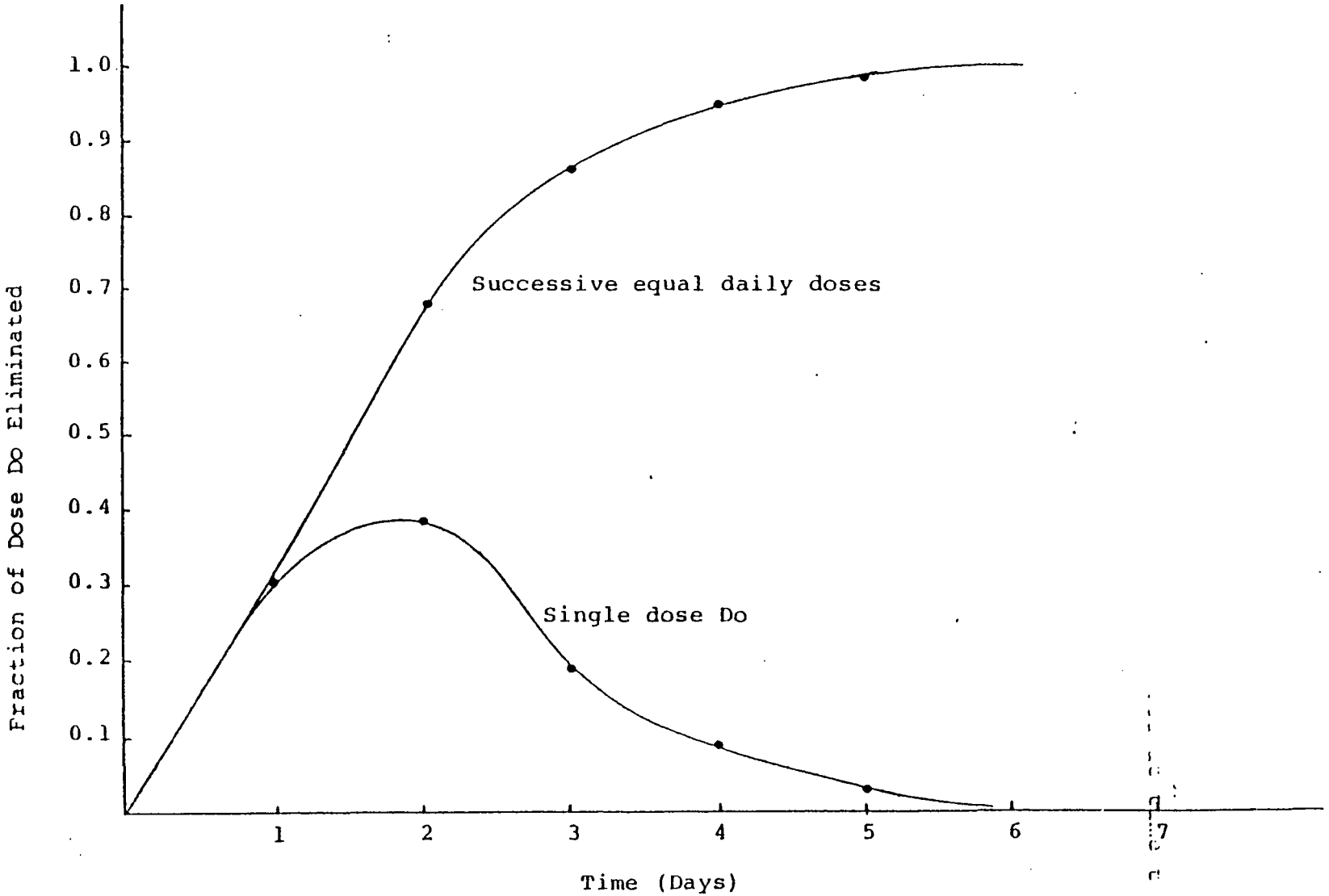
$$ka = \frac{0.693}{t_{\frac{1}{2}}} = \frac{0.693}{0.5\ day} = 1.4\ day^{-1}$$

$$ke = \frac{0.693}{0.7\ day} = 1.0\ day^{-1}$$

Using the integrated form of the equations for the cumulative quantity of 2,4-D excreted as a function of time following exposure the following equation is obtained.

$$E(t) = Do\left(1 - \frac{ke}{ke-ka}\,e^{-kat} - \frac{ka}{ka-ke}\,e^{-ket}\right)$$

Figure 1
Fraction of Applied Dose of 2,4-D Eliminated
in the Urine in Each Day



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Substituting the rate constants above

$$E(t) = Do\ (1 + 2.5\ e^{-1.4t} - 3.5\ e^{-t})$$

Using this equation the plots in Figure 1 were obtained. From this figure the following observations can be made.

The maximum amount of 2,4-D is eliminated during day 2 following an absorption or ingestion of a dose Do of 2,4-D. This equals 38% of the absorbed or ingested dose. This figure indicates that after an absorption or ingestion of an amount Do of 2,4-D then 30% of this amount will be eliminated in the first day 38% in the second day, 18% in the third day, and 9% in the fourth day. In 4 days 95% of the 2,4-D will have passed through the body.

If successive equal doses are applied daily then the body concentration or body burden will build up until the elimination rate equals the dose rate. According to this model and results plotted in Figure 1, this occurs in 4 or 5 days after the first dose. The total body burden becomes approximately 1.5 times Do which is the daily dose exposure.

From the amount of 2,4-D eliminated in the urine an estimate of the dose ingested or absorbed can be made. If it is a single dose then the size of the dose will be approximately 3 times the amount eliminated in the first day after the exposure or 2.6 times the amount eliminated in the second day after exposure. If the exposure is in successive daily exposures then the amount eliminated in the urine will approximately equal the daily exposure after the fourth day. Therefore, if we measure the urinary concentration on Thursdays or Fridays then the amount eliminated will roughly equal the exposure rate during that week assuming the daily exposures are almost equal.

17844

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There are many limitations to this model. First and foremost, the dose rates are never equal on a daily basis. The amount of urine output, the biological half lives and the time of urine sample are several other potential factors contributing to uncertainties in relating 2,4-D in urine concentrations to exposures. With this in mind the elimination levels can only be viewed as a guide to exposure estimation.

4.1.4 Urinary Concentration as Exposure Guides

Urinary 2,4-D concentrations can be transformed to equivalent daily exposure to airborne 2,4-D and an exposure guide developed using the current TLV of 10 mg/m^3. The transformation is based on the standard man who inhales 10 cubic meters of air in an eight hour work day and eliminates 1.4 liters of urine in a day.

The equivalent air exposure calculated assumes the person is subjected to approximately equal time weighted average daily exposures for at least 4 days. Thus the urinary concentrations as exposure guides are as follows:

Urinary Concentration (mg/kg)	Equivalent Daily Exposure to Airborne 2,4-D (mg/m^3)	Exposure Guide
0 - 0.7	0 - 0.1	Normal
0.7 - 7	0.1 - 1.0	Low
7 - 70	1.0 - 10	Moderate
>70	>10	High

4.2 Sampling Strategy

The study was originally designed to cover four spray areas. Two areas would be where 2,4-D/2,4-DP mixture is used and two areas where Tordon 101 is used. Also two types of spray areas were to be considered, i.e. roadside and right of way spray operations. During the study an additional operation was added. This is the mist-blower technique which was used in Kenora area. The table below summarizes the study.

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TABLE 1

Selected Areas and Herbicides Used

Area	Herbicide	Operation	No. of Workers
Walkerton	2,4-D/2,4-DP	Roadside	4
Tweed	2,4-D/2,4-DP	Right of Way/ Roadside	7
North Bay	Tordon	Roadside/ Right of Way	6
Kapuskasing	Tordon	Roadside	4
Kenora	Tordon	Mist-Blower	3

The study period covered the months of June, July and August.

4.2.1 Air Sampling

Air sampling was done daily for each area for five consecutive days or for as long as the weather conditions permitted during that week. Sampling was done only during the actual spray operations.

4.2.2 Urine Sampling

Urine samples were taken generally on Thursdays. The strategy in urine sampling was to obtain

(a) a pre-operation sample (baseline)

(b) 4 weekly samples during spray periods

(c) daily samples during air monitoring weeks.

Each urine sample consisted of an AM and PM sample which were later combined in the lab to form a daily sample. These were then sent to the provincial pesticide lab at Guelph for 2,4-D analysis.

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5.0 RESULTS

The analytical data for the breathing air samples for each of the areas monitored are given in Tables 2-6. The concentrations are all very low so the results have been expressed in micrograms per cubic meter ($\mu g/m^3$). The 2,4-D:2,4-DP ratios are on average equal in the air samples reflecting 1:1 ratio of these components in the mix. The 2,4-D:picloram ratios also roughly reflect the mix being used; the picloram is generally much less than the 2,4-D concentration. The Tordon 101 spray has these components in a 4:1 ratio.

The highest breathing air concentrations were encountered in the mist-blower operations.

The urinary concentrations of 2,4-D, 2,4-DP and Picloram are given in Tables 7-11 for each of the areas monitored. In all cases the 2,4-D and 2,4-DP values are reported as the free acid.

The preseason values were all generally very low except for a few workers. These may have had some pre-exposure to 2,4-D perhaps in spraying it on their lawns for weed control.

The individual urine concentrations show a marked day to day variation in many cases indicating a variable exposure to the herbicides on a day to day basis. In light of this daily variation it is best to consider averages for groups of workers to determine trends.

6.0 DISCUSSION

The air samples provide a measure of the extent of worker exposure through inhalation of aerosols.

The urine samples provide a further measure of worker uptake of the herbicides through dermal, inhalation and possible ingestions routes.

Since daily variations cover a very wide range it is best to consider averages to detect trends and average exposures.

Table 12 shows average air exposures and average urine concentrations grouped as to type of operation. Also the ranges are included for each data set. The broad spread of the data precluded application of normal or log normal statistics.

TABLE 2

2,4-D and Picloram in Breathing Air Samples ($\mu g/m^3$)
Taken in North Bay Area

Worker	Monday			Tuesday			Wednesday			Thursday			Friday		
	24D	Pic*	Time† (min)	24D	Pic*	Time† (min)	24D	Pic*	Time† (min)	24D	Pic*	Time † (min)	24D	Pic *	Time† (min)
N1[1]	25.4	5.0	240	15.8	2.4	285	15.4	2.8	390	11.1	3.3	334			
N2[1]	20.8	2.0	240	18.2	1.0	285	8.0	1.8	300	10.2	1.7	334			
N3[2]	15.6	<0.4	135	8.6	0.3	255	3.7	0.9	268	2.5	0.4	325			
N4[2]	4.4	0.4	135	2.9	<0.2	255	6.2	0.9	210	2.5	0.2	325			
N5[1]	7.8	1.5	270	8.8	0.9	285	9.0	2.2	390	12.0	2.4	334			
N6[2]							7.0	<0.2	270	3.7	<0.2	325			

* Pic = picloram
† Time = Sampling time
1 - Right of Way Spray Operation
2 - Roadside Spray Operation

DOW2155464

TABLE 3

2,4-D and 2,4-DP in Breathing Air ($\mu g/m^3$)
for Walkerton Area

Worker	Monday, June 23			Tuesday, June 24			Wednesday, June 25			Thursday, June 26			Friday, June 27		
	24D	24-DP	Time (min)	24D	24-DP	Time (min)	24D	24-DP	Time (min)	24D	24-DP	Time (min)	24D	24-DP	Time (min)
W1[1]	5.1	5.0	348	9.8	8.6	325	6.7	8.0	348	8.1	10.5	370	13.7	12.3	300
	11.5	11.8	173	15.5	17.0	60	15.6	17.8	90	30.9	37.3	110	23	24	135
W2[1]	3.2	3.1	346	10.8	15.6	255	11.5	14.6	348	8.9	10.0	370	4.0	4.3	300
	6.3	6.7	138	10.3	9.7	155	10.5	11.2	143	3.8	4.8	110	6.3	5.6	120
W3[1]	2.8	3.0	339	3.9	3.3	360	2.6	2.6	355	2.6	3.0	365	5.2	6.3	285
	4.9	5.4	156	9.7	10.1	155	4.1	5.5	150	3.2	3.7	150	4.7	6.1	155
W4[1]	2.4	2.6	334	1.9	1.8	360	3.6	4.2	360	2.6	3.3	365	2.0	2.3	315
	3.0	4.1	140	< .3	< .3	105	5.7	6.9	175	6.5	5.5	170	2.2	3.3	120
W5[2]													2.6	2.1	465
W6[2]													0.6	0.9	270

1 - Roadside Spray Operations
2 - Right of Way Spray Operations

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DOW 2195465

TABLE 4

2,4-D and 2,4-DP in Breathing Air ($\mu g/m^3$)
for Tweed Area

Worker	Monday, July 7			Tuesday, July 8			Wednesday, July 9			Thursday, July 10			Friday, July 11		
	24D	24-DP	Time (min)	24D	24-DP	Time (min)	24D	24-DP	Time (min)	24D (μg)	24-DP	Time (min)	24D	24-DP	Time (min)
T1[1]	11.6	12.7	361	13.3	19.2	120	13.6	19.1	345	30.5	40.4	384	35.3	44.1	218
T2[1]	0.37	0.48	310	4.4	6.1	140	5.3	7.0	342	6.6	7.8	243	11.7	12.7	213
T3[1]	8.1	9.9	322	16.7	22.2	126	13.6	18.8	345	18.6	23.9	376	24.9	32.4	213
T4[1]	8.8	12.3	317	9.4	12.6	127				17.1	19.5	380	15.7	17.1	216
T5[1]	10.7	14.8	337	14.9	19.8	121	23.2	28.4	245	16.5	19.1	382	17.1	23.1	216
T6[2]				12.1	16.4	165				12.1	15.5	330	13.5	19.5	200
T7[2]				6.1	7.9	165	10.1	12.6	325	7.9	10.6	330	7.0	9.5	200

1 - Right of Way Spray Operation
2 - Roadside Spray Operation

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TABLE 5

2,4-D and Picloram in Breathing Air ($\mu g/m^3$)
for Kapuskasing Area

Worker	Monday, July 28			Tuesday, July 29			Wednesday, July 30			Thursday, July 31					
	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)
K1[1]	2.4	0.3	203	2.5	<0.15	333	8.2	10.5	171	7.2	0.6	223			
K2[1]	1.0	<0.2	219	2.7	0.22	369	12.7	1.4	306	13.3	3.6	390			
K3[1]	1.7	<0.2	222	8.7	3.8	368	15.6	3.8	390	8.7	2.4	391			
K4[1]				3.1	0.6	356	5.6	0.6	396	5.7	1.5	383			

1 - Roadside Spray Operation
* Pic = picloram
† Time = Sampling time

DOW2155469

TABLE 6

2,4-D and Picloram in Breathing Air ($\mu g/m^3$)
for Kenora Area

Worker	Monday, Aug. 11			Tuesday, Aug. 12			Wednesday, Aug. 13			Thursday, Aug. 14			Friday, Aug. 15		
	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)	24D	Pic *	Time † (min)
KE1[1]							47.3	11.8	275	61.6	17.4	190	91.3	22.0	195
KE2[1]							75.4	23.2	275	74.7	18.9	190	91	27	200
KE3[1]							18.0	4.8	275	16.2	1.9	210	21.5	6.7	195

1 - Mist-Blower Operation

* Pic = picloram
† Time = Sampling time

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TABLE 7

2,4-D and Picloram in Urine (mg/kg)
for North Bay Area

Worker	May 26		June 19		June 26		July 3		July 10		July 14		July 15		July 16		July 17	
	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic
N1[1]	0.14	ND	2.02	0.05	4.24	0.06	0.63	0.05	1.32	0.04	1.42	0.11	1.46	0.12	3.09	0.10	2.26	0.06
N2[1]	ND	ND	1.19	0.05	0.81	0.01	0.17	0.01	0.43	0.02	0.15	0.01	0.31	0.08	0.60	0.04	0.42	0.01
N5[1]	ND	ND	0.76	0.08	2.57	0.05	0.67	0.15	1.14	0.03	0.90	0.08	3.46	0.05	0.90	0.17	4.30	0.06
N3[2]	ND	ND	0.66	0.04			0.42	0.11	0.50	0.01	0.37	0.38	1.33	0.06	1.67	0.45	2.44	0.14
N4[2]																		
N6[2]	ND	ND	0.30	<0.01	1.28	<0.01	0.17	ND	0.10	0.01					0.07	0.01	0.13	0.01
AVERAGE	0.03								0.70		0.71		1.64		1.26		1.91	

1 - Right of Way Spray Operation
2 - Roadside Spray Operation

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TABLE 8

2,4-D and 2,4-DP in Urine (mg/kg)
for Walkerton Area

Worker	June 2		June 12		June 19		June 23		June 24		June 25		June 26		June 27		July 3	
	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP
W1[1]	0.21	0.06	0.56	0.38	0.68	0.76	0.16	0.24	0.62	0.74	0.99	2.28	1.36	1.40	1.77	1.50	0.79	1.79
W2[1]	0.11	0.08	0.39	0.27	0.75	0.95	0.33	0.71	0.21	0.23	0.27	1.25	0.80	1.90	1.20	1.12		
W3[1]	0.03	0.01	0.18	0.16	0.52	0.53	0.27	0.54	0.28	0.80	1.45	3.38	1.20	1.69	1.54	3.07	0.42	1.09
W4[1]	<0.01	<0.01	0.35	0.22	0.20	0.25	0.07	0.11	0.08	0.17	0.26	0.51	0.22	0.45	0.37	0.51	0.22	0.41
AVERAGE							0.21		0.30		0.74		0.90		1.22			

1 - Roadside Spray Operation

DOW 2159470

TABLE 9

2,4-D and 2,4-DP in Urine (mg/kg)
for Tweed Area

Worker	June 5		June 26		July 3		July 7		July 8		July 9		July 10		July 11		July 17	
	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP	24D	24-DP
T1[1]	0.06	0.05	0.89	2.37	0.56	1.61	0.30	0.47	0.79	0.86	0.21	0.62	0.59	1.96	0.75	1.53	1.25	5.20
T2[1]	0.08	0.18	1.14	2.47	0.61	1.44	0.26	0.54	0.26	0.64	0.27	0.92	1.17	2.47	2.19	3.25	0.46	1.18
T3[1]	0.38	0.47	4.09	27.43	5.75	18.56	2.59	4.81	5.74	4.74	2.54	6.25	4.65	16.3	5.45	10.16	6.62	19.72
T4[1]	0.05	0.08	0.91	3.85	0.76	2.23	1.62	1.33	2.64	4.01	0.68	3.66	2.65	7.69	1.59	3.72	3.46	8.33
T5[1]	0.48	0.37	3.63	9.58	4.07	6.19	0.30	0.99	1.23	2.83	1.62	2.92	2.00	4.13	2.32	4.90	5.51	11.52
T6[2]	1.87	3.86	0.85	2.93	0.56	1.32	0.33	0.24	0.41	0.46	0.43	1.20	0.87	2.27	0.79	1.79	1.54	5.72
T6[2]	0.86	1.13	1.75	3.07	0.62	2.96					0.31	1.40	0.99	3.14	1.29	2.32	2.97	7.44
AVERAGE							0.90		1.85		0.87		1.85		2.05			

1 - Right of Way Spray Operation
2 - Roadside Spary Operation

DOW2155471

TABLE 10

2,4-D and Picloram in Urine (mg/kg)
for Kapuskasing Area

Worker	June 12		July 10		July 17		July 24		July 28		July 29		July 30		July 31		August 1	
	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic
K1[1]	ND	ND			0.35	ND			0.04	ND	0.27	ND	0.63	0.33	2.54	0.44		
K2[1]	0.12	ND	30.55	0.18	6.81	0.84	6.18	1.30	6.11	0.22	3.09	0.14	2.69	0.01	1.92	0.01	8.15	0.03
K3[1]	0.14	0.01	2.69	0.12	0.44	0.28	1.53	0.19	0.96	0.03	2.21	0.12	3.17	0.22	4.71	0.16	7.28	0.21
K4[1]	0.03	ND	1.41	0.03			0.44	0.03	0.16	0.01	0.24	0.08	0.98	0.15	2.24	0.14	3.08	0.08
AVERAGE									2.07		1.45		1.87		2.85		6.17	

1 - Roadside Spray Operation

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TABLE 11

2,4-D and Picloram in Urine (mg/kg)
for Kenora Area

Worker	Aug. 13		Aug. 14		Aug. 15													
	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic	24D	Pic
KE1[1]	2.50	0.36	5.07	0.18	4.67	0.39												
KE2[1]	3.32	0.63	3.40	0.16	2.14	0.47												
KE3[1]	0.44	0.06	0.75	0.11	0.66	0.04												
AVERAGE	2.09		3.07		2.49													

1 - Mist-Blower Operation

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TABLE 12

Average Air and Urine 2,4-D
Concentrations for Different Spray Operations

Operation	No.		Air Samples ($\mu g/m^3$)		Urine Samples (mg/kg)					
					Pre-Spray		During Monitor Week		Season	
	Men	Samples	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Roadside	13	53	6.4	1.0-15.6	0.28	0-1.87	1.2	0.04-8.15	1.6	0.04-30.5
Right of Way	7	38	13.0	0.37-35.3	0.15	0-0.38	1.7	0.15-5.74	1.9	0.04-6.62
Mist-Blower	3	9	55.2	16.2-91.3			2.6			

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6.1 Exposure vs Spray Operation

The data in Table 12 shows that roadside spray operators received the lowest exposure to 2,4-D herbicides and also has the lowest urine concentrations The mist-blower operators on the other hand had the highest air exposure levels. The mist-blower operators encountered 4 to 8 times higher air concentrations but showed only 2 times higher urine concentrations.

6.2 Major Exposure Pathway

To determine the fraction of body 2,4-D intake via inhalation and dermal exposure the roadside and right of way data are considered together. The mist-blower data set is very limited and is considered separately.

The average spray operation is just under 5 hours per day. During this period an average worker will inhale approximately 6 m^3 of air. Using a mean air 2,4-D concentration of 10 µg/m the average worker inhales 60 µg or 0.06 mg of 2,4-D.

The average man excretes 1.4 litres of urine per day. Using an average urine concentration of 1.5 mg/ℓ the average excreted was 2.1 mg/day of 2,4-D. This indicates that the majority of the 2,4-D must enter the body through dermal absorption; air inhalation accounting for a very small fraction. In fact, 35 times as much 2,4-D was excreted via the urine on average as was inhaled. This finding is supported by a study of 2,4,5-T exposures where dermal absorption was estimated at 1000 times the amount of 2,4,5-T inhaled with air. (1)

6.3 Significance of Air Exposure

The current TLV guideline for 2,4-D in air is set at 10 mg/m^3. The average exposure of this study is 0.1% of the TLV and the maximum exposure measured is less than 1% of the current time weighted average exposure for an eight hour day.

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6.4 Significance of Urine Concentrations

The TWA-TLV of 10 mg/m^3 would result in a maximum daily intake of 100 mg for an average man breathing 10 m^3 of air in an eight hour day. This guide does not consider other absorption routes.

Using the urinary model developed earlier the equilibrium dose rate is approximately equal to the excretion rate to maintain a constant body burden. Thus an average excretion rate calculated earlier of 2.1 mg/day represents only 2% of the maximum allowable dose.

The maximum excreted by any man was 30.5 mg/kg, or approximately 43 mg/day. This falls in the range of "moderate uptake" as defined in the exposure guide in Section 4.1.4.

6.5 Tordon vs 2,4-D/2,4-DP Exposures

Table 13 shows the average worker exposure levels using Tordon and 2,4-D/2,4-DP herbicide formulations.

TABLE 13

Tordon and 2,4-D/2,4-DP Worker Exposures

Herbicide	Component	Air (µg/m^3)	Urine (mg/kg)
Tordon	2,4-D	8.6	2.37
	Picloram	1.6	0.34
2,4-D/2,4-DP	2,4-D	10.0	1.37
	2,4-DP	12.5	3.28

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The significant items here are that air exposures to 2,4-D are about equal for both herbicide formulations. The urine concentrations appear significantly different. On average the urine concentrations were higher in 2,4-D when using Tordon, however, the urine concentration of 2,4-DP was more than double that of 2,4-D. In Tordon the 2,4-D is formulated as an amine whereas 2,4-D/2,4-DP are formulated as esters. This may account for differences in absorption rates through skin and elimination rates in urine.

6.6 Weekly Urinary 2,4-D Variations

The daily air and urine concentrations showed wide variations. The duties of driver/sprayer etc. were often rotated among the workers so individual job classifications could not readily be brought out. In a few instances, e.g. Kapuskasing area where two workers were mainly involved in spraying these two had significantly higher exposures to 2,4-D than the other two workers who were drivers.

If the average of all the workers for each area is taken on a daily basis we begin to see the result of cumulative daily doses. The urinary concentrations are generally low on Mondays and increase through the week to reach a maximum on Thursday or Friday. This is illustrated for each of the regions in Figure 2. Variations in type of spraying operation work methods, equipment, herbicide formulation, etc., can account for the different levels observed in each of the areas.

Most of the mixes used were 100 parts of water to 1 part herbicide liquid as received from the manufacturer. The only exception was in the Kenora area where the mix used with the mist-blowers was 16 parts water to 1 part herbicide. This may account for the much higher air concentrations observed for the Kenora area. Table 14 gives the average air and urine concentrations of 2,4-D for each of the areas.

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TABLE 14

Air and Urinary 2,4-D Exposures by Areas

Area	Air $\mu g/m^3$	Urine (mg/kg)	
		Monitor Week	Season
North Bay	10.0	1.40	1.10
Walkerton	5.2	0.67	0.60
Tweed	13.4	1.5	1.8
Kapuskasing	6.6	2.7	3.6
Kenora	55.2	2.55	

6.7 Comparison with 1979 Urinary Levels

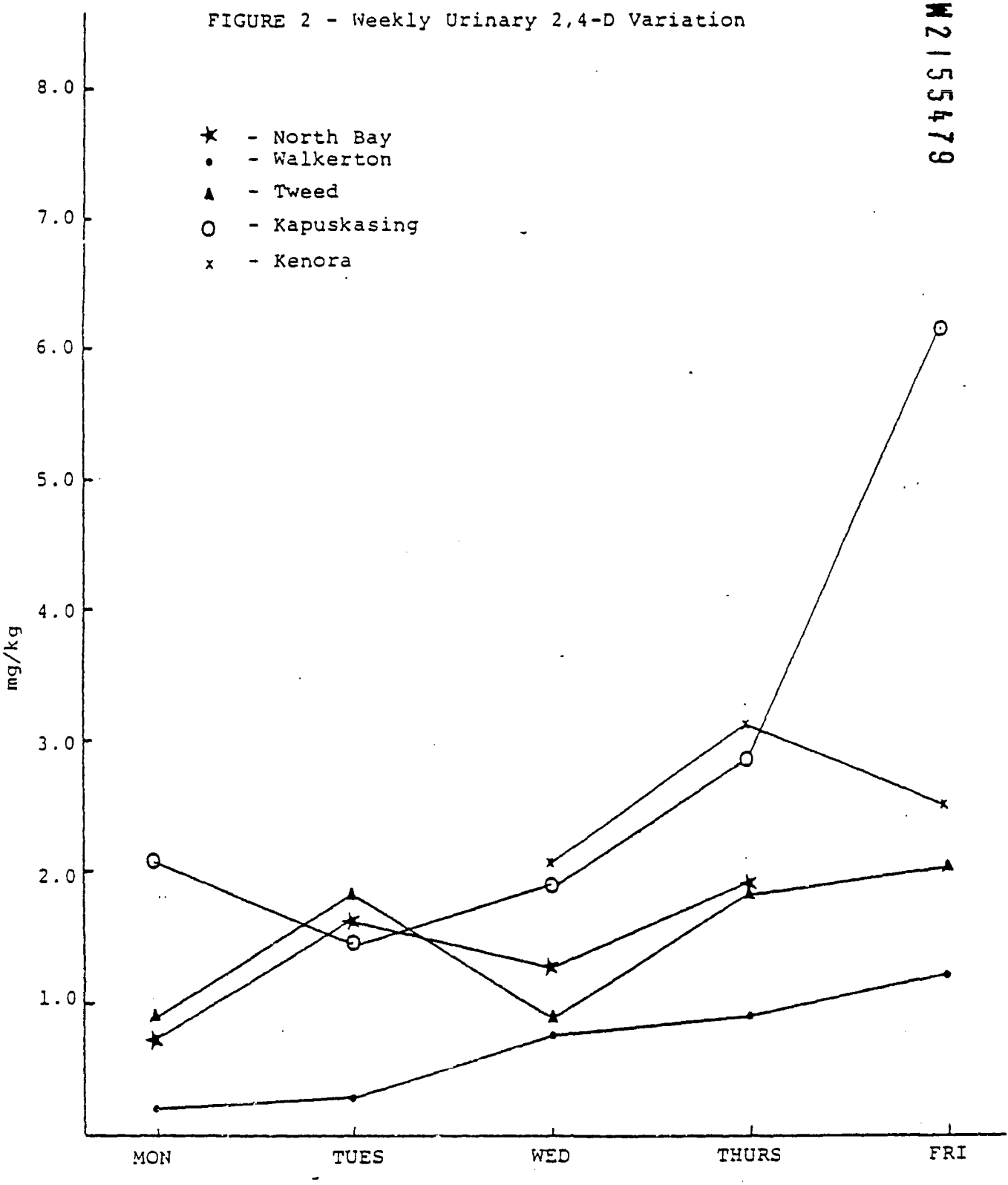
Two weeks of urinary data were taken in 1979 to measure worker exposure to 2,4-D herbicides. That data consisted of a Monday sample and an end of week sample, i.e. Thursday or Friday.

Figure 3 compares the average of the 1980 weekly urinary concentrations for all the areas studied with the two sets of data obtained in 1979. This shows that the improved awareness and improved personnel protection in 1980 has reduced the average urinary concentrations by 3 fold during the course of a week's exposure.

It has been shown that the majority of urinary 2,4-D comes from dermal exposures rather than inhalation. The wearing of gloves and removable coveralls probably accounted for the major reduction of worker exposure to 2,4-D. Now that we know dermal exposure is the major exposure pathway then the protective equipment and operating procedures can be reviewed with this in mind.

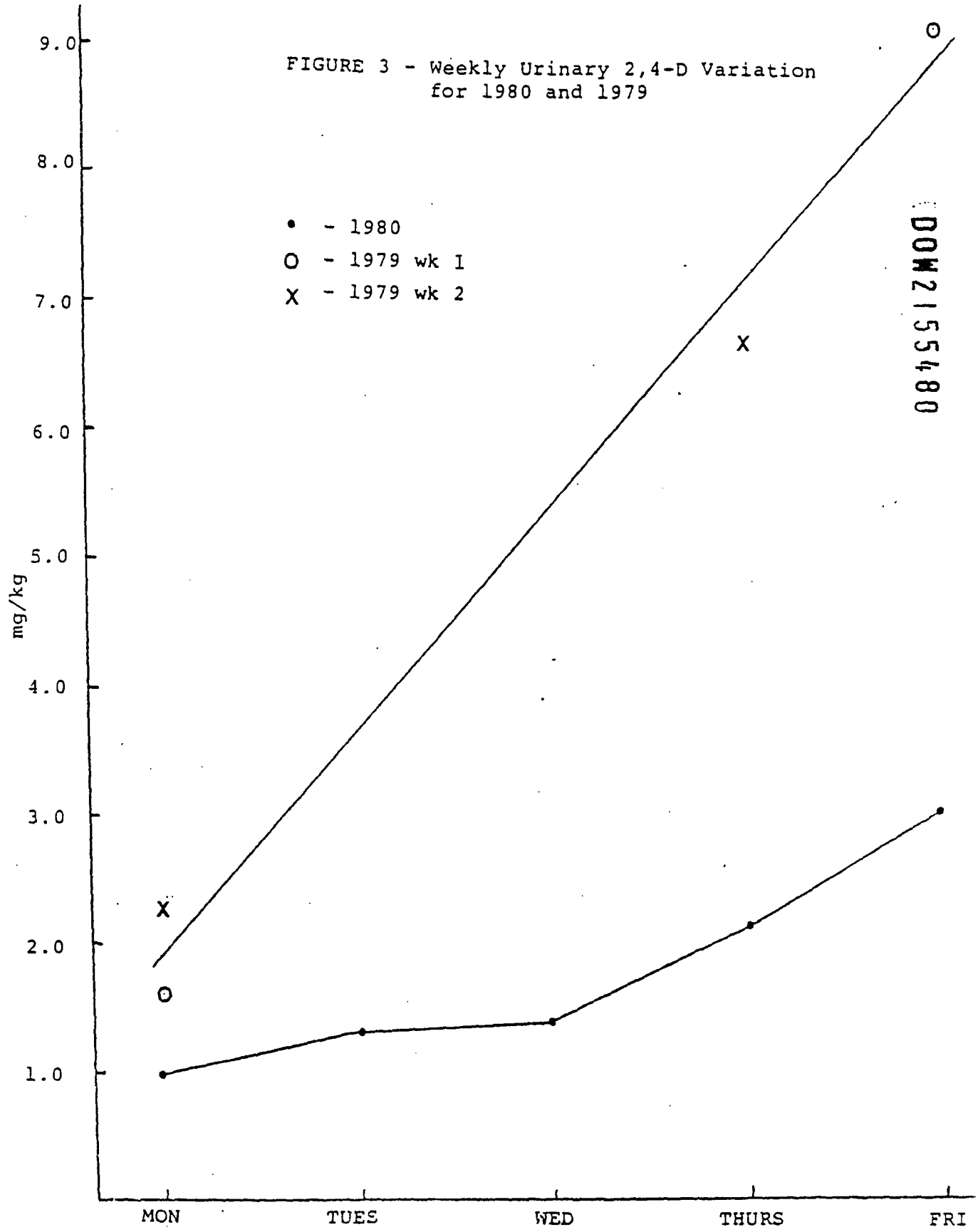
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FIGURE 2 - Weekly Urinary 2,4-D Variation



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FIGURE 3 - Weekly Urinary 2,4-D Variation for 1980 and 1979



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7.0 OBSERVATIONS AND CONCLUSIONS

As a result of this study the following conclusions can be made.

7.1 The major route of worker exposure to 2,4-D herbicides is through dermal absorption. Comparison of the breathing air and urinary data indicates at least 35 times more 2,4-D is absorbed through the skin than is inhaled with the breathing air.

7.2 The average breathing air concentrations were 0.01 mg/m^3 which is 0.1% of the current TLV of 10 mg/m^3 for 2,4-D exposures. The maximum air concentration for a single day was 0.1 mg/m^3 which is 1% of the current TLV.

7.3 The average worker excreted 2.1 mg/day of 2,4-D in urine. This is 2% of the maximum daily allowed exposure via inhalation. The maximum urinary elimination encountered was 43 mg/day. This maximum falls within the range of moderate exposure to 2,4-D.

7.4 Mist-blower operation provided the greatest air exposure to workers at 55.2 $\mu g/m^3$. The right of way spray operations average 13 $\mu g/m^3$ 2,4-D and the roadside operations provided the lowest worker air exposures to 2,4-D at 6.4 $\mu g/m^3$.

7.5 Improved work procedures, protective equipment and training provided to the workers reduced the maximum average weekly urine 2,4-D concentrations by a factor of 3 times over those measured in 1979. This reduction may also reflect increased worker awareness to possible hazards of these herbicides.

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8.0 RECOMMENDATIONS

8.1 Work procedures and protective equipment used by herbicide applicators should be reviewed keeping in mind that the major worker exposure pathway is through skin absorption. Inhalation exposure to 2,4-D has been shown to be minimal.

8.2 Further reductions in 2,4-D intake can be achieved by washing of exposed skin areas and changing of clothing which has been exposed to 2,4-D residues as soon as practically possible after exposures.

8.3 There is a great variability in urine elimination rates throughout the day. Better exposure values can be obtained if 24 hour urine samples are collected rather than spot samples through the day. The 24 hour samples can be better related to the urinary model for total exposure calculations.

8.4 The 1981 herbicide monitoring program can be restricted to urine monitoring as air exposures have been shown to be negligible. Urine monitoring should be continued to check work control methods and to continue our data base.

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University of Arkansas

17587

735

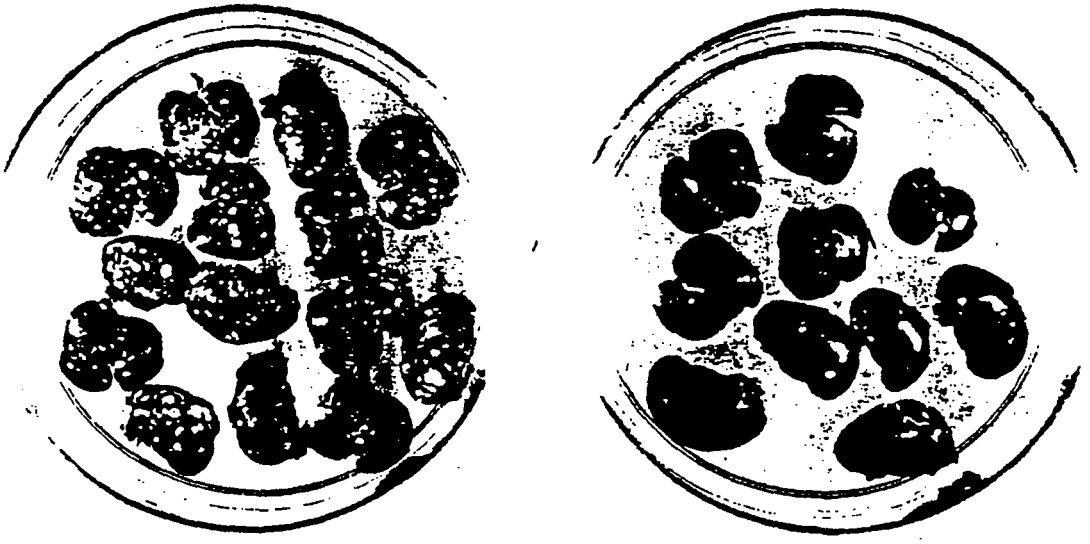
JNCI

Journal of the National Cancer Institute

February 1986
Volume 76
Number 2

FILE: K-2372-(100) 198
K-4568- 198
K-66681- 198
K-5261-

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

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Soft Tissue Sarcoma Risk in Swedish Agricultural and Forestry Workers [1,2]

Kerstin Wiklund, *B.Sc.*, [3] *and* Lars-Erik Holm, *M.D.* [3]

ABSTRACT—The risk of soft tissue sarcoma following possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men, who were employed in agriculture or forestry according to a national census in 1960. This cohort was further divided into six subcohorts, on assumed exposure to phenoxy acid herbicides. The most commonly used phenoxy acid in Sweden was (4-chloro-2-methylphenoxy)acetic acid (CAS: 94-74-6). The reference cohort encompassed 1,725,845 Swedish men employed in other industries. All persons were followed up in the cancer-environment register during the period 1961–79. A total of 331 cases of soft tissue sarcomas was observed in the study cohort and there were 1,508 cases in the reference group [relative risk (RR), 0.9; 95% confidence interval, 0.8–1.0]. No subcohort of agricultural or forestry workers showed any significantly increased RR, nor was there any significant difference in RR between the subcohorts. Despite the greatly increased use of phenoxy acid herbicides from 1947 to 1970, no time-related increase in the RR of soft tissue sarcoma was found in the total cohort or in any of the subcohorts.—JNCI 1986; 76:229–234.

Soft tissue sarcomas constitute a heterogeneous group of malignant tumors arising from the embryologic mesoderm. These tumors are uncommon and often present diagnostic difficulties. The clinical course ranges from slow growth of low-grade cancer to rapid progression of lethal sarcoma (*1*). Soft tissue sarcomas are usually classified according to morphologic similarities with the assumed tissue of origin (*2*).

Increased risk of soft tissue sarcoma has been reported to follow exposure to phenoxy acid herbicides and chlorophenols. Hardell (*3*) observed that several patients with soft tissue sarcoma had been exposed to these substances. His observations prompted two case-control studies in the three northernmost and the five southernmost counties in Sweden. A sixfold increase in risk of soft tissue sarcoma was observed in persons exposed to phenoxy acids or chlorophenols (*4–6*). Division of the material according to duration of exposure to phenoxy acids indicated a dose-response relationship. Hardell found that the risk also related to exposure to those phenoxy acids that are less likely to be contaminated with dioxins (*6*). These Swedish studies (*3–6*) were based on small groups of subjects. The findings have been supported by studies from other countries of workers engaged in the production of phenoxy acid herbicides (*7–11*). Others have, however, failed to observe any relationship between phenoxy acids and the risk of soft tissue sarcomas (*12–22*), although two of these studies found an excess of the total number of cancers (*13, 21*).

In Sweden, most persons occupationally handling phenoxy acid herbicides work in agriculture or forestry or are "licensed applicators." A study of the cancer morbidity among Swedish agricultural workers between 1961 and 1973, on the basis of Swedish CER, showed no increased risk of malignant tumors in connective tissue or muscle (*23*). The CER has been supplemented with data on cancer cases diagnosed in 1974–79 and thus comprises cases between 1961 and 1979. Because of this coverage it is possible to study large cohorts over a long period, which creates opportunities for analyses of time trends.

The aims of the present study were to analyze the risk of soft tissue sarcoma in agricultural and forestry workers in comparison with that in other occupations and to test if there is any time-related trend in this risk, considering the greatly increased use of phenoxy acids between 1947 and 1970 (*24*).

MATERIALS AND METHODS

Cohort.—The study cohort consisted of 354,620 Swedish men who were born between 1891 and 1940 and who were agricultural or forestry workers according to the PH 60. The reference cohort comprised 1,725,845 Swedish men born within the same time and working in economic activities other than agriculture or forestry, according to PH 60.

The cohort was defined by economic activity (industry) instead of by occupation. A previous study (*25*) had shown that the coding of economic activity used in that study and in PH 60 agreed in 97% at a 1-digit level of classification and in 90% at a 3-digit level. The corresponding figures for the coding of occupation were 97% and 88%, respectively.

Subcohorts.—The study cohort was divided into six subcohorts in order to obtain occupational groups with more homogeneous exposure to phenoxy acid herbicides and also to analyze separately occupations within agriculture and forestry with little or no assumed exposure

Abbreviations used: CER=cancer-environment register; CI=confidence interval(s); 2,4-D=(2,4-dichlorophenoxy)acetic acid; MCPA=(4-chloro-2-methylphenoxy)acetic acid; PH 60=population and housing census in 1960; RR=relative risk(s); 2,4,5-T=(2,4,5-trichlorophenoxy)acetic acid; TCDD=2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[1] Received April 8, 1985; accepted September 25, 1985.

[2] Supported by grants from King Gustaf V Jubilee Fund (82:533), the Stockholm Cancer Society (85:91), the Swedish Farmers' Safety and Preventive Health Association, the Swedish Medical Research Council (B84-27X-6885-01A), and the Swedish Product Control Board (14-83/84).

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17569

to these agents. On the basis of the occupational data in PH 60 and of estimated use of different types of herbicides in agriculture, forestry, and horticulture (*24*), the six subcohorts were defined as follows.

Land and/or animal husbandry: Subcohort 1 consisted of men who had stated their occupation as farm worker.

Horticulture: Subcohort 2 was mainly market garden employees (not gardeners in parks, cemeteries, e.g.).

Other agricultural occupations: Subcohort 3 included reindeer or fur farming, veterinary services, miscellaneous other duties, or nonspecified agricultural work.

Silviculture: Subcohort 4 was listed for planting and thinning of young trees and for other care and supervision of forest.

Timber cutting: Subcohort 5 encompassed felling and trimming of trees and transportation of timber from the forests.

Other forestry occupations: Subcohort 6 was involved with rafting and scaling of timber, hunting or gamekeeping, or nonspecified forestry work.

The numbers of persons and person-years in the total cohort, the subcohorts, and the reference group are shown in table 1.

Chemicals.—The phenoxy acid compound used predominantly in Swedish agriculture is MCPA (CAS: 94-74-6). The use of MCPA during 1975 in Sweden was estimated as 1,100 metric tons. Two other compounds have also been used, namely, 2,4-D (CAS: 94-75-7) and 2,4,5-T (CAS: 93-76-5) and some of their esters, though to a lesser extent than MCPA (*24*).

The herbicides used in Swedish forestry since the early 1950's were 2,4,5-T mainly and 2,4-D. Legal restrictions on their use were introduced in the late 1970's, whereupon the use dropped from 180 metric tons to only a few tons in 1971. The use was since increased, however, as some restrictions were lifted. The use of 2,4,5-T, however, has been prohibited in Sweden since 1977. Exposure to these compounds has been mainly in silviculture. Timber felling and trimming may have involved some passive exposure. In horticulture, herbicides other than phenoxy acids have been used. Exposure to these substances sometimes has taken place in hothouses.

On the basis of this information, the exposure to herbicides in the land and/or animal husbandry subcohort was assumed to have happened mainly with MCPA and to some extent with 2,4-D and 2,4,5-T; the horticulture subcohort presumably had exposure to herbicides other than phenoxy acids. The silviculture subcohort was assumed to have been exposed to 2,4,5-T and to some extent to 2,4-D; the timbercutting subcohort presumably had a passive exposure to 2,4,5-T and 2,4-D. The subcohorts other agricultural occupations and other forestry occupations were considered to have been heterogeneous in regard to herbicide exposure.

Phenoxy acids contain contaminants of polychlorinated dibenzo-*p*-dioxins. Only 2,4,5-T among the phenoxy acids is considered to be contaminated with TCDD (CAS: 1746-01-6), which is the most toxic dioxin isomer. Other phenoxy acids are contaminated with dioxins that are less toxic than TCDD. The forestry subcohorts Nos. 4–6 were therefore assumed to have been more exposed to TCDD than were the agricultural subcohorts Nos. 1–3.

Since many Swedish farms include forest land and vice versa, there was undoubtedly heterogeneity in herbicide exposure for the subcohorts; and the assumed exposures can therefore only be very rough estimates of exposure in each group.

Follow-up.—Cases of malignant tumors in the study and the reference cohort were identified in the CER. The observation included tumors notified with the following histologic diagnoses: angiosarcoma, hemangiomatous tumor within the nervous system, leiomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma, myxosarcoma, liposarcoma, rhabdomyosarcoma, malignant synovioma, embryonal rhabdomyosarcoma, and other specified soft tissue sarcoma.

A grouping of tumors was made on the basis of histologic diagnosis in order to test if any subgroup of soft tissue sarcoma showed an increased RR or if there was any difference in RR between subgroups. During the study period there had been changes in the histologic criteria for soft tissue sarcoma, and the tumors were therefore divided into only 5 groups: *a*) angiosarcoma or hemangiomatous tumor of the nervous system; *b*) leiomyosarcoma; *c*) fibrosarcoma, malignant fibrous histiocytoma, or myxosarcoma; *d*) liposarcoma; and *e*) various other specified soft tissue sarcomas.

CER was established by computerized linkage between the nationwide cancer register and PH 60. The reason for this record linkage was that data concerning occupation and domicile in the cancer register often were lacking. CER contains all data in the cancer register, such as: the unique personal identity number, which includes date of birth; name; site and histologic type of tumor; basis and date of diagnosis; and underlying and contributory causes of death for all fatal cases. Most of these data are derived from clinicians' obligatory notifications of cancer. All cases of primary tumors notified to and registered in the cancer register between 1961 and 1979 are currently included in CER. The data supplied by PH 60 to CER concerned occupation, industry, domicile, etc., based on a nationwide questionnaire. CER has the defects inherent in these types of information. The information in PH 60 applies to a particular week in

TABLE 1.—*Number of men and person-years of follow-up in the study cohort and in the reference cohort*

Cohorts and subcohorts	Men in census (1960)	Person-yr of follow-up (1961–79)
Agriculture and/or forestry	354,320	6,079,769
Land and/or animal husbandry	254,417	4,330,717
Horticulture	11,626	199,281
Other agriculture	14,126	243,781
Silviculture	7,215	126,624
Timber cutting	61,153	1,068,318
Other forestry	6,083	104,050
Reference cohort	1,725,845	30,131,674

17570

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TABLE 2.—*Observed numbers of soft tissue sarcomas in 1961–79, RR, and 95% CI for the total period and RR for the three time periods*

Subcohorts and cohort	Observed No. of cases	RR	95% CI	RR* for yr:		
				1961–66	1967–73	1974–79
Land and/or animal husbandry	253	0.9	0.8–1.1	0.9	1.0	0.9
Horticulture	10	0.8	0.4–1.5	—	—	—
Other agriculture	11	0.8	0.4–1.4	—	—	—
Silviculture	5	0.8	0.3–1.9	—	—	—
Timber cutting	49	1.0	0.7–1.3	0.9	1.0	0.9
Other forestry	3	0.6	0.1–1.7	—	—	—
Total study cohort	331	0.9	0.8–1.0	0.9	1.0	0.9

* — = the number of cases in the subcohort was too small to permit division into different time periods.

October 1960 during which the census was made. Concerning occupation, there is no individual information of, for example, the mean period of employment and the occupations before and after this census week. The constitution and deficiencies of CER have been discussed elsewhere (*26*).

In the calculation of person-years, the nationwide cause-of-death register supplemented with information from PH 60 was used in allowing for the decrease of the person-years because of deaths during the follow-up period.

Statistical analysis.—A relative risk estimate, RR (Mantel-Haenszel incidence density ratio), was calculated for the cohorts versus the reference group, with adjustment for effects of confounding variables by stratification according to the Mantel-Haenszel method (*27*). Stratification variables were year of birth (5-yr classes) and domicile in 1960 (county or the three major cities—Stockholm, Gothenburg, and Malmö).

The tumors were divided into three periods according to the year of diagnosis; 1961–66, 1967–73, and 1974–79.

The number of person-years of follow-up for the total study cohort, the subcohorts, and the reference group was calculated from January 1, 1961 until December 31, 1979 or until death if prior to this date. A person who died contributed half a person-year during the year of death.

The size of the subcohorts varied widely and the standard error and CI for RR were therefore calculated according to different principles. For the total cohort (agriculture and forestry) and for the largest subcohort (land and/or animal husbandry), standard errors and CI were calculated according to Breslow (*28*). The other subcohorts were considerably smaller than the reference group and the standard error in the reference group was therefore regarded as negligible. The limits for CI for RR in these subcohorts were calculated with a Poisson distribution table.

Trends in RR for the three study periods were tested according to Breslow and Day (*29*).

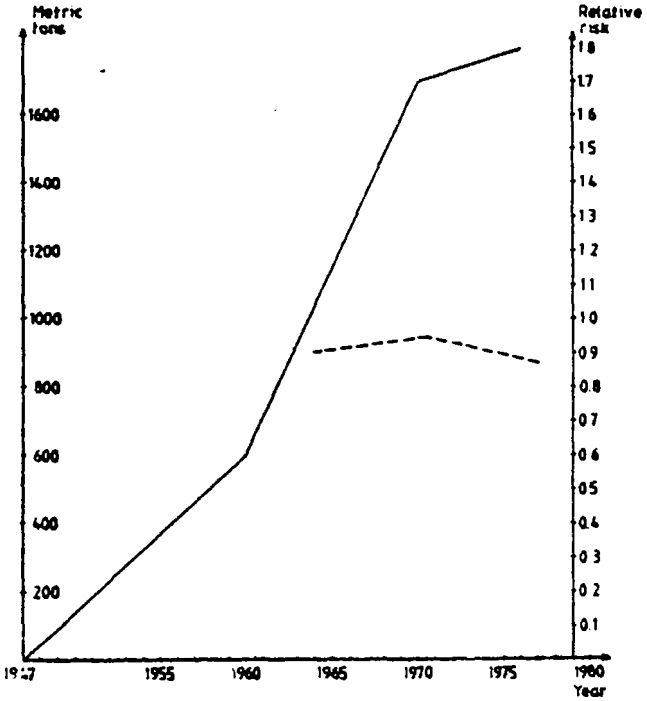
The hypothesis of homogeneity in RR against heterogeneity among subcohorts and among subgroups of soft tissue sarcoma was tested according to Breslow and Day (*29*).

TABLE 3.—*Numbers of soft tissue sarcomas with RR and 95% CI in the total cohort in the three time periods studied*

Time period	No. of cases	RR	95% CI
1961–66	67	0.9	0.7–1.2
1967–73	134	1.0	0.8–1.1
1974–79	130	0.9	0.8–1.1

RESULTS

Between 1961 and 1979, a total of 331 cases of soft tissue sarcoma in the study cohort and a total of 1,508 cases in the reference group were observed. The RR was



TEXT-FIGURE 1.—RR of soft tissue sarcoma 1961–79 in relation to estimated use of phenoxy acids 1947–76. Data on estimated use are from Bäckström (*24*). Estimated use of phenoxy acids (——); RR (——).

17571

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TABLE 4.—*Numbers of cases, RR, and 95% CI between 1961 and 1979 for the two largest subcohorts and total cohort according to histologic type of soft tissue sarcomas*

Histologic diagnosis	Land and/or animal husbandry			Timber cutting			Total cohort		
	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI
Angiosarcoma or hemangiomatous tumor of nervous system	43	0.9	0.6–1.2	7	0.7	0.3–1.5	54	0.8	0.7–1.1
Leiomyosarcoma	55	1.0	0.8–1.4	8	0.9	0.4–1.7	71	1.0	0.8–1.3
Fibrosarcoma, malignant fibrous histiocytoma, or myxosarcoma	75	0.8	0.6–1.1	20	1.2	0.7–1.8	106	0.9	0.8–1.1
Liposarcoma	52	1.0	0.8–1.3	7	0.8	0.3–1.6	63	0.9	0.7–1.2
Various others	28	1.0	0.7–1.5	7	1.1	0.4–2.2	37	1.0	0.7–1.3
Total tumors	253	0.9	0.8–1.1	49	1.0	0.7–1.3	331	0.9	0.8–1.0

estimated as 0.9 (95% CI: 0.8–1.0, table 2). The number of cases, RR, and CI in each subcohort are presented in table 2. None of the RR differed significantly from unity, nor was there any significant difference in RR between the subcohorts.

Tests for time-trend in RR over the three time periods were performed in the total cohort and in the two largest subcohorts land and/or animal husbandry and timber cutting (tables 2, 3). The numbers of cases in the other subcohorts were too small for such an analysis. The hypotheses of a common RR in the three periods could not be rejected, which meant that no time-related trend in any of the subcohorts could be claimed.

In text-figure 1 the RR for soft tissue sarcomas in the total cohort are shown in relation to the estimated use of phenoxy acids in 1947–76. MCPA predominated among these compounds.

The distribution of the various subgroups of soft tissue sarcoma in the total study cohort and the two largest subcohorts is presented in table 4. In the subcohort timber cutting, the RR was 1.2 (0.7–1.8) for fibrosarcoma, malignant fibrous histiocytoma, or myxosarcoma; but this finding was not statistically significant. The hypothesis of a common RR in subgroups of soft tissue sarcoma could not be rejected.

DISCUSSION

The present study failed to observe any statistically significant excess risk of soft tissue sarcoma in any subcohort of agricultural or forestry workers, although their exposure to phenoxy acids can be assumed to exceed that of other occupational groups. No difference in RR was found between the subcohorts, despite probable differences in exposure to the herbicides.

The latency for chemically induced tumors is long, on the average, 15–30 years (*30*). In two earlier Swedish studies (*4*, *5*), the median latency for soft tissue sarcoma after exposure to phenoxy acids was found to be 15 and 20 years, respectively. If an association exists between exposure to these substances and the risk of soft tissue sarcoma, a time-related increase in RR would have been expected in this present study, considering the steep rise in the estimated use of phenoxy acids and the estimated latency (text-fig. 1). No such trend was found, however.

The present study differs structurally from the earlier Swedish investigations (*4–6*), which were case–control studies. A case–control study relies heavily on recall of distant events, sometimes provided by relatives of deceased persons, with consequent risk of uncertainty in assessment of results. The basis for the present study consisted of cohorts with assumed occupational exposure to phenoxy acids. One advantage of utilizing CER and PH 60 is that data can be obtained for large numbers of persons observed over long periods. A major disadvantage of such register-based studies is the frequent lack of individualized exposure data. In our study there was no information on individual exposure to phenoxy acids; nor was there any information on the proportion of workers actually exposed to phenoxy acids, on the extent of this exposure, or on current patterns of herbicide usage.

The possible loss of sensitivity of this study due to the classification of the cohorts being based on only 1 year's employment status can be considered small. Of all men 25–64 years of age and employed in agriculture, forestry, or fishing in 1960, 13% were not in these economic activities in 1950 (*31*). Of those who were employed in other economic activities in the 1960 census, 8% were employed in agriculture or forestry in 1950 (*31*). These figures show the occupational stability in agriculture and forestry in the 1950's.

Hardell (*3*) also found an increased risk of soft tissue sarcoma after exposure to chlorophenols. About 90% of the consumption of chlorophenols has been in sawmills (*3*). Other Swedish studies have shown that among men employed in sawmills or planing mills, the RR of tumors in connective tissue or muscle in the period 1961–73 were 0.5 (*32*) and 0.7 in 1961–79 (*33*).

On the basis of Swedish data (*6*, *34*) we estimate that 15% of agricultural and forestry workers have been exposed to phenoxy acids and 2% of these types of workers have been exposed to chlorophenols, compared to approximately 1 and 3% of workers in other occupations exposed to these respective chemicals. A possible increased risk in a CER-based study would therefore be less than that in earlier reports (*4–6*). On the basis of the above figures and on the assumption of an RR of 6 for

17572

exposed workers, RR in a CER-based cohort study would be about 1.5. An inquiry with the attempt to analyze the use of phenoxy acids and other pesticides in the studied cohort from 1950 onward is currently in progress.

One explanation for the lack of any excess risk of soft tissue sarcoma in the studied agricultural and forestry workers might be underdiagnosis. Agricultural workers are known to utilize the health services less than do other occupational groups (*35*). Some tumors will therefore not be detected or notified to the cancer register to the same extent as in other occupational groups. Moreover, Swedish agricultural workers have been shown to be a group with a decreased risk of cancer in general (*23*). Factors that may contribute to this decreased risk include lower use of tobacco and alcohol, healthier diet and life-style, good health, and more physical activity. The importance of such factors for the development of soft tissue sarcoma is not clear. To reduce "healthy worker effect," the study cohort was compared with all men gainfully employed in industries other than agriculture or forestry, rather than with the total adult male population.

Soft tissue sarcomas often present diagnostic difficulties, which may increase the uncertainty in register-based studies, where the histologic diagnoses are based on cancer notifications and not reviewed by a pathologist. At reviews of such tumor groups in the cancer register, mistakes in diagnosis are fairly commonly found. Altered concepts of malignant potential could have led to inclusion in the register of certain cases that previously were not notified to the cancer register or that were considered benign. Intensified diagnostic efforts and new techniques imply not only that more cases of cancer are detected in early stages but also that there is greater precision in diagnosis, so that a higher proportion of tumors can be assigned to specific coded groups.

Any difference in RR between subgroups of soft tissue sarcoma could not be found.

In an evaluation of the reports (*7–11*) of dioxin exposure and soft tissue sarcoma pathology among chemical workers in the United States, the case and exposure status of some of the 7 cases was discredited (*36*). The authors concluded that their finding does not contradict or confirm the Swedish conclusions by Hardell et al. (*4–6*).

Two other Swedish and some international investigations have not found any association between exposure to phenoxy acids and soft tissue sarcoma (*12–19*). The two Swedish studies were concerned with forestry workers (*12*) and railroad workers (*13*) exposed to herbicides; but the sizes of the studies were small, as in a Finnish study on phenoxy acid applicators (*14*). In an updated mortality study in Washington State for 1950–79, no association was found between soft tissue sarcoma and occupations with exposure to phenoxy herbicides and chlorophenols (*15*). Cancer mortality rates in 150 rural U.S. counties were compared with the use of pesticides between 1950 and 1969, and an association was seen only for use of pesticides with lung cancer (*16*). Workers exposed to 2,4,5-T during an accident in 1949 were found to have no increased cancer risk, as were workers engaged in production of this herbicide between 1948 and 1969 (*17*). A case-control study based on the national cancer register in New Zealand was similarly negative concerning phenoxy acids and soft tissue sarcoma (*18*), as was a British Columbia occupational mortality review based on death registrations in 1950–78 and on cancer mortality among workers in the wood industry, in farming, or in cleaning ground for railroad lines (*19*).

A recent study based on 15 regional cancer registers in England and Wales (*37*) showed an RR of 1.7, which was statistically significant at the 5% level, for soft tissue sarcoma among farmers, farm managers, and market gardeners. The RR were less than unity among agricultural workers, gardeners, and foresters, etc. The authors concluded that the absence of a significantly increased RR in workers in agriculture and forestry as a whole and its presence in 1 of 4 subcohorts may indicate that this was a chance finding.

Studies of professional pesticide applicators in the United States and the German Democratic Republic have not disclosed any association between exposure to phenoxy acids or other pesticides and occurrence of soft tissue sarcoma (*20–22*).

The present study has failed to confirm the results of Hardell (*3–6*). No subcohort of agricultural or forestry workers showed any significantly increased RR. Despite the greatly increased use of phenoxy acids from 1947 to 1970, no time-related increase in RR was found in any of the cohorts.

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2,4,5-T ACID

Trichlorophenoxy Acetic Acid	98.93%
Trichlorphenol	0.76%
Sodium Chloride	0.6%
Freeze Point	150.6°C.
Color	White

2,4-D ACID Batch 7-2 (new acid)

		old acid.
2,4-D	99.35%	98.8
Dichlorophenol	0.18%	0.277
Sodium Chloride	0.035%	0.08
Freeze Point	136.5°C.	135.6
Color	White	

L. C. White

L. C. White
Britton's Division
2-267 Building

LCW/rj
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2372-20 (old method) Part of T.C.C-38-6 IBM

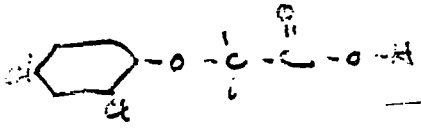
K No. 2372-19 (new method) BIOCHEM. FILE T23.14-11-6

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REQUEST FOR BIOLOGICAL TEST
TO **BIOCHEMICAL RESEARCH LABORATORY**

C.R.I., FILE NAME: 2,4-dichlorophenoxyacetic acid ~~[redacted]~~

STRUCTURAL FORMULA:



MOLECULAR FORMULA $C_8H_6Cl_2O_3$

CONSTITUENTS IN COPOLYMERS OR MIXTURES:

2,4-dichlorophenoxyacetic acid material contains some ____ %

2,4-dichlorophenol - new method material ____ % is essentially free of this material.

DOM2155384

DATE

SUGGESTED SOLVENTS:

DISCUSSED WITH

REQUESTED BY WCB 3-17-50

MADE BY L. White 3-20-50

REFERENCE 23W-122 AMT. SENT 2 lbs. ea

MP ____°C BP ____°C AT ____MM. HG DENS. ____AT ____°C/____°C

HANDLING PRECAUTIONS

PRODUCT STATE: RESEARCH ____ DEVELOPMENT ____ SPEC. CHEM. ____ PLANT ____

USE CONTEMPLATED

TESTS REQUESTED: Skin Irritation & Sensitization Studies - by Stelawski.

CHARGE NO. 12-22-22

LIT. REF. ____ DISCL. NO. ____ PATENT ____

(PLEASE DO NOT WRITE BELOW THIS LINE)

TESTS	BY	DATE
GENERAL SCREENING	[illegible] 3-22-50	

DOM 3-22-50

SAMPLE TO

NOTED BY ____ DATE 3/23/50 BY ____ DATE ____

INITIAL AND DATE ALL ENTRIES - NO ERASURES PERMITTED.

BIOCHEMICAL RESEARCH DEPARTMENT
THE DOW CHEMICAL COMPANY

745

Subject:

RESULTS OF SKIN IRRITATION AND SKIN SENSITIZATION TESTS CONDUCTED ON HUMAN SUBJECTS WITH OLD AND NEW PREPARATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID

File: T23.14-11-6
Charge: 1229-02
Rec'd.: 3/30/50
Fin'd.: 10/11/50
Work by: Industrial-Toxicology Laboratories

To:

Britton's Division (1)
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Check [illegible] 10-12-50

Rept. by: [illegible] 10-12-50

K-2572-61

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DOW 2155380

UNIT INDEX

2,4-D acid produced by the new method of manufacture produced no significant irritation when applied to the skin of humans, nor did it produce any sensitization. A sample of 2,4-D acid prepared by the old method was significantly irritating to the skin of humans but did not produce any sensitization.

INDEX HEADINGS

2,4-Dichlorophenoxyacetic acid

Phenoxyacetic acid: 2,4-dichloro-,

Acetic acid: 2,4-dichlorophenoxy-,

Acetic acid: phenoxy-, 2,4-dichloro-,

PROBLEM

2,4-Dichlorophenoxyacetic acid is a major product of The Dow Chemical Company. A new method for preparing the material which gives a whiter and essentially odorless product is now being used in manufacture. Are there any toxicological advantages between the old and the new material?

17577

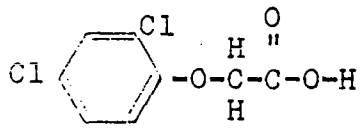
DOW 2155381

MATERIAL

Name: 2,4-Dichlorophenoxyacetic acid

Formula:

Structural:



Empirical: $C_8H_6Cl_2O_3$

Source: L. White, Britton's Division, 267 Bldg.

Reference: 23W-122, 3/20/50

Description of samples:

K2372-19 is a sample of a new product which is essentially white and odorless.

K2372-20 is a sample of the production by the old method. It is slightly off-white in color and has an appreciable odor.

Assays of these two materials revealed the following:

	K2372-19	K2372-20
2,4-D	99.35%	98.8%
Dichlorophenol	0.18%	0.277%
Sodium chloride	0.035%	0.08%
Freezing point	136.5°C.	135.6°C.
Color	White	Off-white
Reference no.	23W-122	23W-122

EXPERIMENTAL PROCEDURE

A sensitizer is a material which has the ability to produce an increase in the susceptibility of an individual to that material; thus, initial contacts with such a material may not produce any appreciable skin irritation, but contacts

at a later date will produce a prompt reaction of the skin. To determine whether or not a material possesses this potentiality, it is customary to apply the material to the skin of the inner surface of the forearm or to the backs of human subjects. The first application is allowed to remain in contact with the subject for four or five days, at which time it is removed, the area washed, and any reaction noted. (This is primary irritation). About three weeks later the same individual is again tested with the material in the same way except that the exposure time is reduced to 24 or 48 hours. Observations are usually made immediately and after 24, 48 and 72 hours, and also after a week or ten days. The occurrence of a more intense reaction after the second application than after the first or a significant rise in the frequency of reactions among the group as a whole is indicative that sensitization has occurred and that the test material is a sensitizer.

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EXPERIMENTAL RESULTS

In these human tests the two samples of 2,4-dichlorophenoxyacetic acid were tested by the technique described in the preceding paragraph. In both instances, the dry solid material was used.

With K2372-19 (new method material) there was no significant amount of primary irritation and no sensitization noted.

With K2372-20 (old method material) an appreciable amount of primary irritation was observed but no sensitization developed. These results indicate clearly that the material prepared by the new process is distinctly superior from a skin irritation standpoint to that material prepared by the old process.

CONCLUSIONS

Although neither the material prepared by the old or the new method is a skin sensitizer, the difference in ability to produce primary skin irritation is substantial and the liklihood of skin irritation developing in handling and use is much greater with the old product.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

751

2,4-D FACT SHEET

November 1982

A. Background

2,4-Dichlorophenoxyacetic acid (2,4-D) is a systemic herbicide widely used to control broadleaf weeds. The term 2,4-D includes the parent acid as well as the 35 derivatives (esters and salts). It has been used in the United States for over 30 years.

There are approximately 1500 products containing 2,4-D registered with the Environmental Protection Agency. Over 60 million pounds of 2,4-D active ingredient were applied domestically during 1980 with the majority used for the control of broadleaf weeds in small grains, field corn and on range and pastureland.

Interest concerning the potential adverse health effects of 2,4-D intensified following EPA's emergency suspension of 2,4,5-T and Silvex in March 1979. This interest was due to the chemical similarities of 2,4-D and 2,4,5-T.

There was in particular concern that 2,4-D would be contaminated with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) as was 2,4,5-T. This dioxin has been linked with potential fetotoxic and carcinogenic effects. However, the 2,3,7,8 TCDD has never been found in any sample of 2,4-D.

The Agency initiated a thorough review of critical toxicological information available on 2,4-D in an attempt to answer questions raised concerning potential health effects associated with the use of 2,4-D in 1979. This review was conducted to determine whether regulatory action was appropriate.

B. Agency Review

After a review of the existing toxicology data base supporting 2,4-D registrations, EPA concluded that the scientifically valid toxicology studies on 2,4-D did not indicate that the continued use of 2,4-D posed a significant health hazard when used in accordance with label directions and precautions. The Agency did conclude, however, that more information on 2,4-D toxicological properties was necessary. An independent Scientific Advisory Panel, consisting of toxicologists and other health experts, agreed with the Agency's conclusion.

17581

C. Agency Actions

The Environmental Protection Agency, under authority of Section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) may request any additional data from pesticide registrants considered necessary to maintain the registration of existing products. On August 29, 1980, after consulting with the Scientific Advisory Panel, the Agency issued an Order and Notice which required all 2,4-D registrants to supply data in the following areas of potential health concern: acute toxicity, tumor formation, reproduction, birth defects, neurotoxicity, and metabolism.

D. Status of 2,4-D Data Call-In

Major registrants of technical 2,4-D products subject to this data call-in have formed the "Industry Task Force on 2,4-D Research Data" to jointly produce the required data. The Task Force has submitted to the Agency acute oral and dermal toxicity testing results for manufacturing-use 2,4-D products. The chronic effects studies will be coming into EPA as they are done, within the next 1 to 4 years.

DOW2155374

E. Dioxin Question

In 1980, the Canadian government announced that some 2,4-D products manufactured in Canada were contaminated with compounds of the dioxin family, although not necessarily the very toxic 2,3,7,8 TCDD. The Agency cooperated with the Canadians to identify the problem, and also began to sample 2,4-D products made in this country. The contamination problem appears to result from the manufacturing processes principally used in Canada rather than the United States, and the Canadian Government has taken appropriate regulatory action to resolve the problem. The U.S. Government concluded that no change in regulatory positions concerning 2,4-D uses or production methods was called for in this country.

Conclusion

EPA's recent evaluations of 2,4-D do not indicate its currently registered uses pose unreasonable risks to public health or the environment. As with any pesticide chemical, if evidence of such risks does arise, the Agency will take appropriate action to reduce or eliminate those risks.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

752

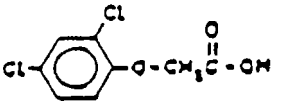
2,4-D FACT SHEET

October 1982

I. 2,4-D DATA CALL-IN

A. Background

2,4-Dichlorophenoxyacetic acid (2,4-D) is a systemic herbicide widely used to control broadleaf weeds. The term 2,4-D includes the parent acid as well as the 35 derivatives (esters and salts). The structure of 2,4-D acid is given below:



There are approximately 1500 products containing 2,4-D registered with the Environmental Protection Agency. Over 60 million pounds of 2,4-D active ingredient were applied domestically during 1980 with the majority used for the control of broadleaf weeds in small grains, field corn and on range and pastureland.

Interest concerning the potential adverse health effects of 2,4-D intensified following EPA's emergency suspension of 2,4,5-T and Silvex in March 1979. This interest was due to the chemical similarities of 2,4-D and 2,4,5-T. The public was concerned that the same toxic effects attributed to 2,4,5-T would also be applicable to 2,4-D. There was also concern that 2,4-D would be contaminated with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) as was 2,4,5-T. This dioxin has been linked with potential fetotoxic and carcinogenic effects. The 2,3,7,8 TCDD has never been found in any sample of 2,4-D. A report of EPA's analysis for dioxins in 2,4-D is presented in part II of this fact sheet. There was also concern because the n-Butyl ester of 2,4-D was used in a 50:50 mixture with n-Butyl 2,4,5-T to produce Agent Orange, the military defoliant widely used during the Vietnam War. Agent Orange has never been registered by EPA for civilian use in the U.S., but its use by the U.S. military in Vietnam has resulted in claims of adverse health effects to American military personnel which are currently under review by the Veterans Administration.

17583

The Agency initiated a review of information available on 2,4-D in an attempt to answer questions raised concerning potential health effects associated with the use of 2,4-D. This review was conducted to determine if 2,4-D should be reviewed under the Rebuttable Presumption Against Registration (RPAR) process or if another regulatory action was appropriate.

B. Agency Review

After a review of the existing toxicology data base supporting 2,4-D registrations, EPA concluded that the scientifically valid toxicology studies on 2,4-D did not indicate that the continued use of 2,4-D posed a significant health hazard when used in accordance with label directions and precautions. The Agency did conclude, however, that more information on 2,4-D toxicological properties was necessary to better assess the potential health hazards associated with the use of 2,4-D.

C. Agency Actions

The Environmental Protection Agency, under authority of Section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) may request any additional data from pesticide registrants considered necessary to maintain the registration of existing products. On August 29, 1980, after consulting with the Scientific Advisory Panel, the Agency issued an ORDER and NOTICE which required all 2,4-D registrants to supply data in the following areas of 2,4-D toxicology: Acute Toxicity, Oncogenicity, Reproduction, Teratogenicity, Neurotoxicity, and Metabolism.

On January 27, 1981 a letter modifying some aspects of the original ORDER and NOTICE was sent to all 2,4-D registrants. This letter stated that the requirement for the studies on acute oral toxicity and acute dermal toxicity of end-use products containing 2,4-D is deferred until the Agency groups the 2,4-D end-use products. Testing will then be required on a representative sample for each group. Registrants of manufacturing-use products are still required to submit acute oral and dermal toxicity studies for these products.

D. Status of 2,4-D data Call-In

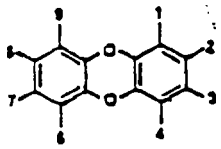
Major registrants of technical 2,4-D products subject to this data call-in have formed the "Industry Task Force on 2,4-D Research Data" to jointly produce the required data. The Task Force has submitted to the Agency acute oral and dermal toxicity testing results for manufacturing-use 2,4-D products.

17584

II. ANALYSIS FOR DIOXINS IN 2,4-D

A. Background

A dioxin is any member of a family of compounds known chemically as dibenzo-p-dioxins and consisting of two benzene rings interconnected by a pair of oxygen atoms. The general structure for dioxins is given below:



Each of the numbered positions can be occupied by a chlorine or other halogen atom, an organic radical, or a hydrogen atom. There are 75 potential isomers of polychlorinated dibenzo-p-dioxin and 22 different isomers of the tetrachlorodibenzo-p-dioxin (TCDD). The term dioxin is often used incorrectly to refer to only one of the 22 isomers of TCDD, the 2,3,7,8 TCDD. This isomer is the highly toxic contaminant of the herbicide 2,4,5-T and has been linked to potential fetotoxic and carcinogenic effects. The 2,3,7,8 TCDD isomer has never been detected in any U.S. samples of 2,4-D tested. For a detailed discussion of dioxins refer to Esposito et al. (1980) which is available through the National Technical Information Service, Springfield, VA 22161.

Canadian 2,4-D technical and formulated products were first reported to be dioxin-contaminated by a group from Agriculture Canada at the workshop on the "Impact of Chlorinated Dioxins and Related Compounds on the Environment" (Cochrane et al., 1980). Sixteen samples of 2,4-D ester and amine formulations as well as ten 2,4-D technical acid samples representing Canadian supplies available in 1980 were analyzed for chlorinated dibenzo-p-dioxins at a detection level of 1 part per billion (ppb). No dioxins were detected in the technical 2,4-D acid samples analyzed. All 2,4-D isooctyl ester samples examined contained dibenzo-p-dioxin contaminants, while 2 of 3 butyl esters and 4 of 7 2,4-D amines showed the presence of chlorodioxin. Levels of dichlorodibenzo-p-dioxin ranged from 5 to 4200 ppb, trichlorodibenzo-p-dioxin ranged from 38 to 2079 ppb and 1,3,6,8 tetrachlorodibenzo-p-dioxin ranged from 20 to 1752 ppb. Levels of all dioxins detected were highest in the isooctyl ester formulations and lowest in the amine salts.

17585

B. Agency Actions

2,4-D Sampling Program

A sampling program of U.S.-produced technical grade 2,4-D products was initiated following Agriculture Canada's announcement of dioxin contaminated 2,4-D products in Canada. Thirty-three samples representing 25 2,4-D technical products produced in the U.S. were collected by EPA Enforcement personnel and delivered to the EPA Chemical and Biological Investigations Laboratory at Beltsville, Maryland for dioxin analysis. Formulations of 2,4-D represented were: acid, sodium salt, various amine salts and several esters. Samples were analyzed using the method of Cochrane et al. (1980). Refinements to the method using appropriate recovery studies validated the technique to a 1 part per billion (ppb) level of sensitivity. All samples were extracted and put through a cleanup and isolation procedure. Final extracts were subjected to low resolution gas chromatograph/mass spectrometer (LR/GC/MS) analysis using software control which allowed detection for ions at mass values of 252, 288 and 322. These ions corresponded to 2,7-dichloro-; 1,2,4-trichloro-; and 1,3,6,8/1,3,7,9 tetrachlorodibenzo-p-dioxins, respectively. The samples were also examined specifically for 2,3,7,8 TCDD and none was found.

Results

Three samples were positive for 2,7 dichlorodibenzo-p-dioxin (Table 1). Levels ranged from 25 to 78 ppb. Two of these three samples also contained the trichlorodibenzo-p-dioxin isomer at levels of 11 to 13 ppb. No tetrachlorodibenzo-p-dioxin was detected in any sample. Sample extracts found to be positive for dioxin were transmitted to the Health Effects Research Laboratory (HERL) at Research Triangle Park, North Carolina for high resolution/GC/MS confirmation. HERL confirmed the presence of the dichlorodioxin and also detected trace amounts (<1 ppb) of 1,3,6,8/1,3,7,9 tetrachlorodibenzo-p-dioxin in two of the three positive samples when analyses were performed at higher sensitivity. The 2,3,7,8 TCDD was never detected in any sample even when analyzed with HR/GC/MS.

Conclusions

Of 33 samples of 2,4-D technical grade acid, ester and salt formulations examined, 30 were free of dioxin contamination. Only three were contaminated with any form of dioxin. The dioxins detected were: 2,7 dichlorodibenzo-p-dioxin, 1,2,4 trichlorodibenzo-p-dioxin and 1,3,6,8/1,3,7,9 tetrachlorodibenzo-p-dioxin. The 2,3,7,8 tetrachlorodibenzo-p-dioxin isomer was never detected in any sample.

17586

January 23, 1981

Fact Sheet on 2,4-D

DOW215536?

Agriculture Canada has taken action to ban the production of some 2,4-D products in Canada, and to control the composition of 2,4-D products sold in the Canadian market in the future. The products which will be affected by this action contain esters of 2,4-D. This action is based on the Canadian findings, announced last fall, of three types of dioxins in 2,4-D products sampled from the Canadian market-place. The dioxins which they found are 2,7 dichlorodibenzo-p-dioxin, 1,3,7 trichlorodibenzo-p-dioxin, and 1,3,6,8 tetrachlorodibenzo-p-dioxin. Some of the samples tested were free of dioxins. When they were present, they ranged in concentration from 5 ppb to approximately 8000 ppb. None of the samples tested contained the highly toxic 2,3,7,8 tetrachlorodibenzo-p-dioxin which is present in 2,4,5-T.

CURRENT EPA ACTION

The Environmental Protection Agency is coordinating its review activities on 2,4-D with Agriculture Canada. When the Agency was informed of the Canadian analytical findings, the Agency began a sampling program to determine whether dioxin contaminents are present in U.S. products. The first phase of this sampling process is nearing completion. The Agency has tested 30 samples of technical 2,4 D acid and ester products and products containing amine salts of 2,4-D. These products were taken directly off the shelves at the production sites. The preliminary findings are as follows: 27 of the samples are free of any dioxin, using a method of analysis sensitive to 1 ppb; 3 of the samples contain the 2,7 dichloro-dioxin in concentrations below 100 ppb.

The EPA sampling program to date has confirmed that there is a significant difference between 2,4-D products sold in the U.S. and in Canada. This may be due to differences between U.S. and Candian manufacturing processes.

The Agency feels that there is no justification for regulatory action to change current uses of 2,4-D products in the U.S., or to prohibit production of 2,4-D products in the U.S., for the following reasons. The products tested from the U.S. market-place are either free of dioxins or contain them at extremely low levels (less than 100 ppb).

There is little data available on the Toxic effects of these dioxins, but existing data indicates they are significantly less toxic than the 2,3,7,8 tetra-dioxin found in 2,4,5-T. Based on the preliminary analyses that the Agency has conducted using available data, the concentrations found in U.S. manufacturing-use products do not appear to pose a health hazard.

ON-GOING EPA REVIEW ACTIVITIES

EPA has a number of review activities under way to assess the safety of 2,4-D. The Agency will continue to sample 2,4-D products to determine whether U.S. manufacturing processes can consistently produce 2,4-D products in the range of the U.S. products which have already been tested.

The Agency will complete a detailed review of the available data on the three dioxins identified in 2,4-D products. In addition, we will make an assessment of additional studies which may be needed and where these studies should most appropriately be conducted.

The Agency also has a long-term effort under way to require additional acute and chronic toxicity data on 2,4-D. On September 2, 1980 EPA sent a data call-in notice under FIFRA 3(c)(2)(B) to all registrants of 2,4-D products. Major registrants of the technical 2,4-D products subject to the notice are negotiating for joint production of the data. They have activities under way to prepare for the testing.

The EPA is developing a long-term work plan for a comprehensive review of the 2,4-D compounds. This work plan will include a complete registration standard review of 2,4-D, to be completed by the time the last studies are submitted. The Agency will review the chronic toxicology studies and the results of our analytical testing as they are submitted to determine whether a change in our regulatory position is warranted, before the entire review is complete.

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72 0840 Ishikawa, K ; Shirahata, R ; Yoshioka, K (Animal Health and Toxicological Lab , Kyowa Chemical Ind Co Ltd , Kitagawa Shizuoka, Japan) **Studies on the behavior of thiocarbamate herbicides in soil. Part I. Gas chromatographic determination of 4-chlorobenzyl N,N-diethylthiolcarbamate (benthiocarb) in soil.** *Jpn Bull Chem Soc* 45 No 1164 1165 1971 (9 references)

Benthiocarb was determined in soils by gas-liquid chromatography using a flame photometric detector Steam distillation was used to remove benthiocarb from the soils followed by liquid chromatography on active charcoal The limit of detection for the residues in soil was 0 02 ppm and for the pure material 0 002 μg When treated with three levels of 0 32, 3 15 and 31 5 ppm, the average recoveries ranged from 70 5 to 93 1 % At the 0 32 ppm level the recoveries were low

72-0841. Bogacka, I (Dept. of Water Protection, Water Economics Res Inst., Gdansk, Poland) **Zastosowanie 4-aminoantypiryny do kolorymetrycznego oznaczania pochodynch kwasow chlorofenoksyalkilowych w wodzie. [Application 4-aminoantipyrine to colorimetric determination of chlorophenoxyalkyl acid derivatives.]** *Chem Anal Warsaw* 16 1 59 64 1971 (9 references) (Polish)

Herbicides such as 2,4-D, MCPA and 2,4-DP can be determined colorimetrically (when concentrations exceed 0 02-0 08 mg/l) by applying 4-aminoantipyrine The herbicide is extracted with ethyl ether from 1 liter of water followed by coupling of the phenyl ether by pyridine hydrochloride to phenol derivatives which are determined colorimetrically with 4-aminoantipyrine This method cannot be used to determine mixtures of herbicides owing to variations in color response Natural waters contaminated with 2,4-D from the environment and natural waters to which herbicides have been added have been tested by this method The standard deviation ranges from 3 5 to 8 5

72 0842. Bogacka, I ; Taylor, R (Dept of Water Protection, Water Economics Res Inst , Gdansk, Poland) **Oznaczanie herbicydow 2,4-DP i MCPP w wodzie metoda chromatografii cienkowarstwowej. [Determination of herbicides 2,4-DP and MCPP in water by thin-layer chromatography.]** *Chem Anal Warsaw* 16 1 215 218 1971 (3 references) (Polish)

The thin-layer chromatographic method reported previously (*Chem. Anal. Ly* 115, 1970) for the determination of 2,4-DP and MCPA has been modified for the determination of 2,4-DP and MCPP The ethyl ether extract is purified on a column of silicic acid and the herbicides are separated on silica gel chromatoplates (0 25 mm) with light petroleum-acetic acid-benzene (10 1 2) as solvent The sensitivity of the compounds is 5 μg/cm² and recoveries of 2,4-DP and MCPP are 85 % and 95 4 % respectively, and the corresponding standard deviations are 1 59 and 1 5 %

72 0843 Zyss, T ; Kaczor, K ; Wyszkowski, M (Office Poznan, Poland) **Oznaczanie pozostalosci chlorowanych weglowodorow w zbozach produktach i paszach zbozowych [Determination of chlorinated hydrocarbon residues in cereal products and feeds.]** *Chem Inst Warsaw* 16 1 1961 1969 (17 references) (Polish)

The procedure of Mills *et al* (*J Assoc Off Agric Chem* 46 186, 1963) was used for the extraction and cleanup of chlorinated pesticide residues in cereal products A sample is extracted with acetone-water mixture, diluted with water, and extracted with petroleum ether The residues are purified by chromatography on a Florisil column by elution with a mixture of petroleum and ethyl ethers Gas chromatography is used to determine the residues Recoveries of lindane, *p,p'*-DDE, and *p,p'*-DDT were measured by electron capture gas chromatography Average recoveries at 0 2 ppm and 0 4 ppm level for lindane were 96 9 and 97 3 % respectively, for *p,p'*-DDE at 0 5 ppm and 1 0 ppm the recoveries were 95 1 and 98 8 % for *p,p'*-DDT at 0 5 ppm and 1 0 ppm were 87 2 and 86 1 % respectively The level of residues only slightly influences the precision of the method

72 0844. Szokolay, A ; Uhnak, J ; Madaric, V. (Geschaftsgesundstitut fuer Hygiene, Bratislava 1, Czechoslovakia) **Gaschromatographische Bestimmung der Rueckstaende von Insektiziden im Milchfett nach einstufigen Isolationsverfahren. [Gas chromatographic estimation of chlorinated insecticide residues in milk fat after one-step isolation procedure.]** *Chem Zvesti* 25 6 456 464 1971 (22 references) (German)

A gas chromatographic method has been developed for the detection of organochloride residues in milk fat The method involves an isolation procedure (extraction and purification with 15 g of sample) and demands 0 3 g of weighed milk fat The yields for DDE, DDT and BHC were 81 0 to 91 8 % The accuracy of the method was assessed by standard deviation was 0 02 to 0 0008 mg/l and variation coefficients ranged from 5 4 to 15 3

72 0845 Sikes, M H ; Sullivan, H C ; Dale, H E (Pesticides Program Res Branch, Dept of Health, Education & Welfare, Res Triangle Park, NC 27514) **A method for determination of low levels of exposure to 2,4-D and 2,4,5-T** *Bull Environ Contam Toxicol* 5 5 417 424 1970 (8 references)

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Fred A. Smith 1-21-81

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DOW CHEMICAL U.S.A.

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LABORATORY REPORT CODE: HET K-002372-(24)

DATE ISSUED: 9 December 1980

DEPARTMENT: TOXICOLOGY RESEARCH LABORATORY

LAB. NO.: 1 5 5 | PROBLEM NO.: 3 1 3 7 2 2 4

CRI NUMBER

TITLE: Pharmacokinetics of 2,4-Dichlorophenoxyacetic Acid in Fischer 344 Rats

31 PAGES IN FULL REPORT

AUTHOR(S): F.A. Smith, R.J. Nolan, E.A. Hermann, and J.C. Ramsey

AUTHOR(S) SIGNATURE(S): F.A. Smith 12-9-80; R.J. Nolan 12-9-80; E.A. Hermann 12-9-80; J.C. Ramsey 12/9/80

REVIEWER'S SIGNATURE: W. H. Braun 12/9/80

This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The pharmacokinetics of 2,4-D, a plant growth regulator and herbicide, was defined in male Fischer 344 rats. First, groups of 3-4 rats were given single oral doses of ^{14}C-UL-2,4-D sodium salt ranging from 10 to 150 mg/kg or intravenous doses of 5 and 90 mg/kg and the concentration-time-profile of radioactivity in the plasma and excretion in the urine were determined. Analysis of the data indicates that 2,4-D is extensively absorbed and rapidly cleared from blood in a biphasic manner. Eighty-nine to 92% of the dose was eliminated via the urine within 24 hr. Second, groups of 6 rats orally administered 10, 25, 50 or 150 mg/kg were sacrificed after 6 hr. At dose levels above 50 mg/kg there was a disproportionate increase in plasma ^{14}C concentration accompanied by a disproportionate decrease in the amount of ^{14}C excreted in urine and in the kidney/plasma ratios of ^{14}C concentration. The K_m for saturable clearance from plasma is approximately 79 µg/ml. When the plasma concentration decreases below this K_m value, plasma clearance is adequately defined by first-order kinetics.

DOW 2155287

[*Contains or relates to trade secrets, confidential or proprietary information of the industry task force on 2,4-D research data.**]

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PHARMACOKINETICS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN FISCHER 344 RATS

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9 December 1980

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SUMMARY

The pharmacokinetics of 2,4-D, a plant growth regulator and herbicide, were defined in male Fischer 344 rats. First, groups of 3-4 rats were given single oral doses of ^{14}C-UL-2,4-D sodium salt ranging from 10 to 150 mg/kg or intravenous doses of 5 and 90 mg/kg and the concentration-time-profile of radioactivity in the plasma and excretion in the urine were determined. Analysis of the data indicates that 2,4-D is extensively absorbed and rapidly cleared from blood in a biphasic manner. Eighty-nine to 92% of the dose was eliminated via the urine within 24 hr. Second, groups of 6 rats orally administered 10, 25, 50 or 150 mg/kg were sacrificed after 6 hr. At dose levels above 50 mg/kg there was a disproportionate increase in plasma ^{14}C concentration accompanied by a disproportionate decrease in the amount of ^{14}C excreted in urine and in the kidney/plasma ratios of ^{14}C concentration. The K_m for saturable clearance from plasma is approximately 79 µg/ml. When the plasma concentration decreases below this K_m value, plasma clearance is adequately defined by first-order kinetics.

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INTRODUCTION

Phenoxyacetic acids as a group are generally absorbed readily from the gastrointestinal tract, are reversibly bound to plasma protein, and in the case of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are excreted essentially unchanged via the urine (Gehring, 1977). Urinary excretion occurs by an active process which can be overwhelmed when large doses are given (Hook, 1974), resulting in Michaelis-Menten rather than apparent first-order kinetics. Evidence for this dose-dependent fate of the phenoxy acids is usually comprised of an increase in fecal excretion, an increasing apparent half-life of elimination and an increasing fraction (though still minimal) of the dose being metabolized.

Khanna and Fang (1966) indicate elimination of 2,4-D may be dose-dependent and their data imply urinary excretion may become non-linear when doses at or above 100 mg/kg are given to Wistar rats. They also show urine is the major route of excretion and the parent compound is excreted unchanged (99%) in the urine of rats. Since 2,4-D is not metabolized to a significant extent, the time-course of ^{14}C activity following the administration of ^{14}C-labeled 2,4-D will reflect the time-course of unchanged 2,4-D.

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Stroo, McCormack and Hook (1979) have shown a single dose of 20 mg/kg 2,4,5-T to Sprague-Dawley rats decreased renal anion and cation transport in vivo 4 hr after treatment but the effect was no longer apparent after 24 hr. Increasing the dose to 100 mg/kg produced effective inhibition of anion transport 24 hr after treatment. Repeated daily administration of the above doses of 2,4,5-T produced effects on renal ion transport similar to those observed after a single exposure. This suggests that renal tissue does not progressively accumulate 2,4,5-T in vivo on chronic exposure. These data also support the conclusions of Kashier and Berndt (1976) that the decrease in anion transport is not a manifestation of toxicity but rather reflects the competition of 2,4,5-T with endogenous materials for the anion transport system.

