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file copy (action)

2,4,5-1
Action 1362

April 22, 1963

Brigadier General Fred J. Delmor (3)
U. S. Army Munitions Command
Chemical-Biological-Radiological Agency
Office of the Commanding General
U. S. Department of Defense
Army Chemical Center, Maryland

Dear General Delmor:

At the request of Dr. Warren C. Shaw, Agricultural Research Service, United States Department of Agriculture, we are supplying herewith three copies of the information available to us concerning the toxicity of 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid).

This information was prepared by Dow for a petition to the Food and Drug Administration requesting a tolerance for 2,4-D in asparagus. (Incidentally, the tolerance of 5 ppm as requested was established by the FDA). Included in this information are references 8 and 9 which give data on the toxicity of 2,4,5-T.

In addition to this information, we would like to also mention that we have been manufacturing 2,4-D and 2,4,5-T for over ten years. To the best of our knowledge, none of the workmen in these factories have shown any ill effects as a result of working with these chemicals.

Further, many millions of pounds of these chemicals have been used here and abroad without causing a single proven case of illness to livestock.

There have been very few complaints and alleged illnesses in humans due to 2,4-D, however, we have never been convinced that these instances were in fact caused by the chemical. In view of the low toxicity observed in other mammalian species, our toxicologists believe these cases have been related to 2,4-D by coincidence.

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Knowing that these products are used by all sorts of people under varied circumstances, we consider the few number of alleged injuries to be phenomenal when related to the large quantities of product manufactured and sold.

We are glad to have this opportunity to serve you, and trust the information we have provided will be of assistance.

Sincerely,

G. E. Lynn
Director of Registration
Eloproducts Department

Jag

cc: Dr. Warren G. Shaw, A.R.S., U.S.D.A.

bc: W. W. Sunderland
V. K. Rowe (No Enclosure)

DOW 534541

Biochemical Research Laboratory

The Dow Chemical Company

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OFFICE COPY

199 BUILDING WIPE SAMPLES
FOR CHLORACNE CONTAMINATION,
1966 TO DATE

File: T17.4-12-3(E)
Date: 5/5/66
By: L. G. Silverstein

Signed *L. G. Silverstein* Date 6/9/66
6-3-66

Checked *H. Boyle* Date 6-6-66

PROBLEM

The chloracne hazard in 199 Building requires continued monitoring of the cleanliness of the reactor room and surrounding areas by means of wipe tests.

PART I

CONCLUSIONS.

1. The positive samples in all three sets of wipes taken in 1966 indicate the definite need for continuing the protective measures which are in force in the building. Proper use of gloves, clothing and shoe covers is still necessary.
2. Wipe testing indicates that the need for continued, frequent, thorough cleanup still exists. There is an improvement from the January to the February and also to the March samples but the latest series indicates that cleanup is still needed and the "ups" and "downs" in cleanliness testify to the need for continued good housekeeping.
3. Because the floors seem to be the most likely spot for significant contamination, the removal of shoe coverings at

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the door between the reactor room and the still room must be observed strictly. Contamination was found on the stairway to the lunch and locker room, although none was found in the lunch and locker room itself and contamination was also found in the foreman's office in the first 1966 wipe samples. This indicates laxity in the observance of shoe cover protection.

4. Wipe samples covering the areas indicated in Table 1 should be continued at least on a monthly basis during trichlorophenate production at 199 Building.
5. Wipe tests for the period 4/19/65 to 7/23/65 are presented in Part II of this report to complete the documentation of wipe testing since the chloracne problem began.

DISCUSSION

The method of taking wipe samples and interpreting them has been described in the previous report on 199 Building contamination, File T17.4-12-3, dated 5/6/65. A positive response on the animal indicates contamination which should be removed. A negative response indicates a clean area. The severity of response may be taken as an index of the dirtiness but any positive response should be considered significant.

Past experience has shown that working areas can be maintained free of detectable contamination by wipe test. Table I gives a detailed description of the locations in which wipe tests were taken and the animal response on wipes taken on 1/17/66, 2/14/66 and 3/29/66.

DOW 1547794

These three series of tests indicate that cleanup procedures do improve the situation generally, although in a couple of instances there was an increase in response in the second series over the first which means that continued frequent cleaning is still in order in this building. The findings of activity in the foreman's office, on the stairway to the lunch and locker room indicates that there has been some laxity in the past in confining the contamination to the reactor room. This probably resulted from not using shoe covers or not removing them as soon as the men left the reactor room. The existence of activity in the reactor room on controls and even on the operator's desk and chair is sufficient evidence that the protective measures now in force must be continued.

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Table 1

199 BUILDING WIPE TESTS
1966

<u>Location</u>	<u>Animal Responses</u>		
	<u>1/17/66</u>	<u>2/14/66</u>	<u>3/29/66</u>
Turbidity meter	Severe	None	
Floor E. panel board	Severe	Moderate	None
Floor W. panel board	Moderate	Moderate	None
Floor inside Door A	Moderate	Slight	None
Floor inside Door 20	Slight-moderate	Moderate	None
Floor inside Door 2	None	None	None
Floor, caustic-methanol pumps	Severe	Moderate	Slight
Floor inside Door D	None	Slight	None
Floor between still and reactor room	Slight-moderate	Slight-moderate	Slight
Floor and door, S. side of still enclosure	Moderate	Slight	Slight
Floor and door, N. side of still enclosure	Severe	Moderate	None
Floor and benches, locker room	None		
Floor and benches, lunchroom	None		
Floor in laboratory	None		
Floor, foreman's office	Slight-moderate	None	
Floor by sink inside Door D	Moderate	Slight	
Operator's desk and chair	Slight	None	
Operator's tool boxes	None		
Valves and floor, oil draw-off station	Slight	Moderate	None
Valve handles, S. side of still enclosure	Slight-moderate	Moderate	None
Valve handles, W. side of still enclosure	None		None
Stairway to bottles	Moderate	None	Slight
Platform above Phenate tank	Moderate-severe	Moderate	Slight

continued on next page

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Table 1 - continued

<u>Location</u>	<u>Animal Responses</u>		
	<u>1/17/66</u>	<u>2/14/66</u>	<u>3/29/66</u>
Plant mechanic's bench	Slight- moderate	None	(Floor) Slight
Pipefitter's bench by still enclosure	Moderate		
Stairway to lunch and locker rooms	Moderate- severe	None	
Laboratory hood	None		
Sovereign's shoes	Slight		

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PART II

WIPE TESTS FOR CHLORACNE CONTAMINATION AT 199 BUILDING APRIL 19, 1965 - JULY 23, 1965

This Part documents the wipe testing done in a period not covered by previous reports on this subject.

CONCLUSIONS

1. The still enclosure in 199 Building was demonstrated to be substantially contaminated by wipe tests on 4/19/65.
2. The operating areas were free of contamination on 6/1/65 indicating that good housekeeping can be attained.
3. A positive wipe test in the oil draw-off area indicates that this area is susceptible to contamination.
4. A rental sparkler filter unit was judged to be free of acne hazard on the basis of VPC analysis only.

DISCUSSION

The wipe samples discussed in this Part were taken during a period of time not covered by other Biochemical Research Laboratory reports on the subject of chloracne wipe testing on 199 Building. The samples reported herein are primarily of historical value because of the changing nature of the situation at 199 Building. This completes the written record of wipe testing in the trichlorophenol area from 1964 to the present time.

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Table 2 describes samples taken in and around the still enclosure. All of these were positive indicating that contamination was present at that time. It has been assumed that work within the enclosure involved the risk of contamination and appropriate precautions are taken at any time a person must enter the enclosure. These samples only substantiate our assumption.

Table 3 describes 20 wipe samples taken on 6/1/65 which pretty well cover the operating area and possible exposure points. All wipe tests in this table showed no response on the animal, indicating that the areas were clean on that date, and also indicating that clean-up procedures can successfully keep the operating area free of contamination.

Table 4 describes wipe tests in the oil draw-off station and oil dumpster. One floor sample showed substantial activity on the animal showing that contamination can get to that spot. The dumpster was free of contamination on 7/1/65.

Table 5 describes both rabbit tests and VPC analysis on a series of wipes taken on a rented sparkler filter which was to be returned to the vendor. Wipe samples on 7/1/65 after the first cleaning of the filter demonstrated that cleaning had been inadequate in terms of removing acne contamination from the interior of the filter. VPC analysis showed agreement with the animal test. Wipes taken on 7/14/65 after another cleaning showed that the inside was still severely contaminated. Wipes taken 7/19/65 showed a substantial decrease in the amount of contamination by VPC analysis only, and those on 7/23/65 showed no detectable dioxin by VPC analysis. The filter unit was returned to the vendor on the basis of the last VPC analysis.

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Table 2

WIPE SAMPLES TAKEN AT 199 BUILDING
APRIL 19, 1965

	<u>RABBIT RESPONSE</u>
1. Floor inside north door of still enclosure	Slight
2. Inside surface, north door of still enclosure	Slight-Moderate
3. Wall panel - first one east of north door	Slight
4. West side of lower decanter	Moderate
5. West wall, north half	Slight
6. Floor sample, filter area	Moderate

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Table 3

WIPE TESTS - 199 BUILDING
JUNE 1, 1966
65

	<u>Rabbit Response</u>
1. Malecki's shoe	None
2. Floor by W. panel board	None
3. Floor by E. panel board	None
4. Floor by N. door still enclosure (outside)	None
5. Floor by S. door still enclosure (outside)	None
6. Floor between still and reactor rooms	None
7. Door (N.) to still enclosure	None
8. S. door to still enclosure	None
9. Floor area inside Door D	None
10. Floor area inside Door 2	None
11. Floor area inside Door 3	None
12. Floor area inside Door 20	None
13. Floor area - locker room	None
14. Floor area - lunch room	None
15. Floor area and first three steps to lunch and locker room	None
16. Valve handles and chain at oil draw-off station - Door 2	None
17. Laboratory floor - 199 Building	None
18. Laboratory hood (inside) - 199 Building	None
19. Door and door handles to laboratory - 199 Bldg.	None
20. Door and bottom of turbidity meter enclosure	None

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Table 4

WIPE TESTS AT 199 BUILDING
JULY 1, 1965

	<u>RABBIT RESPONSE</u>
1. Floor area - oil draw-off station - north of gutter	Moderate-severe
2. Floor area - oil draw-off station - south of gutter	None
3. Oil dumpster - top and sides	None

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WIPE TESTS ON RENTED SPARKLER FILTERS
199 BUILDING

Table 5

Sample Description	7/1/65		7/14/65		7/19/ 65	7/23/ 65
	Rabbit	VPC*	Rabbit	VPC	VPC	VPC
Outside surfaces	None	<0.3				
Inside surface of door	None	<0.3				<0.3
Filter plates	Moderate	18				<0.3
Inside walls (exit end)	Severe	75	Severe	58	8	<0.3
Inside walls (door end)	Severe	16	Severe	42	5	<0.3
Auger and auger trough	Moderate- Severe	30	Moderate- Severe	6	9	<0.3
Movable parts - plate rotator	Moderate- Severe	<0.3**	Moderate- Severe	56	4	<0.3

*Micrograms 2,3,7,8-tetrachlorodibenzodioxin per sample.

**7 γ reported at 1.05 retention time. Probably accounts for severity of response which is not normally produced by this low level of dioxin.

UNIT INDEX

Wipe samples for chloracne contamination in 199 Building revealed the existence of contamination in many areas which indicates that continued intensive clean-up and the continuation of the strict personal hygiene and housekeeping efforts is still required to avoid further chloracne problems.

DISTRIBUTION

G. G. Goergen, Aniline Section, 172 Building
A. F. Lueck, Aniline Section, 199 Building
D. C. Dunkelbarger, Aniline Section, 199 Building
R. E. Walker, Aniline Section, 199 Building
H. L. Gordon, M.D., Medical Department, 607 Building
A. W. Wilson, Safety Department, 401 Building

LGS:sjl

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DOWN 1 547804

Industrial Hygiene Chemical Exposure Data Entry Log
Field Survey TWA Exposure And Excursion Exposure Data

Report no. 17.4-12-3/4851 Date 0765
Building no. 199 Index no. _____
Units of Data W Respirator Worn? _____
Master no. _____

Chemical Name

TCBD

Job Classification

Job No.

DR# 650986

DR# _____

TWA/Exc.

TWA/Exc.

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COMMONWEALTH OF VIRGINIA

1400



GEOFFREY T. MANN, M. D., LL. B.
CHIEF MEDICAL EXAMINER
GEORGE W. THOMA, M. D.
ASS'T. CHIEF MEDICAL EXAMINER
HAROLD L. BEDDOE, M. D.
ASS'T. CHIEF MEDICAL EXAMINER
CHARLES O. BARCLAY, M. D.
ASS'T. CHIEF MEDICAL EXAMINER
NORFOLK DIVISION

DEPARTMENT OF HEALTH
OFFICE OF THE CHIEF MEDICAL EXAMINER
404-406 NORTH 12TH STREET
RICHMOND 19

SIDNEY KAYE, M. SC.
TOXICOLOGIST
J. DOUGLAS REID, SC. D.
MICROBIOLOGIST
MARION V. WALLER, M. SC.
SEROLOGIST
THOMAS D. JORDAN, B. A., LL. G.
ADMINISTRATIVE ASSISTANT

October 22, 1956

The Dow Chemical Company
Midland Division
Midland, Michigan

Dear Sirs:

We have had several cases of sudden death in children with no apparent cause indicated after autopsy. Each of these children had a possible exposure to Esteron 245.

Would you kindly advise us of the relative toxicity of Esteron 245; signs and symptoms developed following exposure, pathology seen at autopsy and also analytical procedure for identity and assay in tissue.

Any assistance you may give us in this matter would be appreciated.

Very truly yours,

Sidney Kaye

Sidney Kaye, Ph.D.

SK/st

DOW 1 278602

17946

WEED KILLER (*Unidentified*)

Aug	1950	Willard Hagle	1526-137	Irritated eyes
June	1950	Ames Manchester	1204-123	Burn left eye
Apr	1950	Willard Hagle	1526-137	Irritated eyes
Mar	1950	Dudley Herman	1204-100	Burn left eye
May	1949	Jack Marden	1527-143	Burn left eye
Nov	1948	Dexter Ames	321-209	Irritation both eyes
May	1948	Ivan Gillis	1204-149	Burn left eye
May	1948	Willard Mosier	1204-170	Bilateral Conjunctivitis
Sept	1947	Michael Cox	315-345	Irritation left eye
July	1947	Richard Baldwin	324-141	Irritation of eyes
June	1947	Alfred Emeott	1204-209	Left eye irritated
Jan	1947	Leonard Hudock	1204-224	Left eye burn
July	1946	Wm. A. Smith	1204-198	Eyes irritated
July	1946	Ed. O'Toole	1204-201	Left eye irritated
July	1946	Edward Smith	1204-111	Left eye burn

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Aug	1951	G. Heintzelman	1204-201	Irritation left eye
Mar	1949	Joe Ortiz	1204-217	Slight irritation
Dec	1948	Joe Ortiz	1204-217	Irritation left eye

2-4 D

Feb	1952	Louis Kocisky	1526-111	Irritation of eyes
Feb	1952	H. Livingston	1526-113	Burn of eye
May	1952	Vincent Mako	315-241	Irritation eyes
Dec	1952	Harold Mahon	1526-125	Burn rt eye (with HCl Ph-1)
Oct	1951	H. Draves	167-117	Burn left eye and nose
June	1950	Leonard Parsons	1526-138	Burns both eyes
Dec	1949	Wm.R. McAnnallen	167-103	Irritation both eyes
Nov	1949	Wm.R. McAnnallen	167-103	Irritation both eyes
June	1949	Lawrence Dice	1527-111	Irritated left eye
June	1949	Floyd Schreiber	1204-151	Irritated left eye
May	1949	Lawrence Dice	1527-111	Irritated left eye
May	1949	Lawrence Dice	1527-111	Irritated right eye
Oct	1948	Irvin Bristol	1204-198	Both eyes irritated
Apr	1948	Pat Walters	1204-220	Eyes irritated, face & neck irritat.
Mar	1948	James Toles	1204-182	Irritated rt eye
Oct	1946	Phillip Sanders	1204-192	Burn left eye
Sept	1946	Andrew Wilson	56-145	Burn eyes
Aug	1946	Clinton Skym	1204-210	Rt eye irritated

DOW 1 278063



THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

March 3, 1953

Dr. E. W. Kinnaird, Administrator
Bourbon County Hospital
Paris, Kentucky

Dear Dr. Kinnaird:

This is in reply to your letter of February 17th asking for information regarding the identification and treatment of injuries which might be caused to persons handling 2,4-D and 2,4,5-T.

2,4-D and 2,4,5-T in their various forms are used as growth control regulators and herbicides. They are among the least hazardous of the agricultural chemicals used in the field today. The LD₅₀ of these materials varies between 500 and 1000 mg./kg. of body weight for several species of animals and hence, the likelihood of systemic intoxication through oral ingestion is quite small. If they should be swallowed in substantial amounts we would expect them to cause irritation of the gastric mucosa, headache, ataxia, and possibly an increased tonus of the extremities. These symptoms would be expected more or less on the basis of animal studies, but insofar as I know - no experiences involving human subjects have occurred. As with any other chemical the first thing to do following ingestion is to induce vomiting. This can be accomplished by any of the well-known means. Symtomatic treatments should then follow.

The compositions of these materials as encountered in agricultural practice do not present appreciable hazards to either the eyes or skin and they are not absorbed through the skin in hazardous amounts. In one or two instances over the past 7 or 8 years, we have heard of a person who has demonstrated a typical hypersensitivity to skin contact, but when one considers the multitude of persons who have had contact with them, this could probably be expected. Patch tests conducted by accepted procedures have not indicated that these materials are likely to induce sensitization of the skin. If some mild irritation should develop through accidental prolonged contact, symtomatic treatment would again be the rule.

During the past seven or eight years we have manufactured and sold millions of pounds of these materials without encountering any difficulty. More millions of pounds have been

17948

Dr. B. W. Kinnaird

- 2 -

March 3, 1953

used in the field and the lack of any serious difficulty whatsoever is, we believe, very excellent evidence that the materials themselves present no hazards of real concern.

If I can be of any further assistance to you in this matter, please let me know.

Sincerely yours,

V K Rowe

V. K. Rowe
Biochemical Research Department
2-280 Building

VKR/bb

cc: H. H. Gay, M.D. ✓

UW 1278665

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NY
OK
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CABLE ADDRESS DOWCHEMCO

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BOSTON

THE DOW CHEMICAL COMPANY

MIDLAND • MICHIGAN

April 17, 1951



Mr. F. V. Reagel
Engineer of Materials
Missouri State Highway Department
Jefferson City, Missouri

Dear Mr. Reagel:

This is in reply to your letter of April 2, 1951 to our Mr. L. L. Coulter requesting our opinion with regard to the possibility of skin irritation occurring from contact with 2,4-D, 2,4,5-T, and TCA (I presume you mean Sodium TCA).

We have studied these materials rather thoroughly in our laboratory on animals. We have had an appreciable amount of work done at an outside consulting laboratory on both 2,4-D and 2,4,5-T using volunteer human subjects.

The results of these tests all show that it is very unlikely that 2,4-D and 2,4,5-T would cause appreciable skin irritation in the normal individual. It is possible, of course, that a rare individual may occasionally exhibit a sensitivity to these as well as to any other chemical. Such occurrences arise everyday with many common household chemicals - soaps, for example.

You have mentioned infection in this case. Certainly, none of these materials would cause a bacterial or fungus infection by themselves. However, irritation of any sort can certainly open the door to infectious organisms.

Sodium TCA is perhaps more likely to cause skin irritation than either 2,4-D or 2,4,5-T for 2 reasons; first, it is used in much higher concentrations; and second, it is inherently more irritating. Appreciable contact with Sodium TCA usually results in a burning sensation which encourages the operator who has carelessly or accidentally gotten the material on him to wash. However, irritation can certainly occur if the material is carelessly handled. This material would not cause infection by itself.

We do not believe that if any of these herbicides are handled in a reasonable manner that they are likely to cause difficulties. This, we believe, has been borne out by their very

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Mr. F. V. Reagel
April 17, 1951
Page 2

widespread use with but an exceedingly low incidence of complaints.

If we can be of further service to you, please do not
hesitate to call on us.

Sincerely yours,

THE DOW CHEMICAL COMPANY



V. K. Rowe
Biochemical Research Department

js

CC: Tech. Serv. & Dev.
Attn: L. L. Coulter, C. B. Branch
Legal Department
Attn: G. J. Williams
Medical Department
Attn: H. H. Gay ✓
Agri. Sales
Attn: E. L. Smith

17951

DUV 12/0000

P.O.Box 469

April 20, 1961

Kelly S. Segars, M.D.
Iuka Clinic
114 S. Pearl Street
Iuka, Mississippi

MN075468

UW 1278795

Dear Doctor Segars:

I have your letter of April 18th, a copy of which I have enclosed so that I can point out to you my difficulties in understanding. You speak of "trichlorophenoxy acetic acid" and I know of no such acid. I assume that you probably mean "2,4,5-trichlorophenoxy acetic acid" which is widely used as a brush killer. We have had a great deal of experience with this compound which we have manufactured in huge quantities and we have done a considerable amount of experimental work on animals and on humans. This acid of itself is mildly irritating and in strong concentrations can produce irritation of the skin, but it takes a considerable length of time of direct, intimate contact even to do this. As a matter of fact, I was not aware that the acid form of this herbicide was on the market, since it is commonly marketed as either the salt or the amine form. If this is indeed the material to which you have reference, then I can only say I am very puzzled as to how the man could have possibly received a burn, unless the contact was with a solution of this material at high temperature.

I would be interested in any further comments you may have, especially to know the circumstances under which the alleged burn was produced.

Very sincerely,

Harold H. Gay, M.D., Medical Director
Medical Department

HHG:ccf

enc.

J. STUART McQUISTON, M.D., F.A.C.P.
R. D. HUNTING, Jr., M.D.
R. M. QUETSCH, M.D.
W. M. KOONTZ, M.D.
1328 2nd Ave., S.E.
Cedar Rapids, Iowa 52403

1433

June 20, 1966

Pow Chemical Company
Midland, Michigan

Gentlemen:

A patient we currently have in Mercy Hospital with symptoms of anorexia, weight loss, joint stiffness and wasting of muscles in the upper extremities attributes these symptoms to a presumed exposure to 2,4,5-T in the form of "Esteron". In the spring of 1965 he used heavy applications of Esteron to the bark of ~~pine~~^{elm} trees that he wanted to kill and in the spring of 1966 he cut these trees down and was cutting them up for fire wood when he developed acute onset of the above symptoms. Approximately three months later, he still complains of some of these symptoms for which no other cause is apparent. The weight loss and some muscle atrophy and weakness in the upper extremities is apparent, without anything else very remarkable on physical examination or laboratory testing. Is there any remote possibility that 2,4,5-T could have left a residual over this long a period of time and that it might have something to do with the symptomatology?

Very truly yours,



Richard M. Quetsch, M. D.

RMQ:ajl

CC: Iowa Poison Information Center
Des Moines, Iowa

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UOW 1 278730

IONS

DOW

COMMUNICATIONS

DOW

COMMUNICATIONS

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Proved

69 LD NY

DR. GAY

607 BLDG

IN TRAVELING WITH SOME OF OUR CUSTOM SPRAYERS I HV BEEN TOLD THAT SOME OF THE PERSONNEL WHO HV BEEN HANDLING THE SPRAYING OF 24D AND 245T FOR THE LAST TWO OR THREE YEARS HV STARTED TO ERUPT IN BLISTERS ABOUT THE SIZE OF A HALF DOLLAR ON EITHER THEIR ARMS, LEGS OR FACES. THESE BLISTERS EVENTUALLY GET LEATHERY ON TOP AND THEN DISAPPEAR. WLD U PLS ADV WHETHER THERE IS ANY RECOMMENDED TREATMENT FOR THIS.

DICK VAN HOUTEN **USE MAIL**

JCM

66

Aug 21

UNLESS INDICATED

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July 17, 1957

W. H. Bucher, M.D.
Director, Poison Information Center
Childrens Hospital Society of Los Angeles
4614 Sunset Boulevard
Los Angeles 27, California

Dear Dr. Bucher:

Your letter of June 4th addressed to The Dow Chemical Company regarding the information on 2,4-D herbicides and their effect upon humans has been referred to me for reply.

As you undoubtedly know, there are quite a number of herbicides based upon 2,4-D (2,4-dichlorophenoxyacetic acid) and its derivatives. We have investigated a number of them quite carefully on animals because of the expected exposure of livestock to them. A reprint of our publication on this is enclosed. It will give you the basic information on ingredients and formulations on the market, whether ours or someone elses.

Unfortunately, in one respect and fortunately in another, I am unable to give you information regarding the effect of human ingestion since I do not know of any such cases. It is conceivable, however, that such could occur and I realize the reason for your inquiry. The information gained by animal studies causes us to comment as follows regarding 2,4-D and related type herbicides:

2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), MCP (4-chloro-o-toloxylacetic acid or 2-methyl-4-chlorophenoxyacetic acid), and Silvex (2,4,5-trichlorophenoxypropionic acid), whether in the form of the acids, salts, or esters, are all low in acute oral toxicity, but if swallowed in quantity, they can cause more or less serious toxic effects. Based on animal studies all of these materials in sufficient doses are capable of causing gastrointestinal irritation, injury to the lungs, liver, and kidney, and greater or lesser degrees of myotonia, particularly of the extremities.

It should be emphasized that many formulations of the basic ingredients listed above contain oils, such as kerosene or fuel oil, which may well cause more serious effects than the so-called active ingredients. This, of course, depends upon concentration.

DOW 1 277945

17955

July 17, 1957

Certainly the first thing that should be done in case an herbicide of the 2,4-D type is swallowed, is to induce vomiting as quickly as possible and call a physician. The physician should consider gastric lavage to remove any unabsorbed material. Further treatment will have to be symptomatic since there are no specific antidotes for these materials known.

If the preparation swallowed contains oil (such as an emulsifiable preparation or one designed for dilution with kerosene or fuel oil before application), consideration must be given to the effect of the oil. The danger from the diluent oils, primarily, is respiratory embarrassment caused by pulmonary edema and pneumonia. In such cases the administration of oxygen most certainly will be beneficial. Chemotherapy would have no effect on the so-called "chemical pneumonia" but it may have some beneficial effect in preventing or controlling possible subsequent bacterial invasion. Oil laxatives should be avoided. Liver damage may be minimized by promptly placing the patient on a diet low in fat and high in protein. The usefulness of lipotropic drugs has not been investigated. Kidney involvement is rarely sufficient to merit special treatment and for those rare cases where the kidneys are seriously involved, the treatment should be the same as for toxic nephritis of other etiology.