As stated, this same dose dependence of urinary excretion also exists for 2,4-D. It is therefore important to define at what approximate dose levels saturation of the elimination process occurs, since the increased body burden of 2,4-D resulting from repeated saturating dose levels would negate the linear extrapolation of toxicity arising from high dose levels to that expected at lower dose levels.

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The purpose of this study was to define the dose-dependent fate of 2,4-D in rats. The major objective was to identify the approximate dose level at which the kinetics of elimination of 2,4-D began to show evidence of saturation effects.

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METHODS

Animals. Male Fischer 344 rats obtained from Charles River Breeding Laboratories, Portage, Michigan and weighing between 200 and 260 grams were used. Upon arrival at the laboratory, the rats were examined for health status by a qualified professional and subsequently acclimated to the test environment according to standard operating procedures. The rats were identified by a metal ear tag inserted within 2 days after their arrival. When required, cannulas were implanted in the left jugular vein using standard surgical techniques (Harms and Ojeda, 1974) at least 2 days prior to initiation of the study. Prior to the initiation of the study, the rats were housed 2/cage in wire-bottomed holding cages. They were individually housed and acclimated for 2 days prior to dosing in all-glass Roth-type metabolism chambers designed for the separate collection of urine and feces. The study was conducted in a room with a 12-hr light cycle, a controlled temperature of 20-22°C and a relative humidity of 40-60%. Ralston Purina Lab Chow and tap water were provided ad libitum.

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Test Material. Unlabeled 2,4-D, reference OC-559-141, was furnished by The Dow Chemical Company, Midland, MI. The compound was purified by recrystallizing once from ethyl benzene and twice from perchlorethylene. This recrystallization process resulted in a compound of 99.9% purity. Uniformly ^{14}C-ring labeled 2,4-D (^{14}C-2,4-D) lot number 71106, with a specific activity of 4.71 mCi/mmole, and a reported purity of >98% was obtained from Pathfinder Labs, St. Louis, MO. Upon analysis in our laboratory the radiochemical purity was found to be only 84%. The following techniques were used to purify the ^{14}C-2,4-D prior to the initiation of the study (L. H. McKendry, Agricultural Products, R&D, 1979).

The major impurities were esters of 2,4-D. The esters were hydrolyzed by treating the residue (dried under nitrogen) with a 10% sodium hydroxide solution at 50°C for 1 hr. This solution was adjusted to neutral pH with addition of concentrated hydrochloric acid and then to a basic pH with the addition of sodium bicarbonate. The basic solution was then extracted three times with petroleum ether and the organic phase discarded. The aqueous phase was then acidified with concentrated hydrochloric acid and extracted four times with petroleum ether. The petroleum ether phase was then dried with magnesium sulfate; the solvent removed under vacuum and the residue redissolved in acetone.

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The solution was spotted on silica gel plates in two separate solvent systems. The resulting chromatograms showed a product of apparently >99% radiochemical purity containing only 0.1% of the methyl ether of 2,4-dichlorophenol. However, 2,4-D and 2-, 3-, and 4-monochlorophenoxyacetic acids co-elute on these two TLC systems used, and analysis by HPLC showed approximately 4% contamination with one or more of these monochlorophenoxyacetic acids.

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Preparative reverse phase liquid chromatography was then used to separate the remaining impurities. A 50-100 µl (maximum) aliquot was injected onto a preparative reverse phase micro Bondapak C_{18} column (Waters Associates Inc., Milford, MA). Eluent was a 30% acrylonitrile/70% H_2O solution buffered to pH 3.0 with H_3PO_4 and NaOH. By monitoring the ultraviolet response and retention time for 2,4-D, the appropriate LC fraction containing the 2,4-D was collected and the impurities discarded with the remaining eluent. The total collected fraction of eluent containing 2,4-D was extracted three times with benzene, the extracts were combined and evaporated to dryness under a stream of dry nitrogen. The compound was then redissolved in acetone. Aliquots of this solution were injected into the Bondapak C_{18} column, fractions collected and counted for ^{14}C activity. The resulting chromatograms showed a 99% radiochemical purity. Two minor uncharacterized impurities comprised the remaining 1%.

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Dosing Solution. Unlabeled 2,4-D was dissolved in a mixture of acetone and water (25:75, v/v) and converted to the sodium salt by the addition of 0.2N NaOH with gentle heating. The solution was neutralized (pH 6.8-7.2) by the addition of 0.1N HCl. The dosing solutions were prepared by mixing appropriate volumes of a 30 mg/kg stock solution containing the sodium salt of 2,4-D, the ^{14}C-labeled 2,4-D and distilled water. The concentration of 2,4-D in the dosing solutions was adjusted such that the targeted dose was administered when 1 and 5 ml/kg of the i.v. and p.o. dosing solutions, respectively, were administered.

Study Design. This study was conducted in two separate segments. The study design of the two segments follows.

1. To determine the time course of ^{14}C concentration in plasma and the rate of excretion of urinary ^{14}C, 3 groups of 3 jugular cannulated rats were administered oral doses of ^{14}C-2,4-D at levels of 10, 50 or 150 mg/kg. The plasma concentration of ^{14}C activity was determined at selected time intervals following administration, and urinary ^{14}C activity was measured for 72 hr after dosing. Total recovery of ^{14}C activity was determined for those groups of rats receiving either 10 or 150 mg/kg. In addition, similar measurements

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were made following intravenous administration to 2 groups of 3 jugular cannulated rats receiving dose levels of either 5 mg/kg or 90 mg/kg.

Blood samples of approximately 75 µl were collected at approximately 1, 2, 3, 6, 9, 12, 15, 18 and 24 hours after dosing and at 12-hour intervals thereafter until radioactivity in the plasma was less than three times background. Additional early sampling times were taken after the 5 mg/kg i.v. dose to more precisely define the distribution phase. The blood samples were collected in heparinized capillary tubes and centrifuged to obtain the plasma. At termination of the experiment the rats were killed by exsanguination, resulting in blood samples of approximately 5 ml or greater. Weighed aliquots of the plasma were added directly to ACS (liquid scintillation fluid; Amersham Corporation) and the radioactivity quantified in a liquid scintillation spectrometer.

Urine samples were collected at 6-hour intervals for the first 24 hours after dosing and at 12-hour intervals for the next 48 hours. The urine was collected in dry ice cooled containers. Weighed aliquots of the urine were added directly to ACS and the radioactivity quantified in a liquid scintillation spectrometer. The pH of the urine was determined using MULTISTIX reagent strips. Fecal samples

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were collected at 24-hr intervals. Skin and aqueous homogenates of feces and carcass were oxidized in a Harvey Instruments Biological materials oxidizer. The ^{14}C-CO_2 produced was trapped and quantified using standard laboratory procedures.

2. To determine the effect of dose level on kidney/plasma ratios of ^{14}C concentration, 5 groups of 6 rats each were given single oral doses of 10, 25, 50, 100 and 150 mg/kg ^{14}C-2,4-D and all rats were anesthetized and killed by exsanguination 6 hr after dosing. Plasma, urine, and kidneys were collected and analyzed for ^{14}C-levels. Whole blood was collected in heparinized containers and centrifuged to obtain plasma. Plasma, urine and kidney samples were analyzed for ^{14}C activity as described above.

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Pharmacokinetic Modeling. Blood plasma concentration data were fit to the pharmacokinetic model shown in Figure 1. The model was defined by the appropriate set of simultaneous differential equations and the values of the time-dependent variables were determined by numerical integration using the Continuous System Modeling Program (CSMP, IBM, 1972). Best estimates of the pharmacokinetic parameters of the model were obtained with an iterative flexible polygon search routine using the least squares criterion. The apparent volume of distribution (V_d) varied with administered dose level, and

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the best estimates for the volumes of distribution were obtained at each dose level using preliminary values for all other pharmacokinetic parameters. Then, the values for the volumes of distribution were fixed and the entire set of blood plasma concentration data at all dose levels (by either route of administration) was used to further refine the pharmacokinetic parameter estimates. Thus, the best parameter estimates were obtained by simultaneously fitting all blood concentration data at all dose levels for either the i.v. or oral route of administration.

Areas under the blood plasma concentration versus time curves (AUC) from 0-72 hours were determined by numerical integration using the values of the pharmacokinetic parameters obtained above. The AUC were extrapolated from 72 hours to infinity as described by Gibaldi and Perrier (1975).

RESULTS

Observations. No changes in appearance or demeanor were seen after oral or i.v. administration of 2,4-D in this study.

^{14}C-Material Recovery. The total recovery of ^{14}C activity following the 10 and 150 mg/kg single oral dose is presented in Table 1. Although not designed as a ^{14}C recovery study,

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the recovery of ^{14}C following the 50 mg/kg oral dose is also presented in Table 1 for comparison. Overall, 97 and 95% of the administered radioactivity was recovered in 72 hr from the 10 and 150 mg/kg studies, respectively.

Plasma ^{14}C-Concentrations. The time-course of radioactivity in the plasma following intravenous and oral administration of ^{14}C-2,4-D is presented in Figures 2 and 3, respectively, in which concentration of radioactivity has been expressed as µg equivalents ^{14}C-2,4-D/g plasma. The data points are mean ± S.D. for three rats, and the solid lines represent the concentration predicted by the best pharmacokinetic parameter estimates for each set of data fit simultaneously to the model shown in Figure 1. The estimates for the pharmacokinetic parameters following both intravenous and oral administration are shown in Table 2. The multiple correlation coefficient for the best fits of these two data sets was 0.90 and 0.86, respectively.

Intravenous Administration. The concentration of radioactivity in the plasma following the 5 mg/kg intravenous dose was linear and did not reveal any indication of saturated clearance. The terminal (or β) clearance phase at this dose was not clearly evident because the concentration of ^{14}C-activity fell below analytical limits.

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The plasma data for the 90 mg/kg i.v. dose was clearly biphasic and showed evidence for the saturable (Michaelis-Menten) clearance of 2,4-D from plasma in the downward curving trend during the first few hours after administration. The half-life for the initial (α) phase was 0.92 hr at both dose levels. The half-life for the terminal (β) phase at both dose levels was 14.4 hr.

Oral Administration. Two hours after the 10, 50 and 150 mg/kg single oral doses, the plasma concentrations were proportional to the administered dose levels of ^{14}C-2,4-D. However, the initial downward curving trend at the high dose levels was indicative of saturable clearance from plasma. The clearance from the plasma was clearly biphasic at all three dose levels with a terminal (β) phase half-life of 18.0 hr. The initial (α) phase half-life was 1.01 hr. The area under the curves (AUC) showed a disproportionate increase from the lowest to the highest dose level. The relative AUC (extrapolated to infinity and normalized to the lowest dose level) were 1.0, 0.6 and 1.5 at the 10, 50 and 150 mg/kg dose levels, respectively.

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Urinary Excretion of Radioactivity. Radioactivity was rapidly excreted via the urine following intravenous or oral administration of ^{14}C-2,4-D and the pharmacokinetic profile was very similar following both routes of administration. Typically, over 85% of the administered ^{14}C-radioactivity was excreted in the urine during the first 12 hr following the dose. The percent of dose recovered in the urine 72 hr after oral administration is shown in Table 1. The percent of dose recovered in the urine 72 hr after intravenous administration was 100.26 and 90.56% from the 5 and 90 mg/kg dose levels, respectively.

The pattern of urinary ^{14}C-radioactivity excreted per 6 hr interval exhibited a rapid log-linear decline for the first 24 hr following the dose, followed by an apparently slower and poorly defined excretion of radioactivity. Since this slower excretion of radioactivity was comprised of widely scattered data points, and represented a total of no more than 1% (mean of all dose levels by either route, range was 0.23 to 2.04% for the 5 mg/kg and 150 mg/kg dose levels, respectively) of the urinary ^{14}C-radioactivity, its contribution to the urinary excretion process was considered to be negligible.

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The predominant portion of the urinary radioactivity was excreted in an apparent first order manner during the first 24 hr, and the resulting slope of the semi-logarithmic plot of percent administered radioactivity excreted per 6 hr interval versus time was determined by log-linear regression. The results for both i.v. and oral routes are shown in Figures 4 and 5, respectively. The mean half-life values for urinary excretion of radioactivity was 1.75 and 2.10 hr for the i.v. and oral routes, respectively. The mean first order rate constant for both routes was 0.367 ± 0.076 hr^{-1} which corresponds to a half-life value of 1.89 hr.

An indication of non-linearity of the urinary excretion of 2,4-D resulting from higher dose levels was seen by the delayed initiation of the log-linear excretion phase at successively increasing dose levels in Figures 4 and 5. The first 6 hr urinary collection interval represented an average of 41, 26, and 6% of the administered oral dose at the 10, 50 and 150 mg/kg dose levels, respectively. Similarly, the first 6 hr interval represented 90 and 8% of the administered i.v. dose at the 5 and 90 mg/kg dose levels, respectively.

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Tissue and Urine Concentration of ^{14}C at 6 Hours. The concentration of radioactivity in the plasma and amount of ^{14}C excreted in the urine as a function of dose are plotted in Figure 6 as the mean ± S.D. for 6 rats at each dose level. The solid lines were drawn by inspection. The plasma ^{14}C concentration 6 hr after dosing increased disproportionately at dose levels above 50 mg/kg. Also, the amount of 2,4-D excreted in the urine during the first 6 hours decreased relative to the dose level at doses above 50 mg/kg and appeared to approach a limiting value. The average amount of 2,4-D excreted at 100 and 150 mg/kg was 8.7±3.3 mg, equivalent to approximately 1.5 mg/hr.

When plasma concentration is plotted vs. kidney concentration of ^{14}C at 6 hr after dosing (Figure 7) the plasma concentration increases relative to the kidney concentration at the higher dose levels.

DISCUSSION

The absorption of 2,4-D by the oral route of administration is complete as evidenced by excretion of over 85% of the dose in 12 hr and recoveries of 97 and 95% of the 10 and 150 mg/kg oral doses, respectively, after 72 hr. In comparison the amount of dose excreted after intravenous administration

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shows 99 and 86% of the dose excreted in 12 hr and recoveries of 100 and 91% of the 5 and 90 mg/kg dose, respectively. The absorption of 2,4-D is also rapid as shown by the absorption rate constant of 1.41 hr^{-1} derived from the plasma data.

Plasma profiles are clearly similar by both routes of administration as shown by the biphasic curves at all doses (Figures 2 and 3). By both routes of administration there was an initial downward curving trend at the high dose levels indicative of saturable clearance from the plasma. The area under the curves (AUC) supported this saturation showing a disproportionate increase of AUC with increasing dose level. The above similarities support that the pharmacokinetic model is the same by both routes of administration and at all doses.

The pharmacokinetic model chosen for plasma incorporates saturable clearance. It is known 2,4-D is not extensively metabolized (Khanna and Fang, 1966) and is excreted almost entirely in the urine. Therefore, this saturation effect probably reflects saturable urinary excretion. Support of this is shown by the plasma/kidney ratios depicted in Figure 7. This clearly shows increasing plasma concentrations relative to kidney concentrations with increasing dose.

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Further support of this saturable excretion is seen in the pattern of urinary ^{14}C-activity excreted during the first 6 hr interval after dosing. A delayed initiation of the log-linear excretion phase at successively increasing doses by either route is seen (Figures 4 and 5). The first 6-hr urinary collection interval by either route represented an average of 41, 26, and 6% of the administered oral dose at the 10, 50 and 150 mg/kg dose levels, respectively, and 90 and 8% of the administered i.v. dose at the 5 and 90 mg/kg dose levels, respectively.

The average half-life for the α phases by the i.v. and oral routes are 0.915 and 1.01 hr, respectively. The average half-life for the β phases by the i.v. and oral routes are 14.4 and 18.0 hr, respectively. Further similarities are seen in the V_m values (46.1 and 53.7 µg/ml hr for i.v. and oral, respectively) and in the K_m values (61.7 and 78.9 µg/ml for i.v. and oral, respectively). These similarities further support that the pharmacokinetic model is the same by both routes and at the dose levels studied. Therefore, toxicity studies at similar doses but by different routes would be expected to be equivalent.

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The model depicted in Figure 1 (Gibaldi and Perrier, 1975) was utilized to calculate α and β and their associated half-lives shown in Table 2. These values are based on the assumption that the first order rate constant for clearance is approximated by the ratio of V_m/K_m. The resulting α and β values are therefore dependent on this constant ratio. Calculation of these α and β values and their associated half-lives is a valid approximation only when the blood concentration is substantially less than K_m. The limiting case for the ratio of V_m/K_m is approached when the blood concentration approaches approximately one-tenth the K_m value.

Based on this study doses greater than 50 mg/kg are needed to bring the plasma concentration above the K_m. At or above this dose saturation of clearance becomes evident. It should be emphasized that even at dose levels above 50 mg/kg plasma is still cleared efficiently and 18-24 hr after dosing, the blood concentration is well below the K_m value.

A multiple dose simulation was performed based on the pharmacokinetic model of Figure 1 and the parameter values shown in Table 2. This model predicts (Figure 8), that upon repeated daily oral dosing, 99% of the steady state will be

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reached in 2 days for either the 10, 50 or 150 mg/kg doses. The rapid elimination of 2,4-D in the urine, the small contribution of the β phase and this multiple dose simulation, all indicate a low potential for accumulation of 2,4-D in the body.

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QUALITY ASSURANCE STATEMENT

This report represents data generated prior to the enactment of the FDA Good Laboratory Practice Regulations. The study was conducted according to standards used in this laboratory at that time. The report accurately reflects all of the data generated. All data and reports are located at the submitting laboratory.

Study Started:	September 1978	Report Issued:	09 December 1980
Protocol Audited:	September 1978	Reported:	September 1978
S.O.P. Data Audited:	28 August 1978	Reported:	28 September 1978
Final Report Audited:	20 November 1980	Reported:	21 November 1980

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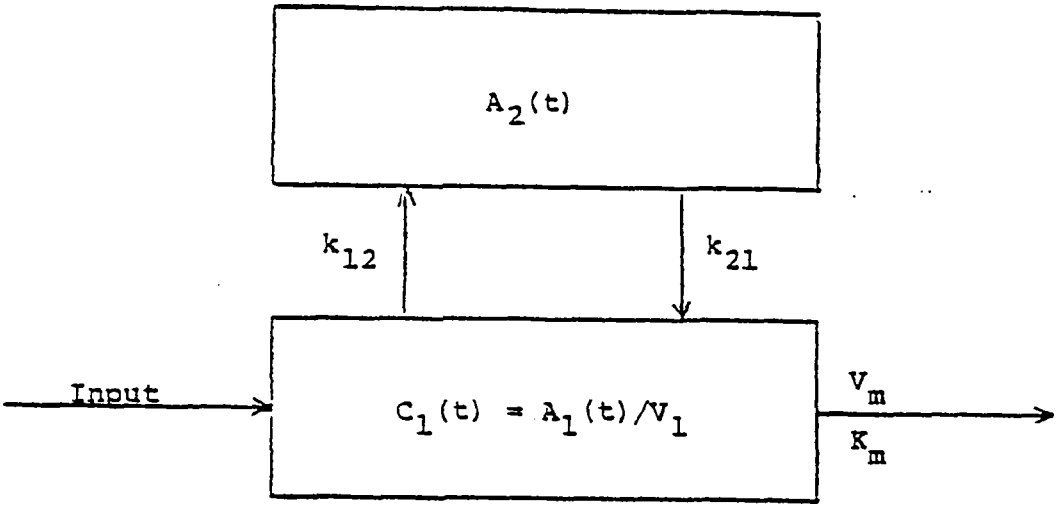


FIGURE 1. Two-compartment pharmacokinetic model describing the distribution and saturable clearance of ^{14}C-2,4-D in the blood plasma of rats. The input function is an instantaneous input for intravenous administration, or a first order rate constant (k_{01}) for oral administration. The transfer coefficient k_{12} and k_{21} between the central (blood) and peripheral compartments are first order rate constants. The clearance of 2,4-D from the central compartment is described as a saturable process with a maximum rate of V_m and one-half the maximum rate at a blood concentration of K_m (the Michaelis constant). See Table 2 for the dimensions of the pharmacokinetic parameters.

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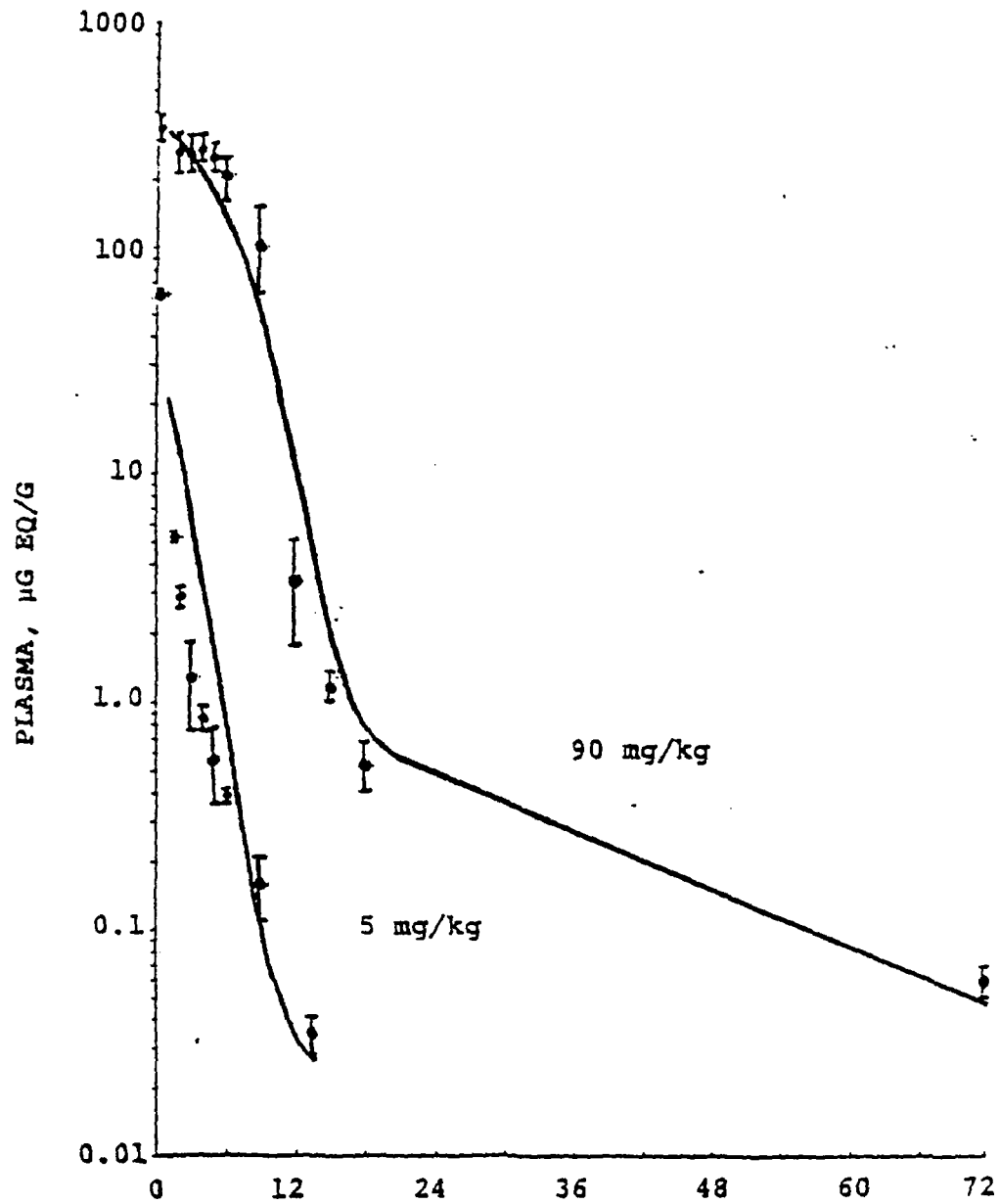


FIGURE 2. Semilogarithmic plot of the plasma concentration of ^{14}C activity, as µg eq/g, resulting from a single intravenous dose of 5 or 90 mg/kg ^{14}C-2,4-D.

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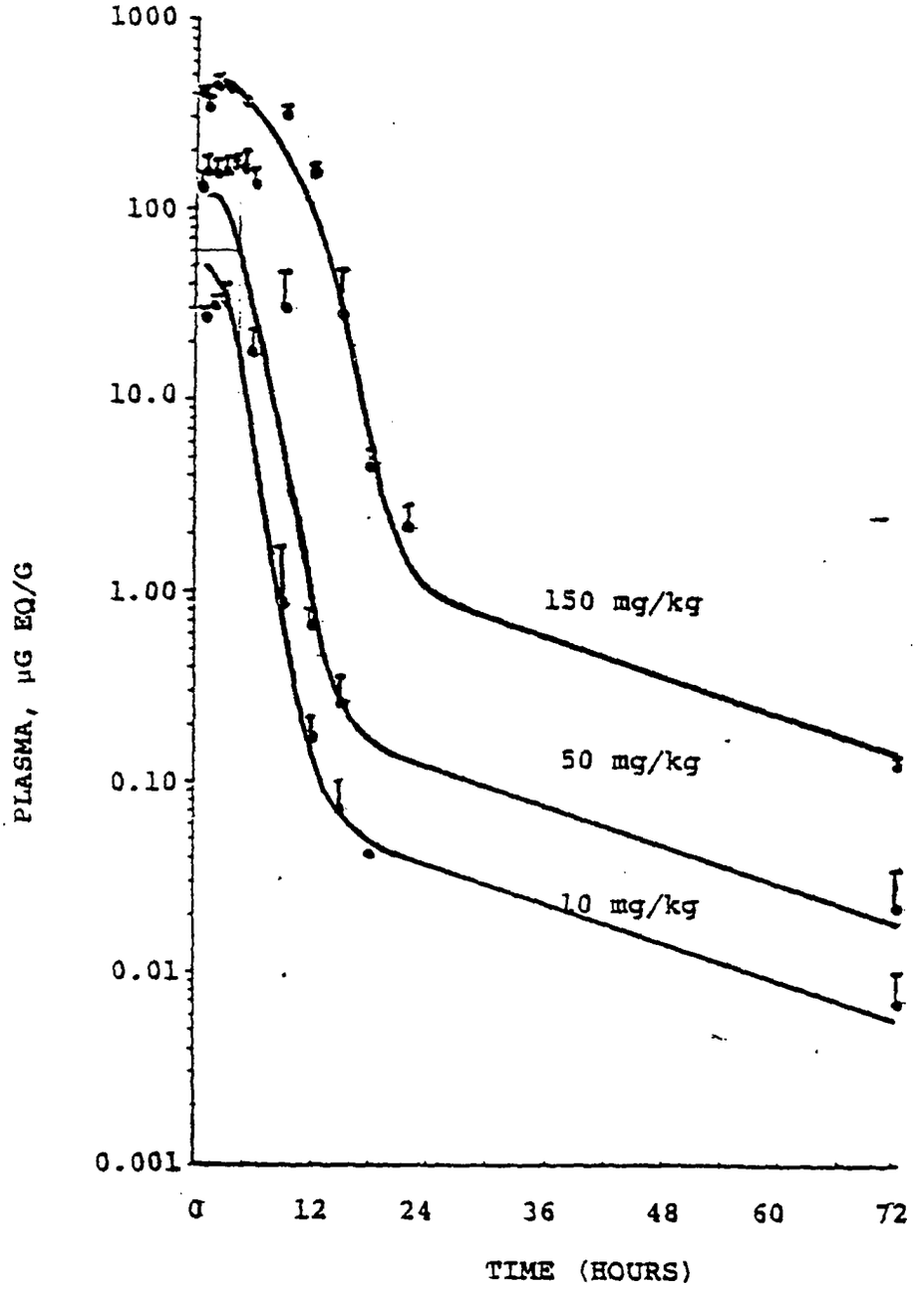


FIGURE 3. Semilogarithmic plot of the plasma concentration of ^{14}C-activity, as µg eq/g, resulting from a single oral dose of 10, 50 or 150 mg/kg ^{14}C-2,4-D.

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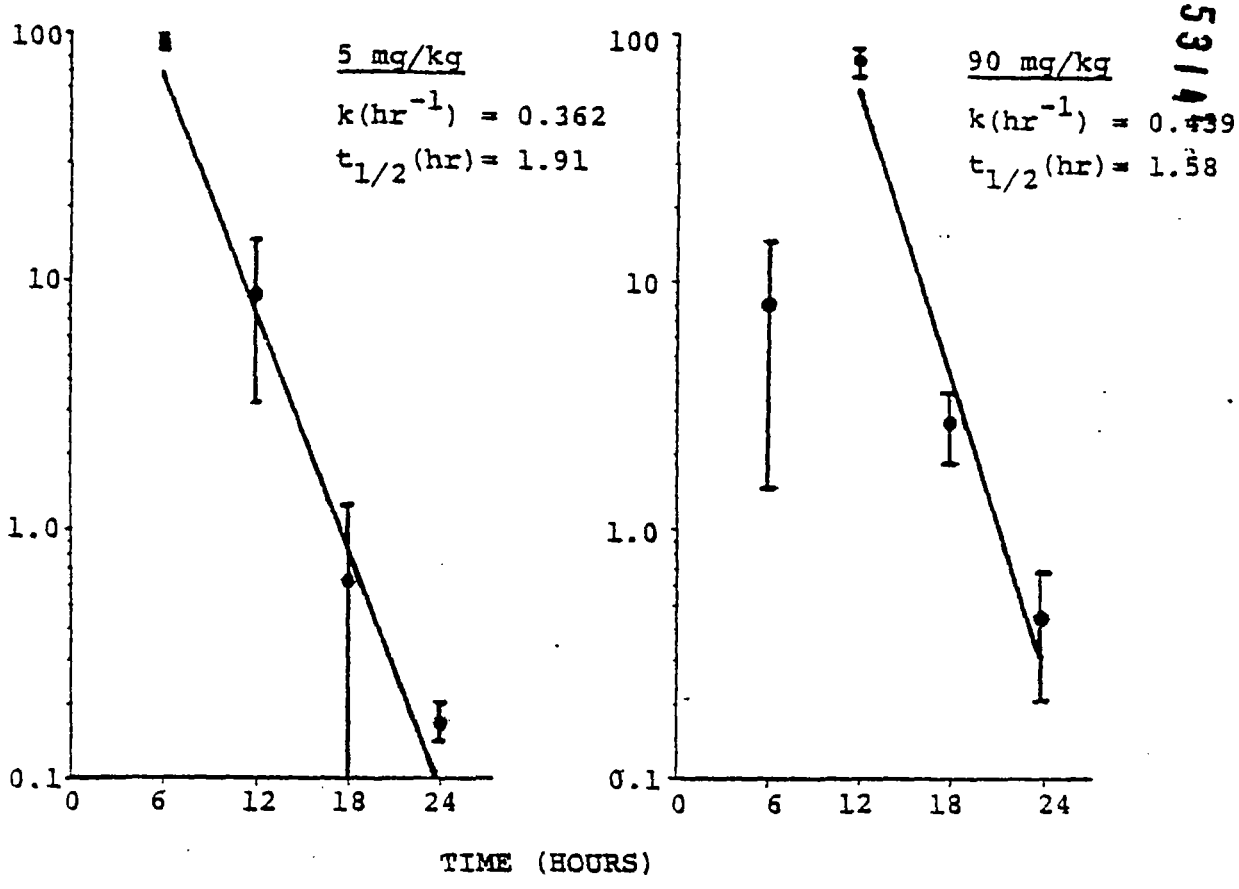
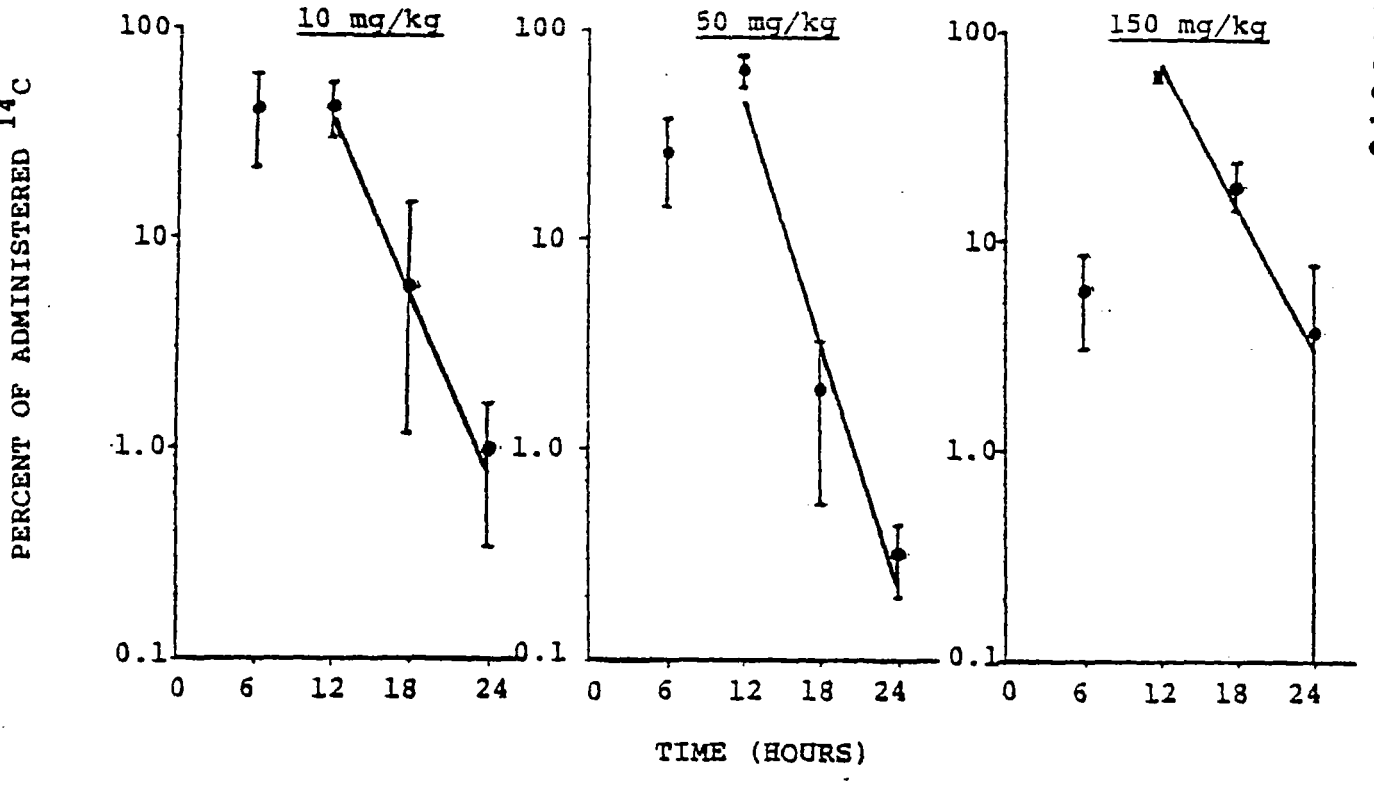


FIGURE 4. Semilogarithmic plot of percent of intravenously administered ^{14}C excreted in the urine per 6-hour interval versus time. Data points are mean ± S.D. for 3 rats. Solid lines were determined by log-linear regression for the time intervals shown (6, 12, 18 and 24 hours at 5 mg/kg; 12, 18 and 24 hours at 90 mg/kg). The percent of administered dose recovered from urine during the 24-72 hr interval was 0.24 and 1.33% for the 5 and 90 mg/kg doses, respectively.

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	10 mg/kg	50 mg/kg	150 mg/kg
$k(hr^{-1})$	0.323	0.444	0.266
$t_{1/2}(hr)$	2.14	1.56	2.61

FIGURE 5. Semilogarithmic plot of percent of orally administered ^{14}C excreted in the urine per 6-hour interval versus time. Data points are mean ± S.D. for 3 rats. Solid lines were determined by log-linear regression for the intervals shown (12, 18 and 24 hours at all dose levels). The percent of administered dose recovered from urine during the 24-72 hr interval was 1.2, 0.44 and 2.04% for the 10, 50 and 150 mg/kg doses, respectively.

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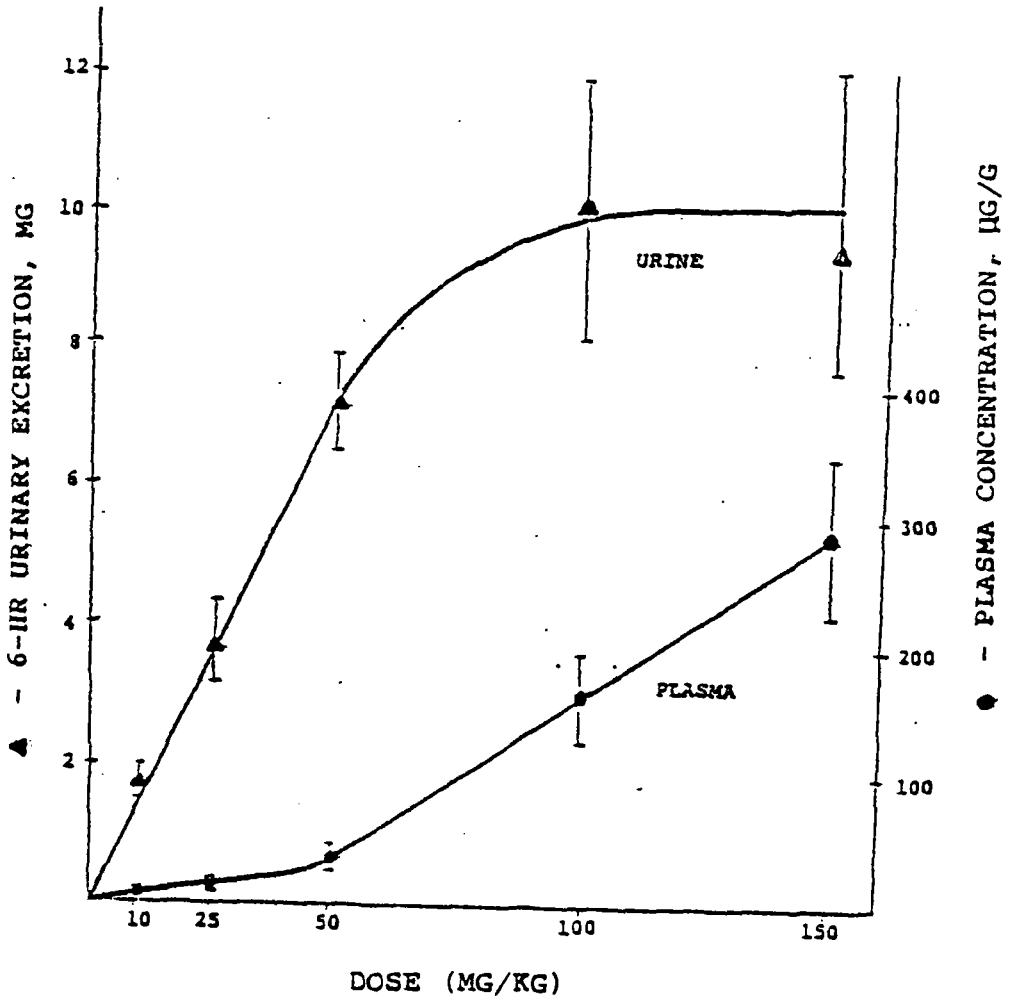


FIGURE 6. Plasma concentration and amount excreted in the urine 6 hr after a single oral dose of ^{14}C-2,4-D. Solid lines are drawn by inspection.

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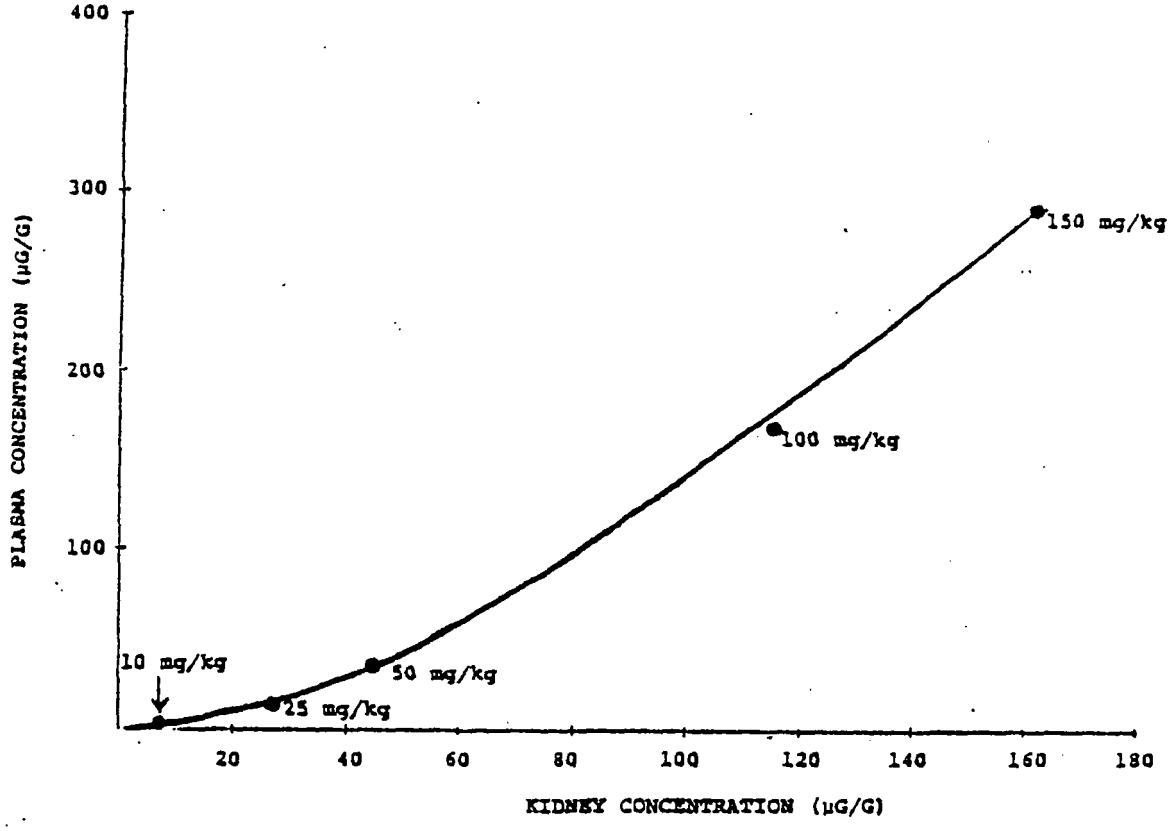


FIGURE 7. Kidney concentrations versus plasma concentrations of ^{14}C 6 hours after an oral dose of 10 to 150 mg ^{14}C-2,4-D/kg. Solid line drawn by inspection.

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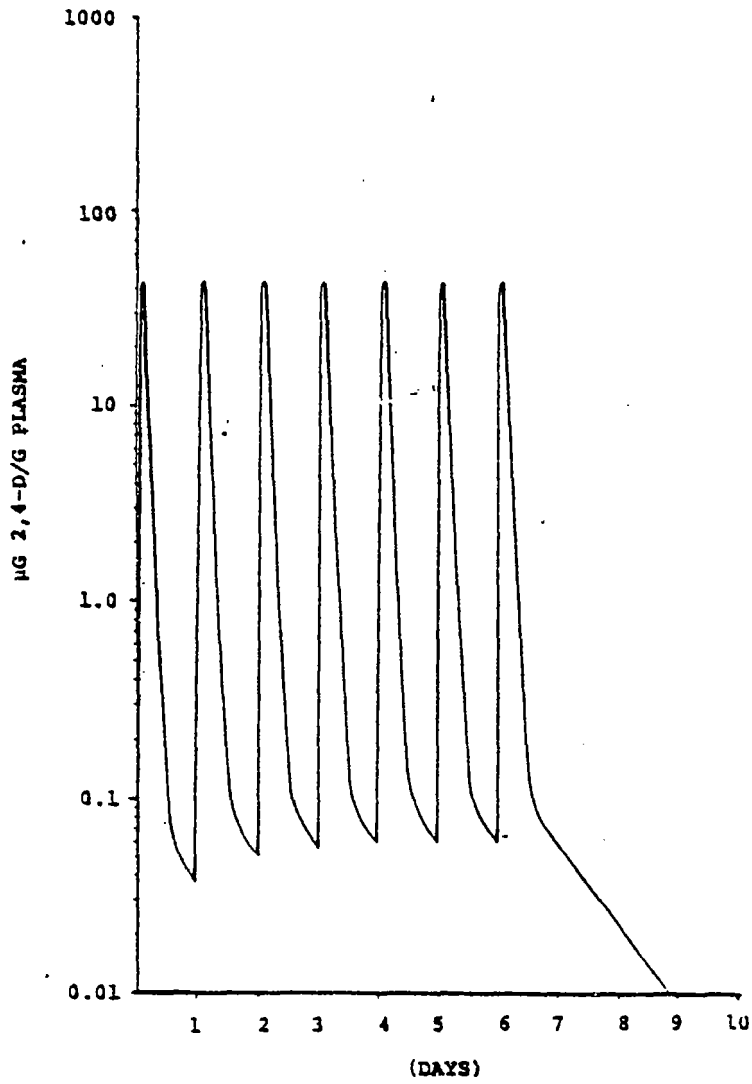


FIGURE 8. Multiple dose simulation, 10 mg/kg, based on the model shown in Figure 1. Seven daily oral doses with a recovery period are shown. This simulation and that of the 50 and 150 mg/kg oral doses (not shown) all predict that upon repeated daily oral dosing, 99% of the steady-state will be reached within 2 days.

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17621

TABLE 1

72-HOUR RECOVERY OF RADIOACTIVITY FROM RATS GIVEN A SINGLE ORAL DOSE OF ^{14}C-2,4-D

	Percentage of a Single Dose (Dose Level, mg/kg)		
	10	50*	150
Urine	92.53	92.59	91.00
Feces	3.22	ND	2.15
Plasma	0.46	0.93	0.68
Cage Wash	0.47	0.52	0.15
Skin	0.55	ND	0.53
Carcass	-	ND	0.37
TOTAL:	97.24±2.94		94.88±1.12

ND = not determined; - = below 3x background.

*50 mg/kg was not designated as a balance study (feces, skin and carcass were not analyzed) and is presented for comparison.

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TABLE 2

PHARMACOKINETIC PARAMETERS DESCRIBING THE DISTRIBUTION AND SATURABLE CLEARANCE OF ^{14}C-2,4-D IN THE BLOOD PLASMA OF RATS FOLLOWING INTRAVENOUS OR ORAL ADMINISTRATION[1]

	Intravenous Administration (5 and 90 mg/kg)[2]	Oral Administration (10, 50 and 150 mg/kg)[3]
k_{01} (hr^{-1})	-	1.41
k_{12} (hr^{-1})	0.0100	0.0081
k_{21} (hr^{-1})	0.0487	0.0391
V_m (µg/ml/hr)	46.1	53.7
K_m (µg/ml)	61.7	78.9
α (hr^{-1})	0.758	0.690
$t_{1/2}$ (α) (hr)	0.915	1.01
β (hr^{-1})	0.048	0.039
$t_{1/2}$ (β) (hr)	14.4	18.0

[1]Based on the pharmacokinetic model shown in Figure 1.

[2]Volumes of distribution were 182 and 252 ml/kg at the 5 and 90 mg/kg dose levels, respectively.

[3]Volumes of distribution were 119, 263 and 265 ml/kg at the 10, 50 and 150 mg/kg dose levels, respectively.

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Biochemical Research Department
THE DOW CHEMICAL COMPANY

K-2372-17 789

File T23.14-11-5
Chg. 5862
Rec'd 12-16-49
Fin'd 8-31-50
Work By Dr.V.A.Dri
Wayne Univ.

Subject: INTERIM REPORT ON THE ACUTE AND SUBACUTE ORAL TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID FOR DOGS.

To J. W. Britton
Executive Research Committee

Check F. C. Spencer 9-5-50

Rept. By V. K. Rowe 8-31-50

UNIT INDEX

A copy of a report of the acute oral studies and a progress report of the 3 month studies being carried out by Dr. V. A. Drill of Wayne University on 2,4-D is given.

IDOW 215246

INDEX HEADINGS

2,4-Dichlorophenoxyacetic acid

Acetic acid: 2,4-dichlorophenoxy-

Phenoxyacetic acid: 2,4-dichloro-

MATERIAL

The material used in this study was designated as K2372-17 It was supplied by L. White of the Organic Semi Plant Laboratory; it bore the reference number 23W-79; and it had a freezing point of 132.8°.

PROBLEM

In order to have some toxicological information relative to the effects of 2,4-D when fed repeatedly in single doses to a larger laboratory animal, the dog, a project was set up with Dr. Dill at Wayne University. A progress report of this work which was filed as an exhibit in evidence at the F.D.A. Hearing for the establishment of residue tolerances on fresh fruits and vegetables comprises a part of this report.

CONCLUSIONS

Dogs appear to be somewhat more susceptible to 2,4-D than rats

17824

K No. 2372-17 BIOCHEM. FILE T 23.14-11-5

REQUEST FOR BIOLOGICAL TEST
TO BIOCHEMICAL RESEARCH LABORATORY

C.R.I., FILE NAME:
2,4-Dichlorophenoxyacetic Acid.

STRUCTURAL FORMULA: MOLECULAR FORMULA

RECEIVED
DEC 16 1949
Biochem. Res. Dept.

DOW 21555250

CONSTITUENTS IN COPOLYMERS OR MIXTURES:

2,4-D	98.5%
2,4-dichlorophenol	1.5%

DATE

SUGGESTED SOLVENTS: DISCUSSED WITH

REQUESTED BY VKR 12-16-49.

MADE BY L. White 12-14-4

REFERENCE 23W-79 AMT. SENT

FP MP 132.8 °C BP °C AT MM. HG DENS. AT °C/ °C

HANDLING PRECAUTIONS

PRODUCT STATE: RESEARCH DEVELOPMENT SPEC. CHEM. PLANT

USE CONTEMPLATED

TESTS REQUESTED: CHARGE NO. 5862

Toxicity of — for dogs.
Acute + 3-month Oral by Gavage.

LIT. REF. DISCL. NO. PATENT

(PLEASE DO NOT WRITE BELOW THIS LINE)

TESTS	BY	DATE
B		
GENERAL SCREENING DNM 12-27-49		

SAMPLE TO

NOTED BY VKR 12-27-49 DATE BY DNM DATE 12/27/49

INITIAL AND DATE ALL ENTRIES - NO ERASURES PERMITTED.

17625

T 23.14-11-第5

DOW2155251

ACUTE AND SUB-ACUTE ORAL TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID FOR DOGS

Victor A. Drill, Ph.D., M.D.
Professor of Pharmacology
Wayne University College of Medicine

17628

DOW2155252

2,4-dichlorophenoxyacetic acid (2,4-D) is known to act as a plant hormone and as a herbicide (1, 2, 3). In one initial report 2,4-D was mentioned to be non-toxic to animals and humans when administered orally (1). However, large doses produced symptoms in mice, rats, rabbits and dogs, which simulated those seen in clinical myotonia(4)More detailed observations were reported by Hill and Carlisle (5) who studied the toxicity of 2,4-D acutely and sub-acutely in various species of animals.

There are no reports in the literature on the acute oral toxicity or chronic oral toxicity of 2,4-D in dogs. The present study was designed, therefore, to observe the acute effects of single doses of 2,4-D and the chronic effects of 2,4-D when administered orally for 90 days. Observations were made on the mortality, symptomatology, body weight, blood count and gross pathology of the animals. A microscopic study of the tissues is under way but at the present time is not completed.

Methods

Adult mongrel dogs of both sexes were used. Most of the animals were housed in the laboratory for a period of 2 to 3 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. The 2,4-D used was commercial material with a purity of 98.5%.

In the acute studies the calculated dose of 2,4-D was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days at which time survivors were autopsied. In the chronic study the 2,4-D was administered orally in capsules, 5 days a week over a 13 week

17627

period. Each capsule was imbedded in a 4 to 5 gram piece of commercial canned dog food. The animals consumed this 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 (6). Upon the death or at the completion of the study, the animals were autopsied, tissues were taken from the lung, heart, liver, kidney, adrenal, spleen, thyroid, intestine and ovary or testes and fixed in 10% formalin.

DOW 2155253

RESULTS

I. ACUTE ORAL TOXICITY

A. Symptoms produced. The effect of various doses of 2,4-D on survival for 14 days is shown in Table 1. The oral LD_{50} is approximately 100 mg/Kgm.

In dogs that died the symptoms produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myotonia. Initial effects were often noted 6 hours after the oral administration of 2,4-D. The animals were more quiet than normal and a slight ataxia was occasionally present at this time. 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 the animals were usually quiet. If the animal is lifted and made to walk the spasm and ataxia are noticed, but when walked for a while the spasm and ataxia tend to decrease. In the later stages of intoxication the animal, when placed on his feet, is

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unable to stand and sits or lies in an awkward position with spastic limbs, particularly spasm of the hind limbs. Other dogs will stumble and roll on the floor in attempts to right themselves. An occasional dog has shown evidence of irritation or pain when the skin over the back of the neck is 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. Although occasional diarrhea was obtained, vomiting was not produced.

Such symptoms may also be seen in dogs that survive the lower acute doses of 2,4-D for 14 days (Table 1). The symptoms in such cases are relatively mild and were limited to spasm in the hindlegs and a slight ataxia.

B. Body weight. The animals dying generally showed a loss of weight, the loss being least in animals that died during the first 4 days of intoxication (Table 1). There was no loss of weight in the animals that received 25 mg. of 2,4-D per kilo. Although food intake was not measured, the dogs ate less food than normal and those that died refused even canned dog food towards the end of their survival.

C. Gross Pathology. The chief gross changes noted were in the small intestine. The intestinal mucosa in some cases showed a mild to diffuse redness (Dogs 58, 73, 75, 81). Dog 86 had a single indurated ulcer in the duodenum. Dog 82 showed what appeared to be occasional small circumscribed hemorrhagic areas in the lungs. In dog 76, 90% of the middle left lobe was a mottled purple-black. Similar changes, but less extensive, were present in the other lobes. Dog No. 75 showed similar gross changes in the middle right lobe and left lobes.

II. CHRONIC ORAL TOXICITY

1. Mortality. All dogs receiving 2, 5 or 10 mgms. of 2,4-D per kilo survived the 90 day test period. Both dogs receiving the 20 mgm. per kilo died during the study. The same total daily dose divided and administered twice a day to two dogs caused death, after a longer time interval, in only one animal (Table 2).

2. General symptoms. Dogs that survived the oral administration of 2,4-D for 90 days were free of any symptoms.

The three dogs that died while receiving the highest dose of 2,4-D showed symptoms and 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 2 to 3 days of their survival 2 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.

3. Body weight. Changes in body weight are listed in Table 2. The only significant change was observed in the dogs that failed to survive the study. The weight loss in these animals began 7 to 12 days before the death of the dog.

4. Blood count. The administration of 2,4-D did not have any effect on the hemoglobin or red cell count of animals that survived or died during the study (Table 3). Of the animals that died, one dog (No. 104) showed a moderate decrease in total white cell count. However, as a similar change was observed in a control dog (No. 62) it was concluded that this was not a significant effect of the drug. The differential count was not

DOW 2155255

significantly affected by 2,4-D with the possible exception of dog 64. This dog showed a marked drop in the per cent of lymphocytes before death. However, a similar change was not present in dog No. 104 just before death.

5. Gross pathology. Gross changes in the internal organs were minor. In dog 61 the renal capsule stripped with difficulty, and a few isolated red areas (1-2 mm.) were present in the duodenum. Dog 91 showed a slight diffuse redness of the duodenal mucosa.

6. Organ weights. There was no significant change in the weight of the thyroid, adrenal, heart, liver or kidney in animals that survived the 90 day test period. An increase in some of the organ weights can be noted in dogs 64, 91 and 104 that received the highest dose of 2,4-D and who died during the experiment.

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Discussion. The symptoms produced by the high single acute doses of 2,4-D are consistent with those observed by Bucher (4). The general symptoms seem to be quite similar in either the mouse, rat, rabbit or dog, and are characteristic of a clinical myotonia. Other effects occasionally observed in the dog are sneezing, and rubbing the eyes, or occasionally diarrhea. Vomiting was not observed in the present study. Some of the dogs dying of the acute doses showed a mild to diffuse redness of the duodenum mucosa and occasionally areas in the lung that appeared to be hemorrhagic. The higher doses of the material produced anorexia and consequent weight loss. Death from large single oral doses of 2,4-D is delayed and occurs days after the initial administration. The irritation and pain observed in some dogs when the skin of the back of the neck is grasped may indicate meningeal irritation, and the extension and crossing of the hind legs in some dogs, when lifted by the shoulders, may point to a spinal or central lesion.

In the chronic study the dogs survived doses of 2, 5 and 10 mg. of 2,4-D per kilo of body weight. Doses of 20 mg. per kilo, however, did

produce death when administered over a period of 18 to 49 days. This is in conformity with sub-acute studies in dogs performed by Hill and Carlisle (5). They administered 2,4-D intravenously in doses of 25 or 50 mg. per kilo for 6 injections. Such animals died or were sacrificed between the 3rd and 16th day of the study. It is evident then that there is some cumulative action of 2,4-D in the studies of Hill and Carlisle and in the present study with the 20 mg. per kilo dose.

The dogs surviving the chronic doses of 2,4-D do not show any significant change in hemoglobin or blood count. The doses of 2,4-D did not significantly affect the white blood count. In one animal that died there was a marked fall in the per cent of lymphocytes in the differential count before death. Hill and Carlisle (5), administering 25 or 50 mg. of 2,4-D per kilo intravenously for six days, have observed a fall in the polymorphonuclear leucocyte count and in the per cent of lymphocytes, although not in all dogs.

DOW 2155257

The symptoms produced in animals dying from the 20 mg. per kilo dose of 2,4-D 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, and bleeding from the gums. Some of the animals also showed difficulty in chewing or swallowing.

Summary. 1. The acute oral LD_{50} of 2,4-D is approximately 100 mg. per kilo. Doses in this range or higher produce a definite myotonia which was accompanied by anorexia and a loss of weight.

2. In chronic oral feeding the animals survived doses of 2, 5 and 10 mg. per kilo for 90 days. Such doses were without effect on body weight, blood count or organ weight. Occasionally upon autopsy some redness of the duodenum mucosa was noted. Three of the 4 dogs receiving 20 mg. per kilo of 2,4-D died during this experiment. Evidence of clinical myotonia

was less in these dogs although such animals also showed some bleeding from the gums and necrotic changes in the buccal mocosa accompanied by difficulty in chewing and swallowing. In the animals that died there was no significant change in the blood picture except for a decrease of lymphocytes in one dog.

DOM2155258

THE HEALTH EFFECTS OF HERBICIDE 2,4,5-T

A Report by the American Council on Science and Health

The American Council on Science and Health is an independent educational association promoting scientifically balanced evaluations of chemicals, the environment and human health.

DOW2155142

The Council is a nonprofit association exempt from federal income tax under Section 501 (c)(3) of the Internal Revenue Code. All contributions are tax deductible as provided by law.

Individual copies of this report are available at a cost of $10.00. Prices for ten or more copies are available on request.

This report on 2,4,5 - T was researched and written by Mary Katherine Hayes, M.S., Research Associate for the American Council on Science and Health (ACSH).

ACSH gratefully acknowledges the comments and contributions of the following individuals who reviewed this report and its contents:

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March 1981: first printing
July 1981: second printing (updated)

17635

Introduction 1

Position Statement 2

Chronology of Events Involving 2,4,5-T 3

Herbicide Production Statistics 5

Uses 6

2,4,5-T Chemistry 10
Chemical Synthesis of 2,4,5-T 11
Chemical Stability of 2,4,5-T 13
Summary 13

Hazards 14
Spray Drift 14
Residues 14
Toxicity 15
Summary 21

Polychlorinated Dioxins 22

2,3,7,8-TCDD 23
Chemistry 23
Summary 28

Toxicity 29
Kociba et al. 1979 33
McConnell and Moore 1979 38
Allen and Van Miller 1977 40
Kociba et al. 1978 41
Summary 45

Teratogenicity 46
Mutagenicity 59
Carcinogenicity 59

Human Experiences with 2,4,5-T and TCDD 65
Dow Chemical Plant Workers 65
Monsanto Plant Accident, Nitro, West Virginia 67
Arkansas Cleft Palate Study 68
Humboldt County 68
Denny, California 70
St. Louis, Missouri, Horse Arena 71
Seveso, Italy 72
Alsea I 75
Alsea II 75
Hanify, Metcalf, Nobbs, and Worsley (1981). 78
Swedish Occupational Epidemiologic Studies 80
Summary 87

Agent Orange 88
History 88
Present Complaints about Its Alleged Effects 89
Agent Orange--Future Plans 89
Scientific Studies 90
Summary 91

EPA Regulatory History 92
Conclusion 94

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17636

INTRODUCTION

This paper by the American Council on Science and Health was prepared as a result of the controversy concerning the suspension and possible cancellation of registration of the herbicide, 2,4,5-trichlorophenoxyacetic acid. Use of the herbicide is currently being questioned and indeed may cease permanently after scheduled hearings by the Environmental Protection Agency (EPA), which began March 14, 1980.

Registered in 1948, the herbicide, 2,4,5-T, is not a newcomer to weed control. But studies within the last ten years involving both human and animal exposure to the herbicide have raised questions regarding potential health hazards accompanying its use. In 1978, the EPA issued a Rebuttable Presumption Against Registration (RPAR) (EPA 1978) and Continued Registration of herbicides containing 2,4,5-T, initiating the process to question the need for continued registration.

However, on February 28, 1979, the EPA exercised its option by citing an "imminent health hazard" and called for an immediate Emergency Suspension of 2,4,5-T (EPA 1979c) on rights of way, forests, and pastures. Exempt from this suspension order were rangeland and rice paddy applications.

Proponents of the ban argue that the herbicide is very toxic to humans. They illustrate their point with a variety of studies that attribute an increase in miscarriages, birth defects, and certain forms of cancer to exposure to 2,4,5-T.

Opponents of the ban defend the use of the herbicide. Interested parties in this camp feel that the scientific evidence does not substantiate the claims made by critics of the chemical. They therefore feel that the EPA's proposed cancellation is unfounded.

At the heart of the matter lies the chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Regarded as one of the most potent toxins known to man, TCDD is a consistent contaminant of 2,4,5-T.

The American Council on Science and Health seeks to present a balanced and unbiased assessment of this current public health issue. In preparation of this paper, the Council has diligently studied the history and past and present uses of this herbicide both in the U.S. and abroad. Furthermore, it has scrutinized available data

on the effects on human and animal health. In addition, it has reviewed information about 2,4,5-T in the popular press and from interested parties on both sides of the issue.

The American Council on Science and Health appreciates the problems encountered in making a definitive decision regarding the use of 2,4,5-T. A thorough analysis of the 2,4,5-T dilemma is complicated by a paucity of human evidence, the need to extrapolate from animal data and a lack of sophisticated detection equipment. The Council recognizes valid arguments submitted by both parties involved in this dispute. The Council presents its paper as its own opinion and interpretation of the information studied and hopes that it addresses all pertinent arguments in this important public health matter.

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POSITION STATEMENT

17837

Based on its review of the scientific evidence, the American Council on Science and Health (ACSH) concludes that there is insufficient evidence to support a ban on 2,4,5-T. No scientific reports presented to date have shown any convincing relationship between the traditional use of 2,4,5-T and adverse health effects in humans.

The toxicity of 2,4,5-T and its contaminant, 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), has been demonstrated under laboratory conditions at doses far higher that those to which humans are exposed. Estimates of human health risk derived from animal experiments are unreliable. Furthermore, those risks which have been calculated for 2,4,5-T and 2,3,7,8-TCDD are extremely small.

ACSH recommends that the current uses of 2,4,5-T in rice fields and rangeland be continued and the suspended uses in forests, railways and highways, and landscaping be reinstated.

ACSH recognizes the problems posed by 2,4,5-T's unavoidable contaminant, 2,3,7,8-TCDD, and urges that every effort be made to further reduce contamination during the manufacturing process. ACSH recommends that applications of 2,4,5-T, particularly aerial spraying, be strictly monitored to minimize unnecessary environmental and human exposure. ACSH believes that stringent safeguards for the manufacture and application of 2,4,5-T will effectively reduce any potential adverse effect of 2,3,7,8-TCDD while allowing for the continued use of the herbicide.

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1945 - developed as an herbicide

1948 - registered with USDA as a pesticide

1949 - accident at Nitro, West Virginia, 2,4,5-T plant

1957 - 2,3,7,8 - TCDD impurity in 2,4,5-T made known

1962 - Vietnam defoliant spraying commenced

1966 - USDA and FDA order residue tolerances be established for most pesticide chemicals in or on food crops

1967 - deadline for obtaining tolerances for residues of most pesticide chemicals including 2,4,5-T on food and feed products and by-products

1969 - 2,4,5-T and 2,4-D were reported to produce birth defects in animals

1970 - USDA requested further teratogenic studies

1970 - Department of Defense cancels use of 2,4,5-T in defoliation procedures in Vietnam

1970 - USDA cancelled all use of granular 2,4,5-T formulations for use around the home, recreational areas and similar sites and all food crops intended for human consumption

1970 - USDA suspends use of 2,4,5-T in lakes, ponds, ditch banks, and all liquid formulations for use around the home, recreational areas, and similar sites

1970 - Congress directed DOD to contract with National Academy of Sciences for study of ecological and physiological effects of use of herbicides in Vietnam

1970 - use of 2,4,5-T banned in Italy; it is also banned in Sweden and the Netherlands

1971 - accident at racecourse in St. Louis, Missouri, involving exposure to TCDD resulting in animal death and human illness; 2,4,5-T not involved

1971 - FIFRA Advisory Committee recommends continued use of 2,4,5-T on forest, range, rice, rights of way; stipulates TCDD (dioxin) content be reduced to

1971 -

1972 -

1972 -

1973 -

1973 -

1973 -

1974 -

1974 -

1974 -

1974 -

1976 -

1977 -

1978 -

year, and applied in a manner that will not contaminate humans

- EPA prohibits use of 2,4,5-T on most food crops

- Amendments to Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) enacted requiring registration of all pesticides and their use plus certification of applicators--Administering Agency for FIFRA is EPA

- Dow Chemical obtains an injunction against EPA enjoining further action against 2,4,5-T

- The study "Primary Carcinoma of the Liver in Vietnam" released in medical journal Chirurgie by Dr. Ton That Tung

- U.S. Court of Appeals overturns injunction; cancellation proceedings continue

- Notice of Intent to hold Hearings on all uses of 2,4,5-T. Public hearing scheduled for April, 1974, following completion of intensive monitoring for TCDD in part per trillion (ppt) range

- National Academy of Science Report - "Effects of Herbicides in South Vietnam - Part A Summary and Conclusions" issued

- information hearings expanded to include all insecticides and herbicides using 2,4,5-Trichlorophenol in their manufacture

- EPA withdraws from cancellation and information gathering proceedings because of its inability to accurately monitor food for TCDD residues

- EPA establishes Dioxin Implementation Plan to identify good analytical methods for detecting TCDD in ppt range

- explosion at ICMESA Chemical Plant in Seveso, Italy, releasing an estimated 1.7 kg. (3.74 lb.) TCDD in an area with population of 100,000

- decision to continue use of 2,4,5-T in New Zealand issued by government

- Ministries of Agriculture, Fisheries and Food (MAFF) of United Kingdom - Advisory committee announce that herbicides containing 2,4,5-T were safe, "if used in the recommended way for the

17638

recommended purposes" -- approval given for their continued use in the U.K.

1978 - Agent Orange Victims, International formed by U.S. Vietnam veteran

1978 - EPA publishes Rebuttal Presumption Against Registration (RPAR) and Continued Registration of 2,4,5-T, citing research that indicates 2,4,5-T and TCDD cause tumors, birth defects, and fetal deaths. RPAR allows continued use pending final decision

1978 - airing of ABC-TV's 20/20 News Magazine two-part program detailing 2,4,5-T and Agent Orange

1979 - suspension hearings commenced on April 19, 1979

1979 - hearings stopped on May 15, 1979, after all registrants withdrew from the hearings

1979 - "Politics of Poison," a documentary on 2,4,5-T shown on NBC San Francisco affiliate KRON-TV

1979 - Dispute Resolution Conference on 2,4,5-T held sponsored by the American Farm Bureau Federation

1979 - availability of EPA Position Document on 2,4,5-T

1979 - hearings before House Sub-committee on Oversight and Investigations on Involuntary Exposure to Toxic Herbicides and Pesticide Products

1979 - Pendulum and the Toxic Cloud by Thomas Whiteside published, dealing with the Seveso, Italy, dioxin accident

1979 - Notice of Intent to Cancel Suspended Uses of 2,4,5-T by the EPA

1979 - Alsea II report submitted to the EPA which provided grounds for initial suspension action by the EPA

1979 - EPA issues Emergency Suspension Order for 2,4,5-T on rights of way, pastures, forests, home gardens, aquatic weeds, ditch banks, and ornamental turf; exempt from this suspension were rangeland and rice paddy applications

1979 - FIFRA Scientific Advisory Panel reviews non-suspended uses of 2,4,5-T and issues opinion

1979 - PBS airs "A Plague on Our Children" as part of its NOVA series dealing in part with 2,4,5-T

1979 - EPA issues Final Determination Concerning the RPAR for certain uses of Pesticide Products and Notice of Intent to hold a hearing

1980 - Dow Chemical filing against U.S. Government claims negligence in misuse of Agent Orange and failure to inform servicemen of potential dangers of exposure to Agent Orange

1980 - Cancellation hearings of 2,4,5-T began March 14, 1980

1980 - Operation Ranch Hand - in depth study of Vietnam veterans scheduled begins

1769

Forty years ago, while researching the properties of naturally occurring plant growth hormones, scientists discovered the phenoxy herbicides (Peterson 1967). Considered as synthetic plant growth regulators, the phenoxy compounds were developed to mimic the properties of a naturally occurring plant growth regulator, indoleacetic acid. Concurrent research in the United States and Great Britain revealed the capabilities of certain phenoxyacetic acids, including 2,4-dichlorophenoxyacetic acid (2,4-D), as active herbicides, selectively killing many broadleaf weeds in cereal grain, grasslands, and coniferous forests (Bovey and Young 1978), (Nutman, Thornton and Quastel 1945), (Blackman 1945). The military furthered their investigation by studying phenoxy herbicide use as a vegetation control weapon in 1944-1945.

By 1946, 2,4-D was used extensively to control weeds in turf, small grains, and corn. However, its efficacy did not extend to brush control. Research for an effective substitute began. Use of 2,4,5-trichlorophenoxyacetic acid began after its registration by the Amchem Products Company, Ambler, Pennsylvania, on March 2, 1948. In the years that followed, 2,4,5-T made its mark as the material of choice for effective brush control on rights of way, rangeland, forestry, and certain weeds in rice, wheat, corn, and sugarcane (CAST 1978).

Although many phenoxy compounds have herbicidal capabilities, most are not commercially available because of prohibitive cost or narrow effectiveness range. Phenoxy herbicides presently in use today in the U.S. include 2,4-D, 2,4,5-T, MCPA, Silvex, 24-DB, MCPB, dichlorprop, mecoprop, 2,4-DEP (Weed Society of America 1974), and dichlorprop-methyl (Federal Register April 7, 1980).

2,4-D predominates in the U.S. today, due to its low cost and high effectiveness. Other phenoxy herbicides are employed only in instances where they exceed the capability of 2,4-D.

In 1975, the United States used 59 million pounds of 2,4-D, 6.7 million pounds of 2,4,5-T, and two million pounds of Silvex (Stanford Research Institute 1976). MCPA, 2,4-DB, dichlorprop and mecoprop find important but limited use for specialized applications (Klingman and Ashton 1975). 2,4-DEP, a chemical related to 2,4-D, acts principally through soil transport (Crafts 1975), but it is not used with the same frequency as 2,4-D.

One hundred companies hold federal registrations and formulate 424 products containing 2,4,5-T. Eleven companies have applied for federal registration of 21 products containing 2,4,5-T previously registered only in certain states (EPA 1974).

In 1969, 11,626,000 pounds of 2,4,5-T acid, esters, and salts were produced in the United States alone. In 1970, the quantity rose to 12,335,000 pounds. Between 1971 and 1974, 738,907 pounds of 2,4,5-T were imported into the U.S. averaging a total of 148,000 pounds per year (EPA 1974).

The phenoxy herbicides gain entrance to the target site by penetrating the plant foliage, roots and soft stem tissue. When applied as a drench in diesel oil, they can soak through the dry bark of trees and enter living tissues (Crafts 1964). Accumulating in the actively growing parts of roots and stems (Crafts 1964), they cause the leaves and buds to twist and curl and new growth of stems and leaves are malformed. Sensitive young plants may die in a few days. Hardy shrubs and trees may succumb only after weeks or months or may survive without injury (Bovey 1977).

A phenoxy herbicide enters a plant in an imperfect and uncontrolled way. Once inside the plant, it interferes with the regulation of growth processes normally governed by the plant's own naturally occurring growth hormones (Ashton and Crafts 1973). The phenoxy herbicides interfere with cell division (mitosis) and cell enlargement, food utilization and various other processes necessary to stabilize life.

Scientists do not know the precise mode of action of either synthetic or natural plant growth regulators. They do know that phenoxy herbicides are far more toxic to green plants than to animals. Plant growth regulating compounds act on plant cell compounds such as cellulose and lignocellulose which are not present in animals.

17840

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Land management personnel utilize phenoxy herbicides in various situations. The following discussion lists kinds of uses and the preferred herbicides for the job.

Timber Management - Foresters have included the phenoxy herbicides among their timber management tools since the early sixties (Bentley 1967).

Forests cover a third of the U.S. (approximately 750 million acres), and two thirds of this land is usable for commercial timber purposes. The remaining, approximately 250 million acres, either produces too little timber for commercial classification or has been utilized for other functions, namely wildlife, water yield, grazing, or recreation (CAST 1978).

According to Walker (1973), competing undesirable vegetation causes about 300 million acres of commercial timber land to operate at less than two thirds of its timber production capacity. Over one hundred million acres of historically high yield areas, now support no significant timber growth because competing vegetation robs trees of vital space, sunlight, and nutrients. Phenoxy herbicides applied here literally shift the advantage to the desired trees, increasing productive land for forest purposes and allowing re-establishment of mature forests.

Traditional forestry methods emphasize hand cutting for thinning and culling of forest stands (Newton 1973), a procedure proven satisfactory as well as economically sound. But nationwide manual attendance of such large forest areas is economically and physically unfeasible.

Kimmins and Fraker (1973) have cited 280 references to herbicide uses in forestry, and Johnson and Lawrence (1977) have listed more than 80 references in a more recent review. In California, herbicides are extensively used to control weeds in Douglas Fir forests, Ponderosa Pine mixed conifer forests, and Red Fir forests.

Personnel used herbicides in two ways in forests: site preparation for stand establishment and conifer release.

The site preparation method involves treatment of land before planting desired conifer seedlings. Fires, logging, and other natural or intentional situations often leave a forest area overgrown with herbaceous and

woody "undesired" growth which must be controlled for successful seedling establishment. This area is first prepared by clearing the site and crushing or burning the waste. A broad spectrum herbicide is applied during the next spring to prevent new growth. After the conifer seedlings are planted, foresters then employ a selective herbicide for continued suppression of rapidly growing plants. This application takes place within one to two years after the desired plantings during the late spring season, a peak time for rapid growth and resprouting of broadleaf competitors.

Some areas may be treated with herbicides prior to the initial burning and planting. With either of these treatments, conifer seedlings establish themselves within six to twelve months. Occasionally land management personnel may find it necessary to re-treat areas because undesired growth has returned (Bentley 1967), (Schubert and Adams 1971), (Gratkowski 1975).

In California, low volatility ester formations of 2,4-D and 2,4,5-T, alone or together, are used for brushfield control, while amitrole, atrazine, dalapon, dicamba, and simazine regulate herbaceous plant or grass growth in site preparation (California Department of Foods and Agriculture 1978).

The conifer release method of herbicide control protects conifer seedlings from competing vegetation which robs young conifers of sunlight, nutrients and moisture.

To accomplish this, aerial application of phenoxy herbicides takes place in early spring or late summer when conifer buds have not broken or hardened, avoiding any damage or injury to new growth (California Department of Foods and Agriculture 1978).

Repeated treatment may be necessary depending on the species of brush or hardwood involved. Because conifers are relatively tolerant of 2,4,5-T, it is the herbicide of choice for broadleaf control. When the control of certain grasses and herbaceous growth is necessary for conifer release, the herbicides atrazine and simazine are used. Conifers are tolerant of these compounds unless their root systems come in contact with them.

In addition to suppressing new growth, conifer release management may also involve control of mature hardwoods, such as oak. On an overstocked plantation or forest, conifers may be thinned out through direct injection of the herbicide into the base of a tree or direct applica-

tion into a basal cut or frill (Fannis 1967). Cacodylic acid, MSMA, and 2,4-D amine are registered specifically for this purpose; picloram is also used.

Type Conversion - Type conversion replaces one type of growth with another. Land management personnel primarily use type conversion to replace brushland with more suitable ground cover. Phenoxy herbicides aid in this transformation.

The object of type conversion is to rehabilitate permanent pasture, range or brushland, create fuelbreaks, maintain wildlife habitats, and manage watersheds. In all cases, workers modify brushland or chaparral, thereby permitting another vegetation to dominate.

Type conversion increases forage for livestock, reduces fire potential and increases water run-off and yield, and provides favorable habitat for wildlife preservation (California Department of Foods and Agriculture 1978).

Range or Brushland Rehabilitation - The United States Forest Service treats approximately 3500 acres per year with herbicides for the purpose of range rehabilitation (USDA 1974). That accounts for only two percent of the possible 120,000 acres of land appropriate for such procedures.

Routinely, areas of sagebrush, chaparral, oak, and other hardwoods are changed via type conversion, to open grassland and meadows for grazing purposes. Herbicides of choice here include the 2,4-D esters, 2,4,5-T, picloram, and Silvex. MSMA and AMS substitute when phenoxy acids cannot be used.

Herbicidal applications are made aerially, often after mechanical clearing of the area. If the terrain permits, ground spraying takes place. Herbicide application can be repeated every five to ten years should the situation warrant it. If the area really lacks a substantial amount of desired plant growth, personnel may elect to re-seed the area with a proper foliage plant (California Department of Foods and Agriculture 1978).

Fuelbreak Maintenance - Fuelbreak maintenance involves the control of specified areas, usually strips, to prevent fire hazards and to avert water run-off problems. This is accomplished by applying herbicides to strategic areas on grassland, particularly shrub border areas. Successful treatment here allows no brush growth whatsoever beyond these borders. In the event of brush fire, firefighters can rely on well marked control areas free

from the threat of brush intervention so they can then plan appropriate strategy.

After mechanical removal of brush or burning, land management personnel aerially spray or treat individual plants with 2,4-D, 2,4-DP, and 2,4,5-T two to three years later. Management goals are accomplished when all brush species are replaced with grassland cover. Maintaining this altered state in fuelbreak and other high hazard areas may necessitate repeated applications of herbicides (California Department of Foods and Agriculture 1978).

Maintenance of Wildlife Habitat - Type conversions for this purpose are not uniformly used. The premise remains the same as for other categories of type conversion. Less productive browse species or brushland give way to preferred grassland. The resulting preferred foliage may provide more palatable browse for various species of wildlife. This effect appears largely as a secondary gain, however, and not as a principal effect of herbicide application.

Rights of Way - Rights of way represent a hallmark of our industrialized society. Whether for a highway, railroad, or utility apparatus, a properly maintained right of way is essential for a problem free operation.

About 3.8 million pounds (41 percent of total use) of 2,4,5-T are used for this purpose in the United States annually (EPA 1979c). 2,4-D, 2,4,5-T, and Silvex provide selective vegetation control here, although 2,4-D alone accounts for about half that use. Of the 50 million plus acres involved, railroads occupy 200,000 miles, and highways account for nearly four million miles. Overhead electric wires require two million miles of air space, with 300,000 wires devoted to high capacity circuits occupying over four million acres (CAST 1978).

Phenoxy herbicides used in rights-of-way control vegetation that represents potential fire hazards, and interferes with access to power, telephone and transmission lines, or obscures roads or makes them inaccessible. Most utility, highway, and railroad rights of way are initially cleared mechanically. Costs vary with labor prices and terrain.

Roads and Highways - Roadway maintenance crews use the phenoxy herbicides to aid in preventing fires and eliminating obstruction of signs and safety signals, thereby providing optimum visual fields for the driver. They also remove tall weeds which promote snow drifts, eliminate weeds declared noxious by appropriate authorities,

allow drainage and aid in aesthetic development, while leaving preferred grassland and low vegetation intact.

After mechanical clearing, booms on large tank trucks distribute the herbicides, although in some areas, spot treatment by hand equipment may be necessary.

2,4,5-T and 2,4-D and other herbicides have eliminated the need for plowing, hand cutting, and burning and also permit fewer mowings per year, thereby reducing fuel costs and conserving gasoline and diesel fuel. Chemicals are particularly useful on steep slopes, wet areas, and areas obstructed by guard rails or other structures hindering conventional machine mowing. Phenoxy herbicides find limited use in those areas where desirable vegetation is so close to the weeds to be sprayed that it is susceptible to damage by drift of 2,4-D or 2,4,5-T.

Utility Rights of Way - Herbicide application on utility rights of way frees various wires and lines from interfering brush and foliage growth. Such maintenance is necessary to control fires and allow access to transmission lines and structural facilities. Workers apply herbicides via ground spray methods primarily, although they may use aerial application in some cases. Ground maintenance includes all applications made with tank spray rigs in accessible areas or made by manually operated backpack spray units.

On power and communication rights of way, control entails use of 2,4-D and 2,4,5-T (Bramble and Byrnes 1972). Newly constructed lines cleared with mechanical equipment may require two herbicide applications initially to control regrowth. Maintenance operations involve the following: (1) selective basal sprays of 2,4-D/2,4,5-T or 2,4,5-T esters at rates of 12-16 pounds per 100 gallons of oil; (2) foliage or stem foliage sprays of 2,4,5-T or 2,4-D/2,4,5-T mixtures in water applied with ground equipment at rates of three to four pounds of chemical per 100 gallons of spray mixture; and (3) aircraft applications of 2,4,5-T or 2,4-D/2,4,5-T mixtures at rates of up to 12 pounds per acre, often as invert emulsions or combined with a thickening agent and applied by special equipment to reduce spray drift (Barnhart et al. 1976).

Silvex and dichlorprop may be injected into tree species resistant to 2,4-D and 2,4,5-T, but they are not generally used because of their limited effectiveness.

Railroads - Vegetation management along railroad rights of way involves applicaton of herbicides directly to the track beds and ballast or to the offtrack right of way parallel to the trackbed (California Department of Fo and Agriculture 1978). Undesired growth limits sight lines, hinders train inspection and yard work, prevents proper drainage of the ballast, presents fire hazard, causes potential train fouling and is aesthetically displeasing (Parker 1965), (Yazell 1965). Administration of the herbicide proceeds by use of spray and tank cars riding directly over the tracks for on-track application. For the surrounding areas, a fire hose nozzle attachment distributes the chemical from the tank car. In areas which are difficult to reach, four-wheel drive vehicles equipped with spray apparatus are used.

Aquatic Management - Herbicides function not only on land, but in water as well where they are used for aquatic vegetation management. Plant life covers most surface water areas. Providing a primary source of nutrients for aquatic life, these plants can also cause problems. Uncontrolled growth and overpopulation of these weeds can clog streams, lakes, reservoirs, and canals; start floods; interfere with recreation and the flow of irrigation and drainage water; restrict navigation; and impair fish and wildlife habitats (Pennsylvania Water Resources Coordinating Committee 1971), (Florida Bureau of Aquatic Plant Control), (Timmons 1960), (Timmons and Klingman 1959).

About two million pounds of 2,4-D and one half million pounds of Silvex are used annually in the United States for the control of aquatic vegetation (Stanford Research Institute 1976). Unlike its close cousin Silvex, 2,4,5-T is not employed for any aquatic management purposes.

Turfgrass - Turfgrass includes lawn, shrubbery, shade trees, and other forms of greenery which can be categorized as ornamental turf. There are 15 million acres of ornamental turf in the United States. About one tenth of this land is devoted to golf courses. Home lawns constitute about one third of all ornamental turf, and homeowners currently use 2,4-D, mecoprop, and dicamba to control weeds.

To manage growth on other ornamental turf, landscapers employ 2,4-D, 2,4,5-T, Silvex, MCPA, and dichlorprop to limit dandelions, plantains, garlic chickweed, and other broadleaf and non-grass weeds. For control of crabgrass and other annual grasses, greenskeepers apply DCPA, benefin, bensulide, arsonates, and siduron among others. Simazine and atrazine control many annual weeds. DCPA and dicamba work on some phenoxy-tolerant broadleaf weeds as well as some of the annual grasses.

Dicamba poses some problems. Since roots readily adsorb this chemical from the soil, nearby shrubs and shade trees

can be injured.

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Phenoxy herbicides are also used in a variety of state, county, and regional public health programs to suppress growth of nuisance weeds. These chemicals can eradicate poison oak, ragweed, poison ivy, and other allergenic plants by direct spot treatments of the toxic foliage.

Agricultural Uses - Four fifths of the 72 million pounds of phenoxy herbicides used annually in the United States are devoted to agricultural management of crop and range land (CAST 1978). In 1975, 55 million pounds of these chemicals were used to treat crop lands in the United States (Stanford Research Institute 1976). Phenoxy herbicides are used primarily on the grain crops, wheat, corn, barley, oats, and rice. 2,4-D application at a rate of less than one pound per acre is standard for many of these crops (USDA 1973). MCPA is used on rice in California.

Non-grain crop production also depends on phenoxy herbicides. They are useful against weeds in apples, asparagus, blueberries, cranberries, flax, pears, potatoes, strawberries, and sugarcane.

Each crop has specific requirements and formulations of the phenoxy herbicides. Variables such as crop rotation, method of application, and selectivity all influence the choice of herbicide used. Farmers prefer a variety of herbicides to combat their agricultural weed problem. Alone, or together with other weed control practices, a change in herbicides can be as effective as a change in crops. By rotating crops, and therefore rotating the herbicides that handle their weed problems, the farmers select different cultural methods and activities for their farmland. This not only helps protect the crop currently being cultivated, but also helps to support growth of the rotation crop not presently grown.

Small Grains - Small grains are almost constantly infested with annual and perennial weeds that respond favorably to the phenoxy herbicides. If left unattended, these weeds substantially reduce crop yields, interfere with harvesting, and reduce grain quality, i.e., wild garlic bulblets harvested with wheat grain give the resulting milled flour a garlicy taste rendering the wheat essentially worthless.

2,4-D is the principal herbicide used for grain crops in the United States today. About 60 million acres of feed and food grains are protected annually by 34 million pounds of 2,4-D (Stanford Research Institute 1976), at a

rate of one quarter to one pound per acre (Crafts 1975), (USDA 1973). When 2,4-D is not effective on certain weed complexes, farmers often choose to use MCPA. Although the cost for MCPA far exceeds that for 2,4-D, approximately four million pounds of MCPA are spread on eight million acres of U.S. land yearly (Stanford Research Institute 1976), (Klingman and Ashton 1975).

Currently, application of the herbicide 2,4,5-T on rice lands survives as one of the uses of the herbicide not suspended by the Environmental Protection Agency. Together with 2,4-D and MCPA, farmers can kill broadleaf weeds that interfere with a successful rice crop. In some instances, a plant such as the curly indigo may be tolerant to 2,4-D or MCPA. In southern states, where these weeds prevail, farmers may choose to use 2,4,5-T exclusively for rice land management.

Farmers have few alternatives to phenoxy herbicides. Mechanical and hand tillage of the land are impractical substitutes. Special herbicides for selective weed grain problems do not act as thoroughly as the phenoxy herbicides. For example, dicamba controls some annual and perennial broadleaf weeds in oats and wheat, but not in barley. Furthermore, it does not regulate woody mustards and other important weeds (Anonymous 1977a). Dicamba has a toxic effect on small seeded legumous plants. It must therefore be ruled out as method of choice in the central and eastern United States where small seeded legumes are often interspersed with small grain crops (USDA 1973). Bromoxynil curbs many annual broadleaf weeds in small grains but is ineffective on many perennials. Again, it cannot be used where small-seeded legumes are sown (USDA 1973).

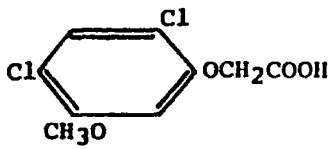
Dinoseb, another herbicide, acts primarily on annual broadleaf weeds in small grains. However, denoseb does not move internally in plants and therefore remains ineffective against perennials. As an added deterrent, dinoseb is far more toxic than the phenoxy herbicides on warm blooded animals (Weed Society of America 1974).

There are essentially no alternative replacements for the phenoxy herbicides, particularly 2,4,5-T, in effective management of rice land weeds.

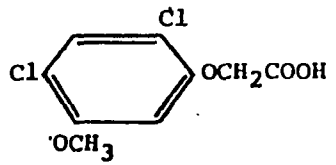
Corn and Other Grains - Alternatives to phenoxy herbicides in agriculture include cultivation supplemented by hoeing. While cultivation holds a significant place in crop management with or without herbicides, it cannot remove weeds directly occurring in the crop row (CAST 1978). For this, farmers must resort to basic handwork.

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Proposed as an intermediate step (Aly and Faust 1964) in the manufacturing of commercial 2,4,5-T, the compound also appears in the product due to its incomplete reaction. 2,4,5-trichloroanisole can also be hydrolyzed under the conditions necessary for step (1) and yield methoxydichlorophenoxyacetic acid as a side product (Aly and Faust 1964).

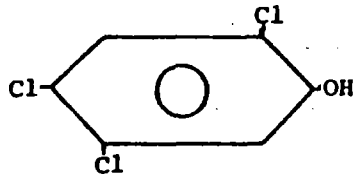


5-methoxy,2,4-dichlorophenoxyacetic acid



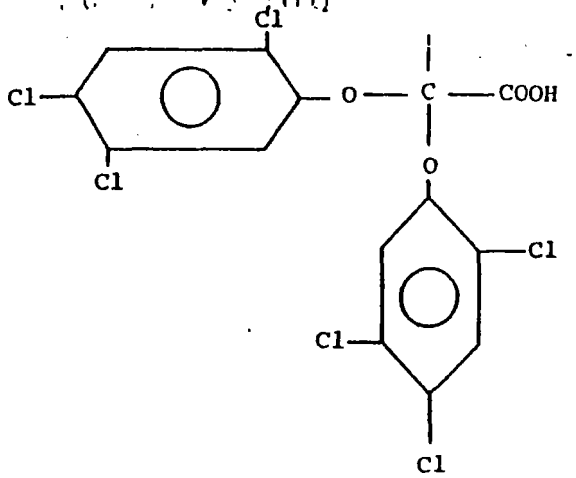
2-methoxy,4,5-dichlorophenoxyacetic acid

2,4,5-trichlorophenol arises from incomplete condensation of the sodium phenoxide with chloracetic acid in step (2).



2,4,5-trichlorophenol

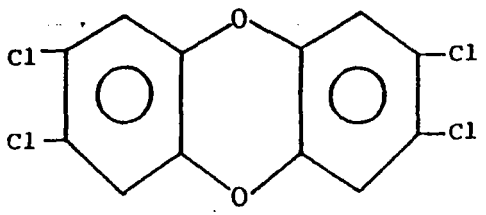
Bis-2,4,5-trichlorophenoxyacetic acid represents a significant impurity since the chloroacetic acid involved in step (2) routinely contains some dichloroacetic acid.



Bis-2,4,5-trichlorophenoxyacetic acid

From the 1,2,5-trichlorobenzene in the tetrachlorobenzene raw material, the by-product 2,5-dichlorophenoxyacetic acid results. Additionally, some sodium 2,4,5-T and sulfate ions appear in the final product due to the acidification process outlined in step (3).

Dioxin, however, is considered the most notorious and noxious by-product formed in the manufacture of 2,4,5-T. It arises as a trace impurity produced under the conditions listed in step (1). Because of its chemical nature and its obvious importance in a thorough review of 2,4,5-T, it will be discussed as a separate entity later in the paper.



2,3,7,8-tetrachlorodibenzo-p-dioxin

Assay methods for 2,4,5-T usually require a simple titration for acid equivalents. A procedure for analyzing the herbicide by gas chromatography exists, e standards for this assay are given although analysts ra .y employ

this method. The marketed 2,4,5-T product generally contains 97 to 98 percent acid equivalent. According to the Department of Agriculture, herbicide titre can run as low as 85 percent in commercial materials and still maintain a 97 percent acid equivalent specification.

Chemical Stability of 2,4,5-T

Photochemical degradation - Many of the phenoxy herbicides such as 2,4-D, readily undergo oxidation, reduction, and substitution (mainly hydrolysis) in aqueous solutions when activated by sunlight in air. Although 2,4,5-T absorbs some ultraviolet light in sunlight, the amount is small and this herbicide is relatively unreactive (seven percent hydrolysis in 48 hours). However, the presence of ferric salts and zinc oxides in the soil water will result in an increase in photolysis rate (Crosby 1976).

Hydrolysis - Esters of 2,4,5-T are readily hydrolyzed to free acid under either acid or basic conditions. Crops rarely exhibit ester residues. The rate of hydrolysis depends basically on the particular ester substituent's stereochemistry, decreasing as the bulk of the ester group is increased closest to the oxygen linkage.

Thermal stability - 2,4,5-T is stable at least to its melting point (156°-158° C).

Biochemical degradation - 2,4,5-T degrades slowly in soil containing organic matter under warm, moist conditions. Half life for the herbicide under these optimal conditions approaches several weeks. Metabolic degradation is caused by two concurrent processes: binding of the herbicide molecule to other compounds, mainly glucose, to form a non-toxic complex, and actual degradation of the molecule. The latter proceeds by the removal of the side chain and replacement of the chlorine atoms with hydroxy (OH) groups, followed by an opening of the benzene ring. Formation of the corresponding phenols is a minor route of breakdown in plants and a major route in soil and rumen. Decay, however, does not follow first order reactions because metabolites of 2,4,5-T greatly increase in number in the presence of the herbicide. If the area is pretreated with 2,4,5-T, degradation lifetime is significantly reduced. Three months is generally accepted as the time span for the herbicide to disappear completely from the soil. Disappearance rate is independent of the amount of herbicide present. The degradation of the herbicide varies with plant species as well as environmental conditions (President's Science Advisory Committee 1971).

Summary

2,4,5-trichlorophenoxyacetic acid is a white crystalline solid with a molecular weight of 255.49 daltons. It melts at temperatures of 156°-158° C and has a solubility of 278 ppm in water. Technical grade 2,4,5-T contains 90 to 92 percent herbicide and eight to ten percent impurities. Preferred formulations of 2,4,5-T are its ester and amine salt derivatives.

2,4,5-T esters are readily hydrolyzed under acidic or basic conditions. Chemically stable at its melting point, the herbicide degrades slowly in warm, organic soil. Its half life approaches several weeks, with a total degradation time nearing three months.

Metabolic degradation occurs by two concurrent processes: the binding of 2,4,5-T to other molecules, i.e., glucose forming a nontoxic complex, and actual degradation of the molecule. This proceeds by the removal of a side chain and replacement of chlorine atoms with hydroxy groups and opening of the benzene ring. Even in the presence of sunlight, 2,4,5-T remains relatively unreactive.

17546

HAZARDS

Hazard is the probability that injury will result when a particular substance is used in a particular quantity and manner. A hazard implies warning. It signals caution before proceeding. It implies potential harm. A hazardous roadway may increase the likelihood of automobile accidents. A hazardous area may mean danger for those not familiar with it. A hazard to one's health may result in bodily injury or even death.

Two kinds of hazards may be considered. The first are hazards that are inherently dangerous. By their very nature, members in this category forebode danger. Circumstances fail to alter underlying jeopardy. No matter how or when you consider them, their presence threatens to disrupt states of synchrony and well-being. Natural disasters such as earthquake, fire, tornado exemplify hazards in this category.

The second are hazards that are situationally dangerous. In these instances, the situation dictates the chance, risk, or danger. Here, the hazard can vary in degrees, and in fact may not even exist at all should the situation change. Automobiles and prescription drugs readily come to mind in describing this category. Hazard implies some quantified risk, and understanding the amount of risk is necessary in evaluating what is a real hazard.

From a public health standpoint, the hazards that concern us are those that affect bodily health and well-being. Increasingly, we are reminded to consider hazards in the environment we live in and in the foods we eat. Sometimes, these hazards take the form of chemicals.

When a chemical is labelled a hazard, it is immediately categorized by pre-conceived notions and handled accordingly. However, specific considerations are needed in evaluation of the hazard. They are toxicity and exposure. Toxicity is the capacity of a substance to injure. It is a universal property of matter varying only in degree. Exposure is the quantification of the degree--in this case, the degree of toxicity. These two elements must be ascertained and related in a thorough analysis of a chemical. A difference exists between an element that is highly toxic but has a low exposure level, and one that has little toxicity but high exposure. In both of these categories amounts are all important factors.

Hazards of the phenoxy herbicides stem from their selective herbicidal ability. These chemicals have the power to kill certain plant life. The beauty in their

17547

action, though, is their lack of effect on certain other plant life, causing them no harm. The phenoxys, as mentioned earlier, direct their killing action towards the broadleaf plants such as weeds encroaching on various agricultural and non-agricultural lands.

A problem arises, however, when these herbicides come into contact with vulnerable but desirable species. This failure of selectivity can cause injury and even death of plants.

Selective mortality is the result of several circumstances. Among them are overdosing a plant. A certain amount of phenoxy herbicide is applied to control populations of plants, but should the plants receive an excessive amount, they will be wiped out all together. This could happen if poorly calibrated spray apparatus is used in spray distribution. Overdosing can also result if the herbicides involved are mismeasured.

Exposing a susceptible plant during its sensitive stages of growth can also result in unwanted injury (Linn et al. 1959). Emergence and budding are usually the vulnerable states.

There are three major potential hazards to be considered when assaying phenoxy herbicides: spray drift, persistence of residues, and toxicity.

Spray Drift

Spray drift to non-target vegetation remains one of the most critical problems of phenoxy herbicide application (Mullison 1970), (Stewart and Gratkowski 1976), (Akesson and Yates 1975). Advanced technology allows up to 97 to 99 percent of phenoxy herbicides to land in the target area either by ground or aerial application (Akesson et al. 1971). However, great care must be taken to assure minimal exposure to non-target areas.

Production of small drops and the discharge point to ground distance with aircraft or ground application affect spray drift (Gratkowski 1976) as do wind velocity and direction. Together with pesticide formulation, these variables can either greatly enhance or reduce exposure of susceptible crops to phenoxy herbicide activity. Air temperature and humidity also alter crop response and influence spray evaporation (Stewart and Gratkowski 1976), (Akesson and Yates 1975), (Butler et al. 1969).

Preferable conditions for application in a hazardous situation require the phenoxy application be made only when a light and continuous wind is blowing away from susceptible crops (Stewart and Gratkowski 1976), (Akesson and Yates 1975).

Phenoxy herbicide application, in fact all pesticide application, may cause problems in instances of atmospheric inversion, when a warm layer of air is overhead under 1500 feet elevation (USDA Forest Service 1977). Stagnant air resulting from atmospheric inversion or lack of wind reduces turbulent mixing or ventilation. During an inversion or when the air is still, build-up of small drops and vapor in the air can drift as a concentrated mass to sensitive crops.

Non-target susceptible vegetation exposed to phenoxy herbicides may exhibit tell-tale symptoms such as bizarre growth. Often, the appearance of injury to the plant is greater than reality (Goodman 1953).

There are several ways to avoid the problems of spray drift. Spraying herbicides in large and heavy droplets (500 to 1500 microns) causes them to fall and settle rapidly in the target area (Akesson and Yates 1975). Droplet size can be increased by using higher spray volumes and lower spray pressures. Droplets may be increased in size through use of nozzles with special design or nozzles with large openings. In addition, aircraft may also be equipped with special systems that closely control droplet size (Stewart and Gratkowski 1976), (Gratkowski 1976). Thickening agents can be added to the spray which act to increase drop size and decrease the risk of fine particles (Stewart and Gratkowski 1976), (Akesson and Yates 1975), (Gratkowski 1976), (Gratkowski and Stewart 1973), (Butler et al. 1969).

There are areas where spraying should be eliminated altogether. Certain crops cannot be protected from spray drift even when conventional methods to eliminate drift have been instituted. When highly susceptible crops such as cotton and grapes are grown within three to five miles of large scale spraying operations (over 100 acres per day), there may be crop injury despite routine precautions. Beans, tomatoes, melons, and certain ornamental plants also succumb easily to damage (Linn et al. 1959).

Federal regulations address these situtations (Akesson and Yates 1975). They forbid the spraying of herbicides at certain times of the year and require special permits for particularly hazardous applications of phenoxy herbicides.

Residues

Residues can be found as an after-effect of almost any product application. A residue is an accumulation of a product for a period following initial application. Dirty wax build-up on wood furniture results from accumulated polish residues. The film inside the coffee pot is the residue formed from the percolation of the coffee beans and water forming that brew.

Residues occur when the initial product is not totally degraded. The persistence of a residue can cause problems. If use of a known toxin leaves sufficient residues in active form, the extent and duration of that toxicity must be recognized and appropriate cautions followed.

It is imperative that we realize the amounts and effects of residues when analyzing an environmental chemical. A chemical's persistence on land, in water, and in the air may profoundly affect the plant or animal that encounters it. Some residues may prove injurious or even fatal to the untargeted species.

Residues do not last forever. Their reduction or disappearance from the environment depends greatly on the half life of the particular chemical, and the conditions where it is deposited. It can range anywhere from hours and days to months and years.

The ill effects of chemical residues can be diminished by avoiding use of potentially hazardous sprays in areas where drift and/or accumulation may be expected or already occur. Common sense dictates that the same care and caution given to areas during spraying should be given to crop areas subject to herbicide residues. Residue accumulation in soil, water, air, and animals should each be considered separately.

Soil Residues - Scientific studies demonstrate that phenoxy herbicides break down in soils (Audus 1960). 2,4,5-T and 2,4-D form carbon dioxide, inorganic chloride, and water as final degradation products (Reigner et al. 1968). Classified as slightly persistent, 2,4,5-T takes anywhere from two to six weeks for a fifty percent disappearance rate. The tendency to volatilize from the soil complex reduces this soil persistence. Volatilization will occur with or without water evaporation (Plimmer 1976). A decrease in soil moisture will lower the soil pH. This favors the undissociated form of both 2,4,5-T and 2,4-D and can possibly enhance vapor loss.

Soil leaching experiments involving 2,4,5-T suggest that amine salts are leached more rapidly than free acid (House et al. 1967).

In 1974, the National Academy of Sciences Report correlated the effect of herbicides on soils of Vietnam with other soil residues (Blackman et al. 1974). Listed are some of the general conclusions:

1) The behavior of herbicides in the soils of South Vietnam was similar to that reported for soils elsewhere.
2) Residues remained only where excessive doses of 2,4-D and 2,4,5-T were applied (Pran Bari). These residues remained ten years after application at levels above the levels likely to induce phytotoxic symptoms in some plant species.
3) When applied to mangrove soils at total doses approaching 10 lb/acre for both 2,4,5-T and 2,4-D, the levels of herbicide residue at the end of 30 weeks had no effect on the establishment of two major mangrove species.
4) In geographical areas subjected to one or two military herbicide missions 1.5 years before sampling, no soil phytotoxic residues could be detected.
5) Soils that received a directed application of Herbicide Orange at a rate of 27 lb/acre safely supported the growth of crops sensitive to 2,4-D or 2,4,5-T four to six months following application.
6) Claims that the herbicides rendered the soil sterile were without any foundation.

The United States Air Force conducted feasibility studies for the disposal of surplus Agent Orange--a 1:1 combination of herbicides 2,4-D and 2,4,5-T. The field study, conducted in 1972 on the Air Force Logistics Command Test Range at Hill Air Force Base in Utah, determined the effects and remaining amounts of herbicides 2,4-D and 2,4,5-T. In replicated sub-surface tests, researchers administered concentrations of either 1,000, 2,000, or 4,000 pounds of herbicide per acre. Stark et al. (1975) took soil samples at three different time periods during 1973 and analyzed soil content for microbial species (bacteria, actinomycetes and fungi). Bacterial counts were higher for soils with greater concentrations of the herbicides and with greater moisture content i.e., those samples collected in mid-winter from the 4,000 pound per acre subplot. Mycoflora experienced no significant effect from the herbicides. Arnold et al. (1976) monitored the herbicide level in these plots. Sampling the plots on eight occasions from 1973 to 1975, they determined the concentrations of the n-butyl esters and the free acids bot' ,4-D and 2,4,5-T. Their research suggested that with massive application rates and soil concentration greater than 10,000 parts per million (ppm), and in an alkaline desert environment, the half life of 2,4-D and 2,4,5-T occurred in the range of 150 to 210 days.

The cooperative studies by Stark et al., and Arnold et al., demonstrated the applications of 2,4-D and 2,4,5-T at massive rates not only did not sterilize the soil, but indeed stimulated the growth of certain microflora, and that this stimulation may have contributed to the degradation of the herbicide (Young et al. 1978).

Norris et al. (1977) examined foliage, forest floor and soil for 2,4,5-T residue. The conclusions, summarized in the journal Weed Science, were that within six months of application, herbicide residues declined 90 percent; within one year, less than six percent of the herbicide remained.

The authors also found little leaching of 2,4,5-T from the forest floor to the soil, and no residues were found deeper than 15 cm. (Maximum residue found was a level of 0.08 ppm despite rainfall of 24 cm the first month and 70 cm the first three months after application.)

Wiese and Davis (1964) found that 2,4,5-T remained in the upper six inches of agricultural soil even after application of 4.5 inches of water over a short period of time.

Helling et al. (1973) found that 2,4,5-T is relatively mobile in soils. However, that movement decreases as organic content increases. 2,4,5-T therefore becomes moderately mobile in clay soils and only slightly mobile in mulch.

Morton et al. (1967) found that both amount and frequency of rainfall affected leaching and microbial decomposition of 2,4,5-T.

Water Residues - 2,4,5-T acids and esters are soluble in water. In addition, 2,4,5-T rapidly dissipates in streams by dilution (USDA/EPA 1979) and photodegradation in water (Crosby and Wong 1970).

Concern for chemical residues found in any water supply stems from fears of contamination of various life forms. If the body of water involved is a reservoir, and were to contain high residues, countless people could conceivably suffer ill effects simply by drinking a glass of water. If a stream, lake, or pond is involved, the resident aquatic life could be jeopardized. The residual effects of chemicals in water therefore can be far ching if concentrations are sufficiently high.

Current information indicates that, although some 2,4,5-T may enter streams flowing through or located adjacent to herbicide spray areas, residues in the streams remain very low (EPA 1974).

The National Academy of Sciences Safe Drinking Water Committee found that contamination of drinking water or other surface water by 2,4,5-T is extremely unlikely (limit of detection/ppt). The report projected the toxicity of 2,4,5-T and TCDD, their acceptable daily intake, and suggested no adverse affect levels (National Academy of Sciences 1977), (Merck Index 1976).

Research reports involving stream contamination from land spraying in Oregon demonstrated that peak concentrations of phenoxy herbicides seldom exceeded 0.1 ppm. These residues rarely lasted for more than a few hours in almost all the streams (Norris et al. 1977). However, herbicide application to marshy areas may result in high-level, long persistent chemical residues in nearby streams.

The Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency stated that all available data indicated that a small amount of 2,4,5-T is present, but does not persist long because clay or the biota absorb the herbicide within a matter of days (EPA 1974). There appears to be little likelihood of 2,4,5-T's presence in alarming quantities in ground water or drainage channels due to local application. Brown and Nishoka (1967) reported that 2,4,5-T was not detectable in samples of water-suspended sediment mixtures from 11 streams in the western United States. Using improved techniques, a subsequent report on the same streams listed positive residues of 2,4,5-T in 28 of the 235 samples taken from 17 rivers as well for the time period during 1966-1968. Twenty-one of these samples were from three rivers: the Arkansas, the Brazos and the Canadian. All seven samples taken from the Canadian River were positive. Actual residue amount remained very small, ranging from 0.01 ppb to 0.07 ppb. Samples collected throughout the year, demonstrated little or no seasonal effect on 2,4,5-T residue levels (Marigold and Schulz 1969). Samples for this experiment were analyzed as they were received. Water and sediment were not separated. The possibility exists that a high percentage of the 2,4,5-T residue was contained in the sediment since there is evidence that phenoxyacetic acid residues persist longer in sediments than in water (President's Science Advisory Committee 1971). (Table 1).

Trichell et al. (1968) studied the effects in runoff water of 2,4,5-T application at a rate of 2.24 kg/ha to

DOW2155157

sod-covered plots of three percent slope. After the application, 1.3 cm of rainfall was simulated to produce the runoff. They recovered 2 ug/ml of the herbicide after 24 hours. Four months after the application, this measurement had diminished to 0.04 ug/ml.

Table 1. Estimated Maximum Level in mg/l (=ppm) of Residues of 2,4,5-T at Various Rates of Application to Bodies of Water Ranging from Three Inches to Ten Feet in Depth

Water depth	Rates of application, pounds per acre							
	1	2	4	6	20	26	80	100
3 inches	1.5	3.0	6.0	9.0	30.0	39.0	120.0	150.0
6 inches	0.75	1.50	3.0	4.50	15.00	19.50	60.0	75.0
1 foot	0.38	0.75	1.50	2.25	7.50	9.75	30.0	37.50
2 feet	0.19	0.38	0.75	1.13	3.75	4.88	15.0	18.75
3 feet	0.13	0.25	0.50	0.75	2.50	3.25	10.0	12.50
4 feet	0.09	0.19	0.38	0.56	1.88	2.44	7.50	9.38
5 feet	0.08	0.15	0.30	0.45	1.50	1.95	6.00	7.50
6 feet	0.06	0.13	0.25	0.38	1.25	1.63	5.00	6.25
7 feet	0.05	0.11	0.21	0.32	1.07	1.39	4.29	5.36
8 feet	0.05	0.09	0.19	0.28	0.94	1.22	3.75	4.69
9 feet	0.04	0.08	0.17	0.25	0.83	1.08	3.33	4.17
10 feet	0.04	0.08	0.15	0.23	0.75	0.98	3.00	3.75

Source: President's Science Advisory Committee 1971.

Following application of 11.2 kg/ha of 2,4,5-T, Edwards and Glass (1971) monitored runoff and percolation of the herbicide for 14 months after application. They determined that 5.5/g/ha (over 0.05 percent) of the herbicide was lost from the treated area. The majority of 2,4,5-T was removed in runoff during the first four months after application. More than half of the loss occurred the first month after treatment.

The method of contamination, i.e. drift or direct application, determines the amount of levels of residues deposited. If a very fine spray is used in an area where dense weeds extend above the water, much of the total herbicide will not reach the water directly. This also holds true for heavily forested spray areas. Much of the foliage intercepts the herbicide before it has a chance to reach the surrounding water. As much as 88 percent of the total herbicide may be intercepted by such plants as liveoak, which are considerably less dense than such water species as water hyacinth or alligator weed (Tschirley 1968). Little information exists on the possibility of release to water of herbicide residues in plants (President's Science Advisory Committee 1971).

Phenoxy chemicals may enter the water but diminish due to the forces of volatilization, degradation, adsorption on sediment, adsorption by biota and dilution as additional stream water passes through the site (EPA 1974). Almost all authorities agree that adsorption on the bottom sediment takes place. Esters of 2,4,5-T are hydrolyzed within a matter of days in all but highly acidic kinds of water (Kenaga 1974). Under laboratory conditions, the 2-ethylhexyl ester of 2,4,5-T was applied to water at a concentration of 1 ppm; 58 percent of the ester remained after four hours; 33 percent after eight hours; and 12 percent after 16 hours.

Air Residues - Prior to 1970, the phenoxy herbicides were used for pre-emergence control of broadleaf weeds in wheat crops. Air samples were collected and analyzed from the State of Washington during the spring and summer seasons where the herbicide was employed for wheat weed control. Results of this analysis demonstrated up to 0.06 ug/m^3 of 2,4-D and 2,4,5-T remained in the air samples taken. Authors extrapolated that a man inhaling 30 m^3/day, at this level of phenoxy herbicide exposure, would be inhaling 1.8 ug phenoxy herbicide per day (or 0.025 ug/kg of body weight/day for a 70 kg man) (Johnson 1971).

The National Air Monitoring Program conducted ambient air monitoring for pesticides in the years 1970 through 1972. Table 2 records the arithmetic mean of residues of 2,4,5-T detected in this program.

Table 2. Air Monitoring Data for 2,4,5-T in 28 State Monitoring Programs (1970-1972)

State	2,4,5-T ester monitored for	ng/m^3 1970	ng/m^3 1971	ng/m^3 1972
Louisiana	Isopropyl ester	-	ND	1.9
Montana		ND	ND	0.8
New Mexico		-	ND	1.0
Idaho		ND	ND	1.7
Illinois	BOEE	ND	3.6	ND
Oregon		ND	0.5	ND
Tennessee		1.1	ND	ND
Tennessee	Isooctyl ester	ND	2.7	ND
Oklahoma		ND	14.6	ND

ND = not detected; ng/m^3 = nanograms per cubic meter

Source: EPA 1978

EPA Air Monitoring Data for 2,4,5-T over a three year period showed that no monitoring location had a detectable herbicide residue in more than one of the three years tested. The iso-octyl ester of 2,4,5-T sprayed in Tennessee and Oklahoma left the greatest amount of residue recorded in the second year of testing.

From these data it would appear that 2,4,5-T residues are neither consistent nor cumulative.

Animal Residues - Perhaps the most important residues are those found in animals. Animal residues accumulate because of herbicide build-up elsewhere. If a residue covers pastureland, then grazing animals ingest these residues. Carrying this one step further, a slaughtered animal, having grazed on residue covered land, can produce residue carrying meat samples--thus demonstrating a satellite effect of an initial chemical accumulation. However, animals rapidly eliminate about 95 percent of ingested 2,4,5-T unchanged in the urine (Sauerhoff et al. 1976), suggesting a low risk of residue accumulation in grazing animals from any but very heavy applications.

Many researchers have studied 2,4,5-T residues in a variety of different animal species. The following summarizes the results of selected papers. (Tables 3,4).

Table 3. Summary of Literature on 2,4,5 - T Metabolism and Loss Rates

Species and Route Administered	Dose	Plasma ½ Life	Urine Clearance	Author
Rat Oral	100 mg/kg	3.0 hrs		Erne (1966)
	50 mg/kg		69% after 7 days	Grunow (1971)
Rat Oral	5 mg/kg	4.7 hrs	13.6 hrs	Piper (1973)
	50 mg/kg	4.2 hrs	13.1 hrs	
	100 mg/kg	19.4 hrs	19.3 hrs	
	200 mg/kg	25.2 hrs	28.9 hrs	
Rat Oral	0.4 mg		75±7% after 24 hrs	Fang et al.
	1 mg	3.5 hrs		
	10 mg			
Rat	5 mg/kg	10.7 hrs		Sauerhoff (1976)
	100 mg/kg	11.0 (from 0-84 hrs)		
		69.3 (from 84-168 hrs)		
Dog Oral	5 mg/kg	77.0 hrs	86.6 hrs,	Piper (1973)
Man Oral	5 mg/kg	23.1 hrs	88.5±5.11% after 96 hrs	Gehring (1973)
	2 mg/kg	18.8±3.1 hrs	over 50% in less than 48 hrs	Kohli (1974)
	3 mg/kg			
	5 mg/kg			

Table 4. Summary of Selected Studies on 2,4,5-T Residues in Animals after Ingesting Feed Containing a Known Concentration of the Chemical

Sample and Species	Dose	Length of Exposure	Residue	Author
Milk or cream: cow	10-30 ppm 100 ppm	oral, total diet for 3 weeks	<0.05 ppm	Leng 1977
Milk: cow	300-1000 ppm	untreated feed	0.15-0.5 ppm	
Calves: muscle	300 ppm	in diet for 28 days	0.12 ppm	
Calves: fat	300 ppm		0.38 ppm	
Calves: liver	300 ppm		0.2 ppm	
Calves: liver	900 ppm		1.0 ppm	
Calves: muscle	900 ppm		0.24 ppm	
Calves: fat	900 ppm		0.38 ppm	
Calves: liver	1800 ppm		*7.9 ppm	
Calves: muscle	1800 ppm		1.2 ppm	
Calves: fat	1800 ppm		2.0 ppm	
Calves	1800-2000 ppm	after 1 wk untreated feed	0	
Sheep: fat	0.15 or 0.75 mg/kg	4 oral doses	0.08 ppm	Palmer,Clark 1971
Sheep: kidney	250 mg/kg	for 4 days	168 ppm	
Sheep: kidney, muscle, fat, liver	0.15 or 0.75 mg/kg	28 days, after 1 wk	0.05 ppm	Clark 1975

*Maximum dose of 1800 ppm caused anorexia and minor weight loss.

weather conditions, poor equipment, temperature, humidity, and improper distribution practices increase the risk of spray drift. Effects of this hazard can be greatly reduced by following certain protocol and deciding if, when, and how herbicide application takes place.

Residues result when immediate chemical degradation does not follow initial application. Residues can accumulate in soil, water, air, and indirectly in animals and humans. Residues do not remain indefinitely. Their loss from the environment can be predicted through the chemical's half life in various media in the environment and a knowledge of the chemical's physical characteristics.

Consideration of toxicity and hazard in relation to 2,4,5-T stems primarily from a contaminant, TCDD, a potent toxin. Direct effects of toxicity will be discussed later in this paper.

DOW2155160

POLYCHLORINATED DIOXINS

The generic term "dioxin" pertains to a family of compounds which are structurally similar but vastly different in toxicity. It is often used incorrectly to designate one member of the family, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). There are 75 possible polychlorinated dibenzo-p-dioxins (PCDDs) with a million-fold spread in the dosage level required to kill laboratory animals. Those with four or five chlorines located at all four lateral positions (2,3,7 and 8) are extremely toxic. The toxicity is progressively decreased by moving one or more chlorines to peri-positions (1,4,6 or 9), and/or by having fewer or more positions substituted.

Low levels of polychlorinated dibenzo-p-dioxins (PCDDs) can be formed as byproducts during the manufacture of chlorophenols under alkaline conditions at elevated temperatures and high pressures. The reaction is a two-step process involving bimolecular condensation of chlorophenates followed by internal ring closure. The number and position of the chlorine substituents on the dioxins depend on the chlorophenates present in the reaction mixture.

In all, 75 possible PCDDs could originate from condensation of chlorophenols with two to five chlorine substituents. Of these, 22 are tetrachloro isomers. However only symmetrically substituted isomers with an even number of chlorines are likely to be formed from reaction mixtures containing predominantly one chlorophenol such as in manufacturing processes.

Other isomers could also result from progressive chlorination or dechlorination of lower or higher analogs. However, photolysis in solution appears to preferentially remove chlorines from the lateral positions (Buser and Rappe 1976). Thus isomers substituted at the 2,3,7,8-positions are not likely to be formed from higher PCDDs by the action of light.

2,4,5-Trichlorophenol is used as a slime control, as a defoaming agent in paper and cardboard manufacture, and in the manufacture of 2,4,5-T. Since tetrachlorodibenzo-p-dioxin arises as a by-product in 2,4,5-trichlorophenol manufacture, it is possible that TCDDs may get into the various products and thence into the environment. On the other hand, researchers have not observed formation of TCDD from trichlorophenol under the action of sunlight, simulated sunlight, or by microbial condensation in soil.

The higher chlorodioxins (6, 7, and 8 chlorine atoms) may be expected to arise in the production of pentachlorophenol. Pentachlorophenol is produced in the U.S. by chlorination of phenol at nearly atmospheric pressure, without alkalinity, and at temperatures of about 200° C (350° F). However, the production of sodium pentachlorophenate involves the same conditions (high temperature, pressure, alkalinity) as that of trichlorophenol. Hexachlorodioxin has indeed been found in almost all samples of commercial pentachlorophenol tested, along with hepta- and octadioxins, the latter two at levels of up to 1000 ppm (Firestone et al. 1972; Plimmer 1973). Formation of hexa- and heptachlorodioxins has been observed when chips of pentachlorophenol treated plywood were burned. Hexa- and octachlorodioxin have been formed from pentachlorophenol under the influence of ultraviolet light.

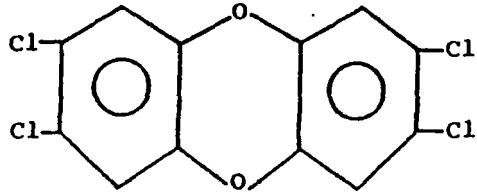
Pentachlorophenol is extensively used for the same purposes as trichlorophenol but also as a wood preservative, for the preservation of hides, starches and glues, in paper designed for contact with aqueous and fatty foods, as antioxidant in rubber articles used in producing, processing or holding food, and in manufacture of certain plastics used in the food industry. Production in the U.S. in 1967 was 44,000,000 pounds and in 1970, 47,000,000 pounds, most of it used as wood preservatives. In comparison, production of 2,4,5-trichlorophenol (including salts) in 1967 was 25,000,000 pounds (no data for 1970), and of 2,4,5-T including salts and esters in 1967, 42,000,000 pounds and in 1970, 12,300,000 pounds (U.S. Tariff Commission 1967 and 1970). Thus, it is likely that hexachlorodibenzo-para-dioxins enter the environment from pentachlorophenol unless production methods are used which reduce the contamination of these substances to acceptable levels.

2,3,7,8-TCDD is the most toxic among the chlorodioxins studied, together with hexachlorodioxin and probably some tri- and heptachlorodioxins. Hexachlorodibenzo-para-dioxin at daily doses of 0.01 mg/kg caused reduction in the rate of body weight increase in pregnant rats and their fetuses and induces edema in chickens. A ten-fold increase in dose caused abnormalities in some fetuses (Schwetz et al. 1973). These doses are roughly 10 to 30 times higher than TCDD doses causing similar effects. In contrast, 2,7-dichloro-, 1,3,6,8-tetrachloro- and octachlorodioxin have very low toxicity, and no demonstrable teratogenicity at the highest doses tested. The LD 50s were over 100,000 times higher than those for TCDD, and no teratogenicity was found at 100 mg/kg/day.

Recognized in 1957 as an impurity in the manufacture of herbicide 2,4,5-T, TCDD is one of the most potent small molecule toxins known to man. Small amounts of the chemical can cause enormous changes in animal cells. Currently, technical grade 2,4,5-T in the U.S. routinely contains TCDD levels at or below 0.1 ppm.

Chemistry

2,3,7,8-tetrachlorodibenzo-p-dioxin exists as a white, crystalline solid, having a molecular weight of 322 grams per mole. It melts at temperatures between 303 and 305° C and decomposes at temperatures between 980 and 1000° C. About 99.5 percent of the dioxin decomposes at temperatures of 800° C.



2,3,7,8-tetrachlorodibenzo-p-dioxin

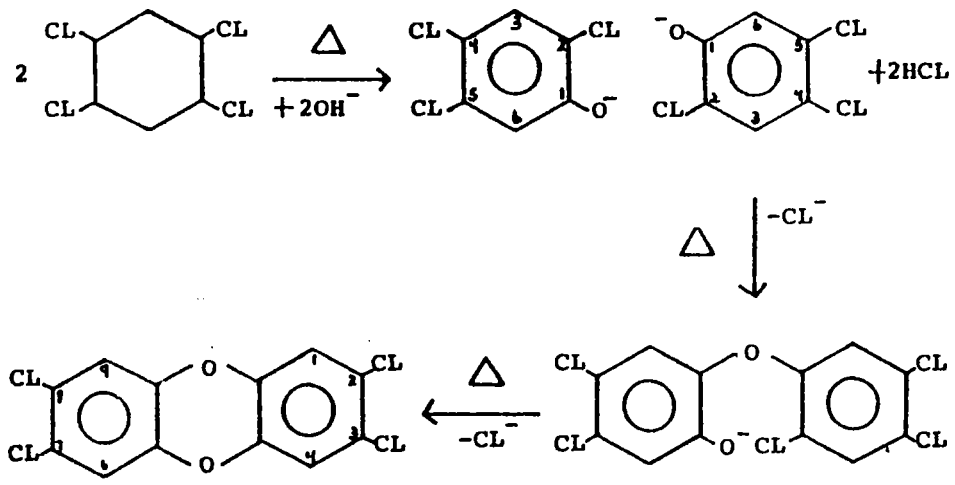
Table 8. Solubility of 2,3,7,8 - Tetrachlorodibenzo-p-dioxin in Various Solvents at 25 ° C

Solvent	Solubility	
	g/100 ml	wt percent
o-Dichlorobenzene	0.18	0.14
Chlorobenzene	0.080	0.072
Perchloroethylene	0.068	0.048
Chloroform	0.055	0.037
Benzene	0.047	0.057
Acetone	0.009	0.011
Dimethylsulfoxide(a)	0.01	0.01
Methanol	0.001	0.002
Water	0.00000002	0.00000002 (0.2 ppb)

(a) DMSO caused detector fouling and a better value could not be obtained. However, solubility does not exceed 0.01 percent.

TCDD is poorly soluble in most organic solvents and [illegible] water. The solubility characteristics with various solvents are shown in Table 8.

2,3,7,8-TCDD arises during the hydrolysis of tetrachlorobenzene to form 2,4,5-trichlorophenol, the industrial precursor of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The hydrolysis occurs in an alkaline environment at high temperatures and under pressure. Each of these conditions favors the formation of the chlorodioxins unless they are very carefully controlled.



2,3,7,8-TCDD "Predioxin"

Since TCDD displays low water solubility compared to its higher fat solubility characteristics, researchers realize the potential for bioaccumulation, described as the uptake or the accumulation less metabolism and excreting by an organism from its surrounding environment. Bioaccumulation processes may concentrate TCDD in lower animals and result in a possibly significant exposure to higher tropic levels.

Prior to 1973, the detection limit for TCDD was 0.1 ppb for soils and 0.05 ppm for biological tissue (Woolson et al. 1973). Kearney et al. (1972) and Dost et al. (1975) ascertained that a one pound/acre application of 2,4,5-T contains 0.1 ppm. TCDD applied directly to the soil could result in a maximum of 0.1 ppt TCDD in the top 15 [illegible] of soil. Baughman and Meselson (1973) have calculated the environmental monitoring of food chains would require a detection level of 1 ppt. For a one gram sample of biological tissue, this would require a limit of detection of one picogram (1 pg or 10^{-12} grams) (Baughman 1976). Such analyses can only take place using ultrasophisticated detection instrumentation and large samples of tissue.

Because of difficulty in detection, model ecosystems are often employed to predict the mechanics of TCDD in the environment.

Matsumura and Benezet (1973) developed an aquatic ecosystem to test TCDD residue on sand affecting brine shrimp, mosquito larvae, and fish. They found that TCDD uptake rates were very low in brine shrimp and fish. Mosquito larvae, which are bottom feeders, showed a faster rate of TCDD pickup. The authors attributed TCDD's failure to accumulate in as many biological systems as DDT to its low solubility in water and its low partition coefficient in lipids.

Isensee and Jones (1972) exposed several organisms to C14-labelled TCDD for up to 31 days to determine the distribution and bioaccumulation potential in an aquatic environment. The authors found that TCDD accumulation was directly proportional to the water concentration of the contaminant, 0.05 to 1330 ppt. Values ranged from 2.0 to 2.6 x 10^4 times the water concentration for snail, mosquito fish and daphnids, and averaged 4.9 x 10^3 for duckweed algae and catfish. No metabolities of TCDD were found in submerged soil, water, snails, mosquito fish, and catfish. Isensee and Jones also reported that 85-99 percent of the labelled TCDD originally added to the model ecosystem at the start of the experiment remained in the soil at the experiment's conclusion. Total recovery for the ecosystem averaged 92.2 percent, illustrating the relative stability of TCDD in this test.

Kearney et al. (1972) analyzed the persistence of TCDD in Hagerstown and Lakeland soils treated with 1, 10, and 100 ppm TCDD. The researchers conducted analyses after 20, 40, 80, 160, and 350 days after chemical application. After one year, 56 percent and 63 percent of the originally applied TCDD were covered from Hagerstown and Lakeland soils, respectively, indicating a TCDD half-life approximating one year. In soils receiving 10, 100, or 1000 ppm 2,4,5-trichlorophenol, no detectable levels of TCDD were found after 70 days. This suggests that TCDD was not biosynthesized by microbial condensation reactions. The long half-life of TCDD suggested to Kearney et al. that it was not readily metabolized by soil microorganisms. TCDD's high toxicity to soil mi[illegible]bes may also account for the chemical's long half-l[illegible].

Nash and Beall (1978) studied the fate of TCDD in plants, soil, water, and air of a microagroecosystem. Tritium labelled TCDD was sprayed on bluegrass turf in a growth chamber at concentrations of 44 and 500 ppb. The isoctyl ester of 20(2,4,5-trichlorophenoxy) propionic acid (Silvex) contained the chemical as a contaminant. The authors determined that:

1) TCDD concentrations in water leached through soil were below the analytical detection limit (10^{-16} g/g water).
2) TCDD concentrations on grass were initially 20 ppt (10^{-12} g/g grass), but after four weeks were at or below 1 ppt. The half life of TCDD was approximately six days.
3) TCDD concentrations in or on soil were less than 0.2 ppt and most (80%) was near the soil surface (0-2 cm).
4) TCDD concentrations in air were (immediately after application) less than 100 fg/m^3 (femtogram - 10^{-15} g/m^3) and after four weeks decreased to less than three fg/m^3.
5) TCDD (or its degradation products) concentration in earthworms were less than 0.3 ppt.
6) The major repositories for TCDD were the soil and thatch.

Authors Nash and Beall concluded that volatilization (approximately ten percent) of TCDD provided a major mechanism for the exit of TCDD from the microagroecosystem. After volatilization, TCDD then was dechlorinated in the direct sun, filtered sun, and also even in shaded areas. They concluded that TCDD is sensitive to photodechlorination in the vapor phase even without the presence of ultraviolet light. This supports the suggestion of Crosby and Wong (1977) that TCDD, once on foliage, degrades in ultraviolet light fairly rapidly, never reaching the soil system.

Indeed the most significant accomplishment in the study of the environmental degradation of TCDD belongs to Crosby and Wong. They found that herbicide formulations with TCDD as a contaminant lose most or all their TCDD within a single day when the leaves, soil, or grass of the treated foliage receive natural sunlight. Photochemical dechlorination causes this degradation of TCDD. While the stability of pure TCDD as a persistent residue in the environment is accepted, it is not stable in the presence of its herbicide vehicle. Appearing on the foliage in a thin herbicide film, the contaminant diminishes as it is exposed to sunlight.

Crosby and Wong have established three requirements for significant dioxin breakdown in the environment. They are: 1) dissolution in a light-transmitting film; 2) the presence of an organic hydrogen-donor, such as a solvent or pesticide; and 3) ultraviolet light. They propose that all three conditions are normally met in applications of 2,4,5-T. Therefore they feel that these data suggest that environmental residues of TCDD often will be considerably less than previously expected.

Numerous studies have demonstrated amount and location of TCDD residue found in various animal species as well as in water and rice. Table 9 represents findings of several investigations.

Table 9.

EPA Dioxin Implementation Plan (1976)

	Sample	TCDD Residue	Level of Detection
	43 beef liver samples of 43 tested	No TCDD found	4-8 ppt sensitivity
	1 beef fat sample of 85 tested	60 ppt	10 ppt
	2 beef fat of 85 tested	20 ppt	10 ppt
	5 beef fat samples of 85 tested	may have between 5-10 ppt	10 ppt
	77 beef fat samples of 85 tested	no detectable TCDD	10 ppt
	Shadoff (1977)		
	Sample	TCDD Residue	Level of Detection
	Arkansas ricelands water, mud, catfish bass from pond in riceland	No detectable TCDD	10 ppt
	Reservoir west Texas water, mud, catfish, walleye pike	No detectable TCDD	10 ppt
	Jensen - Dow Chemical (Dow confidential 1978)		
	Sample	TCDD Residue	Level of Detection
	Rice from high treatment fields and related stores	No detectable TCDD	10 ppt
Cattle	300 ppm 2,4,5-T and 24 ppt TCDD for 28 days	Initially 95 ppt fat	2 ppt
	Liver, kidney	10 ppt	2 ppt
	Muscle	2 ppt	2 ppt
Sheep	300 ppm 2,4,5-T and 150 ppt TCDD for 28 days		
	Fat and liver	Two times the dietary concentration	2 ppt
	Muscle	1/8 times the dietary concentration (20 ppt)	2 ppt

17658

Table 9. (continued)

	Sample	TCDD Residue	Level of Detection
Dairy Cow	Fed 5 to 500 ppt TCDD in diet for 2 to 3 weeks at each level	Residues increased proportionally to increased TCDD levels	
	Milk 5 ppt 500 ppt	Not detectable 89 ppt	ppt
	Cream 5 ppt	10 ppt	ppt
	After diet with TCDD stopped, TCDD content in milk declined rapidly for several weeks and then continued downward at a slower rate.		
	Kocher (1978)		
	Sample	TCDD Residue	Level of Detection
	Range Cattle Fat Grazing on sprayed pasture	3 of 7 samples 3-4 ppt 4 of samples no detectable level	3 ppt
	Baughman and Meselson (1973)		
	Sample	TCDD Residue	Level of Detection
	Viet Nam water samples taken in 1970, analyzed 2½ years later		
	Fish - interior rivers of Viet Nam	70-810 ppt	ppt
	Fish and shellfish coast live of Viet Nam	18-79 ppt	ppt
	Cape Cod Butterfish (control)	Not detectable	ppt
	Dow Chemical Company		
	Sample	TCDD Residue	Level of Detection
	Fish sample from water downstream to Dow's Midland, MI plant		
	Catfish	230 ppt	ppt

17859

Table 9. (continued) DOW2155168

EPA 1980 (unpublished)

Sample	TCDD Residue	Level of Detection
Mother's milk (human) 103 samples from spray and control areas	No detectable residues	1-4 ppt

Meselson, O'Keefe, Baughman (1977)

Sample	TCDD Residue	Level of Detection
Mother's milk 18 samples from humans living on 2,4,5-T treated rangeland	4/18 with 1 ppt	1 ppt
6 controls (Boston)	No detectable residues	

Before completing a discussion of residues, we must consider the most recent reviews of the action of TCDD in the environment. Recently, the Dow Chemical Company of Midland, Michigan, released a report demonstrating the production of TCDD as a by-product of normal combustion processes. Analyzing samples from a number of combustion products, such as refuse incinerator emissions, gasoline-powered automobile exhausts, fireplace soot, charcoal grills, cigarette smoke, and particulate emissions from a chemical plant powerhouse, researchers quantitatively assessed the amounts of TCDD present. Finding measurable amounts of TCDD, they concluded that... "refuse incinerators and fossilfueled powerhouses are sources for both airborne and waterborne particulates which contain chlorinated dioxin..." (Dow Chemical Company 1979). These conclusions have been supported through the research of Olie et al. (1977).

However, Kimble and Gross (1980) have taken exception to all fossil fueled power plants labelled as major sources of environmental dioxin. Analyzing one sample of coal fly ash collected several years earlier, they were unable to detect any TCDD residues at a sensitivity limit of 1.2 ppt. They therefore consider it invalid to extrapolate results from chemical plant powerhouses for application to coal fired power plants.

Should environmental presence of dioxins from combustion processes be further established and supported, the ramifications will be great. For it will become increasingly difficult, if not impossible, to link environmental residues unequivocally and solely to herbicide spraying.

Summary

2,3,7,8-TCDD exists as a white crystalline solid, with a molecular weight of 322 grams per mole. Melting at temperatures between 303 and 305° C, 99.5 percent of TCDD decomposes at temperatures of 800° C.

TCDD can form during the hydrolysis of tetracholorbenzene to form 2,4,5-trichlorophenol in the 2,4,5-T manufacturing process.

Displaying low water solubility and high fat solubility properties, dioxin can accumulate in the environment under some conditions. TCDD is readily decomposed by ultraviolet light. This helps to diminish potential accumulation of the chemical.

Residue experiments have monitored the TCDD levels in land, water, and various species of animals. For the most part, TCDD residue levels directly compare to the amount of initial TCDD application and are not detectable in many instances.

Recently the Dow Chemical Company has suggested that dioxin contaminants result from various combustion processes in the environment. Should this be confirmed, allegations of 2,4,5-T as the primary producer of dioxin in the environment will become increasingly tenuous.

TOXICITY

Different animal species show toxic responses to varying amounts of 2,4,5-T. The usual way of expressing a chemical's acute toxicity is through its LD^{50} defined as the amount of chemical sufficient to cause death in 50 percent of the test animals. Likewise, an LD^{90} signifies a dose potent enough to kill 90 percent of animals tested. For most analytical purposes, an LD^{50} is most commonly used.

An LD^{50} value can differ due to such variables as age or sex of test animals, nutritional state, formulation of toxicant, time of day, route of administration, and temperature. Mode of entrance and chemical form of the substance can also alter an LD^{50}.

On the other end of the LD spectrum from LD^{100} lies a "No observable effect level." Generally a NOEL signifies a toxicological endpoint. Chemicals administered in this concentration cause no observable adverse effects. Toxicologists generally assume that an animal no-effect level can provide the basis for estimation of human safe exposure levels. According to the EPA, a "safe" level for humans is set at some level lower than the animal no-effect level to provide a "margin of safety" that takes into account differences in sensitivities among species and within a single species. This "margin of

Table 10. Summary of Literature on the No Effect, LD^{50} and LD^{100} Levels of the Acute Toxicity of 2,4,5-T

Animal	Route of Administration and Chemical Form	Tox. Dose	Single Dose mg/kg	Author
Mouse	oral - acid	LD^{50}	389	Rowe and Hymas 1954
Mouse	oral - isopropyl ester	LD^{50}	551	Rowe and Hymas 1954
Mouse	oral - mixed butyl esters	LD^{50}	940	Rowe and Hymas 1954
Rat	oral - acid	LD^{50}	500	Rowe and Hymas 1954
Rat	oral - isopropyl ester	LD^{50}	495	Rowe and Hymas 1954
Rat	oral - mixed butyl ester	LD^{50}	481	Rowe and Hymas 1954
Rat	oral - mixed amyl ester	LD^{50}	750	Rowe and Hymas 1954
Guinea Pig	oral - acid	LD^{50}	381	Rowe and Hymas 1954
Guinea Pig	oral - isopropyl ester	LD^{50}	449	Rowe and Hymas 1954
Guinea Pig	oral - mixed butyl ester	LD^{50}	750	Rowe and Hymas 1954
Chick	oral - acid	LD^{50}	310	Rowe and Hymas 1954
Rabbit	oral - mixed butyl ester	LD^{50}	712	Rowe and Hymas 1954
Dog	oral - in capsule acid	LD^{100}	250	Drill and Hiratzka 1953
Dog	oral - in capsule acid	LD^{50}	100	Drill and Hiratzka 1953
Dog	oral - in capsule acid	No effect	50	Drill and Hiratzka 1953
Pig	oral - acid	Anorexia, vomiting, diarrhea, ataxia hemorrhagic enteritis liver and kidney congestion	100	Bjorklund and Erne 1966

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safety" does not represent an infallible indicator of potential hazard to humans. The EPA further asserts that error could be introduced because humans may be more sensitive than the test species by a greater degree than normally allowed, or by the incorrect choice of a no-effect level. Safety factors of 1/100 of a no-effect dose are commonly used in safety assessments.

Since 2,3,7,8-TCDD inevitably contaminates 2,4,5-T, no consideration of the LD^{50} of the herbicide alone can be complete without considering the LD^{50} of the accompanying dioxin (Tables 10 and 11).

Table 11. Summary of Literature Data on the no-effect, LD^{50} and LD^{100} Levels of the Acute Toxicity of TCDD for Animals

Animal	Route of Administration	Tox. Dose	Single Dose ug/kg[1]	Author
Mouse (CD-1 strain, C57Bl/6Sch strain)	oral	LD^{100}	50	Vos, Gupta, et al. 1973
Mouse	oral	A few sporadic deaths	1-130	Schwetz, et al. 1973
Mouse	oral	LD^{100}	150	Goldstein, et al. 1973
Mouse (C57Bl/6 strain)	intraperitoneal	LD^{50}	120	Vinopal, et al. 1973
Rat	oral	No effect	8	Schwetz, et al. 1973
Rat	oral	No effect	16	Schwetz, et al. 1973
Rat	oral	LD^{100}	32	Schwetz, et al. 1973
Rat (male)	oral	LD^{50}	22	Schwetz, et al. 1973
Rat (female, Sherman strain)	oral	LD^{50}	45	Schwetz, et al. 1973
Guinea Pig	oral	LD^{50}	0.6	Schwetz, et al. 1973
Guinea Pig (Hartley strain)	oral	LD^{50}	2.1	Schwetz, et al. 1973
Rabbit	oral	LD^{50}	115	Schwetz, et al. 1973
Rabbit	topically to the skin	LD^{50}	275	Schwetz, et al. 1973
Rabbit	intraperitoneal	No effect	32	Schwetz, et al. 1973
Rabbit	intraperitoneal	2 of 5 died	252	Schwetz, et al. 1973
Rabbit (New Zealand Albino)	intraperitoneal	3 of 5 died	500	Schwetz, et al. 1973
Dog	oral	No effect	300	Schwetz, et al. 1973
Dog	oral	LD^{100}	3000	Schwetz, et al. 1973
Dog	oral	No effect	30	Schwetz, et al. 1973
Dog (Beagles)	oral	No effect	100	Schwetz, et al. 1973
Monkey (Rhesus)	oral	LD^{50}	50-70	McConnell, et al. 1978

[1] 1 mg/kg = 1,000 ug/kg

With increasing amounts of ingested herbicides, animals approach a threshold of intoxication, and from this point on will suffer toxic effects. Initially producing few, if any outward signs, toxic responses from increased or prolonged doses increase in severity until they ultimately cause death (Table 12).

Table 12. Summary of Literature Involving Toxic Effects of 2,4,5-T

Animal	Dose	Length of Study	Effects	Author
Dog	20 mg/kg day,5 days/wk TCDD not slated	65 days	death within 11-75 days in all animals	Drill and Hiratzka 1953
Dog	10 mg/kg/day, 5 days/wk	65 days	overt signs of toxicity weight loss, stiffness of hing legs, muscular weakness, occasional bleeding gums, no fatalities	Drill and Hiratzka 1953
Dog	2.5 mg/kg/day	91 days	no adverse effects	Drill and Hiratzka 1953
Sheep	100 mg/kg/day, repeated doses TCDD not known	acute	no adverse effects	Rowe and Hymas 1954
Steer	100 mg/kg/day repeated doses	acute	no adverse effects	Rowe and Hymas
Steer	500 mg/kg/day	acute 3 days	acute toxicity	Rowe and Hymas 1954
Steer	100 mg/kg/day	acute 3 days	fatal	Rowe and Hymas 1954
Rabbit	acid moistened	application 10X to abraded skin	slight reddening and swelling at application site	Dow Chemical
Rabbit	2000 mg/kg acid cuff technique	1 day	slight reddening and swelling at sight of application cuff	Dow Chemical
Rabbit	butyl ester	10X to abraded skin on belly	slight reddening and swelling - moderate burn in one animal	Dow Chemical
Rabbit	2000 mg/kg butyl ester	1 day	intact skin - no fatalities abraded skin - 1/3 fatalities	Dow Chemical
Mouse 978PF	30-140 mg/kg gavage	6-14 days	varied strain	Highman, et al. 1976 (62)
Mouse CD-1	140 mg/kg gavage	6-14 days	1% moribund	Highman, et al. 1976
Mouse F dihybrid	120 mg/kg gavage	6-14 days	53-82% fatalities	Highman, et al. 1976
Mouse NCTR strain	60 mg/kg gavage	6-14 days	ill or dead	Highman, et al. 1976
Mouse CRBL strain	90-120 mg/kg gavage	6-14 days	no effect	Highman, et al. 1976
Mouse 378	60-120 mg/kg gavage	6-14 days	varied	Highman, et al. 1976 (60)
	60 mg/kg	6-14 days	most animals outwardly normal**	
	120 mg/kg	6-14 days	many animals appeared outwardly normal**	
	TCDD level 0.05 and 0.005 mg TCDD/kg			

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Table 12. Summary of Literature Involving Toxic Effects of 2,4,5-T (continued)

Animal	Dose	Length of Study	Effects	Author
Rat	30 mg/kg dose per animal in feed	90 days	no effect	IARC 1977
Rat	100 mg/kg dose per animal in feed	90 days	anorexia, depressed weight gain, threshold toxicity	IARC 1977
Rat	300 mg/kg dose per diet 1 mg TCDD/kg	90 days	no effect	IARC 1977
Rat	1000 mg/kg dose per diet	90 days	toxicity, no deaths due to treatment, tissue changes noted	IARC 1977
Rat	3000 mg/kg dose per diet	90 days	growth retardation in males, no deaths due to treatment, tissue changes noted	IARC 1977
Rat	10 mg/kg/day	11 days	liver enlargement only	Rip and Cherry 1976
Rat	30 mg/kg/day	2 years	decreased weight gain, increased kidney weight, increased urinary volume, increased coproporphyrin, increased uroporphyrin, slight morphological changes in liver, lungs, kidney, no blood dyscrasias	Kociba, et al. 1979
Rat	10 mg/kg/day	2 years	minimal effects, mineralized deposits renal pelvis male -- increased coproporphyrins	Kociba, et al. 1979
Rat	3 mg/kg*	2 years	no changes	Kociba et al. 1979

*3 mg/kg considered no effect dose level for chronic exposure to 2,4,5-T.
**histopathological changes noted.

Animals suffer toxic effects from 2,4,5-T in dosages ranging from 10 milligrams per kilogram of body weight per day (10 mg/kg/day) in a dog to 140 mg/kg/day in a mouse. However, since several of the studies listed in Table 12 were performed in the 1950s and 1960s, the TCDD level in the 2,4,5-T feed was either not known or not mentioned. TCDD was a significant contaminant in all 2,4,5-T produced at that time, and it could have been a significant factor in the animal toxicity studies. Weight loss and muscle weakness with accompanying tissue changes represent the most common effects of 2,4,5-T toxicity.

Variations are expected by differences within a species (e.g., mice), alternate modes of chemical entrance into the body, and variable lengths of time for the duration of the experiment.

This last consideration expresses a fundamental aspect of any toxicological investigation. A large dose of chemical toxin will profoundly affect an animal in a short time. The results demonstrate acute response. However, thorough investigation also includes a long term chronic exposure response to determine effects of relatively low doses of chemical. Toxicological responses generated from these amounts may not appear within a short period of time; but after longer duration, they indeed may elicit some sort of physical change attributed either primarily or secondarily to chemical exposure.

Both acute and chronic no-effect levels are important in understanding the toxicology of any material.

Kociba et al. 1979

The Kociba et al. study reports the effect of chronic long-term toxicity resulting from exposure to 2,4,5-T (no TCDD found at detection level of 0.33 ppb). Studying the effects of the herbicide on Sprague-Dawley rats, the investigators monitored them for their general health, possible toxicological responses, and change in body weight. Body weight and food consumption provided the basis for determining the test levels of (control) 0, 3, 10, 30 mg/kg body weight distributed in rat feed. Rats were maintained on this diet for a total of two years. Selective killing of a few rats representing all test groups took place on days 118 and 119 of the experiment. Autopsies were performed to determine the health effects after this relatively short period of treatment. These interim findings were later compared to effects noted at the conclusion of the experiment.

Analysis of feed samples indicated the 2,4,5-T concentration level approximated 85 ppm in the 3 mg/kg diet, 230 ppm in the 10 mg/kg diet, and 677 ppm in the 30 mg/kg diet.

Body Weights - There were no statistically significant differences from controls in the mean body weights of any of the rats in the 118-119 day study. Mean body weights of females given 30 mg/kg showed a statistically significant increase four times during the course of the two-year study. Males at this dose showed a trend toward slightly lower values. At the 3 mg/kg or 10 mg/kg dose levels, there were no statistically significant differences from the control values.

Food Consumption - No consistent deviations from the control rate appeared in either sex at any of the experimental dose rates for either the sub-chronic or the chronic study, indicating that consumption remained the same, even in the presence of weight change.

Histopathological Changes - See Table 13

Mortality - Incidence of male deaths at the 30 mg/kg dose level decreased during the latter part of the two-year study. This occurred as a secondary effect of the less severe chronic renal disease seen in these rats. The female mortality rate at the high dose level did not differ from the control rate significantly. Analysis of mortality rates at the smaller dose levels demonstrated no toxicological significance.

Hematological analysis - No statistical differences from the controls appeared in any tissue studied or any dose level during the sub-chronic or chronic parts of the study.

Urinalysis - In males receiving 30 mg/kg dose per day, investigators noted increase in total urine volume, and in excretion of coproporphyrin and uroporphyrin excretion but only in the early phase of the study. In females receiving the larger dose, increased coproporphyrin appeared at most of the sampling times. Uroporphyrin excretion decreased after 119 days but increased after two years. No significant differences in total urinary volume or excretion or urinary creatinine, coproporphyrin, uroporphyrin, or Y-ALA were recorded in males at 3 mg/kg doses or females at either 10 mg/kg or 3 mg/kg dose levels.

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Table 13. Summary of Major Gross and Histopathological Observations in Rats Maintained on Diets Containing 2,4,5-T for 2 Years**

Lesion	No. of Rats Affected/No. Examined							
	Male				Female			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Kidney								
Mineralized deposits in pelvis		NE	NE	NE	1/86	1/50	5/50*	8/50*
Localized reaction adjacent to mineralized deposits		NE	NE	NE	25/86	19/50	22/50	37/50*
Moderate to severe chronic renal disease	56/86	28/50	30/50	22/50*	6/86	6/50	2/50	1/50
Increased pigment in renal tubular cytoplasm		NE	NE	NE	0/86	0/50	0/50	11/50*
Mineralization secondary to renal disease seen in:								
Pulmonary alveoli	13/86	8/50	4/50	0/50*		NE	NE	NE
Myocardium	5/86	3/50	2/50	0/50		NE	NE	NE
Myocardial blood vessels	12/86	6/50	5/50	0/50*		NE	NE	NE
Gastric mucosa and muscularis	25/86	13/49	9/49	1/50*		NE	NE	NE
Liver								
Enlargement	11/86	3/50	7/50	1/50*		NE	NE	NE
Focal biliary hyperplasia	9/86	6/50	8/50	14/50*		NE	NE	NE
Periportal inflammation	29/86	7/50*	9/50	28/50*		NE	NE	NE
Focal aggregates of reticulo-endothelial cells adjacent to degenerate or necrotic hepatocytes		NE	NE	NE	36/86	17/50	21/50	34/50*
Multiple foci of hepatocellular alteration (swollen hepatocytes)		NE	NE	NE	15/86	4/50	6/50	3/50*
Focal hepatocellular cytoplasmic vacuolization	34/86	20/50	12/50*	8/50*		NE	NE	NE
Lungs								
Focal interstitial inflammation	21/86	5/50*	5/50*	27/50*		NE	NE	NE
Focal accumulations of alveolar macrophages in alveoli	28/86	16/50	13/50	29/50*		NE	NE	NE
Focal cholesterol clefts	6/86	4/50	5/50	9/50*		NE	NE	NE
Focal accumulations of secretory material in alveoli		NE	NE	NE	0/86	2/50	2/50	4/50*
Cardiovascular syster								
Dilated flaccid ventricle	28/86	15/50	7/50	9/50	0/86	1/50	2/50	3/50*
Periarteritis	20/86	12/50	8/50	8/50		NE	NE	NE
Left atrial thrombosis	12/86	6/50	4/50	3/50		NE	NE	NE

NE = No treatment-related effect
**Selected from the results of examination of all organ systems to depict the effects considered to be related to treatment The tumour incidence in all the organ systems was considered to be unaffected by any level of treatment.
Values marked with an asterisk differ significantly (by Fisher's Exact Probability Test) from the corresponding control incidence: *P 0.05.

Source: Kociba, et al. 1979

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4736

Table 14. Summary of Literature Involving Toxic Effects of TCDD (dioxin) in Animals

Animal	Single Dose	Length of Study	Effects	Author
Mouse C57Bl/6JFh (J67) strain specific pathogen gastric tube	0.5 ug/kg/wk	4 weeks	no effect on wt gain	Thompson, Faith, McConnell, More 1975
	1.5 ug/kg	4 weeks	no effect on wt gain	
	10.0 ug/kg	4 weeks	no effect on wt gain	
	20 ug/kg	4 weeks	significant decrease in wt gain	
	0.5, 1, 5, 10, 20 ug/kg	4 weeks	no effect on mice challenged with Herpesvirus suis	
	0.5 ug/kg	4 weeks	no effect on mice challenged with Salmonella bern	
	1.0 ug/kg	4 weeks	significant increase in mortality of mice challenged with Salmonella bern	
Mouse CD 1 oral	1, 10, 50	single dose	hematological changes at one wk after dose, normal at 3 weeks	Zinkl, Vos, Moore 1974
Mouse C57Bl/6 strain	25 ug/kg/wk	4 weeks	2000 fold increase in carboxy-porphyrins in the liver	Goldstein, Vos, Hickman, Bergman 1973
Rabbit	1-8 ug on inner surface of ear topical route	single	epithelial thickening, follicular prominance	Dow Chemical
Rabbit	0.4 to 400 ug/kg 5 days/wk applied to inside of ear	4 weeks	acne with increasing severity as dosages increased	Schultz, Norris, Sparsches 1973

Clinical Chemistry - Analyses of serum samples for BUN, SGPT, SAP, bilirubin, total protein, albumin, and glabulin showed no alterations related to 2,4,5-T treatment after either 118 days or two years for any dose level in either sex.

This study concludes that 2,4,5-T given at a 3 mg/kg chronic dose per day signifies a no observable effect dose level for Sprague-Dawley rats. But mention must be given to the discrepancies of TCDD values in this study and commercially available 2,4,5-T.

The herbicide used in this study was "purified." It contained no TCDD limit of detection 0.33 ppb (or 0.00033 ppm + 0.33 g/kg). Commercial samples of that same herbicide routinely contain 0.1 ppm TCDD.

Such alterations in chemical composition must be considered when analyzing true chronic effects of 2,4,5-T (usually from a commercial source).

Toxic effects of TCDD vary from species to species with dosage but invoke a similar response within animal groups. Numerous reports have described TCDD induced lesions in animals at different dose regimens for different time spans. (Tables 14 and 15).

McConnell and Moore 1979

McConnell and Moore reviewed the toxicopathology characteristics of TCDD-like compounds in a 1979 New York Academy of Sciences publication. Generally they observed a commonality of lesions in: 1) the thymus, and to a lesser extent, the lymphoid tissues; 2) bone marrow and hematological dyscrasias arising from it; and 3) monkeys which exhibited cutaneous and gastrointestinal lesions; rabbits displayed some cutaneous abnormalities but fewer than monkeys. In most cases, the degree of tissue destruction is insufficient to determine the cause of death. However, lesions of the immune system may result in secondary disease syndromes, particularly in long term, low level exposures. Animal manifestations ultimately resemble those of a starvation-like syndrome.

The differences between acute high-level and low-level chronic exposures are mainly quantitative. McConnell and Moore detail the process of TCDD intoxication using the monkey as an example. The first indication of toxicity is apparent weight loss. In lethal doses the weight loss is progressive, totalling almost one third of the original weight in monkeys. A cutaneous syndrome, manifested by alopecia and edema, is another characteristic feature of TCDD intoxication. The face and eyelids are the target areas here.

As the toxic response progresses, chloracne develops in the hair follicles of the face and lips. Classically, TCDD poisoning causes chloracne which technically is designated as a comedone formation, with metaplasia of the sebaceous epithelium to the squamous type, often accompanied by thickening of the superficial layers of the skin. A waxy build-up fills the external auditory canal due to lesions in the ceruminous glands. The skin on the remainder of the body, especially on the extremities, becomes dry and scaly. The finger and toenails often fall off with no accompanying pain. In terminal states of the toxicity related illness, the monkey becomes progressively weaker, but this does not interfere with its response to external stimuli.

Overt signs of TCDD intoxication cannot be found as easily in rodents. In the rodent family only rabbits exhibit the acne-like eruptions, which increase in severity in a dose related manner. Other than weight loss, very few clinical signs appear in rats and guinea pigs. These animals usually experience an immediate, dose related and progressive weight loss. Directly preceding death, mice and chicks may exhibit abdominal distension from ascites (fluid in the abdominal cavity)

There seems to be a time threshold for death to be caused by TCDD. Death from an LD^{50} dose of TCDD usually occurs within weeks if it is to occur at all. Administration of superlethal doses does not appreciably hasten the onset of death. Females and neonates tend to be more susceptible to the effects of TCDD than adult males.

While lesions differ, investigators consistently find thymic atrophy and weight loss in all species. At lethal doses thymic involution occurs and the thymus is reduced to almost one fourth its normal size. This reduction occurs by necrosis of cortical lymphocytes, demonstrated by macrophages phagocytizing necrotic debris. After thymic involution, necrotic action is much less conspicuous. Sub-lethal doses of TCDD do not change the microscopic appearance of the organ, but the relative weight may be significantly less than normal (McConnell and Moore 1979).

Reduction in the size of lymphoid tissues, such as in the spleen and gut, may also occur, but not to the extent as that seen in the thymus.

The cutaneous syndrome seen in the monkeys results primarily from changes of the sebaceous glands within the affected tissues. Secretions change from a normally oily consistency to a keratinous form. This can cause plugging and rupture of glands with subsequent inflammatory acne-like changes.

Chicken, mice, and monkeys all exhibit ascites. Hypoproteinemia or direct vascular damage are related to its presence in these animals. Ascites and hydropericardium changes represent such characteristic manifestations in fowl that they can be diagnostic for TCDD-like compounds in that species (McClure et al. 1962).

Monkeys feature proliferative gastrointestinal lesions as a result of exposure, especially chronic, to TCDD and its related compounds. Here, parietal cells give way to mucous secreting cells. Hyperplasia and subsequent hypoplasia of glandular epithelium accompany this alteration. Gastrointestinal lesions in other species are of less diagnostic importance, appearing only in the terminal stages of the disease. In this instance, hemorrhage, usually in the stomach and proximal small intestine, results from loss of integrity of the small vessels of the lamina.

Damage to the liver from TCDD exposure varies much among species. In mice, rabbits, and chicks, hepatic pathology is marked and conspicuous. In monkeys and guinea pigs, damage to the liver remains minimal. In all instances,

however, hepatomegaly (both percent body weight and absolute) consistently appears at exposures considerably below the lethal dose (McConnell and Moore 1979). Researchers attribute the increase in size and weight to hepatocellular enlargement, specifically the endoplasmic reticulum.

In rats and mice, hepatic lesions consist of focal necrosis of hepatocytes with inflammatory infiltrates, cytomegaly, and fatty change. In acutely toxic doses, rabbits characteristically exhibit hepatocellar necrosis with hemorrhage. Bile duct hyperplasia in varying degrees persists in chronic exposure to all animal species. Monkeys, in particular, demonstrate a striking enlargement of the common bile duct.

TCDD causes significant urinary tract lesions only in the guinea pig and monkey. Damage here involves hyperplasia of the transitional epithelium of the renal pelvis, ureter and urinary bladder, with the epithelial lining increasing as much as three times its normal thickness.

Testicular degeneration has occurred in the mouse, rat, guinea pig, and monkey. Seen as restricted degenerative changes in the seminiferous components, the damage here may ultimately result in the remainder of a single layer of germinal tissue. In most cases, though, this occurs near death and has not been demonstrated as a direct response to toxicity.

Hypocellularity of the bone marrow appears in all species although the degree of reduction differs among species. Particularly evident in moribund animals, this change can also take place in animals receiving a sublethal dose of TCDD.

Lesions of the adrenal glands seem to be restricted to animals that die or have been killed while in the moribund condition. Such changes range from adrenal hemorrhage, experienced by many species, to abcess and atrophy of the zona glomerulosa of the adrenal gland in the guinea pig.

Hematological changes resulting from TCDD toxicity not only vary among species, they also differ within species depending on whether the exposure is acute or chronic. Rats and monkeys show no blood alterations as a consequence of acute exposure, but they both show signs of pronounced anemia (especially monkeys) when given chronic doses of the chemical. Mice and chickens also exhibit anemia. The bone marrow depression cited earlier is the most likely cause of this blood dyscrasia.

Leukocytosis (increased white cell count) appears in

monkeys after acute exposure to TCDD but is replaced by leukopenia (low white cell count) in cases of chronic exposure. A decrease in the amount of circulating lymphocytes causes this lessening of the total white cell count in the monkey. Mice, rats, and guinea pigs also experience leukopenia. Rats, however, respond to the toxicity via leukocytosis, especially in long-term studies. By contrast, the leukocytosis in this case results from an increase in neutrophils, not lymphocytes. Thrombocytopenia (a reduction in platelets) is reported in both monkey and rat studies. Specifically, thrombocytopenia with accompanying hemorrhage has been incriminated as a cause of death in chronically exposed monkeys. (Other animal investigations have not released information on this parameter.)

In general, females seem to exhibit more hematologic dyscrasias than males. Their overall susceptibility to intoxication by these chemicals may correspond to this phenomenon, however (McConnell and Moore 1979).

Monkeys, mice, and chickens develop a reduction in total serum protein. Rats and guinea pigs do not. This decrease primarily occurs in the albumin fraction although other specific globulin fractions may also be altered.

Alterations in serum enzymes reflect the histopathologic damage to the various organs involved. Increased SGPT and SGOT values indicate liver lesions. Serum cholesterol levels are increased in rats but decreased in monkeys. Alternately, serum triglyceride levels increase in monkeys but remain normal in rats. Other species investigations have not studied these parameters. Lowered glucose levels are apparent at the terminal stages in all species. This represents generalized debilitation. Parameters of renal functioning such as BUN, creatinine, and serum electrolytes usually remain within normal limits.

Porphyrin levels (particularly in the liver) rise dramatically in mice and chickens, and moderately in rats, rabbits and monkeys but not in guinea pigs. This increase, at dose levels well below the lethal range, substantiates claims calling TCDD the most porphyrinogenic compound ever produced by man (McConnell and Moore 1979).

Two studies on the toxicity of TCDD are particularly noteworthy. They are a two-year study detailing the chronic exposure of TCDD in Sprague-Dawley rats by Kociba et al. and a nine-month study describing long-term toxic effects of TCDD in Rhesus monkeys by Allen and Van Miller.

Allen and Van Miller 1977 DOW2155176

Researchers Allen and Van Miller housed 16 adult Rhesus monkeys, each weighing 5.6 kg, in an environment simulating their natural habitat during mating season. For six months, investigators monitored general health and hematologic values and established control parameters for the study. At the end of this time, they divided the monkeys into two groups of eight animals each. One group was placed on a diet containing 500 ppt TCDD. The remaining group ate an identical diet without TCDD. Food intake was quantitated for each group over a nine-month period of time. After six months on the experimental diet, the females were mated with males from the control group. After nine months, the experimental animals ceased eating the TCDD contaminated diet and ingested a control diet for an additional three months. Researchers estimated total TCDD levels for these animals at 5.8 ± 0.9 ug after three months, 11.3 ± 1.7 ug after six months and 17.9 ± 2.0 ug following nine months on the diet.

After three months on the diet, monkeys developed periorbital edema, loss of facial hair and eyelashes, acne, accentuated hair follicles and dry and scaly skin. After six months, these changes became prominent in six of the eight animals concerned. At the six month analysis, monkeys also experienced a decreased hemaglobin and hematocrit. These lowered hematological parameters became especially exaggerated in six of the seven monkeys that survived nine months on the experimental treatment. Animals in the experimental group lost weight despite no change in feeding habits.

After seven months, one monkey became severely pancytopenic. Prior to death, the animal had virtually no platelets or immature red or white blood cells. In the following four months, four additional monkeys experienced similar clinical changes and died. Of the animals that survived the regimen, all displayed significant hair loss and periorbital edema. During the twelfth and final month of the study, one monkey developed a reduction in both platelets and white blood cells.

Autopsy findings revealed several areas of damage from TCDD exposure. Focal areas of hemorrhage appeared in the skin, heart, lung, stomach, intestine, adrenals, skeletal musculature, brain and bone marrow. Fingernails and toenails grew irregularly, often accompanied by gangrenous necrosis. The animals also suffered from ascites and subcutaneous edema. Hypertrophy, dilatation, edema, and hydropic degeneration of the myocardium occurred in all the animals. The biliary ducts showe

marked dilation, without associated jaundice or obstruction. Animals exhibited moderate hyperkeratosis of the skin and cystic keratosis of the hair follicles and sebaceous glands. Monkeys also demonstrated a marked decrease in the hematopoietic process in the bone marrow and hypocellular lymphoid tissue. Cellular hyperplasia appeared on many levels, especially in the mucous-secreting cells, epithelial lining of the biliary system and the transitional epithelium of the urinary tract and goblet cells of the palpebral conjunctivae. They experienced a concurrent hypoplasia of parietal cells in the gastric epithelium.

Allen and Van Miller conclude from their study that the primary target area for TCDD in non-human primates (monkeys) is the hematopoietic (blood producing) system. As exposure lengthens, cellular degeneration involving the lymphoid system, responsible for the formation of white blood cells (leukocytes) and bone marrow responsible for red blood cell (erythrocyte) formation, increases in severity. In addition, atrophy of the thymus, spleen, and lymph nodes also occurs. The authors further assert that there is considerable difference in the level of TCDD necessary to produce intoxication. While an LD^{50} range of 50-70 ug/kg body weight signifies death in 50 percent of test animals in an acute space of time, a considerably lower dose of 3 ug TCDD per kg body weight will also yield the same results when administered continuously over a nine-month period. Allen and Van Miller also demonstrate that the pathological changes caused by the two dose levels remained quite similar. Therefore, they believe their data indicate that exposure to extremely low concentrations of TCDD can produce widespread deleterious effects in primates. (Table 16).

Table 16. Hemograms and Body Weights of Rhesus Macaques Fed 500 ppt of TCDD for 9 Months

Animal Number	Initial Body Wt. (kg)	Terminal Experimental Values: White Blood Cells X10^3/mm^3	Platelets X10^3/mm^3	Hemoglobin g%	Hematocrit. %	Terminal Body Wt. (kg)
7[a]	6.19	2.3	23	4.0	12.0	5.86
9[a]	6.74	2.4	44	6.9	21.5	5.74
23[b]	6.62	3.8	28	12.6	40.0	5.03
31[b]	6.02	10.4	480	10.7	35.5	5.35
32[a]	5.00	4.1	50	6.0	19.5	4.22
38[a]	5.63	3.8	34	6.6	22.0	5.22
41[b]	5.73	8.0	340	11.5	44.5	5.44
49[a]	5.39	8.7	54	8.5	29.5	4.46
$\bar{X}$ pre-experiment (N = 8)		9.3 $\pm$ 1.2[c]	327 $\pm$ 57[c]	13.9 $\pm$ 0.5[c]	4/29 $\pm$ 1.8[c]	

[a]Values at necropsy.

[b]Values at 12 months on trial.

[c]$\pm$ 1 SD

Source: Van Miller and Allen 1977.

Kociba et al. 1978

Kociba et al. investigated the chronic effects of TCDD using Sprague-Dawley rats. Their study, a long term chronic toxicity and oncogenicity investigation, continued as an outgrowth of their earlier sub-chronic 13 week research describing the toxicological effects in the same species.

The investigators randomly placed male and female Sprague-Dawley rats (Spartan substrain), aged six to seven weeks, into suspended wire-bottom cages. Food and water were available to the animals ad libitum. Groups of 100 rats (50 males, 50 females) ingested a diet that contained 0.1, 0.01, or 0.001 ug of TCDD/kg/day for up to two years. The diet of the control group of 172 rats (86 males, 86 females) contained only the carrier and no TCDD.

The TCDD sample used in this study was provided by the Dow Chemical Company. After purification, the TCDD product had a purity exceeding 99 percent as determined by electron capture gas chromatography. One mg TCDD, dissolved in 40 ml acetone and mixed with 100 g of control feed formed the stock premix. A sufficient amount of stock premix was then mixed with control feed to form a working premix to be used in preparing the test diets. The test diets were prepared by diluting the working premix with control feed to provide dose levels of TCDD as required by body weight and food consumption determinations in order to maintain the designated dosages on a microgram per kilogram per day basis. The concentration of TCDD in the test diet was determined by gas chromatography-mass spectrometry after extraction and suitable clean-up.

All rats were palpated on a monthly basis. Investigators recorded any palpable masses. Body weights and food consumption of 20 male and 20 female rats at each treatment level were routinely recorded for each week of the first three months of the study and at monthly intervals thereafter. All remaining rats were weighed monthly throughout the study.

Eight rats per sex per group provided blood samples for hematological investigation at three, twelve, and twenty-three months of treatment. Studied parameters included: RBC, WBC, thrombocyte count, reticulocyte count, packed cell volume, and hemaglobin. Urine samples were collected from seven to eight rats per sex per group at these same time intervals to determine specific gravity, pH, sugar, protein, ketones, bilirubin, and occult blood. A consulting laboratory analyzed urinary excretion of creatinine, coproporphyrin, uroproporphyrin, and γ amino-levulinic acid in four to five rats per sex per group at months 3-4, 12, and 23.

Automated analysis determined serum levels of BUN, SGPT, bilirubin (both direct and indirect), cholesterol, and triglycerides in seven rats per sex per group at month 22. Similarly, it determined alkaline phosphatase (AP) activity, total protein, albumin and globulin levels in seven rats per sex per group at month 23.

At terminal necropsy, investigators collected serum samples from all survivors or a maximum of 10 rats per sex per group for determination of BUN, SGPT, AP, and total bilirubin. Additionally a consulting laboratory made determinations of gamma-glutamyl transferase activity (GGT).

All rats dying or culled during the course of study underwent gross pathologic investigation and representative portions of major organs and any lesion suggestive of a significant pathologic process or tumor formation were preserved in 10 percent formalin.

Terminal necropsy examination took place at the end of two years of treatment (105th week). The weights of the liver, kidney, brain, heart, thymus, spleen, testes, and ovaries/uterus were recorded for a maximum of 10 rats per sex per group. Researchers also prepared bone marrow smears from most rats. Furthermore they froze portions of fat, liver, and kidney from a maximum of five rats per sex per group for possible TCDD analysis. Subsequent analysis of liver and fat samples from three females/dose level took place using gas chromatography--low resolution mass spectrometry. Additionally, the Kociba group preserved in formalin fixative portions of the esophagus, salivary glands, stomach, small intestine, large intestine, pancreas, liver, kidneys, urinary bladder, prostate, accessory sex glands, epididymis, testes, ovaries, uterus, brain (cerebrum, cerebellum, and brain stem), pituitary gland, spinal cord, peripheral nerve (sciatic), trachea, lungs, spleen, thymus, lymph nodes, heart, aorta, skeletal muscle, mammary tissue (males), adrenal glands, thyroid, parathyroid, tongue

lower jaw, skull (including nasal turbinates and ear canal) and any gross lesion.

All rats from the control and the high dose level, regardless of whether they died during the course of the study or were killed upon its completion, underwent histologic investigation of an extensive list of tissues, intended to include a majority of those tissues listed above as those collected at the time of terminal necropsy. All rats from the two lower dose levels were subjected to histological examination of those selected tissues identified as possible target area organs in addition to gross lesions suggestive of tumor formation.

Statistical evaluation of gross pathology data collated for the entire study compared the data of each of the treatment groups with the data of the control group of that sex. Lower dose levels included the same evaluations as higher dose counterparts at target organ areas (Table 17).

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Table 17. Major Histopathologic Findings in Rats Attributed to Ingestion of Diets Containing TCDD for Two Years

Dose Level of TCDD (ug/kg/day)		
0.1	0.01	0.001
Liver		
Hepatic degenerative inflamatory, necrotic and proliferative changes	Livers had much lesser degree of change	No changes. Livers of females had statistical increase in foci or area of hepatocellular alteration (swollen hepatocytes), and livers of males had statistical decrease in area of hepatocellular alteration (swollen hepatocytes)
Cytomegaly, distortion of lobular pattern and atropy of cords, cytoplasmic vacuolation, fatty change, altered tinctorial properties, hepatic necrosis and inflammation, multinucleated hepatocytes, foci or areas of hepatocellular alteration (swollen hepatocytes), pigment aggregates, bile duct hyperplasia, periportal inflammation fibrosis		
Hepatocellular carcinoma (Fe)		
Hepatocellular hyperplastic (neoplastic) nodules (Fe)	Hepatocellular nodules (Fe)	---
Reproductive		
Decreased incidence of uterine hyperplasia, cyst formation and polyp formation (Fe)	Questionable decreased incidence of uterine polyps (Fe)	---
Mammary Tissue		
Decreased incidence of mammary tumors	---	---
Endocrine		
Pituitary-decreased incidence of pituitary hemangiectasis and adenoma formation (Fe)	---	---
Adrenal-decreased incidence of medullary hyperplasia (M,Fe) and pheochromocytoma (M)	---	---
Increased incidence of cortical necrosis and hemorrhage (Fe), cortical adenoma and hematocyst (M)	---	---

The conclusions found in this study are an extension of a shorter duration thirteen-week investigation performed by Kociba et al. This research also studied the carcinogenicity of TCDD. The results pertaining to that aspect of TCDD response will be dealt with in the Carcinogenicity section of this report.

In this chronic two-year study determining the effect of TCDD on Sprague Dawley rats, Kociba et al. found that: continuous ingestion of diets containing approximately 2200 ppt TCDD (0.1 ug/kg/day) for two years caused multiple toxic effects, including increased mortality, decreased body weight gain, slight depression of certain hematological parameters, increased urinary excretion of porphyrins and γ - amino levulinic acid (γ ALA), increased serum activities of AP, GGT, and SGPT, and morphological changes primarily of the hepatic, lymphoid, respiratory and vascular tissues of the body. This high dose level seemed to reduce the incidence and severity of chronic renal disease in aged male rats. Female rats given this high dose for two years had 24,000 ppt TCDD present in the liver. This compares with 34,600 ppt TCDD present in the liver of female rats given this dose for thirteen weeks, thus indicating attainment of steady state concentration in the early phase of this two-year study.

Ingestion of the intermediate dose level of 0.01 ug TCDD/kg/day (approximately 210 ppt in diet) caused a lesser degree of toxicity. Here, researchers found: 1) increased urinary excretion of porphyrins (females); 2) liver toxicity, including an increased incidence of hepatocellular nodules; and 3) increased incidence of focal alveolar hyperplasia in the lungs. Terminal liver and fat content of TCDD averaged 5100, and 1700 ppt, respectively. This compares with 3700 ppt present in the liver after thirteen weeks at this dose level.

Lifetime ingestion at a dose of 0.001 ug/kg/day (approximately 22 ppt in the diet) caused no effects considered to be of toxicological significance. The liver and fat each contained 540 ppt of TCDD at the end of lifetime ingestion at this low dose level.

To summarize their results, Kociba et al. demonstrated a dose related toxic response to TCDD. At the lowest levels of TCDD tested, 0.001 ug/kg/day, the authors could find no toxic response attributable to the inclusion of TCDD in daily diet given to rats over a two year period of time.

Toxicity Summary

Since TCDD inevitably contaminates 2,4,5-T, no consideration of toxicity of the herbicide can be complete without proper attention to the toxicity of the accompanying dioxin.

Toxic response to both 2,4,5-T and TCDD vary among and, in some cases, within species. The LD^{50} values for 2,4,5-T range from a single 100 mg/kg dose in the dog (acid form) to 500 in the rat (acid form). In the mixed butyl ester form of 2,4,5-T, the mouse exhibits an LD^{50} of 940 mg/kg, single dose.

For TCDD, the LD^{50} for the guinea pig is 0.6 ug/kg. The mouse has a 120 ug/kg LD^{50}. Dogs, however, show no effect at doses as high as 100 ug/kg. LD^{100} for that species is 3000 ug/kg.

Initial toxic response to 2,4,5-T appears at dosages of 10 mg/kg in the dog and escalates to 140 mg/kg in the mouse. Weight loss, muscle weakness, and accompanying tissue changes represent the most common effects of 2,4,5-T toxicity.

In a chronic two-year study detailing the effects of 2,4,5-T on Sprague-Dawley rats, Kociba et al. (1979) ascertained a level (3 mg/kg) where the animals exhibited no toxic effects from chronic exposure to 2,4,5-T. However, the herbicide used in this project was "purified" 2,4,5-T, containing 0.3 ppb TCDD. Commercial samples of the chemical routinely contain 0.1 ppm TCDD. This must be considered when analyzing results from these data and extrapolating from them to populations exposed to the commercial form.

The thymus and, to a lesser degree, the lymphoid tissue and bone marrow (including hematological dyscrasias derived from damage there) are the primary areas which exhibit a toxic response to TCDD. Mice are least sensitive to TCDD's toxic effects. Guinea pigs and chicks suffer the greatest adverse effects. Initial toxic response appears after single doses of 3 ug/kg in the guinea pig and up to a dose of 100 ug/kg in the rat.

Studies of chronic TCDD toxicity include a nine-month exposure of Rhesus monkeys to 500 ppt TCDD in the diet reported by Allen and Van Miller (1977). The authors detailed toxic changes leading to death in five of the eight monkeys studied after what they determined to be

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Table 17. Major Histopathologic Findings in Rats Attributed to Ingestion of Diets Containing TCDD for Two Years (continued)

Dose Level of TCDD (ug/kg/day)		
0.1	0.01	0.001
Pancreas-decreased incidence of acinar adenoma (M)	---	---
Thyroid-occurrence of various isolated follicular changes of questionable significance (M)	---	---
Parathyroid-decreased incidence of parathyroid hyperplasia, secondary to chronic renal disease (M)	---	---
Lymphoid		
Isolated cases of thymic/splenic atrophy (Fe)	---	---
Respiratory		
Focal alveolar hyperplasia, pigment aggregates, accumulation of alveolar macrophages and cholesterol clefts, pulmonary edema, focal interstitial inflammation and fibrosis	Focal alveolar hyperplasia (Fe)	---
Keratinizing squamous metaplasia (Fe)	---	---
Keratinizing squamous cell carcinoma (Fe)	---	---
Cardiovascular		
Isolated cases of hemorrhage in CNA (Fe)	---	---
Increased incidence of mesenteric and thoracic periarteritis, with cases of thrombosis or hematoma formation (M,Fe)	Increased incidence of periarteritis (M)	---
Increased incidence of myocardial degenerative changes (Fe)	---	---

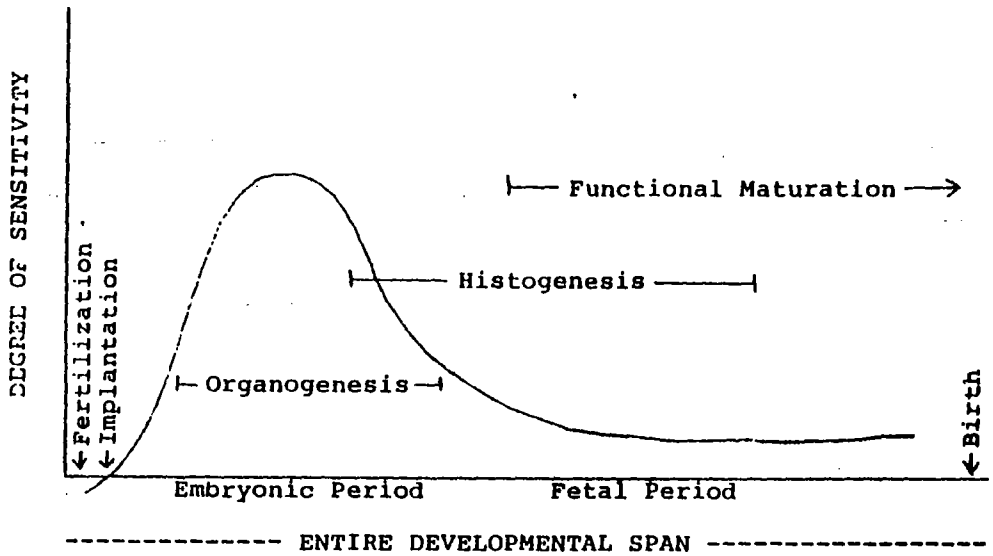
*(-) indicates observation was not noted at that dose level, or incidence was comparable to control data
Tabulation excludes voluminous data considered unrelated to treatment with TCDD

Source: Kociba, et al. 1978.

Up to day 17 after conception in humans there occurs a "refractory period." From day 18 to day 30 the embryo becomes very susceptible to outside interference. At this time, the early stages of tissue and organ differentiation take place (from gastrulation to organ differentiation). Thereafter, the embryo shows an increasing resistance to teratogenic forces as organogenesis advances to completion in days 55 to 60 in man.

In all species, the period of organogenesis remains most fragile to outside teratogenic influences. Upon completion of organogenesis, outside influences can generate an adverse change, but these are usually in the form of growth or functional alterations.

Figure 3. Curve Approximating the Susceptibility of the Human Embryo to Teratogenesis from Fertilization throughout Intrauterine Development



The highest sensitivity, at least to structural deviation, occurs during the period of organogenesis, from about days 18 to 20 until about days 55 to 60, although the absolute peak of sensitivity may be reached before day 30 postconception. As organogenesis is completed, susceptibility to anatomical defects diminishes greatly, but probably minor structural deviation is possible until histogenesis is completed late in the fetal period. Deviations during the fetal period are more likely to involve growth or functional aspects because these are the predominant developmental features at this time (Wilson 1973).

DOW2155184

Wilson (1973) lists six generalizations of teratologic susceptibility:

1) Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which this interacts with adverse environmental factors.
2) Susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence.
3) Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis).
4) The access of adverse influences to developing tissues depends on the nature of the influence (agent).
5) The four manifestations of abnormal development ar death, malformation, growth retardation, and functional deficit.
6) Manifestations of abnormal development increase in frequency and degree as dosage increases, from the no-effect level to the totally lethal level.

Almost any chemical substance possesses a teratogenic potential when administered in excess amounts during a susceptible period of development. A variety of exampl describe a broad spectrum of compounds responsible for myriad of biological and chemical effects.

Table 18. Some Types of Drugs and Other Chemicals that Have Been Shown to be Teratogenic* in One or More Species of Laboratory Animal

Drug Type	Examples
Salicylates	Aspirin, oil of wintergreen
Certain alkaloids	Caffeine, nicotine, colchicine
Tranquilizers	Meprobamate, chlorpromazine, reserpine
Antihistamines	Buclizine, meclizine, cyclizine
Antibiotics	Chloramphenicol, streptonigrin, penicillin
Hypoglycemics	Carbutamide, tolbutamide, hypoglycins
Steroid hormones	Triamcinolone, cortisone, testosterone
Alkylating agents	Busulfan, chlorambucil, cyclophosphamide, TEM
Antimalarials	Chloroquine, quinacrine, pyrimethamine
Anesthetics	Halothane, urethane, nitrous oxide, pentobarbital
Antimetabolites	Folic acid, purine, and pyrimidine analogs
Solvents	Benzene, dimethylsulfoxide, propylene glycol
Pesticides	2,4,5-T, carbaryl, captan, folpet
Industrial effluents	Some compounds of Hg, Pb, As, Li, Cd
Miscellaneous	Trypan blue, tryparanol, diamox, et.

*Teratogenic effects were usually seen only at doses well above therapeutic levels for the drugs, or above likely exposure levels for the environmental chemicals

Source: Wilson, J.G. 1977

There are certain criteria used for recognizing a new teratogenic agent in man. Wilson (1973), in his book, Environment and Birth Defects, lists the necessary criteria:

1) An abrupt increase in the incidence of a particular defect or association of defects (syndrome).
2) Coincidence of this increase with a known environmental change, i.e., widespread use of a new drug.
3) Known exposure to the environmental change early in pregnancies yielding characteristically defective infants.
4) Absence of other factors common to all pregnancies yielding infants with the characteristic defect(s).