I trust that this information will serve your purposes.

Sincerely yours,

VKR

V. K. Rowe
Biochemical Research Department
12-634 Building

VKR/ba

Enc.

cc: H. H. Gay, M.D. ✓

DOW 1 277946

17956

June 24, 1957

1448

Mr. R. L. Keith
General Claims and Land Agent
Rutland Railway Corporation
Rutland, Vermont

Dear Mr. Keith:

It has been requested by our Agricultural Chemicals Sales Department that I write you regarding the toxicology of various herbicides which are alleged to have caused a hemolytic anemia in a man spraying one of your right-of-ways. As indicated before, we have no knowledge or suspicion that any herbicides which we manufacture would have any hematological effects upon persons. In order to reassure you of this, I am enclosing reprints of technical articles having to do with this subject.

One of the articles is 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." This article published in the American Journal of Veterinary Research gives results of a rather exhaustive study. We observed no hematological changes in the studies we made. I am also enclosing a reprint entitled, "Toxicity of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid. A Report of Their Acute and Chronic Toxicity in Dogs," by Drs. Victor A. Drill and Tomiharu Hiratzka. You will note that in their studies on dogs with these two base materials that they also observed no hematological changes, even at dosage levels which produced appreciable mortality. We conclude from these results that 2,4-D and 2,4,5-T are not hematologically active.

Insofar as Radapon is concerned, I am supplying you a data sheet which we have prepared as an interim report of the toxicological work we have done on this material. A detailed report has been filed with the Food and Drug Administration and as a result they have seen fit to set tolerances for its use on several agricultural commodities where it might occur in human food. An extensive report of this work is being prepared for publication. In the brief summary, however, no point is made of the hematological studies although such were made and no effects were observed. I trust that the summary information will be adequate for your purposes. It certainly shows that Radapon (dalapon, sodium salt) is exceedingly low in toxicity, and hence, presents little or no hazards under normal conditions of operation. Certainly no hematological effects would be expected from exposure to this material.

DOW 1 277942

17957

June 24, 1957

I have discussed this problem with H. H. Gay, M.D., our Medical Director, and he concurs with my opinions in this respect. I would suggest that if you have expert medical people who would like to discuss our clinical experience with these materials that you have them contact Dr. Gay at the Medical Department here in Midland. His phone number is TEmple 2-2311, Extension 243.

If we can be of any further assistance to you in this matter, let us know. We would be glad to hear from you at any time.

Sincerely yours,

VKR

V. K. Rowe
Biochemical Research Department
12-634 Building

VKR/vmv

cc: A. A. Lawrence
D. L. Conner
H. H. Gay, M.D.
E. T. Coon
V. K. Rowe (2)

DOW 1277943

17052



DOW CHEMICAL OF CANADA, LIMITED

1459

SARNIA, ONTARIO

March 17, 1966

Mr. L. S. Valberg, M.D.
Assistant Professor of Medicine
Queen's University
Kingston, Ontario

Dear Dr. Valberg:

Your inquiry of the toxicological properties of 2,4,5-trichlorophenol has been forwarded to Dr. H. L. Gordon of our Medical Department, Midland, Michigan for his acknowledgement.

I am certain this arrangement will be to your satisfaction.

Yours very truly,

wjh

M. E. Miller
Technical Service & Development

cc:
Mr. H. L. Gordon, M.D.
The Dow Chemical Company
Building 607
Midland, Michigan

Bremner, N.W.
Baker, G.

DOW 1 277848

17959

Table 9. DOW 1 & 1961

CRUDE FREQUENCIES AND RATES OR RATIOS PER 1000 live births of pregnancy outcomes according to dioxin exposure categories

PREGNANCY OUTCOME	EXPOSED								Control NON- EXPOSED	TOTAL
	MOD- HIGH	TCDD ALONE			OTHER DIOXINS ALONE	ANY DIOXIN				
		ANY LEVEL				ANY DIOXIN				
		< 1YR	> 1YR	TOTAL		< 1YR	> 1YR	TOTAL		
CONCEPTUS DEATHS										
MISCARRIAGE (RATIO)	0 (00.0)	18 (100.4)	25 (123.0)	43 (116.8)	0 (00.0)	36 (112.5)	49 (154.6)	85 (133.4)	213 (119.3)	298 (123.0)
STILLBIRTH (RATIO)	0 (00.0)	3 (18.1)	4 (19.8)	7 (19.0)	0 (00.0)	8 (25.0)	7 (22.1)	15 (23.5)	33 (18.5)	40 (19.0)
TOTAL (RATIO)	0 (00.0)	21 (126.5)	29 (143.6)	50 (135.9)	0 (00.0)	44 (137.5)	56 (176.7)	100 (157.0)	246 (137.8)	346 (142.9)
LIVE BIRTHS										
HEALTH DEFECT (RATE)	2 (181.8)	18 (100.4)	14 (69.3)	32 (87.0)	2 (100.0)	26 (81.3)	26 (82.0)	52 (81.6)	155 (86.8)	207 (85.5)
CONG. MALF. (RATE)	0 (00.0)	14 (84.3)	7 (34.7)	21 (57.1)	1 (50.0)	19 (59.4)	11 (34.7)	30 (47.1)	87 (48.7)	117 (48.3)
IND. MALF. (RATE)	0 (00.0)	1 (6.0)	1 (5.0)	2 (5.4)	0 (00.0)	1 (3.1)	2 (6.3)	3 (4.7)	10 (5.6)	13 (5.4)
INF. DEATH (RATE)	0 (00.0)	4 (24.1)	2 (9.9)	6 (16.3)	1 (50.0)	7 (21.9)	2 (6.3)	9 (14.1)	39 (21.8)	48 (19.8)
TOTAL UNFAVORABLE	0	16	8	24	2	24	12	36	120	156
TOTAL FAVORABLE	11	150	194	344	18	296	305	601	1665	2266
TOTAL	11	166	202	360	20	320	317	637	1785	2422
L. CONCEPTIONS										
TOTAL UNFAVORABLE (RATE)	0 (0.0)	37 (177.9)	37 (160.2)	74 (177.0)	2 (100.0)	60 (186.0)	60 (182.3)	136 (184.5)	366 (180.2)	502 (181.4)
TOTAL	11	187	231	418	20	364	373	737	2031	2760

(2) Rep. Mantel, N.
J. Am Stat. Assoc 58: 690-700 1963

1431

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TABLE 2
ESTIMATED RELATIVE ACUTE TOXICITY HAZARD OF PESTICIDES TO SPRAYMEN*

Most Dangerous	Dangerous	Less Dangerous	Least Dangerous
Demeton (Systox) (QP)	Aldrin (OCC)	BHC (OCC)	Aramite (M)
Di Syston (OP)	Bidrin (OP)	Binapacryl (Moroclide) (N)	Captan (M)
Mevinphos (Phosdrin) (OP)	Carbophenothion (Trithion) (OP)	Chlordane (OCC) 203-390	Carbaryl (Sevin) (C)
Parathion (OP) 3-30	DDVP (OP)	Co-Ral (OP)	Chlorobenzilate (OCC)
Schradan (OMPA) (OP)	Delnav (OP)	Diazinon (OP) 66-600	2,4-D (OCC)
TEPP (OP)	Dieldrin (OCC)	Dicaphon (OP)	Diquat (M)
Phorate (Thimet) (OP)	DNOC (N)	Dichloroethyl Ether (M)	DDD (TDE) (OCC)
Zinophos (Cynam) (OP)	DNOSBF (N)	Dimethoate (OP)	DDT (OCC)
	Endrin (OCC)	Dipterex (Dylox) (OP)	Dilan (OCC, N)
	EPN (OP) 8-36	Endosulfan (Thiodan) (OCC)	2,4,5-T (OCC)
	Ethion (OP) 27-119	Fenthion (Baytex) (OP)	IPC (M)
	Methyl Demeton (Meta-Systox) (OP)	Guthion (OP)	Karathane (N)
	Methyl Parathion (OP) 9-42	Heptachlor (OCC)	Kelthane (OCC)
	Nicotine (M)	Lead Arsenate (M)	Malathion (OP) 885-2800

Pentachlorophenol (M)
Phosphamidon (OP)
Sodium arsenite (M)
Zectran (C)

Lindane (OCC) 76-200
Naled (Dibrom) (OP)
Ruelene (OP)
Toxaphene (OCC) 40-285
V-G13 (OP)
Vapam (M)

Duribon 97-276

Maneb (M)
Methoxychlor (OCC)
Mirex (OCC)
Morestan (M)
NAA (M)
Perthane (OCC)
Phostex (OP)
Piperonyl Butoxide (M)
Ronnol (Korlan) (OP) 906-3025
Rotenone (M)
Simazine (M)
Sulphenone (M)
Tetradifon (Tedian) (OCC)
Thiram (M)
Zineb (M)
Ziram (M)

*From H. R. Wolfe and W. F. Durham, *Proceedings of the Second Eastern Washington Fertilizer and Pesticide Conference*, Washington State University, pp. 14-21 (1960). Key: OCC = organochlorine compound; QP = organic phosphate; C = carbamate; N = nitro compound; M = miscellaneous.

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DOW 1551800

MN073280

MINNEAPOLIS
November 18, 1933

W. W. Boulton
Superintendent
M. & O.

STANDARD GUARANTEE COMPANY 245 3

Refer on the 12th of Nov. of November, 1933, Mr. Boulton advised of the W. W. Boulton Insurance Company, 611 1/2 Court Street, Minneapolis, phone CR 8-4667, called me and informed me that he had just learned that one of their insured, who worked at the Free Service of Minneapolis, Minneapolis had filed with a claim of alleged embezzlement as a result of the operating of the 245 3 near Ironwood, Minneapolis. At that time he had no details. I asked him to get the details and let me know.

I received a call from him yesterday, November 14th, in which he offered this information. The aggrieved former's name is John Nicol of Ironwood. Mr. Nicol's attorney is Arthur Larson of Ironwood. The veterinarian is Dr. Gerald Reed. The county agent is in the net also but we do not have his name. Mr. Gould's investigation revealed that the horseman who gave service allegedly carried some land owned by Mr. Nicol in which he pastured a dairy herd. They are claiming that some of the cows ate the weeds which were sprayed and two of them aborted. He is claiming that the weeds were made more palatable as a result of the spraying, and this is what caused the abortions. Apparently the county agent, the veterinarian, and the attorney have arrived at this conclusion and Mr. Nicol has based his claim on it. They are asking \$100,000 for the loss of calves and the anticipated loss of milk. Apparently this happened back in the early part of September, but Mr. Gould was not sure of the date. I asked him also to check on the kinds of weeds which were believed to be responsible for this action and indicated that I would discuss it with the people concerned here and that we would be in contact with him.

I have discussed this matter with Don Hollinger and he suggested that I get this information into your paper and that probably you would be able to follow up on the case. In view of its nature, I believe that this is a proper procedure.

17962

L. L. Coulter

- 2 -

November 12, 1963

I have sent Mr. Gault a copy of 'Lynas' and my article dealing with the hazards to livestock of 2,4-D type materials. Mr. Gault and I fully anticipate that if this case is carried on that the Dow Chemical Company will be named as a defendant.

There is all of the information that I have at this time. If I can be of any help to you, let me know.

V. K. Howe
Biochemical Research Laboratory
1701 Building
Pheno (W 6-2976

VKH/jd

cc: D. O. Koehlinger
R. D. Martin
O. E. Lynn
V. K. Howe (2)
T2.30-3-30
Correspondence

17963

THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

September 13, 1947

Mr. Fred Karlovetz
 General-Fuel Chemical Company
 Gibsonburg, Ohio

DOW 1551762

MN073280

Dear Fred:

I am writing you at the request of our Sales Department relative to the complaint of Mr. and Mrs. Griffith who alleged that a stream which they use for growing minnows and other live bait was contaminated by one of your trucks to the extent that it killed all of their fish and caused other damage.

There is actually nothing in the literature dealing with the toxicity to fish of the substances which were involved in this case, as I understand it, namely Escher 10-10 and Diuron 745. In 1944 J. H. Harrison and E. W. Reese published an article in the American Journal of Pharmacy, Volume 113, pages 422-423, entitled, 2,4-D Toxicity Towards Certain Species of Fish. I believe in this instance they used the sodium salt and found that concentrations of 500 ppm or more were dangerous to a number of species. It is logical that the oil soluble or emulsifiable preparations such as you were using would be more toxic since oil itself is very toxic to fish especially when dispersed in the water.

Also in 1946 J. H. King and W. T. Penland published in Ecology, Volume 23, No. 4, pages 372-374, an article entitled, The Effect of Two of the New Herbaceous Herbicides on Stream and Large Mouth Bass. They found that 1 ppm was without effect and that 100 ppm caused an appreciable mortality. This work was done in conjunction with a study of the control of certain aquatic vegetation, such as water hyacinth, and the conclusion was drawn that the concentrations applied to such vegetation presented no problem to fish but that the mass of decaying vegetation reduced the oxygen content of the water so that it was detrimental to fish.

In work which has been conducted in our same Biological Laboratory employing the same methods as yours, we have found that 2,4-D ester such as used in Escher 10-10 is toxic to fish at concentrations of about 1 ppm and that 2,4,5-T ester is toxic at concentrations of 1 ppm. It is also interesting to note that oil which has been used as an emulsifier (such as

Mr. Fred Harlovitz

- 2 -

September 13, 1955

Ordinary (heresene) were toxic in concentrations greater than 1 ppm and that the emulsifiers used in preparations such as you are employing are toxic in concentrations greater than 10 ppm. It would seem, therefore, that the appreciable contamination of a small stream could well result in serious injury to aquatic life.

Judging from the copy of the letter from the Griffiths it would appear that there must have been quite a substantial amount of the herbicidal preparation dumped into the stream or there would not have been the foaming and the whitening of the water. It would be my opinion, Fred, that contamination to such a degree as that describe could well have been responsible for the effects described.

I regret that I cannot give you a more favorable report but that is the way it is for me.

Best personal regards.

Sincerely,

V. K. Rowe
Biochemical Research Corporation
15-34 Avenue

VKR/ba

cc: K. L. McCoy
D. H. Gomer
V. K. Rowe (2)
T2.30-3-30
T2.30-4-30
Correspondence

DOW 1 551763

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Check with U.K. Round

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2b regarding
 3-7 type

also death either
 application to
 a farmer has
 1 cherry.
 a policeman to
 been sprayed

For additional information, I would suggest
 contact with Dr. C. G. Johnston of the Veterinary Research Center
 at the University of New Brunswick. Dr. Johnston has worked on this particular
 problem for many years and his studies are very excellent.
 He has also found that these materials are non-toxic.
 Under the current regulations already passed most of our printed
 labels, to help in identifying our own data along with product
 labels to this effect.

It may be of interest to note that we also have weed control
 programs for pasture which can be completed primarily with the
 use of 2,4-D while the cattle are still in the pasture.

To date we have not been able to find one case of all the supposed
 2,4-D - 2,4,5-2 type product deaths or injury that has ever been
 substantiated. Believe me, we have tried -- for with the use of
 these materials on millions and millions of acres of farm land,
 ranch land, materials on utility and roadside rights-of-way, it
 is a problem that has concerned us from the very start. I have
 personally been on thirty-one complaints involving cattle or
 livestock death due to the use of 2,4-D or 2,4,5-2.

17966

THE UNIVERSITY OF MICHIGAN LIBRARY
ANN ARBOR, MICH.
FALL 1963

It has been our procedure in these cases to get the local veterinarian called, as the farmer has already disposed of the animal, and have him immediately perform a post-mortem. Generally, as soon as the animal is opened up, the veterinary can determine immediately what caused the death, and as evidenced, has never in any instance been 0,4,0 or 0,4,5-0 type product.

If a veterinarian is not able to determine the particular cause of death, during the post-mortem, samples of the rumen are sent to Columbia to the Ohio State University School of Veterinary Medicine, where they are analyzed. In these instances we usually find that the product has been highly freshly printed brand or have picked up local estimates of a similar toxic material which a farmer had sold in an area where cattle had access to it.

I feel for the most specific information on timidity, and particularly for timidity, should this case of any others come to court, that you not only contact us, but Dr. Goldstein at Department of Health.

Should you need any further help, please us immediately or contact us by letters.

Very truly yours,

Charles Doolittle
Agricultural Chemical Sales

Chicago

Enclosures

CC: Karl Hummerling, Inc.
P. O. Box 243
Canton, Ohio

17967

THE DOW CHEMICAL COMPANY 1470

MIDLAND, MICHIGAN 48640

May 15, 1968

Mr. N. A. Chamberlain
O. M. Scott and Sons
123 East 6th Street
Marysville, Ohio 43940

Dear Mr. Chamberlain:

I have been asked to provide you with information concerning the health hazards, if any, for the handling of our products 2,4-D acid, Formula 10 herbicide and Kuron herbicide. I hope the following will be adequate for your purposes.

Kuron Herbicide

The enclosed bulletins entitled, "Summary of Toxicological Information and Practical Considerations in the Handling and use of Kuron", "A Brush Filler Formulation", and "Kuron Toxicological Information and Suggestions Regarding Treatment for Persons Swallowing Kuron", should provide you with the desired information.

2,4-D Acid

Both the single dose and the chronic oral toxicity, on the 2,4-D acid is reported in the two additional reprints entitled, "Summary of Toxicological Information on 2,4-D and 2,4,5-T Type Herbicide and an Evaluation of the Hazards to Livestock Associated with Their Use." by V. K. Rowe, and T. A. Hymas, and "Toxicity of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid", by Victor A. Drill, and Lomiharu Hiratzka. Eye and skin irritation studies on rabbits indicate that the undiluted acid may cause marked irritation even corneal injury while strong solutions may cause serious eye damage. A single short skin contact should cause at most only minor irritation. However, prolonged or frequently repeated skin contact may lead to moderate irritation even a superficial burn. The acid apparently is not likely to be absorbed through the skin in acutely toxic amounts. Dusts of the acid are likely to be irritating to the nose if breathed.

DOW i 551640

MANO73280

17968

May 13 1961

FORMULA 40 herbicide

The 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", also gives the single dose oral toxicity on this product. Skin and eye studies on rabbits indicate that the undiluted product is capable of causing serious eye damage. The eye injury should be greatly reduced if the product is diluted with water. The product may cause mild skin irritation upon prolonged or frequently repeated skin contact. FORMULA 40 is not likely to be absorbed through the skin in acutely toxic amounts.

POW 1 551641

Suggested precautions for both 2,4-D acid and FORMULA 40 herbicide.

A study of the above information and long human experience both in the manufacture and use indicate that neither 2,4-D acid nor FORMULA 40 present any unusual health hazards from handling. The precautions, therefore, are those normally recommended for materials with significant eye and skin hazard. Where exposure is likely, suitable eye protection, such as eye glasses with side shields or their equivalent should be worn to protect the eyes and suitable protective clothing, such as clean coveralls with long sleeves should be worn to protect the skin.

Should exposure occur, the eyes should be flushed immediately with plenty of water for at least 15 minutes and medical attention should be obtained promptly. For skin exposure, the contaminated clothing should be removed and the contaminated skin washed with soap and plenty of water. The clothing should be washed before re-use. Any skin irritation or injury should receive medical attention.

In addition to the above, you may find the enclosed reprint entitled, "Health Hazards Associated with Handling and Use of Herbicides", by V. K. Rowe, of general interest.

Thank you for your interest in our products. If we can be of further assistance, please feel free to ask.

Yours sincerely,

Mark A. Wohl
Mark A. Wohl
Research Toxicologist
Biochemical Research
1801 Rockline

Enclosures (1)

17969

1472

MIDLAND
June 15, 1960

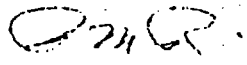
M. G. Norris, Jr.
Oklahoma City, Oklahoma

cc: L. L. Coulter
F. W. Fletcher

Dear Mark:

7-13-167-25
Your ROI Doc. No. 43531, May 12, 1960, regarding preliminary results of toxicology tests with herbicide formulations had several interesting features in it. You mentioned that Esteron 245 at 100 mg/kg fed for a 60-day period to sheep produced no harmful effects. However, Kuron was fatal after 11 daily doses at 100 mg/kg. I am wondering if you have any reasons why Kuron should be so toxic. Do you think this should be rechecked? Of course, I realize that only one test animal was used for each of these experiments. However, we have several new salt formulations of silvex--one in particular, M-1845, which we would also like to have checked by Dr. R. D. Radaleff at Kerrville, Texas, if you think this work can be done. We would be interested in having it done, if you can make arrangements for it, and we will be glad to supply additional samples of chemicals needed. Please let us know.

Sincerely,


Paul M. Ritty
Agricultural Chemicals Development

VM

MN073280

DOW 1551603

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DDW 1 551553

MM073280

December 14, 1961

John H. Risken, Esq.
Riskin and Harron
Attorneys at Law
Power Block
Helena, Montana

Ref: 747

Dear Mr. Risken:

This is in reply to your letter of October 17th inquiring as to what studies we have made on various 2,4-D and 2,4,5-T type herbicides on human subjects. First, I wish to apologize for the tardiness in getting an answer back to you. Your letter arrived about the time I left for Montana on a month's hunting trip and I have had difficulty getting caught up since my return. I was in Helena twice during the trip having stopped to leave some trophies with Bob Miller and to visit with a friend, Harry Joslin. Had I known of this I would have been glad to discuss it with you there.

In direct answer to your question, we have not conducted comparable studies on human subjects. We have, however, conducted tests on human skin with a number of such products and we have had a lot of human experience in manufacturing, handling, sale and use of these materials over many years. They are among the safest agricultural chemicals known. The hazards associated with their use as recommended are not appreciable. This, of course, does not mean that a person could not, if he were a mind to, swallow enough of the material to produce serious injury or even death. To have inhaled enough of the material to have caused death seems impossible.

You note that the claim was made that death was a result of using spray for a very short time of a product called "Low Volatile Brush Killer No. 2". I do not have any specific information on Low Volatile Brush Killer No. 2, but I am told it is a product typical of the commonly available esters of 2,4-D and 2,4,5-T. If this is the case, then the above comments apply; if not, then of course, they do not.

I would certainly appreciate it if you could give me more details of the operations and the alleged ultimate cause of death as this might shed some further light on the case. A detailed description of the claim, any available medical reports or the like would be helpful.

17971

J. H. Nicken, Esq.

- 2 -

December 14, 1961

If we can be of any further help, please let me know.

Sincerely Yours,

V. K. Rowe
Biochemical Research Laboratory
1701 Building

VKR/jd

cc: D. Koehlinger
V. K. Rowe (2)
T2.30-0-30
T2.30-13-30 ✓
Correspondence

DOW 1 551554

THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

May 19, 1953

Mr. G. B. Major
Missouri State Highway Department
Kansas City, Missouri

Dear Mr. Major:

Mr. Hubert B. Crain of our St. Louis Office has informed me that one of your employees has experienced skin irritation of the feet after becoming wet while using a 2,4-D - 2,4,5-T formulation. Mr. Crain also asked me to pass on to you the information we have regarding the skin irritating properties of such materials.

Results of rather extensive laboratory work together with experience in the manufacture and use of these type materials has led us to the following conclusions:

- (1) 2,4-D and 2,4,5-T have essentially the same effect upon the skin.
- (2) Concentrates such as the commercial formulations are somewhat irritating to the skin if exposures are prolonged or frequently repeated. Occasional contacts of short duration are not likely to result in any irritation.
- (3) Material diluted with water for spraying is not appreciably irritating to any except perhaps the hypersensitive individual.
- (4) Material diluted with diesel oil or kerosene will be distinctly more irritating than water sprays. Diesel oil or kerosene alone will cause serious irritation if confined to the skin beneath clothing or shoes.
- (5) Patch tests on 200 human subjects with several commercial formulations, including Dow Esteron Brush Killer, have indicated that these materials are not likely to induce hypersensitivity nor to cause irritation under ordinary conditions of use.

The difficulty you have encountered is of particular interest to me since it is most unusual for skin irritation to result from the use of these materials. I would certainly appreciate very much receiving all the details you can give me as to the materials used, manner of application, to what was the workman exposed, duration of exposure, when with respect to his exposure did he first notice or

MN073280

DOW 1 551491

Mr. C. B. ...

- 2 -

May 19, 1955

feel the irritation, a description of the injury, information with regard to treatment is received, and how serious was the injury.

Inasmuch as you are handling and using herbicides, I am enclosing for your files a reprint of a general article entitled "Health Hazards Associated With Handling and Use of Herbicides", which I prepared for a symposium on the subject at the Northeastern Weed Control Conference a year ago. Perhaps it contains information that will be useful to you.

Again, I would appreciate any information you can give me and if I can be of any help at any time, please do not hesitate to call on me.

Sincerely yours,

V. K. Rowe
Biochemical Research Department
2-220 Building

VKR/jw

Encl.

cc: H. D. Grain, St. Louis Office
H. L. Smith
A. L. Dietzel

DOW 1 551492

17974

DOW CHEMICAL INTER-AMERICAN LIMITED

CABLE: DOWPANAM

1714 EASTMAN ROAD
MIDLAND, MICHIGAN U.S.A.

May 15, 1953

DOW 1 551489

MN073280

Mr. Murray F. Humes
General Purchasing Agent
International Packers Limited
135 S. LaSalle Street
Chicago 3, Illinois

Dear Mr. Humes:

Mr. Ball has turned over to me your letter of May 5,
regarding your request for more information on our
Esteron Brush Killer.

You have seen my letter to your Brazilian representative
and I can add very little to it as to any possible
irritating cause or effect on the operator's stomach
from using either our Esteron Brush Killer or our Esteron
245. These materials have been very widely used in both
this country and abroad and the letter from your Mr.
Irwin is the first case we have ever had reported to our
attention where it was thought there was a nauseating
effect caused by the use of Esteron Brush Killer. In
view of this, I feel certain there is some other factor
which brought about the described effect other than the
use of our brush killer.

It has been the general experience of commercial opera-
tors in this country that the use of Esteron Brush Killer
is more economical than the use of Esteron 245 alone.
This is particularly true when controlling brush in an
area in which you have many different kinds of species
growing. However, it is also true that some species of
brush are more susceptible to Esteron 245 and are almost
completely unaffected by Esteron 1010, which is the 2,4-D
ester in our brush killer mixture. It is also true that
there are some species of brush that are quite susceptible
to Esteron 1010. In general, however, Esteron 245 is more
effective on a wider number of species in controlling
brush than Esteron 1010. Due to the economics in the
cost of these two materials, our Esteron Brush Killer was
developed and has proved very satisfactory.