Since chemical response varies with dosage, route of administration, and particular agent, Wilson(1977) has established criteria and categories for classifying chemicals according to their embryotoxic (causing damage in early pregnancy) potential* in man:

1) Established as being embryotoxic
   a) Unquestionably produces a higher percentage of developmental defects or intrauterine death in infants of exposed women than otherwise similar, nonexposed women
2) Suspected of being embryotoxic
   a) Apparent increase above background level of developmental defects or intrauterine death among infants of exposed women, based on large numbers of accumulated case reports
   b) Retrospective surveys indicate significant increase of defects or death among infants of exposed women, but "memory bias" of mothers introduces uncertainty
   c) Prospective surveys indicate significant increase among infants of exposed women, but total number of exposed cases relatively small in consecutive series
3) Possibly embryotoxic, under usual conditions of dosage or in combination with other unidentified etiologic factors
   a) Questionable increase of defects or death among infants of exposed woman based on large number of accumulated cases or on extensive epidemiologic surveys
   b) Strong indication from studies in more than one animal species that drug has definite embryotoxic potential, especially at high dosage

*Assuming exposure to therapeutic or higher dose during 1st trimester.

4) Not embryotoxic under any known conditions of human usage
   a) No increase in defects or death among infants born to exposed women in well-controlled epidemiologic surveys

   b) Extensively used by pregnant women over many years without substantiation of cause effect relationship between use of the drug and adverse effects on the offspring

Whatever the chemical of effect, however, experimental teratologists generally assume that all embryotoxic agents do have a threshold below which no adverse response can be elicited--a so-called, No-Effect level.

From a No-Effect level, responses multiply as dosages increase. At teratogenic doses, increased malformations and embryolethality appear simultaneously. Beyond that point, these manifestations tend to increase in roughly parallel fashion for a time, although embryolethality eventually overtakes malformations and precludes any survivors at term.

For the initiation of teratogenesis, as with other adverse chemical effects, the primary concern is one of exposure. In man, this translates as the capability of a parental organism to reduce concentration levels of foreign chemicals in the blood. This is accomplished in parental blood by excretion; catabolism and other detoxication in the liver; protein and tissue binding and storage. The rate at which these physical processes occur is all important.

Numerous studies have been conducted detailing the teratogenic, embryotoxic and fetotoxic effects generated by 2,4,5-T and its contaminant dioxin. Researchers experimented with a variety of species and have demonstrated both No-Effect and lethal levels in many cases. The prime target areas for teratogenic response from 2,4,5-T and dioxin are cleft palate and kidney anomalies (Tables 19 and 20).

Table 19. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity of 2,4,5-T

Animal Form and TCDD Content	Dose and Route of Administration	(Gestational Age) Duration	Effects	Author
Mouse acid 1 mg/kg TCDD	20 mg/kg oral daily	days 6-15	increased frequency of cleft palate, fetal growth retardation	Bionetics 1968
Mouse acid 30 mg/kg TCDD	113 mg/kg daily oral or s.c.	days 6-14	increased mortality, cystic kidney, strain variations	Courtney, et al. 1970
Mouse acid 30 mg/kg TCDD	50 mg/kg s.c. daily	days 6-15	no effect	Courtney, et al. 1970
Mouse acid 30 mg/kg TCDD	100,125,150 mg/kg daily s.c.	days 6-15	embryotoxic effect	Courtney, et al.
Mouse acid 0.02 mg/kg TCDD	20 mg/kg daily, oral	days 6-15	no effect	Roll 1971
Mouse acid 0.05 mg/kg TCDD	35 mg/kg daily, oral	days 6-15	cleft palate	Roll 1971
Mouse acid 0.05 mg/kg TCDD	60,90,130 mg/kg daily oral	days 6-15	marked increasing resorptions and 5 dead fetuses	Roll 1971
Mouse acid ester 0.02 mg/kg TCDD	10-15 mg/kg oral, daily	days 6-15	fetal wt reduction	Neubert, Dillman 1972
Mouse acid 0.02 mg/kg TCDD	45,60,90,120 mg/kg daily oral	days 6-15	embryotoxic effect	Neubert, Dillman 1972
Mouse acid 0.02 mg/kg TCDD	150-300 mg/kg single dose	mid gestation	cleft palate, maximum teratogenic effect day 12-13 gestation	Neubert, Dillman 1972
Mouse acid 1.5 mg/kg TCDD	30-60 mg/kg daily, oral	days 6-15	cleft palate	Neubert, Dillman 1972
Mouse acid 0.5 or 0.05 mg/kg	100 mg/kg s.c. daily in DMSO solution 100ml/animal injection	days 6-15	cleft palate, kidney malformations some strains	Neubert, Dillman 1972
Mouse butyl ester 0.02 mg/kg	12 mg/kg daily, oral	days 6-15	no effect	Neubert, Dillman 1972
Mouse butyl ester 0.02 mg/kg	74 mg/kg daily oral	days 6-15	embryotoxic effect	Neubert, Dillman 1972
Mouse acid 1 mg/kg TCDD	50 mg/kg s.c., daily	days 6-14	no effect	Bage, et al. 1973
Mouse acid 1 mg/kg	110 mg/kg s.c., daily	days 6-14	teratogenic, cleft palate, rib and vertebrae anomalies, fetotoxic	Bage, et al. 1973

Table 19. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity of 2,4,5-T (continued)

Animal Form and TCDD Content	Dose and Route of Administration	(Gestational Age) Duration	Effects	Author
Mouse butoxyethyl ester	50,110 mg/kg s.c., daily	days 6-14	embryotoxic effect	Bage, et al. 1973
Mouse acid	10 mg/kg s.c., daily	days 6-15	no effect	Hart 1972
Mouse acid	100 mg/kg s.c., daily	days 6-15	embryotoxic effect	Hart 1972
Mouse acid 0.05 mg/kg TCDD	gavage 60-120 mg/kg daily	days 6-14	retardation in renal A.P. in fetal kidney, reduced fetal wt, cleft palate	Highman, et al. 1976
Mouse acid 0.1 mg/kg TCDD	20,40 mg/kg oral, daily	days 6-15	no effect	Frohberg, et al. 1975
Mouse acid 0.1 mg/kg TCDD	80-120 mg/kg oral, daily	days 6-15	toxic to females, malformations and fetal death	Frohberg, et al. 1975
Mouse acid 0.05 mg/kg TCDD	120 mg/kg oral, daily	days 6-15	teratogenic, fetotoxic (80mg/kg no effect)	Frohberg, et al. 1975
Mouse butoxyethyl ester	20,40,80 mg/kg daily oral	days 6-15	no effect	Frohberg, et al. 1975
Rat acid 0.5 mg/kg TCDD	1-24 mg/kg daily, oral	throughout pregnancy	no effect	Emerson, et al. 1971
Rat acid 1 mg/kg TCDD	24 mg/kg daily, gavage	during pregnancy	no effect	Emerson, et al. 1971
Rat acid "purified"	50-125 mg/kg single injection in utero	single injection on any day from days 12-16	no effect	King, et al. 1971
Rat acid	60-120 mg/kg daily, oral	days 12-16	no effect	King, et al. 1971
Rat acid TCDD not stated	20 mg/kg in diet	throughout gestation	no effect	Hall 1972
Rat acid TCDD not stated	80 mg/kg in diet	throughout gestation	embryotoxic effect	Hall 1972
Hamster acid free of TCDD	20,40,80 mg/kg oral, daily	days 6-10	no effect	Collins, et al. 1971
Hamster acid free of TCDD	100 mg/kg oral, daily	days 6-10	embryotoxic effect	Collins, et al. 1971

1359

Table 19. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity of 2,4,5-T (continued)

Animal Form and TCDD Content	Dose and Route of Administration	(Gestational Age) Duration	Effects	Author
Hamster aicd with oil 0.5,2.9 or 45 mg/kg TCDD	20-100 mg/kg oral, daily	days 6-10	fetal death, teratogenic	Collins, et al. 1971
Hamster acid TCDD not stated	2 mg/kg intravenous single	day 8	no malformed embryos, 9% resorption-test, 6% resorption-control	Gate, et al. 1973
Rabbit acid 0.5 mg/kg TCDD	10-40 mg/kg oral daily	days 6-18	no effect	Emerson, et al. 1971
Monkey acid 0.5 mg/kg TCDD	0.05,1.0,10 mg/kg daily gavage	days 22-38	no effect	Dougherty, et al. 1973
Mouse butoxyethyl ester 0.1 mg/kg TCDD	120 mg/kg daily, oral 216 mg/m^3 aerosol, inhalation	10 exposures	slight maternal toxicity, fetotoxic, teratogenic	Frohberg, et al., 1975
Mouse butyl ester 0.1 mg/kg	aerosol, inhalation, 374 mg/m^3	10 exposures	5-15 females died	Frohberg, et al. 1975
Mouse butoxyethyl ester 0.1 mg/kg TCDD	392 mg/m^3 aerosol inhalation	5 exposures	toxic to females, fetal deaths	Frohberg, et al. 1975
Rat acid 0.5 mg/kg TCDD	50 mg/kg oral daily	days 6-15	minimal fetal effects	Sparschu, et al. 1971
Rat acid 0.5 mg/kg TCDD	100 mg/kg oral, daily	days 6-10	toxic to females, high maternal death, 4 to 25 survived, 3 had complete resorptions, 1 had litter of 13 live fetuses, toxic but no anomalies	Sparschu, et al
Rat acid 0.5 mg/kg TCDD	100-150 mg/kg oral, daily	days 6-15	fetopathy and skeletal anomalies	Khera, et al. 1972
Rat acid 0.4 mg/kg TCDD	25,50 mg/kg oral, daily	days 6-15	no effect	Khera, et al. 1972
Rat butyl ester 0.5 mg/kg TCDD	50-150 mg/kg oral daily	days 6-15	no effect at 50 and 100 mg/kg, at 150 mg/kg, 3 of 8 females died	Khera, et al. 1972
Rat acid 30 mg/kg TCDD Sprague-Dawley strain	4.6,10,46.4 mg/kg oral,	days 10-15	kidney anomalies, embryotoxic, at 46.4 mg/kg, 60% fetal mortality, many abnormalities in survivors	Courtney, et al. 1970
Rat acid, CD strain	10,22,46 mg/kg daily s.c.	days 6-15	no effect	Courtney, et al. 1[illegible]

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17681

Table 19. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity of 2,4,5-T (continued)

Animal Form and TCDD Content	Dose and Route of Administration	(Gestational Age) Duration	Effects	Author
Rat acid, CD strain	80,250 mg/kg daily s.c.	days 6-15	embryotoxic effect	Courtney, et al. 1971
Rat acid purity not stated	100 mg/kg daily, oral	days 1-14	many deformities	Sokolik, Yu 1973
Rat acid purity not stated	400 mg/kg daily, oral	days 1-14	many limb abnormalities	Sokolik, Yu 1973
Rat butyl ester purity not stated	50 mg/kg daily, oral	days 1-16	embryo mortality, cleft palate	Sokolik, Yu 1973
Rat butyl ester purity not stated	200 mg/kg daily, oral	days 1-16	30% embryo mortality and many anomalies	Sokolik, Yu 1973
Rat butyl ester purity not stated	0.01 mg/kg daily, oral	throughout pregnancy	no effect	Konstantinova 1970 Russian
Rat butyl ester purity not stated	0.1 mg/kg daily, oral	throughout pregnancy	threshold level	Konstantinova 1970 Russian
Rat butyl ester purity no stated	0.42 mg/kg daily, oral	throughout pregnancy	toxic to female, irregular embryotoxic effect	Konstantinova 1970 Russian
Rat butyl ester purity not stated	4.2 mg/kg daily, oral	throughout pregnancy	toxic to females, nervous signs embryo deaths	Konstantinova 1970 Russian
Rat acid 0.1 mg/kg TCDD and free of TCDD	25-150 mg/kg daily, oral	throughout pregnancy	no effect	Roll 1973
Monkey acid 0.5 mg/kg TCDD	5,10,20,40 mg/kg, 3 oral doses weekly, 4 weeks	days 20-48	no effect on 12 fetuses removed by hysterectomy 100 days gestation	Advisory Comm. 1971
Monkey acid TCDD content not stated	5,10,20,40 mg/kg 3 times/ week	days 20-48	no effect	Wilson 1971
Sheep acid TCDD content not stated	100 mg/kg daily, oral	days 14-36	no effect	Binns, Balls 1971

Table 20. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity Effects of TCDD (continued)

Animal	Dose and Route of Administration	Duration (Gestational Age)	Effects	Author
Mouse	3 ug/kg daily, s.c.	days 6-15	varied by strain: CD-1: E.D. 50 for cleft palate, DBA/2J: E.D. 50 for cleft palate, E.D. 50 for kidney anomaly, C57Bl/6J: E.D. 50 for cleft palate	Courtney, Moore 1971
Mouse	25,50,100,200,400 ug/kg daily, oral	days 7-16	fetotoxic, teratogenic, increasing with dosage, up to 97% at highest dose	Courtney 1976
Mouse	25,50,100,200 ug/kg daily, s.c.	days 7-16	fetotoxic, teratogenic 97% at highest dose	Courtney 1976
Mouse	0.001,0.01,0.1 ug/kg daily	days 6-15	no effect	Smith, Schwetz, Nitschke 1976
Mouse	1.0 ug/kg daily oral, gavage	days 6-15	increased fetal resorption, 21% cleft palate, 5% kidney anomaly	Smith, Schwetz, Nitschke 1976
Mouse	3 ug/kg daily oral, gavage	days 6-15	71% cleft palate, 28% kidney anomaly	Smith, Schwetz, Nitschke 1976
Mouse	1 ug/kg single, oral	day 10	no effect, cleft palate, 34% kidney anomaly	Moore, et al. 1973
Mouse	1 ug/kg daily, oral	days 10-13	1.9% cleft palate, 58.9% kidney anomaly	Moore, et al. 1973
Mouse	3 ug/kg daily, oral	days 10-13	55.4% cleft palate, 95.1% kidney anomaly	Moore, et al. 1973
Mouse	1,3,10 ug/kg females, oral at parturition	parturition	renal hydronephrosis, 12,71 or 75% depending on dose	Moore, et al. 1973
Rat	0.03 ug/kg	days 6-15	no effect	Sparschu, et al. 1971
Rat	0.125,2 ug/kg daily, oral	days 6-15	depressed fetal wt	Sparschu, et al. 1971
Rat	0.125,0.5,2 ug/kg daily, oral	days 6-15	internal hemorrhages in fetuses	Sparschu, et al. 1971
Rat	8 ug/kg daily, oral	days 6-15	fetal death all	Sparschu, et al. 1971
Rat	0.125 ug/kg daily, oral	days 6-15	no effect	Khera, Rudick 1973
Rat	0.25 ug/kg daily, oral	days 6-15	slight decrease in fetal wt	Khera, Rudick 1973

DOW2155192

Table 20. Summary of Literature Involving the Teratogenicity, Embryotoxicity, and Fetotoxicity Effects of TCDD (continued)

Animal	Dose and Route of Administration	Duration (Gestational Age)	Effects	Author
Rat	0.5,1.0 ug/kg daily, oral	days 6-15	reduced fetal survival, lower body wt, lowered reproductive ability in progeny	Khera, Rudick 1973
Rat	1,2 ug/kg daily, oral	days 6-15	visceral lesions, reduced fetal wt, increased fetal death, maternal toxicity	Khera, Rudick 1973
Rat	4 ug/kg daily, oral	days 6-15	100% fetal death	Khera, Rudick 1973
Rat	0.5 ug/kg daily, s.c.	days 6-15	no effect on fetal mortality or cleft palate, 67% kidney anomaly	Courtney, Moore 1971
Monkey	0.011 ug/kg (estimated daily in diet)	months 6-12	spontaneous abortion, severe maternal toxicity	Allen, et al. 1977
Monkey	0.0017 ug/kg (estimated daily intake)	years 2	4 of 7 aborted, none of controls (zero level TCDD) aborted, one stillborn, 2 normal deliveries maternal toxicity	Schantz, et al. 1979
Monkey	0.0095 ug/kg (estimated dietary equivalent) three times/wk during pregnancy	early pregnancy	1/4 aborted, no maternal toxicity	McNulty 1978
Monkey	0.048 ug/kg (est. dietary equiv.)	early pregnancy	3/4 aborted, 1 showed maternal toxicity	McNulty 1978
Monkey	0.24 ug/kg (est. dietary equiv.)	early pregnancy	2/2 aborted, maternal toxicity proved fatal	McNulty 1978

Teratogenicity Summary

Like many chemicals that have become part of our everyday lives, 2,4,5-T and its contaminant TCDD have produced teratogenic responses in various animal species.

Establishment of a response and its severity depend on dose of the chemical, route of administration, species, and in some cases, sub-strains of species, and finally, period of ingestion during gestation.

Teratologists generally agree that a level exists below which no teratogenic or embryotoxic response can be elicited. Research of 2,4,5-T and TCDD has supported this rationale in many animal species by establishing No-Effect dose levels in test animals.

Embryonic interference by 2,4,5-T and TCDD commonly manifests itself through cleft palate and kidney anomalies at teratogenic doses and embryolethality at higher toxic doses of chemical.

MUTAGENICITY

Analysis of mutagenic effects permits an assessment of the ability of a substance to induce genetic alterations. The resulting information aids in estimating the genetic hazard man, animals, and even plants encounter from exposure to the substance.

Because of the similarities of basic molecular mechanisms by which chemical mutagens and most chemical carcinogens appear to induce genetic effects (i.e., molecular laterations of DNA) researchers postulate that mutagenic effects can be predictive of carcinogenicity (IRLG 1979).

Scientists use a battery of short term tests to indicate the mutagenic potential a chemical possesses. These short term tests may include:

1) tests for mutation in bacteria and eukaryotic micro-organisms;
2) tests for mutations in somatic mammalian cells;
3) tests on chromosomal effects in higher eukaryotes, including mammals;
4) evaluation of DNA repair synthesis (IRLG 1979).

Among the mutagenicity tests on micro-organisms, the one most widely used is the Ames reversion test in Salmonella. E. coli, Saccharomyces, Neurospora, and Aspergillus are also vehicles for mutation induction. Mutagenicity tests also use Drosophila (IRLG 1979).

Tables 21 and 22 summarize the mutagenicity testing for 2,4,5-T and TCDD.

Table 21. Summary of Literature Involving Mutagenic Effects of 2,4,5-T

Species and Type of Test	Results	Reference
Microorganisms		
Salmonella typhimurium	Negative	Anderson, et al. 1972 Ercegovich and Rashid 1977
Saccharomyces cerevesiae	Negative	Siebert and Lamperle 1974 Fahrig 1974
Bacillus subtilis	Negative	Shirasu, et al. 1976
Senatia marcesrens and Escherichia coli	Negative	Fahrig 1974
Drosophila		
Sex-linked recessive lethals	Slight increase	Majamdur and Golia 1974
Sex-linked recessive lethals	No increase	Vogel and Chandler 1974
Somatic cell mutations	No increase	Rasmusen and Svahlin 1978
Mammalian Systems		
Mice, dominant lethal and Micronucleus	Negative	Buselmaier, et al. 1972 Jenssen and Renberg 1976
Cytogenetic effects -- gerbils	Inconclusive	Majamdur and Hall 1973
Cytogenetic effects -- mice	Inconclusive	Davring and Hultgren 1977
Human Data		
Cytogenetics -- workers	Negative	Kilian 1975

Table 22. Summary of Literature Involving the Mutagenic Effects of TCDD

Species and Type of Test	Results	Author
Microorganisms		
Escherichia coli	Increased mutation	Hussain, et al. 1972
Salmonella typhimurium TA-1530	Negative	Hussain, et al. 1972
Salmonella typhimurium TA-1532	Increased mutations	Hussain, et al. 1972
Salmonella typhimurium TA-1532	Increased mutations	Seiler 1972
Salmonella Tester strains	Negative	Communications of B. Ames to USDA 3/74
Mammalian Systems		
Dominant lethal, rats	Negative	Khera and Ruddick 1973
In vivo cytogenetics, rats	Negative	Green 1975 unpublished report
Human Data		
Seveso plant workers, cytogenetics	Inconclusive	Rohrborn 1978
Seveso, fetal material, cytogenetics	Inconclusive	Reggiani 1978

All of the 2,4,5-T mutagenicity studies have drawn either negative or inconclusive results in tests covering primary DNA damage, gene mutations, and chromosome effects. We agree with the Scientific Dispute Resolution Conference, therefore, that reasonably adequate experimental data has been generated regarding 2,4,5-T and that these data are not suggestive of 2,4,5-T induced mutagenic activity (Scientific Dispute Resolution Conference on 2,4,5-T 1979).

Results from several microbial studies have determined that TCDD increases the frequency of gene mutations. Microbial data suggest that DNA repair processes are involved in the mechanism of action since no mutagenic effects can be elicited in studies using repair-defective bacteria strains (Scientific Dispute Resolution Conference on 2,4,5-T 1979).

Mutagenicity Summary

Short term mutagenicity studies have been routinely performed to assess the potential of a substance to cause genetic alteration. The resulting information can then be used as an aid in establishing the risk of a chemical agent's carcinogenic activity.

All of the 2,4,5-T mutagenicity studies have shown either negative or inconclusive results in the various mutagenicity tests performed. In addition, the bacteria data suggest that TCDD acts as a direct initiator of malignant transformation since most mutagenic carcinogens are of the initiator class. Few promoters or co-carcinogens show mutagenic activity.

Mutagenesis studies performed on TCDD show that TCDD has the ability to cause cells to undergo genetic changes.

Human studies involving both 2,4,5-T and TCDD are insufficient and inconclusive.

CARCINOGENICITY

The question of the carcinogenic potential of 2,4,5-T and its associated dioxin (TCDD) has accompanied the herbicide since issuance of the initial RPAR in 1978. Indeed, its possible carcinogenicity, coupled with its potential teratogenicity are the primary points of conflict within the scientific community.

Animal model studies have long been used for predicting carcinogenic potential in man. The species of animals used in these studies must be as carefully considered as the data that ensues from the experiment. As a class, rodents meet certain specifications that make them highly desirable for carcinogenicity experiments. Their lifespan is short which allows the fullest expression of a positive carcinogenic response (these primarily occur late in an animal's life). Rodents are small, relatively inexpensive, and easily bred, all of which encourage large study samples and confirmation of test results by other investigators.

However, researchers may have problems of interpretation when using rodent species. Results obtained using rats or mice cannot be unequivocally adapted to man because of certain biological differences. Second, the Sprague-Dawley rat and several mouse strains noted for their high rate of spontaneous tumor incidence are often used in these experiments, so care must be taken to discriminate between spontaneous tumors and those caused by carcinogenic mechanisms.

Finally, a carcinogenic response can become apparent long after the original cancer causing stimulus has been removed. Manifestations of cellular alterations can take years to develop. This has been a recurring problem encountered with human epidemiologic studies resulting in a Catch-22 situation. By the time a carcinogenic effect expresses itself, many years have passed. Conversely, an immediate cancer response to a stimulus could be viewed with skepticism because that would not fit with the classic carcinogenic mechanism.

All of these factors must be considered when analyzing a chemical for its possible carcinogenic properties.

Animal research has been carried out using mice and rats as primary models for the possible carcinogenic effects of 2,4,5-T and TCDD. Tables 23 and 24 summarize these studies.

Table 23. Summary of Literature Involving the Carcinogenic and Tumorigenic Effects of 2,4,5-T

Animal Form of Herbicide and TCDD Content	Dose and Route of Administration	Effects	Author
Mouse acid TCDD not stated	stomach tube 21.5 mg/kg/day days 7-21, 60 mg/kg in diet for 18 months (27 mg/day)	no effect	Bionetics 1968 Innes 1969
Mouse acid 0.05 mg/kg TCDD XVII-G strain	100 mg/l in drinking water for 60 days then 80 mg/kg of diet for remainder of life span	no effect*	Muranyi-Kovacs 1976
Mouse acid 0.05 mg/kg TCDD C3HF strain	at age 6 weeks, 100 mg/l in drinking water for 60 days, then 80 mg/kg of diet for life span	males - no effect, females - increased incidence of tumors**	Muranyi-Kovacs 1976
Mouse acid 93% pure in DMSO	215 mg/kg single s.c. injection	no effect	Bionetics 1968
Mouse acid 99% pure TCDD level not stated	62 mg/kg i/p injections daily for 6 days	inhibited Ehrlich ascites tumor	Walker, et al. 1972
Mouse acid 99% pure TCDD level not stated	80-85 mg/kg i.p. injections daily for 6 days	doubled inhibition	Walker, et al. 1972
Mouse acid 0.05 mg/kg TCDD	10 mg/kg, 4 single s.c. injections	no effect	Muranyi-Kovacs 1977
Rat acid "pure"	3,10,30 mg/kg in diet for life span	no effect	Kociba, et al. 1979
Rat 0.05 mg/kg TCDD	3,10,30 mg/kg/day in diet	present status: no evidence of carcinogenic response	Leuschner, et al. (in progress)

*XVII-G strain of mice has a known high spontaneous incidence of lung tumors
**C3HF strain of mice has a known spontaneous incidence of hepatoma

Research conducted on animal models for potential carcinogenic effects of 2,4,5-T have failed to yield positive results. The EPA's Carcinogen Assessment Group (CAG) acknowledged those conclusions in their February, 1979, report: "On the basis of the completed mice and rat studies, there is no significant evidence of carcinogenicity for 2,4,5-T." They added, however, that testing has not been done sufficiently in mice (EPA 1979a).

Of the studies listed in this report, only one report (by Muranyi-Kovacs) produced positive results. However, the CAG rejected findings of the Muranyi-Kovacs report, saying, "CAG cannot regard the study as furnishing significant evidence for the carcinogenicity of 2,4,5-T." The CAG then listed their objections to the conduct of the study: 1) inadequate animal husbandry; 2) inadequate dosages; 3) arbitrary exclusion of some test animals from the calculation of tumor incidence; and 4) the unavailability of histology data on some animals. The authors of the report themselves suggest that further and more adequate studies be performed on a greater number of species.

Table 24. Summary of Literature Involving Carcinogenic

Animal	Dose and Route of Administration
Mouse	0.007 ug/kg, gastric intubation weekly for 1 year (beginning at age 10 weeks)
Mouse	0.07 ug/kg, gastric intubation weekly for a year (beginning at 10 weeks)
Mouse	7 ug/kg, gastric intubation weekly, 1 year (beginning at age 10 weeks)
Mouse	2 ug and promoted with 5 ug TPA for 32 weeks
Rat	5000 ng TCDD/g diet in food for 78 weeks. Weekly dose/animal, 2 ug/kg body wt
Rat	1000 ng TCDD/g diet in food for 78 weeks. Weekly dose/animal, 0.4 ug/kg
Rat	50 ng/g in food for 78 weeks Weekly dose/animal, 0.1 ug/kg body weight
Rat	50 ng TCDD/g food in diet for 78 weeks. Weekly dose/animal 0.01 ug/kg body weight
Animal	Dose and Route of Administration
Rat	$5X10^{-12}$g TCDD/g food (5 ppt) in diet for 78 weeks. Weekly dose/animal, 0.001 ug/kg body weight

DOW2155197

Carcinogenic Effects	Author
no effect 2 months after treatment ended	Toth, et al. 1977
no effect 2 months after treatment ended	Toth, et al.
no effect at end of treatment	Toth, et al.
weak tumor initiator in the two-state system of mouse skin tumorigenesis with DMBA	DiGiovanni, et al.
4 squamous tumors (lung), 4 neoplasic nodules (liver), 2 cholangiocarcinomas (liver)	Van Miller, et al. 1977
1 cholangiocarcinoma (liver), 1 angiosarcoma (skin), 1 glioblastoma (brain), 2 malignant histiocytomas (peritoneal)	Van Miller, et al. 1977
1 fibroma (striated muscle), 1 carcinoma (skin), 1 adenocarcinoma (kidney), 1 sclerosing seminoma (testes)	Van Miller, et al. 1977
1 fibrosarcoma (muscle), 1 squamous cell tumor (skin), 1 astrocytoma (brain)	Van Miller, et al. 1977

Carcinogenic Effects	Author
1 ear duct carcinoma, 1 lymphocytic leukemia, 1 adenocarcinoma (kidney), 1 malignant histiocytoma (peritoneal--metastases noted), 1 angiosarcoma (skin), 1 Leydig cell adenoma (testes)	Van Miller, et al. 1977

Table 24. Summary of Literature Involving Carcinogenic and Tumorigenic Effects of TCDD in Animals (continued)

Rat	$1X10^{-12}$g TCDD/g food (1 ppt) in diet for 78 weeks. Weekly dose/animal, 0.003 ug/kg body weight	no effect	Van Miller, et al. 1977
Rat	0.1 ug TCDD/kg/day for 2 years in diet	statistically significant chemically related effects NOTE: Doses sufficient to induce severe toxicity increased the incidence of some types of neoplasms in rats, while reducing the incidence of other types. FEMALES: hepatocellular hyperplastic nodules, hepatocellular carcinoma, squamous cell carcinoma of hard palate and nasal turbinates, keratinizing squamous cell carcinoma of lung. Decreased incidence of benign tumors of uterus, decreased incidence mammary gland neoplasm, decreased incidence pituitary adenoma. MALES: squamous cell carcinoma of nasal turbinates or hard palate, squamous cell carcinoma of tongue, decreased incidence of adenoma of adrenal cortex, decreased incidence of pheochromocytoma of adrenal	Kociba, et al. 1978
Animal	**Dose and Route of Administration**	**Carcinogenic Effects**	**Author**
Rat	0.01 ug TCDD/kg/day for 2 years in diet	FEMALES: hepatocellular hyperplastic nodes, no further difference from controls in all neoplasms MALES: no significant difference from controls	Kociba, et al. 1978
Rat	0.001 ug TCDD/kg/day for 2 years in diet	no significant difference from controls	Kociba, et al. 1978
Monkey	500 ng/kg in diet total exposure between 2-3 ug/kg body wt for nine months	epithelial hypertrophy, hyperplasia, and metaplasia, no neoplastic changes noted	Allen, et al. 1977

TCDD is an extremely potent inducer of hepatic aryl hydrocarbon (AHH) activity in the rat (Poland and Glover 1974). This enzyme system is proposed as a promoter of carcinogenic activity. Furthermore, in the presence of known carcinogen DMBA, TCDD is a weak tumor initiator in the two stage system of mouse skin tumorigenesis.

Investigations of the carcinogenic potential of TCDD (dioxin) have produced dose-dependent results. At relatively high, long term doses, a diet containing TCDD has been found to cause increased incidence of liver and lung tumors, as well as nasal turbinate-hard palate tumors in certain rat species.

Two animal studies predominate in the analysis of the carcinogenic potential of TCDD. They are a two-year chronic study performed by Kociba et al. (1978) using Sprague-Dawley rats and a 78-week investigation conducted on the same species by Van Miller et al. (1977). At highest dosages tested, their experiments yielded similar results. At highly toxic levels of 2200 ppt and 500 ppt, respectively, these reports documented an increase in the incidence of liver and lung tumors in Sprague-Dawley rats. However, the conclusions they drew from the lower dose experiments differed. Kociba's group considered the lower dose ranges negative for carcinogenic effect, citing the appearance of hepatic hyperplastic nodules from a median dose as the only cellular change observed. Van Miller, on the other hand, reported significant carcinogenic potential at doses well below the 500 ppt dose. Tumors, he claimed, appeared with TCDD levels of 1000 ppt, 500 ppt, 50 ppt, and 5 ppt. Only at a level of 1 ppt did Van Miller acknowledge no chemical related effects.

There have been several criticisms of Van Miller's investigation. The Carcinogen Assessment Group (CAG) audited the study and found that it had major shortcomings in design and conduct that limited the reliability of data developed at dose levels lower than 1000 ppt (EPA Position Document 2/3 1979). Therefore, its carcinogenic effects could only be verified at the two highest dose levels. In addition, the investigators in the study reported no tumors in the control animals, a highly unlikely phenomenon when using Sprague-Dawley rats. This strain is particularly susceptible to spontaneous tumor induction, and lack of tumors in controls is highly suspect.

It is also interesting that with one exception the areas of tumor induction at lower dose ranges of TCDD did not correlate with the expected target organs determined from the higher doses (i.e., lung and liver).

Lastly, the experiment only involved 10 animals per test group. This small number is below the minimum number recommended by the National Cancer Institute (1975).

The Kociba study has not escaped criticism either. While there is general agreement that TCDD is carcinogenic at the high dose used in the experiment (2200 ppt), differences exist regarding the middle (210 ppt) dose. Kociba et al. found hyperplastic alterations in the liver at the 210 ppt dose and considered this a negative finding. In an audit of the Kociba study by the CAG, a conflict in the classification of hyperplastic nodules became evident. The CAG re-evaluated this study and concluded that the combined incidence of hepatocellular hyperplastic nodules and the hepatocellular carcinomas is statistically significant for both the 2200 ppt and the 210 ppt level (EPA 1979a).

The disagreement expressed here represents a part of an ongoing controversy regarding interpretation of cellular alterations in rodent liver as a measure of carcinogenic potential. Participants at the Rat Liver Workshop, sponsored by the National Cancer Institute, developed the following guidelines and preferred nomenclature in an attempt to unify interpretation of rat hepatic lesions (National Cancer Institute 1975).

Recommended classification of specific hepatocellular lesions in rats:

1) Foci of cellular alterations
   a) Clear cell foci
   b) Eosinophilic or ground glass foci
   c) Basophilic foci
   d) Mixed cell foci
2) Neoplastic nodules
3) Hepatocellular carcinomas
   a) Well differentiated
   b) Moderately differentiated
   c) Poorly differentiated
   d) With glandular and/or papillary formation
4) Cholangiofibrosis

Based on the experimental and biological evidence available, the members of the workshop have recommended the term "neoplastic nodule" to replace so called "hyperplastic nodules" previously referred to in the literature. They describe these nodules as proliferative lesions which are known to be induced by carcinogens and, at the

least, indicate an increased probability of the development of hepatocellular carcinoma. Although such nodules do occur in control animals, the concensus of those at the workshop felt that such an incidence was very low and comparable to that of naturally occurring hepatocellular carcinoma.

Studies by Hirota and Williams (1979) investigated the persistence and growth of rat neoplasms and concluded that these nodules represent a different type of neoplasm than carcinoma, possibly a benign neoplasm. They assert that these neoplastic nodules are not obligate precursors of carcinoma, and may in fact be at a stage in neoplastic development that is less autonomous than carcinoma. Their interpretation of nodule, therefore, is an end stage, benign neoplasm that is capable of continued growth but is not invasive or metastatic and may only infrequently acquire these properties. In their study, the authors detected no transition of nodules to carcinomas. At intervals after cessation of carcinogen exposure, nodules displayed changes suggestive of phenotypic reversion or degeneration that were not present in carcinomas.

Those in support of the Kociba conclusions favor this interpretation of hyperplastic nodules as non-proof of carcinogenicity. They further support this rationale by citing the lack of carcinogenic change of nodules to tumors within the two year test period of the Kociba study.

The EPA's own Scientific Advisory Panel also has reservations as to whether hyperplastic (neoplastic) nodules are precursors, per se, of hepatocellular carcinoma (EPA 1979d).

Carcinogenicity Summary

Current rat and mouse studies have failed to demonstrate any clear carcinogenic response to 2,4,5-T. However, experts feel that further research is needed to clarify this position.

TCDD, on the other hand, is a potent inducer of aryl hydrocarbon hydroxylase (AHH) activity in the rat. This system is proposed as a promoter of carcinogenic activity. In addition, in the presence of the known carcinogen DMBA, TCDD is a weak tumor initiator in the two stage system of mouse skin carcinogenesis.

At high doses, TCDD does increase the amount of tumors present in Sprague-Dawley rats particularly in the liver and lung. Lower doses have consistently failed to elicit this same response.

Confusion has arisen regarding the classification of hyperplastic nodules as a precancerous condition. Some feel that it is an obligate precursor of malignant alteration, while others consider it a benign condition capable of phenotypic reversion or degeneration not seen in a carcinoma.

15971

HUMAN EXPERIENCES WITH 2,4,5-T AND TCDD

The central theme of the controversy regarding 2,4,5-T and its TCDD contaminant is the fear of adverse effects on human health. The argument is that if these chemicals are capable of producing toxic signs, birth defects, and cancer in laboratory animals, there is no assurance that human beings exposed to these chemicals will not have the same responses. This is the primary concern of government agencies, scientific researchers, and chemical companies alike.

Unfortunately, the biggest problem encountered in analyzing possible ill effects of 2,4,5-T is a basic lack of data. Requiring an "accident" or other form of unintentional exposure to gather their information, researchers are often interrupted in their pursuit of purely chemical related effects by undesirable contributing factors. As a result, no single adverse health effect has been thoroughly investigated. Rather, a potpourri of consequences have been touched upon in a variety of health screenings. This results in a paucity of human evidence, with no firm data on a demonstrable cause/effect relationship. The information already accumulated is at best inconclusive, and at worst, statistically and epidemiologically unsound.

There are some reports of actual, usually non-quantified, human exposure to either 2,4,5-T or TCDD. The following discussion summarizes the relevant "studies" most often referred to in discussions of human epidemiology and 2,4,5-T.

Dow Chemical Plant Workers

This study examines the mortality experience of 204 persons exposed to 2,4,5-T during its manufacture from 1950 to 1971 at the Dow Chemical Plant in Midland, Michigan. Analyzing the health status of 2,4,5-T workers exposed for a period of over one year, the investigators paid particular attention to the crew directly involved in the 2,4,5-T manufacture process: reactor operators, sale wheel operators, acid wheel operators, and dryer operators. One operator from each category routinely makes up the 2,4,5-T manufacture crew.

In general, at exposure concentrations of 2,4,5-T (containing less than 1 ppm TCDD) experienced by the workmen in this study, no adverse mortality effects have been observed attributable to the work environment. Within the scope of this limited survey, mortality compares favorably with that of all U.S. white males, and

1982

also compares well with the background mortality experience in this geographical area (Ott et al. 1980). The single observed cancer death occurred as a result of respiratory malignancy discovered in a 63 year old retiree who had been exposed to 2,4,5-T for eight years and who also had a past history of smoking up to two packs of cigarettes per day. The data appearing on Tables 25 and 26 are point estimates and should therefore only be considered as such. In light of results from present animal experiments, continued data collection should take place to substantiate or dispel current postulates regarding the effects of this herbicide.

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Table 25. Observed and Expected* Deaths Among 204 Employees Exposed to 2,4,5-T, by Cause and Duration of Exposure, 1950-1976

Cause of Death Category	Total Exposed Group		Duration of Exposure			
			1 Year Total Exposure		1+ Year Total Exposure	
	Observed	Expected	Observed	Expected	Observed	Expected
All causes	11	20.3	6	13.3	5	7.0
Total malignant neoplasms	1	3.6	0	2.3	1	1.3
Diseases of cardiovascular system	4	9.1	1	5.6	3	3.5
External causes (accidents and suicides)	6	3.7	5	2.8	1	0.9
All other causes	0	3.9	0	2.6	0	1.3

*Expected numbers of deaths based on U.S. white male mortality rates

Source: Ott, Holder, Olson 1980,

Table 26. Observed and Expected* Deaths Among 204 Employees Exposed to 2,4,5-T, by Cause and Interval Since First Exposure, 1950-1976

Cause of Death Category	Interval Since First Exposure							
	10 Years		10-14 Years		15-19 Years		20+ Years	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
All causes	3	6.6	0	4.7	4	4.6	4	4.4
Total malignant neoplasms	0	1.0	0	0.9	0	0.8	1	0.9
Diseases of cardiovascular system	0	2.3	0	2.2	3	2.3	1	2.3
External causes (accidents and suicides)	3	2.0	0	0.8	1	0.5	2	0.4
All other causes	0	1.3	0	0.8	0	1.0	0	0.8

*Expected numbers of deaths based on U.S. white male mortality rates

Source: Ott, Holder, Olson 1980.

Monsanto Plant Accident, Nitro, West Virginia

This research studied the chronic health effects of exposure to TCDD as reflected in the mortality experience of a cohort of Monsanto Company workers who developed symptoms of chloracne following a trichlorophenol process accident in Nitro, West Virginia, in 1949.

Chloracne is a skin disease characterized by comedones, cysts, pustules, and abscesses. Accompanying this may be hepatic dysfunction, peripheral neuritis, disorders of fat metabolism, and porphyria cutanea tarda, a chronic skin lesion condition caused by disturbances in porphyrin metabolism. Chloracne is primarily associated with exposure to TCDD.

On March 8, 1949, a reaction accident occurred at the plant discharging TCDD into the atmosphere and interior of the building. Employees involved in the preliminary clean-up developed immediate symptoms, including: eye and respiratory tract irritation, headache, dizziness and nausea, and severe irritant reaction of exposed skin (Zack et al. 1980). After these symptoms subsided, chloracne symptoms appeared.

A four-year follow-up revealed the following physical signs (in order of relative frequency): acneform lesions; severe pains in muscles of upper and lower extremities, shoulders and thorax on exertion; fatigue; nervousness and irritability; decrease in libido; dyspnea; vertigo; and intolerance to cold. One year after the accident, four of the six workers examined had liver enlargement and one had sensory loss in one foot. Also found were liver impairment indicated by hepatomegaly (liver enlargement), tenderness in the right upper quadrant and epigastrium, and a delayed prothrombin time.

In 1953, four of the six original workers were again observed. Investigators observed a general regression of both cutaneous and non-cutaneous symptoms. All workers exhibited a marked improvement in their skin lesions. In a few cases, employees still complained of pains in the lower extremities and back, nervousness, excessive fatigue and dyspnea. But no explanation for these symptoms could be made on the basis of physical examination.

The subject of the Journal of Occupational Medicine report in January, 1980, involved the 29-year follow-up of 121 members of the original study cohort--89 members were verified living and 32 were verified deceased by death certificate. The results of standardized mortality ratio for all deaths was 0.69, with 32 deaths observed and 46.41 expected. This was the only statistically significant value determined. There were nine malignant neoplasms, with 9.04 expected. No deaths resulted from stomach or liver cancer. Five lung cancer deaths occurred versus the 3.02 expected. One skin cancer (a fibrous histiocytoma presumably of dermal origin) proved fatal. Exceedingly rare, the expected incidence of its appearance is 0.15. Three deaths from lymphatic and hematopoietic neoplasms resulted with 0.88 expected. There were 17 deaths from diseases of the circulatory system, with 25.01 expected.

Because the study cohort was small and only 32 deaths were observed, the results from this study cannot be considered conclusive. Nevertheless, this is the best long term study of TCDD effects to date. The analysis of mortality experiences of personnel at this plant indicate no apparent excesses of total mortality or of deaths due to malignant neoplasms or circulatory diseases (Table 27).

Table 27. Observed and Expected Deaths Amoung 121 Males Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident

Causes	Observed	Expected
All causes of death	32	46.41
All malignant neoplasms	9	9.04
Buccal cavity and pharynx	0	0.30
Digestive organs and peritoneum	0	2.59
Stomach	0	0.50
Liver	0	0.18
All other digestive organs	0	1.91
Respiratory system	5	3.02
Lung	5	2.85
All other respiratory organs	0	0.17
Skin	1	0.15
Genitourinary organs	0	1.16
Lymphatic and hematopoietic	3	0.88
Other sites	0	0.94
Diseases of the nervous system and sense organs	0	0.36
Diseases of the circulatory system	17	25.01
Arteriosclerotic heart disease, including coronary heart disease	13	17.74
All other disease of the circulatory system	4	7.27
Diseases of the respiratory system	1	2.78
Diseases of the digestive system	0	2.26
All other diseases	2	3.18
External causes of death	3	3.78

Source: Ott, Holder, Olson 1980.

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Arkansas Cleft Palate Study

Because of the teratogenic effects found in animal experiments involving herbicide 2,4,5-T, a study was undertaken to determine what, if any, human effects were experienced after exposure to 2,4,5-T. Performing a retrospective study on the incidence of cleft palate, the investigator sought to correlate the anomaly with agricultural use of the herbicide. Researchers analyzed facial cleft rates and concurrent use of 2,4,5-T in Arkansas during 1948-1974. Estimated level of exposure to the herbicide was categorized as high, medium, or low-exposure on the basis of rice acreage during 6 or 7 year intervals beginning in 1943. A total of 1,201 cases of cleft lip or cleft palate were detected within those 32 years.

The researchers found that facial cleft rates presented by sex, race, time period, and exposure group generally rose over time (Nelson et al. 1979). No significant differences were detected in any race or sex grouping.

Authors of the report concluded that the increase in incidence of this anomaly was due to better case assessment rather than an actual increase in incidence. However, this does not rule out the fact that some causative factors may be playing some role. They further suggest that their crude demarcation of exposure groups may indicate less than accurate results on both the high and low exposure ends of the spectrum. They caution that, "The present study does not indicate any overt causal relationship between 2,4,5-T use and facial clefts." However, they add, "It is important to recognize that such an effect, if it were to exist, would not have been detected, if the increase in facial clefts was less than twofold. A twofold increase in any anomaly would be extremely large. We believe more 'powerful' epidemiological studies of 2,4,5-T are needed to more conclusively resolve questions concerning its developmental toxicity under conditions of regulated use."

Humboldt County

The California Department of Health carried out an epidemiological investigation to assess the claims of abnormal outcome of pregnancies in the Orleans California area, which includes a phenoxy herbicide spray area.

The methods of investigation in Orleans included:

1) Review of Humboldt County vital records for information concerning all live births, fetal and infant deaths, and congenital malformations occurring among infants born in Orleans and nearby areas during the years 1974-1978. (Earlier records not available from the County.)
2) Determination of the dates of spraying phenoxy herbicides within a five mile radius of Orleans.
3) Review of Hoopa Medical Clinic records for live births and spontaneous abortions 1974-1978.
4) Interviews with affected mothers in Orleans for dates and details of pregnancy problems.

Researchers found that, within the study period, 87 pregnancies occurred. Of these, eight (9 percent) ended in spontaneous abortion, one (1 percent) in fetal death (placenta previa), and the remaining 78 in live births (Reed 1979b). Of the live births, six (7.7 percent) children were born with birth defects (two hypospadias, two with incomplete hip socket formation, one cleft lip and palate, one mild talipes equinovarus), and one Sudden Infant Death Syndrome (SIDS) at age seven months (Reed 1979b).

Phenoxy herbicide spraying (almost always with 2,4,5-T) has been carried out in the fall of the year since 1961, with the exception of 1962, 1972, and 1973. Spraying ceased in November, 1976. Therefore, spraying consistenc in this case cannot be established.

At first glance, 16 abnormal pregnancies may seem significant. But when compared to the total pregnancy outcome for that region, the picture comes into clearer focus. The spontaneous abortion rate of 9 percent (8/87 pregnancies) is less than the expected rate of 15 percent. Major congenital malformations occur at the rate of 7 percent of live births, while the inclusion of minor defects brings the percentage up to 15. The Orleans rate of occurrence is 7.7 percent, once again, well within the expected boundaries. One fetal death and one sudden infant death also reflect the patterns established for the state.

Therefore, the investigators conclude that, "it appears unlikely that there has been an unusually high rate of pregnancy wastage in Orleans." (Reed 1979b) (Tables 28 and 29).

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Table 28. Relationship of Abnormal Outcome of Pregnancy and Herbicide Spraying Near Orleans, California

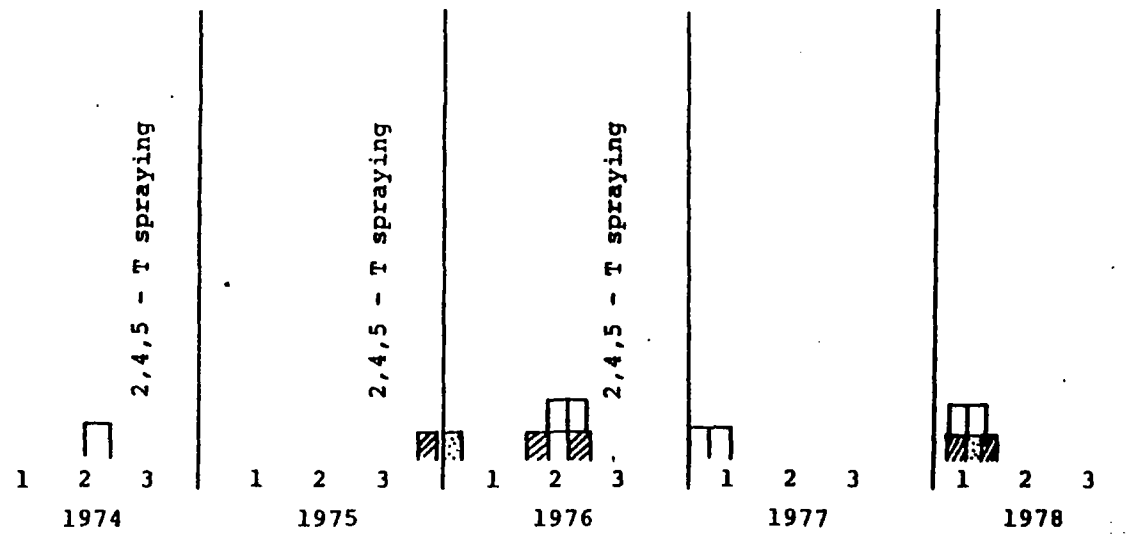


Table 29. Number of Normal Live Births and Abnormal Outcome of Pregnancy Among Residents of Orleans, California 1974-78

Year	Births	Spontaneous Abortions	Fetal Deaths	Infant Deaths	Congenital Malformations	Total Pregnancies
1978	17	2	1		3	20
1977	13	2				15
1976	14	3		1	2	17
1975	16				1	16
1974	18	1				19
Total	78	8	1	1	6	87

Source: Reed, et al. 1979.

DOW2155206

Denny, California

After concern was expressed regarding abnormal outcome of pregnancy in Denny, the California Department of Health Services conducted an investigation to clarify the cause of the health problems of the women of Denny.

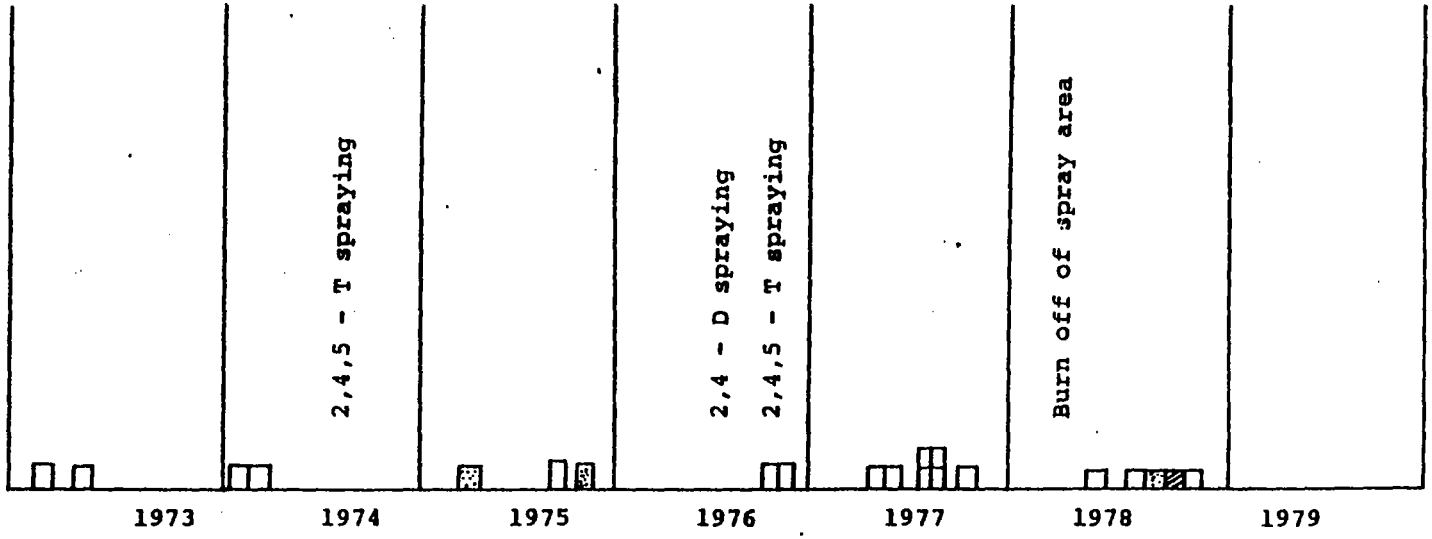
Denny is a small rural community (population as of 1976: 114 persons). There are 25 women of childbearing age in the area. Half of the population lives in Denny, while the others live within ten miles of town. Chief occupations are logging, mining, and ranching.

Methods of the field investigation during this one week study included (Reed 1979a):

1) Discussion with local medical and veterinarian personnel of their knowledge of unusual patterns of abnormal outcome of pregnancy, unusual environmental hazards, and any infectious disease problems.
2) Detailed questionnaire interviews or reviews of medical charts of five of the six Denny women with reported problems (one woman moved out of the state). For the purposes of this study, a miscarriage or spontaneous abortion was defined as a nonviable termination of pregnancy after two missed menstrual periods or a positive pregnancy test.
3) Review of all Hoopa Clinic obstetric admissions for women from the Denny area, 1973-1978.
4) Review of all Hoopa Clinic admissions for spontaneous abortions 1973-1978.
5) Review of all Trinity County vital records of live births as well as infant and fetal deaths, 1973-1978.
6) Determination of dates of spraying and types of phenoxy herbicides used near Denny.

Investigation revealed that during the course of study fourteen women had a total of 21 pregnancies. Four of these women had six miscarriages, and one of the live births had an incomplete cleft lip. Thus, six (29 percent) of 21 pregnancies ended in miscarriage, and one (7 percent) of 15 live births had a birth defect (Table 30).

Table 30. Outcome of Pregnancies and Spraying of Phenoxy Herbicides near Denny, California, 1973-1978



Source: Reed, et al. 1979.

A rough estimate of the incidence of miscarriage yields a figure of 13 percent. This correlates well within the boundaries of an expected 15 percent. However, because of the small number of events and potential sources of missing information, the researchers question the accuracy of this figure.

The occurrence of one malformation in 15 live births is similar to the expected seven percent rate. No fetal deaths for the area were recorded within the study time.

Investigators felt confident that these women were indeed exposed to the herbicide, either by physical presence in direct range of spraying and/or eating local fish and game potentially exposed to the herbicide residue and drinking local stream water originating from spray areas.

Local authorities were unaware of Rubella outbreaks or other infectious diseases which have known associations with congenital malformations or miscarriages. Some practitioners suggested that several Denny residents had nutritional problems, particularly protein deficiency.

In summary four women experienced five medically documented miscarriages in the Denny area between 1975 and 1978. One woman reported an additional unattended miscarriage. Using all sources available, approximately 29 percent of all pregnancies in Denny women ended in miscarriage. However, the quality of the data may be in doubt, and the figure may be inaccurate.

The seven percent incidence of congenital abnormality in this study (one case of cleft lip) falls within the expected range.

The investigators concluded that, "There was no clear temporal relation of the miscarriages to the spraying. However, the pregnant women reported other exposure to sources of herbicides beside the actual spraying." (Reed 1979a).

"The apparent high rate of miscarriages among women in Denny during the past four years, while suggestive, could be a chance cluster of women with medically definable reproductive problems. The lack of temporal association of the miscarriages with the spraying supports this explanation. On the other hand, all of the women report specific exposure to the herbicide spraying as well as to other possible sources of herbicide such as food and water. Thus, we cannot rule out the possibility of toxic effects from the herbicides. From our investigation

we can only conclude that a health problem of pregnancy may exist in this area, and it may be related to environmental toxins."

The authors of the report suggest further studies to clarify this health matter.

1768

St. Louis, Missouri, Horse Arena

In August, 1972, the Missouri Division of Health and the Center for Disease Control (CDC) reported an investigation of a horse arena in eastern Missouri where 54 of 57 horses exposed to the arena had died of an illness characterized by skin lesions, severe weight loss, and hepatotoxicity. Birds, dogs, cats, insects, and rodents were also found dead in and around the arena. A six year old girl, who frequented the arena as a playgound, developed epistaxis, gastrointestinal complaints, and severe hemorrhagic cycstitis characterized by hematuria. Bacterial and viral urine cultures yielded negative results. Three other persons suffered from milder illnesses with symptoms of headaches and nausea. They too, had visited the arena, although for a shorter period than the young girl.

At the time of the trouble, investigators did not know what caused these adverse health effects. However, subsequent soil analysis showed that both TCP (trichlorophenol) and TCDD were the probable toxic substances. The actual levels of TCDD were not known until 1975. It was found that the arena soil contained a TCDD level of 31.8 to 33 ug/g (ppm) of soil. The contamination was traced back to a waste oil used in the area to control dust (Donnell and Phillips 1974).

Beale et al. (1977) have recently reported a reexamination of the young girl who developed hemorrhagic cystitis following exposure to the arena. In the five year interval since exposure, the patient had grown normally. Both her height and weight exceeded the 75th percentile. Detailed physical, neurological, and chemical examinations revealed no abnormalities. The same studies were conducted on the patient's sister and mother, exposed simultaneously, but less extensively to TCDD, and the results were also normal.

The authors concluded, "Our experience demonstrates that people exposed to dioxin can recover completely with no apparent sequela from the toxin. It remains to be determined whether the exposure to dioxin in these

children will result in abnormal pregnancies or affect their offspring." (Beale et al. DON2155208

Seveso, Italy

One of the most publicized chemical accidents in recent times occurred in Seveso, Italy, a small town 40 kilometers north of Milan. The ICMESA (Industrie Chimiche Meda Societa Anonima) Chemical Plant did not produce herbicide 2,4,5-T but made hexachlorophene, an antibacterial agent. The herbicide and hexachlorophene share a common denominator, 2,4,5-trichlorophenol in their manufacturing process.

The ICMESA factory explosion occurred in a reactor that produced 2,4,5-trichlorophenol by combining tetrachlorobenzene with sodium hydroxide in an ethyleneglycol solvent. The manufacturing protocol called for this reaction and a subsequent distillation process to remove the ethylene glycol solvent to be performed each day. However, the Friday before the accident, these steps were not taken. The chemical mixture was left in the apparatus and continued to react, became seriously overheated as a result, and ultimately exploded, blowing the roof off of the reactor. The explosion propelled forth a toxic white cloud which, when cooled, descended slowly over the surrounding area (Whiteside 1979).

Among the chemicals that contaminated the area during cooldown was the chemical dioxin (TCDD). Based on determinations made by production officials, an estimated 4,000 to 5,000 kg of reaction product was lost into the atmosphere (di Domenico 1977) (Anonymous 1977b). Estimates of the TCDD dispersion ranged from 650 grams to 1,700 grams (de Domenico 1977) (Kearney et al. 1973). A sample of the escaped product taken from the reactor head for analysis revealed the presence of 3.5 percent (35,000 ppm) TCDD.

Birds, rabbits, and chickens died soon after the accident (Reggiani 1978). Dogs either died as a result of TCDD poisoning or were selectively killed to ward off further contamination. By the following Monday leaves from plants and trees turned brown and fell off trees in an area measuring 1,000 to 3,000 meters in a triangle with a base of 400 meters and a vertex of around 100 meters centered around the factory (Anonymous 1977b). Measurements of TCDD residue on vegetation in this area ranged from 1 to 15 ppm, with one reading as high as 50 ppm.

Children and adults exposed to the descending dust complained of nausea and developed skin lesions accompanied by redness and swelling. Some experienced the burns and blister common in exposure to caustic material.

As the scientific world descended on the Seveso area, investigators began to map out degress and extent of polluted zones, remaining residues, and effects on animals. Highest TCDD content was determined in the herbivorous animals (Garattini 1977).

People complained of nervous symptoms, mood changes, and above all a serious rash-like skin lesion, later diagnosed as chloracne, the hallmark of dioxin poisoning.

About 1,000 assays led to the Seveso area being divided into two zones. Giovanardi (1976) described the first zone "A" as a triangular area covering approximately 100 hectares (ha). Zone A included land located south, southeast of the ICMESA factory and downwind at the time of the accident.

Table 31. Environmental Concentrations of TCDD Residues in Seveso Area

Zone	Area Km^2 (Acres)	Deg. of Contam. hg/m^2 Average (Range)*	No. of People in Zone
A	0.73 (180)	230 (0 to 5477)	736
B	2.25 (556)	3 (0 to 43.8)	4699
R	12 (2954)	0.0 to 0.5 (5, sporadic)	31800

*Based on measurements of samples collected December 1976 (Homberger, E., et al., "The Seveso Accident: Its Nature, Extent, and Consequences," unpublished (1979)).

Source: Dow Chemical Co. 1979.

Reported soil levels of TCDD here revealed residues greater than 0.001 ppm (1 ppb). Later Reggiani (1978) determined that the same area registered TCDD levels approximating 10 ppb.

The inhabitants of this area were evacuated in three stages: on July 26, July 28, and August 2, 1976.

Zone B had detectable soil levels of TCDD, but they were less than 0.001 ppm. Reggiani later described this area as having residue levels between 0.1 and 10 ppb for TCDD. This area covered approximately 250 ha and was divided between a large urban center and an extensive rural area with some small residential aggregates (Giovanardi 1976). The population of 4,900 was not evacuated. Recommendations were made for the women and children in order to reduce the possibility of exposure (Reggiani 1978).

A third zone, Zone "R", covered a total of about 1,430 ha. Occasional residue concentrations all less than 0.1 ppb TCDD in soil, characterized this zone. The population of Zone R approximated 40,000 people.

An extensive health surveillance system was set up to determine the mid-term and long-range effects of TCDD exposure. Medical surveillance included laboratory tests, medical examination, monitoring of pregnancies and abortions for any abnormal outcomes, rate of stillbirths, population morbidity and mortality, and cancer registry. The medical surveillance program was extended to 11 districts with a population of 216,000.

Reggiani has completed and published the most thorough analysis of health data from the people involved in the Seveso disaster. He concludes:

> "The Seveso accident has not revealed up to now, toxic effects in humans, which have not been observed in other episodes. Chloracne, the typical skin lesion, has occurred in children with the tendency to spontaneous and rapid healing. The peripheral nervous system has perhaps been attacked and reacted with subclinical signs of impairment. Signs of involvement of the liver without apparent functional disorders have occurred. No other organs or functions have been impaired. There has been no derangement of the gestation, no fetal lethality and loss, no gross malformation, no growth retardation at term and no cytogenetic abnormalities. The immunocapability of the population, not even of the children with chloracne, has not been attained."

He warns, however, that certain deficiencies must be addressed--specifically the reproductive data in areas which he feels either do not exist or are deliberately underreported (Reggiani 1978).

At the Dispute Resolution Conference on 2,4,5-T in 1979, Doctors L. Sanchioni and H. Rehder presented data on abortion experiences following the explosion:

1) Twenty-eight of the 35 induced abortions were represented by products of conception that were partially fragmented during curettage and incomplete for morphologic evaluation. The only abnormality detected was in one fetus for the RT Zone, which was trisomic. No morphologic malformations were noted in any of these fetuses.

2) A slight increase in the incidence of spontaneous abortions was determined in the B Zone. These increases might have been due to improved registration (some of these abortions might have been self-induced). Fourteen of the 57 cases of spontaneous abortuses and perinatal mortality examined were malformed structurally (24.6 percent). This incidence was within the range that might be expected generally, but couldn't be compared to the rate in this particular area prior to the accident as no data are available. The types of malformations seen were consistent with those which would be expected spontaneously. All the reported malformations were in the R and RT Zones, where the soil concentrations of dioxin were below detectable limits.

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Table 32. Chloracne Cases (No. and per 1000 Inhabitants) by Pollution Zones

Pollution Zone	(1) Chloracne Sept.-Dec. 1976 "Early"		(2) Chloracne Feb.-Apr. 1977 "Late"		Chloracne (1)+(2)	
	No.	per 1000	No.	per 1000	No.	per 1000
Zone A	46	63.01	15	20.55	61	83.56
Zone B	0	--	9	1.90	9	1.90
Seveso Zone R	1	0.13	28	3.52	29	3.65
Meda Zone R	0	--	20	4.98	20	4.98
Cesano M Zone R	0	--	13	0.87	13	0.87
Desio Zone R	0	--	2	0.43	2	0.43
Seveso Zone non (A,B,R)	0	--	13	1.68	13	1.68
Meda Zone (A,B,R)	0	--	14	0.90	14	0.90
Cesano M Zone non (A,B,R)	0	--	8	0.50	8	0.50
Desio Zone non (A,B,R)	0	--	5	0.18	5	0.18
Other Cities	3		10		13	
Total	50		137		187	

Source: Bisanti, et al. 1979.

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Table 33. Birth Defects/Births by Pollution Zones and Year (1976 to 1978 (I-VI))

Year (months)	Zone A	Zone B	Zone R	3-c.s.z.* (s A,B,R)
1976 (VII-XII)	0/2	0/29	0/221	1/1280 (0.078%)
1977	0/4	0/66	9/376 (2.39%)	29/2328 (1.245%)
1978 (I-VII)	0/--	1/--	5/--	16/--

Admitting a birth defect rate for zone B as for zone R (2.3%), 1.5+0.7 or 0.8 to 2.2 malformed children out of 66 births should be expected (p 0.05)

*Refers to zones outside of contaminated area

Source: Bisanti, et al. 1979.

Alsea I

This study deals with a limited number of women residing in the Alsea Basin area of Oregon. Suspicions arose because a number of women living in this 2,4,5-T treated forest area experienced miscarriages, which they said were correlated with the spraying season. Initially, 10 miscarriages were reported by eight women. Subsequently, 13 miscarriages were reported from nine women. Twelve of the 13 miscarriages occurred within the first 20 weeks of gestation.

The conclusions of this report did not indicate an alarming situation. When the data was compared to the live birth rate in Alsea (86 in the six year period studied), a miscarriage rate of 12-13 lies well within the limits of an expected one miscarriage for every seven live births.

The authors of this report did note a seasonal variation in miscarriage incidence when the Alsea values were compared with those from nearby Corvallis, Oregon. However, insufficient evidence precluded any establishment of proof or non-proof for a relation between miscarriages and 2,4,5-T.

In his analysis of the Alsea I report, Nathan Mantel commented on the miscarriage incidence versus the number of women and suggested that, "the possibility thus arises that at least some of these miscarriages were experienced by 'miscarriage-prone' women." (Mantel 1979). He added that, "it is also possible that publicity about 2,4,5-T spraying and spontaneous abortion could have caused more previous spontaneous abortions to be recalled and reported." (Mantel 1979).

Alsea II

A continuation of the Alsea I study, this epidemiologic investigation further analyzed the possible relation between spraying of herbicide 2,4,5-T and reproduction events. The results of this study provided the basis for the EPA Suspension Order of February 28, 1979 (EPA 1979b).

The Alsea I study described above sought to clarify a possible relation between the herbicide spraying and increased miscarriage incidence reported by nine women in the Alsea basin area. The results of that study were generally inconclusive. The authors suggested that there might be a correlation, but they were unable to prove it.

The Environmental Protection Agency began an epidemiological study to determine if the occurrence of spontaneous abortion in the entire Alsea basin area bore any relation to the use of 2,4,5-T.

The Alsea II investigators collected data from three areas of Oregon: the "study" area, which included an area of approximately 1600 square miles of Oregon coast and coastal mountain range; the "urban" area, which is contiguous with the study area and included the inland towns of Corvallis and Albany, and the rural "control" area which is in sparsely populated eastern Oregon on the Idaho border.

The information gathered in the study came from hospital records and included coded diagnoses, age of patient, spontaneous abortion data, gestation period, and zip code of patient's residence. The investigators limited themselves to spontaneous abortions of less than 20 weeks gestation, treated in hospitals in a six year period from 1972-1977. These data were then compared to live births (in-hospital) for the respective areas. Conclusions reached by the EPA study are summarized as follows:

1) "The spontaneous abortion index (hospitalized miscarriages per 1000 births) for the Alsea study area where 2,4,5-T was used was significantly greater than the index for the urban and control areas where there was no known use of 2,4,5-T.
2) There was a dramatic increase in the spontaneous abortion index for the study area relative to the urban and control areas in the month of June and July; this increase followed, by approximately two months, a period in March and April when 2,4,5-T was used to control vegetation in the study area.
3) Statistical analyses of these data indicate that there was a significant correlation between the amounts of 2,4,5-T used in the study area during spraying season and a subsequent increase in the spontaneous abortion index in the study area." (EPA Position Document 1 1978).

The authors conclude, "The Agency's systematic survey of the occurrence of spontaneous abortions in an area of 2,4,5-T use indicates that there was an unusually high number of spontaneous abortions in the area, and that the incidence of spontaneous abortions may reasonably be related to the use of 2,4,5-T in that area. The data further indicate that the miscarriage experiences which the eight Alsea women reported to the Agency were representative of the experiences of the larger population of women living in the study area." (EPA 1979c).

Numerous critiques of the Alsea II study (Mantel 1979) (Lamm 1979), (Witt 1979), (Wagner et al. 1979) have revealed such flagrant statistical and epidemiological flaws that the majority of the scientific community has accorded little if any weight to the EPA conclusions from the Alsea II study.

Spontaneous abortions or miscarriages as they are commonly called, frequently occur in the first three months of pregnancy, sometimes unbeknown to the mother. Statistics cite a spontaneous miscarriage incidence of 12 to 25 percent, with a 15 percent rate most commonly referred to in the literature. In the United States, hospitalization rate for spontaneous abortion approaches eight percent or 82 per 1000 maternity admissions.

A number of factors influence whether a spontaneous abortion will require hospitalization or receive medical attention. These include:

1) whether the woman recognizes the event as a miscarriage;
2) how anxious the woman is about abortion and the social support system her lifestyle provides her;
3) whether the woman is financially able to secure treatment;
4) whether the treating physician is a specialist who deals regularly with spontaneous abortion cases;
5) the availability of treatment in an out-patient clinic;
6) the standard of health care in the community;
7) the gestational age of the conceptus and the completeness of the abortion (Lamm 1979).

The Alsea II study considered only hospitalized spontaneous abortions. Attended abortions in clinics, unattended abortions, and those events which took place at area hospitals not included in the study were not considered. The study numbers, therefore, do not accurately reflect the miscarriage incidence in either the control or study group.

Second, attention must be given to the physician supervising the miscarriage. The Alsea II study stated that obstetricians in the area hospitalized only 10 percent of persons experiencing spontaneous abortion, while General Practitioners faced with the same problem hospitalized approximately 70 percent of these women.

A further complaint frequently voiced in the literature is the choice of study versus control areas. When the medical effect being measured is common in the general

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population (as are spontaneous abortions), test and control areas must reflect the same historical incidence with common causes of the test event to allow the expression of the effects of the stress agent (2,4,5-T). For the Alsea II study, the test area chosen was the Coast range because that is an area where 2,4,5-T is commonly used in forest management. The control areas appropriately did not have 2,4,5-T sprayed in their locales. But other factors related to the frequency of abortions were not addressed. Among these were such variables as: level of medical practice, age of mother, nutritional state, diet, a variety of infectious agents, anatomic or genetic abnormalities, medicine and other drugs, consumption of alcohol or tobacco, socio-economic status, among others (Witt 1979).

Additionally, towns involved in the study, particularly Alsea (study) and Corvallis (control) do not maintain constant populations. Alsea, a coastal resort town, has an influx of people during the summer months. Corvallis, a college town, experiences a summer exodus at the end of the school year. Further criticisms have questioned the alleged physical similarities of the test and control town which was based on simple assumptions and, in some cases, were completely inappropriate (Witt 1979).

In considering the data produced from the Alsea II study, the EPA has cited "dramatic" increase in miscarriages which have "consistently" occurred at an "alarming" rate. This they attribute to the spraying of herbicide 2,4,5-T (EPA 1979e).

However, when one examines the data more closely, a different picture seems to emerge. The dramatic June peak was apparent in only one of the six years studied. There was no consistency to the abortion pattern, and there was no solid establishment of background miscarriage events to clarify a stress related occurrence. In addition, there was no increase in the number of spontaneous abortions in the later years of the study, even though there was a significant increase in the amount of 2,4,5-T sprayed in the test area. Other peaks and troughs appearing on the Alsea graphs randomly occur throughout the year and could not be attributed to spraying, a strictly seasonal event. When the data are corrected to a common hospitalization rate (100 percent) and the total number of hospitalized spontaneous abortions are expressed as a percentage of live births, the values are (Wagner et al. 1979):

Study area - 11.4 percent
Urban area - 14.0 percent
Control area - 9.3 percent
(Normal range 15 percent) (Wagner et al.

At best, the Alsea II study could only represent the inhospital spontaneous abortion index for its test area. However, in addition to questioning the collection and collation of data, critics are also attacking the statistical tools, particularly application of inappropriate statistical tests. As a result, the entire study has been discredited to such an extent that it is no longer considered a useful study for analysis of the alleged problem, the effects of herbicide 2,4,5-T on human reproduction.

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Hanify, Metcalf, Nobbs, and Worsley (1981).

In 1981, Hanify and her colleagues reported on the relationship between 2,4,5-T spraying and birth defects in New Zealand (Hanify et al., 1981). In this study, hospital records were used to identify the rates of occurrence of all diagnosed malformations, including stillbirths and neonatal deaths, but excluding miscarriages at less than 28 weeks fetal age, in specific areas of Northland, New Zealand. Rates for each month over the period 1960 to 1977 were compared with the densities of monthly aerial 2,4,5-T application in the same area during the same period.

During the study period, 37,751 babies were born in Northland hospitals; 436 of these were stillborn, 264 died shortly after birth, and 510 had diagnosed malformations. It was estimated that well over 99 percent of all births occur in New Zealand hospitals.

The site of each spray job and the quantity and date of each 2,4,5-T application were obtained from various company records. As no aerial spraying took place in Northland in the first half of the 1960s, calendar years 1959 to 1965 were chosen as representative of those without environmental 2,4,5-T. The years 1972 to 1976 were chosen as representative periods of 2,4,5-T spraying. Within that period there was considerable variation in the quantity of 2,4,5-T applied in each year, in each month, and in each spray area.

Two tests of the data were carried out. In the first, babies born between 1 January 1960 and 31 August 1966 were taken as a control group not exposed to 2,4,5-T and those born between 1 September 1972 and 31 August 1977 as a study group. For each of the commoner malformations an incidence ratio and 90 percent confidence interval were calculated. As shown in Table 34, defects of the heart, hypospadias and epispadias, talipes, and the grouping "all birth malformations" had incidence ratios greater than 1.

EPA 5021

Table 34 Incidence ratios for the commoner malformations. Total number of births between 1 January 1960 and 31 August 1966 was 15,000; between 1 September 1972 and 31 August 1977 there were 9614 births. The change in total births is due to regional depopulation.

	Total number		Incidence ratio	Confidence bounds (90 percent)	
	1960 to 1966	1972 to 1977		Upper	Lower
All birth malformations*	148	164	1.73	2.08	1.44
Anencephaly	11	10	1.42	2.90	0.69
Spina bifida	18	13	1.13	2.05	0.62
Cleft lip	16	6	0.59	1.27	0.27
Isolated cleft palate	8	7	1.37	3.19	0.58
All heart malformations	8	20	3.90	7.38	2.06
Hypospadias, epispadias	5	18	5.62	11.73	2.69
Talipes	49	52	1.66	2.29	1.20

*Excludes stillbirths, neonatal death, or dislocated and dislocatable hip.

Source: Hanify et at., 1981.

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In the second test, rates of "all birth malformations" were plotted against the average annual exposure to 2,4,5-T. Regions of high, low, and intermediate spray density are shown in Figure 4, as are the years of spraying and the nonspray period. The incidence rate appears to be positively associated with exposure to 2,4,5-T both across areas and across years.

Binary regression was used to investigate further the correlation between individual exposure and malformations. As shown in Table 35, there was no identifiable association for anencephaly, spina bifida, cleft lip with or without cleft palate, malformations of the heart as a group, or for malformations of the male genetalia. A significant association for talipes was calculated when f, the fixed fractional rate at which 2,4,5-T is assumed to be removed from the environment, was equal to 0.25. The pattern of correlations remained almost unchanged except for some loss of significance when all tests were recalculated omitting data from the Whangarei hospital area. Whangerei City (population 35,000) is the only urban area in the Northland (population 107,000), and the Whangerei area is responsible for about half the total births.

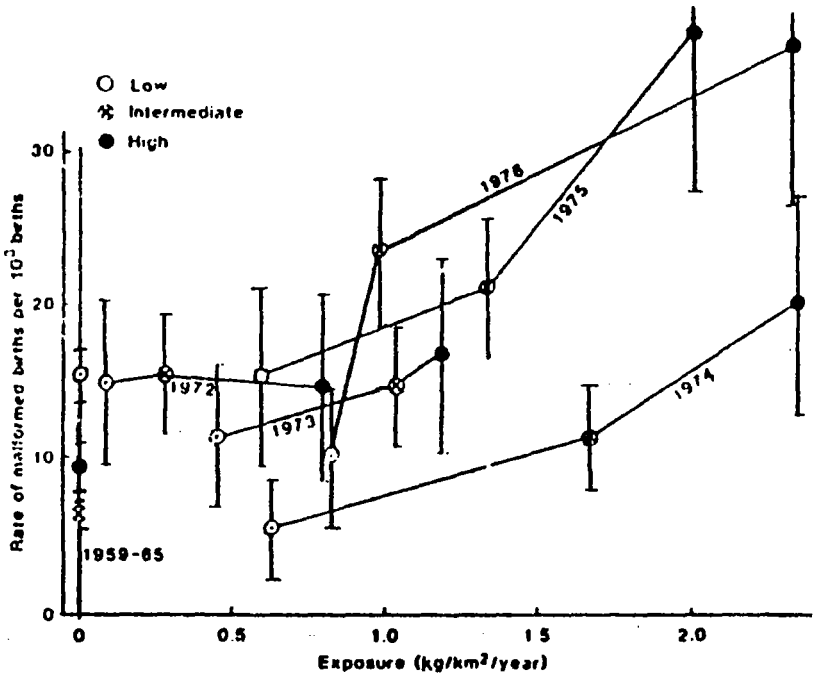


Figure 4 Distribution of birth malformations during years of no spraying (1959 to 1965), which are treated as 1 year, and from 1972 through 1976. Areas of low, intermediate, and high averaged annual spray density correspond to the various hospital areas: low includes Dargaville, Rawene, and Kaitaia; intermediate is Whangarei; and high includes Kawakawa, Kaikohe, and Kaeo. Poisson error bars are shown: decay hypothesis, f=1.00. Source: Hanify et al., 1981.

Table 35 Exposure to putative teratogen for individual malformations.

Malformation	Test	Exposure f = 1.00 T	f = 1.00 μ^*	f = 1.00 $p^\dagger$	f = 1.00 T	f = 1.00 μ^*	f = 1.00 $p^\dagger$
All birth malformations**	T	58	86	.033	231	155	.0000044
	M	103	122	.93	439	462	.89
	A	115	100	.20	639	397	.0089
	TMA	56	42	.037	231	157	.000013
Anencephaly	T	43	43	.60	152	133	.33
	M	85	92	.56	319	356	.70
	A	85	101	.65	319	337	.58
	TMA	41	43	.47	152	157	.49
Spina bifida	T	32	47	.76	135	156	.71
	M	74	89	.77	297	375	.83
	A	89	89	.63	297	332	.73
	TMA	31	42	.69	135	157	.72
Cleft lip	T	5	27	.96	149	156	.91
	M	10	100	.96	547	616	.69
	A	22	33	.60	547	368	.0096
	TMA	5	42	.96	149	157	.92
Isolated cleft plate	T	60	49	.14	271	143	.053
	M	170	109	.12	500	440	.066
	A	170	152	.36	500	322	.27
	TMA	60	42	.16	271	197	.079
All heart malformations	T	75	61	.044	355	152	.00011
	M	90	150	.89	697	535	.74
	A	115	135	.70	697	492	.45
	TMA	70	42	.10	355	157	.00009
Hypospadias, epispadias	T	104	50	.039	307	165	.00029
	M	127	126	.46	494	512	.59
	A	153	137	.30	494	497	.49
	TMA	100	42	.027	307	157	.00054
Talipes	T	52	61	.15	287	153	.035
	M	98	135	.87	481	479	.90
	A	109	93	.14	481	320	.0050
	TMA	51	42	.21	287	157	.050

* The expected value of T if exposure values are assigned randomly to malformed babies, that is, if there is no correlation between malformation rate and exposure. Units of T and μ are kilograms per 10^3 km^2 per year.

† The probability of a higher value of T if the exposure values are assigned randomly to the malformed babies.

**Includes stillbirths, neonatal death, and dislocated and dislocatable hip.

Source: Hanify et al., 1981.

Discussing these findings, the authors noted that the incidence rate of talipes is significantly higher among New Zealand Maoris than among New Zealand Caucasians. Therefore, Maoris and non-Maoris were analyzed separately. When the data were recombined for analyzes which allowed for different malformation rates, the correlations produced results almost identical with those of Table 35. Thus, the authors concluded that race was not primarily responsible for the correlations obtained for 2,4,5-T spraying and the incidence of talipes.

Swedish Occupational Epidemiologic Studies

Axelson and Sundell (1974); Axelson et al. (1979)

In 1974 Axelson and Sundell reported on a retrospective study of mortality and tumor incidence in Swedish railroad workers exposed to herbicides during the period 1957-1972 (Axelson and Sundell, 1974).

Four groups of workers were established: one with a total exposure to herbicides of >45 days and consisting of 348 people contributing 2,978 person-years at observation; another cohort with exposure >45 days to phenoxy acids and combinations and consisting of 207 persons contributing 1,747 person-years at observation; a third cohort with exposure >45 days to amitrol (3-amino-1,2,4-triazol) and combinations consisting of 152 persons contributing 1,285 person-years at observation; and a fourth group with exposure >45 days to other herbicides and combinations consisting of 28 persons contributing 293 person-years at observation.

These cohorts overlap each other with some cases common to more than one group, e.g., in the total cohort there were people with an exposure of 30 days to amitrol and combinations and another 30 days exposure to phenoxy acids and combinations. However, these persons were not included in either the phenoxy- or in the amitrol and combinations cohorts. For each cohort the expected number of deaths during the study period 1957-1972 was calculated from the Swedish average age- and sex-specific death and tumor incidence rates. To account for the latent period in carcinogenesis, Axelson and Sundell considered observed versus expected deaths and incidences without the latent period, or with a latent time of ≥3 and ≥5 years, respectively.

The exposures of the railroad workers were not exactly known in terms of concentrations of different herbicides in ambient air, but many workers reported wet skin on the face and on the hands due to herbicide aerosols. Despite this limitation, the assessment of the different exposures was judged reasonably accurate as the Swedish railway administration was able to provide comprehensive lists of persons engaged in herbicide spraying and good data in terms of exposure days. The study population was restricted to those with a total herbicide exposure >45 days (corresponding to about one spraying season) to avoid "dilution" by those with very low exposure.

As shown in Table 36 there was no statistically significant difference between the observed and expected mortality in the cohort exposed to phenoxy acids and

combinations. In contrast, significant differences were found for the amitrol and combinations cohort and for the other herbicides and combinations cohort.

Table 16 Observed and expected number of total deaths, deaths due to tumors (WHO chap. II) and lung cancer (WHO A 30) during 1957-72 among herbicide sprayers with a total exposure 45 days. Expected number based on Swedish average age and sex-specific death rates.

Exposure	Cause of death	Latent period* 0 yrs: Obs. pers.-yrs	0 yrs: Exp.	0 yrs: Obs.	1 yrs: Obs. pers.-yrs	1 yrs: Exp.	1 yrs: Obs.	5 yrs: Obs. pers.-yrs	5 yrs: Exp.	5 yrs: Obs.
Total	All causes	2,978	28.56	18	1,948	15.25	16	1,362	11.58	14
	Tumors		4.88	6		3.67	6		2.81	6
	Lung cancer		0.83	2		0.63	2		0.58	2
Phenoxy acids and comb.	All causes	1,747	13.48	10	1,128	9.33	8	770	6.88	7
	Tumors		2.97	2		2.22	2		1.68	2
	Lung cancer		0.51	0		0.39	0		0.29	0
Amitrol and comb.	All causes	1,285	9.31	9	839	6.13	8	581	4.62	6
	Tumors		1.96	4[1]		1.47	4[1]		1.11	4[1]
	Lung cancer		0.33	2[2]		0.25	2[2]		0.20	2[2]
Other herbicides and comb.	All causes	293	1.68	4[1]	209	1.34	4[1]	156	1.05	4[1]
	Tumors		0.39	3[1]		0.32	3[1]		0.26	3[1]
	Lung cancer		0.06	1		0.05	1[2]		0.04	1[2]

* [1]$p<0.05$ [2]$p<0.01$ [3]$p<0.001$

Source: Axelson and Sundell, 1974.

The authors noted that the non-significant excess tumor incidence in the cohort exposed to phenoxy acids and combinations might be explained by the additional exposure of four of the eight cases in this cohort to amitrol and combinations.

Axelson and Sundell also conducted a small case-control study as part of their analysis. The tumor incidence in 16 workers with more than 45 days combined herbicide exposure was compared with four age matched (±1.5 years) controls. A relative risk of 1.6 was calculated for men with one or more days of exposure to phenoxy acids and 4.6 for those exposed to one or more days to amitrol.

In a 1979 update of the earlier report, Axelson et al. refined their cohort analysis (Axelson et al., 1979). The deaths occurring in the same group of 348 persons who had worked for more than 45 days with herbicides for some time during the period 1957-1972 were analyzed through October 1978. In this report, the sample was divided into those persons exposed to amitrol and those exposed to phenoxy acids, as well as to those exposed to combinations of both herbicides. The expected number of deaths was calculated by multiplying the age- and sex-specific mortality rates of the official Swedish statistics by the number of observed person-years up to and including 1978. These fractional contributions of

expected mortality were then summarized over the respective calendar year and age strata.

A ten-year latency period factor was included in the calculations based on the time of first exposure to herbicides. (In the earlier study, the latency period was calculated from the time when more than 45 days of exposure had been reached, which sometimes took several years.)

The sample was also analyzed with respect to the time period when the herbicide exposure took place, i.e., whether it occurred during the first five-year period, 1957-61, or later.

Forty-five deaths occurred in the total sample as opposed to an expected 49 (Table 37). Disregarding the latency period, 18 observed tumor cases were found as against an expected 12 cases. In the different subcohorts, an excess mortality from tumors was noted, chiefly among persons with combined exposure to amitrol and phenoxy acids. Tumor related excess mortality in the subgroups with exposure to amitrol or phenoxy acids appeared moderate.

Table 37 Observed and anticipated mortality among 348 herbicide-exposed railroad workers with respect to preparation and combination of preparation. No requirements on induction-latency period.

Population	Death cause	Person years observation	Anticipated	Observed	quotient
Total cohort A+F+(A+F)	Total mortality	5541	49.02	45	0.9
	Tumors		11.85	18	1.5
	Stomach		1.34	3	2.2
	Lungs		2.19	3	1.4
Amitrol (A)	Total mortality	1545	13.77	7*	0.5
	Tumors		3.34	5	1.5
	Stomach		0.37	0	0
	Lungs		0.62	2	3.2
Phenoxy (F)	Total mortality	2265	23.03	20	0.9
	Tumors		5.62	6	1.1
	Stomach		0.65	2	3.1
	Lungs		1.05	0	0
Amitrol + phenoxy (A+F)	Total mortality	1731	12.22	18	1.5
	Tumors		2.89	7*	2.4
	Stomach		0.32	1	3.1
	Lungs		0.52	1	1.9

* $p < 0.05$

Source: Axelson et al., 1979.

When the latency period was considered, a more pronounced excess of tumor mortality appeared, especially in the subcohort with mixed exposure to amitrol and phenoxy acids (Table 38). The total mortality in the mixed exposure group as well as in the phenoxy acids group exceeded the expected values, while mortality in the amitrol group was lower than expected.

There was a significant excess of tumor mortality among those with the earliest exposure to herbicides. However, the validity of this finding is questionable since there was only a relatively small increase in the number of observed person-years for individuals exposed after 1962. The most noteworthy finding was the increased total tumor mortality among persons exposed early (14 cases against an expected 5.44), while those exposed after 1962 showed no clear excess mortality.

The authors noted that tumor mortality among phenoxy-acids-exposed persons increased in contrast to the earlier insignificant excess in the same group. In particular, there was a tendency toward stomach tumors among those exposed during the years 1957-1961 to either phenoxy acids alone or in combination with amitrol. However, the etiologic factors for this group were regarded as uncertain because the persons with mixed exposure appeared to be the most highly affected. The authors suggested that the increase in tumor mortality among those exposed earliest might be explained by more careless handling of the herbicides during that period or a greater contamination with chlorinated dibenzo-dioxines.

Table 38 Observed and anticipated mortality in different subcohorts of herbicide-exposed railroad workers with requirement for ten year induction latency period.

Population	Death cause	Person years observation	Anticipated	Observed	Rate quotient
Subcohort Amitrol	Total mortality	611.0	7.83	4	0.5
	Tumors		1.95	3	1.5
	Stomach		0.20	0	0
	Lungs		0.38	1	2.6
Subcohort phenoxy	Total mortality	816.5	12.53	17	1.4
	Tumors		3.14	6	1.9
	Stomach		0.33	2*	6.1
	Lungs		0.61	0	0
Subcohort Amitrol + phenoxy	Total mortality	782.5	7.25	15**	2.1
	Tumors		1.78	7***	3.9
	Stomach		0.18	1	5.6
	Lungs		0.35	1	2.9

* $p<0.05$; ** $p<0.01$; *** $p<0.0025$.

Source: Axelson et al., 1978.

Hardell (1977)

In 1977, Hardell reported on a series of seven patients with malignant mesenchymal tumors who were exposed to phenoxy acids 10-20 years before diagnosis (Hardell, 1977). These seven cases were drawn from a total of 87 patients with malignant mesenchymal tumors admitted to a Swedish regional hospital during the period 1970-1976. Thirty-two patients were female and 55 were male. Nine of the males were forestry workers, four worked in agriculture and forestry, and six were employed in saw mills or in the pulp industry.

All seven patients reported substantial exposure to phenoxy acids at various times during the 1950s and 1960s. Two patients were smokers and one a nonsmoker; smoking history was not given for the remaining patients in the series. Two tumors were diagnosed in the small pelvis, one in the rectal region, one in the upper leg, and three on the arms of the patients.

Hardell noted that it was impossible to draw conclusions as to the causal relationship between exposure to phenoxy acids and malignant mesenchymal tumors from the cases presented. However, he pointed out that the findings would justify further study because of the rare tumor type involved, the large degree of phenoxy acid exposure, the strong deviation of the sex ratio of this tumor type from the Swedish national average, and the general agreement with the latency period assumed for chemical carcinogenesis.

Hardell and Sandstrom (1979)

In 1979 Hardell and Sandstrom reported a case-control study of soft-tissue sarcomas in patients exposed to phenoxy acids and chlorophenol (Hardell and Sandstrom, 1979). The study consisted of 21 living and 31 dead male patients with histologically confirmed soft-tissue sarcomas. Cases ranged in age from 26 to 80 years and were admitted to a regional hospital during 1970-1977. Four controls matched for sex, age (±5 years), and place of residence were selected for each case. Controls for dead patients were chosen from the National Registry for Causes of Death; deaths from malignant tumors or suicides were excluded. Deceased controls were also chosen so that each was working until 2 years before the retirement of their matched case, or, if he had not retired, until 2 years before his death.

Living cases and controls and the next of kin for deceased cases and controls were initially contacted by telephone. They then received a comprehensive questionnaire by mail which included questions on occupational, environmental, and personal exposure to chemicals, smoking history, etc. Each questionnaire was supplemented as necessary by telephone interview with an individual who was unaware of the respondent's case or control status. To verify employment and phenoxy acids exposure, questionnaires were also sent to employers of those stating work in forestry, saw mills, or pulp industries. Low-grade exposures of less than one day and exposures less than five years before diagnosis of a tumor were not included in the analyses.

Fifty-two patients and 208 controls were included in the study population. Thirty-one patients were deceased. Thirty-six percent of the patients and 9.2 percent of the controls reported exposure to phenoxy acids or chlorophenols.

Questionnaires sent to employers to verify phenoxy acids exposure were judged uncertain and difficult to evaluate because records for individual workers had not been kept. Replies from employers in this group were obtained for 20 of 50 persons involved. In contrast, worker exposure to chlorophenols in saw mills and pulp industries were judged more accurate; data were provided for 97 percent of the study individuals in this group.

As shown in Table 39, the relative risk for soft-tissue sarcomas after exposure to phenoxy acids and/or chlorophenols was calculated as 5.7 for the entire study population. For those exposed to phenoxy acids only,

the relative risk was 5.3 (Table 40). The relative risk for those exposed to chlorophenols only was calculated as 6.6 (Table 41).