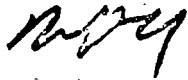
17975

5/15/53

DOW 1 551490

As for the particular situation that prevails in Brazil, I can not answer your question. In the first place, I do not know the species with which you are troubled and probably if I did know then I would have had no experience in trying to eradicate them with any of our materials. I would suggest that if you are interested in determining this exactly, you have your local man carry out some experiments using all three of our materials--Esteron 1010, Esteron 245 and Esteron Brush Killer--to determine which is most satisfactory for the species with which he is bothered under his conditions.

Very truly yours,



W. R. Mullison
Assistant Technical Director

gb

cc: D. B. McCaskey
Montevideo Office
H. R. Hall
Mark A. Wolf

17976



THE DOW CHEMICAL COMPANY

3015 OLIVE STREET
ST. LOUIS 8 MISSOURI

May 1, 1953

MN073280

DOW 1 551488

Dr. V. K. Rowe
BIOCHEMICAL LABORATORYcc:
A. L. DietzelMISSOURI STATE HIGHWAY DEPARTMENT
Kansas City, Missouri

Dear Dr. Rowe:

Mr. G. B. Major, Maintenance Engineer of the subject organization, inquired the other day concerning the effects of 2,4-D or 2,4,5-T in formulations on the skin. One of their employees using our material saturated his clothing to the point where the solution got into his shoes and caused burning effects. This employee has been away from work for several weeks.

We would appreciate very much your writing Mr. Major giving data as to whether the materials mentioned is apt to cause dermatitis which I believe is the injury claimed by their employee. Information as to treatment, et cetera, will also probably be appreciated. We would like to receive copy of your letter.

Kindest personal regards.

Yours very truly,

Hubert D. Crain
Agricultural Chemical Div.

HDC:HG

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UNCLASSIFIED INFORMATION

DOW 1 551234

MN073280

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Various important relationships have alleged by various and other the effects to the environment caused other damage.

are dealing with the those involved in this 10-10 and Esteron 245.

was published an article in the Journal of the American Chemical Society, Volume 111, pages 322-323.

for the purpose of testing the toxicity of 2,4,5-T to certain species of fish. The results of the tests showed that 2,4,5-T was not dangerous to a number of species. It is logical that the oil soluble or emulsified formulations such as you were using would be more toxic to fish. 2,4,5-T itself is very toxic to fish especially when distributed in the water.

Also in 1974, J. E. H. and J. F. P. published in Science, Volume 184, No. 4, pages 372-374, an article entitled, "The effect of 2,4,5-T on the non-Pontogonid herbicides on Duges and large head bass." They found that 1 ppm was without effect and that 100 ppm caused an appreciable mortality. This work was done in conjunction with a study of the control of certain aquatic vegetation, such as water hyacinth, and the conclusion was drawn that the concentrations applied to such vegetation presented no problem to fish but that the mass of decaying vegetation reduced the oxygen content of the water so that it was detrimental to fish.

In work which has been conducted in our water treatment laboratory emulsifying the same chemical mixture, we have found that 2,4,5-T ester such as used in Esteron 10-10 is toxic to fish concentrations of above 1 ppm and that 2,4,5-T ester is toxic above concentrations of 10 ppm. It is also interesting to note that this mixture was commonly used as a herbicide (such as

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Therefore, after 8:00 a.m. I contacted Dr. Hymas, Dr. Morton, Dr. Johnson, Larry Southwick, and Ed Coon. As a result of these discussions, Ed Coon, Dr. Hymas, Dr. Leasure and myself left for Harbor Beach. We arrived there shortly after lunch and talked with the people concerned. It seems that the right-of-way had been sprayed by the Huron County Road Commission with a proper mixture using 5 gallons of Esteron 10-10 and 2 1/2 gallons of Esteron 245 in 1000 gallons of water. The material had been applied to the roadside on the north side of the pasture on July 8th. The cattle became sick and started to appear ill on or about July 15th. Two were stated to have died during the week of July 24th, and one was killed for autopsy just before we had arrived at the Schucker farm. This animal was down and since death was imminent, she was killed for the State Veterinarian and samples taken, presumably for chemical and microscopic analysis. We also visited the carcass and took portions of the liver, kidney, spleen, rumen contents, and lower intestines. The only evidence of abnormalities that we could detect upon gross examination were areas of marked hemorrhage in the small intestine and in the bladder, and an apparent lack of fermentation in the rumen. The urine was not bloody.

Samples of the rumen contents, liver, kidney, and a section of the lower intestines were brought to the laboratory on the evening of July 29th when I personally placed them in our freezer. Appropriate portions of the liver, kidney, and spleen

DOW 1 551213

were placed at this time in formalin for subsequent microscopic examination. On August 1, I personally delivered to Mr. Luce at the Analytical Laboratory the frozen samples for analysis.

We examined the herd which Mr. Schmucker had in the barn and in the feed lot. He had taken them out of the pasture several days before and was feeding them hay. It appeared that three of the sick animals were down in the barn and one of them we were able to get up but the other two never did get up. One was reported to have been up and eating earlier in the morning. Her mouth was clean, her nose was wet, her eyes were dark and somewhat protruding and mucous membranes were a light chocolate color. The rumen was not active. There were no skin lesions visible on any of the animals. Some of the animals obviously were very weak, particularly in the hind quarters and walked stiff legged. The hocks of two of the group had a peculiar stiffness in which the ankles of the hind legs protruded forward and the animals appeared to be walking on their toes. There was considerable unsteadiness in a quite a few of the animals in the herd. The calves appeared to be in good health except for one of the larger ones that apparently was affected the same as the affected cows. The other calves seemed very frisky. About half the cows and the herd bull appeared to be in excellent condition with no signs or symptoms of illness. All of the animals had been examined by a veterinarian in Harbor Beach, Dr. Henry Jurgens,

DOW 1 551214

and a consulting veterinarian as well as a State Veterinarian, Dr. Quinn who had just left when we arrived. We talked with Dr. Quinn and he was at a loss to explain the difficulty. He lost a few minutes, however, and suggested that Mr. Schmucker drench all of the ill animals that evening.

He toured the premises and noted that there was a waste product from the Haron Milling Company in Harbor Beach, which was a white aqueous suspension of flour and middlings which Mr. Schmucker had said that he had been using for years. This material is hauled away from the mill by a number of trucks working continuously making delivery to farmers all over that area. Upon questioning the veterinarian, he stated that there were no other cases of sick cattle around the area and that a number of farmers had been using this waste product as a source of food for their stock. It would seem that if this material were responsible that others would have had similar trouble.

Dr. Leasure toured the pasture along the roadside that had been sprayed and generally over the pasture area and found no evidence of poisonous weeds to which the trouble could be associated. Over grazing or other evidence which would suggest anything unusual was also absent. He stated that if there was any drift from the roadside spray, it was only very slight and in his opinion was of no consequence. He examined the bean field referred to by Mr. Schmucker and said that there was little if any damage to it although a trace of injury could be found in

DOW

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certain spots. There was one circumstance which was chronologically related to the poisoning, and that was the occurrence of heavy rain and a flash flood that went through the pastures on July 19th. It was immediately after this flood that the illness of the cattle became apparent. Whether or not this had anything to do with it is totally unknown.

At the present time, we have no explanation for the illness of a number of Mr. Schmucker's cattle or for the deaths which occurred. In our opinion, these illnesses are in no way related to the spraying along the roadside by the Huron County Road Commission.

Dictated 8-4-55
BY V.K.R.

Transcribed 8-5-55
BY J.C.W.

cc: G. J. Williams
T. A. Hymas
K. Leasure
K. C. Barrons
J. E. Johnson
T. R. Norton

DOW 1 551216

Mr. Henry C. Berry
Director, State Chemist

● DOW 1 551197

Dear Mr. Berry

Allan, Jr. Co. Ltd. had previously suggested that I contact you concerning problems that I have.

Several years ago, I was using Septinon (Liquid) on alfalfa and my wife became allergic to the fumes. This happened two years in succession so I stopped using the material.

In July of 1962 the Power Company gave the right-of-way through my property from our home. The fumes from the 2-4-5-T Ecton grade ether affected my wife to the extent that she was under the doctor's care for thirteen weeks and was away from home for that length of time. Under the doctor's recommendation

My concern is: What is in 2-4-5-T that would affect a person's respiratory system, cause severe breathing difficulty and choking?

MM073280

of the time in his explanation.
I would really appreciate let
me know how you are at your
school business.

My best love
I hope it is
best for you.
Charlotte C. H. R.

DOW 1 551198

April 16, 1958

Mr. P. E. Adley, Manager
Industrial Hygiene Operation
Relations and Utilities Operation
Hanford Atomic Products Operation
General Electric Company
Richland, Washington

UNO73280

Dear Mr. Adley:

Dr. Irish has asked me to reply to your letter of April 7th inquiring as to toxicological data we might have with respect to humans exposed to 2,4-D in the form of our product, Esteron 44.

Frankly, I am very happy to report that I have no toxicity information on humans. So far as I am aware there has never been a case of poisoning in humans from the use of any 2,4-D or 2,4,5-T product. We have had a few cases of skin irritation and with each of these, when it has been possible to study the particular problem, it has been found that the person was hypersensitive to the fuel oil used in diluting the product prior to application. These cases, however, have been rather rare when one considers the extensive use of products of this nature. This experience with humans over a number of years has verified the results of skin irritation and skin sensitization tests we had conducted several years ago by a consulting laboratory. They found that Esteron 44 itself was neither a primary irritant nor a skin sensitizer.

In regard to precautions to be taken when one is applying the material for prolonged periods day in and day out, it would only be reasonable to provide sufficient protection to prevent the continuous wetting of the skin and continuous inhalation of spray mists. We are all aware that oils themselves can cause considerable pulmonary edema and injury to the skin. The type of protection used will be determined by the nature of the particular operation involved. If one were to apply the material where he could not avoid being in the spray drift, then I would recommend the use of protective clothing and a respirator designed to remove mists; further, the operators should clean up afterwards. On the other hand, if the application is of such a nature that there is very little exposure,

Mr. P. B. Alley

- 2 -

April 16, 1958

minimal precautions I am sure would be adequate. I feel quite confident that if you exercise good judgement in these situations that you will encounter no difficulty.

I hope these comments will be adequate for your purposes.

Sincerely yours,

V. K. Rowe

V. K. Rowe
Biochemical Research Laboratory
12-634 Building

VKR/ba

cc: V. K. Rowe (2)✓
T2.30-34-20
Correspondence

DOW 1 551189

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MIDLAND
August 23, 1957

Richard J. Darger
Legal Department
47 Building

TOXICITY INFORMATION ON ESTERON 44 AND ESTERON 245 SUITABLE
FOR TRANSMITTAL TO ATTORNEYS IN THE CASE OF RADEL VS. DOW

The data you asked for in your letter to Mr. Rowe for the
case of Radel vs. Dow may be summarized as follows:

TOXICITY OF ESTERON 44

Active Ingredient

The toxicity information on isopropyl ester of
2,4-dichlorophenoxy acetic acid, the active
ingredient of Esteron 44 may be summarized as
follows:

Ingestion

Single oral doses of this material were administered
by intubation to rats, mice, guinea pigs, and chicks
as an olive oil solution. The doses were calculated
as milligrams of the substance per kilogram of body
weight of the animal.

Table I shows the LD₅₀ values calculated by sta-
tistical analysis of the data obtained using the
method of Litchfield and Wilcoxon.

TABLE I

CALCULATED SINGLE ORAL LD₅₀ VALUES FOR ISOPROPYL
ESTER OF 2,4-DICHLOROPHENOXY ACETIC ACID WHEN FED
TO LABORATORY ANIMALS

<u>Species</u>	<u>LD₅₀ Values In Mgm./kg. of body wt.</u>	<u>19/20 Confidence Limits In mgm./kg. of body wt.</u>
Rats	700	570 to 850
Mice	540	400 to 740
Guinea pigs	550	450 to 670
Chicks	1,420	1,130 to 1,790

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 DOW 1 551183

Skin Contact

Repeated application of this material to the skin of rabbits as the undiluted material resulted in but slight irritation and slight edema on repeated prolonged contact (24 hours a day for 12 days). There was no evidence in these tests that absorption through the skin had occurred.

Esteron 44 Formulation

The toxicity information on this formulation may be summarized as follows:

Ingestion

When Esteron 44 was administered to rats as an olive oil solution, an LD₅₀ value of 650 mgm./kg. of body weight was found. (19/20 confidence limits were 300 to 1,000 mgm./kg.)

It should be noted that the formulation has essentially the same acute oral toxicity as the active ingredient.

Skin Contact

Esteron 44 when applied to the skin of rabbits in undiluted form was found to cause moderate hyperemia, edema, and eventually necrosis. This response is typical of kerosene when tested under similar conditions. The formulation, diluted to use concentration (1%), had no significant effect on the skin of rabbits. Furthermore, there were no indications that this formulation was absorbed through the skin in acutely toxic amounts.

Human skin tests done by the Industrial Toxicology Laboratories of Philadelphia on a similar formulation containing another ester of 2,4-dichlorophenoxy acetic acid indicates that Esteron 44 should be neither a primary irritant nor a sensitizing agent. Very wide experience in the manufacture and use of this product confirms these conclusions.

August 23, 1957

TOXICITY OF ESTERON 245Active Ingredient

The toxicity information on the polypropylene glycol butyl ether ester of 2,4,5-trichlorophenoxy acetic acid, the active ingredient of Esteron 245 is summarized as follows:

Ingestion

When this material was fed to laboratory animals by intubation as a solution in corn oil, the following LD₅₀ values were obtained by calculation.

<u>Species</u>	<u>LD₅₀ values in</u> <u>mgm./kg. of body wt.</u>	<u>95% Confidence Limits</u> <u>in mgm./kg. of body wt.</u>
Rat	480	310 to 740
Rabbit	710	500 to 1,000
Mice	940	670 to 1,310
Chick	1,190	850 to 1,570

Work on guinea pigs resulted in a LD₅₀ value in the range of 500 to 2,000 mgm./kg.

Eye Contact

The instillation of this material onto the eyes of rabbits resulted in moderate pain, slight conjunctival irritation, and slight transient corneal injury.

Skin Contact

When the undiluted material was applied to the skin of rabbits for repeated prolonged periods of time (24 hours a day for 12 days), a mild but definite irritation developed. In these tests no evidence of absorption through the skin was observed.

Inhalation

Rats subjected to essentially saturated vapor of this material generated at 100°C were not observed to be affected adversely.

DOW 1 551185

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Esteron 245 Formulation

The toxicity information on this formulation is summarized as follows:

Ingestion

When rats were fed single doses of this formulation by intubation as an olive oil solution, a calculated LD_{50} value of 800 mgm./kg. of body weight was found. The 19/20 confidence limits were calculated to be 600 to 1,000 mgm./kg.

Eye Contact

The undiluted formulation was found to be slightly irritating and caused slight transient corneal injury when instilled onto the eyes of rabbits. Dilution of this formulation to a 10% concentration resulted in only very slight pain and transient irritation of the eyelids when tested in a similar manner.

Skin Contact

The undiluted formulation caused slight to moderate irritation and slight edema when in repeated prolonged contact with the skin of rabbits, especially when confined. Strong solutions (10%) caused only slight irritation and a "chapping" under similar conditions.

In tests conducted by the Industrial Toxicology Laboratories on 200 human subjects, the formulation was found to be neither a primary skin irritant upon a single prolonged contact, nor a sensitizing agent. (The material was applied once to the backs or forearms of 100 unselected male and female subjects each for an exposure period of five days. The sensitizing dose was applied about three weeks later.)

INTERPRETATION

Based upon the above data, the following interpretations can be made.

DOW 1 551186

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MIDLAND
August 5, 1959

Nels H. Munson
Insurance Manager
Legal Department
47 Building

This is in reply to your letter of August 3 requesting information to send to the Fireman's Fund Insurance Company in regard to alleged injury to cattle from the use of Dow Esteron 2,4,5 Brush Killer.

I believe, at this point, the best thing we can do is to supply the insurance company with reprints of the attached articles. One entitled, "Some Effects of Herbicides on Pasture and on Grazing Livestock" by Grigsby and Farwell, and the second 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" by Rowe and Hymas. You might add in your letter that we concur with the opinions of the claim adjustor, Mr. Tubbs, that it is very unlikely that a brush control treatment had anything to do with the illness of these cattle.

If we can be of any further help, let me know.

V. K. Rowe
Biochemical Research Laboratory
12-534 Building
Phone - ME 6-2375

VKR/vmv

cc: V. K. Rowe (2)
T2.30-3-30
Correspondence

●DOW 1 551797

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THE DOW CHEMICAL COMPANY

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MIDLAND, MICHIGAN
August 3, 1959

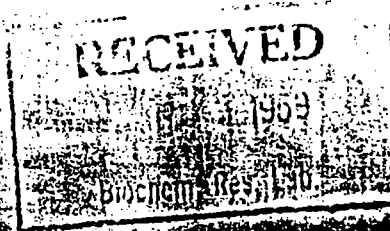
V. K. Rowe
Biochemical Research Lab
634 Building

I am enclosing a letter received from Fireman's
Fund Insurance Company regarding an alledged in-
jury to cattle resulting from the use of Dow's
2-4-5 Brush Killer.

Inasmuch as we are not involved at this state but
possibly could be involved, please tell me what I
can pass on to these underwriters who are attempt-
ing to protect the defendant who used our brush
killer.


Nils H. Munson
Insurance Manager

17984



MIDLAND

July 17, 1988

Jim Johnson
P. O. and L.
Sarnia, Canada

cc: E. L. Wilson
Paul Jilky
Mark Wilcox
V. K. Howe
H. H. McIntyre
Action File-Kurosai SL

V. K. Howe of the Biochemical Research Laboratory has related to me the problem you are having in getting the Department of Lands and Forests to approve the permits to use Kurosai SL as an aquatic herbicide in certain Canadian lakes.

You indicated to Mr. Howe that you needed a statement on the label indicating when the water from such treated lakes would be fit to drink or as an alternative some instructions for treating the water, such as boiling, to dissipate the chemical residues.

We have made several tests to determine the persistence of Kurosai SL in lake water following applications and find that in certain instances the silvex will persist through the entire summer. We have no reason to believe that boiling would reduce the residue of silvex in water.

Although we do not recommend Kurosai SL for potable waters, we do not believe that there is any human or animal health hazard from the occasional use of water treated with the recommended concentrations of Kurosai SL. In support of the conclusion we have available a 90-day dietary feeding study in rats. I am enclosing duplicate copies of this information which can be supplied to the Canadian authorities if you feel that this would be helpful.

We have recently set up a 2-year dietary feeding study of silvex (active ingredient of Kurosai SL) with rats and dogs and, as usual, we will be glad to furnish the Canadian government with the results of these tests when they become available.

We trust this information will be of assistance to you and if you have any further questions, please advise.

Sincerely,

Original Signed By:

C. E. LYNN

C. E. Lynn

Bioproducts Department

abc

UOW 1 551686

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NOT FOR OUTSIDE DISTRIBUTION

ACD Project Status Report - Document No. 21529

SUMMARY OF AQUATIC HERBICIDE INFORMATION
ON KROSAL SL (M-1845) AND KROSAL G (M-1847)

by

Paul M. Ritty

Agricultural Chemical Development

The Dow Chemical Company

Midland, Michigan

February 8, 1961

Copies to:

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D. L. Conner		

DOW 1 551676

MAN 073280

OUTLINE FOR AQUATIC HERBICIDE STATUS REPORT

Summary

Introduction

Compound Identity

Corrosion Studies

Analytical Methods

Toxicology

Mammalian Toxicity

Fish Toxicity

Weed Toxicity

Residue Problems

Water: Odor & Taste

Fish Flesh Effects

Patent Status

Compound Herbicide Activity

Aquatic Weeds Controlled

Speed of Activity

Aquatic Environment Effects

1961 Promotions Plans

Test Marketing Areas

Ag. Chem. Development Plans

Specimen Label

DOW 1581677

SUMMARY OF AQUATIC HERBICIDE INFORMATION
ON KUROSAL SL (M-1845) AND KUROSAL G (M-1847)

by

Paul M. Ritty

DOW 1-551678

SUMMARY

The potassium salt formulations have shown control of a wide variety of submerged aquatic weeds. When applied according to directions, herbicide activity has been equal to the ester form of silvex, Kuron. These salt formulations control a wider range of aquatic species than those which are controlled by 2,4-D formulations.

The toxicity picture of Kurosals SL (M-1845) and Kurosals G (M-1847) are better in regards mammalian toxicity and are considerably better in regards fish toxicity than are those exhibited by Kuron. Residue problems such as water odor and taste thresholds are higher and there is less effect on fish off-flavor.

Dow has no patent status in regards to Kurosals SL and Kurosals G, since these are covered by previous Jones and ICI patents.

INTRODUCTION

The potassium salt formulations have been evaluated in various areas of the United States for the control of submergent and floating aquatic weeds by a number of experiment station, fish and game, and federal and state representatives. Because of many of the inherent safety factors in the potassium salt formulations, interest has grown in various areas of the country. Much investigational work has been completed to date on the potassium salt and, prior to the release of the potassium salt for development, on the sodium salt and the ester formulation of silvex (Kuron). Much of the information is interchangeable, however, the major portion of this report will deal primarily with the potassium salt activity unless otherwise stated.

COMPOUND IDENTITY

1. KUROSAL SL (M-1845)

The composition of Kurosals SL is as follows:

Silvex	53.3 %
KOH	11.1 %
Versene	0.05%
Water	35.6 %

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The formulation contains six pounds of acid equivalent per gallon, and has a specific gravity at 68°F. of 1.351. Kurosai SL, with the pH adjusted between pH 7.3 and pH 13, showed freezing points somewhere between 0 and 3°F. Crystals do not form upon freezing, and the formulation reconstitutes to homogeneous solution at 15°F. to 17°F. Even when the solution has been seeded with potassium salt crystals, no difference in freezing or in reconstitution temperatures has been noted. Stability of Kurosai SL is constant, providing the compound is not allowed to evaporate. If evaporation of the water content occurs, the compound becomes somewhat thickened. Since Kurosai SL is a metal salt, flocculation does occur in hard waters of 200 ppm or more. Flocculation may also occur under some conditions in waters of less hardness than 200 ppm. Therefore, the formulation does not lend itself to pre-mixing before spraying.

Studies with the sodium salt of silvex show this material to be very persistent in water systems in which there is little water exchange. In earlier studies on the sodium salt of silvex, a bioassay experiment indicated the continued presence of the sodium salt of silvex in various waters for the duration of the test whether the systems had been kept under refrigeration or in the warm greenhouse.

CORROSION STUDIES

No problem to date has occurred in tests on tin, galvanized iron, or black iron. The possibility of corrosion in the vapor space above the stored liquid should constitute no problem. The material will be shipped in unlined drums.

2. KUROSAL (M-1847)

The composition of formulation Kurosai G is as follows:

Silvex potassium salt	22.8%
815AA-RVM Attaclay	77.2%

This formulation contains 20% acid equivalent. In physical and chemical property studies on this formulation, the stability of these granules appears to be excellent. There has been no formation of crystals from sublimation. Kurosai G is adsorbed to the attaclay particle surface. Release of chemical upon contact with water is believed to be accomplished almost immediately. There are no problems in regards to storing this formulation. The material will be shipped in fiber drums.

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DOW 1 551680

ANALYTICAL METHODS

A method for determining potassium silvex in water by the use of infrared determination has been perfected by the Dow Spectroscopy Laboratory. The procedure makes it possible to detect 0.02 ppm in one liter of water with an accuracy of plus or minus 0.01 ppm.

There is no simple water analysis that can be made at the treatment site. However, this should not pose a problem since toxic amounts of potassium salt will not occur in a treatment area when directions on the label are followed.

TOXICOLOGY

Mammalian Toxicity

Acute oral toxicity studies on rats, using the potassium salt, showed no marked differences between this salt and the previous work on sodium salt on which extensive tests had been run at an earlier date. According to V. K. Rowe, there is no possibility of skin irritation expected by swimmers in the area where the potassium salt is being applied as an aquatic herbicide.

Reports from the toxicology lab have suggested precautionary labeling for Kurosai SL (M-1845) as follows: "This material has low acute oral toxicity. Eye contact may result in severe irritation and severe damage. It may cause moderate irritation and moderate damage upon prolonged skin contact. It apparently may be absorbed through the skin in acutely toxic amounts. It presents no problem from inhalation of vapor or fumes." The warning statement, which will be on the label of Kurosai SL, the liquid formulation, will be as follows: "Warning - Burns the eyes. May be absorbed through the skin. Do not get in eyes. Avoid contact with skin and clothing. In case of contact, flush eyes immediately with plenty of water for at least 15 minutes and get medical attention; for skin, flush with plenty of water. Wash contaminated clothing before reuse."

In regards to precautionary labels for Kurosai G (M-1847), the following statement has been made: "This material has low acute oral toxicity. Eye contact may result in mild irritation and no damage. It may cause minor irritation and no damage upon prolonged or repeated skin contact. It apparently is not readily absorbed through the skin in acutely toxic amounts. It presents no problem from inhalation of dusts." No additional warning statement will be needed for Kurosai G.

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Fish Toxicity

Twenty-four-hour exposure of Lake Emerald Shiner to 0, 5, 10, 25, 50 and 100 ppm of Kurosai SL gave no mortality at 25 ppm, and 100% mortality at 50 ppm. In additional tests on fish toxicity, an acute 72-hour toxicity tests showed that the toxic level for Kurosai SL appeared to be greater than 150 ppm. However, in another 31-day toxicity test performed in 55-gallon drums, primary dilution of Kurosai SL was fed to the fresh water flow at a rate to give a final concentration of 10 ppm acid equivalent with a continuous flow-through rate of approximately two liters per minute. Results showed a slight indication that Kurosai SL may exhibit some long-term toxicity to brown trout at 10 ppm, however, additional comprehensive studies would be required to verify this. In this 31-day toxicity test, no deleterious effects were exhibited by fathead minnows, brook trout or yellow perch.

Toxicity to Aquatic Weeds

As will be seen later in this report, the weed toxicity of Kurosai SL and Kurosai G is equal to that obtained through standard use of our present Kuron formulation. A wide variety of species of aquatic weeds are controlled or effectively reduced through the use of the potassium salt of silvex.

RESIDUE PROBLEMS

Water: Odor and Taste

It is well known that the Kuron formulation in relatively low parts per million can cause an off-flavor in water. Studies conducted by Winston prior to July 1959 found that the Penola Heavy Aromatic Naphtha used in the Kuron has an odor threshold of 0.2 ppb (billion). Studies completed on February 15, 1961 showed the odor threshold of Kurosai SL to be 1.9 ppm (million), much higher, and safer, than the odor threshold of Kuron, or more specifically, of Penola Heavy Aromatic Naphtha.