Table 39 Exposure to phenoxyacetic acids and/or chlorophenols in the total material

	Exp.	Non-exp.	Total
Case	19	33	52
Control	19	187	206
	38	220	258

Relative risk: 5.7 (95% confidence interval 2.9-11.3).
$x^2 = 24.6$.
$P < 0.001$.

Source: Hardell and Sandstrom, 1979.

Table 40 Exposure to phenoxyacetic acids only. Patients and controls with exposure to chlorophenols are excluded

	Exp.	Non-exp.	Total
Case	13	33	46
Control	14	187	201
	27	220	247

Relative risk: 5.3 (2.4-11.5).
$x^2 = 17.4$.
$P < 0.001$.

Source: Hardell and Sandstrom, 1979.

Table 41 Exposure to chlorophenols only. Patients and controls exposed to phenoxyacetic acids are excluded

	Exp.	Non-exp.	Total
Case	7	33	40
Control	6	187	193
	13	220	233

RR = 6.6.
P < 0.001.

Source: Hardell and Sandstrom, 1979.

Hardell and Sandstrom noted that all those exposed to either phenoxy acids or chlorophenols were also likely exposed to chlorinated dioxins, including 2,3,7,8-TCDD. Thus, they suggested that the increased risk among exposed individuals could be caused by the pure chemical substances, impurities in the commercial preparations, or a combination of both.

Eriksson et al. (1979)

In 1979 Eriksson et al. described a case-control study of soft-tissue sarcomas in those exposed to various chemical substances in the five southernmost states of Sweden (Eriksson et al., 1979). This report was intended to complement a similar study by Hardell and Sandstrom for the three northernmost Swedish states described previously.

In this report, cases included men between the ages of 25 and 75 years with histologically confirmed malignant mesenchymal tumors of the soft parts. Both living and deceased cases were used in the analysis. Each case was matched in terms of sex, age (±5 years), and place of residence with two controls. Deceased cases were also matched for year of death. For those individuals who died in 1978, controls were chosen from death records for 1977. Deceased controls were also required to have been employed up to five years before the retirement or death of their matched case.

Living cases and controls and the next of kin of deceased cases and controls were initially contacted by telephone. This was followed by a mail questionnaire regarding previous and current occupation, exposure conditions in the occupational environment, smoking habits, etc. Special attention directed toward phenoxy acids or chlorophenols was avoided. Questionnaire responses were supplemented by a second telephone interview conducted by an individual who was unaware of the respondent's case or control status.

Additional information on herbicide and pesticide exposure was sought from the employers of those who reported working in agriculture, forestry, or in tree nurseries. Information was also sought from agricultural board members and others to establish a uniform evaluation of occupational exposure to biocides. Low-grade exposures less than one day and exposures less than five years prior to a tumor diagnosis were disregarded.

The study population included 329 individuals, 110 cases and 219 controls. Thirty-eight of the cases died. Exposure to phenoxy acids or chlorophenols was reported by 22.7 percent of the test cases and 5.9 percent of the controls.

As shown in Table 42, the relative risk of malignant mesenchymal tumors of the soft parts following exposure to phenoxy acids or chlorophenols was calculated as 5.1.

When individuals exposed to chlorophenols were excluded, the relative risk for exposure to phenoxy acids alone was 6.8 (Table 43). In a separate analysis to clarify

Table 42 Exposure to phenoxy acids or chlorophenols within triplets (test case and two controls)

Case with exposure to phenoxy acids or chlorophenols	No. of controls with exposure to phenoxy acids or chlorophenols		
	2	1	0
Yes	0	4	21
No	0	9	76

Relative risk = 5.1 $x^2(1) = 20.1$
95% confidence interval = 2.5-10.4

Source: Eriksson et al., 1979.

Table 43 Exposure to phenoxy acids in test cases and controls when those exposed to chlorophenols were excluded and matching was eliminated

	Exposed		
	More than 30 days	30 days or less	Unexposed
Test cases	7	7	85
Controls	2	3	206
Relative risk	8.5	5.7	(1.0)

For exposed compared to unexposed individuals, one obtains relative risk = 6.8; $x^2(1) = 16.2$.
95% confidence interval = 2.6-17.3

Source: Eriksson et al., 1979.

risk for exposure to phenoxy acids without contamination by polychlorinated dibenzodioxins or dibenzo furans, the relative risk for other phenoxy acids (MCPA, 2,4-D, mecoprop, diclorprop) was 4.2 (Table 44). Analysis of exposure to chlorophenols, excluding those exposed to phenoxy acids, gave a relative risk of 3.3 (Table 45). Relative risks of 1.6 and 1.9 were calculated for high- and low-grade exposure to organic solvents, respectively. No risk calculations were reported for individual chemical pesticides other than phenoxy acids because of inadequate numbers of exposed cases and controls.

Eriksson et al. concluded that exposure to phenoxy acids may comprise a risk factor in the development of malignant mesenchymal tumors of the soft parts. They noted that the risk was not limited only to those phenoxy compounds which can contain PCDDs and PCDFs.

Table 44 Exposure to the phenoxy acids MCPA, 2,4-D, mecoprop and dichlorprop when exposure to 2,4,5-T and chlorophenols was excluded and matching was eliminated

	Exposed	Unexposed
Cases	7	85
Controls	4	206

Relative risk = 4.2; $x^2(1) = 5.9$
95% confidence interval = 1.3-13.4

Source: Eriksson et al., 1979.

Table 45 Exposure to chlorophenols in cases and controls when individuals exposed to phenoxy acids were excluded and matching was eliminated

	Exposed	Unexposed
Cases	11	85
Controls	8	206

Relative risk = 3.3; $x^2 = 6.8$
95% confidence interval = 1.3-8.1

Source: Eriksson et al., 1979.

Hardell, Eriksson, and Lenner (1980)

In 1980, Hardell and his colleagues reported the results of a case-control study of malignant lymphomas and exposure to chemical substances (Hardell, Eriksson, and Lenner, 1980). Cases included males between the ages of 25 and 85 with histologically confirmed malignant lymphomas who were admitted to a Swedish regional hospital between 1974 and 1978. Two controls were matched for sex, age (±5 years), and place of residence for each case. For deceased cases, deceased controls were used, also matched for year of death. In order to assure the same possibility for occupational exposure to various agents among cases and controls, deceased controls must have been employed up to five years prior to the last job held by each case individual.

Information on exposures was obtained by mail questionnaires supplemented by telephone interviews. Exposures to phenoxy acids totaling less than one day were not included in the analysis. For chlorophenol exposures, a division was made into high-grade and low-grade exposures. An exposure of at most one week or repeated short-term exposure totaling less than one month was regarded as low-grade. Exposure to organic solvents was divided in similar fashion.

In one analysis, a latency period was included by disregarding exposures less than five years prior to a tumor diagnosis. In a second analysis, no such latency criterion was used.

The study population consisted of 504 men, 159 cases and 335 controls. Of the cases, 60 had Hodgkin's disease and 109 had non-Hodgkin's lymphoma. Sixty-two cases with their corresponding controls were deceased. Of the cases, 36.1 percent reported exposure to phenoxy acids or chlorophenols compared to 9.6 percent of the controls.

As shown in Table 46, the relative risk of malignant lymphomas associated with exposure to phenoxy acids or chlorophenols was 6.0. To analyze for exposure to phenoxy acids alone, all persons with high-grade exposure to chlorophenols were excluded. The relative risk calculated for this groups was 4.8 (Table 47). No significant dose-effect relationship could be established when the study population was divided with different limits for total exposure times.

Among those exposed to chlorophenol or a combination of chlorophenol and phenoxy acids, high-grade chlorophenol exposure gave a relative risk of 9.3; the relative risk

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calculated for low-grade chlorophenol exposure was 2.5 (Table 48).

Table 46 Exposure to phenoxy acids or chlorophenols within triplets (case and two controls)

Cases with exposure to phenoxy acids or chlorophenols	No. of controls with exposure to phenoxy acids or chlorophenols		
	2	1	0
Yes	2	10	49
No	1	16	91

Relative risk = 6.0.
$x^2(1) = 53.3$.
95% confidence interval = 3.7-9.7.

Source: Hardell, Eriksson, and Lenner, 1980.

Table 47 Exposure to phenoxy acids in cases and controls after those exposed to chlorophenols were excluded and the matching was dissolved

	Exposed		
	≥90 days	<90 days	Unexposed
Cases	10	31	108
Controls	4	20	303
Relative risk	7.0	4.3	(1.0)

For exposed as opposed to unexposed, relative risk = 4,8 was obtained.
$x^2(1) = 35.3$.
95% confidence interval = 2.9-8.1.

Source: Hardell, Eriksson, and Lenner, 1980.

Table 48 Exposure to chlorophenols in cases and controls after those exposed to phenoxy acids were excluded and the matching was dissolved

		Exposed		
	Unexposed	Low-grade	High-grade	Total
Cases	83	14	25	39
Controls	277	19	9	28
Relative risk	(1.0)	2.5	9.3	4.6
$x^2(1)$	-	6.1	39.7	34.1
95% confidence interval	-	1.2-5.0	4.6-18.5	2.8-7.8

Source: Hardell, Eriksson, and Lenner, 1980.

Relative risks for exposures to other organic solvents, benzene, trichloroethylene, or styrene were also increased (Table 49).

When the cases were divided into two groups with Hodgkin's disease and non-Hodgkin's lymphoma, no apparent difference could be established for exposure to phenoxy acids, chlorophenols, or organic solvents.

Based on these results, the authors concluded that exposure to organic solvents, chlorophenols, or phenoxy acids present risk factors in the development of malignant lymphoma. They noted, however, that the mechanism for carcinogenesis is unclear and suggested that conceivable modes of action might be immunologic suppression by dioxin contaminants or a direct mutagenic effect of phenoxy acids.

Table 49 Exposure to organic solvents in cases and controls after matching was dissolved and those exposed to phenoxy acids (P) or high-grade to chlorophenols (C) were excluded, disregarding when simultaneous exposure existed. Category division into I = low-grade and II = high-grade solvent exposure

		Exposed				
			II			
	Unexposed	I	Other	Styrene, tri, benzene	I +II total	P, C total
Cases	60	10	30	10	50	23
Controls	222	33	40	8	81	7
Relative Risk	(1.0)	1.1	2.8	4.6	2.3	12.2
(1)	-	0.1	13.7	11.1	13.0	42.5
95% confidence interval	-	0.5-2.4	1.6-4.8	1.9-11.4	1.5-3.6	5.7-25.8

Source: Hardell, Eriksson, and Lenner, 1980.

Summary

The preceding retrospective studies of Swedish workers demonstrate a statistically significant relationship between general types of malignant disease and prior exposure to phenoxy acids and chlorophenols. While questions remain about the comparability of cases and controls, recall bias, and confounding variables, especially the contaminant TCDD, these studies appear to minimize these methodological problems. Thus, they provide consistent, albeit less than definitive evidence of herbicide-related occupational carcinogenesis. However, cohort studies of U.S. workers involved in the manufacture of 2,4,5-T and chlorophenol do not support these findings (Ott et al., 1980; Zack et al., 1980). Although these studies employed a different methodology and, hence, are not strictly comparable, they reported no excess mortality in their study populations. A more precise resolution of the differences between these studies likely depends on a prospective study of herbicide exposure and tumor incidence.

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History

This defoliant, well known because of its use in the jungles of Vietnam, consists of equal parts 2,4-D and 2,4,5-T. One gallon of Agent Orange theoretically contained 4.21 pounds of 2,4-D and 4.41 pounds of 2,4,5-T. Other herbicide formulations which were used as defoliants were:

Agent Blue:	3.10	lb/yd cacodylic acid
Agent Purple:	8.6	lb/gal n-butyl 2,4-D, n-butyl 2,4,5-T iso butyl 2,4,5-T in a 5:3:2 mixture
Agent White:	.54	lb/gal picloram 2.00 lb-2,4-Dx in a 1:4 mixture
Agent Pink:	8.16	lb 2,4,5-T; n-butyl and isobutyl esters in 6:4 mixture
Agent Green:	8.16	lb n-butyl ester of 2,4,5-T

The National Academy of Science (Blackman et al. 1974) reported that from August, 1965, through February, 1971, 2,962 herbicide missions (out of a total 6,237 missions for all herbicides and all uses) for forest defoliation used Agent Orange. These 2,962 missions accounted for 90 percent of all Agent Orange used in Vietnam. From August, 1965, through February, 1971, crop destruction missions with Agent Orange accounted for eight percent of the herbicide applied. The remaining two percent of Agent Orange was used in South Vietnam around base perimeters, cache sites, waterways, and communication lines (Blackman et al. 1974). 2,4-D and 2,4,5-T were used for their selective effects on broadleaf plants and non-effect on grasslike plants such as rice or seed grains.

Reports of the concentration of TCDD in Agent Orange vary. Table 50 summarizes research on the reported range of concentration of various herbicides and TCDD.

Table 50. Concentration, ppm, of TCDD in Samples of Herbicides Orange and Purple[a]

Source of Samples	Number of Samples Orange	Purple	Range of TCDD (ppm)	Mean TCDD, Concentration (ppm)
Johnston Island, Inventory, 1972[b]	200	(4)[c]	0.05-47	1.91
Johnston Insland Inventory, 1974	10		0.07-5.3	1.68
NCBC, Gulfport Inventory, 1972[d]	42		0.05-13.3	1.77
NCBC, Gulfport Inventory, 1975	238		0.02-15	2.11
Eglin AFB Archived Sample		1[e]	-	45
Eglin AFB Inventory, 1972	2		-	0.04

The Weighted Mean Concentration of TCDD in Orange = 1.98 ppm

[a]Analyses for TCDD performed by Interpretive Analytical Services, Dow Chemical U.S.A., Midland Michigan; Aerospace Research Laboratories, Wright-Patterson AFB, Ohio; and The Brehm Laboratory, Wright State University, Dayton, Ohio.

[b]Surplus Herbicide Orange was shipped from South Vietnam to Johnston Island for storage in April 1972.

[c]Four of 200 samples may have been Herbicide Purple.

[d]The Naval Construction Battalion Center (NCBC) Gulfport, Mississippi served as a storage site for Surplus Herbicide Orange from 1969 to 1977.

[e]Herbicide Purple was extensively used in the evaluation of aerial spray equipment on Test Area C-52, Eglin Air Force Base Reservation, Florida, 1962-1964.

Source: Young, et al. 1978.

The weighted mean concentration for all TCDD in Herbicide Orange sprayed in South Vietnam was 1.98 ppm, with individual lots containing higher (15 ppm) or lower (0.02 ppm) concentrations of TCDD (Young et al. 1978).

Herbicide Orange was sprayed on 3.5 million acres from 1965 through 1970. Ninety percent of Agent Orange was sprayed on 2.9 million acres of inland forests and mangrove forests.

Of the 107 million pounds of herbicide aerially disseminated in Vietnam, 44 million pounds were 2,4,5-T. This amount, 41 percent of the total herbicide application, contained an estimated 368 pounds of TCDD. Ninety six percent of all 2,4,5-T was attributed to Agent Orange, while the remaining four percent constituted Herbicides Green, Pink, and Purple. These latter herbicides contained approximately 40 percent of the estimated amount of TCDD disseminated in Vietnam.

If one compares Vietnam to Seveso, Italy, for total amounts of TCDD spread per acre of area as a measure of total exposure, the following analysis can be made.

Seveso (high and median exposure) = 1.3 gm/acre
Vietnam (358 lb on 3 million acres) = 0.05 gm/acre

Even though there is a variability in the concentrations expected in both areas of exposure, it would appear that the Seveso population in the high and median exposure area was exposed to an order of magnitude more TCDD than people in Vietnam who might have been exposed to Dioxin from the use of herbicide sprays.

Agent Orange and the Present Complaints about Its Alleged Effects

Within the last two years, American Veterans of the Vietnam war have expressed concern about their poor health in relation to their individual exposure to Agent Orange. In 1978, after discovering he was suffering from terminal cancer, Paul Reutersham formed Agent Orange Victims, International. Having read of the possible link between Agent Orange and ill health through a UPI newspaper release, Reutersham was convinced that his cancer was a direct result of his exposure to the defoliant. He hoped his organization would inform Vietnam veterans of the situation and force the Veterans' Administration to award compensation for Agent Orange related maladies. To date, the V.A. recognizes only chloracne as an herbicide related illness although several V.A. hospitals have set

up Agent Orange screening clinics to investigate this problem further.

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Agent Orange--Future Plans

Research on the effects of the use of Agent Orange will not only continue, but will also be intensified to determine what, if any, residual effects have resulted from exposure to the defoliant.

To that end, the U.S. Army is initiating Operation Ranch Hand, a research project to conduct complete physical examinations, and determine a wide range of health parameters, on those most directly involved with Agent Orange in Vietnam--the people who handled the herbicides directly.

Although Operation Ranch Hand is slated to begin this year, the results of this study will not be available for several years.

The Vietnam story is far from over. Out of all the 2,4,5-T and TCDD related incidents, the Agent Orange episode has the most gaps, the most unanswered questions. These veterans do suffer from many ailments. Such ailments cannot and should not be disputed. What has not been established, but may be answered by continuing and future research, is the exact cause or causes of their afflictions. Only time will tell if Agent Orange is indeed the culprit. But as the situation rests today, the scientific support for these claims has yet to be established.

Reutersham died of cancer in 1978 at the age of 28. However, his organization survives and is currently filing suit against six major producers of Agent Orange used in the Vietnam war. The class action suit seeks establishment of a $40 billion trust fund as compensation for the adverse health effects afflicting the veterans and their families allegedly caused by Agent Orange. These companies are, in turn, filing suit against the Armed Forces, claiming negligence on the part of the military for failing to warn soldiers of the dangers of exposure to the herbicide.

The issue at hand is a complicated one. These veterans, after having returned from an unpopular war to something less than a hero's welcome, are suffering from a variety of physical and psychological ills. They say their children are born with birth defects because of their father's contact with Agent Orange. There has been no scientific documentation of such a cause and effect relationship.

The illness that afflicted Paul Reutersham, is also alleged to have been a direct result of Agent Orange spraying. In an interview for the documentary, War Shadows, (Eisemann) Paul Reutersham expressed what he saw as the logical course of events leading to his untimely and rapidly approaching death. First there is dizziness and nausea, chloracne, psychoses, neuroses and rapid weight loss. "Within three months, you have cancer."

The problem scientists have had in accepting liver cancer as a direct result of herbicide spraying is one of the calendar. Classically, it takes twenty to twenty-five years for primary liver cancer (an exceedingly rare disease) to manifest itself. In the absence of established scientific documentation, scientists are reluctant to attribute it to an outside influence which occurred, in most cases, less than ten years ago.

Scientific Studies

The first reports linking possible adverse health effects to Agent Orange appeared in Vietnamese newspapers between June 26, 1969 and July 5, 1969 (Advisory Committee on 2,4,5-T). The public response to these reports prompted the retrospective studies of Vietnamese hospital records conducted independently by Cutting (1970) and Meselson (1971).

The President's Advisory Committee on 2,4,5-T (1971) evaluated these studies and concluded the following:

"Summarizing the Vietnam data on human embryotoxicity, it can be said that (1) the sample of births surveyed was from year to year a variable but usually very small fraction of the total number, (2) it was quite unrepresentative of the geographic and ethnic distributions, (3) the heavily sprayed and otherwise exposed areas were greatly under-represented, and (4) the birth records were not trustworthy and, therefore, the rates of stillbirth, and especially of congenital malformation, derived from them were equally unrealizable. For example, the overall congenital malformation rate found in South Vietnam, 4.91 per 1000 livebirths, is about half of what was reported in other studies in various parts of Asia, and possibly a quarter of what might actually exist at term. A further indication that the newborn children were not carefully examined is the absence of Down's syndrome in the list of specific malformations compiled by the Army survey (Cutting, et al.) (1970) despite the fact that some Oriental populations have been reported to have an incidence of this condition not unlike that in Western populations.

Finally there is, and can be, no precise knowledge or reasonable approximation of the exposure to 2,4,5-T (and hence, TCDD) experienced by pregnant Vietnamese women, including what amounts they ingested or absorbed and when this may have occurred during pregnancy. Thus, any attempt to relate birth defects or stillbirths to herbicide exposure is predestined to failure. It can only be concluded that the birth records that have been surveyed, and probably any that will be surveyed in the future, for South Vietnam for the period 1960-1970 cannot answer positively the questions about possible adverse prenatal effects following human exposure to 2,4,5-T. It must be emphasized, however, that the searches that have been made almost certainly would have revealed any marked increase in the incidence of birth defects or the introduction of a striking defect such as that produced by thalidomide. In spite of considerable effort, no such occurrences were found."

A further study on birth defects in South Vietnam was performed by Dr. Ton That Tung, et al. (1971). They reported that out of 903 South Vietnamese seeking shelter in the North, in hospitals and [illegible], 19 adult women, including four mothers and [illegible] children between the ages of 6 and 14 had experienced [illegible] exposure to herbicide sprays while living in the [illegible]. Of the four mothers, two had given birth to children with [illegible] syndrome. Among the 70 children, numerous cases of [illegible] (ocular lesions, lumps on forehead, [illegible] that extended outward) and chromosomal [illegible] were evident. Tung concluded: (translation in [illegible])

> "Though still limited in [illegible], clinical observations confirm the [illegible] on animals by American researchers. The massive and prolonged utilization [illegible] defoliates permanent ocular lesions, [illegible] genomic alterations [illegible] swelling to ancestral [illegible] can provoke among their [illegible] malformations the importance of [illegible] determined. In the abominable [illegible] ever seen such an [illegible] the survivors except [illegible]

A study similar [illegible] performed by Rose and Rose (1972). [illegible] miscarriages, spontaneous abortions [illegible] animals following exposure [illegible]

The linkage of [illegible] to 2,4,5-T suggested by Tung has been [illegible] et al--in a

report by Honoroff (1973)). Grumner's comments after inspection of both North and South Vietnam were:

> "Provided that it be understood that this estimate cannot be anything but a cautious one, it may be assumed that there are at least 25,000 children with hereditary defects in South Vietnam. This does not include all the unborn babies whose mothers were sprayed during the missions that were flown in recent months. It does not include those who were stillborn, or died soon after birth, on account of their serious chromosomatic defects. Even after the war, it will probably be possible to arrive at only an approximation of the entire scale of this crime since one will only be able to examine the survivors when the time comes."

Tung, et al., also reported on another adverse health effect he attributed to defoliant exposure. In 1973, Tung reported an increase in the number of persons diagnosed with primary liver cancers in proportion to all cancer patients admitted to Hanoi hospitals during the period 1962-1968. He compared the 790 liver cases out of 7,911 total cancer cases (10%), with those documented in earlier pre-spraying years, 1955-1961. --- 159 liver cases out of total cancer incidence of 5,492 (2.9%).

In a 1977 International Agency for Research on Cancer (IARC) monograph, (1977), the Tung study was criticized for its limitations in reporting which they considered resulted in an inadequate assessment between herbicide spraying and liver cancer frequency.

Ford, et al., (1978), offered a further factor worthy of consideration, the consumption of aflatoxin-contaminated food in parts of the East. Aflatoxin occurs naturally as a contaminant in cereal grains and has been suggested as an explanation for the increase in liver cancer incidence. War conditions would be expected to increase aflatoxin risks in available food grains.

In 1974, the National Academy of Science (NAS) announced results of its study of Vietnam during 1972-1973. The NAS could find no conclusive evidence of association between exposure to herbicides and birth defects in humans. An analysis of hospital records of three Vietnamese hospitals showed no consistent pattern of association between malformations and annual amounts of herbicides disseminated.

To date there is still a great debate about the harmful effects resulting from human exposure to Agent Orange in Vietnam. Studies performed by Tung have generally been met with skepticism by the scientific community.

Dr. Matthew Meselson, of Harvard, says, "I don't think there is convincing evidence that TCDD is causing serious health problems." (Science, August 1979). He adds, however that since there is no convincing evidence to the contrary, "there is good reason to be concerned."

Dr. Frederick Coulston, of Albany Medical College, state: that "there is no evidence of immediate (or long term) harm in the amount of dioxin people were exposed to" in Vietnam. (Science, August 1979).

Another scientist, Dr. John Moore of the National Institute of Environmental Health Sciences, said there is "no scientific evidence" that "chemical insult to man" could result in birth defects. (Science, August 1979). Even Dr. Tung is not aware of any case where birth defects appeared to have been transmitted through the father.

As for the health effects suffered by the people in South Vietnam, it would appear to be an increasingly difficult task to demonstrate a causal relationship between herbicide spraying and birth defects and cancer. Factors such as nutrition and standard of living are difficult to determine in a country suffering the ravages of war.

In addition, the enforced nomadic existence of the Vietnamese during the war made it almost impossible to quantify actual exposure to the herbicide.

Agent Orange Summary

There is a paucity of human evidence describing the long term adverse effects of 2,4,5-T and TCDD in man because most exposure to these chemicals results from accidents.

Of the evidence that has been accumulated, however, no definitive data have established a direct link between the herbicide and its contaminants with cancer or birth defects. These are the most often cited adverse health effects alleged to be associated with 2,4,5-T or TCDD.

The only demonstrated illness proven to occur in people from exposure to these chemicals is chloracne, a severe acne-like skin eruption. This skin disease is attributed solely to the dioxin contaminant in the herbicide and is such a classic sign that it may be conisdered diagnostic of dioxin poisoning.

The Seveso exposure serves to refute claims of intermediate harm, including birth defects and miscarriages due t

TCDD. If TCDD causes a given response or reaction, it should do so each time a large number of people are exposed. This did not happen in Seveso, which should be strongly emphasized when considering suspected TCDD problems in areas such as Vietnam or Alsea, Oregon.

As further research continues and additional retrospective investigations are performed on the chemical exposure victims, the resulting data may in the future either confirm or deny the adverse health implications of 2,4,5-T and dioxin. However, at present, the data do not exist to make such claims.

EPA REGULATORY HISTORY

2,4,5-T was first developed in the early 1940s. Registered as a pesticide in 1948, (EPA 1978), the herbicide has been the subject of controversy in all parts of the world.

On April 3, 1966, the USDA and the FDA published an announcement covering all pesticides. It abolished the "No Residue and Zero Tolerance" concepts as scientifically untenable. Future registrations would be granted on the basis of "Negligible Residue" or "Permissible Residue". Industry was given until December 31,1967, to comply by obtaining tolerances for residues of 2,4,5-T in all treated food, feed products, and byproducts. (EPA, April 1978).

In the following years, several Pesticide Registration (PR) notices were issued, extending certain "No residue" and "Zero tolerance" beyond the deadline for obtaining residue tolerance. Among uses of 2,4,5-T extended beyond the deadline were uses on grassland pastures and rangeland, McIntosh apples, low bush blueberries, cereal grains, rice, sugarcane, and water in lakes and ponds.

In March of 1970, the USDA issued a report requesting that further teratogenic studies be performed on certain compounds, including 2,4,5-T.

On April 20, 1970, the use of 2,4,5-T was suspended in lakes, ponds, and on ditch banks, and, in liquid formulations around homes, recreation areas, and similar sites. On May 1, 1970, the USDA issued cancellation orders on 2,4,5-T products. Uses listed were for granular formulations for use around the home, recreational area, and similar sites, and all 2,4,5-T uses on food crops intended for human consumption. After this action, Dow Chemical and Hercules Inc., exercised their rights as registrants to petition for referral of cancellation on riceland to an Advisory Committee.

On May 7, 1971, the President's Advisory Committee recommended the following to the EPA: 2,4,5-T be permitted in forestry, range land, and rights-of-way, provided that a limit of 0.1 ppm TCDD content be set for all newly produced 2,4,5-T; that 2,4,5-T be applied no more than once a year to any one site; that 2,4,5-T be applied with caution so that it will not contaminate other areas where it may come into contact with humans. The Committee also requested an additional review once gaps in scientific research regarding the food chain have been closed.

Meanwhile, on September 28, 1970, the USDA issued a report in which they determined that the presence of TCDD as a

contaminant in the manufacturing process of 2,4,5-T was a hazard. The notice stated that TCDD constituted a possible hazard to man since it has been found to be extremely toxic to laboratory animals. Appropriate action would therefore have to be taken by the EPA under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), as amended, since products containing chlorodioxins are considered to be in direct violation of FIFRA.

The Dow Chemical Company obtained an injunction against the EPA, enjoining further administrative action against 2,4,5-T. But the Eighth Circuit U.S. Court of Appeals overturned the injunction in 1973 and the administrative proceedings continued.

On July 20, 1973, a notice of intent to hold hearings on all uses of 2,4,5-T was filed with the Hearing Clerk. All federally approved uses of 2,4,5-T were to be explored in a public hearing scheduled for April 1974, following an intensive monitoring program for TCDD residues in the ppt range.

On May 10, 1974, the informational hearing was expanded to all insecticides and herbicides which include 2,4,5-Trichlorophenol used in their manufacturing process.

On June 24, 1974 the EPA cancelled the information gathering hearings and its other proceedings initiated against 2,4,5-T and related compounds because of its inability to monitor food for TCDD residues with the necessary analytical precision.

On July 25-26, 1974, the Agency held a Dioxin Planning Conference to address the problem of data analysis and retrieval in the areas of analytical methodology, toxicology and monitoring. Of particular concern was a method for monitoring TCDD in the ppt range. As a result of these meetings, the EPA formed the Dioxin Implementation Plan to identify a preferential analytical method for determining TCDD residues in soil, water and biota.

On April 21, 1978, the EPA issued a Rebuttable Presumption Against Registration (RPAR) against 2,4,5-T. The Agency found that the herbicide had exceeded certain risk criteria relating to acute or chronic toxic effects. Under FIFRA the registrants have the opportunity to rebut the presumption and public hearings may ensue. 2,4,5-T use may continue pending outcome.

On February 28, 1979, the EPA issued an Emergency Suspension Order (use prohibited pending outcome of hearing) with the Intent to Cancel uses of 2,4,5-T on rights of way, pastures, forests, home gardens, aquatic weeds, ditch banks and ornamental turf. Exempt from this ban were applications to rangeland and rice paddys. The EPA acted with such speed because of their recently released Alsea II report. But they added that results from animal studies had supported their action. Along with the suspension notice, the EPA issued cancellation hearing notices for each of the suspended uses of 2,4,5-T. Dow and other registrants filed appropriate objections.

Suspension hearings began on April 19, 1979. However, all participants withdrew from the hearings on May 15, 1979.

In July, 1979, the EPA published its intent to issue a hearing notice for all the non-suspended uses of 2,4,5-T. But the EPA's own Scientific Advisory Panel recommended against holding these hearings at this time. "After extensive review of the data, we find no evidence of an immediate or substantial hazard to human health or to the environment associated with the use of 2,4,5-T on rice, rangeland, orchards, sugar cane, and the non-crop uses specified in the decision documents."

The consolidated hearings on the suspended and non-suspended uses of 2,4,5-T commenced in Washington, D.C. on March 14, 1980. A long court battle is expected to take place.

EPA Regulatory History Summary

The EPA course for regulation of the herbicide 2,4,5-T has been a stormy one. With increasing regulation and monitoring of the herbicide, primarily for its contaminant TCDD, the Agency has often been at odds with the registrants of the herbicide.

In February, 1979, the EPA issued an immediate emergency suspension order for certain uses of 2,4,5-T, citing imminent health hazards based on the recently released Alsea II report. Although this report has met with scientific criticisms and repudiation by the scientific community, the cancellation hearings have not been stopped. On March 14, 1980, the cancellation hearings finally began in Washington, D.C. The results of these hearings will not be quickly forthcoming. A long court battle is expected.

CONCLUSION

A thorough analysis of the effects of 2,4,5-T and its contaminant, dioxin, is not possible using the presently available boundaries of finite studies for a select number of species. The bibliography of 2,4,5-T research contains thousands of references, which requires that selected works must be chosen to represent the body of knowledge accumulated on the subject.

It has been our purpose to select those representative papers which have passed peer review and have withstood criticisms directed against them. Indeed, these papers are the ones most often quoted and referred to in many other scientific exercises devoted to 2,4,5-T. If a paper has played an important role in the course of the 2,4,5-T dispute but nevertheless was the object of scientific attack on the data or conclusion, appropriate comments have accompanied the research paper.

There is a wealth of animal research on both 2,4,5-T and dioxin. Toxicity, teratogenic effects and a carcinogenic potential have all been established using these chemicals at high doses. However, an equally important fact is that doses have appeared in all categories from which no observable effect has been found. The authors of this position paper acknowledge these reports to be true No-Effect levels and feel that the establishment of these levels should be accepted as fact in any analysis of the adverse effects of either 2,4,5-T or TCDD.

Human evidence, on the other hand, is not so extensive. In many cases, the gaps in the data remaining to be filled far outnumber what is now known. Analysis of long term effects of dioxin or 2,4,5-T exposure have been negative or inconclusive at best. Researchers have repeatedly failed to show a direct link between exposures to these chemicals and cancer and birth defects, the two main effects often anticipated by researchers.

However, lack of evidence against these adverse health effects cannot be considered to disprove the existence of these health effects. It is impossible to establish unequivocally that 2,4,5-T and dioxin exposure or any other cause does not result in cancer or birth defects. This applies particularly to Seveso and Vietnam, where the exposure to dioxin levels was considerably higher than those normally resulting from the use of the herbicide in land management, and from which, therefore, there would be a greater likelihood of adverse health effects.

The final consideration addressed is that of the economic importance of 2,4,5-T. Formulated as an herbicide which did the job which others had failed to perform, 2,4,5-T fulfills a need which continues today. Because of its effectiveness against broadleaf weeds, 2,4,5-T can outperform alternative herbicides or manual weed management both agriculturally and economically in terms of cost effectiveness. A substitute would mean higher costs for alternative chemicals or for manual labor to accomplish the same results achieved by 2,4,5-T. The increased cost would, in turn, have to be passed on to the consumer, if the land manager could survive the added expense. Otherwise the U.S. might see the bankruptcy of many agricultural producers who rely on this herbicide to perform successfully and cost-effectively.

If the Administrative Court of the EPA decides in favor of the EPA, in the current EPA cancellation hearings, to ban all uses of 2,4,5-T, the information presented in this position paper may remain academic. Whatever the outcome of the hearings, the decision will have far-reaching consequences, not only for the principals involved in herbicide use, but for the American-consuming public as well.

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1729

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17738

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R & D REPORT

DOW CHEMICAL U.S.A.

CRI NUMBER:

LABORATORY REPORT CODE: HET K-002372-043 P

DATE ISSUED: February 26, 1986

DEPARTMENT/CITY: Mammalian and Environmental Toxicology Research Laboratory

LAB. NO. | PROBLEM NO.

TITLE: 2,4-D ACID: ACUTE TOXICOLOGIC PROPERTIES

PAGES IN FULL REPORT: 1

AUTHOR(S)/MASTER NUMBER(S): J. M. Wall/077592

AUTHOR(S) SIGNATURE(S): J M Wall 26 Feb 86

REVIEWER'S SIGNATURE: K. S. Rao 2/26/86 K. S. Rao

This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

A sample of 2,4-D Acid, identified as batch number 850914/20865/1, was submitted for acute toxicologic evaluation. The source of this buff-colored solid was Ivon Watkins, Dow Ltd., New Plymouth, New Zealand. The test material, which is in commercial production, contains 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol.

Acute oral toxicity testing was conducted with Fischer 344 rats (Charles River Breeding Laboratories, Inc., Kingston, NY). Eye and skin irritation tests were conducted with New Zealand White rabbits (Hazleton-Dutchland, Inc., Denver, PA).

The acute oral toxicity of the test material was low. Range-finding tests indicated the single dose oral LD50 for female rats was greater than 1000 mg/kg. Three rats/dose group received the test material as a 10% suspension in corn oil, by oral gavage, at dose levels of 250, 500, or 1000 mg/kg. Lethargy, the only in-life sign of toxicity, was observed in the 1000 mg/kg dose group. All rats survived and steadily gained weight during the two week observation period.

Test material was instilled into the conjunctival sacs (eyes) of a male rabbit at a dosage of 0.1 g/eye. The single exposure resulted in slight to moderate discomfort, severe conjunctival redness and swelling, severe reddening of the iris, and severe corneal opacity. Ocular effects, which were still present in both eyes 21 days after exposure, were more pronounced in the eye where washing was not conducted immediately after treatment.

Test material was applied topically to intact and abraded skin of two male rabbits under dry or moistened conditions. The material was applied to the abraded sites for three applications and to the intact sites for eight applications at a dosage of 0.5 g/application. Essentially no irritation was observed after prolonged (24 hours) or repeated contact with the test material. Results of the skin irritation tests indicated the test material is unlikely to be absorbed in acutely toxic amounts.

DOW 2455429

ARE MATERIALS DESCRIBED IN THIS REPORT ON TSCA INVENTORY? YES [X] NO [ ]
IF NO, LIST MATERIALS ON REVERSE SIDE OF THIS PAGE THAT WE WILL USE OR PRODUCE COMMERCIALLY THAT WILL REQUIRE A PMN.

17736

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DOW CHEMICAL U.S.A.
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MAMMALIAN & ENVIRONMENTAL TOXICOLOGY RESEARCH LABORATORY
MIDLAND, MICHIGAN

FILE NO.
MET – K-002372-043

NAME OF CHEMICAL OR FORMULATION: 2,4 – D Acid

MOLECULAR FORMULA:

SYNONYMS: B/N 850914/20865/1

SAMPLE REFERENCE AND SOURCE:

MOLECULAR WEIGHT:

STRUCTURAL FORMULA - OR COMPOSITION

	gram/Kg	Batch No:
2,4 – Dichlorophenoxyacetic Acid	859	850914/20865/1
2,4 – Dichlorophenol	3	
Moisture	12g	
Inerts	Balance	

PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT °C mmHg.	EXPLOSIVE LIMIT (% BY VOL. IN AIR)	FLASH POINT °F	IGNITION TEMP. °C	MELTING PT. °C	VAPOR PRESSURE mmHg. @ °C

CORROSIVENESS (TO COMMON METALS): NONE	pH	PHYSICAL STATE: SOLID	COLOR: BUFF	PURITY (%)

CHEMICAL REACTIVITY: NOT HAZARDOUS	ODOR (INCLUDE CONCENTRATION IN AIR): TYPICAL PHENOXY PRODUCT
STABILITY (TO pH CHANGE, HEAT, LIGHT): NOT HAZARDOUS	SOLUBILITY: INSOLUBLE IN WATER

CAS NUMBER:

STAGE OF DEVELOPMENT:

☐ RESEARCH ☐ BENCH ☐ PILOT PLANT ☐ SEMI PLANT ☑ PLANT ☐ REGISTRATION

PROPOSED AND POTENTIAL USE:

PRODUCT IS IN COMMERCIAL PRODUCTION.

METHOD OF HANDLING: (PLEASE STATE ANY NECESSARY HANDLING PRECAUTIONS WHICH NEED TO BE TAKEN)

Batch-wise production. Some manual handling in plant. Potential for eye/skin contact and ingestion/inhalation user and to lesser extent by manufacturing personnel.

AMOUNTS TO BE HANDLED:

Large quantities. Package containers of 200 Kgs.

HUMAN EXPERIENCE IN HANDLING:

No Problem. Has been handled for many years.

PURPOSE(S) FOR TOXICOLOGICAL TESTS:

- ☑ DATA SHEET FOR SAFE HANDLING
- ☑ MATERIAL SAFETY DATA SHEET/LABEL
- ☐ IS TOXICITY COMPATIBLE WITH INTENDED USE
- ☑ HAZARD CLASSIFICATION FOR SHIPPING
- ☑ REGISTRATION
- ☐ PRE MANUFACTURE NOTIFICATION (PMN)
- ☐ OTHER — PLEASE EXPLAIN

NOTE: FOR ALL CIA STUDIES A CHEMICAL ANALYSIS ON THE ACTIVE INGREDIENT (AT THE MINIMUM) OF EACH TEST SAMPLE MUST BE SUBMITTED.

IS THE MATERIAL SUBMITTED FOR TESTING ON THE TSCA INVENTORY? YES ☑ NO ☐

CHARGE NO.: Charge IWD, New Zealand

DEPARTMENT NAME:

ESTIMATED COST FOR TESTS:

AMOUNT OF MATERIAL SUBMITTED: 400 GRAMS ____ MILLILITERS

ADDITIONAL NAMES & ADDRESS FOR REPORTS:

R.W. MOFFAT
IVON WATKINS-DOW LTD,
PRIVATE BAG,
NEW PLYMOUTH,
NEW ZEALAND.

TO AVOID DELAYS PLEASE FILL OUT AS COMPLETELY AS POSSIBLE!

- ☑ CLASS I SCREEN FOR TOXICITY
- ☑ CLASS IA FOR REGISTRATION
- ☐ OTHER

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[signature]	10/10/95	Ivon Watkins-Dow Ltd New Plymouth NZ	

FORM C-22551 R-6-95

rec'd 11/6/95 439gm Nil

17737

ACUTE ORAL TOXICITY

TOXICOLOGY RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN

FILE NO. K-002372-043

MATERIAL: 2,4-D Acid

CC + SYRINGE ____ g
EMPTY SYRINGE ____ g
CC ____ g
SP. GRAVITY ____

☐ SOLUTION ☒ SUSPENSION
1.0 gm IN 10 ml volumetric flask brought up to volume
☐ WATER ☒ CORN OIL
☐ OTHER ____ 12/4/85

SPECIES: ☒ RAT ☐ CAVY ☐ RABBIT ☐ MOUSE
AGE: 7 week
633 RACK
SEX: ☐ M ☒ F

DATE/TIME FED	CAGE	ANIMAL MARKING	WEIGHT GM.	DOSE GM./KG.	DOSE CC.	ANIMAL WEIGHT (GM.) 12-4-85	12-11-85	12-18-85
11:00 12-4-85	3	858-0963	72	~~.25~~	.18	81	98	110
		0964	92		.23	104	118	125
		0965	77		.19	88	103	113
	4	0966	98	.50	.49	110	124	130
		0967	90		.45	106	121	128
		0968	90		.45	103	121	127
	5	0969	85	1.0	.85	90	110	117
		0970	100		1.00	108	125	130
		0971	86		.86	92	111	122

Stc 12/13/85

DOW215429

17738

OBSERVATION BY: [illegible]

COMMENTS ON BACK OF THIS PAGE

SIGNED [illegible]

DATE 12/11/85

METHODOLOGY

k-2372-(43)

Animals used for acute oral toxicity tests are fed by single-dose gavage using a calibrated syringe and a blunt feeding needle. The animals are fasted overnight (except rabbits and mice) and are weighed before dosing, the day after dosing, and at 1 and 2 weeks after dosing. The animals are observed closely the day of treatment, then, daily* for the 2 week observation period. Unless otherwise noted, 7-10 week old Fischer 344 rats, 6-8 week old CF_1 mice, 6-8 week old Hartley guinea pigs, or young adult New Zealand albino rabbits are used. A minimum of 3 animals of one sex per dose level are used. Non-corrosive liquids are dosed as received, corrosive liquids are diluted in water or corn oil. Pastes and solids are prepared as solutions or suspensions in water, corn oil, or 0.5% methocel.

DATE	COMMENTS
12-4-85 12:00	Rats appear alert Jm
1:00	Rats appear alert Jm
2:00	Rats appear alert Jm
3:00	Rats appear alert Jm
4:30	Cage 5 1/3 rats appears slightly lethargic, all others appear alert Jm
12-5-85	Cage 5 rats appear slightly lethargic, all others appear alert Jm
12-6-85	Rats appear alert Jm
12-9-85	Rats appear alert Jm
12/10/85	Rats appear alert Jm
M 12/11/85	Rats appear alert Jm *error Jm 12/11/85
12/12/85	all rats look good Rc
12/13/85	Rats look fine. Mmj
12/14/85	all rat look good Rc
12/17/85	Rats look fine. Mmj
12/18/85	Rats look fine. Mmj

DOW215426

Animals were: ☒ Euthanitized ☐ Sent to necropsy

Date: 12/18/85 Jm

*except weekends & holidays

Compound will not dissolve in water. A 25% suspension in corn oil is too viscous. Can use a 10% suspension in corn oil. Mmj 11/27/85

BACK OF FORM C-40581 R-7-85

17739

TOXICOLOGY RESEARCH LABORATORY

EYE IRRITATION TEST — DOW METHOD DOW2155422

FILE NO. K-002372-043

MATERIAL: 2,4-D-Acid

RABBIT NO.: 85A-6425

SEX: [X] MALE [ ] FEMALE

CONCENTRATION: % W/V [ ] UNDILUTED

CAGE NO.: 600-8

COMMENTS	RESPONSE	NOT Washed (Left)	Washed (Right)	DATE AND INITIALS
IMMEDIATE:	PAIN	3	4	
	CONJUNCTIVA	3	3	Jw 12/3/85
	CORNEA	2	1	
	IRIS	1	1	
AFTER ONE HOUR (~~OR _____ HOURS~~)	CONJUNCTIVA	4	5	
	CORNEA BEFORE STAIN	2	1	Jw 12/3/85
	CORNEA AFTER STAIN	4	4	
	IRIS	3	3	
AFTER 24 HOURS	CONJUNCTIVA	5	5	
	CORNEA BEFORE STAIN	2	1	Mmj 12/4/85
	CORNEA AFTER STAIN	4	4	
	IRIS	5	5	
AFTER 48 HOURS	CONJUNCTIVA	5	4	
	CORNEA BEFORE STAIN	3	1	Jw 12/5/85
	CORNEA AFTER STAIN	4	3	
	IRIS	5	4	
AFTER 72 HOURS	CONJUNCTIVA	5	4	
	CORNEA BEFORE STAIN	3	1	Mmj 12/6/85
	CORNEA AFTER STAIN	4	3	
	IRIS	5	4	
AFTER 7 DAYS	CONJUNCTIVA	5	3	
	CORNEA BEFORE STAIN	4	1	Mmj 12/10/85
	CORNEA AFTER STAIN	4	1	
	IRIS	5	3	
AFTER 14 DAYS * Iris can't be evaluated due to cornea opacity of	CONJUNCTIVA	5	3	
	CORNEA BEFORE STAIN	4	1	Mmj 12/17/85
	CORNEA AFTER STAIN	3	2	
	IRIS	*	2	
AFTER 21 DAYS	CONJUNCTIVA	5	3	
	CORNEA BEFORE STAIN	5	1	Jw 12/24/85
	CORNEA AFTER STAIN	2	1	
* cannot adequately visualize iris due to corneal opacity Jw 12/24/85	IRIS	*	3	

SIGNED: [signature]

DATE: 12/24/85

17740

K-2372-(43)

METHODOLOGY

Twenty-four hours prior to use, the eyes of the rabbits were examined and established as being without defects or irritation. The test procedure consisted of instillation of approximately 0.1 milliliter or 0.1 gram of the test material into the conjunctival sac of the right eye of a rabbit. Following a 30 second exposure period the eye was washed for 2 minutes with tepid, flowing tap water. The test material was then instilled in a similar manner in the left eye which was left unwashed. At various time intervals both eyes were examined for conjunctival irritation, corneal injury and irritation of the iris. The behavior of the animal was observed for indications of pain or discomfort. Both eyes were examined at intervals to 7 days post-instillation. If injury or irritation persisted, examinations were conducted at 14 and 21 days post-instillation. A drop of 5% aqueous fluorescein was used to help assess corneal injury at the 1-hour reading and at all subsequent readings.

DOW215428

Grading of Response:

A. Immediate Response (Pain)

1. No response.
2. Very slight – No more than a few blinks – Normal in a minute or so.
3. Slight – blinks – tries to open but can't. Reflexes close eye.
4. Moderate. Holds eye shut and puts pressure on lids, may rub eye with paw.
5. Severe. Holds eye shut vigorously, rubs eye with paw – may squeal.
6. Very severe. Holds eye shut vigorously. May squeal, claws at eye, jumps, tries to escape.

B. Conjunctival Response

1. None.
2. Very slight. Capillaries prominent. Very slight swelling &/or redness.
3. Slight. Conjunctival membranes somewhat inflamed. May be some sclero-corneal inflammation. Similar to response from a mild soap.
4. Moderate. Conjunctival membranes inflamed and sore, lids swollen and reddened.
5. Severe. Conjunctival membranes red and swollen. Nictitating membrane sore and inflamed.
6. Very severe. Conjunctival membranes markedly reddened and swollen. Nictitating membrane edematous. Pussy exudate – eye may be stuck shut.

C. Corneal Response (Before staining)

1. No response.
2. Very slight haziness.
3. Slight. Cloudy in part.
4. Moderate. Cloudy over whole but not opaque.
5. Severe. Cornea up to 50% opaque.
6. Very severe. Cornea completely opaque.

D. Corneal Response (After staining)

1. None.
2. Very slight. Few diffuse areas of epithelial injury. Probably heal in 24-48 hours.
3. Slight. Up to 50% of eye covered with shallow epithelial injury. Probably heals.
4. Moderate. 50-100% of eye injured but lesions are shallow. Doubtful if vision will be impaired.
5. Severe. Marked injury over whole area which may result in loss of eye.
6. Very severe. Eye lost.

E. Irritation of the Iris

1. No response.
2. Very slight.
3. Slight. Just perceptible.
4. Moderate. Definitely perceptible.
5. Severe. Marked change.
6. Very severe. Complete loss of function.

Comments

Observations recorded in this space may include unusual types of severity of responses not fully covered by the descriptions under Grading of ponse. Particular remarks may be made concerning the lids, nictitating membrane, iris, pupil, lens, or anterior chamber.

TOXICOLOGY RESEARCH LABORATORY

SKIN IRRITATION — DOW METHOD

DOW2155429

FILE NO. K-2372-(43)

MATERIAL: 2,4-D Acid

RABBIT NO.: 85A9463 ♂

CAGE NO.: 608-2

TEST BY REPEATED APPLICATION ☐ UNDILUTED; ☒ WET PATCH ☐ DRY PATCH

DATE		11/18/85	11/19/85	11/20/85	11/21/85	11/22/85	11/25/85	11/26/85	11/27/85	12/4/85						
DAYS ON EXPOSURE		0	1	2	3	4	7	8	9	14						
ON EAR	APPLICATION NO.															
METHOD A	HYPEREMIA															
	EDEMA															
	NECROSIS															
	EXFOLIATION															
	HAIR LOSS															
	SCAB															
	SCAR															
ON ABDOMEN INTACT	APPLICATION NO. (erythema)	1	2	3	4	5	6	7	8	Use Q						
METHOD B	HYPEREMIA		2	1	2	2	1	1	1	1						
	EDEMA		1	1	1	1	1	1	1	1						
	NECROSIS		1	1	1	1	1	1	1	1						
	EXFOLIATION		1	1	1	1	1	1	1	1						
	SCAB		1	1	1	1	1	1	1	1						
	SCAR		1	1	1	1	1	1	1	1						
ON ABDOMEN ABRADED	APPLICATION NO. (erythema)	1	2	3												
METHOD C	HYPEREMIA		1	1	1	1	1	1	1	1						
	EDEMA		1	1	1	1	1	1	1	1						
	NECROSIS		1	1	1	1	1	1	1	1						
	EXFOLIATION		1	1	1	1	1	1	1	1						
	SCAB		1	1	1	1	1	1	1	1						
	SCAR		1	1	1	1	1	1	1	1						
WEIGHT IN KG		2.67					2.72			2.78						
OBSERVATION BY		Mmj	Nel	Qw	Qw	Mmj	Qw	Qw	Qw	Mmj						

(x 0)

SIGNED: Michelle M. Jeffreys

DATE: 12/2/85

17742

FORM C-2560 R-11-84

K-2372-(43)

SKIN CONTACT-IRRITATION

METHODOLOGY

New Zealand albino rabbits are prepared for this test by shaving the hair from the entire abdomen with a straight razor and barber soap. Animals are then rested for several days to allow any abrasions to heal completely and to be sure skin is suitable for use. Applications are made according to the methods described below and reactions and observations are recorded daily during the application period and at 2 weeks from initial exposure. Applications are usually discontinued and the animal humanely euthanatized upon production of an irreversible skin burn.

METHOD A (EAR)

Liquids and pastes (no solids) are applied to the ear. Ten ~ 0.1 ml applications are made over a period of 14 days. These applications are left uncovered.

METHOD B (INTACT ABDOMEN)

Liquids (0.5 ml) or solids (0.5 g) are applied under an ~ 1 by 1 inch cotton pad held in place by a cloth bandage taped to the marginal hair. Ten applications are made over a period of 14 days. This allows continuous, intimate contact with the skin for a 2-week period. If sufficient solid sample is available, another rabbit is treated in the same manner except the cotton pads are moistened with distilled water.

METHOD C (ABRADED ABDOMEN) 1 ml Mmj 11/9/85

An area of skin about 1 by 1 inch is cross-hatched with a sharp hypodermic needle to penetrate the stratum corneum but to produce no more than a very slight trace of bleeding. Liquids and solids are applied as in method B. Three consecutive daily applications only are made, which allows for 3 days of intimate, confined contact with the abraded skin.

DOW215543O

GRADE	HYPEREMIA (erythema)	EDEMA	NECROSIS	EXFOLIATION	HAIR LOSS	SCAB	SCAR
1	NONE	NONE	NONE	NONE	NONE	NONE	NONE
2	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT
3	SLIGHT	SLIGHT	SUPERFICIAL	SLIGHT	SLIGHT	SLIGHT CRUSTATION	SLIGHT
4	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE CRUSTATION	MODERATE
5	MARKED	MARKED	DEEP	MARKED	MARKED	MARKED SCAB	MARKED
6	VERY RED	SEVERE	VERY DEEP	SEVERE	SEVERE	DEEP SCAB	SEVERE

DATE: COMMENTS:

11/25/85 Rabbit appears alert but is not eating Q.
12/2/85 *Rabbit appears alert but is not eating much. Mmj
Applications were not made 11/28/85 or 11/29/85 due to Thanksgiving Holiday. Mmj 12/3/85

17743

used 10/1 Mmj

TOXICOLOGY RESEARCH LABORATORY

SKIN IRRITATION — DOW METHOD

DOW2155431

FILE NO. K-2372-(43)

MATERIAL: 2,4-D Acid

RABBIT NO.: 85A9462 ♂

CAGE NO.: 608-1

TEST BY REPEATED APPLICATION ☐ UNDILUTED; ☐ WET PATCH ☒ DRY PATCH

DATE		11/18/85	11/19/85	11/20/85	11/21/85	11/22/85	11/25/85	11/26/85	11/27/85	12/2/85						
DAYS ON EXPOSURE		0	1	2	3	4	7	8	9	14						
ON EAR	APPLICATION NO.															
METHOD A	HYPEREMIA															
	EDEMA															
	NECROSIS															
	EXFOLIATION															
	HAIR LOSS															
	SCAB															
	SCAR															
ON ABDOMEN INTACT	APPLICATION NO.	1	2	3	4	5	6	7	8							
METHOD B	HYPEREMIA (erythema)		1	1	1	1	1	1	1	1						
	EDEMA		1	1	1	1	1	1	1	1						
	NECROSIS		1	1	1	1	1	1	1	1						
	EXFOLIATION		1	1	1	1	1	1	1	1						
	SCAB		1	1	1	1	1	1	1	1						
	SCAR		1	1	1	1	1	1	1	1						
ON ABDOMEN ABRADED	APPLICATION NO.	1	2	3												
METHOD C	HYPEREMIA (erythema)		1	1	1	1	1	1	1	1						
	EDEMA		1	1	1	1	1	1	1	1						
	NECROSIS		1	1	1	1	1	1	1	1						
	EXFOLIATION		1	1	1	1	1	1	1	1						
	SCAB		1	1	1	1	1	1	1	1						
	SCAR		1	1	1	1	1	1	1	1						
WEIGHT IN KG		2.72					2.81			2.89						
OBSERVATION BY		Mmj	Nel	Rw	Rw	Mmj	Rw	Jw	Rw	Mmj						

SIGNED: Michelle M. Jeffrey

DATE: 12/2/85

17764

k-2372-(43)

SKIN CONTACT-IRRITATION

METHODOLOGY

New Zealand albino rabbits are prepared for this test by shaving the hair from the entire abdomen with a straight razor and barber soap. Animals are then rested for several days to allow any abrasions to heal completely and to be sure skin is suitable for use. Applications are made according to the methods described below and reactions and observations are recorded daily during the application period and at 2 weeks from initial exposure. Applications are usually discontinued and the animal humanely euthanatized upon production of an irreversible skin burn.

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Liquids (0.5 ml) or solids (0.5 g) are applied under an ~ 1 by 1 inch cotton pad held in place by a cloth bandage taped to the marginal hair. Ten applications are made over a period of 14 days. This allows continuous, intimate contact with the skin for a 2-week period. If sufficient solid sample is available, another rabbit is treated in the same manner except the cotton pads are moistened with distilled water.

METHOD C (ABRADED ABDOMEN)

An area of skin about 1 by 1 inch is cross-hatched with a sharp hypodermic needle to penetrate the stratum corneum but to produce no more than a very slight trace of bleeding. Liquids and solids are applied as in method B. Three consecutive daily applications only are made, which allows for 3 days of intimate, confined contact with the abraded skin.

DOM215543 2

GRADE	HYPEREMIA (erythema)	EDEMA	NECROSIS	EXFOLIATION	HAIR LOSS	SCAB	SCAR
1	NONE	NONE	NONE	NONE	NONE	NONE	NONE
2	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT	VERY SLIGHT
3	SLIGHT	SLIGHT	SUPERFICIAL	SLIGHT	SLIGHT	SLIGHT CRUSTATION	SLIGHT
4	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE CRUSTATION	MODERATE
5	MARKED	MARKED	DEEP	MARKED	MARKED	MARKED SCAB	MARKED
6	VERY RED	SEVERE	VERY DEEP	SEVERE	SEVERE	DEEP SCAB	SEVERE

DATE: COMMENTS:

11/25/85 Rabbit appears alert Rm

12/2/85 Rabbit appears alert Mmj

Applications were not made 11/28/85 or 11/29/85 due to Thanksgiving holiday Mmj 12/3/85

17745

used 10/: Mmj

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

File T23.14-11-1
Chg. 1219-02
Rec'd 6-13-47
Fin'd 6-25-47
Work By D. R. Mussell

Subject RANGE-FINDING TOXICOLOGICAL TESTS ON CHICKENS WITH 2,4-DICHLOROPHENOXYACETIC ACID

To File report Check Dudderson 6-24-47 Rept. By V. K. Rowe 6-25-47

Problem:

Some information regarding the toxicity of Dow 2,4-D on fowls was desired.

Experimental:

Four groups of 3 chickens each were set up and fed diets containing given amounts of 2,4-dichlorophenoxyacetic acid. These groups were maintained on these diets for a week and then discarded. At the end of the experiment, all of the birds looked and acted in an entirely normal fashion. The setup and data obtained are summarized in Table I.

Conclusions:

2,4-Dichlorophenoxyacetic acid can be tolerated better by chickens than by rats and there is no danger of chickens, and presumably other birds, obtaining lethal quantities of 2,4-D from forage treated in the manner recommended for weed control.

rl-2

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DOW 215541

Table I

Results of Feeding of 2,4-D to Chickens with Their Diet

ate	Weight in Grams															
	Controls				0.05% 2,4-D in Diet				0.1% 2,4-D in Diet				0.3% 2,4-D in Diet			
	White	Green	Blue	Ave.	White	Green	Blue	Ave.	White	Green	Blue	Ave.	White	Green	Blue	Ave.
-13-47	215	290	340	262	325	260	290	292	210	230	260	233	275	300	230	268
-17-47	265	360	400	342	375	320	355	350	255	295	330	293	330	320	260	303
-19-47	285	385	410	360	385	330	370	362	275	320	360	318	370	350	280	333
ve. wt. gain		98				70				85				65		
otal food onsumed		800				820				785				675		

DOW 1551 S

741

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

File T23.14-11-1
Chg. 1219A
Rec'd 9-27-44
Fin'd 10-19-44
Work By H. A. Wyse

Subject

PRELIMINARY INFORMATION RELATIVE TO THE TOXICITY OF 2,4-DICHLOROPHENOXY-ACETIC ACID (K2372-3)

Check Edwin E. Munn Rept. By V. K. Rowe 10-23-44

To Technical Service & Development Div.
(W. W. Allen)
W. R. Veazey
Organic Research Laboratory

Preliminary tests have indicated that 2,4-dichlorophenoxyacetic acid has a moderate acute oral toxicity and that it does not produce marked skin irritation.

We would not expect serious difficulties to result from handling this material in a reasonable manner.

Similar tests will be made on 2,4,5-trichlorophenoxyacetic acid when a sample is available.

1a-4 copies

IDOW 21 55413

17-8

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1219 25 25C 244

742

K-2372-(1)

ACUTE ORAL TOXICITY OF 2,4-DICHLORO-PHENOXYACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS

DOW2155407

V. K. Rowe
D. D. McCollister
and
H. C. Spencer

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

17749

The 2,4-dichlorophenoxyacetic acid was administered by oral intubation to rats, mice, guinea pigs and chicks as a solution in olive oil emulsified with 5-10% gum arabic solution. For comparison, the sodium salt of 2,4-dichlorophenoxyacetic acid was fed in aqueous solution to rats and guinea pigs.

The guinea pigs and the white rats were mature, young adult animals selected from the stock colonies of this laboratory. Young adult white mice were obtained from the Carworth Farms, Rockland, New York. The chicks (New Hampshire Red) were purchased from a commercial hatchery and used when about three weeks of age. Mixed sex groups of each species were used in these tests.

Table I summarizes the concentrations and volumes of olive oil solutions of 2,4-dichlorophenoxyacetic acid administered to each species.

TABLE I

SINGLE ORAL ADMINISTRATION OF
2,4-DICHLOROPHENOXYACETIC ACID

Species	Per cent Material in Olive Oil	Volume Given by Stomach Tube	
		Minimum (cc.)	Maximum (cc.)
Rats	5, 10	0.4	4.0
Guinea Pigs	10	0.9	2.9
Mice	5, 10	0.1	0.2
Chicks	3	0.3	5.2

Mortalities resulting from the various single oral dosages of 2,4-dichlorophenoxyacetic acid and its sodium salt are given in

DOW 2155408

Tables II and III. 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.

TABLE II

MORTALITIES RESULTING FROM ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID

Dosage (gm./kgm.)	Rats		Guinea Pigs		Mice		Chicks	
	No. Fed	No. Died	No. Fed	No. Died	No. Fed	No. Died	No. Fed	No. Died
0.1	10	0					6	0
0.2	10	0			6	0		
0.3	10	3	6	0	5	1	6	1
0.4	10	6			6	4		
0.5	10	7	6	4	6	6	6	6
0.6	10	10						
0.7	3	3	6	6	6	6	6	4
1.0							6	6

TABLE III

MORTALITIES RESULTING FROM ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID, SODIUM SALT

Dosage (gm./kgm.)	Rats		Guinea Pigs	
	No. Fed	No. Died	No. Fed	No. Died
0.3	10	0	6	0
0.5	10	2	6	4
0.7	10	4	6	6
1.0	14	14		

The acute oral LD_{50} values with their 19/20 confidence limits were determined for each according to the method described by Litchfield and Wilcoxon[1]. The results of the statistical treatment of the dosage-response data are presented in Table IV.

1. Litchfield, J. T., Jr. and Wilcoxon, F.: J. Pharm. and Expt. Ther. 96:99, 1949.

DOW2155409

TABLE IV

THE ACUTE ORAL LD_{50} VALUES FOR 2,4-DICHLOROPHENOXYACETIC ACID WHEN ADMINISTERED TO RATS, MICE, GUINEA PIGS AND CHICKS

Species	Form	LD_{50} (19/20 Confidence Limits) gm./kgm.	Slope Function*
Rats	Oil-acid	0.375 (0.302-0.465)	1.62
Mice	Oil-acid	0.368 (0.312-0.434)	1.23
Guinea Pigs	Oil-acid	0.469 (0.397-0.553)	1.17
Chicks	Oil-acid	0.541 (0.358-0.817)	1.81
Rats	Aq.-Na salt	0.805 (0.610-1.063)	1.77
Guinea Pigs	Aq.-Na salt	0.551 (0.417-0.727)	1.55

*The fold change in dosage required to produce one unit standard deviation in response along the line.

DOW 2155410

The Litchfield and Wilcoxon test for parallelism of two lines revealed that the dosage-response line for the rats (oil-acid) deviated significantly (19/20 probability) from those for the mice and the guinea pigs receiving the 2,4-dichlorophenoxyacetic acid in olive oil. The line for the chicks also deviated significantly in parallelism from those for the guinea pigs and mice. The tests for parallelism of two lines and estimate of relative potency indicated no statistically significant differences in comparison of rats versus chicks and mice versus guinea pigs.

The dosage-response curves for guinea pigs (oil-acid) and guinea pigs (aqueous-sodium salt) did not deviate significantly in parallelism or relative potency. A comparison of the rats (oil-acid) versus rats (aq.-Na salt) revealed no significant difference in parallelism of the two lines, but the test for relative potency indicated that the free acid was significantly more toxic to rats than was the sodium salt.

2,4 - Dichlorophenoxyacetic Acid

T-60.14-2

T 23.14-11-1

T 22.14-11-1

Called

2-4-D

Sent copy of article right to W.W. Allen

DOW2155411

T 23.14-11-1

Repeated Oral Feeding of 2,4-Dichlorophenoxyacetic Acid [20 Doses].

Purpose: Since 2,4-dichlorophenoxyacetic acid has been shown to be an effective weed killer, it is necessary to have some information concerning its oral toxicity, particularly upon repeated ingestion.

Material:

The material used in this experiment was obtained from 267 Building on 10-3-44 and designated as K 3372-3.

DOW 2155412

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

Subject: THE TOXICOLOGICAL PROPERTIES OF 2,4-DICHLORO-PHENOXYACETIC ACID, AND ITS SODIUM AND ALKANOLAMINE SALTS, AS REVEALED BY EXPERIMENTS UPON LABORATORY ANIMALS

File T23.14-11-1
Chg. 1219-02
Rec'd
Fin'd 6-12-47
Work By V. K. Rowe
L. F. Ney
H. C. Spencer

To W. R. Veazey Check V. K. Rowe 6-24-47 Rept. By Dmadams 6-24-47

SUMMARY

The acute systemic toxicity, as shown by the administration of single doses to rats and cavies, was the same for the free acid and the sodium salt, and was of the same order as that of phenol. The studies on chronic systemic toxicity, using repeated oral administration to rats, showed but very little evidence of summation (or accumulation) of injury. The LD_0 for single oral doses was 0.3 g./kg., whereas the largest daily dose without detectable effect was less than 0.1 g./kg. but greater than 0.03 g./kg.

The outstanding pathological effect was seen in the gastro-intestinal tract, consisting of inflammation, sloughing, and hemorrhage; but minor changes were found in the liver and kidney.

The free acid and its salts are but slightly irritating to the skin. Only strong solutions were capable of causing irritation.

There was no evidence of absorption through the skin.

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CONCLUSIONS

1. 2,4-Dichlorophenoxyacetic acid and its salts are moderately toxic by single and repeated oral administration.
2. The principal toxic action on repeated administration was exerted upon the gastro-intestinal tract.
3. The acid and its salts are but very slightly irritating to the skin, and are not absorbed to an appreciable degree.
4. This material presents but little hazard when used as a weed killer. The use concentrations are sufficiently low to offer no hazard of systemic toxic effects and of skin irritation to man or animals. Concentrated solutions and powdery solids might

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Materials tested:

2,4-Dichlorophenoxyacetic acid: The regular plant product containing at least 98% of the acid and less than 1% of dichlorophenol. Fusion point at least 132°C.

2,4-Dichlorophenoxyacetic acid, sodium salt with one molecule of water of crystallization: The regular plant product containing at least 98% of the salt.

40% Liquid Weed Killer Concentrate: A 40% solution of the acid in water as a mixture of alkanol amine salts.

Acute oral toxicity for rats and guinea pigs:

The 2,4-dichlorophenoxyacetic acid was administered as a water solution of the sodium salt (pH 7.2), one ml. of the solution containing 50 mg. of the acid. This solution was administered by stomach tube to young adults of both sexes, the rats receiving volumes of solution up to 5.9 ml. and the guinea pigs receiving volumes up to 14.8 ml. The largest single dose given to guinea pigs was 8.9 ml., larger amounts being given in 2 doses at a 2-hour interval.

All deaths occurred within 48 hours, most of them within 24. The survivors were gaining in weight and of normal appearance within 7 days after feeding. The results given in Table I, may be summarized as follows:

Animal	LD_0	LD_{100}
Rat	0.3 g. per kg.	1.0 g. per kg.
Guinea pig	0.3 g. per kg.	1.0 g. per kg.

Table I

Acute Oral Toxicity of 2,4-Dichlorophenoxyacetic Acid for Rats and Guinea Pigs when Fed as the Sodium Salt

Dose in g./kg.	Rats		Guinea Pigs	
	No. Fed	No. Died	No. Fed	No. Died
0.3	10	0	10	0
0.5	10	2	10	6
0.7	10	4	10	5
1.0	14	14	10	10

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Table I

Results of Feeding of 2,4-D to Chickens with Their Diet

ate	Weight in Grams															
	Controls				0.05% 2,4-D in Diet				0.1% 2,4-D in Diet				0.3% 2,4-D in Diet			
	White	Green	Blue	Ave.	White	Green	Blue	Ave.	White	Green	Blue	Ave.	White	Green	Blue	Ave.
-13-47	215	290	340	262	325	260	290	292	210	230	260	233	275	300	230	268
-17-47	265	360	400	342	375	320	355	350	255	295	330	293	330	320	260	303
-19-47	285	385	410	360	385	330	370	362	275	320	360	318	370	350	280	333
ve. wt. gain		98				70				85				65		
otal food onsumed		800				820				785				675		

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A preliminary or range-finding acute oral study was conducted on the 40% Liquid Weed Killer Concentrate using rats. The 40% concentrate was diluted with an equal volume of water for feeding. The results are shown below:

Dose in g./kg. (of the 40% concentrate)	Rats	
	No. fed	No. died
0.3	2	0
0.5	2	0
0.7	2	0
1.0	2	2
3.0	2	2

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From these data, it appears that this liquid concentrate may be slightly more toxic than the 2,4-dichlorophenoxyacetic acid content would account for. This is not at all surprising.

Irritating action upon the skin of rabbits:

Tests were made with albino rabbits using daily applications upon the ear and shaven belly. The applications on the ear consisted of a thorough wetting of the inner surface once a day, 5 days a week. The applications on the belly were made by a small cotton pad, wet with the test solution, which was held in place by a cloth bandage.

Tests made with 5% and 1% water solutions of the acid as the sodium salt and with a 5% solution of the acid in butyl carbitol acetate and in 50% ethanol resulted in no significant response. The ear usually showed nothing; the belly showed very slight hyperemia and scaliness, a reaction frequently seen in control experiments.

A 40% concentrate of the acid, present as a mixture of alkanolamine salts, seemed to be strongly irritating when bandaged on the skin of the rabbit, producing a severe inflammation and edema with sloughing. After this reaction subsided, however, daily applications were made with but a very slight irritation

resulting. Daily applications of this concentrate upon the uncovered ear resulted in no response. When diluted to the concentration recommended for use, this mixture of alkanolamine salts produced no response in the rabbits' skin.

In none of the tests on skin irritation was there any evidence of systemic toxic effects.

DOM2155394

Repeated oral administration to rats:

Experiment I

Experiment design: Olive oil solutions of 2,4-dichlorophenoxyacetic acid, suspended in 2 to 3 cc. of gum acacia solution, were administered by stomach tube 5 days a week until death or serious intoxication occurred, or until 20 doses had been given. The concentration of the olive oil solution varied according to dosage of the acid so that each experimental rat received not more than 1 ml. of the oil daily; the control rats received 1.5 ml. of oil daily.

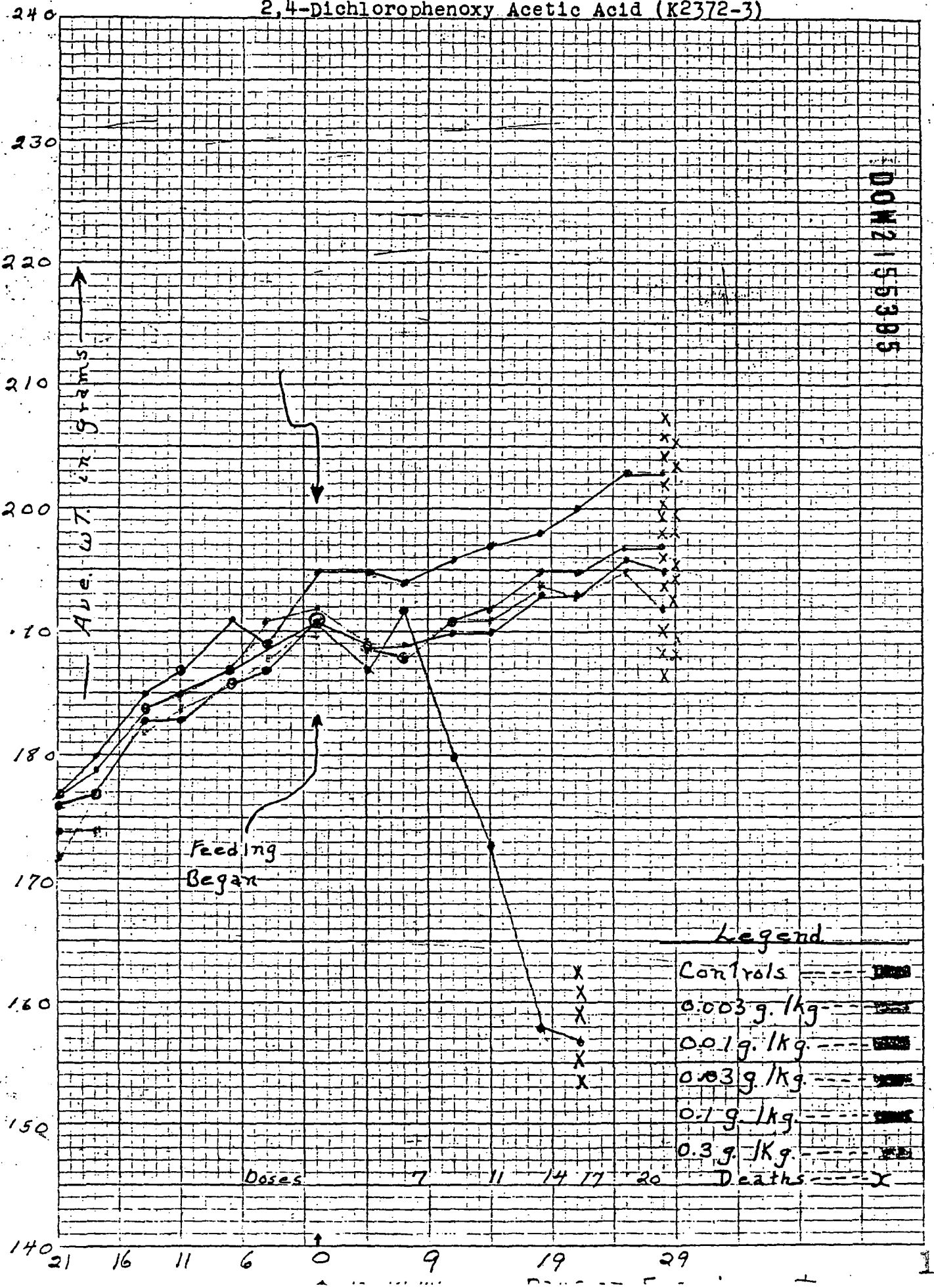
Groups of 5 female rats received each of the following doses in g. of acid per kg. of body weight: 0.003, 0.01, 0.03, 0.1, and 0.3. The control group consisted of 6 rats. The rats were weighed for 21 days prior to the beginning of the experiment, then matched according to body weight and started on the experiment, the ages being 60 to 80 days. The various groups had average weights of 174 to 176 g.

All animals were weighed twice a week during the period of feeding and were observed for evidence of ill effect.

The examination of the animals consisted of gross observation of organs, determination of starved body weight, and weights of liver, kidney, heart, and spleen, determination of blood urea concentration, and microscopic examination of tissue sections from heart, liver, kidney, spleen, adrenal, pancreas, and stomach. Rats on doses of 0, 0.01, and 0.03 g. per kg. were also examinad fro blood hemoglobin, total red cell count, total white and differential cell count.

FIGURE I

Body Weight Curves of Female Rats That Received Repeated Oral Doses of 2,4-Dichlorophenoxy Acetic Acid (K2372-3)



MADE IN U.S.A.

Results: Three rats receiving 0.3 g. per kg. died after two feedings, so that the two survivors were killed and autopsied. Of the 5 rats receiving 0.1 g. per kg. two maintained their weight, three lost weight to a marked degree and had diarrhea. All 5 were killed and autopsied after receiving 15 doses. The rats on doses of 0.03, 0.01 and 0.003 g. per kg. showed a slight depression of growth at first, but recovered, so that at the end of the feeding period the greatest difference in average weight from that of the controls was 6%. This was not considered significant.

DOW 2-55396

Hematological examination of the rats receiving 0.01 and 0.03 g. per kg. revealed no differences from the control group. See Table II.

The measurements of organ weights revealed an increase in liver weight in the two rats that maintained their body weight on 0.1 g. per kg., and possibly a slight increase in kidney weights. See Figure III.

Table II

Summary of Hematological Studies on Female Rats that Had Received 20 Doses in 27 Days of 2,4-Dichlorophenoxyacetic Acid

Dose, g./kg.	Controls	0.03	0.01
No. of rats in group	6	5	5
Erythrocytes per cumm.	11,400,000	10,500,000	10,000,000
Hemoglobin, g./100 cc.	13.9	13.3	12.9
Leucocytes per cu. mm.	18,800	18,300	17,400
Differential count:			
Neutrophils - Filamented	10	15	9
Lymphocytes - small	86	83	87
- large	4	2	4
Mean cell diameter (microns)	6.4	7.0	6.8

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Urea-nitrogen determinations were made on oxalated blood obtained at autopsy from the control and experimental animals. The results are presented graphically in Figure II.

It is evident that, except for 3 animals, the urea-nitrogen concentration was essentially the same in the animals that had received repeated oral doses of 2,4-dichlorophenoxyacetic acid as in the controls. The three high values (2 in animals that received 0.01 g./kg. and one in an animal that received 0.03 g./kg.) may indicate slight impairment of kidney function in these animals. It is impossible to evaluate properly these findings without more corroborative work, but at least they should serve as an indication that repeated ingestion of 2,4-dichlorophenoxyacetic acid might lead to kidney dysfunction in some instances.

Gross examination of the two rats killed after 2 doses of 0.3 g. per kg. revealed a severe irritation in the stomach and gut. There was a great deal of blood throughout the gastro-intestinal tract, with inflammation and ulceration of the mucosa. This irritant effect was probably the cause of the 3 early deaths at this dosage.

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The 3 rats that showed diarrhea and marked weight loss at 0.1 g. per kg. had a moderate irritation in the gastro-intestinal tract. The two rats that maintained their weight showed no irritation.

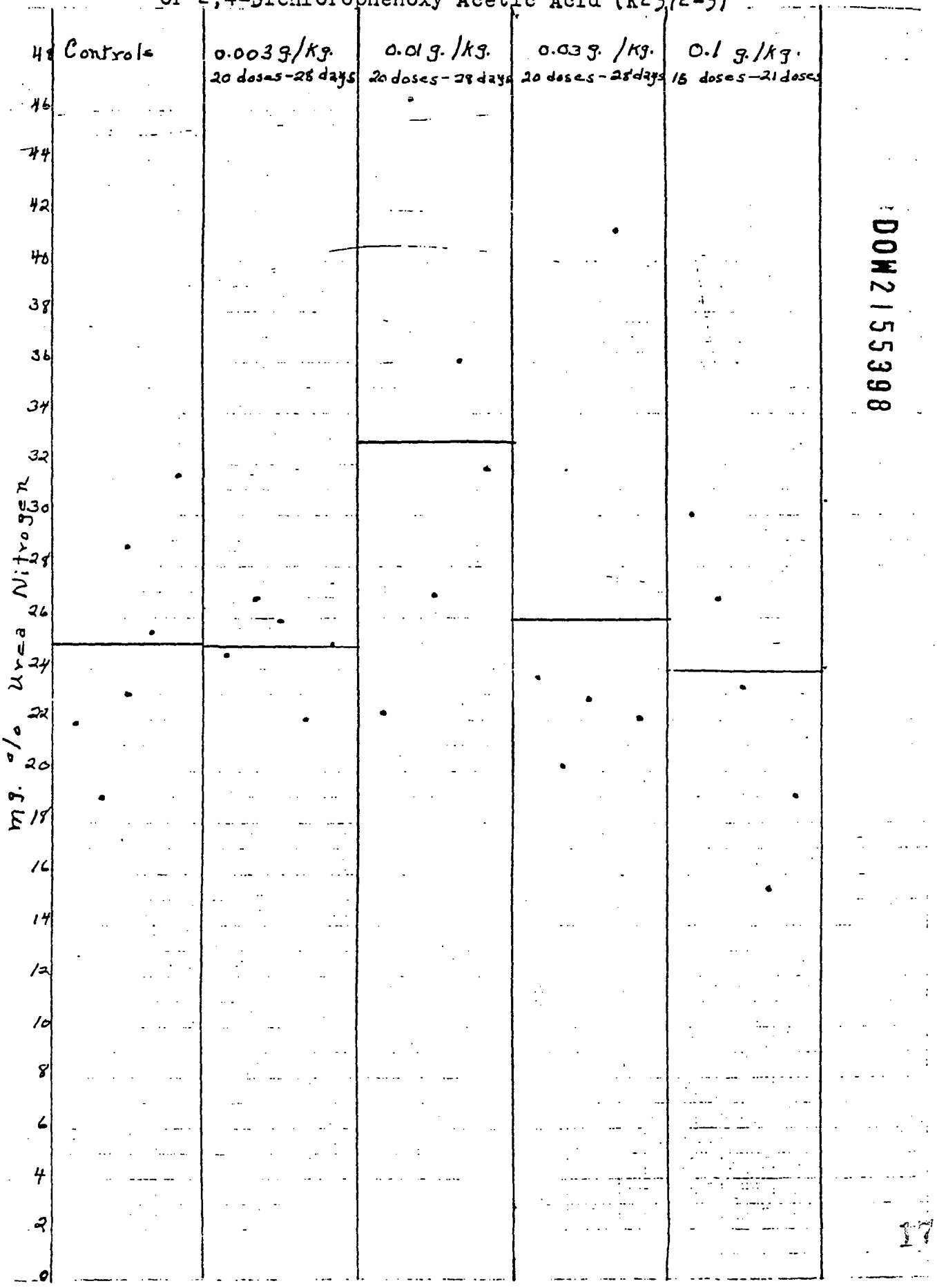
The control rats and those receiving 0.03, 0.01, and 0.003 g. per kg. showed no gross evidence of ill effect.

Histopathology: Sections of the heart, liver, kidney, spleen, adrenal, pancreas, and stomach were prepared for microscopic examination. The results of this work are presented in Table III.

No histopathological changes were observed in the tissues from the control animals or in those that received 20 doses in 28 days of 0.003, 0.01 or 0.03 g./kg. 2,4-dichlorophenoxyacetic acid.

FIGURE II

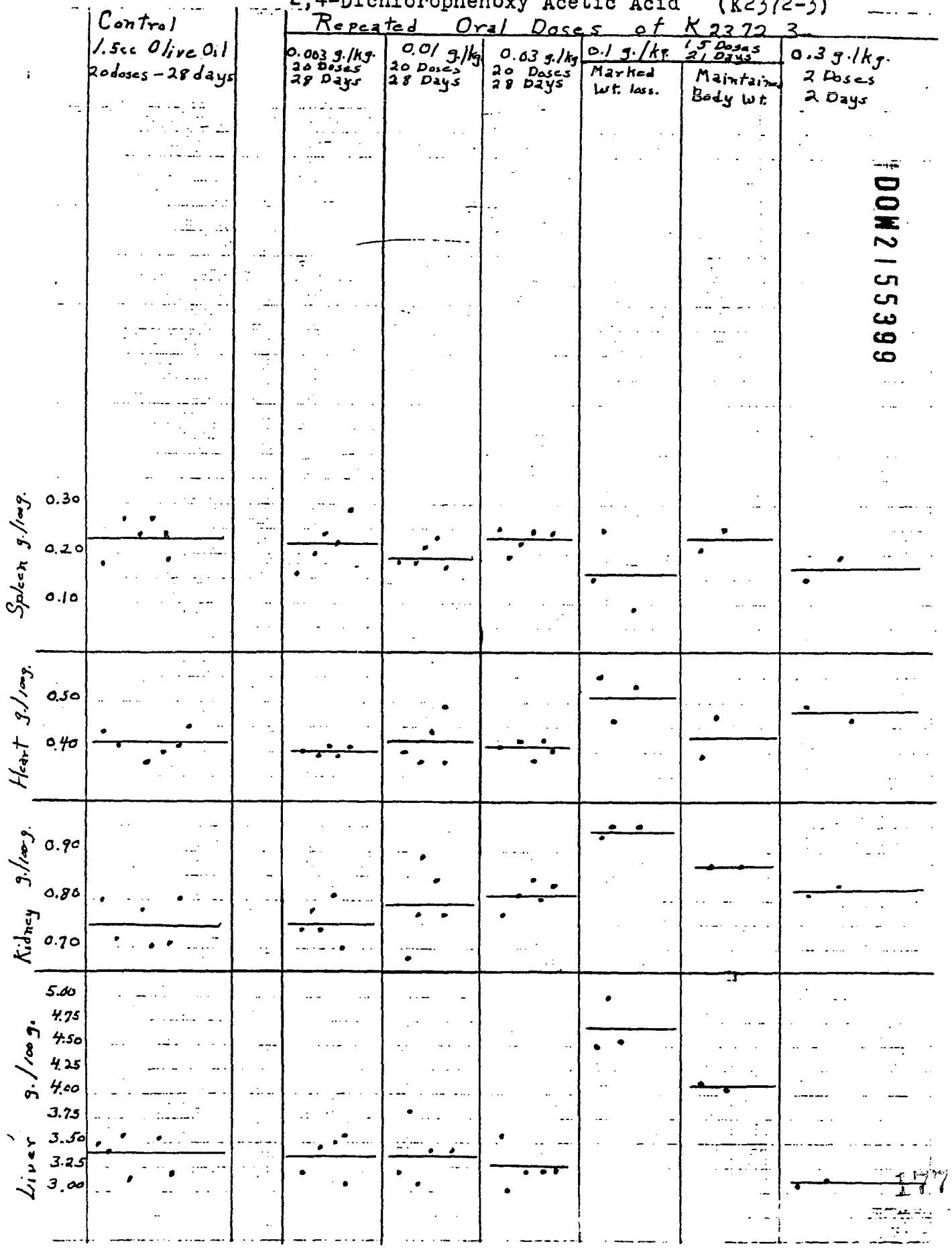
Blood Urea - Nitrogen of Female Rats That Received Repeated Oral Doses of 2,4-Dichlorophenoxy Acetic Acid (K2372-3)



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FIGURE III

Organ Weights of Female Rats That Received Repeated Oral Doses of 2,4-Dichlorophenoxy Acetic Acid (K2372-3)



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Except for the gastrointestinal irritation, the only significant change found in the rats that received 15 doses in 21 days of 0.1 g./kg. was slight cloudy swelling of the liver. Such a reaction is not severe and is commonly believed to be reversible.

The same type of change in the liver was also found in the rats that received 2 doses of 0.3 g./kg. These rats also showed slight kidney involvement.

DOW 2155400

Summary of Experiment 1:

The results of repeated administration by stomach tube are summarized in Table IV. The only toxic effect of importance was local irritation of the gastro-intestinal tract. This action was produced by doses of 0.1 g. per kg. or larger but not by doses of 0.03 g. per kg. or less. The injury to liver and kidney at doses of 0.1 and 0.3 g. per kg. seemed to be of a minor nature.

Species: Rat
Sex: female

Table III
BIOCHEMICAL RESEARCH LABORATORY
Summary of Pathological Findings

Problem No. T23.14-11-1
Expt. No.
Run No. K3372-3
Material: 2,4-dichlorophenoxy-acetic acid

Repeated oral doses: _____g./kg.
Repeated vapor exposures: _____mg./l.

Animal No.	Dose g./kg.	No. of doses	No. of days	Killed or Died	Condition	Lung	Heart	Liver	Kidneys	Spleen	Adrenals	Pancreas	Stomach Tissues	Bone Marrow	
3506	0	20	28	K	Normal		N	N	N	N	N	N	N		)
3508	0	20	28	K	"		N	N	N	N	N	N	N		) 1.5 cc. olive
3661	0	20	28	K	"		N	N	N	N	N	N			) oil daily
3498	0	20	28	K	"		N	N	N	N	N	N	N		) controls
2517	0	20	28	K	"		N	N	N	N	N	N	N		)
3493	0	20	28	K	"		N	N	N	N	N	N	N		)
34950	.003	20	28	K	"		N	N	N	N	N	N			
3491	0.003	20	28	K	"		N	N	N	N	N	N			
3516	0.003	20	28	K	"			N	N	N	N	N			
3511	0.003	20	28	K	"		N	N	N			N			
3706	0.003	20	28	K	"		N	N	N						
3519	0.01	20	28	K	"		N	N	N	N	N	N			
3520	0.01	20	28	K	"			N	N	N	N	N			
3497	0.01	20	28	K	"		N	N	N	N	N	N			
3499	0.01	20	28	K	"		N	N	N	N	N	N	N		
3492	0.01	20	28	K	"		N	N	N	N	N	N	N		

N = Normal tissues as compared with tissues for control animals.

1219-97-5C-1246

DOW 1554 01

Page 8

Species: Rat
Sex: female

Table III (cont.)
BIOCHEMICAL RESEARCH LABORATORY

Summary of Pathological Findings

Problem No. T 23.14-11-1
Expt. No.
Run No.
Material: K2372-3
2,4-Dichlorophenoxyacetic acid

Repeated oral doses: _____g./kg.
Repeated vapor exposures: _____mg./l.

Animal No.	Dose g./kg.	No. of doses	No. of days	Killed or Died	Condition	Lung	Heart	Liver	Kidneys	Spleen	Adrenals	Pancreas	Stomach	Bone Marrow	Gross Exam. Stomach	Gross Exam. Intestine
3518	0.03	20	28	K	Normal		N	N	N	N	N	N	N			
3490	0.03	20	28	K	"		N	N	N	N	N	N	N			
3688	0.03	20	28	K	"		N	N	N	N	N	N	N			
3695	0.03	20	28	K	"		N	N	N	N	N	N				
3689	0.03	20	28	K	"		N	N	N	N	N	N				
3489	0.1	15	21	K	Marked loss in wt. Diarrhea - thin		N	+	N	N	N	N			+	+
3503	0.1	15	21	K	"		N	+-	N	N	N	N	+		+	+-
3502	0.1	15	21	K	"		N	+-	N	++	N	N	+		+	N
3694	0.1	15	21	K	Normal app.		N	+	N	N	N	N			N	N
3505	0.1	15	21	K	" "		N	+-	N	N	N	N			N	N
3521	0.3	2	2	K	Moribund		N	+-	sl.c.	N	N	N			++	++
3584	0.3	2	2	K	"		N	+	+	N	N	N			++	++

N = Normal tissues as compared with tissues for control animals.