Fish Flesh Effects

Test fish (perch) from a 31-day toxicity test were subjected to routine taste procedure tests for the detection of off-flavor in fish flesh. Fish exposed to Kurosai SL had a very slight off-flavor that was detected only by the most sensitive tasters. On the whole, it was the consensus of the taste panel that what off-flavor there was was not objectionable and that it would not be noticeable if there were no control fish with which to be compared.

DOW 1651681

18001

PATENT STATUS

A literature search has found that the potassium salt is covered in some early Jones and ICI patents covering the aryloxy, aliphatic and phenoxy propionic acid derivatives. Therefore, Dow does not have a patentable position on the potassium salt of silvex. We are licensed to sell herbicides under both the Jones and ICI patents, however, and no royalties would have to be paid. This means, however, that any company that can obtain silvex acid could manufacture and sell the potassium salt. At the present time, The Dow Chemical Company enjoys the position of being the only manufacturer of silvex acid. Should the demand for silvex acid become increasingly great, however, there is a possibility that other chemical companies could manufacture silvex acid in this country or that greater pressure could be applied from foreign manufacturers. Until such occurrences happen, even though other companies would purchase silvex acid for the purpose of manufacturing potassium salt, Dow would enjoy increased sale of silvex acid.

DOW 1551682

COMPOUND HERBICIDE ACTIVITY

Aquatic Weeds Controlled

Numerous reports of tests by co-operators have reported that the following aquatic weeds have been controlled at various rates. Listed below are the aquatic weeds, scientific and common names of only those weeds reported from tests which gave accurate reporting. Many other reports have contained comments concerning the "control" of other aquatic weeds, not mentioned, but the remainder of the data in the reports were subject to question. Hence, only those listed below are results of accurate testing and reporting.

Scientific Name

Common Name

Anacharis spp.
Cabomba spp.
Ceratophyllum spp.
Eichhornia crassipes
Eleocharis sp.
Jussiaea grandiflora
Jussiaea sp.
Leersia sp.
Myriophyllum spp.
Najas spp.
Nasturtium officinale
Nelumbo pentapetala
Nitella sp.
Nuphar advena
Nymphaea odorata
Potamogeton capillaceus
Potamogeton crispus

Elodea
Fanwort
Coontail
Water hyacinth
Spikerush
Water primrose
Primrose willow
Cutgrass
Water milfoil
Naiad
Watercress
Water lotus
Nitella
Spatterdock
White water lily
Variable pondweed
Pondweed

18002

Scientific Name

Common Name

Potamogeton epihydrus	
Potamogeton pectinatus	Sago pondweed
Potamogeton sp.	Southern pondweed
Ruppia sp.	Widgeon grass
Salix sp.	Waterwillow
Sparganium eurycarpum	
Sparganium spp.	Burreed
Utricularia spp.	Bladderwort

Speed of Activity

Reports received from 1959 and 1960 test results showed great variability in the time needed to control any of the above species. Time varied from one week to three months before complete control was obtained. On an average, it appears that control can be expected in approximately six weeks after treatment.

Aquatic Environment Effects

No accurate results have appeared from the tests conducted prior to this report in regards to extremes of herbicide activity due to pH. We have pH ranges in the reports ranging from 5.5 to 9.0 with apparently little noticeable effect on herbicide activity. We do have numerous reports, however, indicating that any time that Kurosol SL was pre-mixed and applied then as a diluted spray, the activity was not as good as occurred where the material was introduced directly into the lake as the concentrated chemical. Hose proportionaters or Venturi metering systems apparently can be used without seriously affecting the activity of the chemical.

Water temperatures were reported on tests applied at 65°F. water temperature and above. We have no Reports of Outside Information indicating chemicals applied to colder waters than this. However, undoubtedly, some of the northern experiments were applied to waters cooler than 65° F. without deleterious effects. Generally speaking, laboratory information shows water temperatures of 55°F. and above should be safe minimal limits for application of the potassium salt of silvex.

1961 PROMOTIONAL PLANS

Test Marketing Areas

At the present time, test marketing of Kurasol SL and Kurasol G will be conducted in the following areas of the country:

1. Michigan - Test marketing in three regional fish commission areas of southern Michigan in recreational

DOW 1551683

18003

waters. Main emphasis will be establishing dealers and users who will be do-it-yourself applicators, that is, lakefront lot owners. Kurosals G will be promoted for this purpose. Also in Michigan, we will be working with a custom applicator who will sell Kurosals SL on a custom applicator basis for the treatment of large portions of, or total, lake areas.

2. In the Northeast - We will work directly with two large custom applicators, one from Boston, Massachusetts and the other from Philadelphia, Pennsylvania, whose areas cover the North Atlantic seaboard states. Kurosals SL will be the major product moved through them; however, they will have access to Kurosals G if their custom application work calls for it.
3. State of Georgia - SPADE will work directly with the State Game and Fish Commission who are the major custom applicators for the State of Georgia. Probably both granular and liquid formulations will be promoted through them in the State of Georgia.
4. Possible distribution may be set up through present Dow distributors in San Antonio, Texas, and Norman, Oklahoma, working with custom applicators and with both formulations.

Agricultural Chemical Development Plans for 1961

1. Nation-wide investigations of Kurosals SL (M-1845) and Kurosals G (M-1847) will continue as was conducted during 1960 in order to obtain more meaningful data from the test areas. Reporting forms will be sent to each of the Ag. Chem. Development field specialists for distribution to co-operators. Continued effort, soliciting state and federal game and fish commissions, public health agencies, and fish and wildlife personnel, as well as experiment station investigators, should be continued for this year so that a more complete group of data can be obtained for possible nation-wide sales in 1962.
2. CRI Intra-Company Sampling - A plan is now underway for sampling to Midland employees of The Dow Chemical Company who have lakefront lots in an effort to obtain additional information on the use of the granular formulation by lakefront cottage owners. Results from such a test program through the Midland employees should give a wide range of aquatic conditions in cold and warm recreational waters within the State of Michigan.

DOW 1551684

18004

CONCLUSION

As will be seen from the foregoing information on Kurosai SL and Kurosai G, we have two formulations which have shown considerable ability as aquatic herbicides, increased safety factors over presently available formulations of Kuron or competitive compounds, and a reduction in these formulations of some of the inherent hazards found in presently commercially available aquatic herbicides. We need additional good records of field successes with these two formulations. We should aggressively pursue the development of these products during 1961, so that we can obtain additional information as to the market desires of the aquatic herbicide field.

DOW 1 551885

1500
DOW 1 551274

MN073280

MIDLAND
May 22, 1959

Milton Irvine
Agricultural Chemical Sales
Abbott Road Building

TOXICOLOGICAL INFORMATION ON KURON FOR OUTSIDE DISTRIBUTION

This is the information I promised you over the phone. It is my understanding that it is to be sent to a company who will be using Kuron to formulate new products. I hope the following information proves adequate for your purposes.

KURON

Toxicity

Kuron has a low acute oral toxicity. It does not present a hazard from ingestion under the usual conditions of handling and use. However, injury could occur if large amounts were swallowed. The LD₅₀ values for a number of species are as follows:

<u>Species</u>	<u>LD₅₀ in g/kg of body weight</u>
Rat	1.07
Guinea pig	0.85
Rabbit	0.85
Mouse	2.14
Chicks	2.0

Kuron presents no unusual hazards from skin and eye contact. It may be expected to cause, at most, only mild irritation when in contact with the eye. Skin contact, for repeated prolonged periods of time, may cause, at most, only a mild irritation. It apparently is not absorbed through the skin in toxic amounts. In addition, results of human skin tests with Kuron diluted to 1.0 percent with water indicate a high degree of assurance that Kuron should not cause skin irritation nor skin sensitization when used as directed.

Suggested Precautions for Handling Kuron Safely

Suitable eye protection such as safety glasses may be worn to protect the eyes simply to avoid the discomfort that may occur upon contact. Reasonable care and personal cleanliness should be practiced to avoid skin difficulties.

Milton Irvine

- 2 -

May 22, 1959

UW 1 551275

Recommended First Aid Measures

Should the eyes become contaminated with Kuron, they should be flushed with plenty of water. Any irritation or injury that develops should receive medical attention.

Grossly contaminated skin should be flushed with plenty of water. Grossly contaminated clothing should not be re-used until cleaned. Any skin irritation that occurs should receive medical attention.

We plan to write up a suitable bulletin on Kuron for outside distribution. It should be available in about 3 to 5 weeks.

- Mark A. Wolf -

Mark A. Wolf
Biochemical Research Laboratory
634 Building
Phone ME 6-2776

MAW/jd

cc: V. K. Rowe
M. A. Wolf
T2.30-40-20

18007

January 2, 1959

Dr. J. V. Klauder
1934 Spruce Street
Philadelphia 4, Pennsylvania

Dear Dr. Klauder:

As per our telephone conversation, I am writing you regarding your doing for us a skin irritation and skin sensitization study on humans with a product of ours known as Kuron. This product is an emulsifiable composition of the propylene glycol butyl ether esters of 2(2,4,5-trichlorophenoxy)propionic acid. This material has shown considerable promise as an aquatic herbicide which may be used in lakes, etc. where swimmers might come in contact with it in diluted form. It will be used for such purposes in concentrations of 3.5-4.0 ppm.

As you know, our general policy for marketing a product which may come into contact with the general public, even in dilute solutions, is to have data in our files regarding its skin irritation and sensitization properties on humans. Our laboratory tests on rabbits with this material have shown that a concentration of 1% may cause a trace of irritation when contact is prolonged and repeated. At 10%, it is mildly irritant upon prolonged and repeated contact and undiluted, it is moderately irritating. It is not absorbed through the skin in toxic amounts. This is typical of most herbicides of this type which are commonly used and handled today. This material is not markedly different than some of those which you tested some years ago for us in conjunction with the late Dr. Shelanski.

As we agreed, I suggest a pilot study at concentrations of 1% (10,000 ppm), 0.1% (1,000 ppm), and 0.01% (100 ppm), all in water, plus whatever additional concentrations need be checked on humans to determine the maximum concentration not likely to cause primary irritation. I want the sensitizing applications to be made with as high a concentration as practicable so as to establish as large a margin of safety as possible.

As we agreed further, the study will be of the repeated insult type on 50 human subjects and the cost to us will be \$750.00.

Dr. J. V. Klauder

-2-

January 2, 1959

As you know, I usually do not send single compounds out but wait until we have a group to study, however, our people are in a hurry for studies on this one and I am wondering if you could do it promptly. We want to have a report by March 15, 1959 as some decisions regarding it must be made then.

If you have any questions, please do not hesitate to call me.

With best personal regards.

Very truly yours,

V. K. Rowe
Biochemical Research Laboratory
12-634 Building

VKR/vmv

cc: V. K. Rowe (2)
T2.30-40-20 -
T2.30-40-3
Correspondence

DOW 1 551265

18009

● DOW 1 551262

MN073280

December 5, 1958

Albert M. Kligman, M.D., Ph.D.
Associate Professor of Dermatology
Hospital of the University
of Pennsylvania
36th and Spruce Street
Philadelphia 4, Pennsylvania

Dear Dr. Kligman:

Some months ago you visited our laboratory and discussed skin work with Dr. Irish and others. I am sorry I was not here at that time for I would have enjoyed such a discussion. Since then we have been planning to use your services when the opportunity arose. With this background, I am writing to discuss with you the possibility of your doing for us a skin irritation and skin sensitization study on humans with one compound.

This material is a product of ours known as Kuron. It is an emulsifiable composition of the propylene glycol butyl ether esters of 2(2,4,5-trichlorophenoxy) propionic acid. This material has shown considerable promise as an aquatic herbicide which may possibly be used in lakes etc. where swimmers might come in contact with it in diluted form. As a general policy before marketing a product which may come in contact with the general public, even in dilute solutions, we want to have data in our files regarding its skin irritation and sensitization properties. Our laboratory tests on rabbits with this material have shown that a concentration of 1% may cause a trace of irritation when contact is prolonged and repeated. At 10%, it is mildly irritant upon prolonged and repeated contact and undiluted, it is moderately irritating. This is typical of most herbicides of this type which are commonly used and handled today.

In thinking about what might be an appropriate study, it seemed to me that we should check decreasing concentrations of Kuron in water to find the maximum concentration which will not cause primary irritation in humans and call this concentration, (X). I would propose then a repeated insult study by the Shefiandsky and Shefiandsky method on 50 humans applying 20% of (X) 9 times in 3 weeks and then challenging two weeks later with 10% of (X). This would give us basic information on the primary irritant properties and also on the possible capacity to produce sensitization.

18010

When used as an aquatic herbicide, this material is applied so as to give a concentration in the water of about $3\frac{1}{2}$ - 4 ppm. Hence, concentrations, which I suspect can be tolerated repeatedly by humans, will be far above this figure but I believe this is desirable. In other words, I would like to test it in as high a concentration as we can without showing irritations.

I would like your suggestions as to the appropriateness of such a testing program. If you have a program which you think might be better, do not hesitate to suggest it. In any event, I would like your suggestions and an estimate of the charges for such a study together with an estimate of when it could be completed and reported to us.

I might inform you that it is not our usual procedure to send one compound for testing. Usually I save these up until I have several so that they can all be done at once but this time our folks are in a hurry and I do not have any backlog for such testing.

Your early reply will be appreciated.

Sincerely yours,

V. K. Rowe
Biochemical Research Laboratory
12-634 Building

VKR/vmv

cc: V. K. Rowe (2)
T2.30-40-20
Correspondence

*He wants \$1,000
for this - so am
sending it to Klander.
- VKR.*

DOW 1 551263

18011

THE DOW CHEMICAL COMPANY

MIDLAND
October 10, 1958

1506

AN073280

● DOW 1 551258

V. K. Rowe
Biochem Lab
634 Bldg

cc:
Harry McIntyre
C. E. Otis

Subject: REACTION OF SWIMMERS TO KURON

Thank you for your comments on our proposed label copy for use of Kuron in the control of aquatic weeds. We have forwarded a revised proposed label to Harry McIntyre and we anticipate that he will apply for registration in Washington very shortly. Once we know how we stand with the folks in Washington, we can better determine what our approach should be in regard to skin reaction tests. If we get acceptance reasonably soon so that we can forsee a good program in 1959, we will then contact you and it would seem to be a good thing then to take whatever steps are necessary to put us in a better position to defend ourselves. Meantime, until we have a little more assurance of how this thing might go, it might be just as well to postpone these tests.

Yours very truly,



L. L. Coulter
Agricultural Chemical Development

wb

181

MIDLAND

January 31, 1958

L. L. Coulter
Agricultural Chemical Development
American Legion Building

KURON FOR AQUATIC WEED CONTROL

This is in reply to your letter of January 24th in which you asked for our comments on the subject problem. Frankly, we are concerned about the possible liability which may well result from the use of Kuron or any other herbicide for aquatic weed control. I will try to put the various problems as we see them in the following paragraphs.

1. Fish.

The data that has been obtained by A. W. Winston, Jr. in the Waste Disposal Laboratory indicates that a concentration of 5 p.p.m. is probably tolerable for at least a few days to the Lake Emerald Shiner. We have no data that I am aware of relative to the affect upon fish, and other valuable aquatic life for that matter, from prolonged exposure to concentrations of 2 p.p.m. Whereas a concentration of 5 p.p.m. might be suitable for certain species for a few days, we have no data to assure us that 2 p.p.m. would be safe for desirable species over a prolonged exposure such as would occur in the use of Kuron. If a lot of fish were to die subsequent to treatment with Kuron, I feel quite confident that it would be blamed regardless of whether it was, in fact, responsible or not.

2. Bathers.

You have raised a question about bathers. I do not believe that there is much likelihood of bathers being adversely effected by 2 p.p.m. of Kuron. However, we do not have data which could be used advantageously to prove such a point if someone were to claim skin irritation, rash, or whatnot from water which had been treated. In my opinion the probability of difficulty occurring is rather small but if an allegation were made I am afraid we would not be in a strong defensive position.

DOW 1 551251

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January 31, 1958

DOW 1 551252

3. Formulation.

I do not know how the material will be applied but I would make every effort to avoid oil, particularly oil which has a low odor threshold. One of the earliest ways of creating questions is to treat water with a material which can readily be detected by odor. Furthermore, most oils are far more toxic to fish than are the herbicides of the 2,4-D type. In fact, concentrations in the neighborhood of 1 p.p.m. of oils are in some instances quite lethal to fish.

4. Municipal Water Systems.

I do not know whether it is planned to use Kuren on impounded waters designed for human consumption or not. I would be somewhat apprehensive about such waters, not because I believe they will constitute a real hazard, but because of the furor throughout the country today in regard to contamination of various foods and drinks with chemicals. In certain areas impounded waters are used directly for drinking purposes; in others they are filtered and chlorinated prior to use. The effect of such treatment upon a water containing Kuren is unknown. Whether or not it would produce offensive odors should be investigated. Secondly, it could be alleged that the treatment of such waters amounts to undesirable contamination of the drinking water of the public. Again, I do not believe we would be in a good position to defend ourselves should such allegations be made.

5. Livestock and Wildlife.

I would also wager that the question will come up as to whether water from a treated lake would be suitable or safe for domestic livestock and wild animals to drink. Again, there is no question in my mind but that such water would be safe. However, we have no data to prove it and our opinions would be our only defense. I doubt, however, if this will be as serious a problem as that of water being used for human consumption.

I do not wish to be an alarmist in regard to the use of Kuren for aquatic weed control but I do think that we should all give serious thought to the problems which may arise as a result of such use. My own feeling is that we should be very cautious about practicing this product for such use until

L. L. Caulter

- 3 -

January , 1948

sufficient experience has been gained by governmental agencies and the like who wish to promote its use to answer some of the questions which I have raised in the paragraphs above. In any event, I believe we should recommend against the treatment of potable water systems.

I will be glad to discuss these problems with you at any time.

V. K. Rowe
Biochemical Research Laboratory
12-634 Building
Phone - 8771

VHR/ba

cc: P. W. Fletcher
D. L. Connor
K. C. Farron
J. W. Eritton
D. D. Irish
V. K. Rowe (2)
T2.30-40-20✓

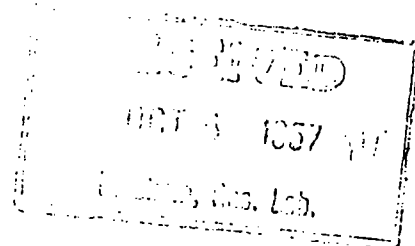
DOW 1 551253

18015

RECEIVED
October 3, 1957

V. L. Lane
Biol. Control Lab.
330 Building

cc: S. H. Davidson
F. U. Fletcher



EVALUATION OF USES INVOLVED IN CONJUNCTION WITH AQUATIC WEED CONTROL WITH KURON

During the last two years we have had some very excellent results in the Eastern states using Kuron (Dowanol ester of 2,2,4,5-trichlorophenoxypropionic acid) at a concentration of two parts per million for control of submergent aquatic weeds. The normal procedure is to apply Kuron to the pond surface or just below the surface in sufficient quantities to build a concentration of two parts per million in the entire volume of pond water. We do not know how long Kuron persists, however, we do have some tests from actual field applications made this year and know that it may last as long as a month and probably last longer. Laboratory work is continuing on this aspect.

In any event, it is apparent that we will want to intensify our efforts in developing this use, at least, for certain phases of aquatic weed control during 1958. Actually there are a lot of questions that develop in conjunction with the addition of any toxicant to water. These deal with the effect of Kuron on the taste of water in the event that it is used in a reservoir, the effect on chlorination, the taste of fish, the toxicity to other organisms, etc. Since the performance of this product seems to be sufficiently established to promise at least some commercial use ultimately, we wonder if the Robert A. Taft Sanitary Engineering Center might be a good organization for us to contact in conjunction with some of these problems. Before doing so we, of course, should decide what work can be done here and what specific projects we would be interested in having them work on. Since you

DOW 1 551248

MN073280

*Smith/Lang revision
of 7/24/78 draft*

7/31/78

RESPONSE OF DOW CHEMICAL U.S.A.

TO NOTICE OF REBUTTABLE PRESUMPTION

AGAINST REGISTRATION AND CONTINUED REGISTRATION

OF PESTICIDE PRODUCTS CONTAINING

2,4,5-T

①
DOW025961

SECTION IV. Exposure Analysis - Humans

A. Realistic Evaluation of Pesticide Application and Use

The following is quoted from Position Document 1, Federal Register, April 21, 1978, p. 17137, Section IIIB (3) *space*
"in order to determine whether a rebuttable presumption should be issued based on reproductive and fetotoxic effects pursuant to Section 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."

~~It is also interesting to note that~~ *The Working Group has commented that they have reconsidered*
~~points out that they have been required to reconsider the~~ *the*

the potential exposure to that portion of the population at risk (women of child-bearing age) because of social changes which have given these women the opportunity to now be employed in occupations such as "pesticide applicators, operators of highway construction and maintenance equipment, foresters and chemical formulators." With respect to reproduction and

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D.C., E.D. MI. 4-4-73; DOW/EPA AGREEMENT 9-79

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animal studies, and (4) judgment ~~based upon~~ ^{in judging} both logic and scientific perspective, whether an ample margin of safety indeed does or does not exist, *it can be concluded that:*

~~In the presentation given below it is concluded:~~

Neither 2,4,5-T nor TCDD occur as residues in the food supply

- ~~2,4,5-T (or TCDD) does not occur in the human~~

~~feed supply. Thus the margin of safety is even greater than that estimated by EPA.~~
so this is not a source of exposure in humans.

- EPA's estimate for exposure of applicators is grossly exaggerated.
- An ample margin-of-safety does indeed exist between the levels of 2,4,5-T and/or TCDD which produce ~~reproductive and/or~~ ^{"reproductive and/or"} fetotoxic effects and the levels to which humans can reasonably be exposed.

stop

B. Oral Exposure

EPA's Criteria and Evaluation Division (CED)

CED considered range and pasture grass uses with their "worst case" assumptions ~~from~~ ^{for} meat and milk derived from laboratory type studies (EPA #37) in which cattle were fed 300 ppm 2,4,5-T in the diet for ~~2-3 weeks~~ ^{4 weeks (incorrectly cited as 2-3 weeks)}. Although the calculations resulting therefrom were deemed as ample margins of safety by the Working Group, it is important to note that the margin of safety is even greater when one considers the levels reasonably to be anticipated.

This means nothing without the Table from the Position Document Table 25

EPA said 2-3 but it was 4 weeks in most animals.

What about TCDD

B. Oral Exposure

EPA's Criteria and Evaluation Division (CED) concluded that an ample margin of safety existed for ~~estimated oral exposure to 2.4~~ oral exposure to 2,4,5-T, even for their "worst case" estimate of exposure. However, the margin of safety is actually many times greater, ~~than~~ based on residue levels of that could reasonably be anticipated to be present in meat and milk.

EPA's assumed that ^{all meat and milk eaten by} ~~one cow~~ ^{on individual cows} ~~was~~ ^{maintained} continuously on freshly treated pasture ^{containing 300 ppm 2,4,5-T} ~~without a~~ ~~one continuous basis,~~ and ~~was~~ milked or slaughtered without withdrawal.

These assumptions are erroneous for the following reasons:

- few pastures are treated with 2,4,5-T;
- the rate of treatment in pastures is 0.5 lb/A on or broadcast basis, ^{for weed control, or at} ~~or possibly~~ 2 lb/A for spot treatments to control brush;
- residues of 2,4,5-T in or on grass would ^{average about} ~~not be only about~~ 100 ppm per pound applied per acre and would decline to about 27 ppm

(5)

Furthermore, labels for 2,4,5-T products specify that meat animals must be withdrawn for 2 weeks ~~before~~ ^{prior to} slaughter after grazing in treated areas, and that dairy animals must ~~be~~ not be grazed in treated areas for 6 weeks after treatment. These label limitations were

derived from data submitted to EPA ^{by Dow} in a petition for ~~tolerance for meat~~ negligible residue tolerance ~~for~~ ^{for} 2,4,5-T at the sensitivity of the analytical methods for ~~in meat and milk~~ meat and milk (PP No. 1F1102, ~~File~~)

The residues assumed by EPA to be present in meat and milk could only result from illegal use of ~~the~~ 2,4,5-T, contrary to label directions.

Thus, there is ~~not~~ no possibility of a ~~residue~~ ^{of 2,4,5-T in these food items,} being detectable residue ~~in these food items~~, and ~~the~~ EPA's estimates for oral exposure are grossly exaggerated.

Table IV SUMMARY: NORMALIZED (SPECIFIC) RESIDUES OF 2,4,5-T ON TREATED GRASS

Location	Formulation Applied	lb 2,4,5-T/A Applied a.e.	Average Specific Residue ppm/lb 2,4,5-T/A						
			Application-Harvest Interval Weeks**						
			0	1	2	4	8	16-18	52-54
California	ESTERON* 245	12	27	42	27	39	11	7	--
	ESTERON BK	6	65	--	--	15	12	4	--
	VERTON* T	6	63	6	23	16	9	2	ND
	VERTON T	1	49	32	26	21	10	2	ND
Michigan	ESTERON 245	12	112	63	34	13	6	4	--
	ESTERON BK	6	178	91	41	18	8	5	--
North Carolina	ESTERON 245	12	123	34	30	4	2	<1	--
	ESTERON BK	6	96	25	17	5	1	<1	--
	VERTON T	6	46	21	5	2	<1	<1	ND
	VERTON T	1	109	24	13	35	1	<1	ND
Texas	ESTERON 245	12	100	48	38	27	13	<1	--
	ESTERON BK	6	107	53	39	24	12	<1	ND
	VERTON T	12	96	122	--	8	3	<1	--
	VERTON T	2	112	142	--	48	7	<1	ND
Average			92	54	27	20	7	2	0

*Trademark of The Dow Chemical Company

**A few intervals are approximate.

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(7)

ment

Evidence is also presented in Section II and in depth in Appendix II that TCDD is not concentrating, persisting, or accruing in man or the environment. Studies have shown that TCDD residues decline rapidly due to photodegradation ~~after application~~ on foliage treated with 2,4,5-T so little is available for ingestion or accumulation by animals. No TCDD has been found in milk and only occasional low part per trillion levels ^{under conditions most likely for it to occur} have been detected in beef fat. No TCDD was found in rice, nor in fish, mud or water from an area where TCDD had been used on rice for many years. Similarly, no TCDD was found in a reservoir containing water from a watershed which had been treated with 2,4,5-T, nor in human milk collected from the same area.

Thus, there is convincing evidence that neither 2,4,5-T nor TCDD are occurring as residues in the food supply of the United States as a result of the present uses of 2,4,5-T.

18022

(8)

States as a result of the present uses of 2,4,5-T. The margin of safety implied by EPA, even though considered by them as ample, in reality ~~is~~ even much greater.

What about TCDD? Need to refer back to Section II and repeat conclusions about TCDD not necessary etc.

C. Dermal and Inhalation Exposure

In considering exposures by dermal or inhalation routes, ~~the Criteria and Evaluation Division~~ ^{CED} and the Working Group assumed conditions in which a pregnant woman, weighing 60 kg during the critical days of organogenesis, is carrying a backpack sprayer treating a right-of-way or spot treating a pasture or rangeland, or is clearing brush with tractor-mounted low boom spray equipment in rangeland or right-of-way, or during aerial application of 2,4,5-T is exposed directly beneath the spray plane.

EPA's estimates for exposure of applicators to 2,4,5-T are greatly exaggerated due to a series of erroneous assumptions. The major source of error is the linear extrapolation of data for contamination by each of two thiophosphate insecticides applied as fine spray for mosquito control, or by a contact-type herbicide applied for broadcast "burn down" of ground cover, as representative of what might occur with 2,4,5-T applied as a systemic auxin-type herbicide to weeds, brush or trees.

The calculations also assume that all major contamination is due to contact with diluted spray during application,

(9)

whereas
However the greatest potential for dermal exposure occurs during handling of the concentrate, such as in loading or mixing. EPA's error is compounded by assuming that values for total exposure to the concentrate in the dilute spray are due only to the dilute spray, and by extrapolating from the dilute spray (e.g., 0.06% fenthion) to a 4% 2,4,5-T solution.

Consideration of such mediating factors would appear to be essential to make reliable estimates of the potential for exposure of applicators of 2,4,5-T. The information summarized in Appendix V gives the following reasons for the gross exaggeration of human exposure analysis presented in the Position Document 1.

- (1) the linear extrapolation from data for contamination with totally different chemicals applied under dissimilar conditions;

- (2) assumption that all applications of 2,4,5-T were at the high rate of 4 lb/acre although most are at ^{0.5}~~2.5~~ to 2 lb/acre; *assumption that the dilution rate was 1 gallon of product to 10 gallons with no dilution of 1:10 with water whereas although ground applications are generally at 1 to 3 gallons in 100 gallons most 2,4,5-T is diluted 1:100 and is often applied in oil or oil/water rather than in water alone*

- (3) assumption that applicators would be wearing only very light clothing although most states require pesticide applicators to wear minimum protective clothing, and the fact that even wearing simple protective clothing

*city
refuses
to sign
carbon*

as would be required by following the label precautionary statements would decrease potential exposure down to as ^{little} ~~low~~ as 2% of the "unprotected" values;

- (4) disregarding consideration that the greatest potential exposure is on the hands particularly when handling the concentrate which can be avoided by simply wearing gloves;

(5) assumption that exposure would be continuous during 8 hours each day despite the physical nature of the operation such as in backpack spraying, and that the intermittency of all spray operations, particularly aerial, precluded against such exposure;

(6) assumption based on application of malathion as a fine spray in oil that as much as 2% of the droplets from ^{aerial} ~~2.4.5-T~~ spraying would be inhalable (< 60 ~~mils~~ ^m in diameter);

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(7) assumption that exposure by inhalation would be 3% of the estimated dermal exposure during ^{use of} ~~application of~~ ground equipment, although data in numerous studies showed it would be less than 0.5% in ~~applying~~ directed application of coarse sprays such as ~~in~~ row crops with this type of equipment.

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In summary, reevaluation of the procedures and calculations undertaken by the EPA Working Group leads to the following modifications:

- in* - Errors is assuming rate of application and dilution of spray -- ~~2~~^{up} to 8 times ;
- Disregard for protection supplied by even simple clothing -- up to 50 times ;
- Over-estimation of number of hours per day exposed -- ~~2 to 4 times~~ ^{up to 8 times} ;

Error in quantity assumed absorbed through skin
2 to 4 times

Can't use this one for TCDD

- Erroneous assumption of exposure by inhalation of spray droplets, particularly for ground application -- ~~2 to 10 times~~ ^{up to} 10 times.

long with measurements

The above factors, along with data from indirect measurements for ~~2,4,5-T~~ 2,4,5-T in applications and others, indicate that EPA has over-estimated exposure to this herbicide by at least two orders of magnitude (i.e. more than 100 times too high).

[Signature]

*off that EPA is...
H exposure...
to high by...
not two orders
of magnitude.*

Part II of Appendix V presents a review of ingestion studies with 2,4,5-T in humans, data from the exposure of workers manufacturing 2,4,5-T and data from workers obtained during actual application of 2,4,5-T. From the metabolism and pharmacokinetic studies it is demonstrated that urinary levels (amount excreted in urine within 24 hours) can be used as a measure of the effective dosage absorbed by ex-

estimate

new

(12)

Consideration of the above factors ^{along with} ~~along with~~ calculations based on actual data ~~from studies with R.Y.S-7~~ ^{from studies with R.Y.S-7} ~~calculations based on indirect measurements for R.Y.S-7~~ in applicators and other exposed populations, ~~in applicators and other exposed populations~~, indicate

that EPA has over-estimated exposure to this herbicide
by at least two orders of magnitude (i.e. more than 100 times too high). Their estimates were all based on ^{data for} direct measurements ~~using data for~~ ^{on} other pesticides under dissimilar conditions, ~~which led~~ to a series of erroneous assumptions. However, in-depth evaluation based upon indirect measurements of exposure (Appendix V) provides a clearer, more accurate basis for estimating exposure from data on spraying operations of R.Y.S-7, including in one study conducted in an EPA Laboratory (Shafik et al., EPA #33).

Part II of Appendix V presents a review of data from ingestion studies with 2,4,5-T and other phenoxy herbicides in humans, data from exposure of workers manufacturing 2,4,5-T, and data from workers applying 2,4,5-T ~~and~~ under actual field conditions. Using data from metabolism and pharmacokinetic studies, it is demonstrated that ~~urinary excretion~~ ~~of 2,4,5-T~~ measurements of 2,4,5-T in urine can be used to estimate the effective dosage level absorbed by exposed individuals, either from ~~dermal~~ dermal contamination or inhalation.

Such indirect measurements ~~provide a~~ ^{afford a} more more realistic estimate of exposure to 2,4,5-T. For example, data from two studies in applicators spraying 2,4,5-T with backpack equipment reveal that the actual exposure is only about 18028 0.06 mg/kg under "worst case" conditions, and about 0.03 mg/kg for applicators wearing gloves but without due caution to avoid skin contact

Thus, EPA's estimate of 7 mg/kg is too high
by at least two orders of magnitude. ~~As pointed out~~
~~some of the~~ (P) A similar conclusion was drawn
from a review of the assumptions used by EPA
in extrapolating data from direct measurements
of other pesticides. As discussed in Appendix
V, the effective dose would likely be less than
0.01 mg/kg from exposure during application
of 2,4,5-T with even minimum protective equipment
and reasonable care to avoid skin contact.
Exposure to TCDD present at 0.05 ppm
in 2,4,5-T would be 20 million times less
than for 2,4,5-T. Thus the effective dose of TCDD
would be only about 1×10^{-6} ~~mg~~ micrograms per
kilogram of body weight in an applicator
applying 2,4,5-T with a backpack sprayer.

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Available data for exposure in applications using back-drawn equipment consistent EPA's ^{estimation} that the effective dose would be known with this type of equipment than with a backpocket sprayer. Similarly, ~~there~~ ^{during aerial application} ~~independent exposure for a blower~~ ^{would occur} ~~application would~~ ^{occur} only if ~~the~~ he or she did not move over to the next swath once the pilot had a fix on his/her flight path. Thus, the fact that the ~~upper~~ effective dose in backpocket applications was only 0.06 mg/kg under "worst case" conditions is ample evidence that EPA's estimate of 0.077 mg/kg for aerial application is grossly exaggerated.

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Another factor which EPA has not considered is that most teratogenic studies with 2,4,5-T involved daily administration of the herbicide to pregnant animals ~~for a large portion of the pregnancy~~. Higher doses could be tolerated without effect if administered only on one day of pregnancy, even if that day was critical ~~for development~~ in the development of the embryos. Thus, comparison of ~~the~~ estimated dosages to the dose which caused ~~no~~ effect on repeated daily exposure in animals ~~also~~ provides ~~an~~ ^{an} ~~adequate~~ ^{adequate} built-in margin of safety. EPA

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Table I ^{lists} ~~is a summary of~~ the dosage levels used in a number of teratology studies with 2,4,5-T ~~as~~ summarized in Section C.6.0.b of PP 1F1102 submitted to EPA in May 1973². The circled ~~rate~~ dosage levels are those which ^{caused} ~~no~~ effect in the pregnant females or their offspring when administered daily on specified days during pregnancy. Additional studies are discussed in Section III.C.1 of this Rebuttal ~~Rebuttal~~ Document ~~which~~ ^{from which} substantiate the conclusion that EPA's selection of 20 mg/kg as the no-effect level is ~~very~~ ^{very} conservative, ~~and that 50 mg/kg would be~~. Moreover, the dosage levels required to cause even a minor fetotoxic effects ^{in various species} are substantially higher, ~~which provides a factor which also~~ ~~also provides a built-in safety factor not~~ ~~considered by EPA.~~ a factor which also ^{provides} ~~adds~~ a built-in margin of safety not considered by EPA. Such excessive conservatism is not warranted in the case of 2,4,5-T where the effects ^{not teratogenic, except possibly in} ~~are produced as at lower levels and are not~~

18032

TABLE V

TERATOLOGY -2,4,5-T

Section C.6.D.(b) of PP 1F1102, submitted May 1973.

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add

AUTHORS	SPECIES	FORM OF 2,4,5-T	ROUTE	DOSE LEVELS MG/KG/DAY
COLLINS & WILLIAMS, 1971	HAMSTER	ACID	ORAL	20, (40), 80, 100
COURTNEY ET AL., 1970	RAT MOUSE	30 PPM 2,3,7,8-TCDD		
COURTNEY & MOORE, 1971	MOUSE RAT		SC SC	(50), 100, 125, 150 10, 22, (46), 80, 250
KHERA & MCKINLEY, 1972	RAT	ACID, SALT, ESTERS	ORAL	25, (50), 100, 150
NEUBERT & DILLMAN, 1972	MOUSE MOUSE	ACID BUTYL ESTER	ORAL ORAL	8, 15, (30), 45, 60, 90, 120 12, 74, (<12)
POLL, 1971	MOUSE	ACID	ORAL	(20), (35), 60, 90, 130
EMERSON ET AL., 1971	RABBIT RAT	ACID ACID	ORAL ORAL	10, 20, (40) 1, 3, 6, 12, (24)
SPARSCHU ET AL., 1971	RAT	ACID	ORAL	50, 100, (<50)
BINNIS & BALLS, 1971	SHEEP	ACID ESTER	ORAL	100, (>100)

○ = NO-EFFECT DOSE LEVEL FOR EMBRYO AND FETAL TOXICITY.

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E. Margin of Safety Evaluation

~~Although~~ The Position Document states that 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 levels to which humans can reasonably be anticipated to be exposed. However, the Working Group has not stated what they would consider an "ample" margin of safety.

According to Table 32 in the Position Document, ~~a~~ an estimated dose of 0.023 mg/kg by inhalation in a flagger during 8 hours of aerial spraying does not offer an ample margin of safety. In comparing this dose to the no-adverse effect level of 20 mg/kg for 2,4,5-T, it appears that a margin of $20/0.023 = 870$ -fold is not ^{considered} ~~considered~~ adequate. Similar calculations for dermal and cumulative exposures during aerial application

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(20)

indicate that the margin of safety for these situations are 392 and 270-fold, again not considered adequate.

On the other hand, the corresponding doses of TCDD estimated to ~~be~~ range from 2×10^{-6} mg/kg/day ^{for inhalation} to 7×10^{-6} for cumulative exposure, do offer an ample margin of safety. When compared to the no-effect level of 0.03 mg/kg for this toxic impurity, the the

margin of safety for TCDD ~~is~~ ~~at~~ range
from 4300 for cumulative exposure to 15,000 for
exposure during ~~8 hours~~ ^{by} of inhalation of ~~spray~~
~~droplets~~ of spray droplets while standing directly
beneath the spray path for 8 hours during

airial spraying. ~~There~~ ~~the~~ ~~margin~~ ~~of~~
~~indefinitely~~ ~~small~~ The margin of safety
would be still greater for dosages reasonably
expected to be received by flaggers under actual
exposure conditions, and would be (infinitely)
greater for those exposed to trace amounts of
spray drift downwind from the treated area.

7. 18035

With regard to other spraying situations, let us assume that a backpack applicator is wearing adequate protective clothing and is exercising reasonable care to avoid skin contact while applying

2,4,5-T containing 0.05 ppm TCDD. ~~As discussed in Appendix I, the doses of 2,4,5-T and TCDD received would be, respectively, no more than~~

Under such circumstances, ~~as discussed in Appendix I, the doses of 2,4,5-T and TCDD received would be, respectively, no more than~~ 0.01 mg/kg ~~(2,4,5-T)~~

~~with a safety margin~~ and $0.01 \times 0.05 \times 10^{-6} =$

0.0005×10^{-6} mg/kg ~~or~~, or 5×10^{-7} mg/kg.

The margins of safety compared to the dosage levels which caused no-adverse-effect would

be $20/0.01 = 2000$ -fold for 2,4,5-T and

$0.03/5 \times 10^{-7} = 60,000$ fold TCDD. The margin

would be even greater for an applicator ~~applying~~ using tractor drawn equipment which offers even greater protection from spray drift during the application.

One further point should be mentioned. There would appear to be no precedent for requiring such large safety factors, nor for comparing the ~~values to~~ estimated doses to levels which caused no adverse effect on repeated administration to pregnant animals. ~~Back in~~ 1971-72 At the time EPA was considering taking action against 2,4,5-T in 1971-72-73, one of the ten ^{issues} ~~points~~ delineated by the Administrator was the need for dose-response data for 2,4,5-T (Dow # 150). Such data has been obtained as outlined in Appendix I. ~~It is already demonstrated that 2,4,5-T is and data has also been obtained~~ (P) More significantly, studies have also been conducted in ^{man} ~~humans~~ which clearly show that 2,4,5-T is handled in much the same way by humans as by rats. Thus, there is no basis for assuming that humans might be uniquely susceptible to the "teratogenic", or more correctly, "fetotoxic" potential of 2,4,5-T, such as occurred with thalidomide. 18037

Since 2,4,5-T is absorbed into the body following ingestion, and ~~probably~~ ^{apparently} dermal exposure, and is excreted unaltered via the urine by both man and the rat, there is no reason to suspect a situation analogous to the one claimed for thalidomide. Thus the margin of safety needed for exposure to 2,4,5-T is no more than for most chemicals and drugs, including aspirin, caffeine, vitamin A, etc. (See Tables VI (a) and (b) for safety factors for selected drugs. Furthermore, rats have been shown to handle large doses of 2,4,5-T at a different rate than smaller doses which caused no effect. Thus, it is inappropriate to predict what would happen from exposure to low doses on the basis of tests at high doses above the threshold (Appendix I,

Conclusions

- 2,4,5-T is not a teratogen in species other than mice which are uniquely sensitive to any stress during pregnancy;
- TCDD ~~causes~~ ~~not a teratogen~~ fetotoxic and embryotoxic effects rather than teratogenic effects;
- Use of 2,4,5-T containing trace levels of TCDD does not pose a teratogenic risk to "women of child bearing age", whether pregnant or not;
- There is ~~no reason to~~ no evidence to indicate that man is more sensitive than rats to the "teratogenic" potential of 2,4,5-T and/or TCDD;
- The margin of safety for applicators using backpack equipment to apply 2,4,5-T is ~~very~~ estimated to be at least 2000-fold if reasonable care is taken to avoid skin contact.

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TABLE VIa

Safety Factors For Selected Drugs

<u>Drug</u>	<u>Safety Factor*</u>
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 Table for further data on the drugs listed.

TABLE VII

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. <i>Tox. Appl. Pharmacol.</i> 1978, In Press
Valium (tranquilizer)	170	Mice	Oral	100 mg/kg	-	140 mg/kg	Cleft palate	Miller et al. <i>TAP</i> ^b 12, 53-61, 1973
Phenobarbital (anticonvulsant)	10	Rabbit	Oral	-	-	50 mg/kg	Skeletal defects	McColl et al. <i>TAP</i> 10, 244-252, 1967
Meclizine (antihistamine)	12	Rat	Oral	5 mg/kg	-	25 mg/kg	Cleft palate microstomia	King, C.T.G., <i>Science</i> 141, 353-355, 1963
Norethindrone (progestin)	50	Mice	Oral	0.5 mg/kg	-	1.0 mg/kg	-	Andrew et al. <i>Teratology</i> 5, 249, 1972
Norethindrel (progestin)	4	Mice	Oral	0.15 mg/kg	-	0.6 mg/kg	Cranial retardation	Gidley et al. <i>Teratology</i> 3, 339-344, 1970
Vitamin A	40	Mice	Oral	-	-	400,000 IU/kg	Exencephaly Cleft palate Microphthalmia	Giroud et al. <i>C. R. Soc. Biol.</i> 156, 449-450, 1962
Acetazolamide (diuretic)	7	Rat	Dietary	40 mg/kg	-	150 mg/kg	Limb deformities	Layton et al. <i>Science</i> 149, 306-308, 1965
Morphine (analgesic)	666	Mice	SC	200 mg/kg	-	300 mg/kg	Exencephaly	Johannesson et al. <i>Acta Pharm. Toxicol.</i> 31, 105-113, 1972
Aspirin: teratology	3	Pat	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. B. <i>TAP</i> 11, 280-292, 1967
Acetaminophen (analgesic): reproduction	<6	Mice	Dietary	Effects at lowest dose, 130 mg/kg				Wright, H. B. <i>TAP</i> 11, 280-292, 1967

^aBased on the no-effect dose level in teratology studies.^bTAP = Toxicology and Applied Pharmacology.

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Corrected working copy

CONFIDENTIAL - SUBJECT TO INJUNCTION
D.C., E.D. MI. 4-4-78; DOW/EPA AGREEMENT 9-79

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WHAT THE 2,4,5-T

R P A R

MEANS TO YOU

18042

On April 21, 1978 the United States Environmental Protection Agency scheduled a Rebuttable Presumption Against Registration (RPAR) review of the continued use of products containing the herbicide 2,4,5-T.

In view of the importance of 2,4,5-T for the control of unwanted brush and weeds in forests, rangelands, and rights-of-way areas, as well as for weed control in rice, we know you are interested in information on this RPAR action, and the threat its possible loss represents to you as a farmer, rancher, rights-of-way manager, or forester.

The RPAR Process

An RPAR is EPA's formal review of the benefits versus risks of continued use of a pesticide. This action begins when EPA concludes it has found some evidence of a potential health or environmental hazard (usually from laboratory studies) on the basis of certain risk criteria as defined in the Code of Federal Regulations. Notice of an RPAR does not represent a ban or cancellation of the product's use.

Once an RPAR is issued, producers and users alike have a period of time--usually 105 days--to both refute the risk evidence cited by EPA and provide information (usually in the form of letters) illustrating the importance of continued availability of the product for agricultural or other uses.

Producers of a product have the primary responsibility for assembling evidence to challenge EPA's allegations of possible health or environmental risk.

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Evidence attesting to economic or other benefits derived from use of a pesticide must be provided primarily by those experienced in its use including scientists, grower organizations, agribusiness, and individual users.

After assembling all available risk/benefit information, EPA will determine whether the compound is reasonably safe as used, whether additional limitations are needed, or whether it should be taken off the market. In order for the agency to make an intelligent decision, EPA must have information from users.

2,4,5-T Background

Since its introduction in 1948, 2,4,5-T has been a mainstay in the battle to control trees, brush and weeds which 1) threaten the growth of rice, conifer seedlings, or rangeland grass, and 2) create hazardous situations on road shoulders and utility rights-of-way. During this period, the toxicity of 2,4,5-T has been exhaustively studied by scientists worldwide. The consensus of these scientists is that it is not hazardous to human or animal life under normal conditions of use and even under conditions of substantial misuse.

Despite this enviable safety record, special interest groups which oppose the use of virtually any of modern agriculture's chemical tools have been exerting strong pressure to have 2,4,5-T banned. What these groups fail to understand is that so far there is no alternative--chemical or non-chemical--that achieves the public benefits of 2,4,5-T.

The 2,4,5-T RPAR - - -

Dow believes that the 2,4,5-T RPAR proceeding provides an effective means to resolve--once and for all--the issues raised by those who oppose the use of this important herbicide. Dow is dedicated to working towards a scientific resolution of these issues.

Consistent with our belief that 2,4,5-T does not pose a hazard to either human or animal life, Dow will present a great deal of scientific evidence to EPA to rebut alleged health risks.

and You

Economic and other benefits information must come primarily from you, the user. To keep these products on the market, it is essential that letters be sent with information showing the importance of 2,4,5-T to your farm, ranch, forest operation, or the rights-of-way you manage. Detailed information on the following points will be most helpful:

1. Usage of 2,4,5-T containing herbicides by crop, acreage, or weed control objective.
2. 2,4,5-T Effectiveness in your programmed use.
3. Availability, comparative effectiveness, and cost of alternative herbicides or cultural practices.
4. Effects that loss of 2,4,5-T herbicides would have on your ability to grow rice, raise livestock, or manage forests or rights-of-way.

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5. Your estimate of the dollar impact the loss of 2,4,5-T might have on your local economy.

Where and when to send Benefits Information

Benefits information should be sent to EPA. Although the deadline is not clear, information should be sent as soon as possible in order to have the greatest impact. Don't assume "the other guy will save it."

If possible it is best to send three (3) copies with the reference notation: OPP-30000/26.

Mail to: Federal Register Section
Technical Services Division (WH-569)
Office of Pesticides Programs, EPA
Room 401 East Tower
401 M Street, S.W.
Washington, D. C. 20460

Your input is vitally important to
a correct resolution of this issue.

ACT NOW !

18046



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D.C., E.D. MI. 4-4-78; DOW/EPA AGREEMENT 9-79

DOW CHEMICAL U.S.A.

May 30, 1978

MIDLAND, MICHIGAN 48640

J. N. Bean - Seattle
D. W. Cummins - Geneseo
C. E. Jentes - St. Louis
C. T. Lichy - Philadelphia
J. C. Ryder - Minneapolis
R. E. Ullom - Minneapolis
D. M. Baldwin - Phil.
D. B. Hale - Atlanta
J. R. Huffman - Atlanta
R. W. Steele - Lubbock
P. D. Ludwig - 9001 Bldg.

B. C. Byrd - Wayside
L. E. Hammond - Chicago
J. B. Grumbles - Dallas
M. L. Smith - Seattle
P. M. Ritty - Kansas City
L. E. Warren - Davis
R. D. Fears - Cincinnati
R. P. Harrison - Memphis
S. M. Hunter - Charlotte
P. VanDerSchaaf - Geneseo

cc: M. L. Traynor - 2030 Bldg. R. D. Moss - 9001 Bldg.
W. H. Mullison - 9001 Bldg. R. W. Colby - 9008 Bldg.
R. C. Hunter - 9008 Bldg. J. H. Davidson - 9001 Bldg.
O. K. Jantz - 9008 Bldg. M. L. Leng - 9008 Bldg.
J. A. Gray - 2030 Bldg. D. D. McCollister - 9008 Bldg.
R. E. Hefner - 9008 Bldg. W. J. Stearns - 9008 Bldg.
C. S. Williams - 9008 Bldg. W. G. Wright - 9008 Bldg.

2,4,5-T RPAR - BACK-UP LITERATURE

As mentioned in my communication of May 15, 1978, literature pieces are being developed to support your efforts towards (1) informing cooperators, users, etc. of the issues involved in EPA's RPAR action and (2) soliciting benefits letters.

While some of the material is still being printed, I thought you might be interested in seeing "proofs" as well as completed materials so you can begin developing strategies for use of these materials. Copies are enclosed with this letter and include:

- 1) A set of transparencies which briefly covers the RPAR for use in talks before user groups, etc. The same format is being used for preparation of 2x2 slides which will be sent in the near future.
- 2) A copy of a 2,4,5-T "backgrounder" entitled "RPAR Review of 2,4,5-T Herbicides". Prepared by Wendell Mullison, this piece was designed primarily for university/extension personnel, but can be distributed to anyone who might have interest in the material contained therein. This literature piece is being copied in quantity and additional copies can be obtained by contacting Mazie Sowles (517-636-1020).

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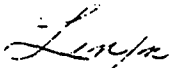
- 3) A proof copy of "What the 2,4,5-T RPAR Means to You". This release, which is being formatted into brochure form is intended primarily for farmers and other users as a solicitation for benefits letters. As such, it can be distributed at meetings, through grower organizations, etc. It is anticipated this brochure will be available in quantity within one week. Again, place your requests through Mazie Sowles.
- 4) Also enclosed is a proof copy of "Fact Sheet 2,4,5-T RPAR". This is a press release and is being distributed to various media from Midland. However, you may wish to use it as a supplement to or in lieu of the above materials. This item is also being printed in quantity.

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Best of luck in your efforts towards stimulating those benefits letters. A good idea of the types of comments needed in benefits letters is contained in the enclosed DuPont release "Update in the Benomyl RPAR".

As to possible strategies for generating letters - in addition to direct solicitations by mail and personal conduct, one idea which has substantial merit is to give a brief talk before users or user groups followed by passing out pens and paper so that individuals can prepare a response during the meeting. Letters can then be collected and posted to EPA. Needless to say, the paper used for such letters should not contain Dow letterhead and the mailing to EPA should be through a third party sponsor. According to reports, this technique has worked quite well on occasion with other RPARed compounds.

One final comment - on no occasion should benefits letters show Dow as a "cc" on the letter. Some letters of this type are inevitable, but it is important that we do not encourage Washington supporters to copy us (or show that they are copying us). We will be able to track the volume of the response by direct inquiries of EPA in Washington.



L. L. Smith, Jr.
Product Technical Specialist
Agricultural Products Department

nbe
enc.

18048

2,4,5-T RPAR - REVIEW OF EPA'S RATIONALE
AND THEIR CALCULATIONS FOR EXPOSURE TO 2,4,5-T AND TCDD

by Marguerite L. Leng, May 1, 1978

EPA's rationale for issuing a Rebuttable Presumption Against Registration for 2,4,5-T is presented in their Position Document No. 1 dated April 21, 1978^{1/}. We have compiled a file of the 179 cited references including EPA memos from their Cancer Assessment Group and of their exposure estimates as calculated by their Criteria and Evaluation Division.