DOW 21 55402

1219-97-5C-1246

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Page 9

Table IV

Summary of Experimental Results Obtained on Female Rats that Received Repeated Oral Doses of 2,4-Dichlorophenoxyacetic acid (K2372-3)

Dose g./kg.	Percent in olive oil sol'n	No. of doses	No. of days	Growth and Survival	Hema-tology	Blood urea	Gross appearance of viscera	Organ weights	Histopathology
0	0	20	28	Normal	Normal	Normal	Normal	Normal	Normal
0.003	0.06	20	28	Normal in appearance; 3-6% below controls in final weight	--	Normal	Normal	Normal	Normal
0.01	0.2	20	28		Normal	3 normal; 2 sl.high	Normal	Normal	Normal
0.03	0.6	20	28		Normal	4 normal; 1 sl.high	Normal	Normal	Normal
0.1	2.0	15	21	3 lost wt. rapidly; had diarrhea	--	Normal	Gastro-intestinal irritation	Liver+ Kidney+	Liver ± Kidney: normal
				2 maintained weight; no diarrhea	--	Normal	Normal	Liver+ Kidney+	Liver ± Kidney: normal
0.3	6.0	2	2	3 out of 5 died; others moribund	--	--	Gastro-intestinal irritation	Normal	Liver + Kidney ±

Biochemical Research Laboratory
T23.14-11-1
Page 10

DOW 2155403

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Experiment 2

Design of experiment: Dichlorophenoxyacetic acid was incorporated in the rat food in an attempt to avoid the acute irritation in the gastro-intestinal tract. The normal rat food was a finely ground powder of fresh ground whole wheat, 55%; dried whole milk, 25%; dried meat residue (liver), 12%; dried brewer's yeast, 5%; calcium carbonate, 1%; and iodized salt, 2%. Weighed amounts of dry, ground dichlorophenoxyacetic acid were added to this diet to give concentrations of 0.01, 0.03, 0.10, 0.30, and 1.0% by weight.

Groups of 5 female rats were started on these concentrations, and on the normal diet, when 96 to 108 days of age. The rats were selected and put in matched groups according to growth made over a period of about 3 weeks just prior to the experiment. Whenever possible during the course of the experiment, rats that were obviously quite sick and losing weight were killed and autopsied in order to determine the cause of the ill condition. All animals found to have ear infections were promptly killed in an attempt to minimize the spread of this infection. The examination of animals at autopsy was the same as that made in Experiment 1.

Growth and survival: The rats on diets containing 1.0 and 0.3% 2,4-dichlorophenoxyacetic acid lost weight at a very rapid rate so that they were killed and autopsied after 16 days (one rat died on the 0.3% diet).

Rats receiving a diet containing 0.1% of the 2,4-dichlorophenoxyacetic acid grew poorly. Only one rat survived for the duration of the experiment (108 days) and two deaths were unexplained except as the result of the experimental procedure.

Rats receiving concentrations of 0.03 and 0.01% grew as well as did the controls, the maximum difference between group average weights being less than 5%. All deaths in these and the control group were due to ear infections or pneumonia.

Food consumption was extremely low at concentrations of 0.3 and 1.0%, the exact amount eaten being uncertain because of the large amount wasted by the rats. The amount of food eaten at the lower concentrations was essentially normal.

Blood urea concentrations, made at autopsy of the survivals, were somewhat lower in the experimental animals than in the controls. This difference is of questionable significance; however, since all values were in the normal range, the control values being grouped at the high end.

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Table V

Urea-Blood Concentrations at Autopsy of Diet Animals

Concentration of Dichlorophenoxyacetic Acid in Diet	No. of Animals	Blood urea mg./100 ml.
0 %	4	23.2,24.7,26.9,37.1
0.1 %	1	12.5
0.03%	3	16.0,17.2,18.3
0.01%	4	14.3,18.6,20.5,22.1

Results of autopsy: The rats killed after 16 days on diets containing 1.0 and 0.3% dichlorophenoxyacetic acid were thin and poor, with depleted fat depots.

The results of organ weight determinations in rats on the 1.0, 0.3 and 0.1% diets are uncertain because of the decreased body weights. The weights of heart, liver, kidney, and spleen of rats on 0.03 and 0.01% diets were normal.

Microscopic examination of the tissues revealed only a slight parenchymatous degeneration of the liver cells, in the rats on the 1% diet. Two of the 4 rats on the 0.3% diet showed a slight cloudy swelling in the liver and in the proximal convoluted tubules of the kidney. The one rat surviving on the 0.1% diet showed a very slight cloudy swelling in the liver. None of the other animals showed any pathological changes referable to the treatment.

Conclusion on Experiment 2:

When 2,4-dichlorophenoxyacetic acid was incorporated in the diet of rats over a period of 103 days, adverse effects were observed at concentrations of 0.1% and higher but not at concentrations of 0.03% and lower. The pathological changes, found only in the liver and kidney, were of a minor nature and were insufficient to explain the marked gross response observed. Extreme weight loss at concentrations of 0.3 and 1.0% were associated with an almost negligible intake of food.

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Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

File T23.14-E1-1
Chg. 1219-02
Rec'd 6-13-47
Fin'd 6-25-47
Work By D. R. Mussell

Subject RANGE-FINDING TOXICOLOGICAL TESTS ON CHICKENS WITH 2,4-DICHLOROPHENOXYACETIC ACID

To File report Check D.W. Adams 6-24-47 Rept. By E. K. Rowe 6-25-47

DOW 2155388

Problem:

Some information regarding the toxicity of Dow 2,4-D on fowls was desired.

Experimental:

Four groups of 3 chickens each were set up and fed diets containing given amounts of 2,4-dichlorophenoxyacetic acid. These groups were maintained on these diets for a week and then discarded. At the end of the experiment, all of the birds looked and acted in an entirely normal fashion. The setup and data obtained are summarized in Table I.

Conclusions:

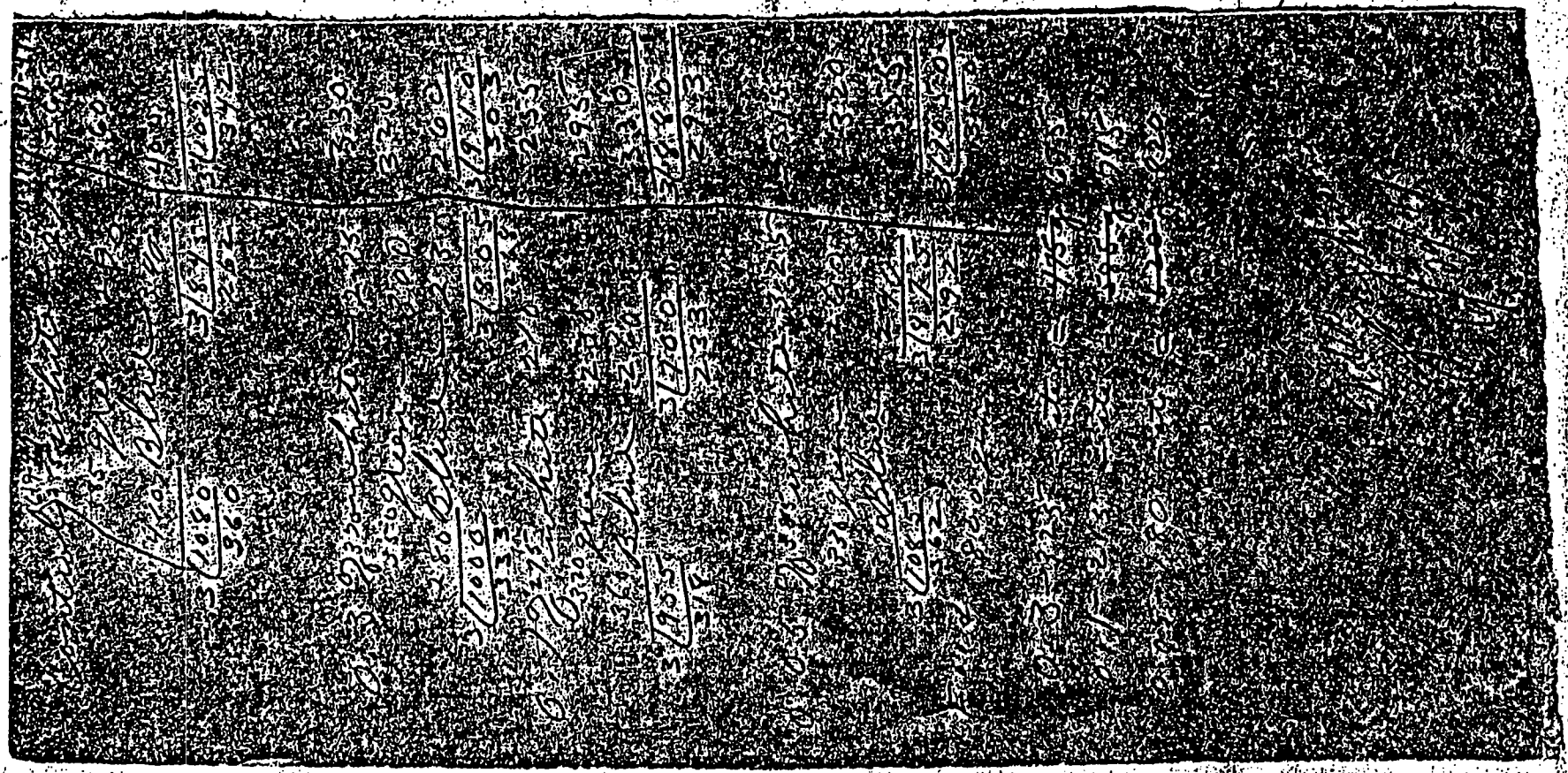
2,4-Dichlorophenoxyacetic acid can be tolerated better by chickens than by rats and there is no danger of chickens, and presumably other birds, obtaining lethal quantities of 2,4-D from forage treated in the manner recommended for weed control.

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Biochemical Research Department
THE DOW CHEMICAL COMPANY

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File T23.14-11-1
Chg. 1219-02
Rec'd 9-27-44
Fin'd 8-31-50
WorkBy V.K.Rowe

Subject: RESULTS OF SEMICHRONIC ORAL TOXICITY STUDIES ON 2,4-DICHLOROPHENOXYACETIC ACID.

To Agricultural Projects Committee
Att'n: W. C. Dutton
W. W. Allen
D. D. Irish
J. F. Kagy
G. E. Lynn

Executive Research Committee

Britton's Division
Att'n: J. W. Britton
R. C. Dosser

Legal Department
Att'n: W. W. Sunderland

Check H. Spencer 9-3-50

Rept.By V.K.Rowe 8-31-50

K-2372-(

DOW 2155386

UNIT INDEX

The effects of feeding 2,4-D repeatedly to rats either by stomach tube or as a part of their diet are described.

INDEX HEADINGS

2,4-Dichlorophenoxyacetic acid

Acetic Acid: 2,4-dichlorophenoxy-

Phenoxyacetic Acid: 2,4-dichloro-

MATERIAL

The samples of material used in these tests were designated as K2372-3 and K2372-4. They were regular production samples obtained from 267 Building in 1944 and 1945.

DOW 21 553897

PROBLEM

The toxicological work reported in the attached summary was carried out some time ago. The purpose of doing the work was to acquire toxicological information upon which to base conclusions with regard to the health hazards encountered in manufacture and handling.

The attached summary of this work was submitted as exhibit in evidence on August 2, 1950 to the F.D.A. Hearing for the establishment of residue tolerances on fresh fruits and vegetables.

CONCLUSIONS

It appears from these experiments that 2,4-D is moderately toxic when fed repeatedly to rats. Doses of 30 mg./kg. by stomach tube were well tolerated and a dietary concentration of 300 p.p.m. was well tolerated.

This information together with the other information available led us to suggest that a residue tolerance of 20 p.p.m. 2,4-D on fresh fruits and vegetables would provide a wide margin of safety.

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K-4568-(100) 1971

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4237 CASSAN, G., J. KOLMAN, J. GILLY and J. BOES. Pulmonary siderosis in electric welders: Diffuse interstitial fibrosis and complex nodular form. ARCH MAL. PROF. 33/4 Sup. 193-194. 1972.--Two cases of pneumoconiosis with iron deposits in the lungs are reported in welders. Though usually nodular, siderosis pneumoconioses may be pseudotumoral and difficult to diagnose. There are diffuse mesh or less sclerosing forms, with a functional deficit. The problem of pathogenesis is not wholly resolved. The questions of exogenous Fe metabolism and the harmfulness of associated dusts are involved. In the case of a locksmith engaged in arc welding for 17 yr, a left pulmonary opacity developed, shown by tomography to be situated in the lingula. This mass was surgically removed. Histologic examination showed numerous hemosiderin-charged cells. Several months postoperatively a moderate ventilatory deficit with an obstructive syndrome was found. --P. H. V.

4238 GIRARD, R., G. H. PROST and C. BARTHELEMY. Total number of compensated occupational diseases in France. ARCH. MAL. PROF. 33/4 Sup. 201-202. 1972.--Of 48 reported cases of benzene poisoning 21 were acquired during paid work and thus qualified as occupational disease indemnifiable by Social Security. Only 6 were indemnified however, partly because of the omission by the physician to report the accident as indemnifiable and partly because the poisoning occurred on premises which were not registered by the employer as hazardous to human health. In 1968 in a French work force of 11,735,638 persons, 1,028,325 accidents occurred of which 2041 were fatal. Of the more than a million accidents, only 4124 resulted in indemnification of the worker by Social Security. Of this number, 2043 cases were due to cement dust inhalation.--R. K.

4239. ROITEAU, H. L., M. ROBIN and S. GELOT. Polycyclic hydrocarbons in various asbestos products. ARCH. MAL. PROF. 33/6: 261-270. 1972.--Different hypotheses put forward to explain the origin of carcinogenic potentialities of asbestos are examined. Results of determinations of polycyclic hydrocarbons carried out in 12 samples of materials composed of chrysotile are reported. In 1-3 of the samples analyzed, carcinogenic compounds are observed. These compounds probably penetrate the asbestos during transport or industrial treatment, as raw chrysotile does not contain polycyclic hydrocarbons. Their presence may confer a carcinogenic potentiality to the fibers which already contain Ni and Cr. These metals can inhibit benzopyrene-hydroxylase in the lungs and thus retard the metabolic degradation of carcinogenic hydrocarbons. Further research needs to determine the origin of carcinogenic hydrocarbons in order to suppress this form of pollution.--C. M. M.

4240 MARTINET, P. Working in noise, a program for the protection of hearing faculties, a judicious distribution of periods of noise-exposure to noise is necessary. ARCH. MAL. PROF. 33-e: 285-291. 1972.--Above a certain sound level, the exposure to noise during the whole working period entails a risk to the hearing acuity of the workmen. When periods of non-exposure to noise are interspersed, the total exposure length remaining the same, noises of higher intensity can be heard without increased risk. A practical method, based on the works of Kryter, was established which allows the determination of the length and distribution of the periods during which it is advisable to withdraw the workers from noise aggression. In many cases, this method would be less stringent than the one based upon the "noise rating number" and thus more easily accepted by those responsible for the organization of work. Nevertheless, it would in general be more stringent in the case of shrill noises (central frequency range 2000 Hz).--C. M. M.

K-17590

4241. ASSOULY, M., G. SIOU and A. CAVIGNEAUX. Polyneuritis caused by n-hexane. ARCH. MAL. PROF. 33/6: 309-310. 1972.--Two new cases of polyneuritis of the sensory-motor type were observed in young leather factory workers using glues with a petroleum C base having a high content of n-hexane. Tests on the factory air showed a solvent level higher than 500 ppm (maximum levels of 100 ppm and 500 ppm were set in Japan and the USA respectively). Although the causative agent is not yet determined, the dangers of petroleum C and n-hexane is certain and preventive measures involving atmospheric controls should be taken to guard against this new source of toxic polyneuritis.--S. L. R.

4242 TOPPING, G. and J. M. PIRIE. Determination of inorganic mercury in natural waters. ANAL. CHIM. ACTA. 62/1: 200-203. 1972.--A recently developed procedure incorporating a $KMnO_4$-H_2SO_4 solution and flameless cold vapor atomic absorption spectrophotometry was used for the determination of Hg in sea waters enriched with Hg at levels of 0.1, 1.0 and 10.0 g l^{-1} as part of studies on the effects of sublethal amounts of trace metals on marine food chains. The technique described below permitted water samples to be analyzed for Hg down to 0.002 g l^{-1}, with a 4:1 sample. Scale expansion was not required, so that an extremely steady base line on the chart record was ensured.--C. M. P.

4243 SIEGEL, N. J. and T. J. SPACKMAN. Chronic hypercalcaminosis A with intracranial hypertension and low cerebrospinal fluid protein concentration. CLIN. PEDIATR. (PHILA.) 11/10: 580-584. 1972.--An unusually high dietary intake can lead to vitamin A intoxication. These 2 children had clinical and radiologic evidences of increased intracranial pressure and low cerebrospinal fluid protein concentrations. Intoxication of these siblings was induced by administration of large amounts of vitamin A by a health-faddist mother.--C. M. M.

K-1338

4244 ANONYMOUS. Toxicity of thallium. BR. MED. J. 3/5829: 317. 1972.--Tl is an extremely toxic and cumulative poison. It constitutes the main hazard to the general public in the form of rodenticides and insecticides. Tl salts can enter the body by inhalation as a dust, by ingestion either from contaminated foods or from the hands or by absorption through the skin. Several poisoning cases, the importance of iodine in the differential diagnosis when early hazards in accidental cases are observed and the therapy of this kind of toxicity are discussed.--C. M. M.

4245 BRESSON, J. B., J. BOITEUX-LOLON and R. GIRARD. Dermatitis caused by phenoplastic resins. ARCH. MAL. PROF. 33/4 Sup. 199-201. 1972.--Twelve cases of dermitis were observed over a 4 yr period in 80 bakelite factory workers using phenoplastic resins. The lesions consisted of erythematous papules usually located on exposed parts of the body. Many were complicated by scratching. No generalized lesions were observed. This may have been a result of the fact that affected workers were quickly moved to other jobs in the factory. The lesions appeared from a few weeks to a few months after the beginning of exposure in most cases, except in 2 more slowly-developing cases of eczema in subjects not actively manipulating the resins). Skin tests could unfortunately not be conducted. Preventive measures take included the transfer of workers with histories of allergy problems and workers presently suffering from exposure to other plastics, the reduction of handling to a minimum, recommendations for careful personal hygiene, the wearing of protective gloves and reduction of exposure to dusts and vapors.--S. L. R.

4246 COMMITTEE ON GOVERNMENT OPERATIONS, SUBCOMMITTEE ON EXECUTIVE REORGANIZATION AND GOVERNMENT RESEARCH. HEARINGS. Chemicals and the future of man. U.S. CONGR. SENATE 92ND 1ST SESS. APRIL 4-6, 1971. 1-301.--Studies were made of the safety of synthetic and natural chemicals in food, drugs, air and drinking water. Attempts were made to learn what effect these compounds have on our health and on the health of future generations. Pollutants, carcinogens, mutagens and teratogens were discussed. The effectiveness of Federal efforts in research, regulation and testing in this area were assessed.--C. M. M.

K-4568

4247 COMMITTEE ON COMMERCE, SUBCOMMITTEE ON ENERGY, NATURAL RESOURCES AND ENVIRONMENT. The effects of 2,4,5-trichlorophenoxyacetic acid on man and the environment. U.S. CONGR. SENATE 91ST, 2ND SESS. SER. 91-43: 1-471. 1970.--Some chemical data concerning the synthesis and reactions of 2,4,5-T, estimated phenoxy pesticides and defoliation studies are included in this report. Recommendations are given for the immediate suspension of registration of economic poisons containing 2,4,5-trichlorophenoxyacetic acid.--C. M. M.

4248 LOGSDON, J. E. and J. L. ROBINSON. Radioactive waste discharges to the environment from nuclear power facilities. U.S. OFF. RADIAT. PROGRAMS, SURVEILLANCE, NSP. DIV. 1971(TECH. REP. ORP/NSD-71-1): 1-29. 1971.--Discharges through 1968 of liquid and gaseous radioactive wastes from 9 nuclear power facilities are summarized in this report. A general discussion of the sources, types, and amounts of liquid and gaseous wastes, the administrative controls and the operating experience for the 9 nuclear power stations are also included. Various tables and charts summarize the characteristics of the facilities and their discharges. This addendum to the report updates the tables and charts through 1970. The report includes an appendix for each facility, which describes the characteristics of the plant and its discharge limits and provides a brief history of its operation. The appendices also include graphs of discharges, power production and maximum operations as a function of time.--C. M. M.

4249 U.S. PUBLIC HEALTH SERVICE. The health consequences of smoking. A report to the Surgeon General, 1971. U.S. PUBLIC HEALTH SERV. PUBL. 1971(DHEW HSM-71-7513): 1-458. 1971.--Hazards of cigarette smoking including cardiovascular diseases, chronic obstructive bronchopulmonary disease, cancer, peptic ulcer and tobacco amblyopia are discussed in detail. The dangers of maternal smoking during pregnancy on fetal growth are also presented.--C. M. M.

4250 MARTIN, G. B., D. W. PERSBING and E. E. BERKAU. Effects of fuel additives on air pollutant emissions from distillate oil fired furnaces. U.S. ENVIRON. PROT. AGENCY, PUBL. AP-SER. 1971(AP-47): 1-46. 1971.--The Office of Air Programs of the U.S. Environmental Protection Agency studied the use of fuel additives to control air pollution from distillate oil burning systems. The available literature was surveyed, and samples of all known additives procured.

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B 12 in the Geneva and Nantua Lakes. Consequences of these variations for the biological evolution of the lakes.] C R HEBD SEANCES ACAD SCI SER D SCI NATUR PARIS 272(6): 608-611. 1970[rec'd. 1971].--The vitamin B 12 contents of water from Lake Geneva from June to Nov. 1970, was determined. Comparative measurements were done on Nantua Lake. An increase in this vitamin contents in water samples of Lake Geneva, influenced by urban waste water pollution, has the threat of causing the unfavorable development of algae such as was the case for Lake Nantua. The increased levels in deep water are probably due to the activity of B 12 producing bacteria present in the sediments or in clear water. Surface B 12 level is lower in summer. The reduction of this vitamin in sewer water by an appropriate treatment may be a remedy in the case of Lake Geneva and other lakes presenting the same problem.--A. B. C.

543. FOSTER, P., and A. W. MORRIS. (Mar. Sci. Lab., Menai W Bridge, Anglesey, N. Wales, UK.) The use of ultra-violet absorption measurements for the estimation of organic pollution in inshore sea water. WATER RES 5(11): 19-27. Illus. 1971.--Changes in the UV absorption spectra of sea water at an inshore station were monitored over an annual period. These changes are interpreted in terms of variable supply of organic matter from the major sources of land drainage and autochthonous biological activity. Differences in the molecular nature of the organic matter derived from these sources result in seasonal variations in both the degree of absorption per unit of dissolved organic C and the relative absorption within different wavelength regions. Hence, only a poor correlation between the UV absorption and the dissolved organic C content is obtained. Appreciable short term variations in spectra were also recorded. These variations severely limit the use of UV absorbance measurements for the indication of marine organic pollution.

544. STIRN, JOZE. (Mediterr. Mar. Sorting Cent., Salammbo, W Tunis.) Further contributions to the study of the bioproductivity in polluted marine ecosystems. REV INT OCEANOGR MED 18/19: 21-27. 1970[rec'd. 1971]. [Fr. summ.]--A water mass which is under influence of non ionic organic sewage changes its chemical composition and also its ecological conditions with respect to normal marine ecosystems. It results in a higher density of primary production with predominance of certain phytoplanktonic species. Some observations in the Lake of Tunis in comparatively highly polluted and in slightly polluted area show a high rate of Nannochloris oculata in highly polluted areas.--A. B. M.

545. BOURCIER, MICHEL. (Sta. Mar. Endoume, Marseille, Fr.) S Ecoulement des boues-rouges dans le Canyon de la Cassidaigne (December W 1968). [Running red mud in the Canyon of Cassidaigne (December M 1968).] TETHYS 1(3): 775-782. Map. 1969[rec'd. 1971]. [Engl. summ.]--Since March 1967 aluminum works have been discharging residues into the submarine canyon of Cassidaigne (near Marseilles) by means of a pipe ending at a depth of 350 m. Up to Dec. 1968 the residues in fine red colored mud spread in the canyon and reached a depth of 1800 m. Two different zones can now be distinguished: a central zone zone near the mouth of the pipe with a thick layer of red mud, and a thin layer peripheral zone with normal benthic life.--A. B. M.

546. COOPER, JAMES M. and JERRY L. WILHM. (Zool. Dep., W Okla. State Univ., Stillwater, Okla., USA.) Longitudinal variation of periphyton productivity in Skeleton Creek, Oklahoma. PROC OKLA ACAD SCI 49: 19-22. Illus. 1970[rec'd. 1971].--Periphyton biomass was sampled using artificial substrata over a series of increasing exposure times in an enriched stream. A growth curve was plotted and analyzed by a discontinuous linear regression model. The maximum instantaneous growth rate was used to estimate productivity. Productivity decreased downstream from the pollution outfall.--N. F. G.

547. KRIPPELOVÁ, TEREZIA. (Bot. Inst., Slovak Acad. Sci., A Bratislava, Czech.) Puccinellio-Chenopodietum glauci assoc. nova. S ENVIRON POLLUT 1(2): 185-[illegible]. [Engl. summ.]--Puccinellio-M Chenopodietum glauci assoc. nov. represents a new plant community of the Polygonion avicularis Br.-Bl. 1931 association. It was first observed in Kosice in eastern Slovakia and is important to determine the plant zones. It develops here with trampling from the association Plantagino-Lolietum in an area suffering from air pollution. It is characterised by trampled soils with high Mg concentrations which come from the aerial effluent of the magnesite works in Tahanovce.

548. BAKER, JENIFER M. (Univ. Coll., Swansea, Wales, UK.) Sea-W sonal effects of oil pollution on salt marsh vegetation. OIKOS 22(1): S 106-110. Illus. Maps. 1971.--Salt marsh transects near Pembroke, SW Wales, were experimentally sprayed with fresh Kuwait crude oil at different times of year, and recovery measured using a points frame. There was little long term vegetative damage to most perennial species, but in the case of the annual Suaeda maritima damage was severe, and was further investigated using density and dry weight measurements. Flower density measurements for Juncus gerardii, Festuca rubra, Plantago maritima, and Spartina x townsendii, showed that marked reduction of flowering can occur if plants are oiled when the flower buds are developing. Flowers, if oiled, rarely produce seeds. Oiling of seeds during winter may reduce germination in the spring.

549. STEBBINGS, R. E. (Nature Conservancy, Coastal Ecol. Res S Sta., Norwic. Engl., UK.) Recovery of salt marsh in Brittany sixteen W months after heavy pollution by oil. ENVIRON POLLUT 1(2): 183-187. 1970.--Two visits separated by 16 mo. were made to salt marshes and a shingle spit in Brittany, where oil was stranded in the spring of 1968, to assess macrochanges in floral composition and the overall effects of heavy contamination by weathered oil. Most salt marsh plant species survived all but the heaviest contamination. Oil appeared to prevent gaseous interchange between soil and air, yielding reducing conditions which led to chlorotic symptoms in plants. As the oil was rapidly denatured salt marshes can be important for retaining oil during this process and preventing pollution elsewhere.

550. SHCHOLOKOVA, I. P., and V. P. BYKOVA. (Inst. Microbiol. W Virol., Acad. Sci. Ukr. SSR, Kiev, USSR.) Dryzhdzhova flora vodoemov, zabrudnenykh naftoproduktami. [Yeast flora of reservoirs contaminated with oil-products.] MIKROBIOL ZH (KYYIV) 32(5): 442-444. 1970 [Russ. and Engl. summ.]--The yeast flora was studied in contaminated and uncontaminated reservoirs. Yeasts of the genera Candida, Torulopsis, Rhodotorula, Trichosporon, Saccharomyces and Hansenula were isolated. Uncontaminated reservoirs supported species which did not assimilate n-alkanes more often. The strains of yeast from the reservoirs contaminated with oil-products assimilate oil hydrocarbons more than strains of the same species isolated from the Dneper water and from lakes. Oil-products influence the selection of active strains of hydrocarbon oxidizing yeast.--N. F. G.

551. PARSONS, ALICE B., and PATRICK R. DUGAN. (Fac. Micro-W bial and Cell. Biol., Ohio State Univ., Columbus, Ohio, USA.) Production of extracellular polysaccharide matrix by Zoogloea ramigera. APPL MICROBIOL 21(4): 657-661. 1971.--Z. ramigera 115 synthesized large amounts of matrix polymer [which might be exploited as means of pollutants from water] from fructose, galactose, glucose, lactose, mannose, soluble starch and sucrose when these carbohydrates were used as supplements to a chemically defined medium. All of them supported polymer synthesis to the extent that cultures thickened to a gel. Concentration of carbohydrate nutrients in the range 0.5 to 2.0% was not a critical factor in determining eventual total thickening to a gel, except in relation to the incubation time required. Glucose disappeared from the growth medium rapidly and correlated with increasing cell growth and poly-β-hydroxybutyrate (PHB) accumulation. PHB concentration decreased as extracellular polymer was synthesized, suggesting a link between PHB and extracellular production.

552. MONTGOMERY, MARVIN L., and LOGAN A. NORRIS. P (Inst. Forstl. Standortsforsch., Hochsch. Bodenkultur, Vienna, Austr.) W Eine vorläufige Bewertung der Gefahren, die mit der Anwendung von S 2, 4, 5-T im Wald verbunden sind. [A preliminary study of the dangers involved in the use of 2, 4, 5-T in the forest.] BODENKULTUR 21(4): 425-431. 1970.--The use of 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) causes a slight temporary water pollution. There is no significant danger to fish or other animals. The extensive absorption and degradation of 2, 4, 5-T in forest soils hinders its later displacement from treated areas to open water and subsoil water. Rainfall, dilution by growth and degradation reduce the residue of the herbicide in the vegetation within several weeks. 2, 4, 5-T has a low toxicity; it is not permanent in the soil or in plants and is quickly eliminated by animals. --E. K.

553. ENVIRONMENTAL POLLUTION. Vol. 1, No. 1. 1970. Z Quarterly. Kenneth Mellanby [Editor]. Elsevier Publishing Co., L.T.D.: Essex, England. Pr. $15.60.--This international journal is concerned mainly with the biological effects of pollution and includes research papers on the ecological effects of all types of environmental pollution and pollution control. The journal provides a vehicle for the widest dissemination of original papers discussing the ecological implications of pollution problems from many points of view including the international, political, economic, social, medical, managerial, planning and engineering aspects. Research papers must be factual and original, and must not have been published elsewhere, though papers describing work whose chemical aspects have already been published may be accepted if previously unpublished biological information and conclusions are included. Types of contributions will include: research papers; review articles; short communications; book reviews; conferences and meetings; and letters to the editor. The principle language of the journal is English, but papers in French and German will also be included. Correspondence should be addressed to the Elsevier Publishing Co. LTD, Ripple Road, Barking, Essex, England.--B. J. S.

554. GLOYNA, EARNEST F. and W. WESLEY ECKENFELDER, W Jr. [Edited by]. Water Resources Symposium, No. 3. Water quality D improvement by physical and chemical processes. xv-[illegible]. Illus. University of Texas Press: Austin, Tex., USA; London, England. 1970. Pr. $15.00.--This volume contains the papers prepared in a special lecture series for the 3rd Water Resources Symposium, sponsored by the University of Texas Center for Research in Water Resources in April 1969 at Austin, Texas. Emphasis is on the most recent develop-

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analyzing biological materials by various methods is discussed and the biological methylation routes of mercury in the environment are presented and illustrated by schematics.

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71-2070. Anonymous. Herbicides: "Secret" 2,4,5-T report. *Chem. Eng. News 49(29): 15; 1971.*

The registration of 2,4,5-T for use on crops for human consumption and in granular form around homes and recreational areas was cancelled on May 1, 1970. Its use around water and in liquid form around houses and recreational areas had already been suspended on April 15. Under the Federal Insecticide, Fungicide, and Rodenticide Act, the Dow Chemical Co. and Hercules, Inc., petitioned for referal of the cancellations to an advisory committee. The committee's report, submitted to the Environmental Protection Agency (EPA) for action on May 7, 1971, recommends that all uses of 2,4,5-T in effect before April 1970 be allowed with the provisions that permissible residual levels in food be established and that residues of the contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), be kept at a specific low level. The report was criticized by several prominent scientists. The report itself contains a dissenting opinion by EPA advisory committee member Dr. Theodor D. Sterling. Sterling said that the data considered by the committee failed to justify its judgment that there is a level at which TCDD is not teratogenic. He also said that the report did not consider the consequences of the admitted uncertainty about the fate of TCDD in food chains and animal tissue. Dr. Samuel S. Epstein, speaking for the Committee for Environmental Information, said that "there are recent experiments, inexplicably discounted by the EPA committee, that show even low doses of 2,4,5-T do cause teratogenic effects in offspring." He said that the report contained "clearly technical errors and unsubstantiated statements" and that the EPA committee had not shown a compelling need for the continued use of 2,4,5-T.

71-2071. Marechal, G.; Demonzay, D. (Biologie-Chemiegruppe, Fa. Pechiney-Progil (PEPRO), Lyons, France). **Moegliche Rueckstaende von Lindan in Milch und Milchprodukten sind ohne praktische Bedeutung.** [The possible residues of lindane in milk and milk products are without practical significance.] *Dent. Milchwirt. 21(33): 1420-1; 1970.* (4 references) (German)

Arguments are presented to show that lindane (at least 99.5% pure *gamma*-isomer of hexachlorocyclohexane), one of the chlorinated hydrocarbon insecticides, should not be condemned along with the other insecticides in the group. Recent publicity about the residues of chlorinated hydrocarbon insecticides has brought about mass condemnation of this entire group of insecticides. If DDT were used as the only indicator, this condemnation might be just. DDT is slowly degraded and is easily stored in lipids. DDT residues are readily found in the fatty tissues and milk of cows that have eaten feed containing DDT. Lindane, which is initially applied in much lower concentrations (80 to 350 g/ha lindane as compared to 1200 to 2500 g/ha DDT), is more rapidly degraded and is quickly expelled from the bodies of mammals. Tests with milk showed that residues of DDT were still detectable 3 weeks after the cows were treated with DDT (Claborn, 1960), whereas no residues of lindane were detected in the milk after 1 week (Radelett *et al.*, 1952). As lindane is rapidly degraded on the surface of plants (Huddleston *et al.*, 1960) and Trace and Ware, 1965) and it is rapidly excreted by animals, one can assume that future use of lindane will result in residues below the tolerance levels.

71-2072. Crosley, D. G., Sieber, J. N. (Dept. of Environmental Toxicology, U. of California, Davis, CA 95616). **California symposium bridges pesticides communications gap.** *Farm Chem. 134(5): 38, 64-5; 1971.*

The Symposium on Agricultural Chemicals: Harmony or Discord for Food, People, Environment was held February 16-17, 1971, at Sacramento, California. The meeting, sponsored by the University of California Division of Agricultural Sciences, was attended by about 350 participants from industry, government and the University. Twenty-four papers were presented on the themes of the need for agricultural chemicals, problems associated with their use and solutions to these problems. One paper pointed out that the problem of using chemicals in agriculture involves food supply, the displacement of agricultural workers, increased urbanization, decreased net farm income and inequitable distribution of food. Another participant stated that despite increasing food production, output is only 6% above national needs and that chemicals must be supplemented with resistant plants and the use of predators and pheromones in order to increase the margin between output and consumption. One paper noted that losses in California of wildlife and fish in 1970 were the lowest of any year in the preceeding ten. The view was expressed that the greatest danger of pesticide use lies in direct exposure of agricultural workers. Remedies such as mandatory protective clothing, respirators and periodic checks of blood cholinesterase levels were mentioned. The idea was put forth that the most serious problem associated with placing sole reliance on chemical control of pests is pest resurgence and emergence. Doubt was cast on the belief that synthetic compounds could be developed that would not induce resistance in pests. As for the future, the participants concluded that there will be more attention paid to environmental side effects, more restrictions of continued scrutiny and expanded use of agricultural chemicals.

71-2073. Shepard, H. H. (Author address not given). **NAS symposium examines pest control strategies.** *Farm Chem. 134(6): 46, 48; 1971.*

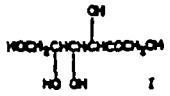
Some of the papers presented at the symposium on "Pest control strategies for the future" are briefly reviewed in this article. The symposium, attended by about 300 delegates, was organized by the Agricultural Board of the National Research Council-National Academy of Sciences and held in Washington, D. C. Several government and private institutions cooperated in sponsoring the meeting. The papers dealt with such topics as "Pest control in health and agriculture: international challenges," "Ecology of agriculture in the world of today and tomorrow," "Implementing pest control strategies," "Basic principles of ecosystems analysis," "Hormonal

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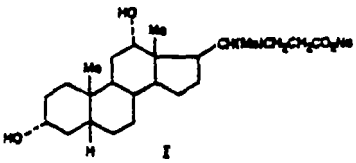


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LIPOLYSIS. The plasma insulin level was raised by 56 and 114%, resp., by infusion with 1.0 and 1.5 g I/kg/hr. The respective plasma I levels at these infusion rates were 87 and 140 mg %. Infusion with 0.5 and 1.0 g I/kg/hr decreased the plasma FFA level to 57 and 31%, resp., of the initial value. The plasma triglyceride concn. decreased to a min. at 105 min, after which it gradually rose and eventually exceeded the initial level.

15063s **Antiviral properties of some polysaccharide preparations and RNA from yeast.** Kovalenko, A. G. (Inst. Microbiol. Virol., Kiev, USSR). *Mikrobiol. Zh. (Kiev)* 1972, 34(1), 86–7 (Ukrain). Polysaccharide prepns. from *Candida tropicalis*, *C. arborea*, *Saccharomyces cerevisiae*, and *S. carlsbergensis*, and prepns. of *glucan* [9012-72-0] and *mannan* [11118-75-5] from *C. viswanathii* and *C. tropicalis* decreased the PLANT SENSITIVITY to the POTATO VIRUS X and TOBACCO MOSAIC VIRUS when tested at 1 mg/ml on *Solanum tuberosum*, *Nicotiana tabacum*, *Datura stramonium*, *Gomphrena globosa*, and *Chenopodium amaranticolor*. A prepn. of RNA from yeast was less effective.

15069t **Glycoprotein receptors for concanavalin A isolated from pig lymphocyte plasma membrane by affinity chromatography in sodium deoxycholate.** Allan, David; Auger, Judy; Crumpton, M. J. (Natl. Inst. Med. Res., London, Engl.). *Nature (London), New Biol.* 1972, 236(62), 23–5 (Eng).



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The specific *concanavalin A* [11028-71-0]-receptor(s) of pig lymphocyte plasma membrane were glycoproteins that could be solubilized in 1% *sodium deoxycholate* (I) [302-95-4] and purified by adsorption to and elution from concanavalin covalently attached to *Sepharose 4B* [9036-81-7]. This purification method may be applicable to the purification of those membrane receptors whose biol. activities are retained after solubilization in DETERGENT.

15070m **Metabolism of Choline and Transmethylation Reactions.** (Metabolizm Kholina i Reaktsii Peremetilirovaniya) Cielens, E. (Zinatne: Riga, Lat. SSR). 1971. 208 pp. 1.04 r.

For papers of related interest see also Section:
1 13790x Mechanism of action of antibiotics.
13801b Biological properties of selected fluorine-containing organic compounds.
13877f Analogs of adenosine 5'-diphosphate. New platelet aggregators. Influence of purine ring and phosphate chain substitutions on the platelet-aggregating potency of adenosine 5'-diphosphate.
13948e Induction of benzpyrene hydroxylase by flavone and its derivatives in fetal rat liver explants.
13950z Potentiation of the antiviral activity of interferon by cycloheximide.
13951a Cyclic nucleotide analogs as carcinostatic agents.
13977p Halothane inhibition of phytohemagglutinin-induced transformation of lymphocytes.
14006h Inhibition of urea biosynthesis and conversion of L-citrulline-6-^{14}C to [^{14}C]-urea by α-methyl aspartic acid and β-methyl aspartic acid.
14380a Effect of chemical substances on platelet functions.
2 14509z Conductance changes produced by l-norepinephrine on lipid membranes containing a proteolipid from the bovine spleen capsule.
14539j In vitro effect of hypothalamic extract and other stimulating agents on glucose oxidation and luteinizing hormone (LH) release from rat anterior pituitary glands.
14594y Comparative action of aminazine and insulin on oxidation processes in the brain of rats after the administration of serum from schizophrenics.
14646s Analysis of the blocking effect of serotonin on the glossomandibular reflex.
4 15090t Induction in different species.
15166x Selective inhibition of the synthesis of mouse satellite DNA by dehydrobeliotridine.
15284j Preferential in vivo inhibition of ribosomal ribonucleic acid synthesis in mouse liver by the exotoxin of Bacillus thuringiensis.
5 Agrochemicals.
10 Microbial Biochemistry.
11 Plant Biochemistry.
12 Nonmammalian Biochemistry.
13 Mammalian Biochemistry.
14 Mammalian Pathological Biochemistry.

4—TOXICOLOGY

K4568

T. R. TORKELSON

15071n **The problem of using 2,4,5-T** [2,4,5-trichlorophenoxyacetic acid]. Maier-Bode, H. (Pharmakol. Inst., Friedrich-Wilhelms-Univ., Bonn, Ger.). *Anz. Schaedlingsk. Pflanzenschutz* 1972, 45(1), 2–6 (Ger). A REVIEW with 20 refs. on the safety factors involved in the use of *2,4,5-T* [93-76-5], since *2,3,6,7-tetrachlorodibenzo-p-dioxin* [1746-01-6], present as an contaminant, is teratogenic.

K6665

15072p **Cycle of carcinogenic polycyclic aromatic hydrocarbons in plants and in man.** Wagner, K. H.; Wagner-Hering, E. (Inst. Ernährungsphysiol. II, Justus Liebig-Univ. Giessen, Giessen, Ger.). *Wein-Wiss. Vitae* 1971, 16(6), 260–6 (Ger). A REVIEW with 17 refs.

15073q **Formation, pharmacology, and toxicology of the cyclohexylamines, starting product for the synthesis of cyclamates.** Classen, H. G.; Marquardt, P. (Abt. Exp. Ther., Univ. Freiburg, Freiburg/Br., Ger.). *Ernaehr.-Umsch.* 1972, 19(1), 13–17 (Ger). A REVIEW with 61 refs.

15074r **Statistics vs safety factors and scientific judgment in the evaluation of safety for man.** Weil, Carrol S. (Mellon Inst., Carnegie-Mellon Univ., Pittsburgh, Pa.). *Toxicol. Appl. Pharmacol.* 1972, 21(4), 454–63 (Eng). A REVIEW and discussion with 19 refs. on carcinogens, suggesting that safety should be evaluated by appropriate toxicol. tests, statistical comparisons of data from treated and control animals, delineating min. and max. no ill-effect levels for these animals, and applying a safety factor to its use.

15075s **Research on teratogenic effects in the mouse.** Groupe d-Etude des Risques Teratogenes Universite-Industrie (Lab. Pharmacodyn., Fac. Pharm., Montpellier, Fr.). *Proc. Eur. Soc. Study Drug Toxicity* 1971, 12, 307–31 (Fr). Exptl. factors influencing teratogenic tests in mice were discussed; 39 refs. Variables pertaining to the test mice, such as genetics and conditions of copulation, to the drug treatment used, such as dosage and duration of treatment, or to the interpretation of the test results were discussed.

15076t **Fluorometric procedures for illicit drug detection.** Passwater, Richard A. (Am. Instrum. Co., Silver Spring, Md.). *Fluorescence News* 1971, 6(3), 8–11 (Eng). A REVIEW with 7 refs. and a selected BIBLIOG. with 37 refs. Methods of choice, esp. that of L. Goldbaum et al. (1971), for the detection of drugs, such as *morphine* [57-27-2], in biol. fluids are discussed.

15077u **Toxicology of basic materials in washing and cleaning agents.** Glozhuber, Chr. (Toxikol. Lab., Henkel und Cie. G.M.B.H., Duesseldorf, Ger.). *Fette, Seifen, Anstrichm.* 1972, 74(1), 49–57 (Ger). A REVIEW with 60 refs.

15078v **Toxicology of food additives.** Villanua, L. (Spain). *An. Real Acad. Farm.* 1971, 37(4), 497–510 (Span). A REVIEW with 42 refs. of the mechanism of accumulation, direct effects, and indirect, long-lasting toxicity of food additives.

15079w **Lead poisoning. Disease of our time.** Hankin, Lester (Biochem. Dep., Connecticut Agric. Exp. Stn., New Haven, Conn.). *J. Milk Food Technol.* 1972, 35(2), 86–97 (Eng). A REVIEW discussing the *lead* [7439-92-1] poisoning problem among children as regards sources of contamination as well as testing methods used to find potential victims of this disease. The amts. and sources of Pb in foods, both raw and processed, and in air are detailed. Implications of daily ingestion of small amts. of Pb from food, water, and air are discussed; 128 refs.

15080q **Biological detection of mycotoxins.** Gedek, Brigitte (Inst. Mikrobiol. Infektionskr. Tiere, Univ. Muenchen, Munich, Ger.). *Zentralbl. Veterinaermed., Reihe B* 1972, 19(1), 15–32 (Ger). A REVIEW with 92 refs. on the sensitivity of biol. methods of detection for mycotoxins.

15081r **Metal-induced granulomatous hypersensitivity in man.** Epstein, William L. (Sch. Med., Univ. California, San

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een reached.

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2776 MAIER-BODE, H. (Pharmakol. Inst. Rhein, Friedrich-Willhelms-Univ., Reuterst. 2b, 53 Bonn/Rh., West Ger.) The 2,4,5-T question. ANZ SCHAEDLINGSKD PFLANZENSCHUTZ 45(1): 2-6. 1972. [Engl. summ.]--The teratogenic effect of 2,4,5-T, proved in lab animals in the USA in 1969, is attributed to an impurity of 30 ppm of the highly toxic 2,3,6,7-tetrachloro-dibenzo-p-dioxin. The sample tested originated from a factor which stopped 2,4,5-T production years ago. The manufacturers today can guarantee that the dioxin content of the technical 2,4,5-T acid is not higher than 0.1 ppm. For humans, domesticated animals and wildlife, no danger should occur from dioxin residues resulting from 2,4,5-T uses in agriculture and forests. High oral doses of 2,4,5-T with a dioxin content not higher than 0.1 ppm can cause a teratogenic effect in extremely susceptible lab animals. The no-effect level of 2,4,5-T in NMRI-mice was 20 mg/kg per day. There are no indications that man is 2,4,5-T-susceptible. The relatively low persistence of 2,4,5-T indicate that the herbicide in food or in the environment is probably not reaching a contaminating level for men, domesticated animals, fur-bearing animals or wild-fowl. Concerning the toxicological aspects, at the moment there is no fundamental reason for suspension of the use of 2,4,5-T-preparations officially approved and recommended in agriculture and forests. The BRD-Bundesrat [a West German government agency] decided to allow the use of 2,4,5-T-preparations in general (agriculture, forests, park and garden lawns), with the exception of aerial sprayings which can be done only with the special permission of the regional officials concerned.

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plasma membrane were glycoproteins that could b solubilized in 1% *sodium deoxycholate* (I) [302-95-4] an purified by adsorption to and elusion from concanavali covalently attached to *Sepharose 4B* [9036-61-7]. Thi purification method may be applicable to the purification o those membrane receptors whose biol. activities are retained afte

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15071n **The problem of using 2,4,5-T** [2,4,5-trichlorophen= oxyacetic acid]. Maier-Bode, H. (Pharmakol. Inst., Friedrich-= Wilhelms-Univ., Bonn, Ger.). *Anz. Schaedlingsk. Pflanzens= chutz* 1972, 45(1), 2-6 (Ger). A REVIEW with 20 refs. on the safety factors involved in the use of *2,4,5-T* [93-76-5], since *2,3,6,7-tetrachlorodibenzo-p-dioxin* [1746-01-6], present as an contaminant, is teratogenic.

15072p **Cycle of carcinogenic polycyclic aromatic hydroca= rbons in plants and in man.** Wagner, K. H.; Wagner-Hering, E. (Inst. Ernaehrungswiss. II, Justus Liebig-Univ. Giessen, Giessen, Ger.). *Prot. Vitae* 1971, 16(6), 260-6 (Ger). A REVIEW with 11 refs.

15073q **Formation, pharmacology, and toxicology of the cyclohexylamines, starting product for the synthesis of cyclamates.** Classen, H. G.; Marquardt, P. (Abt. Exp. Ther., Univ. Freiburg, Freiburg/Br., Ger.). *Ernaehr.-Umsch.* 1972, 19(1), 13-17 (Ger). A REVIEW with 61 refs.

15074r **Statistica vs safety factors and scientific judgment in the evaluation of safety for man.** Weil, Carrol S. (Mellon Inst., Carnegie-Mellon Univ., Pittsburgh, Pa.). *Toxicol. Appl. Pharmacol.* 1972, 21(4), 454-63 (Eng). A REVIEW and discussion with 19 refs. on carcinogens, suggesting that safety should be evaluated by appropriate toxicol. tests, statistical comparisons of data from treated and control animals, delineating min. and max. no ill-effect levels for these animals, and applying a safety factor to its use.

15075s **Research on teratogenic effects in the mouse.** Groupe d-Etude des Risques Teratogenes Universite-Industrie (Lab. Pharmacodyn., Fac. Pharm., Montpellier, Fr.). *Proc. Eur. Soc. Study Drug Toxicity* 1971, 12, 307-31 (Fr). Exptl. factors influencing teratogenic tests in mice [illegible]

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FIG. 3

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Toxicology Letters, 14 (1982) 175–181
Elsevier Biomedical Press

BILIARY EXCRETION OF 2,4,5-TRICHLOROPHENOXYACETIC ACID IN THE RAT

(HPLC; hepatic transport; phenoxyacid herbicides)

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(Received June 9th, 1982)
(Accepted June 11th, 1982)

SUMMARY

Urethane-anesthetized, male Sprague–Dawley rats were administered 25 mg/kg 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) intravenously. 6 h after administration, 15.7% of the dose was recovered in the bile. Reversed-phase high-performance liquid chromatography (HPLC) analysis of bile indicated that more than 85% of compound excreted in bile was unchanged 2,4,5-T. Since renal function was apparently compromised by the urethane anesthesia, only 4% of the dose was recovered in urine in 6 h. These data demonstrate that biliary excretion can be a slow but significant route of elimination under conditions of impaired renal function. Since 2,4,5-T is eliminated largely as the unchanged acid, reabsorption and thus enterohepatic circulation may significantly prolong the half-life of 2,4,5-T in the body.

INTRODUCTION

Although numerous studies have examined the toxicity, fate and disposition of phenoxy acid herbicides [1–10], none have examined directly the biliary excretion of 2,4,5-T. Sauerhoff et al. [9] examined the urinary and biliary excretion of Silvex in the rat and found that saturation of the renal transport system for Silvex occurred at doses above 50 mg/kg, and biliary excretion became relatively more important in the total elimination of Silvex as the renal system was saturated. Based on this and other plasma elimination data, Colburn [10] developed a two-compartment pharmacokinetic model of chlorophenoxy acid herbicides which suggested that

Abbreviation: 2,4-D,2,4-dichlorophenoxyacetic acid; HPLC, high-performance liquid chromatography; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

0378-4274/82/0000–0000/$02.75 © Elsevier Biomedical Press

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enterohepatic recirculation of these compounds would be expected to be extensive at higher doses. Since none of these compounds is appreciably biotransformed in the liver [2, 5], reabsorption of the fraction excreted in the bile may significantly prolong the plasma half-life of these compounds without reducing the total amount eventually excreted in the urine.

This study investigated directly the biliary excretion of 2,4,5-T for 6 h following an i.v. injection of [^{14}C]2,4,5-T to determine the extent and nature of biliary excretion of this particular phenoxy acid herbicide, and to determine the relationship between plasma and bile concentrations of 2,4,5-T.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats (200–250 g) were anesthetized by intraperitoneal administration of urethane (1 g/kg), and the bile duct and left femoral vein were cannulated with PE-10 and PE-50 tubing, respectively. The penis was tied off just prior to i.v. administration of 2,4,5-T to facilitate collection of urine at the end of the bile collection period.

Technical grade 2,4,5-T (92%, Sigma Chemical Company) was purified by recrystallization from benzene. The sodium salt for i.v. injection was prepared and combined with uniformly ^{14}C-ring-labeled 2,4,5-T (13.3 mCi/mmol, Mallinckrodt Inc., St. Louis, MO) such that an injection of 5.0 ml/kg body weight provided a dose of ^{14}C-labeled sodium salt of 2,4,5-T of 10 μCi/kg, 25 mg/kg. Chemical and radiochemical purity of this solution was >98% as determined by reverse-phase HPLC analysis.

Bile collection

A 15-min pre-injection bile sample and 12 consecutive 30-min bile samples were collected into pre-weighed glass test tubes following i.v. injection of 2,4,5-T. Blood samples (100 μl) were collected every 30 min in heparinized microhematocrit tubes by the tail-clip method and a 25-μl aliquot of plasma was collected for scintillation counting. Bile samples were weighed and 50 μl aliquots of bile were placed in scintillation minivials containing 100 μl of 30% H_2O_2 to reduce color quenching from bile pigments.

HPLC analysis

Reverse-phase, HPLC analysis of bile samples were conducted with a Hewlett-Packard 1084B automated HPLC equipped with a Brownlee C-18 microbonded 20 cm column. The two solvents used were 0.05 M phosphoric acid (solvent A) and methanol (solvent B).

Bile samples were diluted 1:3 with acidified methanol, centrifuged at 10000 × *g*

17784

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for 2 min, filtered to remove remaining particulates, and a 50-μl aliquot was injected onto the column.

Elution of samples was initiated at 30% solvent B, with a flow rate of 3 ml/min. A two-step gradient changing solvent B to 60% at 3 min and 75% B at 8 min, respectively, was used. After 10 min a linear gradient from 75% to 98% B at 15 min was included to ensure elution of nonpolar bile components from the column. UV absorbance at 235 nm was monitored for detection of 2,4,5-T in the complex bile matrix. Fractions of effluent were collected every 30 s (1.5-ml fractions), and 1.0-ml aliquots of each of these fractions were added to 10.0 ml of scintillation cocktail for liquid scintillation counting of ^{14}C radioactivity.

RESULTS

[^{14}C]2,4,5-T-derived radioactivity appeared in the bile within 10 min after i.v. injection of the compound. The excretion rate of 2,4,5-T in bile decreased continually throughout the 6-h bile collection period (Fig. 1A). Bile flow remained relatively constant throughout the 6-h collection period, indicating that liver function remained adequate during this period. A total of $15.7 \pm 1.3\%$ of the injected dose was recovered in bile in 6 h. The decline in the biliary excretion of [^{14}C]2,4,5-T-derived radioactivity was associated with a decline in the concentration of 2,4,5-T in bile (Fig. 1B). However, the decline in concentration of 2,4,5-T in bile was not a result of elimination of 2,4,5-T from plasma, since the concentration of 2,4,5-T in plasma decreased by only 25% over the 6 h of bile collection (Fig. 1B). A bile to plasma ratio of 2.2 was obtained during the first 30-min sampling period, but the concentration of 2,4,5-T in bile declined to the plasma 2,4,5-T concentration by 3 h after injection.

The volume of urine obtained by bladder puncture at killing was 1.23 ± 0.32 ml, and contained $3.9 \pm 1.6\%$ of the injected dose of 2,4,5-T.

To determine the chemical nature of the 2,4,5-T derived radioactivity in the bile, aliquots of bile were analyzed by reverse-phase HPLC. With the solvent system and programmed gradient used in this study, authentic 2,4,5-T standard eluted from the column at 10.1 min, and was completely resolved from the structurally similar phenoxyacids 2,4-D and 2,4,5-trichloropropionic acid (Silvex) which eluted from the column at 7.9 and 11.0 min, respectively. Bile samples collected from animals prior to injection of 2,4,5-T produced characteristic and reproducible HPLC spectra (Fig. 2A). Peaks eluting from the column between 5.2 and 7.0 min were not identified, but most likely represent various conjugated bile acids and bile pigments. When bile from rats receiving 25 mg/kg [^{14}C]2,4,5-T was subjected to HPLC analysis, a new peak at 10.1 min was observed (Fig. 2B). Approx. 85% of the radioactivity recovered from the column was associated with the peak eluting at 10.1 min. The peak at 8.2 min appeared quite variable in magnitude with bile from different animals, and did not appear to be related to the 2,4,5-T administration, as only a small amount of radioactivity was ever associated with this peak (Fig. 2B).

17785

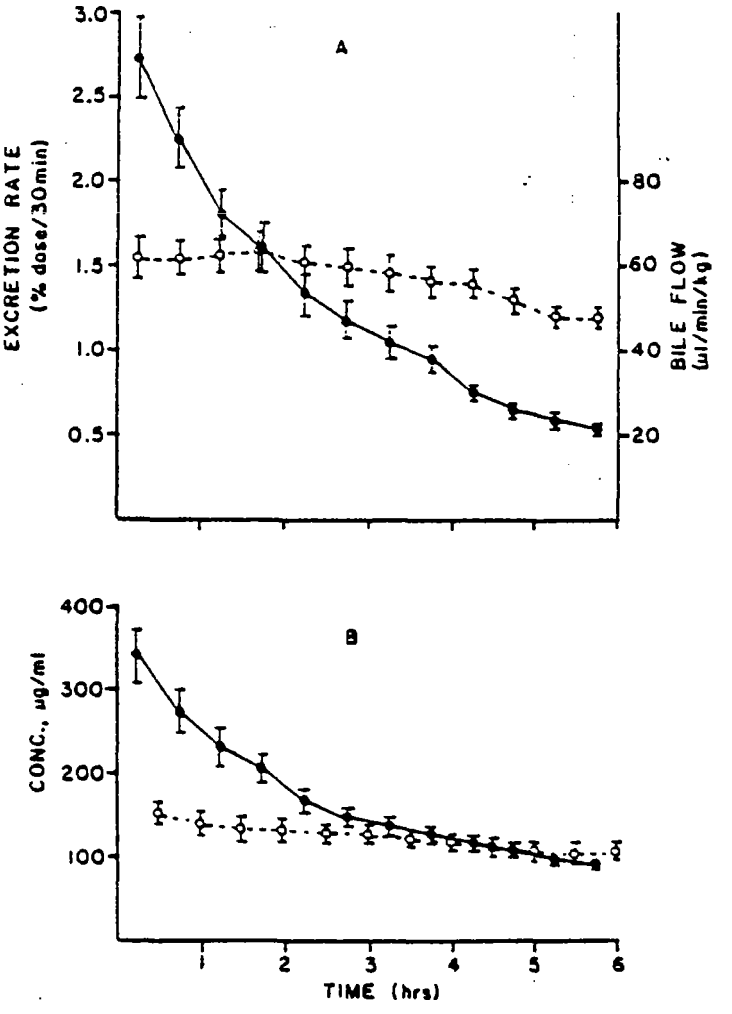


Fig. 1. (A) Bile flow (o—o) and biliary excretion rate of 2,4,5-T (o——o). Bile samples were collected at 30-min intervals for 6 h following i.v. administration of 25 mg/kg [^{14}C]2,4,5-T. (B) Concentration of 2,4,5-T in bile (o——o) and plasma (o—o). Plasma samples were collected at the end of each 30-min collection period. Each value represents the mean ± SE of 5 rats.

DISCUSSION

The results of this study demonstrate that the phenoxyacid herbicide 2,4,5-T undergoes significant biliary excretion in the rat; 15.7% of the administered dose

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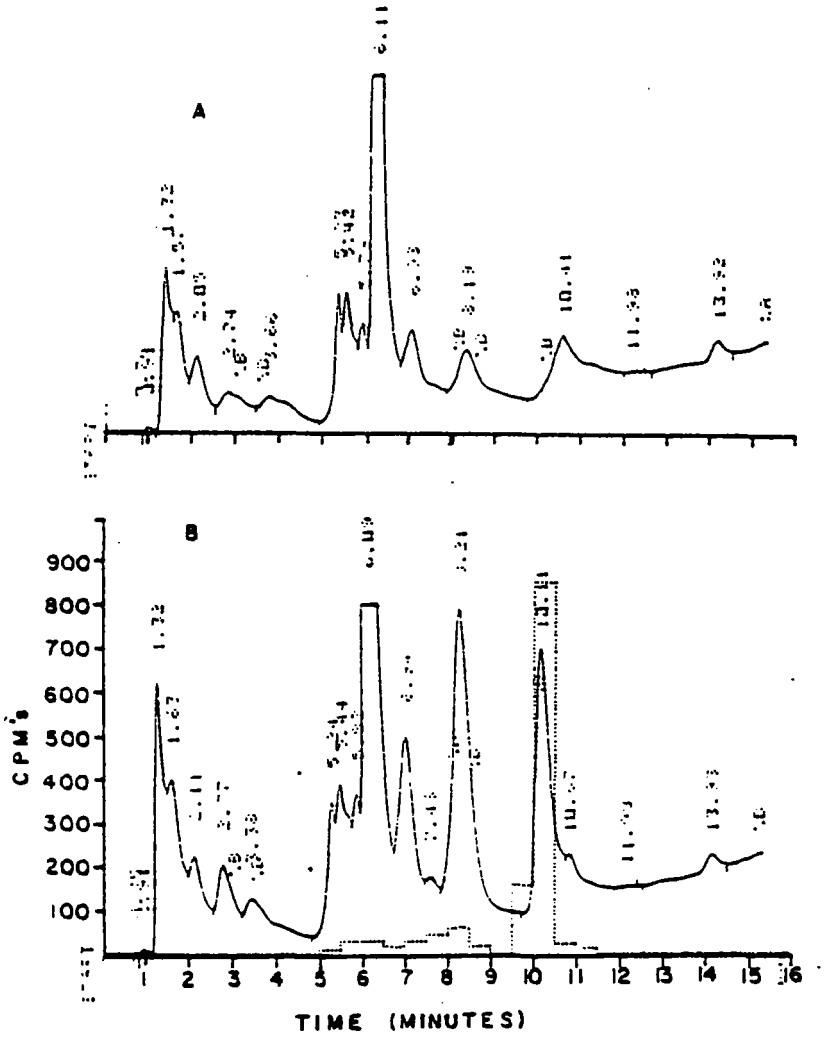


Fig. 2. Representative reverse-phase HPLC analysis of bile from an untreated rat (A) and bile from a rat which received 25 mg/kg [^{14}C]2,4,5-T (B). Bile sample in B was taken from the first 30-min collection period. Serial 0.5 ml fractions were collected and analyzed for radioactivity (B, dotted line).

was recovered in the bile in 6 h. Other studies in the rat have demonstrated that 75–80% of orally or i.v. administered 2,4,5-T is ultimately eliminated in the urine, with a plasma half-life of about 5 h at doses less than 50 mg/kg [5, 6, 8]. In the present study, elimination from the plasma compartment was slower than expected;

17787

the plasma concentration of 2,4,5-T 6 h following administration was app 75% of the initial value. The low urine output and small per cent of the eliminated in the urine suggest that renal function was compromised in urethane-anesthetized rats. Severs et al. [11] recently reported that uret anesthesia severely impaired renal function secondarily to a toxic effect or mesenteric vasculature after intraperitoneal administration of urethane. T biliary excretion of 2,4,5-T in the urethane-anesthetized rat possibly represente maximal biliary excretory capacity for this compound, since the preferred rou elimination may have been impaired.

The rate of decline in biliary concentration of 2,4,5-T with time did correspond with the much slower decline in plasma concentration. The decre rate of biliary elimination did not relate to either a decrease in bile flow or lo biotransformation of 2,4,5-T, since bile flow declined only slightly, and most o 2,4,5-T excreted in bile was not biotransformed.

Since concentration of 2,4,5-T in bile was initially greater than that in plasm active transport system for 2,4,5-T may be present in the liver, as has been desc for renal elimination of 2,4,5-T [12–14]. However, if such a system exists, considerably less efficient than hepatobiliary transport processes responsibl biliary excretion of other organic anions, cations, and neutral compounds [1 appears that the mechanisms responsible for biliary elimination of 2,4,5-T 'fati with time, since biliary concentration of 2,4,5-T declined until it reached th plasma. The biochemical mechanism responsible for this phenomenon remains elucidated.

ACKNOWLEDGEMENT

This work was supported by a PMA Foundation Starter Grant.

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17789

Volume 20 Number 6 December 1982

Contents of Volume 20 (1982) included loose in this issue

Food and Chemical Toxicology

An International Journal published for the British Industrial Biological Research Association

(formerly Food and Cosmetics Toxicology)

RESEARCH SECTION

Effect of dietary butylated hydroxyanisole on methylazoxymethanol acetate-induced toxicity in mice (*B. S. Reddy, K. Furuya, D. Hanson, J. DiBello and B. Berke*) 853

Lack of carcinogenicity of butylated hydroxytoluene on long-term administration to B6C3F$_1$ mice (*T. Shirai, A. Hagiwara, Y. Kurata, M. Shibata, S. Fukushima and N. Ito*) 861

An investigation of the genetic toxicology of irradiated foodstuffs using short-term test systems. III—*In vivo* tests in small rodents and in *Drosophila melanogaster* (*H. W. Renner, U. Graf, F. E. Würgler, H. Altmann, J. C. Asquith and P. S. Elias*) 867

No volatile *N*-nitrosamines detected in blood and urine from patients ingesting daily large amounts of ammonium nitrate (*G. Ellen, P. L. Schuller, P. G. A. M. Froeling and E. Bruijns*) 879

The influence of food flavonoids on the activity of some hepatic microsomal monooxygenases in rats (*M. H. Siess and M. F. Vernevaut*) 883

Inactivation of aflatoxin B$_1$ mutagenicity by thiols (*M. Friedman, C. M. Wehr, J. E. Schade and J. T. MacGregor*) 887

The effects of patulin and patulin–cysteine mixtures on DNA synthesis and the frequency of sister-chromatid exchanges in human lymphocytes (*R. Cooray, K.-H. Kiessling and K. Lindahl-Kiessling*) 893

The subchronic toxicity and teratogenicity of alternariol monomethyl ether produced by *Alternaria solani* (*G. A. Pollock, C. E. DiSabatino, R. C. Heimsch and D. R. Hilbelink*) 899

Continued on inside back cover

ISSN 0278-6915

FCTOD7 20(6) 853–994 (1982)

Pergamon Press

17790

pool and excrete it (Wynder & Reddy, *Dig. Dis.* 1974, 19, 957) and that changes limited to dietary cholesterol have a small effect on serum cholesterol (Key *et al. Metabolism* 1965, 14, 759). They therefore suggest that the level of serum cholesterol may not be the most appropriate parameter for future investigations of colon carcinogenesis; additional dietary studies of cholesterol intake along with further analysis of steroid metabolites in the large bowel may be of more value.

Another look at the carcinogenicity of [redacted]

Eriksson, M., Hardell, L., Berg, N. O., Möller, T. & Axelson, O. (1981). Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. *Br. J. ind. Med.* 38, 27.

Phenoxy acids are present in many of the herbicides used in agriculture, horticulture and forestry. A case-control study in northern Sweden suggested that exposure to phenoxy acids and chlorophenols may be associated with a six-fold increase in the risk of developing soft-tissue sarcoma although the role played by impurities such as dioxin or dibenzofuran could not be ascertained (*Cited in F.C.T.* 1980, 18, 541). The same group of workers has now conducted a further case-control study of soft-tissue sarcomas with the aim of confirming the earlier findings and obtaining information on the effects of different phenoxy acids. This study was carried out in southern Sweden where exposure to phenoxy acid herbicides is likely to be through their agricultural use rather than through forestry, as in the north.

Each of 110 cases (38 deceased) with soft-tissue sarcomas was compared with two carefully selected controls for exposure to phenoxy acids other than 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), to phenoxy acids including 2,4,5-T and to chlorophenols. Exposure was assessed initially by a self-completed questionnaire which was followed up by a telephone interview by a 'blind' interviewer and contact with employers etc. if this was judged necessary. Individuals exposed to phenoxy acids for a total of less than 1 day were considered to be unexposed, and separate analyses were performed for individuals exposed to phenoxy acids for more or less than 30 days. Risk ratios for soft-tissue sarcomas calculated for the various exposure groups were as follows: phenoxy acids or chlorophenols 4·7; phenoxy acids except 2,4,5-T 4·2; phenoxy acids including 2,4,5-T 17·0 (>30 days 8·5, ≤30 days 5·7); all exposures to phenoxy acids 6·8; chlorophenols 3·3. The authors conclude that their results indicate that exposure to phenoxy acids and chlorophenols might constitute a risk factor in the development of soft-tissue sarcomas and that this risk relates not only to 2,4,5-T which, like certain chlorophenols, may contain polychlorinated dibenzodioxins and dibenzofurans but also to other phenoxy acids such as 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid and the analogous phenoxypropionic acids which are less prone to contain such contaminants. They also conclude that individuals with low exposure to chlorophenols (defined as a maximum of one continuous week or repeated short-term exposures amounting at the most to 1 month in all) are not subject to an increased risk of developing soft-tissue sarcomas. Smoking habits and exposure to organic solvents, asbestos, glass fibre, power saws and various pesticides did not appear to be associated with an increased risk of developing soft-tissue sarcomas.

[The results of this study are similar to those of the previous investigation (*Cited in F.C.T.* 1980, 18, 541) which was criticized on a number of counts by the UK Advisory Committee on Pesticides (*Further Review of the Safety for Use in the U.K. of the Herbicide 2,4,5-T*; December 1980; pp. 72). The methods used in the present study were very thorough in many respects and the authors have gone to considerable lengths to assess exposure to phenoxy acids and other compounds. They have also taken great care in selecting the controls. However some of the Committee's criticisms are probably also applicable to the present study. The accuracy with which workers (or their relatives or colleagues) can recall the type or duration of exposure to chemicals is likely to be limited, and, once again, the types of tumours observed covered a wide range.

In a recent review Coggon & Acheson from the MRC Environmental Epidemiology Unit (*Lancet* 1982, I, 1057) also point out the possibility of bias resulting from differences in the recall of distant events, particularly between patients and healthy controls. They report, however, that one of the Swedish authors (Hardell, *Scand. J. Work envir. Hlth* 1981, 7, 119) has compared the cases with soft-tissue sarcomas with a control group of patients with colonic cancer and obtained a similar (five-fold) increased risk of soft-tissue sarcoma after exposure to phenoxy acids and chlorophenols. Coggon & Acheson (*loc. cit.*) conclude that "there is suggestive evidence of a biological association between phenoxy herbicides (or their contaminants) and soft-tissue sarcomas".]

Aluminium and impaired breeding in wild birds

Nyholm, N. E. I. (1980). Evidence of involvement of aluminium in causation of defective formation of eggshells and of impaired breeding in wild passerine birds. *Envir. Res.* 26, 363.

Severe eggshell defects and reduced clutch sizes indicated a breeding impairment among birds nesting along the shores of two lakes in Swedish Lapland. Such impairment was not noticed in birds breeding further from the shores of these lakes nor in birds breeding along the shore of a lake in a similar environment further north. Extensive analysis of various tissues indicated that neither biocides such as DDT or polychlorinated biphenyls nor heavy metals such as cadmium, chromium, copper, lead or mercury were likely to be causal factors.

The medullary tissue of humeri from nineteen female pied flycatchers (*Ficedula hypoleuca* Pallas) were subjected to multi-element analysis. Specimens were obtained during the laying phase (when the birds were about to lay their second egg) or during the incubation period. Thirteen of the birds laid at least one egg with a defective shell and were classified as

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PESTICIDE RESIDUES AND EXPOSURE
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678

11 DOW 215509

Review of Studies with 2,4,5-Trichlorophenoxyacetic Acid in Humans Including Applicators Under Field Conditions

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Studies in animals and humans have shown that oral doses of phenoxy herbicides are rapidly absorbed and are excreted virtually completely as phenoxy acids in urine with a half-life of less than 1 day. The rate of absorption of 2,4,5-T into the body appeared to be slower after external exposure than after oral administration in humans. Pharmacokinetic modeling indicated 97% of the 2,4,5-T absorbed through the skin would be cleared within 1 week. Measurement of 2,4,5-T excreted in urine of spray crews demonstrated that the maximum absorbed dose is not likely to exceed 0.1 mg per kg of body weight per work day. Urinary excretion provided a more reliable measure of dose than analysis of patches worn by the workers. Exposure was highest in mixers who handled the spray concentrate and in sprayers using backpack equipment. Some absorption of 2,4,5-T was apparently due to wearing contaminated clothing by field workers.

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5,T) has received considerable public attention in recent years. Concern that it might cause birth defects, or miscarriages, or even cancer, resulted in numerous investigations during the past decade (Table I). In April 1978 the U.S. Environmental Protection Agency (EPA) issued a Rebuttable Presumption Against Registration (RPAR) for all uses of 2,4,5-T (1). In February 1979 EPA issued a temporary suspension of uses in forestry, rights-of-way and pastures (2). Uses in rice and rangeland were not affected. Hearings began in March 1980 to evaluate all available data on potential risks, and to balance these risks against benefits from each use. No decision had been reached when this review was completed in August 1981.

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1978 - EPA issues Rebuttable Presumption Against Registration for all uses of 2,4,5-T.

1979 - EPA temporarily suspends uses in forestry, rights-of-way, and pastures.

1980 - Cancellation hearings begin in March, to review all available evidence on risk, then to weigh benefits vs. risks for each use of 2,4,5-T.

1981 - Decision?

It should be noted that hazard or risk is a function of exposure as well as of toxicity. Thus, an important aspect of this continued debate on 2,4,5-T is the need for representative data on real exposure in humans, not only for applicators of the herbicide but for the populace at large. EPA's RPAR Position Document 1 (1) included estimates of exposure to 2,4,5-T based on direct measurements of external contamination by other pesticides applied with equipment similar to that used for 2,4,5-T. However, in most cases, application conditions were quite different from those used for 2,4,5-T.

The response submitted by The Dow Chemical Company included a more realistic evaluation of human exposure to 2,4,5-T (3). This evaluation relied on data from several studies in humans which showed that 2,4,5-T was rapidly excreted in the urine with nearly complete clearance from the body within less than a week after exposure. Based on these data, the dose received by a person using a backpack sprayer was estimated to be less than 0.1 milligram per kilogram of body weight per day of exposure spraying brush. This estimate was subsequently confirmed by an extensive study conducted in forest workers applying 2,4,5-T under field conditions in Arkansas.

The following review summarizes the findings in all reported studies on excretion of 2,4,5-T by humans given oral doses, or exposed to the herbicide under normal field conditions, including careless work habits.

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After Ingestion (hr)	(day)	% of Dose Excreted	% of Dose Excreted	Daytime Collection[2]
0-12		26.8		0.70
12-24	1	14.1	38.1[1]	
24-36		20.7		0.70
36-48	2	8.7	67.5	
48-60		9.9		0.69
60-72	3	4.4	81.8	
72-84		4.8		0.72
84-96	4	1.8	88.4	
				av. 0.70

[1]One subject pooled the 0-12 and 12-24 specimens so the mean excretion for day 1 is not the sum of the mean excretions for 0-12 and 12-24 hours.

[2]Calculated from 26.8/38.1 = 0.70; 20.7/29.4 = 0.70; 9.9/14.3 = 0.69; 4.8/6.6 = 0.72.

Although considerable variation was noted among individuals in each study, particularly in those conducted by Kohli, the overall agreement is very good. As summarized in Table III, 26 to 57% of the 2,4,5-T was recovered in urine collected the first day after administration of a single oral dose, compared to the 50% predicted by pharmacokinetic modeling (6).

Dermal Application Studies

In a study by Feldmann and Maibach (13), only about 6% of the 2,4-D applied to the forearm of human volunteers was recovered in urine collected during a week after exposure. Also, the rate of excretion was slower after dermal exposure than after intravenous injection (13), or after oral administration (8) of 2,4-D in humans.

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Summary of Excretion [illegible]
After Administration of Single Oral Dose

Reported by	Dosage mg/kg	Number of Subjects	Mean % of Dose in Urine Day 1	Day 2	Day 3	Day 4
Gehring (6)	5	5	38	29	14	7
Matsumura (10)	1.5	1	45	30	5	
	1.9	1	45	30	5	
Kohli (12)	2	1	26	22	18	7
	3	1	57	16	3	3
	5	6	27	23	10	4
Calculated[1]			50	25	13	6

[1]Approximate values assuming instant absorption of the total dose and a half-life of 1 day.

Similarly, a slower rate of excretion was noted after application of 2,4,5-T esters to the shaved skin of rats than after oral administration in this species (Ramsey, unpublished data). The rate controlling step was shown to be absorption of the ester through the skin. Once inside the body, the esters were rapidly hydrolyzed to the acid, and the acid was excreted at the same rate as following oral administration of 2,4,5-T in rats.(29)

A controlled dermal exposure study was conducted recently at Oregon State University in which 2,4,5-T ester formulation was applied to the skin of humans (14). Four concentrations of 2,4,5-T ester emulsion were applied to the point of runoff on bleached denim patches, 900 square centimeters in area. The patches were then held in close contact with the skin on the upper thigh of four volunteers, including one woman. The patches were removed after 2 hours and total urine was collected for 5 days. As shown in Table IV, less than 0.5% of the applied 2,4,5-T was absorbed from the cloth, even when soaked with concentrated spray solutions.

Studies in 2,4,5-T Applicators

All available studies on exposure to 2,4,5-T in applicators are reviewed herein, with special emphasis on the "state of the art" studies by Lavy in forest workers under normal work conditions in Arkansas.

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100 gal[1]	excreted in 5 Days[2]	mg 2,4,5-T Absorbed[3]	per kg bw[4] per cm² per hr
2	0.38	0.44	0.000003
4	0.72	0.84	0.000007
16	0.66	1.14	0.000009
32	1.38	2.34	0.000011

[1]Patches soaked with 40 ml of solution containing 2.4, 4.8, 19.2, and 38.4 grams of 2,4,5-T acid equivalent per liter, amounting to about 100, 200, 800 and 1600 mg per patch.

[2]Urine collection began 24 hours before application of treated patch and continued for a total of 5 days.

[3]Corrected for incomplete excretion in 4 days after exposure.

[4]For 70 kg person.

Dow Study. In a review of direct and indirect measurements of exposure in applicators, Leng (3) reported an unpublished early study by Dow in which 2,4,5-T exposure was measured in eight field personnel and two observers. Direct measurements of exposure were based on analyses of air samples and of patches simulating skin exposure. Indirect measurements of actual dose received were based on analyses of blood samples and 24-hour urine collections from these individuals.

The workers were applying a low-volatile 2,4,5-T butoxyethanol ester formulation diluted with fuel oil at 3 gal/100 gal (2% 2,4,5-T acid equivalent). The spray was applied selectively to the lower 3 to 4 feet of trees and brush in a utility right-of-way using hand-pressurized backpack sprayers (2.5 gal capacity). The applicators wore short-sleeved shirts open at the neck, long pants, no gloves and no hat. One of the backpacks leaked as evidenced by soaking of the applicator's clothing at the lower back.

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have prevented the relatively high exposure experienced by this individual, estimated as a dose of about 0.2 mg/kg of body weight.

Table V

2,4,5-T in Urine of Spray Applicators: Backpack Crew, 24 Hour Collection (3).

Subject	Occupation	kg bw[1]	2,4,5-T in Urine ppm	2,4,5-T in Urine mg/24 hr	mg 2,4,5-T per kg bw[2]
1	Applicator	73	0.85	1.45	0.02
2	Applicator	73	4.30	7.06	0.10
3	Applicator	59	3.00	3.58	0.06
4	Applicator	68	17.00	13.18	0.20[3]
5	Foreman	77	0.75	1.25	0.02
6	Foreman	87	3.80	4.79	0.06
7	Foreman	70	0.07	0.09	0.001
8	Gen. Foreman	78	0.03	0.08	0.001
9	Ind. Hygienist	75	0.02	0.03	0.0004
10	Clin. Chemist	73	<0.01	<0.03	<0.0004

[1]Kilograms of body weight for each subject.

[2]Calculated from the urinary levels using the pharmacokinetic model developed from an oral study in humans (6), assuming that repeated daily exposure had resulted in a steady state body burden of 2,4,5-T.

[3]Subject 4 wore a leaking backpack sprayer which soaked his shirt and the small of his back.

EPA Study. The first published report of studies on 2,4,5-T applicators was by Shafik et al. of EPA in 1971 (7). They analyzed urine samples collected from people occupationally exposed to 2,4-D and 2,4,5-T, and reported higher exposure in spray operators than in those who had less direct contact with the herbicides.

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taken to avoid skin contact.

A total of eight urine samples were collected, one from each individual on the afternoon after spraying since 7 a.m. that morning, and one in the early morning the following day. The 2,4,5-T found in the urine samples ranged from 0.5 ppm to 3.6 ppm. Assuming a steady state body burden and an average 2.0 ppm 2,4,5-T in 1500 ml of urine per day, the estimated daily excretion in these applicators was 3 mg of 2,4,5-T. For workers weighing 75 kg, this corresponds to an effective dose of about 0.04 mg/kg which is similar to the doses observed in the Dow study.

Lavy Studies. The first study was in Arkansas in 1978 using 2,4,5-T under field conditions for forestry applications in that area. Complete details of the study were reported to EPA in February 1979 (15) along with kinetic analyses of the data by Ramsey et al. (16).

The study involved monitoring a total of 21 crew members applying 2,4,5-T with four types of equipment (Table VI). Two applications were made 1 or 2 weeks apart, and exposures were reported separately. One backpack sprayer did not take part in the second phase, making a total of 41 exposures.

Table VI

2,4,5-T Study in Forest Workers, 1978:
Total Urine Collection for One Week (Lavy, 15, 19).

Type of Application	No. of Subjects
Backpack spraying	7
Tractor driven mistblower	4
Helicopter using	
- microfoil boom	5
- raindrop nozzles	5
Total: 21 subjects, 41 exposures[1].	

[1]One member of the backpack crew did not take part during the second spray period.

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Monitoring during the application included measuring the 2,4,5-T level in air and on patches fastened to the clothing of the workers. Total urine collections were made over 12-hour periods before, during, and for up to 5 days after each exposure, and each sample was analyzed separately for 2,4,5-T.

The product used was a low-volatile 2,4,5-T propylene glycol butyl ether ester formulation containing 0.04 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It was applied as an emulsion in water at a rate of 1.6 pounds of 2,4,5-T acid equivalent per acre (lb/A) in the backpack study, and 2 lb/A in the other studies. These were low-volume applications at 10 gal/A in the ground studies and 5 gal/A by air. Thus, the workers in the aerial studies were exposed to about 5% 2,4,5-T spray solutions compared to the 2 or 3% used in the earlier studies by Dow and EPA described above (3, 7).

The four crews were selected from a group who normally did this type of work in that area of Arkansas. They were instructed to perform their routine duties in their usual manner with as little influence as possible from the test situations. Prior to the spray program each worker filled out a form which provided personal information regarding his or her vital statistics and history of any previous involvements with 2,4,5-T. Workers were selected who indicated they had not worked with 2,4,5-T for 2 weeks prior to the study (15).

For the most part, the workers did not wear gloves or special protective clothing. Typical attire for members of the spray crews included long trousers, long or short sleeved shirt, and cloth sneakers, leather shoes or field boots. All wore hats except four members of the backpack crew.

Each crew member wore six patches consisting of 10 cm x 10 cm squares of gauze, backed by filter paper, fastened to clothing on the chest, back, upper arms, and upper thighs. In addition, each worker wore a portable air pump which drew a known volume of air through a column of XAD-2 resin which trapped the 2,4,5-T from the air.

Total urine collection began the day prior to spraying (day 0), and was continued the day of spraying (day 1), and for at least 4 or 5 days following each of two spray operations 1 or 2 weeks apart. The total urine excreted by each worker throughout the entire test period was collected for 12-hour periods and kept refrigerated until analyzed. Analyses were conducted at Northrop Services, Inc. in Little Rock, Arkansas, using a modification of the gas chromatographic method of Kony et al. (17). The urine samples were hydrolyzed to release possible conjugates although 2,4,5-T has been shown to occur as the free acid in human urine (6, 17).

For most workers, the highest concentration of 2,4,5-T was in urine collected the first day after spraying. By the end of the second day, all but two workers had excreted more than 50% of the total amount they excreted in the 4 to 5 day post-spray

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exposure than after oral administration, probably due to slower absorption of the 2,4,5-T ester from the skin than 2,4,5-T acid from the gut. This is in agreement with observations made by Feldmann and Maibach for 2,4-D and other pesticides applied to the forearm of human volunteers (13). Calculations by Ramsey et al. using three methods showed that 97% of the 2,4,5-T absorbed by forest workers would be excreted in urine within 7 days following dermal exposure under typical field conditions (16).

The study demonstrated the variability in exposure of the 21 crew members depending on the job being performed and on the care taken by some individuals to avoid exposure. Tables VII through X report the apparent total dose absorbed by each test subject, based on the total 2,4,5-T recovered in urine during each test period divided by the person's body weight. Figures 1 through 4 illustrate the excretion patterns for those with the highest apparent exposure.

The backpack crew was made up of seven members, including two couples working as pairs in which the female sprayers weighed about 50 kg (110 lb). The total absorbed dose of 2,4,5-T ranged from 0.01 mg/kg in the mixer-supervisor to around 0.09 mg/kg for the first exposure in each of three sprayers (Table VII).

Table VII

Total 2,4,5-T Excreted in Urine per Exposure in a Backpack Crew (adapted from Lavy, 15, 19).[1]

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw[1]
1	M	72.6	Mixer/Supervisor	0.011, 0.012
2	M	68.1	Sprayer	0.086, 0.074
3	F	49.9	Sprayer	0.087, 0.053
4	M	95.3	Sprayer	0.030, 0.033
5	F	52.2	Sprayer	0.031, 0.017
6	M	65.8	Sprayer	0.038, ———
7	M	74.9	Sprayer	0.093, 0.037

[1]Based on excretion on day 1 of exposure and 5 days after the first exposure and for 4 days after the second exposure, excluding excretion on day 0 before each exposure.

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habits. Wearing of contaminated clothing between sprayings might account for the increased excretion observed in these two male workers (Nos. 2 and 4) at the end of the first spray period.

Figure 2 gives the excretion pattern for the other two male backpack sprayers, one of whom did not take part in the study during the second test period.

Exposure in the mistblower study was somewhat less than in the backpack study. The highest apparent dose was about 0.08 mg/kg in the mixer (No. 11) during the first test period (Table VIII). The values shown in the last column of Table VIII were calculated by Ramsey et al. (16), and represent the maximum dose absorbed by each worker in the two sprayings 2 weeks apart. Figure 3 reveals that the mistblower driver (No. 10) was being exposed at a constant rate throughout the test period, possibly from wearing contaminated clothing.

Table VIII

Total 2,4,5-T Excreted in Urine, and Calculated Dose per Exposure in a Crew Using a Tractor Mounted Mistblower (adapted from Lavy 15, 19, and Ramsey et al. 16).

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw	
				Measured	Calculated[1]
8	M	95.3	Supervisor	0.029	0.032
				0.008	0.012
9	M	84.0	Driver	0.042	0.046
				0.032	0.037
10	M	106.7	Driver	0.033	0.041
				0.040	0.054
11	M	79.5	Mixer	0.078	0.086
				0.043	0.053

[1]Maximum value for total absorbed dose for each individual calculated by three methods based on the pharmacokinetic model for clearance after dermal exposure to 2,4,5-T (16).

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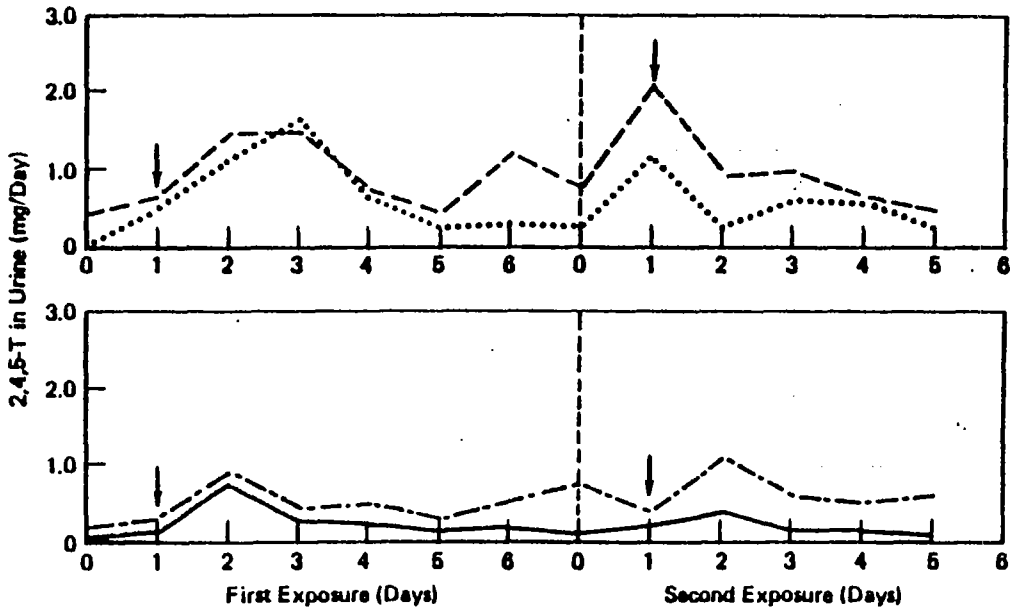


Figure 1. Excretion of 2,4,5-T in two couples applying the herbicide with backpack sprayers (arrows indicate application dates). Key: Couple 1: – – –, male (2); · · ·, female (3); Couple 2: — — —, male (4); ——, female (5).

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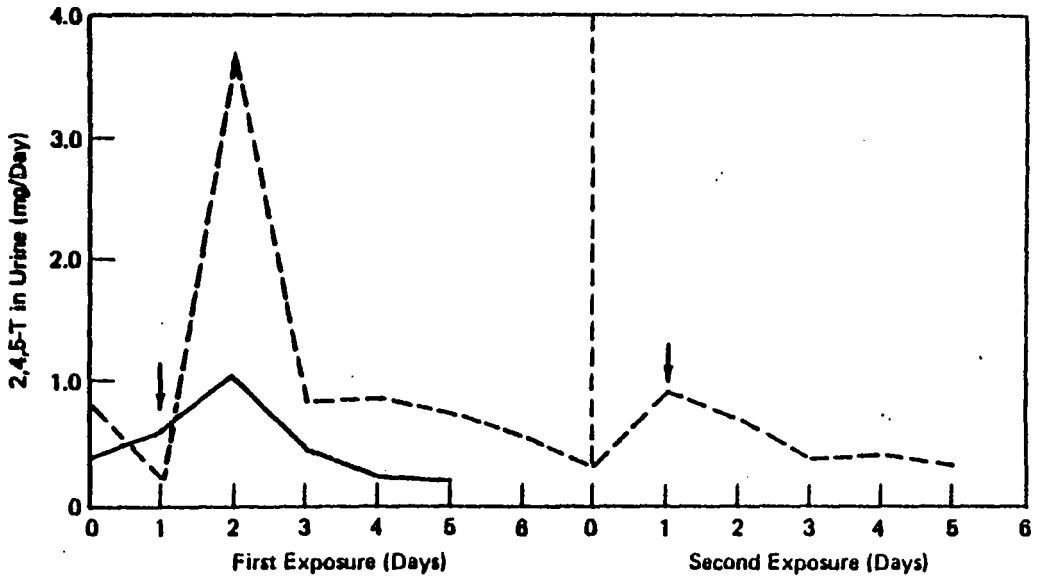


Figure 2. Excretion of 2,4,5-T in two men applying the herbicide with backpack sprayers (arrows indicate application dates). Key: ——, male (6); – – –, male (7).

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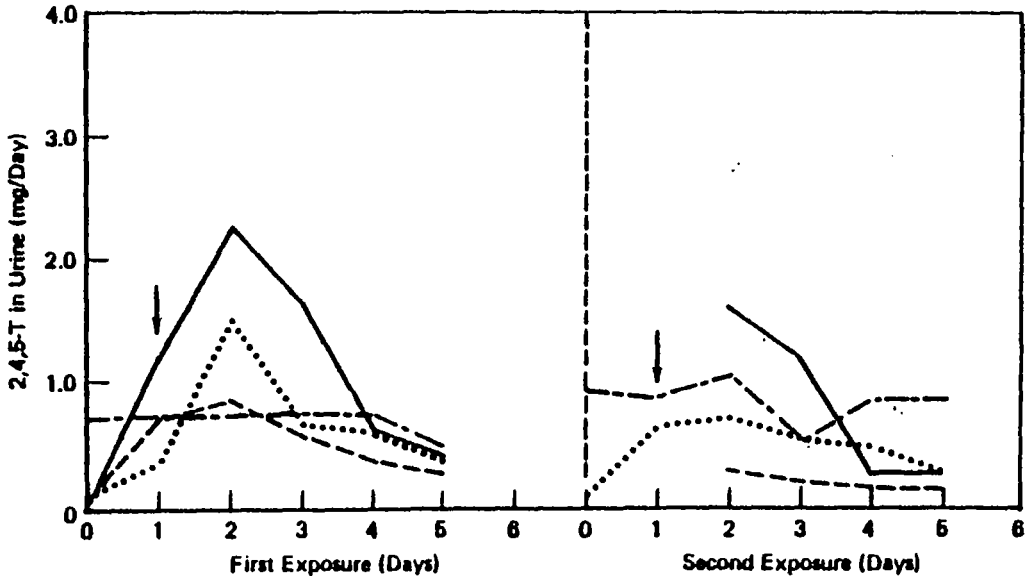


Figure 3. Excretion of 2,4,5-T in four men applying the herbicide with a tractor drawn mistblower (second application was 2 weeks later). Key: – – –, supervisor (8); · · ·, driver (9); — · —, driver (10); ——, mixer (11).