In their exposure analysis, EPA states that *"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 population at risk with potential exposure to 2,4,5-T and or TCDD."* (p. 101a - 102 of typescript copy received by certified mail on April 24, 1978)

Teratogenic Effects

EPA's press release issued on April 12, 1978 stated that *"the pesticide and its dioxin contaminant have killed fetuses or caused birth defects, such as cleft palate, among laboratory mice, rats, hamsters and birds in tests conducted by the National Institutes of Health and other researchers."* On the basis of many cited studies, they concluded that no-effect levels had been established for teratogenic/fetotoxic effects at 20 mg/kg for 2,4,5-T and 0.03 µg/kg for TCDD. Although it was mentioned occasionally in the text that the doses were administered daily, few of the tables specified that dosage rates were in milligrams or micrograms of chemical per kilogram of body

^{1/} Federal Register Vol. 43, No. 78, p. 17116 to 17157, April 21, 1978.

weight per day, nor that the treatment was continued during up to half of the animal's pregnancy.

Exposure Levels for Pesticide Applicators

To simplify calculation of exposure by skin absorption and inhalation, EPA assumed that all applications were made using a product containing 4 pounds of 2,4,5-T per gallon diluted 10-fold with water. However 2,4,5-T low volatile esters such as in ESTERON* 245 Herbicide are relatively insoluble in water and require addition of surfactants and/or oil to form emulsions with water. Some applications are made in oil alone, particularly for low volume sprays.

Dermal exposure was estimated for different types of application equipment based on available data for other products, chiefly insecticides. Workers were assumed to be wearing very light clothing with little protection from the spray. However, 2,4,5-T is generally applied to foliage in early spring when target brush and trees are growing vigorously, or in the fall with thorough coverage of dormant stems. Use in forests is limited to a brief time in the spring between leafing-out of deciduous trees and bud-break in conifers, or during the dormant period in late fall to early winter. Female applicators would not likely be wearing shorts and halter tops at these times, as apparently assumed by EPA. The letter of transmittal accompanying EPA's memo on exposure^{2/} stated: *"We have modified the Working Group's description of bikini clad persons entering into sprayed areas. In general, spray applicators of 2,4,5-T are very sensitive to releasing spray deposits into populated sections of either rights-of-way or forests. This is in part due to intense pressures which have been brought to bear by the private sector on public utilities and federal and state foresters. The modified exposure estimate we have provided names no specific exposed population because in our opinion, identification of an exposed sub-population to 2,4,5-T spray, even under rangeland situations where responsibility is*

*Trademark of The Dow Chemical Company

^{2/} Reference No. 164. Exposure Analysis, 2,4,5-T, dated February 1978 from Gunther Zweig, Chief of Chemistry Branch, Criteria and Evaluation Division to Harvey Warnick, Project Manager for 2,4,5-T in the Office of Special Pesticide Reviews. The calculations were made by Carroll Collier.

more difficult to affix, is at the best, very difficult."

The following calculations for different spray situations are also presented in a summary table attached to this review.

1. Backpack Sprayer

EPA's worst case calculation is for use of a hand-pressured backpack sprayer to treat brush in right-of-ways or for spot application in pasture or rangeland. Based on data for fenthion insecticide applied as a dilute spray in water (0.06%) by workers wearing short-sleeved, open-necked shirts with no gloves or hat^{3/}, they estimated that a woman weighing 132 lb (60 kg) would be contaminated by 0.18 pints (1/3 cup or 86 ml) of 10% 2,4,5-T spray solution in an 8-hour day. If 10% of this was absorbed even after washing, she would receive a dose of 6.8 milligrams of 2,4,5-T and 0.0007 micrograms of TCDD per kilogram of body weight. It does not seem likely that a woman would continue to get this much on her skin each day, particularly if the formulated product is applied in oil or as an oil/water emulsion. Furthermore, she would not likely be doing this work, day after day, during several months of her pregnancy. (In the Dow animal studies, dosing was done daily on days 6 through 15 of the 20-21 day gestation period in rats).

2. Tractor Mounted Low Boom Sprayer

EPA next estimated a somewhat lower exposure in or on a woman operating a tractor-mounted low-boom sprayer for clearing brush on rangeland or rights-of-way. Based on exposure studies for paraquat applied with this type of equipment as a 0.12% solution at a rate of 100 gallons per acre,^{4/} EPA concluded that a female applicator would be contaminated by 0.048 pint (23 ml) per 8-hour day. Using the above 10% absorption rate for 2,4,5-T applied as a 10% solution, her dosage level would be 1.8 mg 2,4,5-T and 0.00018 µg TCDD per kg per day on this job. Again, no

^{3/}Reference No. 166. Wolfe et al. Mosquito News 34(3): 263-267 1974.

^{4/}Reference No. 147. Steiff et al. Bull. Environ. Contam. Toxicol. 14(3): 334-340, 1975

consideration was given to how frequently nor for how long she would be doing this during her pregnancy.

3. Aerial Application

For exposure during aerial application, EPA assumed the woman was standing directly beneath the spray plane. Estimation of dermal exposure was for contamination on all areas of bare skin, listed as head, neck, shoulders, forearms, hands, and thighs (such as when wearing shorts and a halter top which is not likely during 2,4,5-T spraying in early spring and late fall). Based on data for "malathion in oil sprays, applied at 0.46 pounds per 0.76 gallons of water(?) per acre,"^{5/} EPA calculated that application of 2,4,5-T at 4 pounds in 10 gallons of water per acre would deposit 31 mg 2,4,5-T on her skin. If 10% was absorbed, she would receive 0.051 mg 2,4,5-T and 5×10^{-6} μ g TCDD per kg of body weight. Compared to no-effect levels of 20 mg/kg for 2,4,5-T and 0.03 μ g/kg for TCDD in teratogenic studies, daily exposure at this rate represents a margin of safety of almost 400-fold for 2,4,5-T and 6000-fold for TCDD. If the 2,4,5-T was applied at usual rates of treatment (0.5 to 2 lb/A) the margin of safety would be proportionally greater. Nevertheless, EPA concluded that this exposure is not safe enough for 2,4,5-T although it is safe enough for TCDD (in spite of the fact that TCDD is always present in 2,4,5-T).

EPA also calculated the inhalation exposure for aerial spraying, again based on data for malathion.^{6/} Since 2,4,5-T is applied as a medium to coarse spray, they assumed that only 2% of the spray droplets would be small enough for inhalation, but that all the inhaled material would be 100% absorbed. They also assumed that the spray would be inhaled for 8 hours per day rather than for only 2 hours after application as in the malathion model. Even so, the daily dosage level by inhalation was only 0.023 mg 2,4,5-T and 2×10^{-6} μ g TCDD per kg of body weight, with safety factors of 870 and 15,000 respectively. Again, this margin of safety was inadequate for 2,4,5-T but adequate for TCDD.

^{5/}Reference No. 167. Caplan et al. AMA Arch. Indust. Health 14: 326-332, 1956

^{6/}Reference No. 168. Akesson and Yates, undated and unpublished.

Cumulative Exposure

EPA also estimated the cumulative doses for dermal and inhalation exposure in each situation. Exposure by inhalation was judged to contribute only negligibly to the dose received by dermal exposure from application with a backpack sprayer or a tractor mounted sprayer. However, the contribution by inhalation from aerial sprays was deemed to be additive. The cumulative dose was estimated to be 0.074 mg/kg for 2,4,5-T and 7×10^{-6} for TCDD, with margins of safety of 270-fold for 2,4,5-T and 4000-fold for TCDD. This was deemed adequate for TCDD but not for 2,4,5-T. Once more, no consideration was given to the fact that the woman would have to be standing directly beneath the spray plane and inhaling the spray for 8 hours, each day, during the critical stage of pregnancy.

Oncogenicity

EPA's 4/12/78 press release also stated that *"2,4,5-T and dioxin have caused leukemia or lung, liver, or other tumors among mice and rats in studies conducted by the National Institutes of Health, Dow Chemical, and others."* They did not differentiate between effects alleged to have been caused by 2,4,5-T and those caused by TCDD *"since manufacturers are unable to produce 2,4,5-T without TCDD."*

It should be noted that the RPAR was issued before EPA's Cancer Assessment Group had even reviewed the two complete reports for Dow's 2-year feeding study with TCDD in rats, as delivered to them by me more than 6 months ago. On the other hand, they apparently accepted at face value all the "data" from poorly conducted and reported studies by others. For example, they concluded that Allen's study^{1/} demonstrated a 46% incidence of tumors in a total of only 50 rats in five groups fed 5 to 5000 parts per trillion TCDD for up to 65 weeks. In that study none of the 10 control rats developed tumors but 6 of them died before the study was terminated at only 22 months.

^{1/} Reference No. 109 VanMiller et al. Chemosphere 9: 537-544, 1977

The French study with 2,4,5-T in two strains of mice was also discussed extensively in spite of the fact that the authors stated their results were inconclusive.^{8/} We need to submit a progress report on the Dow 2-year feeding study with 2,4,5-T in rats to supplement the negative study in mice reported by Innes et al. of NCI.^{9/}

EPA also cited two preliminary epidemiological studies from North Vietnam^{10/} and Sweden,^{11/} *"neither of which 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."* Unfortunately, they did not have time to include comments on the Dow Study submitted in March 1978 which demonstrated no adverse effect in 204 employees exposed in the 2,4,5-T plant for intervals of 1 to 10 years. All exposures were during the years 1950 - 1971 when the TCDD level was higher than the current 0.1 ppm limit in 2,4,5-trichlorophenol and all products derived from it.

Conclusion

EPA's appraisal of the hazard of 2,4,5-T is exaggerated. Your comments are solicited on the above review, and on any portions of the position document not covered herein. Please send any comments or additional information no later than June 15, 1978 to Marguerite L. Leng, Building 9008, The Dow Chemical Company, or Box 1706, Midland, Michigan 48640.

^{8/} Reference No. 105 Muranyi-Kovacs et al. Br. J. Cancer 33: 626-633, 1976.

^{9/} Reference No. 107 Innes et al. J. Natl. Cancer Inst. 42: 1101-1114, 1969.

^{10/} Reference No. 120 Tung et al. Chirurgie 99: 427-436, 1973.

^{11/} Reference No. 108 Hardell, L. Lakartidningen 74: 2753-2754, 1977.

SUMMARY OF EPA ESTIMATES OF EXPOSURE TO 2,4,5-T AND TCDD IN A 60 KG WOMAN

<u>Situation</u>	<u>Treatment</u>	<u>Daily Exposure</u>	<u>2,4,5-T (Margin of Safety)</u>		<u>TCDD (Margin of Safety)</u>	
			<u>mg/kg/day</u>	<u>vs</u> <u>20 mg/kg</u>	<u>µg/kg/day</u>	<u>vs</u> <u>0.03 µg/kg</u>
Backpack sprayer; rights-of-way; spots in pasture or rangeland	4 lb/gal diluted 10-fold with water applied to wet.	0.18 pint; 1/3 cup; 86 ml.	6.8	(2.9)*	0.0007	(43)*
Tractor mounted low boom sprayer; rights-of-way; rangeland.	4 lb/gal diluted 10-fold with water applied to wet	0.048 pint; 3/4 fl. oz; 23 ml.	1.8	(11)*	0.00018	(167)*
Aerial application on person directly beneath spray plane, wearing very light clothing, and who remains there all day.	4 lb a.e. in 10 gal water per acre as medium to coarse spray.	3.1 mg (dermal)	0.051	(392)*	5×10^{-6}	(6000)
		0.34 mg (inhalation for <u>2 hr.</u>)	0.023 <u>per 8 hr</u>	(870)*	2×10^{-6}	(15000)
		Cumulative	0.074	(270)*	7×10^{-6}	(4286)

*Inadequate margin of safety (for daily exposure at these rates, during critical stage of pregnancy).

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9008 Building
May 1, 1978

MIDLAND, MICHIGAN 48640

To: E. H. Blair, 2020
G. E. Blau, 1707
R. B. Bumb, 47
W. B. Crummett, 574
J. H. Davidson, 9001
J. E. Donalds, 9008
P. J. Gehring, 1803
C.A.I. Goring, 9008
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R. D. Hart, 2030
R. E. Hefner, 9008
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P. G. Stamison, 9008
W. J. Stearns, 9008
M. J. Traynor, 2030
J. R. Vaccaro, 1803
C. S. Williams, 9008

cc: R. H. Schroeter, Los Angeles
M. R. Wessel, 2030

From: Marguerite L. Leng, Senior Registration Specialist
Health and Environmental Research, 9008 Building

2,4,5-T RPAR - A GAME BY EPA'S RULES

The Rebuttable Presumption Against Registration (RPAR) process is a game played under EPA rules with a stacked deck. Contrary to David Menotti, EPA's new Associate General Counsel, it is not an open two-way debate of all available information in which the pros and cons are reviewed in public before a regulatory decision is reached. Instead, EPA publishes all the "risk" information in the Federal Register whereas rebuttal comments and information eventually become available for public inspection only in an obscure Washington office during normal working days. Meanwhile, a chemical such as 2,4,5-T is indicted by the press and public outcry continues. What happened to DDT can happen again. The EPA Administrator still has the last say, regardless of the outcome of "public" review of data or of legal decisions following public hearings.



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As noted in the attached EPA press release, issuance of the 2,4,5-T RPAR was prompted by *"some 450 letters from citizens and environmental groups such as the Citizens Against Toxic Sprays (CATS), protesting use of the chemical."* The U.S. Department of Agriculture is also succumbing to the same pressure due to complete absence of similar input from the other side. Positive action is needed now to avoid a moratorium on continued use of this valuable herbicide pending a final decision by EPA to possibly restrict it or ban it.

Jack Anderson's column on Monday, April 24 (attached) exemplifies how the RPAR action can be distorted. EPA's press release also provided fuel for our opponents. Their *"calculations show that an ample margin of safety may not exist for persons applying the pesticide or people exposed to aircraft spraying of it."* Costle's assistant Barbara Blum stated *"Finding of birth defects and cancerous tumors among laboratory animals exposed to 2,4,5-T indicate that it may cause the same problems among people."*

EPA's conclusions about carcinogenicity are based on poorly conducted and reported animal studies and on "preliminary" epidemiological studies in North Vietnam and Sweden. However, they still have not reviewed two extensive reports on Dow's two-year study with TCDD delivered to them six months ago, nor a recent study demonstrating no adverse effect in Dow production workers.

Appended hereto is a review of EPA's rationale for issuing this RPAR and of their calculations on exposure of applicators to 2,4,5-T and its TCDD contaminant. Although their Position Document admits that these chemicals are not accumulating in the environment, they reserve judgment on the hazard of TCDD in food until more data are available from their monitoring studies. With regard to teratogenic/fetotoxic effects, they concede that no-effect levels have been established in animal studies with 2,4,5-T and TCDD, but ignore that these dosage levels were administered daily for up to half the duration of pregnancy. They also appear to require a 1000-fold margin of safety even for their exaggerated estimates of exposure in women of "child-bearing age" during actual application of the herbicide.

I urgently solicit comments from all recipients of this letter, either for incorporation in our formal rebuttal or for independent submission to EPA before the final deadline of August 4, 1978. Other Dow products are also at stake in this "game." RPAR's are scheduled to be issued shortly for 2,4,5-trichlorophenol (TCP) and pentachlorophenol (PCP). Next on their list are silvex (2,4,5-TP), erbon and ronnel. 2,4-D and dinoseb (DNOSBP) are currently under pre-RPAR review and others are likely to follow in domino fashion.

Copies of EPA memos or other cited documents are available by calling Agnes at 636-1350.

M. L. Long

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REBUTTAL TO EPA'S INTERPRETATION
OF DATA REGARDING THE HEALTH
HAZARDS OF 2,4,5-T

Dr. D. G. Crosby
University of California, Davis
Davis, California

and

Dr. J. B. Knaak
California Department of Food and Agriculture
Sacramento, California

July 1978

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I. Introduction

2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and its derivatives long have been important to California agriculture and forestry. According to the 1972 California Pesticide Use Report (1), almost 108,000 lbs. of the chemicals were applied annually in dozens of uses throughout the state at that time. In recent years, permit use has declined to less than 20,000 lbs. (2), but "2,4,5-T" still remains the herbicide of choice for forest and range applications.

For both practical and toxicological considerations, it is important to specify the exact chemical species referred to as "2,4,5-T." The free acid 2,4,5-T seldom is used, but rather its amine salts or low-volatility esters. The physical and chemical properties of these compounds differ widely, although the derivatives eventually are converted to true 2,4,5-T in the environment. The molecular weight change can be especially significant: 2,4,5-T (acid) containing 0.1 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) would produce a commonly used propylene glycol butyl ether ester containing only 0.07 ppm of TCDD. Unfortunately, the RPAR generally leaves doubt regarding which form of 2,4,5-T is being referred to.

During several decades of widespread use, 2,4,5-T herbicides were considered undistinguished in their toxicity to animals--so much so, that they were sold extensively for farm and home use and have remained without any reported episodes of human harm in the state. However, in the late 1960's, observations of teratogenicity and fetotoxicity in laboratory animals fed TCDD-contaminated 2,4,5-T introduced a decade of public confusion and furor. The deep resentment over the Vietnam War (in which 2,4,5-T butyl ester was used as a defoliant), a widely publicized industrial accident in Seveso, Italy, and the susceptibility of TCDD's unusual toxicity to dramatic and imaginative reporting combined to set the stage for the present regulatory controversy.

Indeed, there is no question that TCDD is an extremely toxic and dangerous substance, as demonstrated by the extensive (although variable) investigations of the past few years and outlined in the RPAR document. However, present-day manufacturing methods produce 2,4,5-T generally containing less than 0.05 ppm of TCDD, and the present commercial herbicide and its derivatives can be--and presently are--used safely in California. This document is not a recommendation for the use of phenoxy herbicides or for any proprietary product but is intended to offer alternative interpretations of data used by EPA for a Rebuttable Presumption Against Registration and continued registration of pesticide products containing 2,4,5-T.

II. The "Triggers"

A. General Comments.

An RPAR (Rebuttable Presumption Against Registration and Continued Registration) has been issued against products containing 2,4,5-T because there is evidence that 2,4,5-T and/or its dioxin contaminant

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(TCDD) may cause a significant increase in benign or malignant tumors and teratogenic and/or fetotoxin effects in certain sensitive species of laboratory animals. This section of the response, dealing with the toxicology of 2,4,5-T and TCDD, will attempt to bring about a clearer understanding of the relationship between published toxicological studies conducted in the laboratory and the use of 2,4,5-T in the environment.

B. Oncogenicity.

At present, most 2,4,5-T oncogenicity studies must be considered to be primarily academic. Of the four investigations cited in the RPAR, one represented daily oral dosing of 2,4,5-T (containing 30 ppm of TCDD) at 60 mg/kg via gelatin capsule or subcutaneous injection of the 2,4,5-T to very aged mice, and two others were concerned with feeding rats pure TCDD. The fourth and most pertinent study (3) involved ad libitum feeding of 2,4,5-T containing less than 0.05 ppm TCDD to two separate strains of mice at 80 ppm in the diet over most of their lifetimes. The results showed little or no significant difference between treated and control animals, although there is indication that 2,4,5-T actually may protect against oncogenicity. The most notable feature among XVII/C mice was the very significant increase in longevity among the treated animals. This longer life-span clouds the results, as any increase in tumor incidence could be attributed to the greater susceptibility of old individuals to cancer (in mice as well as people).

The TCDD oncogenicity data are widely considered to be preliminary and very controversial (4), although they show that both TCDD and 2,4,5-T exhibit no-effect levels. Unfortunately, the key question of oncogenicity by dermal exposure--essential to the RPAR argument--remains unanswered.

C. Teratogenicity/Fetotoxicity.

There seems to be little controversy over the fetotoxic effects of TCDD. In fact, it was this property of contaminated 2,4,5-T which was brought to light by the early studies (5). As with oncogenicity, however, most investigations have been more academic than practical--high level or pure TCDD, DMSO injection, etc. However, a more realistic test with 2,4,5-T containing 0.5 ppm TCDD (about 10 times present levels) at 1-24 mg/kg/day to rats or 10-40 mg/kg/day to rabbits during key periods of pregnancy showed no effects; both fetotoxicity and maternal toxicity were observed at the environmentally unrealistic dosage of 100 mg/kg/day, however.

Unfortunately, the maternal toxicity observed in a number of the tests where toxicant levels produced fetotoxicity obscures the results, as poisoned mothers generally are not expected to produce healthy offspring. Again, no-effect levels for both TCDD and 2,4,5-T were observed, but tests utilizing dermal exposure have not been reported.

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D. No-effect levels.

Data in the RPAR document reveal no-effect levels for TCDD and/or TCDD-containing 2,4,5-T in relation to mortality, oncogenicity, and teratogenicity/fetotoxicity. Significant mortality increases (compared to controls) did not occur with pure TCDD below oral doses of 0.5 ppb (0.1 ug/kg/week); the oncogenic no-effect levels in lifetime feeding studies were between 0.001 and 0.01 micrograms/kg/day for pure TCDD (corresponding to 20-200 mg/kg/day of 2,4,5-T containing 0.05 ppm TCDD); and no teratogenicity or fetotoxicity were observed with pure TCDD at 0.03 microgram/kg/day (rats) and 0.1 microgram/kg/day (mice) or with 2,4,5-T (0.05 $\pm$ 0.02 ppm TCDD) at 25-150 mg/kg/day (rats) and 20 mg/kg/day (mice).

Although close extrapolation of toxicity data from rats to humans generally is unsatisfactory, the lowest of the NOEL values--corresponding to daily ingestion of 20 mg/kg of pure 2,4,5-T (1.2 grams/day in a 60 kg person)--seem likely to be acceptable. Dermal and inhalation exposure data are inadequate to set no-effect levels.

III. Human Exposure

A. General.

Principal applications of 2,4,5-T in California in 1977 (2) were on forest timberland and on range land. The maximum application rate was on the order of 2 lbs/acre of actual 2,4,5-T. In a typical application--for brush control on forest land ("conifer release")--herbicide is applied as a single aerial spray of emulsified low volatile esters of 2,4-D and 2,4,5-T in water (corresponding to 2,4,5-T at 1 lb/acre) (Table I). There are no "flagmen"; application is by enclosed helicopter onto largely uninhabited areas, spray is mixed and loaded semi-automatically (although limited contact is possible), and the herbicide is applied only under county permit and under inspection.

TABLE I. COMPOSITION OF TYPICAL HERBICIDE SPRAY.

Constituent	lbs/acre
Water	100
2,4-D propylene glycol butyl ether ester	1.52
2,4,5-T propylene glycol butyl ether ester	1.45 ^a
solvent and emulsifier	1.26

^aEquiv. to 1 lb/acre 2,4,5-T (<45 ug TCDD)

Despite a permitted TCDD level of 0.1 ppm (based on 2,4,5-T content), the concentrate actually contains less than 0.05 ppm TCDD (typically 0.035 ppm in our analyses). Spray contains less than 0.5 parts per billion for each lb/acre of 2,4,5-T, corresponding to less than 25 micrograms of TCDD per acre as released from the sprayer.

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In herbicide spray, TCDD is very unstable to sunlight on leaves, soil, and other surfaces (6). [The persistence referred to in the RPAR document was due to a laboratory study with pure TCDD and without the energetic ultraviolet portion of sunlight (7)]. The EPA-cited 1969 work of Getzendaner and Hummel (unpublished) also demonstrated rapid TCDD loss from 2,4,5-T-treated grazing land (grass). TCDD becomes tightly bound to soil due to its unusual electropositive nature (8). Consequently, dispersal, environmental degradation, and adsorption reduces the concentration of TCDD to well below the 25 microgram per acre figure and allows the rough estimate of potential exposure shown in Table II (9).

TABLE II. ESTIMATED MAXIMUM PROBABLE TCDD LEVELS RESULTING FROM A NORMAL HERBICIDE APPLICATION (9)

TCDD		Maximum Conc. (ppt) ^a	Probable Conc. (ppt)
Leaf	10	(1 pg/cm ²)	0.2
Soil/litter	1.1	(100 pg/kg)	0.05
Water	10 ⁻⁴	(0.1 pg/l)	10 ⁻⁸
Biota (fish)	1	(1 mg/kg)	10 ⁻⁴
Milk	0.1	(100 pg/l)	0.01

^aFrom a 1 kg/ha application of herbicide (0.1 ppm TCDD)

The chemical analysis for environmental and food residues of 2,4,5-T is routine and allows reliable measurements at part-per-billion levels (10). Such methods have been used to demonstrate the environment dissipation and breakdown of 2,4,5-T (10,11). While TCDD analysis at these levels also is relatively straightforward (6), the methods required to detect and to overcome interfering substances at part-per-trillion (ppt) levels of TCDD become extremely complex (12,13,14,15). Despite isolated and poorly verified claims to the contrary, the best analytical methods presently available anywhere generally are conceded to be reliable only down to about 10 ppt. This would preclude measurement of TCDD in most environmental samples (Table II), and indeed, surveys have failed to find any TCDD which might be present as a result of normal applications of 2,4,5-T (14).

Considering the points presented above, the restriction of 2,4,5-T applications in California to professional (licensed) applicators, principal application to uninhabited locations, and cancellation of uses on food crops, around the home, in recreation areas, and near water, it is most unlikely that the public will receive measurable exposure to TCDD, let alone exposure above the no-effect level. The two cited instances of epidemiological investigations of 2,4,5-T effects in humans involved massive exposure and questionable circumstances and data base, and justify the RPAR document's conclusion that "Neither of these studies is sufficient to be the basis of any firm conclusions concerning connection between 2,4,5-T and cancer." However, a more detailed epidemiological study involving railroad workers who had used only phenoxy herbicides in comparison to those who had used other herbicides revealed no relation of tumorigenesis to exposure to the phenoxy (16).

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B. Occupational Exposure.

1. Oral Exposure. The continuing FDA nationwide "Market Basket Survey" has failed to show 2,4,5-T present in any food composite at or above -- 0.02 ppm, and a specific search for TCDD in milk with the best existing method also was negative (16). The estimate provided in the RPAR is based on a single highly exaggerated and artificial animal-feeding experiment (17), so the RPAR document's "theoretical exposure of an average woman" at 0.7 micrograms/kg/day is meaningless. The Working Group correctly considers that the difference between the no-adverse effect level of 2,4,5-T for teratogenicity (20 mg/kg/day) and this exaggerated oral exposure constitutes an "ample margin of safety" (a safety factor of about 30,000).
2. Dermal Exposure. In the absence of published data, the estimated dermal exposure levels cited in the RPAR necessarily are based on application of chemicals other than 2,4,5-T. However, the RPAR exposure estimates are both simplistic and unrealistic when applied to 2,4,5-T. First, 2,4,5-T is a restricted-use chemical in California and must be applied under a county permit and direct supervision of a licensed applicator: this means appropriate spraying conditions and personal protection--the example of "exposed shoulders, forearms, hands, and thighs" is not applicable. There will be no "exposed population directly beneath the spray plane" except in an unforeseen accident when virtually no pesticide may be considered safe. The description of the other applications is equally unrealistic: no more than 15% of the body surface will normally be exposed to spray (18).