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Values obtained for helicopter crews are presented in Tables IX and X for applications with a microfoil boom and raindrop nozzles, respectively. Flagmen in both studies excreted only 0.001–0.003 mg/kg indicating they received very little exposure even though they were standing under the spray path during repeated passes by the helicopter. The pilot in the raindrop nozzle study (No. 17, Table X) received a higher dose than the pilot in the microfoil study (No. 12, Table IX), possibly because he routinely checked and unplugged nozzles with his fingers at each fill-up time, and also helped to change the spray boom on the helicopter before and after each spray period.

Table IX

Total 2,4,5-T Excreted in Weekly Urine per Exposure in a Helicopter Crew Using a Microfoil Boom (adapted from Lavy, 15, 19).

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw
12	M	95.3	Pilot	0.001, 0.011
13	M	109.0	Mixer	0.075, 0.061
14	M	84.0	Supervisor	0.004, 0.004
15	M	61.3	Flagman	0.001, 0.001
16	M	74.9	Flagman	0.002, 0.001

Table X

Total 2,4,5-T Excreted in Weekly Urine per Exposure in Helicopter Crew Using Raindrop Nozzles (adapted from Lavy, 15, 19).

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw
17	M	72.6	Pilot	0.034, 0.039
18	M	86.3	Mixer	0.068, 0.127
19	M	81.7	Supervisor	0.007, 0.001
20	M	86.3	Flagman	0.003, 0.001
21	M	95.3	Flagman	0.001, 0.001

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Figure 4 illustrates the discrepancy in doses received by mixers. Mixer No. 1 (backpack crew) had very low levels of 2,4,5-T in his urine, possibly due to the fact that he wore gloves and was cautious in his work habits. Mixer No. 13 (microfoil study) had relatively high levels of 2,4,5-T in his urine on day 0 before each spraying, indicating he might have been wearing contaminated clothing. Mixer No. 18 (raindrop nozzle study) exhibited a much higher peak urinary level in the second test, and also received almost twice the dose (0.127 mg/kg) than he did in the first test (0.68 mg/kg), or than mixer No. 13 in both microfoil boom tests (0.075 and 0.061 mg/kg). These data support an observation made years ago by Wolfe (18) that the likelihood of serious contamination is greatest during handling of the pesticide concentrate and could be largely avoided by wearing impermeable gloves during mixing.

A summary is presented in Table XI showing the total 2,4,5-T excreted by the 21 forest workers in two exposures under typical field conditions. The highest dose was received by mixers who handled the herbicide concentrate, followed by applicators using backpack sprayers, spray tractor drivers, helicopter

Table XI

Summary of Total 2,4,5-T Measured in Urine of Spray Crews per Exposure Under Field Conditions in Forests in Arkansas (adapted from Lavy, 15, 19).

Job Description	Number of Subjects	Range of mg 2,4,5-T per kg bw[1]
Mixers	4	0.012 - 0.138
Backpack Sprayers	6	0.019 - 0.104
Spray Tractor Drivers	2	0.033 - 0.049
Helicopter Pilots	2	<0.001 - 0.044
Supervisors	3	0.002 - 0.030
Flagmen	4	<0.001 - 0.003

[1] Includes 2,4,5-T excreted on day 0 before each spraying due to wearing contaminated clothing or exposure of fingers during mixing of spray, and 2,4,5-T excreted on day 6 after first spraying (i.e. on day 0 of second spraying).

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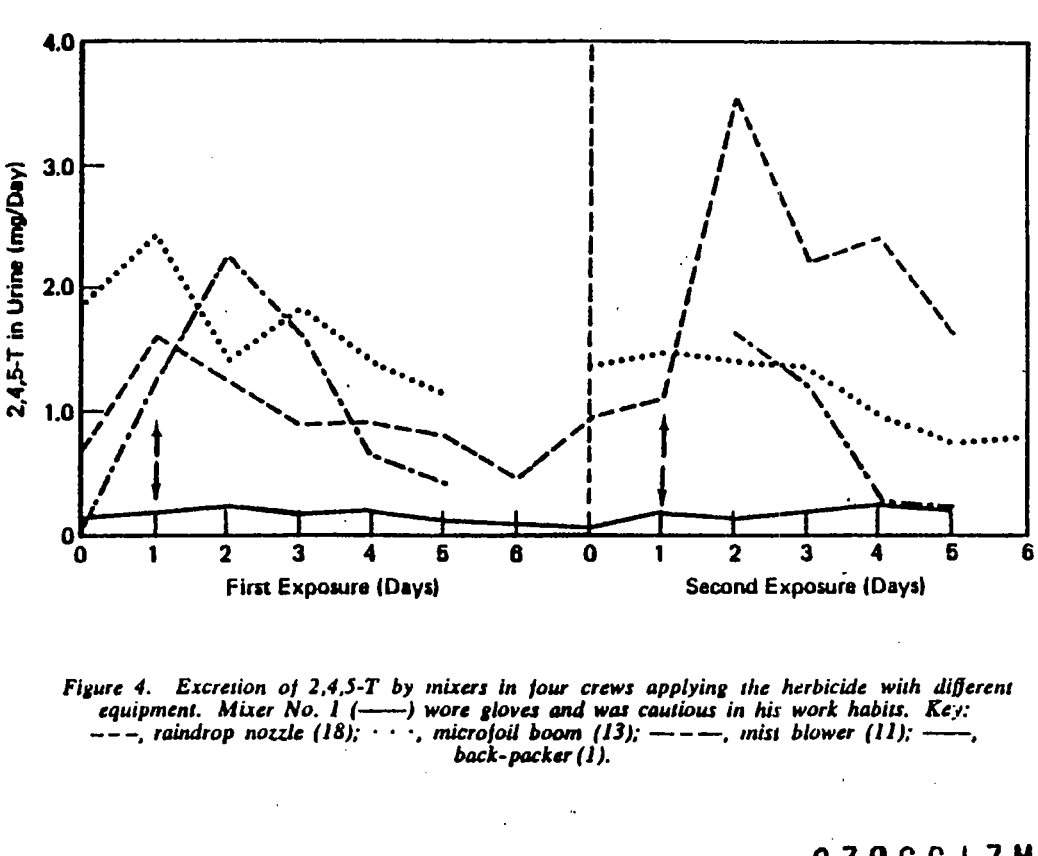


Figure 4. Excretion of 2,4,5-T by mixers in four crews applying the herbicide with different equipment. Mixer No. 1 (——) wore gloves and was cautious in his work habits. Key: – – –, raindrop nozzle (18); · · ·, microfoil boom (13); — — —, mist blower (11); ——, back-packer (1).

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long sleeves, and gloves, and had practiced good work habits like mixer No. 1.

A subsequent study by Lavy et al. (20) demonstrated the effectiveness of wearing protective clothing in decreasing exposure to 2,4-D in spray crews during aerial applications in forests in the Pacific Northwest. Each of three crews, made up of six members per crew, were engaged in two helicopter applications of low volatile ester formulations. During one treatment the workers wore normal clothing and followed their own individual work habits and precautions during the spray operation. In the second treatment the same crews wore protective clothing and were supervised in their work to maintain more stringent protection from exposure to the herbicide. Comparisons of the two treatments indicated that, although low levels of exposure did occur, less exposure was evident when precautions were taken.

Data from analyses of air samples in Lavy's 2,4,5-T study (15, 19) and 2,4-D study (20) indicate that exposure via inhalation was insignificant during application of low volatile ester formulations of these herbicides.

Data obtained by direct measurements of contamination of patches worn by workers have been used for estimating exposure to pesticide sprays (1). However, such data are of limited value for predicting exposure to phenoxy herbicides applied as emulsions (3). For example, in Lavy's study on 2,4,5-T in forest workers (15, 19), the total exposed skin area was estimated for each worker according to the method of Wolfe (21), using photographs taken at the spray site. Total dermal exposure was based on the average amount of 2,4,5-T detected on the six patches worn by each crew member multiplied by the total estimated exposed skin area for that worker. As shown in Table XII, the observed dose (i.e. amount excreted in urine) was generally much lower than the estimated external dose. Also, patch data would not predict exposure from contamination of fingers while handling the concentrate during mixing of sprays.

The potential for exposure in bystanders has also been investigated. In a study by Lavy et al. (22) an individual wearing gauze patches stood directly under the spray path of a helicopter during application of 2,4,5-T at a rate of 2 lb/A. Based on finding an average 2 mg per 100 square centimeters of patch area, the external exposure calculated by Wolfe's method was 0.86 mg/kg for a person weighing 76 kg who was wearing shoes, short sleeves, long pants, and no hat. Although the urinary excretion of 2,4,5-T was not measured in this individual, flagmen exposed repeatedly during field applications of 2,4,5-T

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Table XII

Apparent External Exposure Compared to
Actual Dose of 2,4,5-T Absorbed by Forest Workers
(adapted from Lavy et al. 19, 22).

Job Description	Number of Exposures	mg 2,4,5-T per kg body weight	
		gauze patches[1]	excreted in urine[2]
Mixers	7	0.04 - 1.62	0.011 - 0.127
Backpack sprayers	11	0.20 - 2.54	0.017 - 0.093
Mistblower crew	5[3]	0.20 - 1.58	0.029 - 0.042
Helicopter crews	12[4]	nd - 0.02	<0.001 - 0.004
Pilots	4	nd - 0.11	<0.001 - 0.039

[1]Based on average 2,4,5-T found on six patches per subject for each exposure, and estimated total skin area exposed on each subject as calculated by method of Wolfe et al. (21).

[2]Excluding amount found in urine on day 0 before spraying, possibly due to wearing contaminated clothing.

[3]Excluding mixer listed separately.

[4]Excluding mixers and pilots, listed separately.

excreted only <0.001 to 0.003 mg/kg (Table XI). This is a fraction of a percent of the exposure predicted by patch data, despite the fact that the flagmen were under the spray path for several passes of the helicopter.

Lavy also reported that no 2,4,5-T was detected on patches worn by an individual who walked through an area sprayed 2 hours earlier (22). Also, 84% of the 2,4,5-T applied by helicopter was found within 80 feet of the spray path and 99% of the spray was delivered within 160 feet of the spray path (22). Thus persons near or entering sprayed areas shortly after application are not likely to receive measurable exposure to 2,4,5-T.

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Swedish Study. Occupational exposure to 2,4-D and 2,4,5-T was also studied in a forest situation in Sweden (23). The materials used were butoxy ethyl ester formulations of 2,4-D or of a 2:1 2,4-D/2,4,5-T mixture applied as a 2% emulsion in water using tractor drawn equipment. The spray was applied at a rate of 2 to 3 kilograms total active ingredient per hectare (1.8 to 2.7 lb/A) to a distance of about 20 meters from both sides of the tractor. Two areas of about 70 to 80 hectares were sprayed during the test week. The temperature was about 20°C from Monday through Thursday with little wind, and was 10-15°C on Friday with sporadic wind.

Four spraymen took part in the study, working in pairs of two, one driving the tractor and the other walking ahead to mark the areas to be sprayed. One tractor had a cab with a windshield but was open on the left side. The other tractor was completely open and both men got sprayed when the tractor turned.

Three of the four men took part in preparing the sprays from the herbicide concentrates. They wore jeans and shirts, with rubber boots or leather shoes and leggings. However, they had bad work habits, contrary to advice and recommendations outlined in the publication "Pesticides" published by the Council of Europe (24). They wore the same clothes all week as they had worn the week before doing the same work. Two of them lived in a house trailer in the spray area and took their rest periods in the trailer where the odor of the herbicide was noted. All were smokers and smoked during the day without washing their hands.

Monitoring consisted of measuring the 2,4-D and 2,4,5-T in the air during spraying, and in blood and urine of the workers during the 5-day work week and for 36 hours afterwards. The levels found in urine are shown in Table XIII, compiled from data given in the Swedish paper. In the first pair, urinary levels ranged from 1.0 to 6.3 ppm 2,4-D, and from 0.4 to 1.2 ppm 2,4-5-T during the whole week, with little difference between the two workers. Generally higher levels were found in the second team, peaking at 11.4 ppm 2,4,5-T in the urine of one worker on Tuesday morning, and 14.0 ppm 2,4-D in the urine of the other worker on Saturday evening.

The estimated mean 24-hour excretion in the Swedish study was 12 mg 2,4-D and 7 mg 2,4,5-T, amounting to 0.16 and 0.09 mg/kg in workers weighing 75 kg. In a later experiment, the authors calculated that exposure ranged from 0.10 to 0.60 mg/hr for 2,4-D and 0.03 to 0.07 mg/hr for 2,4,5-T in the four workers, based on 3 to 4 hours spent spraying each day during the week. The highest levels found in urine were lower than the 17.0 ppm found in the early Dow study in which one of the workers had a leaking backpack sprayer and whose estimated exposure was only about 0.2 mg/kg of body weight (Table V). Thus, despite poor work habits, the doses of 2,4-D and 2,4,5-T received in the Swedish study (23) were comparable to those received by the backpack sprayers in the studies done by Dow (3) and by Lavy in Arkansas (15, 19).

17810

Table XIII

Levels of 2,4-D and 2,4,5-T Found in Urine of Swedish Forest Workers (adapted from Kolmodin-Hedman et al. 23).

Worker Herbicide[1]		Test 1 KK D	Test 1 KK T	Test 1 LJ D	Test 1 LJ T	Test 2 JG D	Test 2 JG T	Test 2 LEO D	Test 2 LEO T
Mon.	pm	1.0	0.5	1.9	0.5	4.5	3.1	1.0	1.3
Tues.	am					3.4	11.4	3.0	4.9
	pm	2.4	1.0	1.6	0.4				
Thurs.	pm					3.4	6.5	1.9	3.7
Fri.	am					4.1	4.2	2.2	2.3
	pm	6.3	1.2	3.0	1.2	5.6	3.0	4.0	3.3
Sat.	pm	3.6	0.9	3.1	0.9	6.4	2.7	14.0	4.3
Sun.	am	2.1	0.4	3.1	1.0	3.4	2.2	5.3	3.5
	pm	1.5	0.7	1.4	0.7	1.6	2.1	4.2	2.5

[1]D = 2,4-D; T = 2,4,5-T, as ppm found in urine.

Toxicological Significance of Exposure to 2,4,5-T Sprays

Sporadic exposure resulting in doses of less than 0.1 mg/kg of body weight per day spent spraying 2,4,5-T would not appear to pose a significant risk to applicators of the herbicide, even if the 2,4,5-T contains trace amounts of the impurity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Perspective on the potential risk can be gained by comparison of this estimated maximum dose (0.1 mg/kg) with doses which caused no effect in animals treated daily for extended periods.

Numerous studies have demonstrated that the no-effect-level for 2,4,5-T in pregnant rats and mice is about 20 mg/kg of body weight when given daily for 10 days during the critical stage of development of the fetuses (1). In Dow studies with specially purified 2,4,5-T, no effect was observed in rats fed 3 mg/kg of body weight per day continuously for 2 years (25), or over three generations of reproduction (26). In studies in Germany with

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17811

technical 2,4,5-T containing 0.05 ppm TCDD, the no-observed-effect level was 30 mg/kg/day in rats which received this dosage prior to conception, through pregnancy, and for 30 months after birth (27), or during three generations of reproduction (28). Thus, all no-effect dosage levels are considerably higher than the estimated maximum dose from exposure to 2,4,5-T in humans, including applicators and mixers.

Conclusions

1. The maximum exposure expected under relatively careless field conditions for use of 2,4,5-T would be less than 0.1 mg/kg of body weight per day spent spraying.
2. In the study in forest workers, the highest exposure was in mixers who handled the herbicide concentrate, followed by applicators using backpack sprayers, spray tractor drivers, helicopter pilots, and flagmen. (See Table XI.)
3. Exposure would be considerably reduced if the workers wore gloves, particularly for the mixers during preparation of the spray. (See Figure 4, mixer No. 1, solid line.)
4. Measurement of contamination of patches worn by field workers is of limited use for estimating actual exposure to herbicides such as 2,4,5-T. (See Table XII.)
5. Bystanders or persons entering treated areas shortly after spraying are not likely to receive measurable exposure; thus exposure in the general population is extremely unlikely.

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RECEIVED October 28, 1981.

17814

K-4568-C100
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Bulletin of Environmental Contamination and Toxicology

Volume 24 • Issue 1 • January 1980

Identification of Chlordanes and Related Compounds in Goby-Fish from Tokyo Bay by T. Miyazaki, K. Akiyama, S. Kaneko, S. Horii, and T. Yamagishi 1

Histopathology of Kidney of Albino Rat Poisoned with Uranyl Nitrate by K. A. Goel, V. K. Garg, and V. Garg 9

Influence of Pesticides and Some of the Oxidized Analogues on Microbial Populations, Nitrification and Respiration Activities in Soil by C. M. Tu 13

Flux of Aroclor 1254 Between Estuarine Sediments and Water by D. J. Wildish, C. D. Metcalfe, H. M. Akagi, and D. W. McLeese 20

Incorporation of Labeled Pesticides and Environmental Chemicals into Nuclear Fraction of Cultured Human Cells by M. Murakami and J. Fukami 27

Toxicant Effects on Reproduction and Disruption of the Egg-Length Relationship in Grass Shrimp by A. L. Buikema, Jr., B. R. Niederlehner, and J. Cairns, Jr. 31

Toxic Effects of the Phytoalkaloid Colchicine on Oviposition and Neurosecretion of the Vector Snail (*Indoplanorbis exustus*) by M. M. Hanumante, D. P. Vaidya, and R. Nagabhushanam 37

Fate of Diisopropyl Methylphosphonate (DIMP) in a Lactating Cow by G. W. Ivie 40

Characterization of the Contamination of Marijuana with Paraquat by J. A. Liddle, L. L. Needham, Z. J. Rollen, B. R. Roark, and D. D. Bayse 49

Effect of Di-2-ethylhexyl Phthalate in the Female Rat: Inhibition of Hepatic and Adrenal Sterologenesis *In Vitro* by F. P. Bell 54

Contents continued on back cover

BECTA6 24(1) 1-176 (1980)
ISSN:0007-4861

Springer-Verlag

17815

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Bull. Environm. Contam. Toxicol. 24, 90-96 (1980)

Field Worker Exposure and Helicopter Spray Pattern of 2,4,5-T[1]

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The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has proved to be very effective in the control of broadleaf species in rice fields, grasslands, and forests. The herbicide breaks down rapidly in the soil (WHITESIDE and ALEXANDER 1960) and leaves little or no residual effects upon the environment. However, because questions have arisen on the safety of direct exposure to 2,4,5-T, the herbicide has been placed on the Environmental Protection Agency's (EPA) list for Rebuttable Presumption Against Registration, a process designed to allow clear evaluation of the benefits and risks involved with a compound before it is released for future use. Position Document 1 on 2,4,5-T issued by the EPA indicates that data are inadequate on measurement of 2,4,5-T exposure to field workers involved in actual spraying operations. Some earlier research suggests that the herbicide is fetotoxic and possibly teratogenic for mice in doses above 20 mg/kg/day (ROLL, 1971) and is fetotoxic for rats at 25 to 150 mg/kg/day (SPARSCHU et al. 1971).

The objectives of this study were to measure the direct exposure encountered by people in the actual spray area and to determine the distribution range of 2,4,5-T sprayed from a helicopter.

MATERIALS AND METHODS

Applicators and other crew members in the following operations were used to gather the human exposure data: helicopter (5-man crew), a tractor driver spraying rice levees, tractor mist blower (4-man crew), and forest backpack spray workers (12-man crew).

The spray materials used were low volatile ester formulations of 2,4,5-T. The actual materials included Thompson Hayward's DedWeed LV-6 and Dow Chemical Company's ESTERON 245. These materials were applied

[1]Published with approval of the Director of the Arkansas Agric. Exp. Stn.

0007-4861/80/0024-0090 $01.40
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at rates of 2 lb/A (active ingredients) in water. Applications by helicopter and tractor used 5 gallons of water per acre, and the backpack spray contained 10 gallons per acre. The rice levee was sprayed in the afternoon and other operations were begun in early morning when wind was less than 5 mph. Studies were completed between July and October, 1978, with air temperatures ranging from 18 to 32 C. Time in the field for each operation included: helicopter, 116 min; tractor rice spraying, 65 min; tractor mist blower in forest, 245 min; and backpack, 180 min. All crew members were instructed to work in a manner no different from their normal operations.

An attempt was made to calculate respiratory exposure. Vapors and air borne particles of 2,4,5-T in the spray material were collected through a face mask respirator. This triangular shaped device was placed over the nose and mouth to filter all air breathed by each worker. A polyurethane filter set in the mask had been preextracted before use to prevent any interfering peaks on the analytic chromatogram. After these filters were exposed to the spraying operation, they were taken from the masks and stored in methanol. In spite of the preextractions, interfering peaks occurred that prevented effective analyses.

External exposure was measured by attaching 100-cm^2 gauze patches to the outer garments of all crew members. The patches were constructed by modifying a procedure used by WOLFE et al. (1975). Nine layers of cotton gauze, backed with two layers of Whatman No. 1 filter paper, were taped along the borders with masking tape. At spray time in the field the patches were attached in six locations for each worker: upper back, upper chest, bicep-deltoid area of each arm, and upper front of each thigh. Immediately after the spray application, the patches were removed from the clothing. To minimize contamination, only project supervisors were permitted to remove patches. As the patches were removed, the masking tape was cut from the edges to prevent the glue on the tape from interfering with subsequent gas chromatographic determinations and to allow each patch to be disassembled as it was placed in an individual amber-colored jar containing methanol as a storing, extracting solvent. The jars were kept at a controlled room temperature (4 C) until analyzed. For calculation of exposure based upon these patches, each worker was weighed and photographed in his work clothes, his exposed skin area was noted, and a formula derived from WOLFE et al. (1975) was employed.

An additional effort was made to calculate exposure to individuals who might inadvertently be in an area

17817

being sprayed with 2,4,5-T. For this test one individual with gauze patches attached to clothing on his head, shoulders, arms, chest, and front and back thighs, stood directly under the path of the helicopter from which crewmen sprayed. Another individual equipped with six patches, arranged as on crew members, walked through the area that had been sprayed with ESTERON 245 two hours before.

To measure the width of a helicopter spray pattern and to obtain information on drift (with the helicopter at an 80-ft height applying 2,4,5-T with a microfoil boom), mylar sheets (9 X 9 in) were placed on the ground at intervals of 80, 160, 320, 640, and 960 feet on each side of the spray path. A total of three replications were included in the study. The mylar sheets were attached to 9 X 9-in floor tile to keep them from being moved by the wind or helicopter. Immediately after the spraying was completed, the mylar strips were removed from the tile and placed in jars of methanol in a manner similar to that used with the gauze patches.

Analyses for 2,4,5-T employed the ^{63}Ni gas chromatographic analytical procedures of MATTICE et al. (1979). All values in this study are reported as the methyl ester of 2,4,5-T unless otherwise stated.

RESULTS AND DISCUSSION

Helicopter crews received low levels of 2,4,5-T on their patches (Table 1). In general their patch exposures were relatively uniform, except for the mixer of

TABLE 1

Milligrams of 2,4,5-T Detected on Individual 100 cm^2 Gauze Patches of Crews Applying the Herbicide.

Crew and Duty	Body wt	Chest	Back	Left arm	Right arm	Left thigh	Right thigh	Ave./ $\bar{X}$
	(kg)				(mg)			
Helicopter pilot	72.6	ND	ND	ND	0.010	0.034	0.094[a]	
flagman	86.3	0.052	0.058	0.046	0.067	0.014	0.010	
flagman	95.8	0.024	0.023	0.031	0.028	0.006	0.007	
mixer	86.3	0.051	0.039	0.012	0.011	0.263[a]	0.088	
super-visor	81.7	0.028	0.103	0.058	0.064	0.037	0.018	
								0.043

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Crew and Duty	Body wt (kg)	Chest	Back	Left arm	Right arm (mg)	Left thigh	Right thigh	Ave./ $\bar{X}$
Rice Levee driver		0.02	0.02	0.03	0.03	0.59[a]	0.03	
Mist blower driver	84.0	0.31	1.02	0.44	0.57	0.17	1.01	
driver	106.7	0.58	1.11	1.56	0.66	0.58	0.77	
mixer	79.5	0.74	0.08	0.02	4.49	1.08	8.76[a]	
supervisor	95.3	0.27	0.38	0.52	0.47	0.64	1.30	
								0.94
Backpack sprayer		0.07	0.65	0.27	0.20	0.55	0.75	
sprayer		0.75	[b]	2.33	2.40	6.10	22.47[a]	
sprayer		5.61	1.86	1.77	1.47	0.18	68.93[a]	
sprayer		0.79	0.91	2.01	1.19	2.82	6.19	
sprayer		0.95	17.96	2.11	54.85[a]	1.80	1.06	
sprayer		2.41	2.46	1.23	1.19	24.18	25.65	
sprayer		1.31	0.63	0.55	0.76	0.68	1.79	
sprayer		2.25[a]	0.01	0.40	0.53	0.04	0.08	
sprayer		1.57	[b]	2.00	4.01	31.76	2.73	
sprayer		0.54	0.65	2.92	2.53	0.30	2.47	
sprayer		1.52	2.57	0.98	0.60	1.98	11.09[a]	
sprayer		2.26	1.34	8.01	4.32	3.61	15.32	
								5.43

[a]This value exceeds 50% of the total 2,4,5-T detected on all six patches, thus indicating that non-uniform deposition occurred.
[b]Sample was lost.

Right thigh	Ave./ $\bar{X}$
0.094[a]	
0.010	
0.007	
[a] 0.088	
0.018	
	0.043

the mist blower crew who received considerably more exposure than did his fellow workers. The average exposures for the operators of backpack sprayers were greater than those for the other studies. The members of this crew who received the highest 2,4,5-T amounts on their patches received more than twice the patch exposure of workers with highest amounts in the other studies.

Calculations based upon the formula derived from WOLFE et al. (1975), the photographs revealing exposed skin areas, and the 2,4,5-T analyses from the gauze patches indicated that external exposure values ranged from 0.0036 to 0.0136 mg/kg for helicopter crews. Values for the mist blower crew were from 0.046 to 0.205 mg/kg, while those of the backpack crew ranged from 0.081 to 1.85 mg/kg. The exposure of the tractor driver spraying the rice levees was 0.046 mg/kg.

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17819

patches or that hands habitually were rubbed on this area to clean or dry them off. Thus, extrapolations of values based on any one patch or on an average patch concentration in relation to dermal exposure would not necessarily result in the correctly calculated exposure.

The exposure received on different portions of the body of a person directly under the spray path of the helicopter varied from 0.01 on the chest to 6.48 mg on the left front thigh. If we assume that the sprayed individual weighed 76 kg and was wearing shoes, short sleeves, long trousers and no hat, the calculated potential exposure for this individual is 0.86 mg/kg. There was considerable variation in amounts of 2,4,5-T on the patches worn by this individual; however, the average amount detected per 100-cm^2 patch was 2.07 milligrams on a acid equivalent basis. Calculations for 2 lb/A active ingredient (acid) indicate that a helicopter should deliver to the land surface a total of 2.23 milligrams per each 100 cm^2. The exposure received by this individual directly under the spray path of the helicopter should represent the maximum 2,4,5-T external exposure that could occur to someone who was inadvertently exposed to the forest operation.

Although 2,4,5-T was detected on the gauze patches, it should be pointed out that in the recent human exposure study conducted by LAVY et al. (1979) there was relatively low correlation (0.45) between the amount of 2,4,5-T detected on gauze patches and the 2,4,5-T excreted in the urine of exposed individuals. In that study a value of 0.04 was obtained when the milligrams of 2,4,5-T excreted in the urine of each worker was divided by the milligrams of 2,4,5-T body exposure as estimated from analyzing the gauze patches from each worker. Using this information, if we assume that 4% of the 2,4,5-T which was predicted to come in contact with exposed dermal area was actually absorbed and consequently excreted in urine, the individual directly under a helicopter spray path would have absorbed and excreted 0.034 mg/kg 2,4,5-T.

The worker walking through a field which had been sprayed with ESTERON 245 two hours earlier was outfitted with a set of six patches. Analysis of these patches revealed no detectable 2,4,5-T. Sensitivity limits of the technique would have detected 2,4,5-T in excess of 10 micrograms.

17820

2,4,5-T Deposition Pattern When Applied by Helicopter using a Microfoil Boom.

Distance from Spray Path (ft)					
0	80	160	320	640	960
		(mg/pad)*			
2.78	0.30	0.06	0.03	ND	ND
0.98	0.82	0.08	0.70	0.02	0.01
1.65	0.13	0.08	ND	ND	ND
	ND	ND	ND	ND	ND
	0.01	ND	ND	ND	ND
	ND	ND	0.02	ND	ND
		Averages ($\bar{X}$)			
1.80	0.21	0.04	0.13	ND	ND

*Values below 0.01 mg/pad were below detection limits (ND).

Although wind speed was nearly negligible at the time of the spraying, some drift is indicated. The first three analyses listed in Table 2 at all distances were all lying to the leeward side of the helicopter. The last 3 analyses were on the windward side. Only two of the twelve samples taken beyond 320 feet of the spray path showed trace levels of 2,4,5-T. All other samples were below the 10-microgram detection level.

These data indicate that workers applying 2,4,5-T are receiving much less than the 20 mg/kg levels of 2,4,5-T reported to have caused fetotoxicity in mice (ROLL, 1971). This information in conjunction with the 2,4,5-T excretion studies (GEHRING et al., 1973) and a recent human exposure study conducted by LAVY in November, 1978, indicates that 2,4,5-T quantities received by applicators should be below any known possible health hazard levels.

ACKNOWLEDGMENTS

Appreciation is expressed to the Southern Region Pesticide Impact Assessment Program and to National Forest Products Association for support of the study and to Martha Davis for her assistance in manuscript preparation.

17821

REFERENCES

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17822

MATERIAL SAFETY DATA SHEET

DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 24 SEP 79 PRODUCT CODE: 87120

PRODUCT NAME: TORDON (R) 101 MIXTURE WEED AND BRUSH KILLER MSD: 0381

INGREDIENTS (TYPICAL VALUES-NOT SPECIFICATIONS)	: % :
4-AMINO-3,5,6-TRICHLOROPICOLINIC ACID, (PICLORAM), TRIISOPROPANOLAMINE SALT	: 10.2 :
2,4-DICHLOROPHENOXYACETIC ACID, TRIISOPROPANOLAMINE SALT	: 39.6 :
SEQUESTERING AGENTS, WATER, AND ISOPROPANOL	: 50.2 :

SECTION 1 PHYSICAL DATA

BOILING POINT: IBP >180F, 82C : SOL. IN WATER: INFINITE
VAP PRESS: APPROX. 32 MMHG @ 20C : SP. GRAVITY: 1.143 68/68F, 20C
VAP DENSITY (AIR=1): NOT APPLIC. : % VOLATILE BY VOL: 50% @ APPROX. 100C
APPEARANCE AND ODOR: DARK BROWN LIQUID.

SECTION 2 FIRE AND EXPLOSION HAZARD DATA

FLASH POINT: 114F, 46C* : FLAMMABLE LIMITS (STP IN AIR)
METHOD USED: TCC : LFL: NOT DETER. UFL: NOT DETER.
EXTINGUISHING MEDIA: WATER FOG, ALCOHOL FOAM, CO2, DRY CHEMICAL.
SPECIAL FIRE FIGHTING EQUIPMENT AND HAZARDS: SELF-CONTAINED AIR SUPPLY.
AVOID GETTING WATER FROM FIRE FIGHTING INTO WATER SUPPLIES.
KEEP AWAY FROM POSSIBLE SOURCES OF IGNITION.
AUTOIGNITION 698F, 370C.

*NO FLASH POINT BY TOC WHEN TESTED TO 200F, 93C.

SECTION 3 REACTIVITY DATA

STABILITY: COMBUSTIBLE. KEEP AWAY FROM IGNITION SOURCES.
INCOMPATIBILITY: CONSULT MANUFACTURER FOR SPECIFIC CASES.
HAZARDOUS DECOMPOSITION PRODUCTS: HYDROGEN CHLORIDE AND NITROGEN OXIDES UNDER FIRE CONDITIONS.
HAZARDOUS POLYMERIZATION: WILL NOT OCCUR.

SECTION 4 SPILL, LEAK, AND DISPOSAL PROCEDURES

ACTION TO TAKE FOR SPILLS (USE APPROPRIATE SAFETY EQUIPMENT): ABSORB SPILLS

(CONTINUED ON PAGE 2)

(R) INDICATES A REGISTERED OR TRADEMARK NAME OF THE DOW CHEMICAL COMPANY

DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 24 SEP 79 PRODUCT CODE: 87120
PRODUCT (CONT'D): TORDON (R) 101 MIXTURE WEED AND BRUSH KILLERMSD: 0381

SECTION 4 SPILL, LEAK, AND DISPOSAL PROCEDURES (CONTINUED)

ACTION TO TAKE FOR SPILLS (USE APPROPRIATE SAFETY EQUIPMENT): (CONTINUED) WITH INERT MATERIAL SUCH AS SAND OR SAWDUST. IN CASE OF LARGE SPILLS, DIKE AREA TO CONTAIN. CONSULT MANUFACTURER FOR CLEAN-UP.
DISPOSAL METHOD: BURY WASTE IN NON-CROP LAND AWAY FROM WATER SUPPLIES.

MN0123332

SECTION 5 HEALTH HAZARD DATA

INGESTION: LOW IN SINGLE DOSE ORAL TOXICITY; LD50 (RAT) APPROXIMATELY 3000 MG/KG OF BODY WEIGHT.
EYE CONTACT: CAUSES MODERATE IRRITATION, MAY EVEN CAUSE TRANSITORY CORNEAL INJURY.
SKIN CONTACT: MAY CAUSE SLIGHT IRRITATION, EVEN A MILD BURN UPON PROLONGED REPEATED EXPOSURE.
SKIN ABSORPTION: NOT LIKELY TO BE ABSORBED IN TOXIC AMOUNTS.
INHALATION: LOW VAPOR TOXICITY; ACGIH TLV FOR ISOPROPANOL 400 PPM (1978).
EFFECTS OF OVEREXPOSURE: GASTRIC UPSET. LIVER, KIDNEY DAMAGE AND CENTRAL NERVOUS SYSTEM DEPRESSION SECONDARY TO ISOPROPANOL.

SECTION 6 FIRST AID--NOTE TO PHYSICIAN

FIRST AID PROCEDURES:

EYES: IRRIGATE WITH FLOWING WATER IMMEDIATELY AND CONTINUOUSLY FOR 15 MINUTES. REFER TO MEDICAL PERSONNEL.
SKIN: CONTACT WILL PROBABLY CAUSE NO MORE THAN IRRITATION. WASH OFF IN FLOWING WATER OR SHOWER.
INHALATION: REMOVE TO FRESH AIR IF EFFECTS OCCUR. CONSULT MEDICAL PERSONNEL.
INGESTION: IF SWALLOWED, INDUCE VOMITING IMMEDIATELY BY GIVING TWO GLASSES OF WATER AND STICKING FINGER DOWN THROAT. CALL A PHYSICIAN.

NOTE TO PHYSICIAN:
EYES: MAY CAUSE MODERATE IRRITATION. STAIN FOR EVIDENCE OF CORNEAL INJURY. IF CORNEA IS BURNED, INSTILL ANTIBIOTIC STEROID PREPARATION FREQUENTLY. CONSULT OPHTHALMOLOGIST.
SKIN: MAY CAUSE MILD IRRITATION. TREAT AS ANY CONTACT DERMATITIS. IF BURN IS PRESENT, TREAT AS ANY THERMAL BURN.
RESPIRATORY: INJURY IS UNLIKELY.
ORAL: LOW IN TOXICITY.
SYSTEMIC: LIVER AND KIDNEY ARE PROBABLY TARGET ORGANS FOR OVER-EXPOSURE. NO SPECIFIC ANTIDOTE. TREATMENT BASED ON THE SOUND JUDGMENT OF THE PHYSICIAN AND THE INDIVIDUAL REACTIONS OF THE PATIENT.

SECTION 7 SPECIAL HANDLING INFORMATION

(CONTINUED ON PAGE 3)
(R) INDICATES A REGISTERED OR TRADEMARK NAME OF THE DOW CHEMICAL COMPANY

DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 24 SEP 79 PRODUCT CODE: 87120
PRODUCT (CONT'D): TORDON (R) 101 MIXTURE WEED AND BRUSH KILLERMSD: 0381

SECTION 7 SPECIAL HANDLING INFORMATION (CONTINUED)

VENTILATION: RECOMMEND LOCAL VENTILATION. RECOMMEND CONTROL OF ISOPROPANOL TO SUGGESTED GUIDE.
RESPIRATORY PROTECTION: NONE NORMALLY NEEDED; FOR ISOPROPANOL USE A NIOSH APPROVED ORGANIC VAPOR RESPIRATOR.
PROTECTIVE CLOTHING: CLEAN, BODY-COVERING CLOTHING.
EYE PROTECTION: SAFETY GLASSES WITHOUT SIDE SHIELDS.

MN012332

SECTION 8 SPECIAL PRECAUTIONS AND ADDITIONAL INFORMATION

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: SEE LABEL - KEEP OUT OF REACH OF CHILDREN. DO NOT SWALLOW. AVOID CONTACT WITH SKIN, EYES, AND CLOTHING. WASH WELL AFTER HANDLING. KEEP CONTAINER CLOSED. KEEP AWAY FROM HEAT AND OPEN FLAME. PROVIDE EYE FOUNTAIN AND WASHING FACILITIES NEAR WORK AREA. DO NOT STORE NEAR FOOD, FEEDSTUFFS, FERTILIZERS, SEEDS, INSECTICIDES, FUNGICIDES OR OTHER PESTICIDES. DO NOT CONTAMINATE WATER TO BE USED FOR IRRIGATION.

ADDITIONAL INFORMATION: REVISIONS 9/24/79 -- CHANGED SPECIFIC GRAVITY, STABILITY, EYE CONTACT, SKIN CONTACT, SKIN ABSORPTION, INHALATION, FIRST AID PROCEDURES, NOTE TO PHYSICIAN, SPECIAL HANDLING INFO., ADDED CENTIGRADE TEMPERATURES, FLASH POINT AND METHOD USED.

LAST PAGE

(R) INDICATES A REGISTERED OR TRADEMARK NAME OF THE DOW CHEMICAL COMPANY

THE INFORMATION HEREIN IS GIVEN IN GOOD FAITH, BUT NO WARRANTY, EXPRESSED OR IMPLIED, IS MADE.

ALBERT M. KLIGMAN, M. D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA, PA. 19104

812

NO72992 UO1200324

August 22, 1967

Mr. V. K. Rowe,
Biochemical Research Laboratory,
1803 Building,
The Dow Chemical Company
Midland, Michigan 48640

Dear V. K.:

I am anxious to report my work with the acnegens. I hope that continued support will be possible. I can come almost any time in September, or early October. I prefer to come on a Tuesday evening and to return to Philadelphia on Wednesday afternoon. How about calling at your convenience to set-up an acceptable time? I believe I have much that will be of interest to you.

With every good wish, I remain

Sincerely yours,

Albert M. Kligman, M. D.

AMK/a

17826

THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN 48640

MN0729921 DOW 00822412

August 9, 1967

Albert M. Kligman, M.D., Ph.D.
Department of Dermatology
Hospital of the University of Pennsylvania
36th and Spruce Streets
Philadelphia, Pennsylvania 19104

Dear Dr. Kligman:

I have your note of a few weeks ago and apologize for not answering it sooner. We do want you to come to Midland to present a progress report on the work that you have done with the acnegens. At the present time I do not know when will be a good time. I hope we can work it in early this fall. Is there any time when it would be best for you or any time when it would be impossible for you? If you will let me know the answer to these questions, I will do my best to fix a date.

In answer to your question I do not know whether acnegens have been found to be carcinogens and I did not know that carcinogens were potent acnegens. I am very interested, however, and should like to discuss this matter further with you. We're looking forward to your visit.

My best personal regards.

Sincerely yours,

V. K. Rowe
Biochemical Research Laboratory
1803 Building

ks

cc: Correspondence
V. K. Rowe (2)

17827

ALBERT M. KLIGMAN, M. D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA 4, PA.

DEPARTMENT OF DERMATOLOGY

EVERGREEN 2-4600
EXTENSION 451

814

July 8, 1966

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

RECEIVED
JUL 12 1966
Biochem, Res. Lab.

00120682411

Dear Mr. Rowe:

Supplementing data submitted by Dr. Kligman on the tetrachlorodibenzo-p-dioxin study, enclosed is additional information on Groups #1 through #6. We trust this information will be of help to you.

Yours truly,

Adele F. Allen
Secretary to Dr. Kligman

afa
Enclosure

17828

ALBERT M. KLIGMAN, M. D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA 4, PA.

815

DEPARTMENT OF DERMATOLOGY

EVERGREEN 2-4600
EXTENSION 451

RECEIVED
JUN 27 1966
Biochem, Res. Lab.

June 22, 1966

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

DOW 00720238

Dear V. K.:

Now that I have collected the detailed data, it is evident that the tetrachlorodibenzo-p-dioxin study was by no means a tiny or trivial affair. This study was supervised by one of my graduate dermatologists; he kept giving me a negative read-out throughout the many months of study. I personally saw the subjects in the last two groups which received the highest doses.

The skeletal report I sent was further reduced from the very bony summation he gave me. I was hopeful that the report might be sized to the significance of the results. I almost sent you a letter saying, "We did the work you ordered and found nothing". (I guess those happy days are gone).

Wherever the subject could not be followed for six weeks owing to sudden discharge, or to institutional decisions, detailed data are deleted.

I am grieved that so little has been learned, especially since practically the whole grant was spent in laboratory work. This was a discouraging project.

With every good wish, I remain

Very sincerely yours,

Albert M. Kligman, M.

AMK/a
Enclosure

17829

816

ALBERT M. KLIGMAN, M. D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA, PA. 19104

DEPARTMENT OF DERMATOLOGY

EVERGREEN 2-4600
EVERGREEN 6-3459
EXTENSION 2451

RECEIVED
JUN 1 1966
Biochem, Res. Lab.

May 31, 1966

AN072992 UU:20682237

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

Dear Doctor Rowe:

In my dioxin report, I omitted mention that CBC's and SGPT's were also done on the volunteers during the long period over which the agent was topically applied. No abnormalities were found. This further strengthens our resolve to use larger quantities in an attempt to produce acne.

Respectfully submitted,

Albert M. Kligman, M. D.

AMK/a

17830

817 48640

DOW 780401
MN073280

July 9, 1965

Albert M. Kligman, M.D., Ph.D.
Department of Dermatology
Hospital of the University of Pennsylvania
36th and Spruce Streets
Philadelphia 4, Pennsylvania

Dear Dr. Kligman:

I am sending you under separate cover a small amount of 2,3,7,8-tetrachlorodibenzo-p-dioxin. This is the material which is a potent acnegen and is highly toxic. I have checked back on our figures and find that the single dose oral LD_{50} for rabbits is in the neighborhood of 100 micrograms/kilogram, and we had one animal die which had received a single dose of 16 micrograms/kilogram. It is safe to say, however, that doses of 0.5 to 1.0 mg/kg are always fatal, although deaths may be delayed for 10 to 20 days post treatment. The typical clinical picture is severe liver and kidney injury.

In regard to the skin response on rabbits, we have attempted to quantitate this by applying 0.1 ml of test solution to one to two square inches of the surface of the inner face of the rabbit ear. We find that when the total dose does not exceed about 0.2 of a microgram of the acnegen, no follicular prominence or epithelial hyperplasia develops. When the total dose is about 0.5 of a microgram on this area, the response is marginal; 1 to 2 micrograms almost always produces a response, and 4 to 8 micrograms usually produce a severe response. We have not as yet been able to quantitate the dose required to cause 50% mortality from skin exposure, but we are sure it is well above the total dosages noted above.

In view of this information, it does not seem probable that the dosages shown in the accompanying suggested protocol for the human work would be likely to constitute any serious systemic hazard because the dose on a per kilogram basis would be far below that which produces any significant effect systemically in the rabbit. I might add that the rabbit is far more sensitive than the rat to this type of compound. Nevertheless, the seriousness of the consequences that might develop from testing with this type of compound require that we approach the matter in a highly conservative manner. It

17831

DOW 780402

is with this thought in mind that I have developed the attached protocol. The number of persons per experiment is your decision; I would suggest two as a starter. When applications are repeated, I would like to have them made on consecutive days, if it is convenient to do so. Although the time required to conduct these experiments will require several months, I believe it is the safe way to proceed, using a few people at a time with careful observations on each. The observations are to be made at your discretion, but I would urge routine SGOT's and alkaline phosphatases as a minimum.

There is another item upon which comment should be made. I have indicated in the suggested protocol that a two week observation period should be used prior to starting the next series of experiments. This is because our experience with both animals and man indicates that there is an induction period. In a few instances, we believe an eruption in the human has developed four to six weeks post exposure. Also, we have had a few serious flare-ups which have developed within a matter of days, post exposure. I have compromised on a two-week observation period, but of course, any treated individual should be watched for at least two months post test.

You asked about materials in which this test substance is soluble. I have indicated it is quite soluble in chloroform and benzene and slightly soluble in alcohol. I believe that a solution in 50/50 alcohol and chloroform would be quite appropriate for your work. In regard to covering the exposed area, I would suggest that when the treated area has dried that it be covered lightly with a gauze simply to keep the material from being brushed away or having a person contaminate his hands or clothing inadvertently.

I hope I have answered your questions, but if you have any others, please do not hesitate to contact me.

Sincerely yours,

V. K. Rowe
Biochemical Research Laboratory
1701 Building

VKR/jd

Attach.

cc: Medical Department:
Gordon, Holder, Kramer
J. E. Peterson
L. Silverstein
H. R. Hoyle
V. K. Rowe (2)
136.25-66681-7

819

ALBERT M. KLIGMAN, M. D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA, PA. 19104

January 23, 1968

Copies to Montville Libertini Holden

MN0697799

DOW 7405393

Mr. V. K. Rowe
Biochemical Research Laboratory
1803 Building
The Dow Chemical Company
Midland, Michigan 48640

Dear V. K.:

This note is a follow-up to my report of July 8, 1966. In that study, you will recall that 6 groups of healthy adult subjects received small and increasing doses of tetrachlorodibenzo-p-dioxin. We followed a specific protocol laid down by you. Unfortunately, not a single subject developed acne nor was there any evidence of toxicity.

This encouraged me to proceed more vigorously. We then assembled a new panel of 10 subjects and applied 0.05 ml of a 1% solution in alcohol chloroform to a one inch square on the back. These applications were made every other day for one month. The treated sites were covered with a non-occlusive gauze square. Each week for 6 weeks, the following laboratory tests were done: Urinalysis, CBC, BUN, SGOT, Alkaline phosphatase, and Creatinine clearance.

Clearly, this exposure was immensely greater than the former one. 8 of 10 subjects showed acne form lesions usually beginning 3 to 4 weeks. This began as with a typical development of comedones. In 3 instances, the lesions progressed to inflammatory pustules and papules. These lesions lasted for 4 to 7 months, since no effort was made to speed healing by active treatment. Biopsies were obtained in 5 instances at various stages. The histologic and clinical manifestations were in every way comparable to classical chloracne. The lesions were indistinguishable from those obtained by Dow 6X and Halowax.

In no instance was there laboratory or clinical evidence of toxicity. The subjects remained well throughout the study.

17833

These results implement the conclusions formerly drawn, namely: it is much more difficult to induce acne in the human than in the rabbit ear. The process begins more slowly and the doses required are very much greater. However, unlike the rabbit ear, the comedones often become inflammatory. It is a certainty that the rabbit ear is exceptionally sensitive to acneigenic chemicals and is an excellent system for the detection of such chemicals. Finally, it may be said that chloracne closely mimics acne vulgaris. The only difference is the paucity of anaerobic organisms in the comedo. One may conclude that bacteria are not very significant in the pathogenesis of chloracne.

Very sincerely yours,

Albert M. Kligman, M. D.

AMK/klf

UCW / HCCH

DOW520379

17835

MASTER COPY

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

Technical Information In Support of Application for Registration of Revised Labeling for the Use of TORDON 101 Mixture Weed and Brush Killer on Highways, Railroad and Utility Rights-Of-Way and Other Non-Crop Areas

VOLUME I

JAN 16 1970

Registration Section
Agricultural Department

DOW 520380

17836

DOW 520382

CONTENTS

		Color Section
I	FOREWORD	Brown
II	LABEL COPY	Gray
III	PERFORMANCE	Green
IV	TOXICOLOGY	White
V	RESIDUES	Yellow

Technical Information In Support

of

Application for Registration

of

Revised Labeling for the Use

of

TORDON 101 Mixture
Weed and Brush Killer

on

Highways, Railroad and Utility Rights-Of-Way

and

Other Non-Crop Areas

VOLUME I

Registration Section
Agricultural Department

DOW 520381

17038

DOW520383

TSCA

I FOREWORD

TORDON 101 Mixture Weed and Brush Killer has been extensively used since it gained Federal acceptance under USDA Registration Number 464-306 on June 14, 1963. During the use period from then to now there have been no unusual adverse incidents experienced by the users. In fact it has been widely accepted as being one of the most satisfactory herbicides available with a broad range of utility against numerous weed and brush pests not readily controlled by other herbicidal products commercially available.

Since the initial registration date of June 14, 1963, an uncommon amount of research has resulted in extending the utility of the product with the result that labeling has been revised several times to provide for several uses in addition to those for which the initial application was made. The following tabulation "Chronological Evolution of Registered Uses for TORDON 101 Mixture" gives a summary history of registration activities with this product. This current application contains a proposed revised label along with a two volume compilation of technical information in support of the proposed revisions.

In addition, it should be noted there is a proposed name change for the product - from TORDON® 101 Mixture Brush Killer to TORDON® 101 Mixture Weed and Brush Killer.

DOW 0003

Chronological Evolution of Applications for Registration of TORDON 101 Mixture-January 5, 1969

Dates and Contents of Applications by Dow			Dates of Actions by USDA	
Submitted	Submission	Uses Requested	Labeling Accepted	Notification to Dow
March 28, 1963	Original application for registration and supporting technical information	Control of unwanted brush on utility rights-of-way	June 14, 1963	June 17, 1963
Sept. 18, 1964	Proposed label revision and supporting technical information	To provide for above use with NORBAK particulating agent in aerial applications	Oct. 14, 1964	Oct. 21, 1964
April 29, 1965	Proposed label revision and supporting technical information	To provide for killing unwanted trees by injector and frill treatment	June 8, 1965	June 10, 1965
August 4, 1965	Proposed label revision to include additional uses as shown by supporting technical information	To provide for extended uses with NORBAK particulating agent in aerial application	Oct. 29, 1965	Nov. 12, 1965
Sept. 8, 1965	Application for Temporary Permit for investigational purposes with required protocol	To control noxious perennial weeds on roadsides	Nov. 10, 1965 (USDA Temporary Permit No. 464-EXP-17G)	Nov. 10, 1965
Feb. 4, 1966	Application and supporting technical information	To provide for use with NORBAK particulating agent on highway rights-of-way	March 30, 1966	April 1, 1966
March 5, 1968	Application for Temporary Permit for investigational purpose with required protocol	To provide for extended use on highways and use on rail-	March 5, 1968 (USDA Temporary Permit No. 464-EXP-31G)	April 22, 1968

DOW520385

Williams
9/22/70

SEP 22 1970

~~J. E. Johnson~~
R. E. Naegele

see suggested changes.

Unless you have suggestions to the contrary, I propose to answer the Surgeon General in this fashion. (letter attached)

Jim
G. J. Williams

DOW 424717
MN007023

THE DOW CHEMICAL COMPANY

BARSTOW BUILDING
2020 DOW CENTER
MIDLAND, MICHIGAN 48640

DOW 47418

Jesse L. Steinfeld, M. D.
Surgeon General of the
Public Health Service
Department of Health, Education, and Welfare
Washington, D. C. 20201

Dear Dr. Steinfeld:

Thank you for your letter of September 15 relative to the use of TORDON 101 by the Department of Defense and other federal agencies.

We think that the statement of the Interdepartmental Working Group of the President's Cabinet Subcommittee on Pesticides is an accurate and reasonable statement and certainly accords with our own judgment on the use hazards of TORDON 101. We also respect and agree with your position concerning any subsequent information that may become available concerning hazards associated with the use of TORDON 101.

The Dow Chemical Company ~~feels~~ a great responsibility in the production and sale of ~~potentially hazardous materials~~ and we always try to base our decisions on sound scientific data. As indicated in our letter of June 3, 1970 we feel that this ~~is the only proper~~ basis for decision-making and we would hope that it would continue to be used by ~~industrial~~, as well as federal, state and local governments.

Sincerely,

G. J. Williams
Vice President

GJW:llm

17843

AG FORMULATIONS

JAP Dowco 233 Coral

INTERIM REPORT NO. 181

DOW CONFIDENTIAL

RECEIVED
JUN 9 19/5
C.W. SIMON

38323

AUTHOR: R. L. Van Horn

K-NO. 42085

95

DATE: June 2, 1975

DATA BOOK 1-5480-19

FORMULATION DESCRIPTION: Water Soluble and Wettable Powder Herbicides

TITLE

Compatibility of Tordon® 101 Mixture and Dowco 233 (M-3724) with Karmex 80 WP and Paraquat CL.

PROBLEM

Ag-Organics Development personnel wanted to know the feasibility of tank mixing Tordon 101 Mixture or M-3724 with Paraquat CL plus Karmex 80 WP.

DOW 349211

MN 348600

COPIES TO

FORMULATIONS RES. MGR. K. G. Seymour, 9001
TECH. CENTER FORM. SPEC. C.A. Highhill/E.S. Parsey, 834
PLANT SUPT.
PMT R&D REP. T. Haagsma, 9008
FORMULATIONS FILE: Dowco 233

Area ABCT's
Ag Formulations L
F. N. Keeney, 900
B. Byrd, Charlott
Sales Office

DATA AND CONCLUSIONS

The compatibility of Tordon 101 Mixture and M-3724 (3.0 lb Dowco 233) was tested in combination with Paraquat CL and Karmex 80 WP as field spray dilutions in water.

Both Tordon 101 Mixture and M-3724 produced satisfactory tank mixes with Paraquat CL and Karmex 80 WP. All spray dilutions settle out upon standing but redisperse easily with mild agitation. No flocculation was observed with any of the spray mixtures after twenty four hour storage.

For commerical use, good continuous agitation will be required during all mixing and field application of these herbicide tank mixes.

Copies sent 7/9/75
Grumbles
Johnson
L. Jones
V. Dill
A. Ruiz
O.A. Knudtsen

RECEIVED
JUN 10 19/5
JUAN A. PAEZ

17044

ALBERT M. KLIGMAN, M.D., PH. D.
HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA
36TH AND SPRUCE STREETS
PHILADELPHIA, PA. 19104

901

IN072892 DOI120682245

January 23, 1968

Mr. V. K. Rowe
Biochemical Research Laboratory
1803 Building
The Dow Chemical Company
Midland, Michigan 48640

Dear V. K.:

This note is a follow-up to my report of July 8, 1966. In that study, you will recall that 6 groups of healthy adult subjects received small and increasing doses of tetrachlorodibenzo-p-dioxin. We followed a specific protocol laid down by you. Unfortunately, not a single subject developed acne nor was there any evidence of toxicity.

This encouraged me to proceed more vigorously. We then assembled a new panel of 10 subjects and applied 0.05 ml of a 1% solution in alcohol chloroform to a one inch square on the back. These applications were made every other day for one month. The treated sites were covered with a non-occlusive gauze square. Each week for 6 weeks, the following laboratory tests were done: Urinalysis, CBC, BUN, SGOT, Alkaline phosphatase, and Creatinine clearance.

Clearly, this exposure was immensely greater than the former one. 8 of 10 subjects showed acne form lesions usually beginning 3 to 4 weeks. This began as with a typical development of comedones. In 3 instances, the lesions progressed to inflammatory pustules and papules. These lesions lasted for 4 to 7 months, since no effort was made to speed healing by active treatment. Biopsies were obtained in 5 instances at various stages. The histologic and clinical manifestations were in every way comparable to classical chloracne. The lesions were indistinguishable from those obtained by Dow 6X and Halowax.

In no instance was there laboratory or clinical evidence of toxicity. The subjects remained well throughout the study.

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These results implement the conclusions formerly drawn, namely: it is much more difficult to induce acne in the human than in the rabbit ear. The process begins more slowly and the doses required are very much greater. However, unlike the rabbit ear, the comedones often become inflammatory. It is a certainty that the rabbit ear is exceptionally sensitive to acneigenic chemicals and is an excellent system for the detection of such chemicals. Finally, it may be said that chloracne closely mimics acne vulgaris. The only difference is the paucity of anaerobic organisms in the comedo. One may conclude that bacteria are not very significant in the pathogenesis of chloracne.

Very sincerely yours,

Albert M. Kligman, M. D.

AMK/klf

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Evaluation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in C57BL/6 mice:

Reproduction and Fertility in Treated Male Mice and Evaluation of Congenital Malformations in Their Offspring

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NTP-80-44

TABLE OF CONTENTS

	Page
Abstract	v
Introduction	1
Materials and Methods	5
Test Chemicals and Purity	5
Animals and Husbandry	8
Preparation of Diets	8
Experimental Design	10
Toxicopathology	10
Fertility and Reproduction	11
Statistical Evaluation	13
Results	15
Feed Consumption and Body Weight	15
Organ Weights and Histopathology	15
Fertility	26
Teratological Examinations	31
Postnatal Litter Examinations	36
Discussion	46
Bibliography	52
Acknowledgements	57

DOW 1136193

17848

LIST OF TABLES

		Page
Table 1.	Analysis of 2,4-D Sample for Dioxin.	6
Table 2.	Analysis of 2,4,5-T Sample for Dioxins	7
Table 3.	2,4-D, 2,4,5-T and TCDD Concentrations in Stock Corn Oil Solutions and Projected Dosages in Feed	9
Table 4.	Fertility and Mating Efficiency in Treated and Control C57Bl/6 Mice, 8 Week Total	27
Table 5.	Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development, Group I Control.	32
Table 6.	Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development, Group II.	33
Table 7.	Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development, Group III.	34
Table 8.	Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development, Group IV.	35
Table 9.	Summary of Most Frequently Occurring Defects in Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD	37
Table 10.	Percent Malformed Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD	38
Table 11.	Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males, Group I Control.	39

17849

LIST OF TABLES (continued)

Page

Table 12. Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males, Group II 40

Table 13. Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males, Group III. 41

Table 14. Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males, Group IV 42

Table 15. Summary of Malformation Rates (%) in Postnatal Study[1] 43

Table 16. Summary of Specific Malformations in Postnatal Study. 44

Appendix Table 1. Summary of All Malformations Observed in Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD. 49

Appendix Table 2. Incidence of Fused Sternebrae in Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD. 50

DOW 11361195

17850

LIST OF FIGURES

		Page
Figure 1.	Average Weekly Food Consumption.	17
Figure 2.	Mean Body Weights for Male Mice.	18
Figure 3.	Average Weekly Weight Gain	19
Figure 4.	Average Liver Weight	20
Figure 5.	Average Thymus Weight.	21
Figure 6.	Average Spleen Weight.	22
Figure 7.	Average Testis and Epididymis Weight	23
Figure 8.	Average Kidney Weight.	24
Figure 9.	Average Brain Weight	25
Figure 10.	Average Sperm Concentration.	28
Figure 11.	Average Percent Motile Sperm	29
Figure 12.	Average Percent Abnormal Sperm	30

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ABSTRACT

This study was undertaken to determine the effects of mixtures (simulated Agent Orange) of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on reproduction and fertility of treated male mice.

Male C57BL/6 mice were given feed containing varying concentrations of 2,4-D, 2,4,5-T and TCDD such that daily doses of approximately 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 2.4 µg/kg TCDD (Group II) or 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 0.16 µg/kg TCDD (Group IV) or 20 mg/kg 2,4-D, 20 mg/kg 2,4,5-T and 1.2 µg/kg TCDD (Group III) would be achieved. Controls (Group I) were given a diet with only the corn oil vehicle added to the feed. In the treated animals, dose-related liver and thymus toxicity were found and body weight gain was significantly reduced. Liver and thymus toxicity showed significant or complete recovery when the mice were returned to a control diet. Sperm concentration, motility and percent sperm abnormalities were evaluated and no significant effect was noted during or after the dosing period.

At the conclusion of an eight week dosing period treated males were mated to untreated virgin females (three per male per week for eight weeks). Mating frequency, average fertility, percent implantation and resorption sites and percent fetal malformations were all measured in relation to the treatment. No significant decrement in fertility or reproduction was noted in the study. There was no evidence of germ cell toxicity. Survival of offspring and neonatal development were apparently unaffected by paternal exposure to the simulated mixtures of Agent Orange.

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INTRODUCTION

Chlorinated phenoxyacetic acid compounds are used extensively as herbicides in forestry and agriculture. The Department of Defense tested and used a number of different herbicides containing chlorinated phenoxy acids in Vietnam as defoliants; these included Herbicide Orange, Herbicide White, Herbicide Purple, Herbicide Pink and Herbicide Green (Young et al., 1978). The herbicide most extensively used was Herbicide Orange, a 1:1 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). It has been estimated that 107 million pounds were sprayed with the majority used in the years 1967 to 1969 (77% of total herbicide sprayed) (Young et al., 1978).

During the synthesis of 2,4,5-trichlorophenol (TCP) and subsequently 2,4,5-T, but not 2,4-D, a highly toxic contaminant is formed. This contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has been found in Herbicide Orange at an average concentration of 2 ppm with individual analysis of up to 47 ppm reported (Young et al., 1978). Occupational or environmental exposure to humans to TCDD has been associated with a number of clinical disorders (Firestone, 1977; IARC, 1978). The heaviest exposures have involved industrial accidents which occurred in plants synthesizing TCP. The most consistently documented clinical manifestation has been chloracne, a severe form of pustular folliculitis which is most frequently observed on the face, neck and upper extremities. Other less common clinical findings following TCDD exposure include porphyria cutanea tarda, central and peripheral nervous system disorders, depression and irritability, hepatic dysfunction and altered serum lipid concentrations (Firestone, 1977; IARC 1978; Young et al., 1978).

A number of Vietnam Veterans have expressed concern as to health effects that may have resulted from exposure to Herbicide Orange either through application of the herbicide or through inhabiting defoliated areas (Holden, 1979; Rawls, 1979). A particular concern is that Herbicide Orange exposure may be related to reported decreases in both libido and fertility (low sperm counts and abnormal sperm forms) and that it may also be responsible for birth defects observed in offspring sired by veterans who were exposed to Agent Orange (Bogen, 1979; Holden, 1979).

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The toxicity of TCDD and the phenoxy acids 2,4-D and 2,4,5-T has been studied in some detail. The biological effects of these chemicals are well documented in a number of mammalian test systems (Gehring and Betso, 1978; Moore, 1978). 2,4-D, 2,4,5-T and TCDD have all been investigated for teratogenicity and fetotoxicity when given to pregnant females. 2,4-D acid and 2,4-D esters show signs of fetotoxicity and embryotoxicity in hamsters and rats at high dose levels, but it is unclear whether the compounds are actually teratogenic (Collins and Williams, 1971; Khera and McKinley, 1971; Schwetz et al., 1971). Exposure of mice to 2,4,5-T during pregnancy results in congenital malformations (Courtney and Moore, 1971; Neubert and Dillman, 1972; Hood et al., 1979). Studies in rats (Sparschu et al., 1971) and monkeys (Dougherty et al., 1975), indicate that the teratogenicity of 2,4,5-T may be a species-dependent phenomenon, since gestational exposure to this compound produced fetotoxic but not teratogenic effects (Gehring and Betso, 1978).

Early studies with 2,4,5-T samples which were contaminated with 30 ppm of TCDD indicated that the herbicide was teratogenic in rats (Courtney et al., 1970). Subsequent studies by Courtney and Moore (1971), using

purified 2,4,5-T, showed that both 2,4,5-T and TCDD were teratogenic in three strains of mice but in rats only TCDD was fetotoxic and possibly teratogenic. TCDD has been shown by other laboratories to be teratogenic and/or embryotoxic in mice at levels above 0.1 µg/kg/day (Smith et al., 1976) and rats at 0.125-2.0 µg/kg/day (Sparschu et al., 1971).

Although fetotoxicity and teratogenicity associated with gestational exposures to these compounds have been extensively studied, there is a paucity of data as to the effects of male exposure on fertility and development of their offspring. Investigations in male rats undertaken to determine whether dominant lethal mutations could be caused by TCDD were negative. However, the incidence of fertile matings was decreased but it was not determined whether this was due to the systemic toxicity of TCDD or a direct effect on reproduction (Khera and Ruddick, 1971). Other studies that have considered reproductive competence in males have generally been multigeneration studies of animals treated during their entire lives with one of the compounds of interest. In those studies neither 2,4-D (Hansen et al., 1971) nor 2,4,5-T (Smith et al., 1978) significantly reduced fertility when males and females were given feed containing the herbicides. Three-generation studies with TCDD demonstrated that ingestion of levels greater than 0.1 µg/kg/day decreased fertility and litter survival in the f_0 generation; exposure to 0.01 µg/kg/day decreased fertility in the f_1 and f_2 generations, but not the f_0 generation (Murray et al., 1979). In that case, an increase in the percentage of resorbed implantation sites could be related to female exposure to TCDD, but not to male exposure (Murray et al., 1979).

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The consequences of chemical toxicity on male reproductive capabilities might include loss or decrease in fertility, abnormal sperm morphology, decreased sperm concentration and/or motility, or lesions in the reproductive tract and accessory sex glands (Gomes, 1970; Manson and Simons, 1980). In addition to effects on reproduction or fertility, chemical exposure might cause genetic mutations in the male germ cells which could be expressed in the offspring as an inherited anomaly, or embryo and fetal death (Joffe, 1979; Manson and Simons, 1980). Another mechanism to explain fetal effects via the male would be that the chemical might actually be transmitted to the female in the seminal plasma which could then result in a direct exposure of the ova.

The dominant lethal (Epstein, 1973; Generoso, 1973) and sperm morphology (Wyrobek, 1979) assays, used routinely in mice to evaluate potential chemical mutagenicity in male germ cells, were employed in this study. Both of these test systems involve chemical exposure followed by fertility testing or sperm evaluation of the animals for the duration of the spermatogenic cycle (approximately 35 days in mice). This approach is necessary since male germ cells are constantly dividing and differentiating during transit from spermatogonia to spermatozoa with each developmental stage varying in its sensitivity and susceptibility to chemical toxicity or mutagenicity. Experimental designs which are directed at determining male germ cell toxicity must consider this sperm maturation process to assure that all stages of development are tested.

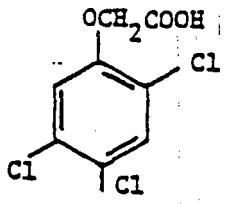
The following investigations were undertaken to determine if composite exposure to 2,4-D, 2,4,5-T plus TCDD (i.e., Herbicide Orange), could affect reproductive function in male mice.

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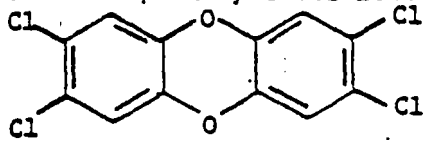
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2,4-dichlorophenoxyacetic acid (2,4-D)



2,4,5-trichlorophenoxyacetic acid (2,4,5-T)



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

2,4-dichlorophenoxyacetic acid (2,4-D) (AGR 171114, 98.5% pure) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (AGR 133711, 98.7% pure) were supplied by the Dow Chemical U.S.A., Midland, Michigan. Both samples were analyzed by Dow Chemical for TCDD contamination who reported no TCDD or other dioxin detected in the samples (Tables 1 and 2).

The free acid was used to eliminate the volatility problem associated with the butyl ester which would compromise quantification of dose administered and pose an exposure risk to laboratory personnel. The free acid form is readily absorbed from the gastrointestinal tract.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was synthesized by the Environmental Chemistry Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. The TCDD was reported to be of greater than 98% purity by gas chromatographic analysis

TABLE 1

Analysis of 2,4-D Sample for Dioxins

	Concentration	Detection Limit
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Not detected	1 ppb
Hexachlorodibenzo-p-dioxin	Not detected	1 ppb
Heptachlorodibenzo-p-dioxin	Not detected	20 ppb
Octachlorodibenzo-p-dioxin	Not detected	5 ppb

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TABLE 2

Analysis of 2,4,5-T Sample for Dioxins

	Concentration	Detection Limit
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Not Detected	0.5 ppb
Hexachlorodibenzo-p-dioxins	Not detected	0.03 ppm
Octachlorodibenzo-p-dioxin	Not detected	0.3 ppm

performed by NIEHS. The principal contaminate was 2,3,7-trichlorodibenzo-p-dioxin.

Animals and Husbandry

Four week old male and 10 week old female C57BL/6N inbred mice (Cesarean-Originated, Barrier Sustained) were purchased from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Upon arrival the mice were eartagged and housed in plastic cages with stainless tops (males, one per cage; females, ten per cage). Absorb-dri hardwood bedding (Barnes Supply, Durham, NC) was used, and cages were cleaned once each week. The animals were kept in constant temperature (20 ± 2°C) and humidity (R.H. 50 ± 10%) on a fixed cycle of 12 hours light-12 hours darkness. The mice were allowed food and water ad libitum. The powdered diet was open formula NIH-31 prepared by Zeigler Bros. Co. of Gardners, Pa.

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Preparation of Diets

Stock solution of the test chemicals were prepared in a corn oil vehicle. The calculated and analytical values are given in Table 3. The test diets were prepared each week for 8 weeks by adding the appropriate stock solution into the feed (2% vol/wt). The concentration of chemicals in the feed was not changed during the 8 week exposure period study. Feed consumption (gm/mouse) was measured once a week. Approximate dose levels were projected using consumption of 5 gm feed/day by a mouse weighing 25 gm. The controls (Group I) were given a diet containing 2% corn oil. Group II consisted of mice that received about 40 mg/kg/day of 2,4-D, 40 mg/kg/day of 2,4,5-T and 2.4 µg/kg/day of TCDD for a total dose of 2.24 gm/kg of 2,4-D and 2,4,5-T each and 0.13 mg/kg of TCDD over the entire 8 weeks. Group III mice were treated at a rate of 40 mg/kg/day

TABLE 3

2,4-D, 2,4,5-T and TCDD

Concentrations in Stock Corn Oil Solutions*

and Projected Dosages in Feed

Treatment Group	2,4-D[1] ppm*	2,4-D[1] mg/kg/day**	2,4,5-T[1] ppm*	2,4,5-T[1] mg/kg/day**	TCDD[2] ppb*	TCDD[2] µg/kg/day**
I (Control)	0(0)	0	0(0)	0	0(0)	0
II	10,000(9380)	40	10,000(9590)	40	600(505)	2.4
III	10,000(9310)	40	10,000(9480)	40	40(39)	0.16
IV	5,000(4880)	20	5,000(4830)	20	300(271)	1.2

*Calculated prepared concentrations and in ( ) amounts detected by analysis.

**Dose levels based on ideal concentrations in feed and an average feed consumption of 5 gm feed/day/25 gm mouse.

[1]Samples of oil collected for 2,4-D and 2,4,5-T analyses were extracted with ethyl ether, derivatized with diazomethane, extracted with hexane and analyzed on an electron capture gas chromatograph (ECGC) by the Midwest Research Institute.

[2]Samples of oil collected for TCDD analysis were saponified in ethyl alcohol and potassium hydroxide, then extracted with hexane and analyzed with an ECGC by the Midwest Research Institute.

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of 2,4-D and 40 mg/kg/day of 2,4,5-T (same as Group II), but received only 0.16 µg/kg/day of TCDD; the total dosages of 2,4-D and of 2,4,5-T were 2.24 gm/kg and 0.009 mg/kg of TCDD. The mice in Group IV received 20 mg/kg/day of 2,4-D, 20 mg/kg/day 2,4,5-T, and 1.2 µg/kg/day of TCDD, resulting in a total 8 week exposure of 1.12 gm/kg of 2,4-D and of 2,4,5-T and 0.067 mg/kg of TCDD. After the 8 week exposure period all mice were fed standard pelleted NIH 31 diet.

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Experimental Design

Two hundred male mice were weighed and sorted (by weight) into eight groups (25 per group). One half of the male mice (4 groups of 25 each) were used for toxicity evaluation, while the other half (4 groups of 25 each) were used for fertility and reproductive studies.

All males were then acclimatized on NIH-31 laboratory chow for three weeks before the chemical exposures were begun. Chemical exposure began when the males were eight weeks old. Body weights and food consumption were recorded on a weekly basis. The males were assigned to experimental and control groups such that weight differences between groups were minimized. The mice were then treated with one of the three treated or control diets for eight consecutive weeks.

Toxicopathology

One hundred of the mice were studied during and after the 8 week feeding period. Four animals from each of the four dose groups were killed by decapitation at 1, 4, 5, 8, 12 and 16 weeks after first receiving treated feed. Each mouse received a gross autopsy examination; body and organ (brain, liver, spleen, kidney, thymus and testis/epididymis) weights were measured. These organs as well as lung, duodenum, ear,

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prostate, seminal vesicle, coagulating gland and urinary bladder were fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin blocks. Six μm histologic sections were prepared and stained with hematoxylin and eosin. The tissues were examined for evidence of histopathologic change.

Also at sacrifice, the vas deferens were removed and spermatozoa milked into a 1.0 ml volume of 0.9% saline. The concentration of sperm per vas deferens was estimated with a hemocytometer immediately after collection. Additionally, the percent motile (any movement vs. no movement) sperm was enumerated. The sperm sample was then stained with 0.25% eosin Y for 30 minutes. The sperm were evenly distributed within the staining solution using a Pasteur pipette and four slide preparations were prepared from each sample. The smears were allowed to air dry, were cover slipped and were examined at 400 X magnification. Three hundred sperm were studied for each sample and sperm were classified as normal or abnormal using the criteria of Wyrobek and Bruce (1975) and Soares et al (1979).

Fertility and Reproduction

Fertility and reproduction assessments were conducted on the remaining 100 mice (four groups of 25).

After the male mice had been treated with the test chemicals for eight weeks they were returned to control feed. Beginning the next day, each male was housed with three virgin female mice (fourteen weeks of age) for up to 5 days each week for eight weeks. Each female was examined each morning for evidence of mating by detection of a vaginal plug (Day 0 of pregnancy). Each mated female was removed from the cage, weighed and placed in a cage with other mated females in that group. Females which did not appear to have mated during the 5-day cohabitation period

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were observed for three more weeks to permit detection of possible pregnancies for which vaginal plugs were not observed. Apparent failure of a female to mate or conceive was verified three weeks after cohabitation by killing the animal, removing the uterus, and staining it with ammonium sulfide to better identify the presence of implantation sites (Kopf et al., 1964).

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For each weekly mating trial one female bred to each male was put in a group to be sacrificed on day 18 of pregnancy for teratology examination. A second bred female from each male was placed in a group which was allowed to deliver and rear her offspring. All remaining dams found to have plugs were placed in a "teratology backup group" to be subjected to teratological evaluation if the dam selected for day 18 sacrifice was found not to have any live fetuses. The above pregnant mice were systematically distributed to the teratology, postnatal or "backup" groups such that no one group was biased with dams which mated first, second or third. However, if less than three dams had mated, priority was generally given to the teratology group. All mated dams were weighed on days 0, 7, 11, 15 and 18 of gestation.

On day 18 of gestation, the dams designated for teratology examination were coded to permit identification only by number so that laboratory personnel conducting the teratogenic analysis did not know the test group. The mice were killed by cervical dislocation and their reproductive status was determined. Implantation sites in each uterine horn were counted and the general condition of each conceptus was recorded. The detection of implantation sites in the uteri of apparently nonpregnant females was achieved through use of ammonium sulfide (Kopf et al., 1964). Live fetuses were weighed individually, sexed internally (surgical incision below navel), and examined for external malformations. Live

fetuses weighing <0.5 g, or weighing less than two-thirds the mean of their larger littermates, were designated as being "stunted". At least one-half of the fetuses of each litter, all "stunted" fetuses and fetuses having external malformations, were examined for visceral alterations (Staples, 1974). The bodies of all fetuses were then processed for skeletal examination (Staples and Schnell, 1964). The heads of each fetus subjected to visceral examination (with the exception of any fetuses which had external head malformations) were cut off at the base and examined by the free-hand sectioning technique described by Wilson (1965).

The remaining dams (postnatal group) were allowed to deliver their litters. Live and dead offspring as well as birth weight were recorded (day 0). The pups were reweighed on days 4, 7 and 21 and viability also was recorded. The dams and their offspring were killed on day 21.

The above procedures were repeated weekly for eight weeks resulting in a total of eight sets of data. Four weeks after the conclusion of the breeding study, (week 20 of the experiment), the male mice were killed and autopsied in a manner identical to that described for the males sacrificed for toxicopathologic evaluation.

Statistical Evaluation

Statistical evaluations of possible pairwise treatment-control differences in food consumption, body weights, organ weights, fertility, mating efficiency, and sperm number, motility and abnormalities were made by Dunnett's test (Miller, 1966). Analysis of variance procedures were employed to assess the significance of differences among groups, week-to-week variability, and week by group interactions. Analyses of abnormalities among the offspring were carried out employing pairwise

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comparison of control versus treated groups with the Mann Whitney U test. The analysis of malformations considered the average percent malformed fetuses per litter.

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RESULTS

Feed Consumption and Body Weight

The projected food consumption of 35 gm/week (5 gm/day) proved to be a conservative estimate in all groups throughout the period of chemical exposure (Figure 1). Lower food consumption for all groups is indicated in week-2 because only a fraction of the week (5 days) was measured. The addition of 2,4-D, 2,4,5-T or TCDD did not significantly decrease feed consumption during the full eight week dosing period in any treatment group, as compared to the controls. Statistically significant changes in feed consumption were only found in sporadic cases and no general trend of decreased feed consumption could be attributed to the addition of either phenoxy acids or TCDD.

Body weight and weight gain, however, showed significant reductions in the treated animals when compared to controls (Figures 2 and 3). This reduction in body weight was most pronounced in group II (2.4 μg TCDD/kg/day and 80 mg phenoxy acid/kg/day) from weeks 3 through 8 of the study. The Group II animals recovered most of their weight deficit when returned to control diet.

Generally all of the mice appeared healthy throughout the course of the study. Only two animals died during the twenty weeks, one in group IV at 5 weeks and one in group II at 19 weeks. Their death did not appear to be treatment-related.

Organ Weights and Histopathology

The mean organ weights of animals killed on weeks 1, 4, 5, 8, 12, 16, and 20 are shown in Figures 4-9. Statistically significant increases in liver weight (Figure 4) were observed in all treated groups and was positively correlated with the amount of TCDD exposure (i.e., 2.4 > 1.2

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> .16 µg/kg TCDD/day, groups II, IV and III respectively). After conclusion of exposure the liver weight returned toward normal, although Groups II and IV continued to show significantly elevated values even at week 20. The livers of treated mice were enlarged, lighter in color than normal and mottled. The thymus was decreased in weight, which also appeared to be a function of the level of TCDD rather than phenoxy acid exposure (Figure 5). Although the thymus weights were significantly ($p<.01$) reduced in Groups II and IV relative to controls throughout the treatment period (weeks 1-8), thymic recovery appeared complete and weights were not statistically different from the controls by 4 weeks after the last exposure. No significant treatment-related effects were observed in the spleen (Figure 6), testis (Figure 7), kidney (Figure 8), or brain (Figure 9). Histopathological evaluation showed no treatment-related changes in any organs, with the exception of the liver. Even the thymus, which had decreased in size to as much as one-third that of the control thymus, appeared histologically normal. The mild toxic effects observed in the liver included hepatocellular swelling, scattered single cell necrosis, increased numbers of mitotic figures, excess extramedullary hematopoiesis, and leukocytic infiltration. These changes were most apparent in group II (2.4 µg/kg/day TCDD; 80 mg/kg/day phenoxy acids) and least apparent in Group III (0.16 µg/kg/day TCDD; 80 mg/kg/day phenoxy acid). These signs of toxicity diminished substantially by the end of the twelfth week of the study (four weeks after chemical exposure was concluded).

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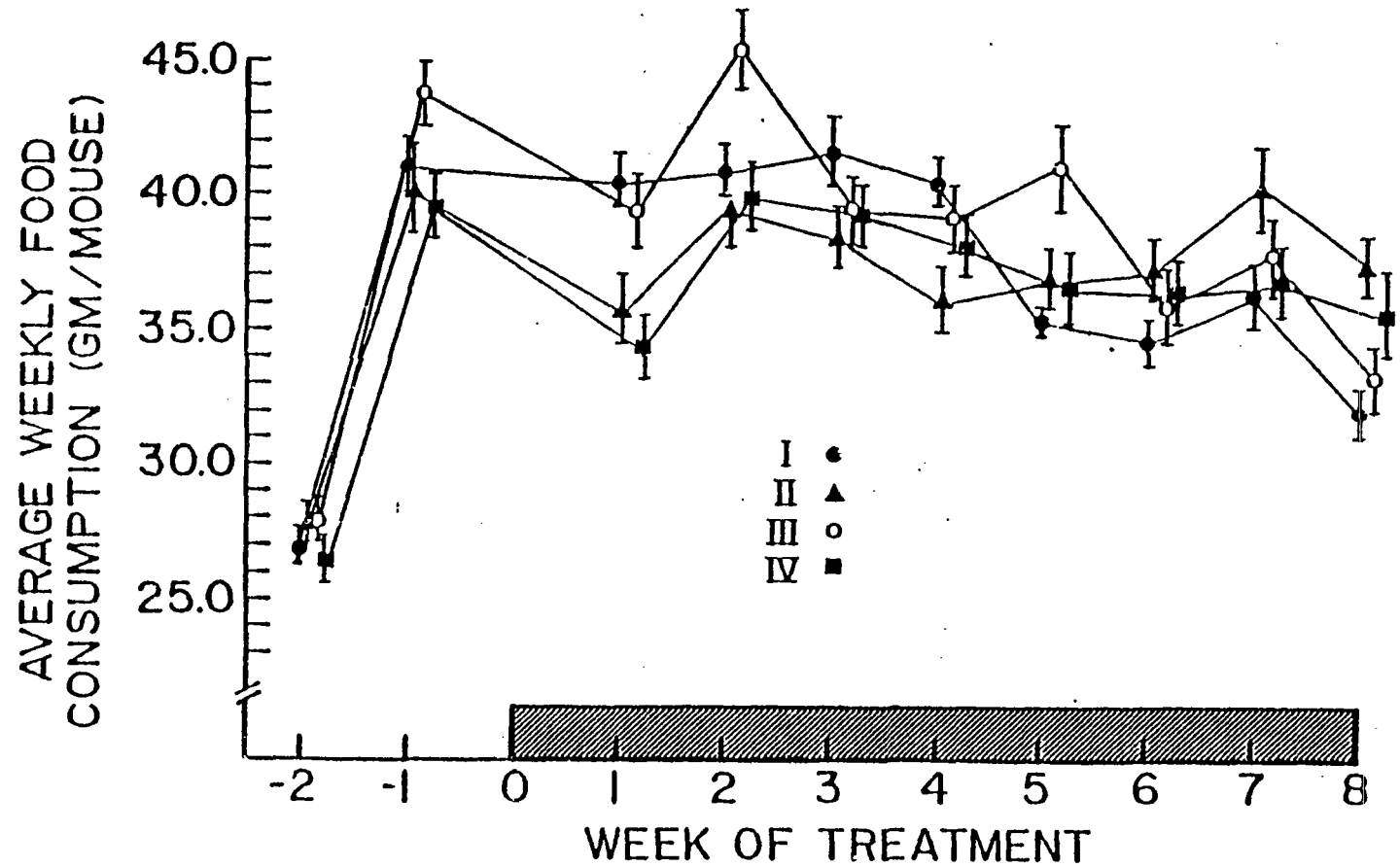


Figure 1. Average weekly food consumption before and during the treatment period of the study for the males. Values are mean ± S.E.M.

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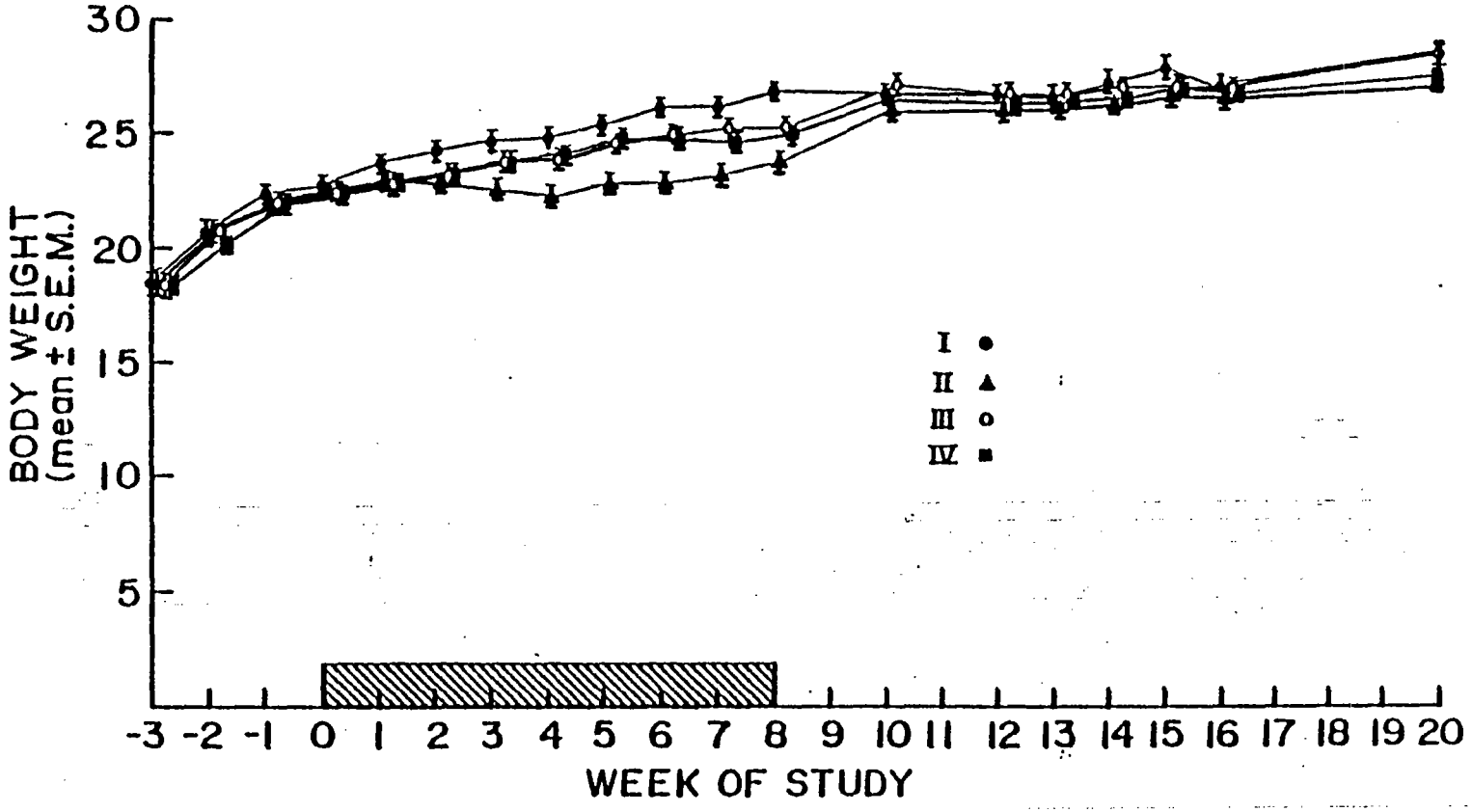


Figure 2. Mean body weights for male mice in all four treatment groups. Significant reductions ($p<0.05$) in weight, as compared to control, for groups II and III were present in weeks 3-8, group IV in weeks 6-8. Values are mean ± S.E.M.

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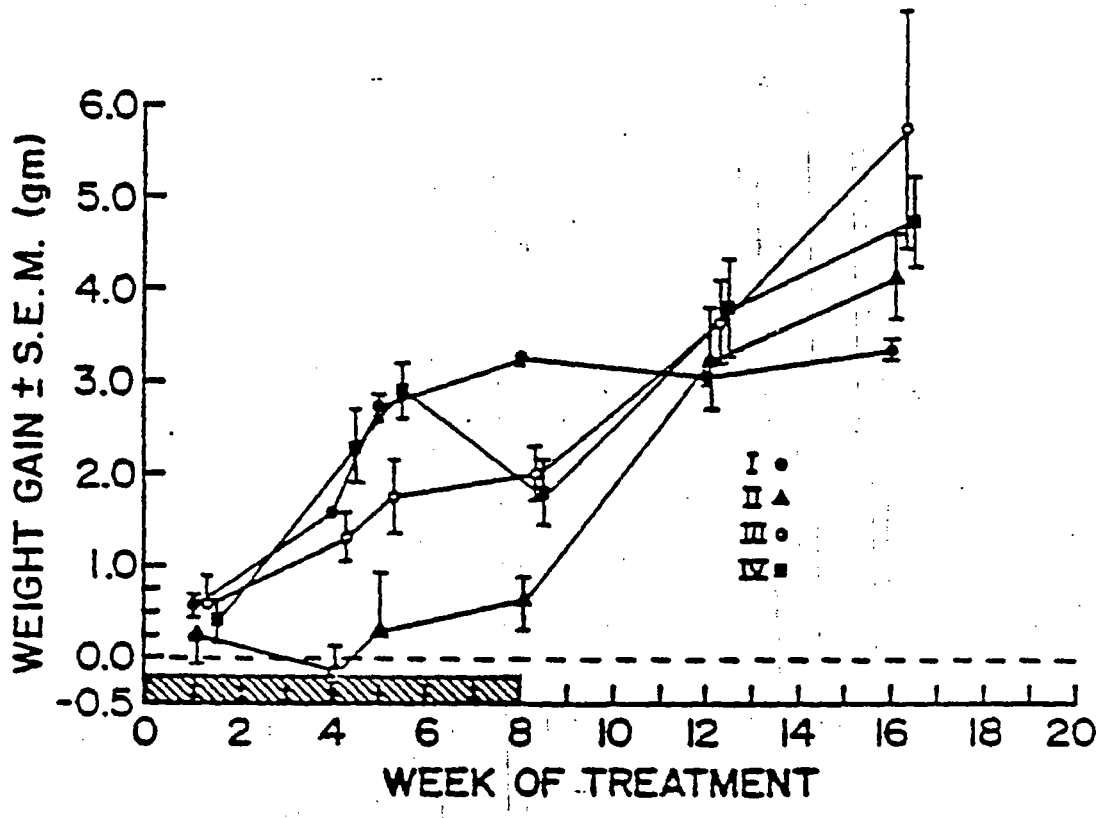


Figure 3. Average weekly weight gain. Weight gain was significantly ($p<0.01$) reduced in Group II relative to control during weeks 4, 5 and 8. Rapid recovery was observed such that no significant difference was present by week 12 in these animals. Values are mean ± S.E.M., n = 4 per group.