While data on skin-absorption of 2,4,5-T by man have not been found, Maibach (18) determined the penetration of the closely-related 2,4-D to be 5.8% of the dose (administered to the forearm). A liquid ester of 2,4,5-T might be expected to penetrate more readily, but malathion was absorbed to an extent of 6.8% and ethion to only 3.3%. It is probable that the Working Group's estimate of 2,4,5-T skin absorption is high by a factor of at least two.

Considerable effort has been expended toward the development of reliable data for estimating the amount of pesticides to which a worker is exposed during mixing-loading and spraying operations. Exposure is measured by using cloth patches placed on the skin or clothing of the worker and by measuring the amount of metabolites excreted in urine or feces. Cholinesterase activity before and after application of D-2 insecticides also provides an estimate of both exposure and absorption. In all cases, the measurements must be made under actual use conditions. According to the estimates of Houghton (26) an orchard sprayman collects pesticide residues on his clothing equivalent to roughly 6 times the level deposited on foliage during spraying. For example, azinphosmethyl applied at a rate of 3 lbs. per 100 gallons of water to peaches gives a deposit on the foliage of 3.0 micrograms per cm² of leaf surface (27), so the applicator would collect about 18 ug of azinphosmethyl per cm² of skin or clothing. If we assume that only 15% of the dermal surface of a 60 kg woman is exposed and a maximum of 10% of the deposit is absorbed from the exposed area, the calculated dose amounts to 0.09 mg/kg. This is only 1/2 of the dose estimated in the Position Document for spray applicators using a low-boom sprayer.

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However, in a field study by Knaak et al. (28), workers mixing and loading phosdrin with closed systems and applying the insecticide with a tractor-mounted low-boom spray rig, actually collected only 0.5 ug/cm² on cloth patches attached to their clothes during a 7 hr. work-day. On a mg/kg basis, this amounts to 0.16 mg/kg total deposit for the 60 kg woman, or an actual dose of 0.002 mg/kg through exposed skin rather than the 0.09 mg/kg calculated value or the RPAR value of 1.8 mg/kg.

The estimates made by the Working Group for tractor-mounted, low-boom spray equipment also are inconsistent with the study of Staiff, et al. (25), cited in the Position Document. The Working Group assumed that 0.048 pints of diluted material would be spilled on the skin of the worker. According to the RPAR, this amount of 2,4,5-T spray (Table 26) would contain 1089 mg of 2,4,5-T and less than 0.1 ug of TCDD, although the study cited in the Position Document states that a maximum of only 27.2 mg of pesticide was actually found (3.4 mg/hr. x 8 hrs. + 27.2 mg).

In 1976, Knaak and Schlocker (23) extracted the coveralls of mixer-loaders at the end of the working day and analyzed the extracts for organophosphate pesticides. The total amount of pesticide extracted from each coverall varied between 1.5 and 10.5 mg. The residues found on the clothing of these workers are typical of those found when workers use open systems for mixing and loading. The 4,086 mg of 2,4,5-T suggested in the Position Document (Table 27) would result in 200 ug of 2,4,5-T per cm² of clothing if the amount were equally distributed over the coveralls, whereas the analysis of clothing from men mixing and loading gave actual values of only 2 to 3 ug/cm². These gross discrepancies between the values calculated by the EPA and those actually found emphasize the inaccuracies which can result when "worst-case" estimates become multiplied.

According to the cited study by Wolfe et al. (24), maximum fenthion exposure amounted to 6.3 mg/hr. using a backpack sprayer. Because a worker's exposed skin amounts to only 15% of his total skin area, the total pesticide collected over an 8 hour work-day must be multiplied by 0.15 (6.3 mg/hr x 8 hr x .15 = 7.5 mg/day) to give the total amount of pesticide available for absorption. The 7.5 mg/day must be multiplied by 0.1 to give the actual amount absorbed (0.75 mg/day) or 0.0125 mg/kg/day of 2,4,5-T according to the Position Document's method of calculating dose. This value is substantially lower (544x) than the 6.8 mg/kg proposed in the Position Document. On the basis of this new estimate, the safety factor would be 1600 for 2,4,5-T and 25,000 for TCDD, certainly adequate to protect the health of the worker.

The exposure estimates for aerial application presented in the Position Document were based on a study involving malathion (30). In a recent study conducted by the California Department of Food and Agriculture, 2,4,5-T was applied by helicopter to forest areas at a rate of 3 lbs. per 10 gallons of water per acre (29). Under these conditions, foliage residues amounted to 10 ppm (0.11 ug/cm²) immediately after spraying. A woman working in this area during spraying would collect about 2 mg of 2,4,5-T on exposed skin and clothing. Since the exposed skin represents only 15% of the total

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body surface, the amount available for absorption represents 0.3 mg, and even if 10% of this residue is absorbed, the 2,4,5-T dose received amounts to 0.0003 mg/kg. The safety factor for 2,4,5-T under these conditions amounts to 40,000, and the factor for TCDD is approximately 1 billion.

3. Dermal Toxicity. The Working Group distinguishes between teratogenic effects of "2,4,5-T" and those of TCDD in estimating margins of safety. As the 2,4,5-T values already represent herbicides containing usual levels of TCDD, the additional data on pure TCDD are extraneous and misleading. Unfortunately, no data appear to be available on the teratogenic effects of either TCDD or 2,4,5-T applied dermally, and so the oral no-effect levels are used in the RPAR to calculate margins of safety.

However, a large store of experience as well as any handbook of toxicity values indicate that chronic oral toxicity figures cannot be extrapolated successfully to dermal exposure, except that the dermal values always show distinctly less effect. The acute LD₅₀ of TCDD in the rabbit has been measured for both the oral (0.115 mg/kg) and dermal (0.225 mg/kg) routes (19). The oral toxicity of technical 2,4,5-T butyl ester in the rabbit is 712 mg/kg (range 500-1,000) (21), but corresponding dermal values apparently have not been reported. The oral LD₅₀ of 2,4,5-T acid in most mammals is on the order of 500 mg/kg, while the dermal LD₅₀ of the closely-related 2,4-D is 1400 mg/kg (20). Toxicity values from injection in DMSO would bear no relation to surface application of an aqueous solution to intact skin.

At low levels, TCDD becomes bound tightly to protein and would be expected to find difficulty in penetration. Evidence of persistence in skin may be found in the toxic skin reaction--called chloracne--which appears even at very low dermal exposures to TCDD [detectable on a rabbit ear with a few nanograms (19)]. Although chloracne has been observed in factory workers exposed to massive levels of 2,4,5-T (22), the lack of a record indicates it is not prominent even among high-risk workers such as aerial applicators.

Following an industrial accident in Seveso, Italy, chloracne was observed among the population exposed to very high levels of TCDD, as might be expected. However, abnormal births (or aborted fetuses) among some 200 (allegedly) directly-exposed pregnant women were "not disproportionate to the usual incidence of abnormal births" according to the RPAR document. Thus, even vastly greater exposure than ever could be envisioned for the RPAR's "60 kg woman" did not produce the effects feared in Position Document 1.

Considering the safety factors already incorporated into 2,4,5-T use in California, the undoubted large overestimate by the Working Group of possible exposure and dermal toxicity, and the lack of teratogenic or fetotoxic responses among even highly exposed humans, the highly conjectural calculations of the RPAR document are obviously erroneous.

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4. Inhalation Exposure. Many of the problems which plague calculations of dermal exposure also apply to inhalation, although oral toxicities perhaps are more reasonably extrapolated to the respiratory route; again, use of "unprotected persons directly beneath the spray plane" as the basis of all safety calculations is simply unrealistic. Other assumptions also are probably quite inaccurate for a 60 kg person: 2,4,5-T is not applied at 4 lbs/acre but at one to three lbs/acre; the effective diameter of respired pesticide particles is generally no more than about 10 microns (rather than 60 microns), so the availability from a coarse spray would be greatly diminished; respiration rate would generally not exceed 1 m³/hr for a 60 kg woman (31); and the lung absorption rate certainly would not be 100% of the available particles (31). Thus, the dose level calculated by the Working Group could be as much as 100 times too high.

Toxicity values for pure TCDD again have little meaning compared to those of TCDD-containing 2,4,5-T. If we assume a dose level of even 2 ug/kg/day compared to a no-effect level of 20 mg/day, the safety factor becomes 10,000. Even the RPAR document's value of 0.023 mg/kg/day for 2,4,5-T provides a daily dose of only 10^{-12} g/kg of TCDD—why is this considered unacceptable when 5 times this amount of pure TCDD would provide "an ample margin of safety"?

Inhalation exposure to 2,4,5-T certainly is not a new phenomenon, either during application or manufacture, and there is no history of complaint. Based on toxicity values and practical experience, OSHA has recently set the time-weighted average air standard for human inhalation of 2,4,5-T at 10 mg/m³ (33).

5. Cumulative Exposure. It is apparent that oral and inhalation exposure to both 2,4,5-T and TCDD will be negligible compared to possible dermal exposure. The greatest dermal exposure may be expected from use of a back-pack sprayer (0.0125 mg/kg/day of 2,4,5-T and 1×10^{-9} mg/kg/day of TCDD as "worst cases"). Considering the Working Group's no-adverse cumulative effect values of 20 mg/kg/day and 0.03 mg/kg/day for 2,4,5-T and TCDD respectively, the argument of acceptability seems to revolve about what (unspecified) margin of safety EPA considers acceptable. We propose that the minimum safety factors of 1,000 for 2,4,5-T and 30,000 for TCDD represented here are acceptable.

C. California Experience.

2,4,5-T and its derivatives have seen large-scale use in California for about 40 years. While analytical figures are not available, it must be assumed that for most of this period, TCDD levels were much higher than they are at present.

The California Department of Public Health has maintained records for herbicides in general during much of this period (Table III) and for specific herbicides during the most recent reporting periods (1975-77) (33). California physicians are required by law to report in detail any pesticide illness. The reports for 1975, 1976, and 1977 show no observable illness attributed to use of 2,4,5-T, despite the fact that chloracne is a readily recognized symptom. Likewise, neither the other state agencies applying 2,4,5-T nor the professional applicators themselves have observed toxic effects in workers.

TABLE III. SUMMARY OF PHYSICIAN'S OB REPORTS^a

Year	Total Pesticides Reports	Total Herbicide Reports ^b	Total 2,4,5-T Reports
1977	1828	225	0
1976	1752	216	0
1975	1762	218	0
1974	—	—	—
1973	1451	203	—
1972	1505	243	—
1971	1477	201	—
1970	1493	214	—
1969	727	76	—
1968	834	65	—
1967	1400	189	—

^a State of California, Department of Public Health, Center of Health Statistics, Berkeley, California, 1978.

^b Estimate.

The Wildlife and Natural Resources Committee of the California Senate heard extensive testimony in March and April, 1978, on a bill (SB 1357) to prohibit aerial application of 2,4,5-T and "other TCDD-containing materials" and then voted the bill down. In view of the California use restrictions, the conditions of actual registered and permitted uses, and the complete lack of verifiable evidence of deleterious effects even among high-risk human populations, California experience with 2,4,5-T does not presently justify suspension of use.

D. Current Research.

The University of California, California Department of Food and Agriculture, and the U.S. Forest Service presently are engaged in research on the environmental distribution and fate of 2,4,5-T and TCDD in both laboratory and practical field experiments. The principal purpose is to assess the possible exposure levels of humans and wildlife, and to determine possible bioaccumulation and food chain transfer, especially of TCDD. Preliminary results suggest that TCDD may not survive normal spraying operations.

IV. Conclusions

During the past few months, a number of careful, thorough, detailed reviews of 2,4,5-T and TCDD hazards have become available (4,34,35,36). Their consensus is that, under present restrictions, use levels and patterns, and TCDD levels, 2,4,5-T and its derivatives can be applied safely. The concern of the regulatory agencies and the emphasis of the RPAR document on occupational exposure is appropriate--there is, after all, no evidence of public danger under normal use conditions--but calculated "worst-case" extrapolations based on other "worst-case" estimates soon become unrealistic.

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There is no question that TCDD is itself a very dangerous substance. With the lack of explicit human-exposure data for 2,4,5-T, prudence demands close control over its release, occupational awareness of the hazards, and free choice by individuals--especially women of child-bearing age--of their occupational exposure to it. However, in view of the facts and interpretations presented here, this should not preclude continued important use of such a valuable chemical tool as 2,4,5-T.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Date: August 26, 1977

SUBJECT: TCDD - dioxin: mutagenicity

FROM: Ruth Pertel, Ph.D., OSPR. *R. Pertel*

TO: Mary Reece and Harvey Warnick

Attached is a review of the mutagenicity of TCDD and related herbicides. The evidence is weak for heritable genetic effects since the level of testing is meager and the reported tests have major deficiencies. However, TCDD appears to have the potential to act as point (gene) mutagen (Hussain, et. al., (1972) and a chromosomal mutagen from the in vivo cytogenetics studies.

Of concern, in the light of the DBCP problems, are similar effects on testicular atrophy in rats due to chronic exposure to the butyl, ether 2, 4, 5-T (Yefimenko, 1974).

The review of the Hussain paper by Dr. Malcolm is of the quality of validations that I would like to see for all validations.

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JUNE 1979
ENVIRONMENTAL SCIENCE & TECHNOLOGY

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ENVIRONMENTAL SCIENCE & TECHNOLOGY

**Safeguarding
clean air at
Canada's tar sands**
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James Martin (R, N.C.), a former professor of chemistry at Davidson College, proposed that this zero-risk concept has its root in the Delaney Amendment (1958) to the Pure Food, Drug, and Cosmetics Act. That amendment essentially adds, to requirements that a food, drug, or cosmetic be safe and effective, the mandate that the substance also be generally free of risk.

Now suppose that to achieve zero risk, regulations state that an effluent contain a no detectable (N.D.) level of Chemical A. Suppose, further, that the latest monitoring instruments, capable of showing concentrations in parts per billion, indicate "N.D." levels of this Chemical A in the effluent; thus, the discharger is in compliance. Then, XYZ Instruments Corp. announces a new generation of instruments that can detect Chemical A to one part per trillion, and this instrument, which now must be used as the monitor, detects two parts per trillion, maximum. The discharger is now in violation, and perhaps subject to adverse actions, regardless of what, if any environmental, health, or welfare risk such a minute concentration of Chemical A may pose.



Rep. Martin
the Delaney syndrome

This is one of the many problems that the chemical industry faces—problems brought about, and aggravated by what Congressman Martin called "the Delaney Amendment mentality that pervades regulators." Naturally, one would like to minimize risk as much as possible. However, whether zero risk is a desirable goal, even if it were technically and economically achievable, is a question that could have philosophers in deep discussion for a long time. But no matter how one may feel about the matter of risk, one thing is certain—the present regulatory trend certainly has the chemical industry going through interesting times! JJ

EPA suspends the major uses of two herbicides

A link between forestry sprayings and miscarriages in women living in Alsea, Ore., prompted the emergency suspension of 2,4,5-T and Silvex

In March, the EPA took a step unprecedented in its nine-year history: it placed the herbicides 2,4,5-T and Silvex under an emergency suspension order, temporarily banning the major uses of these chemicals.

Both herbicides are unintentionally contaminated with low levels of the most deadly chemical known to man—TCDD (dioxin)—during their production. It is suspected that dioxin contamination, and not the principle ingredients in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex), is the cause of the spontaneous abortions that prompted the agency to take this "most drastic" step.

Long history of use

Dow Chemical, the major manufacturer of both herbicides, introduced 2,4,5-T to the marketplace in 1948; it soon found its place in forestry management, and clearance of rights-of-way, uses that are now temporarily banned.

As a component of Agent Orange, 2,4,5-T was widely used by U.S. troops carrying out defoliation operations during the Vietnam War. Reports from Vietnam of miscarriages and deformed babies forced the U.S. to withdraw Agent Orange from use. The culprit was the teratogen dioxin—2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the same chemical that erupted from a chemical plant in 1976 and showered the residents of Seveso, Italy.

In 1970, the EPA restricted the uses of 2,4,5-T to forestry and pasture management and clearance of rights-of-way, banning its use in homes, gardens and at recreational sites. The agency also directed manufacturers to reduce the dioxin content to 0.1 ppm, reasoning that this reduction would

render the herbicide less toxic, more safe.

However, ongoing animal studies continued to document the deadly potency of even very low levels of dioxin. EPA again held hearings in 1973-74 to consider cancelling 2,4,5-T's registration. These hearings ended inconclusively, and the EPA withdrew its proposed cancellation order.

Now, 10 years after Agent Orange was withdrawn as an accessory war weapon, 2,4,5-T and its close chemical cousin, Silvex, have been suspended for most uses. This is only a temporary ban until a full review determines their health effects and their benefits.

Dow and the three other manufacturers claim that the herbicides are safe. To the contrary, the EPA in its suspension order and notice of intent to cancel cites 40 animal studies that show that the herbicides cause spontaneous abortions, birth defects and malignant tumors. But, until the Alsea, Ore., study, no tangible correlation could be made between the dioxin-contaminated 2,4,5-T and its effect on human health.

TCDD, the suspected culprit, has never been found in human tissues, although dioxin has been found in a few samples of beef fat and human milk. The unintended human experiment at Seveso is marred by under-reporting of pregnancies, legal and illegal abortions, and probably even spontaneous abortions.

The Alsea studies

Pregnancy and abortion records were better for the Alsea basin. In 1978, nine women wrote to the EPA stating that their 13 miscarriages could be correlated with peak forestry sprayings. Scientists who examined their medical histories and the spraying periods could not pinpoint a

cause-and-effect relationship. However, the EPA pursued the matter further.

The agency called upon scientists from Colorado State University and the University of Miami Medical School to study rates of miscarriages over a six-year period. Miscarriages in the Alsea area where heavy spraying of 2,4,5-T occurs annually in March and April were compared to miscarriages in a similarly rural area in eastern Oregon where spraying does not occur. The findings: the miscarriage rate in the Alsea basin was significantly higher than that in the control area, and the miscarriages in Alsea peaked in June and July, two to three months after the annual Spring sprayings.

Study links miscarriages to herbicide sprayings



EPA's Blum
"alarming correlation"

"This alarming correlation," deputy administrator Barbara Blum stated at the press conference announcing the suspension, "comes at a time when 7 million pounds of 2,4,5-T are about to be used to control weeds on power line rights-of-way and in pastures, and to manage forest lands across the Nation." The suspension, Blum said, would protect 4 million people from exposure to the Spring sprayings, and have minimal short-term economic impact.

Blum cautioned that the epidemiological study is highly suggestive, though "not proof of a cause and effect relationship." Dow and the 10-12 other plaintiffs seeking legal and administrative relief agree. Dow calls the study "seriously flawed."

The herbicide manufacturers withdrew from an administrative hearing which was to decide on the correctness of the emergency suspension. The hearing was dissolved last month.

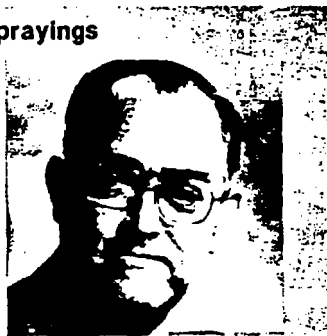
Dow then asked for immediate cancellation hearings before an EPA administrative law judge. Such a judge was assigned to the cancellation hearings. He called for a prehearing conference early this month. The hearings themselves, which will prob-

ably begin in July, are expected to take several years to complete.

Safe or not?

Dow claims that it is fighting EPA's action, not because 2,4,5-T is such a money-maker for the company—it only accounts for 0.2% of Dow's annual revenues—but because "it's time to take a stand." Dow president David Rooke queried at a press conference: "If you can wipe a product with this much proven safety off the market, then what is safe?"

The safety of 2,4,5-T is now being more carefully determined. The EPA is analyzing samples of soil, water, game meat and human milk from the Alsea basin for TCDD. The University of Illinois Medical Center is planning



Dow's Rooke
"seriously flawed"

a computerized (medical) case history study of Vietnam veterans, and their spouses and offspring. Another Illinois scientist, M. M. Yokoyama, is looking for alterations in levels of human white blood cells and antibodies caused by exposure to Agent Orange.

Should dioxin show up in EPA's collected samples, or should alterations in circulating levels of leukocytes and antibodies be traceable to dioxin exposure, the environmental agency would have little difficulty in cancelling the herbicides' registrations—something it had attempted to do with 2,4,5-T in 1973.

The cancellation hearings will determine whether the risks outweigh the benefits, or whether the risks are acceptable. However, the fact that fewer than a dozen of the several hundred potentially hazardous pesticides have been banned by the EPA in its nine-year existence speaks to the circuitous and ineffectual registration and cancellation procedures laid down by the pesticide law. Manufacturers can skillfully manipulate these regulations to their advantage. And they have. The 2,4,5-T case is not unique: it is simply a microcosm of pesticide politics.

LRE

AIR SAMPLING SYSTEMS



Air Quality Sampler

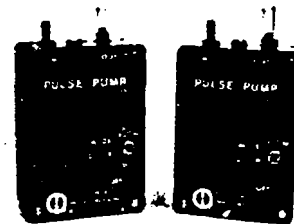
The EMI AQS-II, an economical alternative to continuous air monitoring, collects unattended samples for up to 96 hours. Equipped with a low power electronic timer/controller, the AQS-II can be programmed to gather 4 to 24 sequential or random hourly samples. Leasing is available.

Radon Sampler

Similar to the AQS, the Radon Sampler collects a 12 to 48 hour sample in a single bag. Designed to satisfy NRC requirements, the Radon Sampler includes an electronic controller, filter, regulated piston pump, and battery, all in an environmentally enclosed housing.

Air Sampling Bags

These pretested, chemically inert, highly retentive duPont Tedlar™ bags successfully collect many gases. In-stock sizes of 2, 5, 10, 15, 30, and 50 liters are supplied with fitting, tubing, and pinch clamp. Custom sizes and hardware are available.



Personal Sampling Pumps

These small and versatile Pulse Pumps have flow rates adjustable from 1 to 20 liters per hour and a high flow rate of 90 liters per hour. As both filter sampler and bag filler, they adapt to air sampling applications from industrial hygiene monitoring to ambient air collection. Rechargeable batteries optional.



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SAN FRANCISCO, CA 94111
TELEPHONE 415/398-7664
TWX 910/372-7270 EMI SFO

CIRCLE 1 ON READER SERVICE CARD

Volume 13, Number 6, June 1979

18074

DOW 0966608

I. Background

2,4-D is one of the most widely used herbicides in the United States. There are approximately 1,500 products containing 2,4-D registered with EPA, and more than 70 million pounds of the active ingredient are distributed annually. The term "2,4-D" refers to the phenoxy herbicide 2,4-dichlorophenoxy acetic acid and its 35 derivative salt and ester forms. 2,4-D is used to control broadleaf weeds in a variety of places including home lawns, cereal and grain crops, commercial areas, commercial turf, rights-of-way, and forests.

Public concern about the potential adverse health effects of 2,4-D has intensified since the emergency suspension of 2,4,5-T and Silvex in March 1979. This concern stems primarily from 1) the chemical similarity of 2,4-D and 2,4,5-T as phenoxy herbicides, and 2) the question of 2,4-D dioxin-contamination, especially contamination with tetrachlorodioxin, a manufacturing contaminant in 2,4,5-T, which causes cancer and miscarriages. Due to the chemical similarity of 2,4-D and 2,4,5-T, the public has expressed concern about the potential for cancer and miscarriages from the use of 2,4-D. There is also concern because the controversial military defoliant Agent Orange, used in Viet Nam, was composed of 2,4,5-T and 2,4-D. Agent Orange was never registered by EPA for civilian use in the United States. Its use in Viet Nam by the U.S. military has resulted in claims of adverse health effects to American military personnel. The Veterans Administration is studying these claims.

Prompted by these concerns and EPA's need to resolve the questions surrounding the use of 2,4-D, the Agency initiated a review of the available information on the potential health effects of 2,4-D. This review was conducted in part to determine if the herbicide should be reviewed under the RPAR process (Rebuttable Presumption Against Registration) or if another regulatory action was appropriate.

II. Agency Review and Conclusions

Based on the results of this review, EPA has concluded that a) the presently available information on the potential adverse health effects of 2,4-D does not support a regulatory action to remove 2,4-D products from the market; b) information from scientifically valid studies does not indicate that the continued use of 2,4-D poses an imminent hazard or unreasonable adverse effect when used according to label precautions and direction for use; and c) the Agency should act quickly and vigorously to obtain better toxicological information on 2,4-D.

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These conclusions are based on these following considerations:

1. There is no evidence available at this time that indicates 2,4-D contains any form of dioxin. This includes the tetrachloro-dioxin (TCDD), which is a manufacturing contaminant of 2,4,5-T and causes cancer and miscarriages.

TCDD is not theoretically expected to be found in 2,4-D. The manufacturing processes and starting chemicals from which 2,4-D and 2,4,5-T are made are not the same. Although other much less toxic dioxins are theoretically possible in 2,4-D, they have not been found despite thorough chemical analyses.

2. Because products containing 2,4-D have been registered for use since the 1940's, most of the scientific data submitted to support the product registrations now on the market were developed many years ago. While some of these studies are scientifically valid, many others do not meet today's standards for scientific testing. As a result, there are significant information gaps in several areas including cancer-potential, reproductive effects, neurotoxicity, and metabolism in animals.

3. The studies most pertinent to the question of tumor-causing potential (oncogenicity) of 2,4-D were considered inadequate and inconclusive. No valid conclusions could be drawn one way or another from the data.

4. Almost all animal tests conducted on the potential reproductive effects of 2,4-D show that, unlike 2,4,5-T with its contaminant TCDD, there is a no-effect level for injury to the fetus (fetotoxicity) from 2,4-D. A no-effect level in animal studies is the dose level below the lowest dosage that produces observable adverse effects. At comparable dose levels, 2,4-D induces less serious fetotoxic effects than 2,4,5-T contaminated with TCDD.