17871

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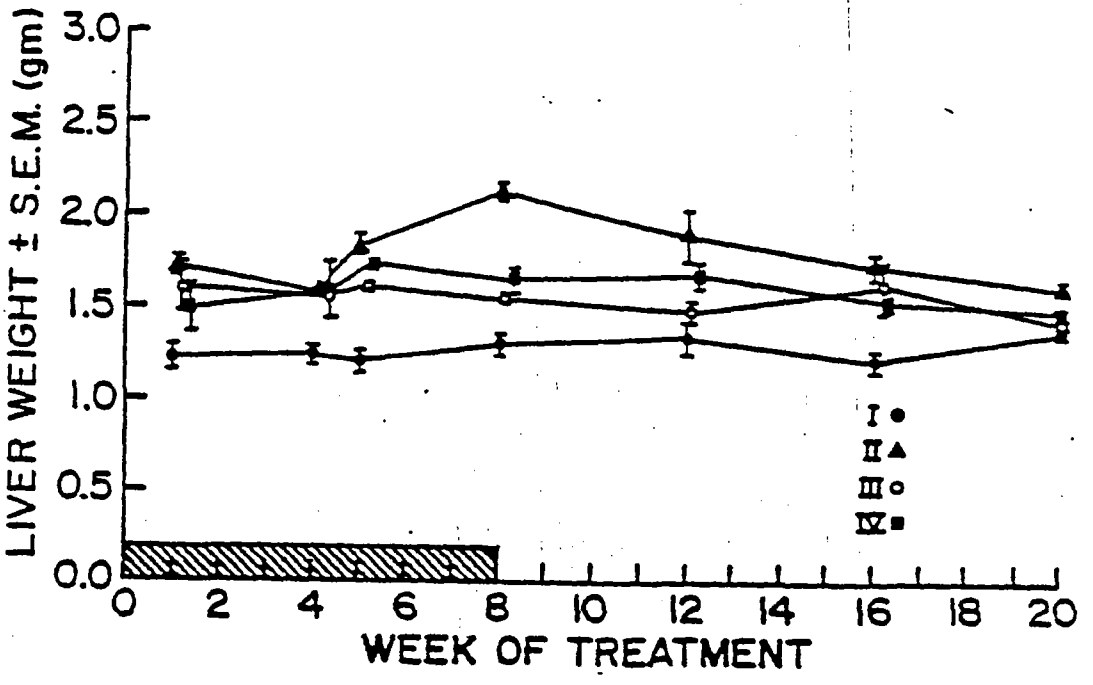


Figure 4. Average liver weight. Liver weight was significantly ($p<.05$) increased in Group II (weeks 1-20), Group III (weeks 1-5, week 16) and Group IV (weeks 4-20) relative to controls. Values are mean ± S.E.M., n = 4 per group, weeks 1-16, n = 25 per group in week 20.

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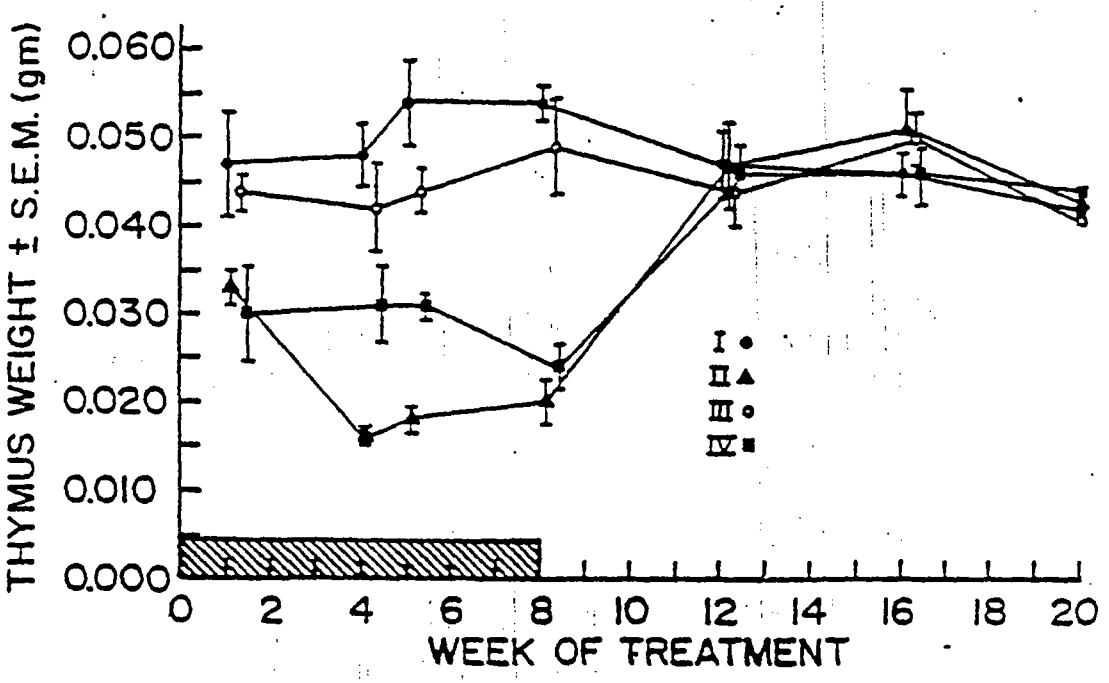


Figure 5. Average thymus weight. Thymus weight was significantly ($p<.01$) reduced in Groups II and IV relative to controls (weeks 1-8). Complete recovery was observed in all groups by week 12. Values are mean ± S.E.M., n = 4 per group on week 1-16, n = 25 per group in week 20.

17873

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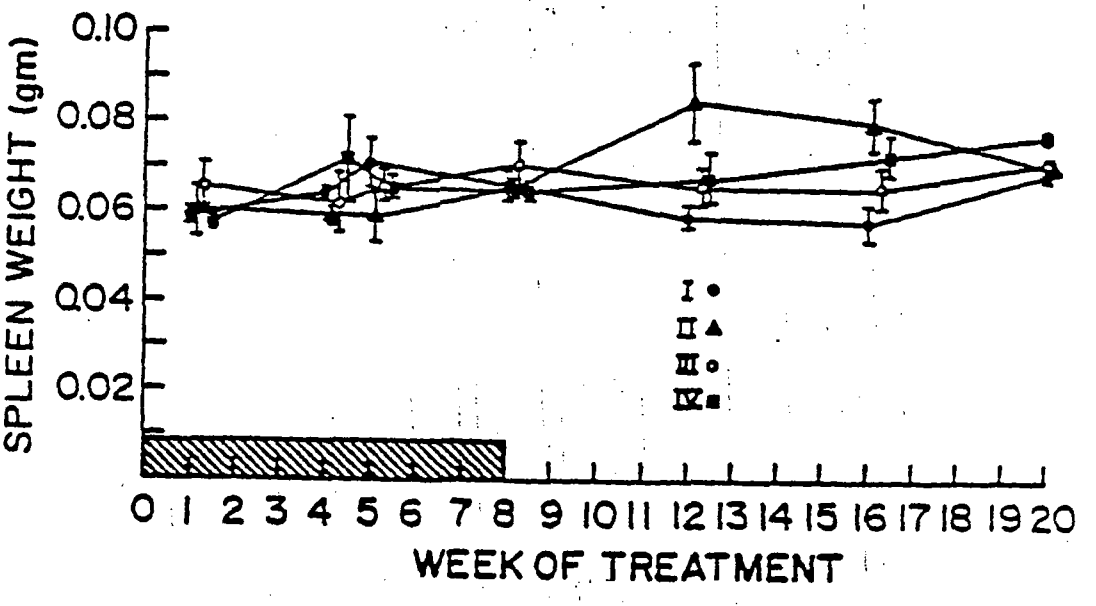


Figure 6. Average spleen weight was not significantly changed by treatment. Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

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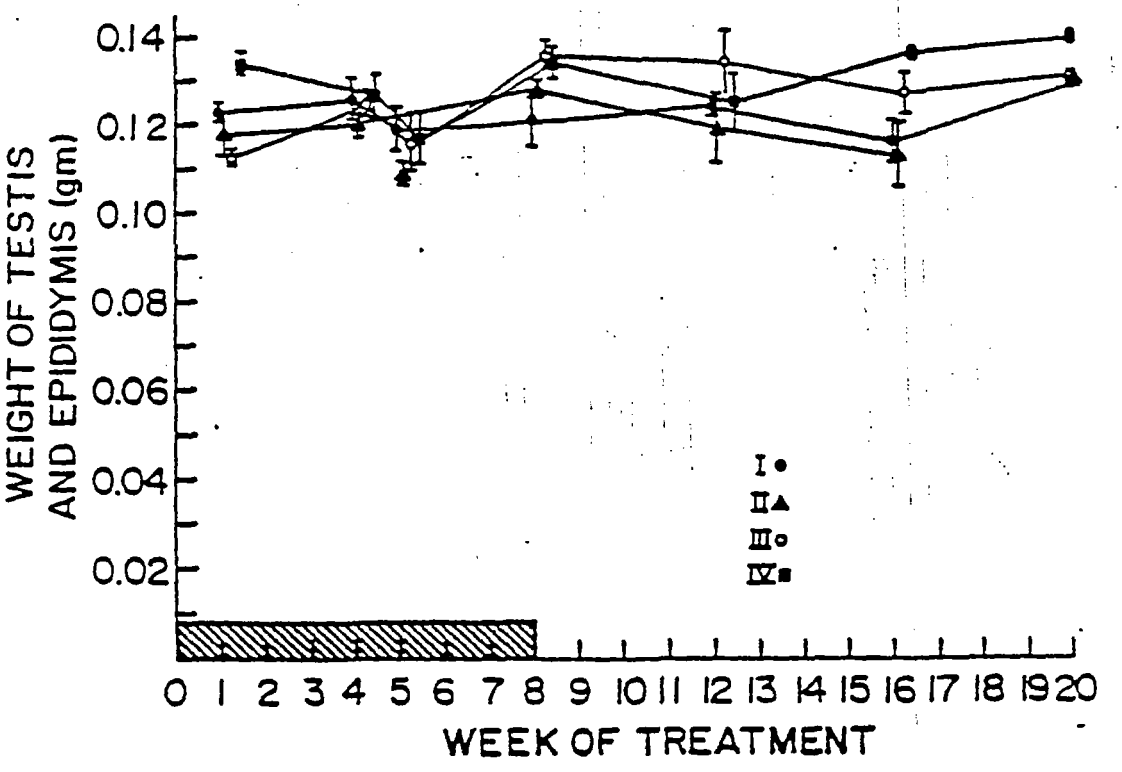


Figure 7. Average testis and epididymis weight was not significantly affected by treatment. Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

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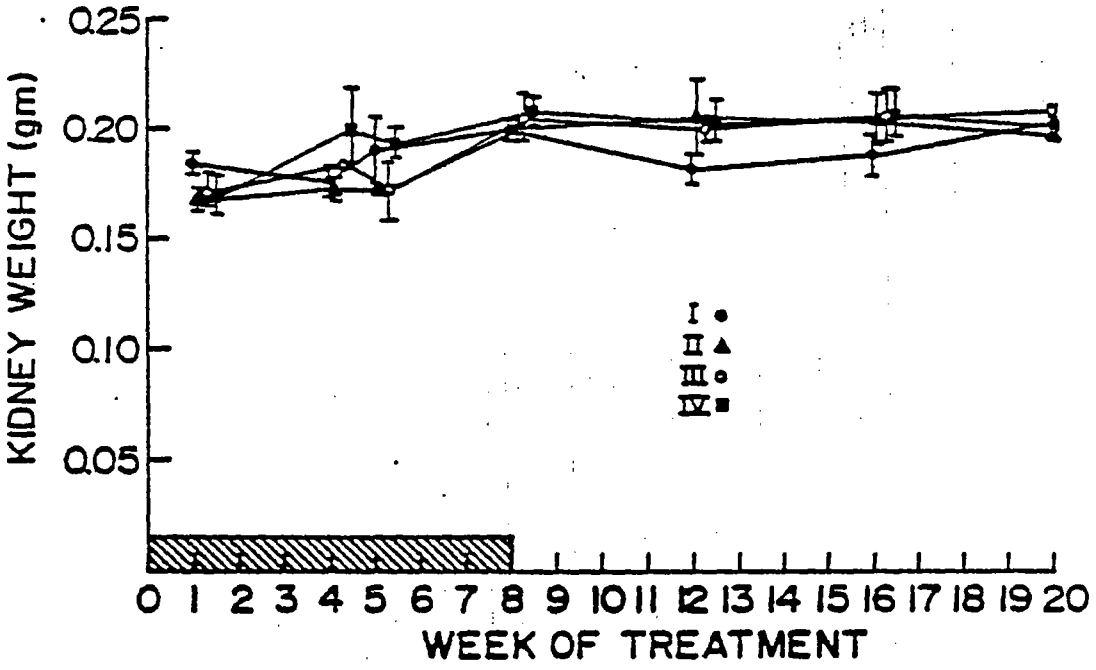


Figure 8. Average kidney weight was not significantly changed by treatment. Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

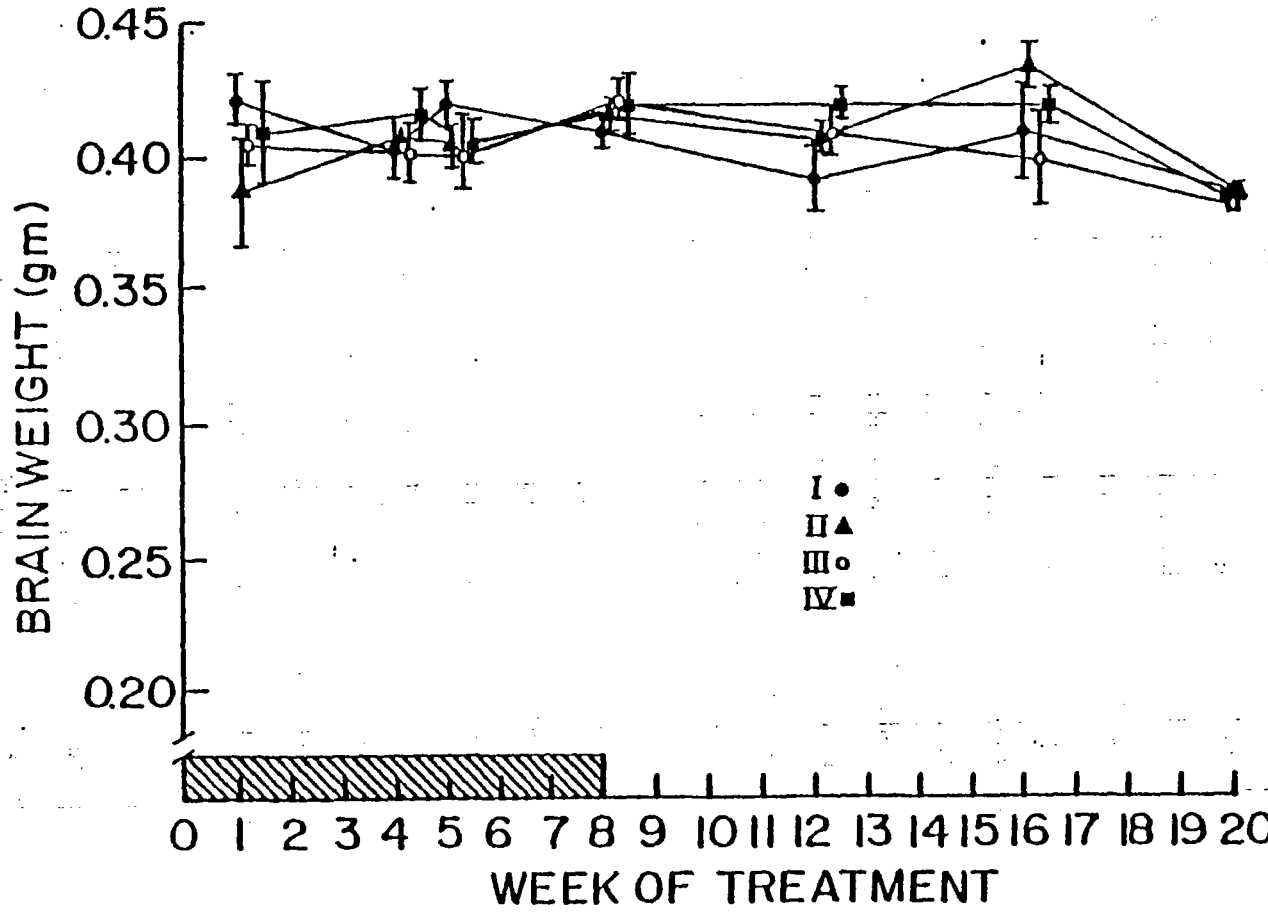


Figure 9. Average brain weight was not significantly altered by chemical treatment. Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

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Fertility

The average percent matings and overall fertility of the males through 8 weeks of the study are given in Table 4. There was a significant reduction in the mating frequency in males from group III (80 mg/kg/day phenoxy acid, 0.16 µg/kg/day TCDD). This effect was not significant in Group II whose phenoxy acid exposure was similar and whose TCDD exposure was 15-fold greater. Therefore, no dose-related effect could be attributed to this decrease. Also, the percent fertile matings and total fertility were not significantly reduced in Group III or in any group when compared to the control.

When fertility was evaluated on a week by week basis, no treatment-related changes were observed. Fertility was also studied on an individual per male basis and no significant changes were detected within treated males as compared to controls. At the conclusion of the study sperm concentration and motility and percent abnormal sperm were measured (week 20, Figures 10-12). These values were analyzed, on an individual male basis, to determine whether any correlation existed between low fertility performance (plug frequency, percent fertile matings and total fertility) and low values for sperm quality (concentration, motility, percent abnormal). In all groups there was no correlation between the parameters measured. This would indicate that, even though there was considerable variability within these parameters, variations in fertility could not generally be attributed to specific changes in sperm quality. The values for sperm concentration (Figure 10) and sperm motility (Figure 11) fluctuated considerably from week to week. Percent abnormal sperm were less variable (Figure 12). No treatment-related changes were observed in these parameters. The marked reductions in sperm concentration (Figure 10) and increase in sperm motility (Figure 11) which were

DOW 1136224

TABLE 4

Fertility and Mating Efficiency in Treated and Control C57Bl/6 Mice
8 Week Total

Treatment Group[1]	Mating[2] Frequency	Fertile Matings (percent)[3]	Total[4] Fertility (percent)
I(Control)	74.6 ± 1.6	56.2 ± 2.2	42.0 ± 1.9
II(80;2.4)	70.3 ± 2.4	58.3 ± 2.9	41.0 ± 2.6
III(80;0.16)	67.8 ± 2.4*	55.3 ± 2.3	37.7 ± 2.2
IV(40;1.2)	73.0 ± 2.0	60.8 ± 2.9	44.2 ± 2.3

[1]Calculated daily exposure is given in parentheses as total mg phenoxy acids/kg/day; μg TCDD/kg/day
[2]Percent plugs observed/total females housed with males.
[3]Percent fertile matings/females with plugs.
[4]Percent fertile matings/total females housed with males.
*P<.05 relative to controls

Values are mean ± standard error of the mean, n = 25 per group.

17879

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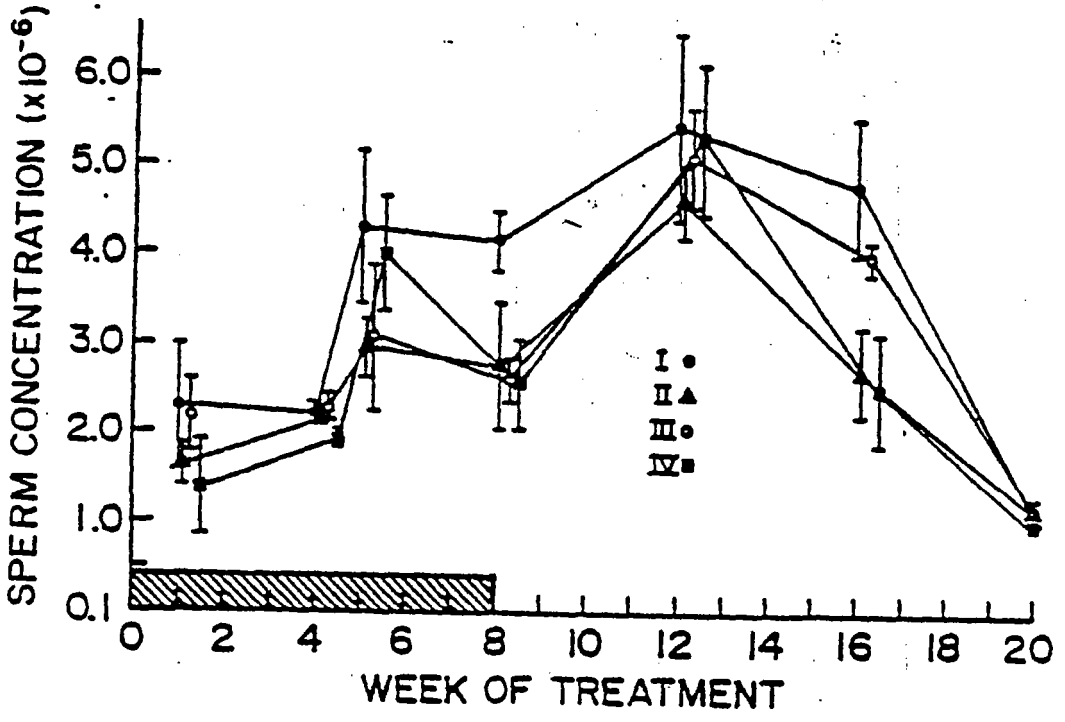


Figure 10. Average sperm concentration. Sperm concentration was quite variable and the only significant reduction relative to controls was in week 16 ($p<.05$, Group II; $p<.01$, Group IV). The marked reduction in all groups at 20 weeks may have been related to the mating of those animals. Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

17890

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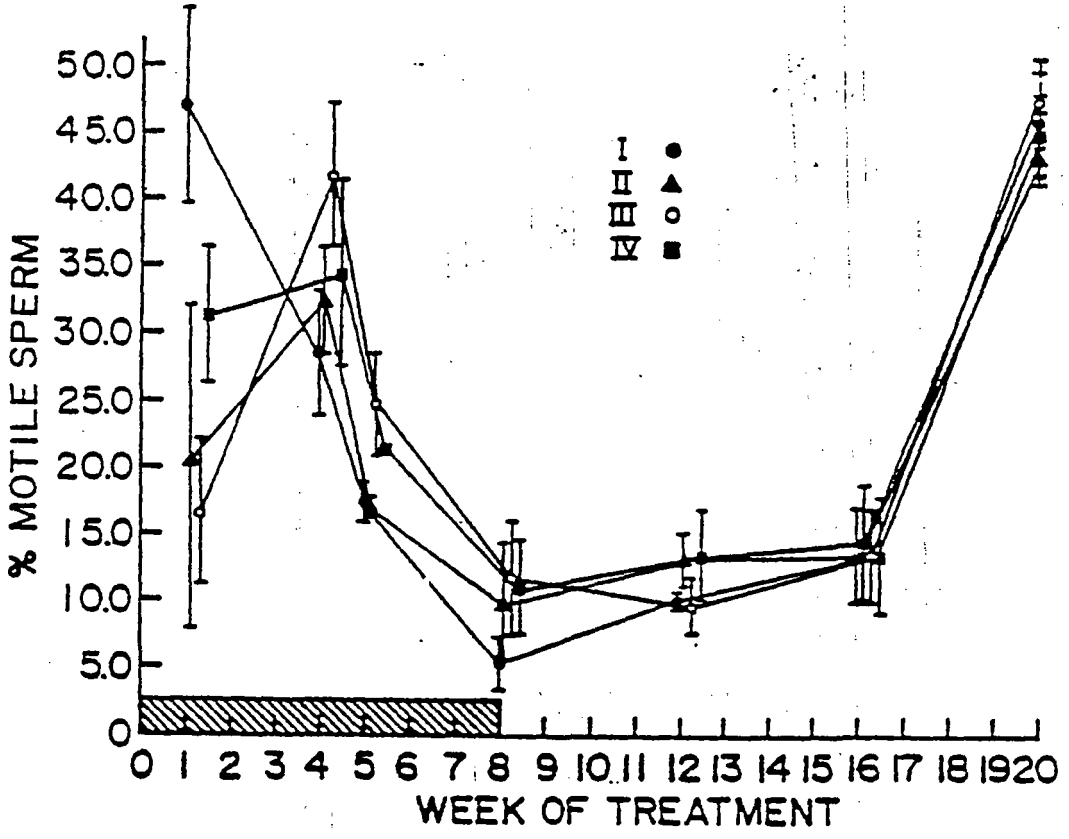


Figure 11. Average percent motile sperm. No significant change was observed in the percent motile sperm ($p<0.05$) when treated and control values were compared. The drop in motility seen from week 4 to week 16 may have resulted from not breeding those males; in contrast, the week 20 (mated) values were much higher. Values are mean $\pm$ S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

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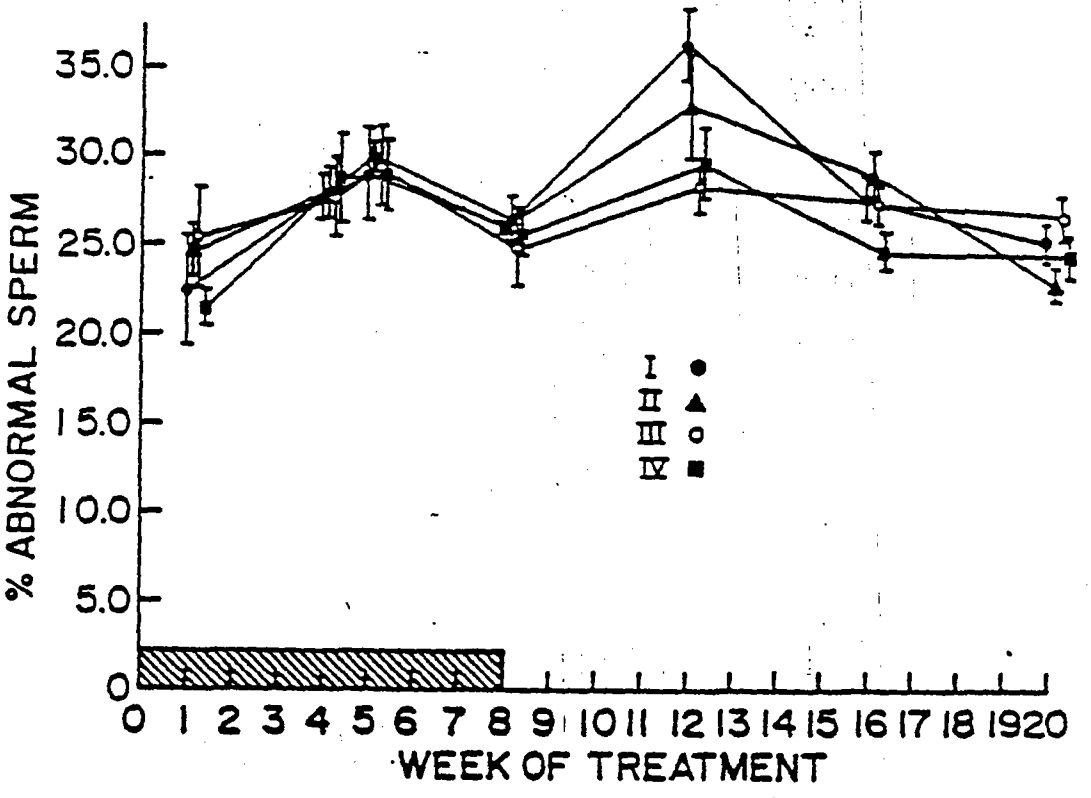


Figure 12. Average percent abnormal sperm. Treatment had no significant influence on the percent abnormal sperm ($p<0.05$). Values are mean ± S.E.M., n = 4 per group in weeks 1-16, n = 25 per group in week 20.

17088

observed from weeks 16 to 20 of the study might be at least partly explained by the fact that the males whose sperm were checked on week 20 had been through an 8-week intensive mating program whereas the males monitored in the earlier weeks were virgins.

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Teratological Examinations

The results of the teratology examination of the dams mated with treated or control males for each group by week are detailed in Tables 5-8. A comparison of the tables indicates that the average number of implants per litter, average number of resorptions per litter or average number live fetuses per litter (Tables 5-8) were unaffected by the male's chemical exposures. For example, the mean values for control and Group II (most heavily exposed) were 7.1 vs 7.4 implant sites per litter; also in Groups I and II there were 4.9 vs 5.0 live fetuses per litter and 2.18 vs 2.37 resorptions per litter. The average fetal weight was significantly ($p<0.05$) greater in all treatment groups as compared to controls. The total number of dead fetuses (i.e., offspring which weighed more than 0.3 gm; offspring weighing <0.3 gm were listed as a resorptions) was 1 in Group I, 2 in Group II, 1 in Group III and 0 in Group IV for the entire 8 week study. The ratio of male to female fetuses was also determined; no treatment group exhibited any significant change ($p<0.10$) in this ratio as compared to the control.

Congenital malformations were not significantly increased in the offspring of treated versus control males (Tables 5-10). Visceral malformations were observed with less frequency than external and skeletal malformations in all groups. The incidences of the most frequently observed malformations are summarized in Table 9: eye defects (anophthalmia and microphthalmia), jaw anomalies (agnathia, micrognathia), were observed in 1.4 to 2.4 percent and 1.2 to 1.6 percent of the fetuses,

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Table 5

Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development
Group I Control

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Number of females examined	19	22	22	25	23	19	22	19	171
Maternal weight gain	13.1 ± 1.0	12.8 ± 0.7	12.7 ± 0.7	12.0 ± 0.8	10.0 ± 0.7	10.1 ± 0.7	11.4 ± 0.8	12.1 ± 0.8	11.8 ± 0.3
Number of implants per litter	8.1 ± 0.5	7.7 ± 0.4	8.0 ± 0.4	7.4 ± 0.6	5.4 ± 0.5	5.8 ± 0.4	7.2 ± 0.5	6.6 ± 0.6	7.1 ± 0.2
Number of resorptions per litter	2.16 ± 0.38	2.41 ± 0.32	2.18 ± 0.32	2.16 ± 0.25	2.39 ± 0.25	2.16 ± 0.33	2.27 ± 0.3	1.68 ± 0.30	2.18 ± 0.11
Number of live fetuses per litter	5.9 ± 0.7	5.3 ± 0.5	5.9 ± 0.5	5.2 ± 0.6	3.0 ± 0.5	3.7 ± 0.4	4.9 ± 0.5	4.9 ± 0.6	4.9 ± 0.2
Average fetal weight per litter	1.04 ± 0.03	1.06 ± 0.02	1.05 ± 0.03	1.00 ± 0.02	1.03 ± 0.02	1.04 ± 0.03	0.99 ± 0.03	1.01 ± 0.03	1.03 ± 0.01
Male/female	59/52	47/68	63/64	69/60	38/32	32/38	52/53	42/52	404/419
Visceral Malformations:									
Number of fetuses examined	60	62	68	73	39	39	62	52	455
Number with visceral malformations	0	0	0	1	1	0	0	0	2
Skeletal and External Malformations:									
Number of fetuses examined	113	117	129	129	70	70	108	94	830
Number with malformations	2	3	4	4	4	0	4	5	26

Values are mean ± standard error of the mean.

Table 6

Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development
Group II

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Number of females examined	19	18	21	17	22	20	17	14	148
Maternal weight gain	14.1 ± 0.5	12.7 ± 0.9	12.2 ± 0.8	11.5 ± 1.0	10.8 ± 0.7	10.4 ± 0.9	12.6 ± 0.9	12.0 ± 1.0	12.0 ± 0.3
Number of implants per litter	9.2 ± 0.4	7.4 ± 0.6	8.2 ± 0.4	6.2 ± 0.6	7.1 ± 0.4	6.2 ± 0.5	7.5 ± 0.4	7.0 ± 0.7	7.4 ± 0.2
Number of resorptions per litter	2.37 ± 0.27	1.94 ± 0.37	2.71 ± 0.40	1.76 ± 0.22	2.55 ± 0.36	2.30 ± 0.45	1.88 ± 0.38	3.43 ± 0.71	2.37 ± 0.14
Number of live fetuses per litter	6.8 ± 0.3	5.5 ± 0.7	5.6 ± 0.5	4.5 ± 0.7	4.5 ± 0.4	3.9 ± 0.6	5.6 ± 0.6	3.6 ± 0.7	5.0 ± 0.2
Average fetal weight per litter	1.00 ± 0.02	1.14 ± 0.03	1.08 ± 0.03	1.09 ± 0.02	1.02 ± 0.03	1.11 ± 0.03	1.02 ± 0.02	1.10 ± 0.03	1.08 ± 0.01
Male/female	62/67	39/60	61/48	36/39	45/54	43/34	42/54	28/20	356/376
Visceral Malformations:									
Number of fetuses examined	66	53	61	42	54	42	47	28	393
Number with visceral malformations	0	0	0	0	0	0	2	0	2
Skeletal and External Malformations:									
Number of fetuses examined	129	99	115	78	100	77	96	50	744
Number with malformations	2	6	4	0	5	1	7	1	26

Values are mean ± standard error of the mean.

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Table 7

Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development
Group III

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Number of females examined	18	15	21	17	19	22	21	12	145
Maternal weight gain	12.9 ± 0.9	12.9 ± 0.9	11.9 ± 0.7	12.0 ± 0.9	10.7 ± 0.8	10.9 ± 0.8	10.2 ± 0.6	12.2 ± 1.1	11.6 ± 0.3
Number of implants per litter	8.4 ± 0.6	7.6 ± 0.5	7.5 ± 0.4	6.6 ± 0.6	7.2 ± 0.5	6.6 ± 0.6	6.8 ± 0.4	7.0 ± 0.6	7.2 ± 0.2
Number of resorptions per litter	2.65 ± 0.58	2.27 ± 0.44	2.14 ± 0.35	1.65 ± 0.28	2.84 ± 0.37	2.14 ± 0.34	2.05 ± 0.30	2.33 ± 0.72	2.26 ± 0.1
Number of live fetuses per litter	5.7 ± 0.7	5.4 ± 0.6	5.3 ± 0.5	5.0 ± 0.5	4.3 ± 0.4	4.5 ± 0.5	4.8 ± 0.4	4.7 ± 0.7	4.9 ± 0.2
Average fetal weight per litter	1.06 ± 0.03	1.11 ± 0.02	1.15 ± 0.02	1.09 ± 0.03	1.09 ± 0.02	1.05 ± 0.03	1.00 ± 0.02	1.09 ± 0.03	1.08 ± 0.01
Male/female	47/50	41/39	58/53	33/50	46/35	51/47	51/49	29/26	356/349
Visceral Malformations:									
Number of fetuses examined	53	44	60	44	47	58	56	32	394
Number with visceral malformations	1	3	0	0	0	0	0	1	5
Skeletal and External Malformations:									
Number of fetuses examined	97	81	112	85	82	98	100	56	711
Number with malformations	2	1	4	2	5	0	5	1	20

Values are mean ± standard error of the mean.

Table 8

Effect of 2,4-D, 2,4,5-T and TCDD on Fetal Development
Group IV

	Week of Study								Total 8 week
	1	2	3	4	5	6	7	8	
Number of females examined	18	23	20	24	21	21	18	19	164
Maternal weight gain	11.6 ± 0.8	12.9 ± 0.8	11.5 ± 0.7	12.2 ± 0.8	9.9 ± 0.8	9.9 ± 0.8	11.9 ± 0.8	11.9 ± 0.9	11.5 ± 0
Number of implants per litter	8.9 ± 0.7	7.3 ± 0.6	7.0 ± 0.6	7.6 ± 0.5	6.0 ± 0.5	6.5 ± 0.5	7.5 ± 0.6	5.9 ± 0.7	7.0 ± 0.
Number of resorptions per litter	4.00 ± 0.50	1.77 ± 0.29	1.30 ± 0.28	1.88 ± 0.30	1.95 ± 0.37	2.43 ± 0.35	2.39 ± 0.48	1.58 ± 0.30	2.11 ± 0
Number of live fetuses per litter	4.9 ± 0.6	5.6 ± 0.6	5.7 ± 0.5	5.8 ± 0.6	4.1 ± 0.5	4.1 ± 0.4	5.1 ± 0.6	4.3 ± 0.7	5.0 ± 0.
Average fetal weight per litter	1.12 ± 0.04	1.16 ± 0.03	1.09 ± 0.02	1.11 ± 0.02	1.10 ± 0.03	1.07 ± 0.03	1.05 ± 0.03	1.07 ± 0.03	1.10 ± 0
Male/female	33/45	59/69	58/54	63/75	45/41	40/46	42/46	36/44	376/420
Visceral Malformations:									
Number of fetuses examined	41	67	62	77	50	50	50	46	443
Number with visceral malformations	0	2	1	1	1	0	0	0	5
Skeletal and External Malformations:									
Number of fetuses examined	78	129	113	139	86	86	92	82	805
Number with malformations	2	5	5	3	2	0	4	4	25

Values are mean ± standard error of the mean.

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respectively. Cleft palate and heart or major blood vessel anomalies were observed in all groups in somewhat lower incidences (Table 9). The total percent malformed fetuses (Table 10) ranged from 3.1 to 3.6 percent and the weekly percentage did not show any treatment-related increases in congenital malformations. A comprehensive listing of malformations observed during the study has been included (Appendix Table 1).

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Postnatal Litter Examinations

When females were allowed to carry their litters to term, the survival and development of their offspring were studied. The number of live pups and their mean body weight were compared in treated and control offspring (Tables 11-14). The lack of a toxic effect is graphically demonstrated by comparing values for control (Group I) animals to those for the animals exposed to the highest dose of phenoxy acid and TCDD (Group II). There was a marked reduction in the number of litters from day 0 to day 4 in all groups. In group I the number of litters fell from 80 to 44 and in group II from 90 to 60. This was accounted for in all groups by cannabalism by the mothers. After day 4, the loss of litters was greatly decreased. Other parameters show little difference between Groups I and II; on day 0, the number of live pups per litter were 4.40 and 4.19, number of dead pups per litter was 0.92 in both groups, and the average pup weights were 1.37 and 1.39. At day 21 the number of live pups per litter were 5.15 and 4.59 and the average pup weights were 7.48 and 7.60, respectively, for groups I and II. No effect on postnatal viability or growth could be attributed to the exposure of the adult male mice to phenoxy acids or to TCDD.

The pups were examined externally on day 0 for malformations (Tables 15 and 16). During the entire eight weeks the total malformation

17888

TABLE 9

Summary of Most Frequently Occurring Defects in
Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD

	Treatment Group			
	I	II	III	IV
Anopthalmia/ micropthalmia	1.4(12/830)	1.9(14/744)	2.0(14/711)	2.4(19/805)
Agnathia/micrognathia	1.3(11/830)	1.2(9/744)	1.4(10/711)	1.6(13/805)
Cleft Palate	0.6(5/830)	0.7(5/744)	0.7(5/711)	0.7(6/805)
Heart/Vessels Anomalies	0.2(1/455)	0.5(2/393)	1.0(4/394)	0.7(3/443)

Values are percent incidence of specific anomalies; no. specific malformations per no. observed is given in parentheses.

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TABLE 10

Percent Malformed Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD

Week	Treatment Group I	II	III	IV
1	1.8(2/113)	1.6(2/129)	3.1(3/97)	2.6(2/78)
2	2.6(3/117)	6.1(6/99)	3.7(3/81)	5.4(7/129)
3	3.1(4/129)	3.5(4/115)	3.6(4/112)	5.3(6/113)
4	3.1(4/129)	0.0(0/78)	2.4(2/85)	2.2(3/139)
5	5.7(4/70)	5.0(5/100)	6.1(5/82)	3.5(3/86)
6	0.0(0/70)	1.3(1/77)	0.0(0/98)	0.0(0/86)
7	3.7(4/108)	8.3(8/96)	5.0(5/100)	4.3(4/92)
8	5.3(5/94)	2.0(1/50)	1.8(1/56)	4.9(4/82)
Total	3.1(26/830)	3.6(27/744)	3.2(23/711)	3.6(29/805)

Values are percent malformed fetuses, number malformed per number observed is given in parentheses.

No values are significantly different from controls ($p<0.05$).

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Table 11

Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males
Group I Control

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Day 0:									
Number of litters	17	15	15	10	5	10	5	3	80
Number live pups per litter	3.94 ± 0.70	4.67 ± 0.80	6.00 ± 0.62	2.60 ± 0.85	4.00 ± 1.10	3.70 ± 0.91	5.40 ± 0.51	5.00 ± 2.51	4.40 ± 0.32
Number dead pups per litter	1.47 ± 0.43	1.33 ± 0.45	0.20 ± 0.20	1.10 ± 0.41	1.00 ± 0.78	0.44 ± 0.18	0.20 ± 0.20	1.33 ± 1.33	0.92 ± 0.16
Average pup weight	1.39 ± 0.03	1.34 ± 0.02	1.37 ± 0.48	1.41 ± 0.02	1.36 ± 0.04	1.38 ± 0.05	1.33 ± 0.03	1.30 ± 0.03	1.37 ± 0.01
Day 4:									
Number of litters	8	5	10	4	4	6	5	2	44
Number live pups per litter	5.00 ± 1.20	6.60 ± 0.93	5.30 ± 0.75	5.50 ± 0.50	4.75 ± 0.75	4.00 ± 0.78	5.40 ± 0.51	7.50 ± 0.50	5.30 ± 0.33
Average pup weight	1.87 ± 0.10	2.22 ± 0.19	2.04 ± 0.15	2.43 ± 0.10	2.71 ± 0.24	2.32 ± 0.28	2.35 ± 0.19	2.25 ± 0.29	2.22 ± 0.07
Day 7:									
Number of litters	8	5	9	4	4	5	5	2	42
Number live pups per litter	4.29 ± 0.99	6.60 ± 0.93	5.78 ± 0.64	5.50 ± 0.50	4.75 ± 0.75	4.00 ± 0.95	5.40 ± 0.51	7.50 ± 0.50	5.32 ± 0.31
Average pup weight	3.13 ± 0.29	3.64 ± 0.33	3.60 ± 0.19	4.07 ± 0.18	4.71 ± 0.39	4.67 ± 0.26	4.03 ± 0.24	3.59 ± 0.35	3.83 ± 0.12
Day 21:									
Number of litters	8	4	9	4	4	5	5	2	41
Number live pups per litter	4.75 ± 0.98	6.00 ± 0.91	5.78 ± 0.64	5.25 ± 0.63	3.50 ± 1.04	4.00 ± 0.95	5.40 ± 0.51	7.50 ± 0.50	5.15 ± 0.32
Average pup weight	6.43 ± 0.51	7.02 ± 0.75	7.34 ± 0.32	8.74 ± 0.21	8.62 ± 0.54	7.71 ± 0.65	8.07 ± 0.51	6.41 ± 0.12	7.48 ± 0.21

Values are mean ± standard error of the mean.

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Table 12

Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males
Group II

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Day 0:									
Number of litters	17	16	14	5	16	11	6	5	90
Number live pups per litter	4.76 ± 0.53	4.88 ± 0.68	5.29 ± 0.74	2.80 ± 1.20	3.55 ± 0.59	1.55 ± 0.74	3.67 ± 0.92	4.00 ± 1.41	4.19 ± 0.29
Number dead pups per litter	0.88 ± 0.40	1.06 ± 0.30	0.64 ± 0.27	1.00 ± 0.78	0.79 ± 0.26	1.25 ± 0.45	1.33 ± 0.80	1.00 ± 0.45	0.92 ± 0.14
Average pup weight	1.39 ± 0.03	1.39 ± 0.04	1.33 ± 0.02	1.47 ± 0.05	1.42 ± 0.03	1.42 ± 0.09	1.38 ± 0.07	1.36 ± 0.04	1.39 ± 0.01
Day 4:									
Number of litters	11	11	11	3	14	3	3	4	60
Number live pups per litter	4.63 ± 0.46	5.25 ± 0.56	5.36 ± 0.61	4.67 ± 0.67	4.64 ± 0.58	4.50 ± 0.50	3.67 ± 0.33	4.25 ± 1.44	4.79 ± 0.25
Average pup weight	2.21 ± 0.11	2.30 ± 0.14	2.11 ± 0.06	2.59 ± 0.19	2.47 ± 0.10	2.44 ± 0.18	2.36 ± 0.18	2.47 ± 0.21	2.33 ± 0.05
Day 7:									
Number of litters	11	11	11	3	14	3	3	4	60
Number live pups per litter	4.20 ± 0.44	4.73 ± 0.65	5.36 ± 0.61	4.67 ± 0.67	4.64 ± 0.58	3.67 ± 0.88	3.33 ± 0.68	4.25 ± 1.44	4.58 ± 0.25
Average pup weight	3.61 ± 0.19	3.65 ± 0.25	3.58 ± 0.10	4.12 ± 0.29	4.08 ± 0.22	3.03 ± 0.90	4.19 ± 0.21	4.04 ± 0.24	3.79 ± 0.09
Day 21:									
Number of litters	10	10	10	3	13	3	3	4	56
Number live pups per litter	4.00 ± 0.49	5.00 ± 0.58	5.10 ± 0.64	4.67 ± 0.67	4.92 ± 0.55	3.67 ± 0.88	3.33 ± 0.67	4.25 ± 1.44	4.59 ± 0.25
Average pup weight	7.28 ± 0.48	7.71 ± 0.24	7.20 ± 0.39	8.11 ± 0.45	8.43 ± 0.21	5.30 ± 0.14	8.43 ± 0.11	7.58 ± 0.52	7.60 ± 0.16

Values are mean ± standard error of the mean.

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Table 13

Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males
Group III

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Day 0:									
Number of litters	14	11	12	9	10	11	11	3	81
Number live pups per litter	5.64 ± 0.52	3.91 ± 1.01	5.67 ± 0.85	6.11 ± 1.03	3.80 ± 0.80	2.73 ± 0.75	3.82 ± 0.89	1.67 ± 1.67	4.44 ± 0.32
Number dead pups per litter	0.57 ± 0.34	1.55 ± 0.43	0.27 ± 0.14	0.11 ± 0.11	0.60 ± 0.34	0.50 ± 0.27	0.90 ± 0.41	1.00 ± 0.58	0.67 ± 0.13
Average pup weight	1.32 ± 0.02	1.39 ± 0.06	1.37 ± 0.27	1.36 ± 0.04	1.32 ± 0.06	1.39 ± 0.05	1.37 ± 0.02	1.38	1.36 ± 0.01
Day 4:									
Number of litters	12	3	8	4	8	5	7	1	48
Number live pups per litter	5.30 ± 0.47	4.33 ± 0.67	5.25 ± 0.10	5.25 ± 0.75	4.25 ± 0.49	4.60 ± 0.40	5.57 ± 0.53	5.00	4.91 ± 0.25
Average pup weight	2.03 ± 0.09	1.76 ± 0.89	2.19 ± 0.16	2.25 ± 0.16	2.50 ± 0.10	2.79 ± 0.08	2.73 ± 0.07	2.81	2.37 ± 0.06
Day 7:									
Number of litters	12	3	8	4	8	5	7	1	48
Number live pups per litter	5.00 ± 0.44	4.33 ± 0.67	5.13 ± 1.04	5.25 ± 0.75	4.25 ± 0.49	4.60 ± 0.40	5.57 ± 0.53	5.00	4.92 ± 0.24
Average pup weight	3.25 ± 0.21	2.79 ± 0.17	3.52 ± 0.93	3.67 ± 0.23	4.16 ± 0.13	4.67 ± 0.14	4.54 ± 0.13	4.66	3.82 ± 0.12
Day 21:									
Number of litters	12	3	7	4	8	5	6	1	46
Number live pups per litter	5.00 ± 0.44	4.00 ± 0.58	5.43 ± 0.97	5.00 ± 0.71	4.25 ± 0.49	4.60 ± 0.40	5.50 ± 0.62	4.00	4.87 ± 0.23
Average pup weight	6.83 ± 0.22	7.43 ± 0.64	7.12 ± 0.37	8.39 ± 0.25	8.15 ± 0.18	7.66 ± 0.24	8.59 ± 0.28	10.31	7.67 ± 0.15

Values are mean ± standard error of the mean.

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Table 14

Effect of 2,4-D, 2,4,5-T and TCDD on Postnatal Development of Offspring of Treated Males
Group IV

	Week of Study								Total 8 weeks
	1	2	3	4	5	6	7	8	
Day 0:									
Number of litters	19	23	14	11	15	10	11	5	108
Number live pups per litter	4.84 ± 0.64	5.35 ± 0.68	5.29 ± 0.83	3.82 ± 1.06	3.67 ± 0.56	2.60 ± 1.19	4.45 ± 0.71	3.80 ± 1.66	4.41 ± 0.29
Number dead pups per litter	0.95 ± 0.29	0.74 ± 0.20	0.79 ± 0.38	1.09 ± 0.32	0.79 ± 0.28	1.50 ± 0.50	0.27 ± 0.14	0.80 ± 0.49	0.84 ± 0.11
Average pup weight	1.36 ± 0.02	1.36 ± 0.03	1.39 ± 0.03	1.44 ± 0.05	1.39 ± 0.03	1.43 ± 0.05	1.38 ± 0.03	1.43 ± 0.06	1.39 ± 0.01
Day 4:									
Number of litters	10	10	8	4	9	4	8	3	56
Number live pups per litter	5.57 ± 0.43	5.40 ± 0.67	6.00 ± 0.66	6.25 ± 1.03	3.33 ± 0.50	5.75 ± 1.49	4.88 ± 0.55	4.67 ± 0.88	5.13 ± 0.27
Average pup weight	2.24 ± 0.16	2.41 ± 0.08	2.60 ± 0.97	2.30 ± 0.13	2.40 ± 0.19	2.94 ± 0.50	2.54 ± 0.16	2.38 ± 0.28	2.41 ± 0.08
Day 7:									
Number of litters	10	10	8	4	9	4	8	3	56
Number live pups per litter	5.00 ± 0.47	5.40 ± 0.67	6.00 ± 0.66	6.00 ± 1.08	3.33 ± 0.50	5.75 ± 1.49	4.88 ± 0.55	4.67 ± 0.88	5.04 ± 0.26
Average pup weight	3.28 ± 0.31	4.08 ± 0.15	4.06 ± 0.18	3.88 ± 0.24	3.98 ± 0.41	3.80 ± 0.33	4.24 ± 0.25	3.99 ± 0.46	3.94 ± 0.10
Day 21:									
Number of litters	10	10	8	4	9	4	8	3	56
Number live pups per litter	5.00 ± 0.47	5.30 ± 0.63	5.75 ± 0.59	6.00 ± 1.08	3.33 ± 0.50	5.75 ± 1.49	4.75 ± 0.59	4.67 ± 0.88	4.96 ± 0.25
Average pup weight	6.99 ± 0.34	8.15 ± 0.33	7.53 ± 0.40	8.20 ± 0.09	8.53 ± 0.68	6.66 ± 0.74	8.34 ± 0.37	7.38 ± 0.97	7.80 ± 0.18

Values are mean ± standard error of the mean.

TABLE 15

Summary of Malformation Rates (%) in Postnacal Study[1]

Week	Treatment Group I	II	III	IV
1	5.4(5/92)	2.1(2/96)	1.1(1/87)	1.9(2/105)
2	2.2(2/90)	3.2(3/95)	5.0(3/60)	0.0(0/140)
3	1.1(1/93)	6.0(5/83)	6.6(5/76)	2.4(2/85)
4	0.0(0/37)	5.3(1/19)	8.9(5/56)	13.0(7/54)
5	4.0(1/25)	1.1(1/88)	4.5(2/44)	2.7(2/73)
6	2.2(1/45)	0.0(0/32)	2.1(1/48)	2.1(1/47)
7	7.1(2/28)	13.3(4/30)	3.8(2/53)	3.8(2/52)
8	5.3(1/19)	8.0(2/25)	0.0(0/8)	4.3(1/23)
Total	3.0(13/429)	3.8(18/468)	4.4(19/432)	2.9(17/579)

[1] All fetuses (live or dead) were assumed to be at risk.

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TABLE 16

Summary of Specific Malformations in Postnatal Study

	I	II	III	IV
Anophthalmia/ microphthalmia	1.9(8/429)	2.6(12/468)	2.5(11/432)	1.7(10/579)
Agnathia/micrognathia	1.4(6/429)	1.3(6/468)	1.9(8/432)	1.4(8/579)
Cleft Lip/palate	0.2(1/429)	0.4(2/468)	0.0(0/432)	0.0(0/579)
All else	0.0(0/429)	0.0(0/468)	0.5(2/432)[a]	0.0(0/579)

[a]One exencephaly; one clubbed right hind limb.

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rate in these mice was between 2.9 and 4.4 percent. The only individual values which approach statistical significance are the malformation rates for week four group IV (40 mg/kg/day phenoxy acid, 1.2 µg/kg/day TCDD) (Table 15) versus control. In that case the control animals had no malformations and the treated had 13% malformations; all of these were either eye or jaw anomalies.

As seen in the prenatal evaluations, eye and jaw malformations accounted for the majority of the defects noted (Table 16). Anopthalmia or micropthalmia were seen in 1.7 to 2.6 percent of the pups and agnathia or micrognathia were seen in 1.3 to 1.9 percent of the pups for all treatment groups.

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DISCUSSION

In these studies we have evaluated the toxicity of three mixtures of 2,4-D, 2,4,5-T and TCDD on reproduction in male mice. Mating frequency, fertility, germ cell mutagenesis, and fetal or postnatal development were all considered in the selection of toxicological endpoints. Male mice were continuously exposed to high doses of 2,4-D plus 2,4,5-T mixed with 2 or 30 ppm TCDD in the phenoxy acid for 8 weeks. These TCDD levels represent average and high contamination levels within Herbicide Orange used in Vietnam (Young et al., 1978).

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This study employed the free acids of 2,4-D and 2,4,5-T rather than the butyl esters which were components of Herbicide Orange because of the lower volatility of the acids. The esters are rapidly metabolized to the free acid in both plants and animals, and therefore, the systemic toxicity can be attributed to the free acid (Gehring and Betso, 1978) and should be comparable on a molar basis. The dose levels employed exhibited moderate to low direct toxicity in exposed male mice as evidenced by decreased body weight gain, changes in thymus and liver weights and morphologic changes in the liver. The mortality, however, was quite low. The severity of these toxic effects appeared to be primarily related to the TCDD content of the simulated "Herbicide Orange" mixture.

Despite the use of continuous, moderately toxic, chemical exposures throughout the complete period of spermatogenesis, no significant increase in reproductive abnormalities in the 2,4-D, 2,4,5-T or TCDD exposed groups were observed. TCDD has previously been demonstrated to alter spermatogenesis in C57BL/6 mice (McConnell et al., 1978), however, that study used a single high (lethal range) dose of TCDD. Additionally,

17898

testicular lesions were only found in clinically ill animals, as opposed to those which survived exposure to similar doses (McConnell et al., 1978). Altered spermatogenesis has also been reported in rats (Kociba et al., 1976), guinea pigs (McConnell et al., 1978), and monkeys and chickens (Norback and Allen, 1973), although these were again toxic exposures. In the present study, morphological changes were not observed in the testis of treated mice. Throughout this study testis weight was not affected, nor was sperm motility or percent abnormal spermatozoa. Mean sperm concentration, however, was slightly reduced after five and eight weeks of dosing, although the effect was not statistically significant ($p>0.10$).

The levels of TCDD chosen for this study were within the range that had already been shown to result in cleft palate and kidney anomalies when given to pregnant C57BL/6 female mice (Moore et al., 1973). Mixtures of phenoxy acids plus specific levels of TCDD were used in order to better mimic human exposures to Herbicide Orange. Additionally, previous investigators (Neubert et al., 1973) showed that adding as little as 0.1 µg/kg TCDD to 2,4,5-T increased the teratogenicity in mice of 2,4,5-T in offspring of exposed mothers above that expected by a simple additive effect. The dose of TCDD in this study is at or above the 0.1 µg/kg/day level. Also, it should be emphasized that human exposures involved mixtures of 2,4-D, 2,4,5-T and TCDD (Herbicide Orange).

Certain chemicals, when given to adult males, can cause fetal death or alter normal development in offspring sired by those males (Joffe, 1979; Manson and Simons, 1980), however, such effects were not elicited in the experiments we report by exposing male mice to the 2,4-D, 2,4,5-T and TCDD mixtures. As evidenced by the numbers of implants and resorptions, neither embryo toxicity nor dominant lethal mutations could be

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attributed to exposure to these chemicals. The unaffected values for percent abnormal sperm, which is a test for mutagenicity (Wyrobek, 1979) also leads one to the conclusion that the chemicals, as given, were not mutagenic towards the male germ cells. This correlates well with previous multigeneration studies (Murray et al., 1979) and dominant lethal assays (Khera and Ruddick, 1971).

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The values for percent malformed fetuses also indicated that 2,4-D, 2,4,5-T and TCDD had no influence on the offspring of exposed males. The study was designed such that, if the overall percent malformed fetuses (3%; see Appendix Table 1) was doubled in one of the experimental groups, there was a 90% chance that it would have been detected. There was a 70-80% chance of detecting a four-fold increase in congenital defects in any one week. For any specific malformation or class of malformations the corresponding powers would be somewhat less. For example, for visceral defects (background rate 0.44%; see Table 5) the experiment had approximately a 90% chance of detecting an overall rate as high as 3% in any particular treatment group. The only variation which we could find elevated in treated versus control offspring, in the entire study, was the incidence of fused sternebrae (Appendix Table 2). In that case, we observed a statistically significant ($p<0.05$) increase in fused sternebrae in Group III at week 3 and in Group IV at week 4. The incidence in the controls at those times, however, was unusually low (0 in both weeks 3 and 4). This type of skeletal variation has been described as occurring as often as 5-15% or more in offspring from untreated pregnancies in mice and although it is frequently observed, the incidence is quite variable and this anomaly is considered a variation and not a malformation (Wilson, 1973).

APPENDIX TABLE 1

Summary of All Malformations Observed in Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD

	Treatment Group			
	I	II	III	IV
Visceral Malformations[1]				
Heart/Vessels Anomalies	0.2(1/455)	0.5(2/393)	1.0(4/394)	0.7(3/443)
Kidney Agenesis	0.2(1/455)	0.0(0/393)	0.3(1/394)	0.0(0/443)
Liver-two lobes only	0.0(0/455)	0.0(0/393)	0.0(0/394)	0.2(1/443)
Lung-lobes 2/3 normal size	0.2(1/455)	0.0(0/393)	0.0(0/394)	0.0(0/443)
Right kidney-1/2 normal size	0.0(0/455)	0.0(0/393)	0.0(0/394)	0.2(1/443)
Skeletal and External Malformations[1]				
Anopthalmia/micropthalmia	1.4(12/830)	1.9(14/744)	2.0(14/711)	2.4(19/805)
Agnathia/micrognathia	1.3(11/830)	1.2(9/744)	1.4(10/711)	1.6(13/805)
Cleft palate	0.6(5/830)	0.7(5/744)	0.7(5/711)	0.7(6/805)
Cleft lip/nose	0.0(0/830)	0.0(0/744)	0.3(2/711)	0.0(0/805)
Open eye	0.5(4/830)	0.0(0/744)	0.0(0/711)	0.0(0/805)
Exencaphaly/hydrocephaly	0.2(2/830)	0.4(3/744)	0.1(1/711)	0.2(2/805)
No tongue	0.0(0/830)	0.0(0/744)	0.1(1/711)	0.0(0/805)
Umbilical hernia	0.1(1/830)	0.3(2/744)	0.3(2/711)	0.1(1/805)
Ribs fused/missing	0.1(1/830)	0.3(2/744)	0.0(0/711)	0.0(0/805)
Spinal centra doubled/misaligned	0.1(1/830)	0.0(0/744)	0.1(1/711)	0.0(0/805)
Spinal arches fused	0.0(0/830)	0.0(0/744)	0.1(1/711)	0.0(0/805)
Mandibles fused	0.0(0/830)	0.0(0/744)	0.1(1/711)	0.0(0/805)
Skull bones missing	0.0(0/830)	0.1(1/744)	0.1(1/711)	0.0(0/805)
Eye bones missing	0.0(0/830)	0.1(1/744)	0.0(0/711)	0.0(0/805)
Facial bones fused	0.2(2/830)	0.5(4/744)	0.4(3/711)	0.0(0/805)
Kinked Tail	0.1(1/830)	0.0(0/744)	0.0(0/711)	0.0(0/805)
Total Malformations[2]	5.2(43/830)	5.8(43/744)	6.6(47/711)	5.7(46/805)
Total Malformed Fetuses[2]	3.1(26/830)	3.6(27/744)	3.2(23/711)	3.6(29/805)

[1] Values are percent incidence of specific anomalies; no. specific malformations per no. observations is given in parentheses.

[2] Values are percent incidence of malformations or malformed fetuses; no. malformations or malformed fetuses per no. observations is given in parentheses.

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APPENDIX TABLE 2

Incidence of Fused Sternebrae in Fetuses Sired by Males Treated with 2,4-D, 2,4,5-T and TCDD

Week	Treatment Group I	II	III	IV
1	0.9(1/113)	1.6(2/129)	1.0(1/97)	1.3(1/78)
2	1.7(2/117)	3.0(3/99)	3.7(3/81)	1.6(2/129)
3	0.0(0/129)	0.9(1/115)	7.1(8/112)*	2.7(3/113)
4	0.0(0/129)	2.6(2/78)	2.4(2/85)	5.0(7/139)*
5	1.4(1/70)	2.0(2/100)	3.7(3/82)	2.3(2/86)
6	1.4(1/70)	5.2(4/77)	3.1(3/98)	3.5(3/86)
7	3.7(4/108)	2.1(2/96)	0.0(0/100)	2.2(2/92)
8	2.1(2/94)	2.0(1/50)	0.0(0/56)	1.2(1/82)
Total	1.3(11/830)	2.3(17/744)	2.8(20/711)	2.6(21/805)

*p<.05 vs. controls.

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Certain anomalies are associated with embryotoxicity, not teratogenicity or mutagenicity. However, with chemical exposure only to the males, not pregnant females, embryotoxicity would not be expected in their offspring. If exposure of the embryo directly to the chemicals had occurred, via seminal plasma or sperm, one would expect to observe increased embryotoxicity in the first weeks of the study, when the chemical levels in the body (or ejaculate) were the highest. One would have also anticipated that during the first weeks of the teratology study germ cell toxicity would most likely have been detected, because the spermatozoa that were evaluated in the first week of mating had been exposed to the chemicals throughout all stages of the spermatogenic process. If the spermatogonia had been affected by the exposure, the effect would have been most apparent in the last weeks of the mating, because at that time, we were evaluating spermatozoa which were spermatogonia during the entire 8 week dosing period and only began to proceed through the spermatogenic cycle near the end of chemical exposure.

Thus, there does not appear to be a residual or transient effect of 2,4-D, 2,4,5-T and TCDD at the concentrations in this study, on the fertility of exposed male mice. In addition, exposure to these chemicals did not appear to influence the fetal or neonatal development or the viability of offspring sired by these mice.

DOW 11362248

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17904

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DOW 11362251

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DOW 11362252

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DOW 11362253

ACKNOWLEDGEMENTS

We acknowledge the valuable statistical consultations of Dr. K. Abeywickrama, Dr. B. Gladen and Dr. J. Haseman, the assistance of Dr. E. E. McConnell in evaluating histopathological specimens and editing the manuscript, the technical assistance of Ms. M. L. Dellinger, Ms. D. Frazier and Ms. M. Ross, and Ms. W. Peterson for preparing the manuscript.

We thank Thomas Mangum for his valuable technical support during this study. We also wish to thank Melissa Marr and Patricia Fisher for performing the statistical analyses of the teratological and postnatal data. The technical assistance of Freida Gerling, Betty King, Loretta Langhoff, Burnes Ray, Lynn Smith and Vickie Wilson is gratefully acknowledged.

This study was partially supported by contract number N01-ES-2127 from the National Institute of Environmental Health Sciences and the National Toxicology Program.

DOW 1136254

17909

THE DOW CHEMICAL COMPANY

MIDLAND DIVISION
MIDLAND, MICHIGAN

January 28, 1965

CONFIDENTIAL

DOW 1568535
MN0012332

Herrn Dr. Hans Merz
Director of Production
C. H. Boehringer Sohn
Ingelheim, am Rhein
West Germany

Dear Dr. Merz:

We very much appreciated the opportunity to talk with you yesterday and I have summarized below our understanding of the essential elements of our conversation, as you suggested.

##

At the outset I explained to you that there were four Dow people on three telephones here. I then introduced Mr. Silverstein, Dr. Henry Tolkmith, who would be on hand to handle any necessary translation, and finally, Mr. Dylewski, an engineer concerned with the development of a new process for 2,4,5-Trichlorophenol. We then began with a series of medical questions posed by Mr. Silverstein.

Silverstein:

1. We have isolated and identified a symmetrical and an unsymmetrical isomer of tetrachlorodibenzodioxin. The symmetrical isomer is "2,3,6,7" (German notation) and the unsymmetrical is "2,3,6,8". Limited animal experimentation indicates that the unsymmetrical isomer
   active. Have you any information on the
   vity of these two isomers?
   we stopped research - it was too dangerous".

A147

   ny liver biopsy specimens available for
   study?
   since we have had no new cases for seven
   s.

2. ... liver function tests or kidney function tests used on your chloracne cases been published anywhere? Answer: "Do not know".

4. Did you note any increased incidence of diabetes in your chloracne patients, or did any diabetics with chloracne suffer aggravation of their diabetic condition?
   Answer: "No cases of diabetes were found".

5. Did you study catechol amines or ketosteroids in your chloracne cases?
   Answer: "Do not know".

Dr. Merz said that he would check further into those questions which were answered above as "Do not know".

- - - - - - - - -

Dylewski (Engineer): He explained that we are considering a distilled grade of 2,4,5-Trichlorophenol (2,4,5-T) as part of our product requirement.

Dr. Merz reiterated that Boehringer's management had decided not to distill this material because of the danger of the formation of chloracne inciting materials.

Mr. Dylewski was nevertheless interested to hear more about Boehringer's experience with the distillation and thereupon proceeded to describe his proposed procedure and to invite Dr. Merz's comments.

The procedure involves acidification of the aqueous phenate with either HCl or H_2SO_4 to a pH of 4.5-5.5 followed by separation of the 2,4,5-T oil from the salt brine, washing of the phenolic oil with water, drying by vacuum distillation and finally distilling of the 2,4,5-T without fractionation in a graphite still.

Dr. Merz said that they formerly distilled 2,4,5-T in copper equipment operating with a sump temperature in the range of 130-150°C. Chloracne inciters were formed presumably because of residual cations (Na) in the phenol which produced Na trichlorophenate thus leading to the formation of dioxin compounds. Mr. Dylewski asked if Dr. Merz could name a particular concentration of salt below which it would be safe to operate a distillation. Dr. Merz would not name a tolerable level but suggested that a water extraction could be better affected by use of a retainer solvent such as methylene chloride or chlorobenzol for the 2,4,5-T.

DOW 1 568536

Dr. Trapp asked Dr. Merz to describe how $Ba(OH)_2$ functions to produce a better quality product. Dr. Merz replied that $Ba(OH)_2$ traps carbonate which is an impurity in the caustic.

Dr. Merz was asked if he knew what the material of construction of the reactor autoclave was, and he replied, "Iron, or common steel".

In closing, Dr. Trapp informed Dr. Merz that we had not yet received the secrecy agreement papers from Boehringer and Dr. Merz said that he would check immediately on this because he was certain that they were completed and had assumed that they had been mailed.

##

Wir danken Ihnen nochmals für Ihre weiterer Unterstützung, Dr. Merz.

mit freundlichen grüssen,

Walter B. Trapp
Assistant Director
Benzene Research Laboratory
474 Building

bc: Hamburg Office (Grote/Kube)
G. A. Griess
S. W. Dylewski
I. Silverstein

DOW 1568537

1236

C. H. BOEHRINGER SOHN

CHEMISCHE FABRIK

Postanschrift: C. H. Boehringer Sohn, (24) Ingelheim am Rhein

Ingelheim 7111
bei Selbstwahl 06132-7111
Boehringer Ingelheimrhein
Fernschreiber 4-17777
Alle Codes in Gebrauch
Landeszentralbank Mainz 48/81
Conrad Hinrich Donner, Hamburg 1
Deutsche Bank AG, Filiale Mainz
Deutsche Bank AG, Filiale Frankfurt (M.
Kreissparkasse Ingelheim am Rhein Nr. 3
Postscheckkonto: Frankfurt (Main) Nr. 16

DOW Chemicals Company

Midland / Michigan

U.S.A.

Ihre Zeichen	Ihre Nachricht vom	Unsere Nachricht vom	Unsere Zeichen	
			Dr.Mz/Oe	(24) Ingelheim am Rhein den 15.12.1964

Betrifft

Sehr geehrte Herren,

während eines kürzlichen Besuches Ihrer Herren Dr. Trapp, Lueck und Silverstein erwähnten wir einen wissenschaftlichen Bericht, in welchem die Isolierung des chlorakneaktiven Wirkstoffs sowie die Folgerungen für die betriebliche Arbeitsweise ausführlich beschrieben sind. Herr Silverstein zeigte daraufhin Interesse an der Überlassung eines Exemplars dieses Berichtes.

Bis heute haben wir den Inhalt dieses Berichtes ausserhalb unseres Hauses niemandem zur Kenntnis gegeben, da wir besonderen Wert darauf legten, die ausserordentliche Gefährlichkeit des Tetrachlorbenzodioxin nicht allgemein bekannt werden zu lassen. Da Sie aber den gleichen Wirkstoff aus den Nebenprodukten Ihrer Trichlorphenol-Produktion isolieren konnten, haben wir uns entschlossen, Ihnen diesen Bericht zu überlassen. Wir möchten Sie aber bitten, diesen ebenso wie die übrigen, Ihnen bereits übergebenen Verfahrensunterlagen streng vertraulich zu behandeln und ausserhalb Ihrer Firma niemandem zur Kenntnis zu bringen.

Wir hoffen, dass der beiliegende Bericht (Versuche zur Auffindung des bei der 2,4,5-T-Säure-Herstellung auftretenden Chlorakne-Erregers) vom 12.9.1956 Ihnen bei der Lösung Ihres Chlorakneproblems nützlich sein wird und verbleiben

mit freundlichen Grüssen
C.H. BOEHRINGER SOHN
ppa.

(Dr. Kudszus) (Dr. Merz)

Anlage

17913

December 15, 1964

Dow Chemical Company
Midland, Michigan
U.S.A.

MN0123332 DOW 1568605

Honored Gentlemen:

During a brief visit with your messers. Dr. Trapp, Lueck and Silverstein we mentioned a scientific report, in which the isolation of chloracne active substances as well as conclusions concerning operating procedures are fully described. Mr. Silverstein showed an interest in this and asked for a copy of this report.

Until now we have disclosed the content of this report to no one outside of our company, as we attach a special value thereto, because the extraordinary danger of the tetrachlorobenzodioxin is not generally known. However, since you have isolated the same material from the by-products (oil) of your Trichlorophenol process we have chosen to turn this report over to you. We would ask you, however, that you commit yourselves to a management policy of strict confidence with respect to this information and that you would disclose it to no one outside of your firm.

We hope that the enclosed report (Experiments on the determination (isolation) of chloracne exciters in the production of 2,4,5-T acid) of Dec. 9, 1956 will be of use to you in the solution of your chloracne problem and we remain

with friendly greetings

C. H. BOEHRINGER SOHN

Signed by

Dr. Kudszus Dr. Merz

Attached papers

Translated by: W.B. Trapp

THE DOW CHEMICAL COMPANY
CHEMICAL PHYSICS RESEARCH LABORATORY
1603 BUILDING

PROBLEM # SL 151874

REPORT NO. SL 151874

MN0123320 DOW 1569203

A. J. Erbel 6-15-65

EFFECT OF 1,2,4,5-TETRACHLOROBENZENE PURITY ON FORMATION OF CHLORACNEGENS IN 2 4,5-TRICHLOROPHENOL PROCESS

Three identical experiments were run using different grades of 1,2,4,5-tetrachlorobenzene. The products were analyzed and tested on rabbits to determine if there were any differences in chloracnegenic activity.

TYPE OF REPORT: Research PAGES: 2 STAGE

DISTRIBUTION: ("COMPLETE REPORT)

*MIDLAND CRI (4 Copies)

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17915

FOR USE OF DOW EMPLOYEES ONLY

FORM C-4300 PRINTED IN U.S.A. 57-42

EFFECT OF PURITY OF 1,2,4,5-TETRACHLOROBENZENE ON CHLOROACNIC ACTIVITY IN 2,4,5-TRICHLOROPHENOL PROCESS

PURPOSE:

As a part of the program to identify and minimize the formation of toxic by-products in the 2,4,5-trichlorophenol process, three experiments were run to determine the influence of 1,2,4,5-tetrachlorobenzene purity.

EXPERIMENTAL:

Ten pounds of 1,2,4,5-tetrachlorobenzene were obtained from 636 Building and divided into three portions. One third, designated as 783-74-1 was used as it came from the plant. One third (783-74-2) was distilled and the remaining one third (783-74-3) was distilled and then recrystallized from CCl_4.

These three grades were then hydrolyzed under identical conditions to 2,4,5-trichlorophenol in a one liter stirred Parr reactor as follows:

1 mol (216 g) 1,2,4,5-tetrachlorobenzene
3 mols (138.5 g) 97% Flake NaOH
510 ml of anhydrous methanol

Heated to 180°C for 30 minutes, quenched in an ice bath until no pressure showed on gage, opened and transferred to a still with 500 ml of hot water. The methanol was flashed off and the 2,4,5-trichloro anisole extracted with CCl_4. The aqueous residue then acidified with H_2SO_4 to a pH of 3.5 and the 2,4,5-trichlorophenol extracted with CCl_4.

The CCl_4 was then removed by evaporation to obtain the anisole and phenol fractions. Conversions to 2,4,5-trichlc phenol were uniform, being 88%, 89% and 88%. Tetrachloro dibenzo-p-dioxin, measured by V.P.C. in micrograms per milliliter were 30, 30 and 20 respectively. (Attached report AL-W7-768 Analytical Lab. - 574 Bldg. H. H. Gill).

Samples of the anisoles were submitted to the Biochemical Research Laboratory for rabbit tests. They were applied to the rabbit ear as 10% aqueous solutions in Dowanol DPM at the rate of 0.1 cc/ear/day, being equivalent to 20 applications over a period of 37 days.

In no case was folliculitis noted and it is considered unlikely that the materials would produce chloroacne in humans. (Letter report from K. J. Olson - Biochem. Res. Lab. - May 27, 1965).

DOW 1 589204

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CONCLUSION:

The results show that any impurities in the plant grade 1,2,4,5-tetrachlorobenzene as used at present, have no measurable effect on the chloroacnegenic activity in the 2,4,5-trichlorophenol process..

A. J. Erbet

6-15-65
AJE:scb

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DOW 1 5692

SUBJECT TCP — Acne SHEET NO. 1398 OF

Task Force Bldg #1 DATE 11/23/64 BY N. G. D.

1) Check inerting of chemical sTiCl – N_2 or sTeon Cool ? – Check Richards on their experience

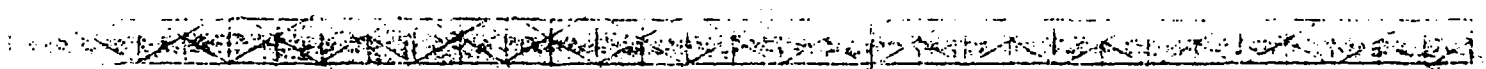
2) VPC e Turbidity on Steam to 349 Bldg.

3) A.W. Try Lower temp reaction – Cu Catalyst Copper Cable ??

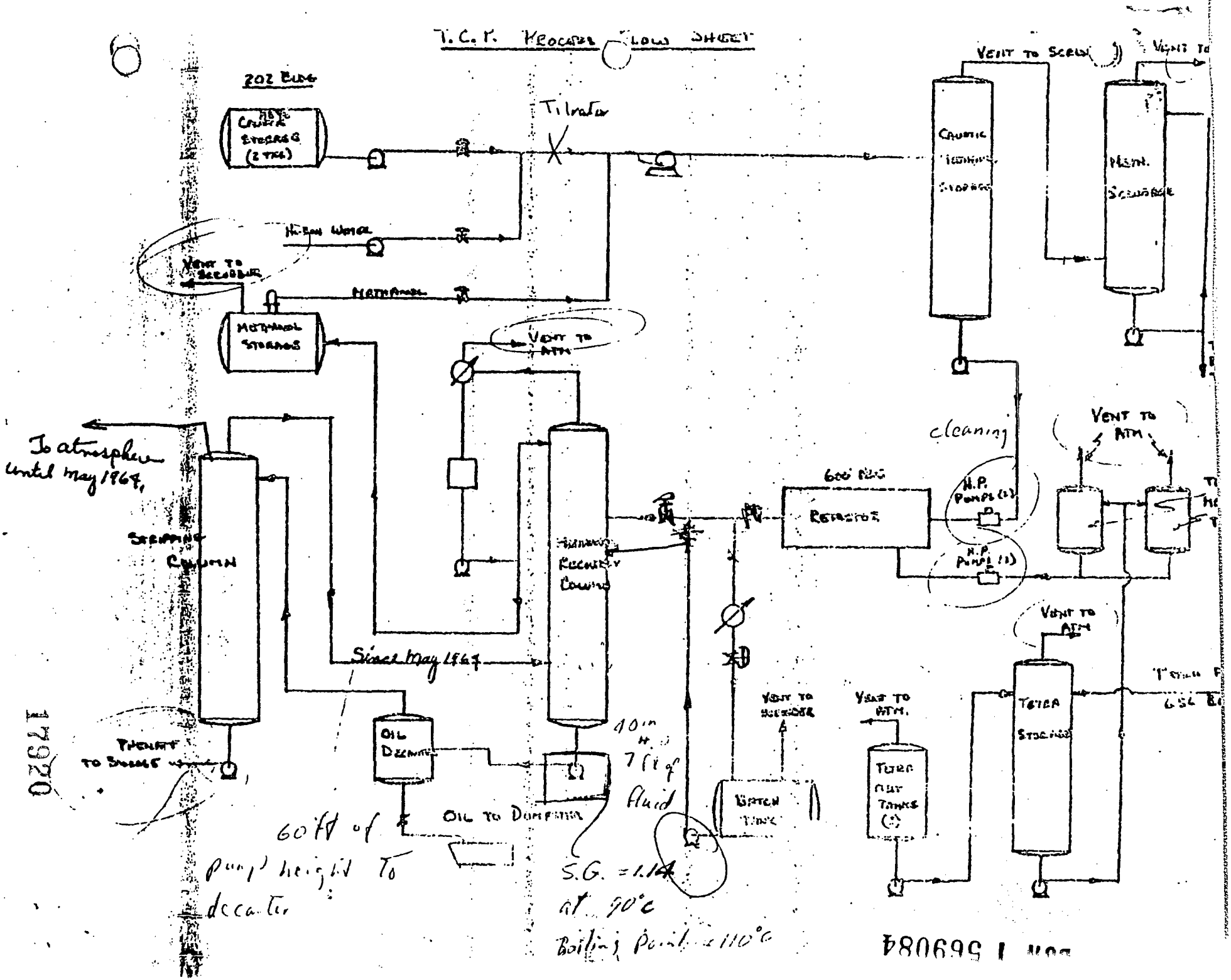
4) Submit question for Trip –
245 T plant – Dooser – Leach

5) Flake Pilot – Reen – Trial – Check Eng – cooling coil

DOW 1569083



17919


T. C. P. Process Flow Sheet
202 Bldg
Titrator
Caustic Heating (2 tks)
Hi-Sol Water
Methanol
Vent to Scrubber
Methanol Storage
Vent to Atm
To atmosphere until May 1964
Stripping Column
Since May 1967
Oil Decanter
Phenate to Storage
Oil to Dumping
60 ft of pump height to decanter
40 in H2O
Fluid
S.G. = 1.14 at 90°C
Boiling Point = 110°C
Caustic Heating Storage
Vent to Scrubber
Neut. Scrubber
cleaning
600 Psig
Reactor
H.P. Pumps (2)
Vent to Atm
Vent to Atm
Vent to Scrubber
Vent to Atm.
Tower Neut Tanks (2)
Tower Storage
17920

DOW 1 551622

MN0732280

MIDLAND
November 1, 1962

Mr. Paul H. Ribby
Bioproducts Department
Abbott Road Building

TOXICOLOGICAL INFORMATION ON A NUMBER OF AGRICULTURAL FORMULATIONS SUITABLE FOR SENDING TO R.S. Dunham of the U. S. Dept. of Agriculture, 2371 Buford Avenue, St. Paul 8, Minnesota

Here is the information that Mr. Dunham has asked for in his letter of October 19. I shall take up the materials as he lists them in his letter.

2,4-D, 2,4,5-T and MCPA

2,4-D (2,4-dichlorophenoxy acetic acid), 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) and MCP (4-chloro-o-tolony acetic acid) and their salts and esters form the basis for a wide variety of herbicidal formulations. The LD_{50} values for both the active ingredients and, in several instances, the formulations are given in the enclosed reprint entitled, "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". The hazards involved in their handling and use are covered in the paper entitled "Health Hazards Associated with Handling and Use of Herbicides".

Silvex

The toxicological information on Silvex is summarized in the enclosed bulletin entitled, "Silvex Technical Bulletin No. 1". One additional statement may be made. Silvex is not absorbed through the skin in acutely toxic amounts.

Dalapon

The information desired on this product and its commercially available formulations is given in the enclosed reprint entitled "Toxicology of Dalapon Sodium" and the ACD data sheet HG-10 entitled "Toxicological Information Relative to Dowpon or Radapon".

Dinitro

The dinitro herbicides that Dow sells are formulations containing alkyldinitrophenols. The toxicity of these herbicides has been shown to be directly dependent upon the content of the available free alkyl dinitro phenol and in the case of Dow Selective Weed Killer, upon the methyl alcohol content. The enclosed reprint

entitled "Toxicological Studies on Laboratory Animals of Certain Alkyldinitrophenols Used in Agriculture" summarizes the toxicological information on 4,6-dinitro o-sec butyl-phenol used in Dow General Weed Killer, Dow Selective Weed Killer and Dow's Premerge Weed Killer. The toxicological information on Methyl Alcohol is summarized in the enclosed chemical Safety Data Sheet SD-22 entitled "Methanol". The hazards involved in handling Dinitro herbicides are discussed in the previously mentioned paper entitle "Health Hazards Associated with Handling and Use of Herbicides".

Erbon

Please refer to the enclosed Technical Bulletin entitled "Erbon", page 3 for the summary of toxicological information on Baron which is the emulsifiable formulation of Erbon.

TCA Sodium

The toxicological information on this product is given in the enclosed bulletin on Sodium TCA. Of interest is the disucssion of health hazards given on this herbicide in the paper entitled "Health Hazards Associated with Handling and Use of Herbicides".

Garlon

Garlon is an emulsifiable formulation containing Dalapon and Silvex esters as the active ingredients. The toxicological information on Garlon may be summarized as follows: Garlon has a low acute oral toxicity; the LD_{50} for rats being in the range of 4 g./kg. of body weight. It may cause only very mild eye irritatio when in contact with the eye and mild skin irritation if skin contact is prolonged or frequently repeated. It is not absorbed through the skin. When diluted with water to recommended use concentrations, Garlon presents no hazard from handling. When handling Garlon reasonable care and cleanliness should be practiced to avoid skin and eye contact.

Zytron

The toxicological information on Zytron and on Dow Crab Grass Killer are summarized in the enclosed data sheet on "Dow Crab Grass Killer".

Paul, one further idea has come to me that may be useful. How about collecting labels from representative formulations and sending them on to Mr. Dunham? They may prove helpful to him. They can be obtained from the Labeling Section of the Legal Department, phone 6-4136 or 6-4128.

-Mark A. Wolf-

Mark A. Wolf
Biochemical Research Laboratory
1701 Building

cc: T23.14-11-20
T23.14-23-20
T2.30-193-20
T2.30-62-20
T2.30-133-20
T2.30-111-20
T2.30-18-20

17922

1324

Dr. Joseph V. Klauder
1934 Chuck Street
Philadelphia 3

MAR 24 1959
Biochem. Res. Lab.

March 19, 1959

Dr. V. K. Rowe
Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

Dear Dr. Rowe:

I herewith report results of repeated insult method of patch test with Kuron in accordance with your letter of January 2, 1959.

As reported in my letter of January 16, 1959, the initial application of Kuron in the patch test study was 5% aqueous solution, subsequent applications were 1%, except for the final or challenging application; 5% solution was used.

Fifty male subjects were employed. The tested solution saturated a Johnson and Johnson lintine disc, 1½ inches in diameter. This was applied to the back of the subject and allowed to remain for twenty-four hours. A freshly prepared disc, or patch, was again applied in twenty-four hours. This procedure was repeated every other day for twelve consecutive applications. Following the last application, a final or challenging application was made after two weeks time. After each removal of the patch, I personally examined its site for cutaneous reaction.

The result of the study is shown in Table 1.

As seen in the Table no cutaneous reaction was observed after the first application of 5% solution. To determine what concentration of Kuron exerted primary cutaneous reaction, patch tests were performed on subjects other than the fifty studied subjects. This was reported in my letters of February 3, 1959 and February 16, 1959. As stated in these letters, none of the subjects reacted to 5, 10, 20, 30 and 50 per cent concentration. As a final test Kuron undiluted was poured on the forearm and allowed to remain for two hours. No cutaneous reaction resulted. From this study it could not be concluded that Kuron exerted any cutaneous reaction.

As seen in the Table, all but one of the subjects showed negative reactions to all applications, including the challenging one. The exception was Subject Number 36, who showed one and two plus reactions on the 10, 11, 12 and challenging application. As reported to you by telephone, to exclude a fatiguing action, it was proposed to re-test this subject after a rest period of several weeks. Accordingly, (your letter of March 11, 1959) the first re-test was performed with the following concentrations of Kuron, as well as with four other samples that you sent.

First Test

Kuron 0.1 per cent in water
Kuron 131 0.1 per cent in sesame oil
Kuron 135 0.05 per cent in sesame oil
Kuron 133 0.01 per cent in water
Kuron 137 0.01 per cent in water

MN073280 DOW 7819221

17923

Second Test
(24 hours later)

Kuron 1.0 per cent in water
Dowco 134 1.0 per cent in sesame oil
Dowco 135 0.5 per cent in sesame oil
Dowco 136 0.1 per cent in water
Dowco 137 0.1 per cent in water

The result of both those re-tests was negative. This evidences that the aforementioned reactions on the last four applications of Subject Number 36 in the patch test study was a fatiguing one, and not allergenic. Considering the concentration in actual use of Kuron, three to four parts per million, a fatiguing action is inconceivable.

The study showed no primary irritant action of Kuron, a fatiguing action only in one subject and no evidence of allergenic action.

Yours very sincerely,

Joseph V. Klauder

JOSEPH V. KLAUDER, M. D.

JVK/mmck
Enclosure

DOW 781922

DOW "KUKON"

Subject NO.	1	2	3	4	5	6	7	8	9	10	11	12	*C
1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-
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49	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-

*C - challenging application
- - negative reaction

DOW 781923

17925

C. R. I. Name: Kuzan

Common Name:

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MN057107

MIDLAND
May 12, 1958

T. Hymas
Agricultural Chemical Research
47 Building

TOXICITY INFORMATION ON SOME RECENTLY RELEASED DOW AGRICULTURAL CHEMICALS

V. K. Rowe has asked me to send you the toxicity information on some agricultural chemicals recently released to Sales. I hope the following is of help to you with your paper.

DOW 1430004

	LD_{50} INFORMATION
Esteron 2,4,5-OS	Rat, in range of 1.0 and 2.0 gm./kg.
Veon 100	No test run
Reddon concentrate	Rat, in range of 0.5 to 1.3 gm./kg.
Esteron Brush Killer OS	No tests run.
DowMCP amine Weed Killer	Rat, in range of 0.63 to 2.0 gm./kg.
Garlon	Rat, range not determined but > 2.0 gm./kg.
Erbon	Rat 1.12 gm./kg. Guinea pig 0.85 gm./kg. Rabbit 0.71 gm./kg. Chick 3.17 gm./kg.
Inverton	Rat, range not determined but > 1.0 gm./kg.
Esteron 99	No test run.
Dalapon	Rats, mice, guinea pig, rabbit and chick 3.86 to 9.33 gm./kg.
Novon	Rat, range not determined but > 2.0 gm./kg.
Baron	Rat, in range of 1.0 to 4.0 gm./kg.
Brush Killer X	No test made.

17927

EYE CONTACT

Veon 100, Dow MCP amine Weed Killer, Inverton, and Dalapon are markedly irritating and damaging to the eye. Eye protection such as face shields, goggles with side shields or their equivalent should be worn.

Esteron 2,4,5 OS Reddon concentrate, Garlon, Erbon, Novon Baron, and Brush Killer X are only very slightly to slightly irritating to the eye. Minimum eye protection should be worn.

SKIN CONTACT

Most of these products are moderately irritating to the skin on prolonged or repeated contact. None of them present a problem from skin absorption.

Baron, in strong solutions and as the undiluted formulation may cause acme-form dermatitis upon repeated skin contact so that special attention should be given to its handling. They may all be handled safely if suitable precautions are taken to avoid prolonged or repeated skin contact.

At use concentrations most of these materials do not present a problem from skin or eye contact.

The following table on the toxicity of various products to fish may be of some use to you.

DOW 1430005

Material	Highest Concentration Showing No Effect ppm	Lowest Concentration Causing Complete Kill ppm
Diluent Oil	1.0	10.0
Diluent Oil Plus Emulsifiers	1.0(1)	10.0(1)
Emulsifiers Alone	10.0(2)	40.0(2)
Esteron 2,4,5-T	2.0	4.0
Esteron Brush Killer	2.0	5.0
2,4-D, Dowanol 97B Ester	7.0	20.0
2,4,5-T, Dowanol 97B Ester	10.0	15.0
2,4-D, Sodium Salt	4,000	>4,000
2,4,5-T Sodium Salt	1,000	1,500
Dalapon, Sodium	2,500	>6,000
Trichloroacetic Acid, Sodium Salt	4,000	>6,000
Silvex, Sodium Salt	20.0	25.0
Silvex, Polypropylene Glycol Butyl Ether Ester	1.0	5.0
Kuron	5.0	9.0
Baron	6.0	15.0

DOW 1430006

(1) Concentration is on oil basis.

(2) Concentration is that which could be contained in this much oil. (Emulsifier to oil ratio 15:85).

V. K. Rowe suggested also that you might find the enclosed summaries on Dalapon useful.

If there is any further help we may give, let me know.

Mark A. Wolf

Mark A. Wolf
Biochemical Research Laboratory
12-634 Building
Phone - 8991

17929

MAW/mo

cc: V. K. Rowe

1349
from Texas Rept for Sept, Oct. Nov. 1957

MN057107

A program for the evaluation of potential aquatic herbicides as to their toxicity to fish has been initiated. A total of five compounds has been tested. The results of those evaluations are as follows:

Compound tested	Fish species tested	Percent Kill 05 ppm	10 ppm	50 ppm	100 ppm	200 ppm	Control
Kuron	Sunfish	28.5	71.4	85.7	100.0	100.0	00.0
Kuramine	"	08.3	08.3	09.1	16.6	50.0	16.6
2,4,5-T ethanol	"	100.0	100.0	100.0	100.0	100.0	00.0
2,4,5-T ester	"	50.0	92.0	100.0	100.0	100.0	00.0
ethynylcyclo-hexanol	"	41.6	58.3	75.0	-	-	00.0

Each compound was tested in a minimum of ten fish. The fish were trapped in Lake Jackson, Texas and placed in one-gallon tanks, five fish per tank. The tanks were aerated by a Saxton aerator and the fish were not fed during the course of the trial. The herbicides were introduced into each tank and the fish exposed for a period of twenty-four hours.

Kuron exhibited considerable toxicity above 05 ppm. Kuramine exhibited low toxicity at all concentrations whereas the remaining three compounds tested were considerably toxic.

DOW 1429984

Chemical Formulations

J. N. Lipscomb

Approximately 710 compound were received and filed in the compound room.

A. Experimental Veterinary Formulations - One experimental Trolene screwworm smear TF-30 was made. This is a modification of TF-28, made to prevent settling of the thickener. Ten injectibles were made and are as follows:

TF No.	Description
31	Pure ethyl succinate to check for irritation
32	Contains the inactive ingredients of TF-29 to check for irritation
33	K-19,236 in Robane, fatty acid
34	K-19,236 in ethyl succinate
39	K-26,019 in ethyl succinate

17930