In tests with rats, 2,4,5-T with its TCDD contamination caused resorptions of the fetus (dissolution of the unborn animal) at very low levels. This effect was virtually non-existent in rats fed 2,4-D at the same dose levels. Because of the significance of fetotoxic effects and because several of the reproductive tests on 2,4-D were found to be scientifically deficient, new tests will be needed before sound conclusion can be made.

5. The scientific evidence available at this time does not indicate the potential human exposure is sufficient to result in human health effects.

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6. The most vigorous authority available to EPA under the pesticide law to fill information needs is a new section of FIFRA (Federal Insecticide Fungicide Rodenticide Act) passed in 1978. This provision, known as 3(c)(2)(B), allows EPA to request any additional data from pesticide registrants that is considered necessary to maintain the registration of existing products. The Agency can immediately require the

manufacturers to develop the data where gaps exist. The registrants have 90 days to show that they are complying. Their product registrations may be summarily suspended if they fail to meet the Agency's conditions. No other action could obtain this information any faster. EPA is putting the data requirements into final form and they will be issued to the registrants after review by our Scientific Advisory Panel. These scientific experts will review and comment on the data requirements to assure that they will provide the information EPA needs to more definitively answer the questions on potential health effects of 2,4-D.

7. Based on a review of the toxicology data (see section IV below), and a review of the risks of other pesticide chemicals now undergoing regulatory action, the Agency believes that the risks of several other pesticides are higher and better documented than those associated with 2,4-D. To put the review of these other higher priority chemicals aside in order to devote EPA resources to taking action against 2,4-D would not, in the Agency's opinion, best serve the public interest.

III. Additional Actions

In addition to requiring several important studies of the manufacturers on 2,4-D, EPA will also:

1. Conduct several tests on reproductive effects (through our Office of Research and Development) of several derivatives of 2,4-D in order to quickly get new information and have a good basis for comparison with the company-produced data.

2. Continue its ongoing review of forest pest control practices. This review will evaluate all chemical and non-chemical controls to identify the most environmentally protective ways to control forest pests. The Agency believes that a piecemeal approach to forest chemical regulation only leads to confusion, both to the industry and to the public. Unless we review the whole range of possible controls, examining one chemical at a time only gives rise to questions about the chemicals which would be used to replace those examined and prohibited from use.

3. Review all new data as it comes in to determine if a change in our regulatory posture is warranted. This includes evaluating the results of new animal tests as well as looking into reported incidents involving human exposure to the chemical.

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4. Continue to support field tests to measure exposure to 2,4-D during the present growing season.

5. EPA is informing the Inter-Agency Work Group, established by the White House to study the possible long-term effects of Agent Orange, of the actions being taken. EPA will also share its scientific findings with this committee.

IV. Toxicology Background

The potential hazard of a chemical is usually measured in laboratory animal tests. Animals are given doses of a chemical over a specific time period. Scientists attempt to derive from most of these tests a "no observable effect level" (NOEL) -- the dose level below the dosage where effects are first observed. From the animal tests and NOEL's, the potential effects on humans and other animals can be estimated. A set of brief definitions is provided below to permit better understanding of the subsequent discussion of toxicological findings.

A. General terms

1. Acute oral toxicity (LD50) - this test determines the dose level which produces death in half the test animals after a single oral dose (short-term test). Used to predict the near-term toxicity of the chemical immediately upon contact with people or other non-target animals.
2. Chronic feeding tests - animals are fed for most their life span (usually greater than 18 months in rodents) in order to determine the dose level which shows no toxic effect in test animals. This is the test from which the NOEL is (usually) derived.
3. Oncogenicity testing - animals fed relatively large doses of the test chemical for their life span (usually 18 months to 2 years in rodents) to try to induce tumors. These tests are used to predict whether the chemical may pose a cancer hazard.
4. Reproductive testing - these tests evaluate the effects of the chemical on the fertility of both the male and female parents by exposing the animals for a period of time before breeding. The tests also measure the possible effects of the chemical on the pregnant female and the fetuses through several generations. (The test with rodents through 3 generations runs approximately 14 months.)

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5. Teratology testing - these tests evaluate the effects of the chemical on fetuses by exposing pregnant females during the short period of time that the fetus is most susceptible to congenital malformation. Teratogenic effects include cleft palate, central nervous system deformities, eye and limb deformities, and internal organ malfunction. These are considered to be life-threatening effects that put the animal at a disadvantage for surviving in its environment.
6. Fetotoxicity - fetotoxic effects can be seen in either the reproduction or teratology tests. Toxicity may be seen in the extreme form as fetal death or as less severe problems, such as delayed formation of bones, reduced body weights at birth, or edema (abnormal fluid accumulation in the tissue). Most fetotoxic effects appear to be reversible once exposure to the test chemical is curtailed. Therefore most fetotoxic effects are considered to be less serious than teratogenic effects, with the exception of fetal death.

8. Summary of Toxicology Review

Most of the data in EPA files on the potential health effects of 2,4-D are centered on the acid form, even though there are many derivatives, such as salts and esters. This is because the many forms of 2,4-D metabolize to the acid form in the environment and in the body. The discussion of animal data below, therefore, concerns the acid form of 2,4-D unless otherwise noted.

1. Acute toxicity - low to moderate. The potential for immediate poisonings from contact with the chemical is unlikely.
2. Neurotoxicity - There is little definitive information on the possible neurological effects of 2,4-D. In several reported cases of impaired nerve function, it was not known if the individuals were peculiarly sensitive to that type of effect or were exposed to other toxic materials.
3. Reproductive effects (effects on the unborn) - Tests have been conducted on rats, mice and hamsters to evaluate the possible reproductive effects of 2,4-D. In almost all tests, a NOEL has been established. 2,4-D causes some of the less serious fetotoxic effects, such as edema (swelling of tissues) at the lower dose levels tested, and causes life-threatening birth defects (skeletal malformations) and cleft palates only at the very high levels tested.

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Based on the NOELs in the animal studies, EPA estimates that the level of exposure in a "worst-case" situation (eg. a person standing directly under a spray plane) would be 500 to 1000 times less than the dose level that might cause an effect.

Much of the data available to judge these effects was generated by old study protocols, has deficiencies in the test methods, and needs clarification by further study.

EPA also reviewed summaries of tests conducted in Russia which state that some derivatives of 2,4-D produced adverse effects on unborn animal fetuses at much lower levels than indicated by the data in EPA's files. These summaries could not be used in the Agency's review because the identity of the test material, and its impurities, was unclear, and because there were no numerical data to back up the summary conclusions. In some cases tests need to be done on specific derivatives of 2,4-D.

3. Oncogenicity (potential for causing tumors) - Several rodent studies have been conducted to date, but none of these studies produced data that showed 2,4-D was oncogenic in test animals. The tests were conducted a decade ago and are considered to be inadequate by today's scientific standards. New studies on rodents are needed.
4. Mutagenicity (inheritable effects) - The vast majority of the mutagenicity studies conducted on 2,4-D are negative. However, there are three positive studies. Taken as a group, the results of the studies can best be described as inconsistent and inconclusive. A new series of tests being conducted by the Department of Health, Education, and Welfare will be reviewed by EPA when they are completed.
5. Epidemiology - No epidemiological studies of human health effects from 2,4-D exposure have been completed. However, EPA is currently investigating reports about alleged adverse effects from potential chemical exposure in several parts of the country. EPA will be looking at the results of those studies and will decide in the near future about additional field work.

V. Exposure to 2,4-D

There are at least three ways that the average citizens might come into contact with 2,4-D - through the diet, during home use, and drift of the herbicide from nearby use.

a) Diet

The EPA has set tolerances for residues of 2,4-D in various food crops. The Food and Drug Administration (FDA) routinely samples a variety of foods (the Market Basket Survey) which FDA considers to be representative of the average American diet. Samples are analyzed for pesticide residues. During the period of 1974 to 1977, no 2,4-D residues were found in any of the products surveyed. However, during the 1965 to 1977 period, a variety of other food products were analyzed, of which about 1.1% were positive for 2,4-D in very minute quantities that were well below EPA's tolerance (allowable residue) levels.

b) Home use

There are currently a number of registered home-use products which contain 2,4-D in a variety of formulations. Exposure to the herbicide in home-use situations will depend to some extent on the specific formulation used. If care is exercised by the homeowner in adhering to the directions for use and precautionary statement on the label, exposure to 2,4-D should be low.

c) Drift

"Drift", the airborne transport of pesticide materials to a non-target area, is a common source of exposure. Sometimes, a pesticide will drift during application, depending on climatic conditions (temperature, wind speed), type of formulation used, terrain (forests, mountains), and type of application method used (aerial, ground spray). Several States have imposed restrictions on 2,4-D use in order to cut down on drift potential.

Once on the ground or target crop, the herbicide may become airborne again by the process of vaporization. This particular type of drift has been the subject of intensive research by the producers of 2,4-D. Since the introduction of less volatile forms of the herbicide over the last few years, this kind of drift has become much less extensive.

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VI. Environmental Persistence

2,4-D is not a persistent pesticide. Breakdown of the herbicide begins almost immediately after application at a rate dependent on several environmental factors such as temperature, humidity and medium (air, soil, crop, water). The rate of loss (commonly referred to as the half-life) is a measure of the time required for half of the substance to be degraded or lost.

On sprayed vegetables, the half-life varies from 1-3 weeks depending on geographic location, climatic conditions, vegetation type, application technique and formulation used.

In soil, the half-life varies from several days to 2 weeks, depending on acidity, soil type and amount of rain.

In water, the half-life varies from a few days to several months depending on factors such as oxygen concentration, acidity, light intensity, water temperature and formulation used.

April 22, 1980

18002

Identification of Three Neutral Contaminants in Production Grade 2,4-D

Beverly L. Huston

*Letter copy in M. Long
away - included file*

The structure of a major neutral contaminant in production grade 2,4-D is derived by chemical and spectroscopic data and confirmed by synthesis. Two other neutral impurities are tentatively identified and shown to be isomeric with the major

neutral contaminant by combined glc mass spectroscopy. Evidence is presented to show that these impurities can interfere with the glc analysis of 2,4-D for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin content.

Although the toxicology of pesticides is extensively studied, little research has been devoted to the nature and toxicological significance of trace amounts of impurities in pesticide chemicals. However, the observation (Courtney *et al.*, 1970) that samples of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) used in a teratogenic study contained significant amounts of the highly toxic compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Higginbotham *et al.*, 1968) has led to an increased interest in the nature of impurities in pesticides.

Recently, we had occasion to analyze, by gas-liquid chromatography, the neutral extracts of some samples of production grade 2,4,5-T and 2,4-D (2,4-dichlorophenoxyacetic acid). The glc tracings of the extracts from both pesticides indicated the presence of several neutral contaminants and in the case of the 2,4-D extract three of these impurities had very similar retention times to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The present paper describes the isolation and characterization of the major of these three impurities and the tentative identification of the other two. Furthermore, this work points out the need for caution on the part of the analyst in interpreting glc data alone when analyzing samples of pesticides for impurities.

EXPERIMENTAL

Materials. All solvents were pesticide grade and used as received. Production grade 2,4-D and the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were generously supplied by Dow Chemical Company, Midland, Michigan. 2,6-Dichlorophenol, 2,4-dichlorophenol, and methylene iodide were obtained from Aldrich Chemical Company, Eastman Organic Chemicals, and Fisher Scientific, respectively.

Instruments. Mass spectra were obtained with a Hitachi Perkin-Elmer (RMS-4) mass spectrometer coupled with a Perkin-Elmer (Model 990) gas chromatograph, fitted with flame ionization detectors. The stainless steel column, 6-ft $\times$ 1/8-in. o.d., was packed with 3% XE-60 on 100-120 mesh Chromosorb W (HP), Chromatographic Specialties Ltd. Operating conditions were: detector and injector temperatures, 220°C; oven temperature, 200°C; carrier gas, helium at a flow rate of 34 ml/min. Nmr spectra were obtained with a Varian A-60A spectrometer in deuteriochloroform solution with tetramethylsilane as internal standard. Ultraviolet spectra were obtained with a Unicam model SP 1800 spectrometer. Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. Microanalyses were performed by A. B. Glynn, Microanalysis Laboratories Limited, Toronto, Ontario.

Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Tunney's Pasture, Ottawa K1A 0L2, Ontario

Chromatography. Baker analyzed reagent silica gel was used for column chromatography. Preparative thin-layer chromatography was performed on plates coated with 0.5 mm silica gel (MN-Silica Gel G/u.v.-254, Macheney, Nagel & Co.). A mixture of hexane-benzene (4:1) was used as developing solvent. Glc analyses were obtained on several instruments using the columns and conditions listed in Table I.

Extraction of 2,4-D Neutral Impurities. Production grade 2,4-D (1 kg) was extracted by shaking with hexane (1 l.) and filtering through a medium porosity sintered glass funnel. The extraction process was repeated with four additional (1 l.) portions of hexane. A total of 8 kg of 2,4-D was processed in this manner and the combined extracts were evaporated to dryness on a rotary evaporator at 40°C. The residue was taken up in ether (100 ml) and extracted with two 50-ml portions of 10% sodium hydroxide solution, washed with water (50 ml), and the ether layer was dried with anhydrous sodium sulfate. Evaporation of the ether gave the neutral impurities as a pale yellow semi-solid.

Purification of Impurity III. The neutral residue was dissolved in a minimum amount of hexane and chromatographed on a column of silica gel (1.5 cm $\times$ 42 cm). Elution with hexane (200 ml) gave a fraction that contained impurity III as the major component as indicated by glc. Preparative tlc of this fraction gave a band (R_f 0.62) which was eluted with ether and shown by glc to contain impurity III with a trace of impurity II. Three recrystallizations of this material from hexane gave pure III as colorless crystals, mp 101-101.5°C; nmr δ 5.76 (s, 2 H), 7.25-7.41 (m, 6 H); uv λ_{max}^{EtOH} 228 nm (ϵ 19,900), 281 (2740), 289 nm (2240); mass spectrum m/e 336 (parent ion).

Anal. Calcd. for $C_{12}H_4O_2Cl_2$: C, 46.42; H, 2.38; Cl, 42.26. Found: C, 46.34; H, 2.35; Cl, 41.24.

Synthesis of Bis(2,4-dichlorophenoxy)methane and Bis(2,6-dichlorophenoxy)methane (3 and 5). The appropriate dichlorophenol (16.3 g, 0.1 mol), anhydrous potassium carbonate (14 g, 0.1 mol), methylene iodide (4 ml, 0.05 mol) and dry acetone (100 ml) were combined and refluxed for 8 hr. The mixture was cooled, filtered, and the solvent removed *in vacuo*. The residue was dissolved in methylene chloride (100 ml), washed with two 75-ml portions of 10% sodium hydroxide solution, one 75-ml portion of water, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a pale yellow solid which was recrystallized twice from 95% ethanol to give colorless crystals of 3 and/or 5. Bis(2,4-dichlorophenoxy)methane, mp 100-102°C [lit. mp 100.4-100.8°C (Miron and Lowy, 1951)]. Mixture melting point with III 101-102°C; nmr δ 5.75 (s, 2 H), 7.25-7.41 (m, 6 H); mass spectrum m/e 336 (parent ion). Bis(2,6-dichlorophenoxy)methane, mp 78.5-80°C; nmr δ 5.68 (s, 2 H), 6.85-7.40 (m, 6 H); mass spectrum m/e 336 (parent ion).

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Table I. Glc Parameters

Column	Stationary phases	Support	Dimensions	Injector/detector temperature, °C	Oven temperature, °C	Detection	Carrier gas flow rate, ml/min
Glass	QF-1/SE-30 6%/4%	80-100 mesh Chromosorb W (HP)	5-ft × 1/8-in. (o.d.)	225/210	190	Tritium foil ^a electron capture	120 ^a
Glass	SE-52 2.5%	80-100 mesh Gas Chrom Q	3-ft × 1/8-in. (o.d.)	220/220	185	Tritium foil ^a electron capture	60 ^a
Stainless steel	SE-30 5%	80-100 mesh Chromosorb W	6-ft × 1/8-in. (o.d.)	230/230	200	Flame ionization ^c	30 ^c
Stainless steel	XE-60 3%	100-120 mesh Chromosorb W (HP)	6-ft × 1/8-in. (o.d.)	220/220	200	Flame ionization ^d	35 ^d

^a Carrier gas, nitrogen. ^b Carrier gas, helium. ^c Varian Aerograph Model 600-D. ^d Varian Aerograph Model 2100. ^e Hewlett-Packard Model 700. ^f Perkin-Elmer Model 990.

Table II. Gas Chromatographic Comparison of Three 2,4-D Neutral Impurities and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Column	Retention time, min 2,3,7,8-tetrachlorodioxin	2,4-D Impurities		
		I	II	III
QF-1/SE-30	11.5	9.2	10.4	11.6
SE-52	7.4	5.6	6.5	7.4
SE-30	7.9	6.0	6.7	7.7
XE-60	5.8	5.7	6.3	7.6

Anal. Calcd. for $C_{12}H_4O_2Cl_2$: C, 46.42; H, 2.38. Found: C, 46.56; H, 2.41.

Synthesis of 2,2',4,6'-Tetrachlorodiphenoxymethane (6). α -2,4-Trichloroanisole (2 g, 0.01 mol) [prepared according to the method of Lashua and Ranck (1966)] was stirred and refluxed for 8 hr with a suspension of the potassium salt of 2,6-dichlorophenol in dry benzene. The cooled mixture was washed with water and the benzene layer dried over anhydrous sodium sulfate. Evaporation of the solvent gave a colorless solid which, upon four recrystallizations from 95% ethanol, gave colorless needles, mp 85-86°C: nmr δ 5.75 (s, 2 H), 6.88-7.61 (m, 6 H); mass spectrum *m/e* 336 (parent ion).

Anal. Calcd. for $C_{12}H_4O_2Cl_4$: C, 46.42; H, 2.38. Found: C, 46.53; H, 2.48.

RESULTS AND DISCUSSION

The analysis of the neutral extracts of production grade 2,4-D was investigated by glc using the columns and conditions listed in Table I. It is apparent, from an examination of Figure 1, that several neutral impurities are present and, as indicated in Table II, three of these components, labeled I, II, and III in Figure 1, have retention times similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin under the glc conditions stated in Table I.

Isolation and Characterization of Component III. Since component III appeared to be present in relatively substantial quantities, it was decided to isolate and identify this material. Hexane extraction of production grade 2,4-D followed by column chromatography, on silica gel, gave a fraction enriched in component III as estimated by glc. Further purification of this fraction by preparative thin-layer chromatography, on silica gel, gave a pale yellow solid which, after repeated recrystallizations from hexane, gave pure III as colorless crystals, mp 101-101.5°C. The mass spectrum of III shows a parent ion at *m/e* 336 and an isotopic distribution pattern typical of four chlorine atoms (McLafferty, 1967). Consideration of this data together with the elemental analysis (experimental) gives rise to $C_{12}H_4O_2Cl_4$ as a

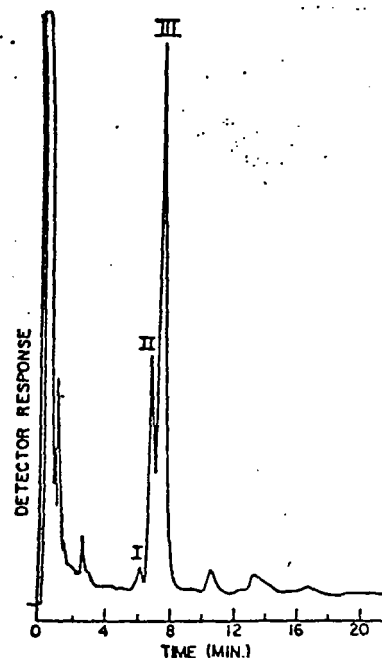
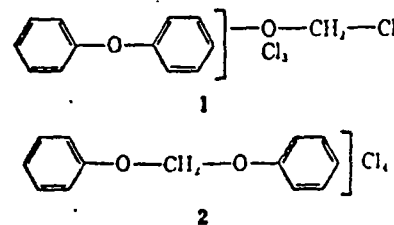


Figure 1. Chromatogram of 2,4-D neutral extract. Column 6' SE-30; temperature, 200°C; nitrogen flow rate 30 ml/min

probable molecular formula for compound III. The ultra-violet spectrum of III exhibits absorption bands at 228 nm ($\epsilon = 19,900$), 281 nm ($\epsilon = 2740$) and 289 nm ($\epsilon = 2240$), suggesting a substituted phenol ether (Scott, 1964). The nuclear magnetic resonance spectrum of III consists of a complex multiplet between 7.25 and 7.41 ppm (6 H) and a singlet at 5.76 ppm (2 H), indicating the presence of six aromatic protons and a substituted methylene group. These facts suggest two possible part structures 1 and 2 for compound III.



A choice between part structures 1 and 2 was made when it was observed that an acetone solution of III did not react with aqueous silver nitrate. Since part structure 1 contains an ionizable halide function it would be expected to give a precipitate with silver nitrate. Part structure 2, on the other hand, contains only aromatic halogen and would not be

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Table III. Comparison of Physical Properties of 2,4-D Impurities and Synthetic Diphenoxymethanes

	Compound					
	I	Bis-(2,6-dichlorophenoxy)methane	II	2,2',4,4'-Tetrachlorodiphenoxymethane	III	Bis-(2,4-dichlorophenoxy)methane
Retention time, min	6.0,* 9.2*	6.0,* 9.2*	6.7,* 10.4*	6.7,* 10.4*	7.7,* 11.6*	7.7,* 11.6*
Mass spectrum (parent ion and main fragmentation ion) <i>m/e</i>	336 175	336 175	336 175	336 175	336 175	336 175
Nmr, ppm	...	...	...	...	5.76 (2 H) 7.25-7.41 (6 H) 101-101.5	5.75 (2 H) 7.25-7.41 (6 H) 100-102
Melting point, °C	...	78.5-80	...	85-86	...	...

* SE-30 column. † SE-30/QF-1 column.

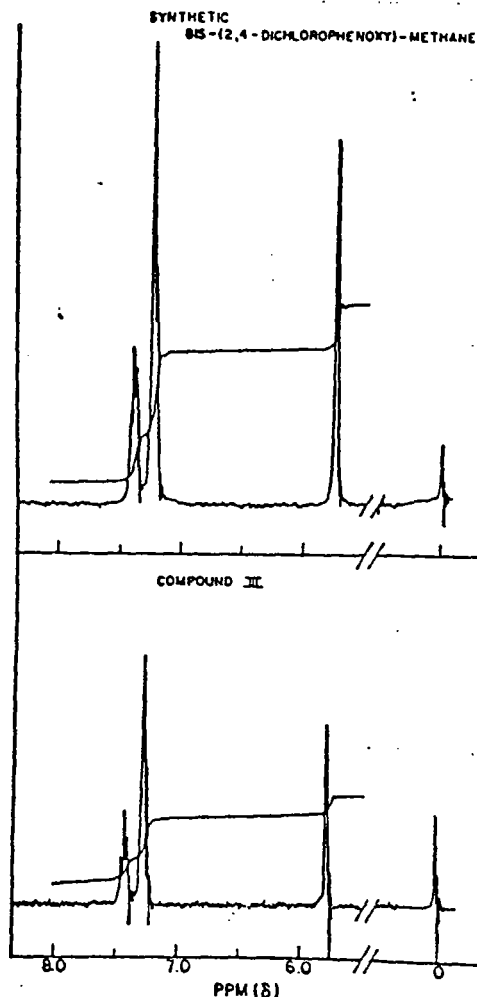
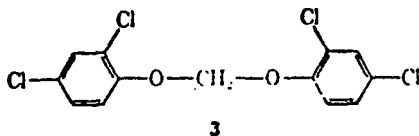


Figure 2. Nmr spectra of compound III and synthetic bis(2,4-dichlorophenoxy)methane

expected to react with silver nitrate. Thus, part structure 2 was favored for compound III and furthermore, since the most reasonable precursor of III is 2,4-dichlorophenol, structure 3 could be written for compound III.



The mass spectrum of compound III supports structure 3 since the main fragmentation ion at *m/e* 175 represents the loss of C_6H_4OCl from $C_{12}H_8O_2Cl_2$ and can be readily accom-

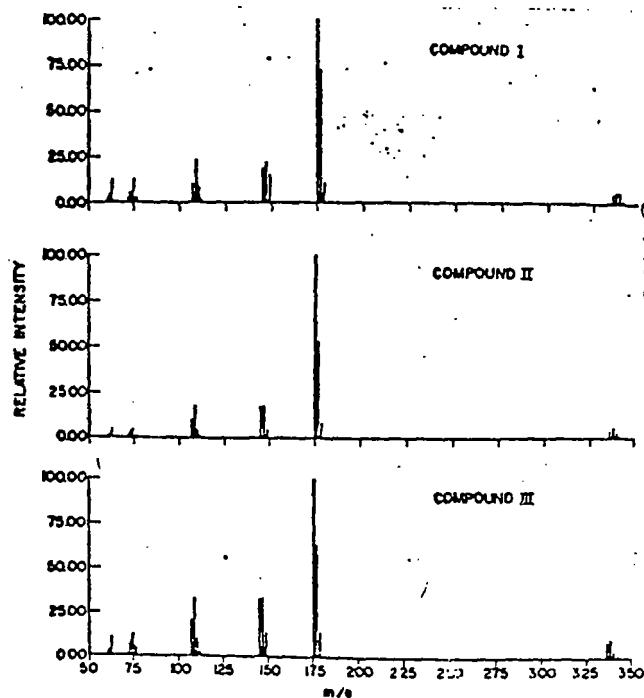
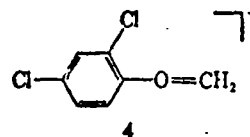


Figure 3. Mass spectra of compounds I, II, and III

modated by cleavage of one of the $O-CH_2$ bonds in 3 to give the ion 4, analogous to the loss of an alkoxyl group of a ketal (Budzikiewicz *et al.*, 1967).



Structure 3 was proven to be correct by synthesis of bis-(2,4-dichlorophenoxy)methane, and its identity with III confirmed by mixture melting point, glc retention time, and comparison of nmr spectra (Figure 2) and mass spectra (Table III).

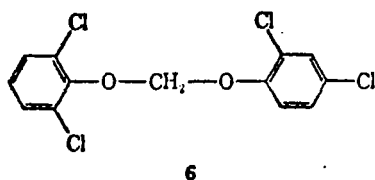
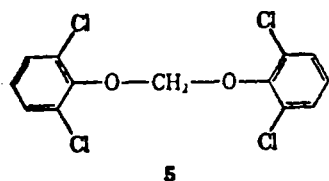
The Identity of Impurities I and II. Since impurities I and II are present to a lesser extent than III, as indicated by glc, no attempt was made to isolate them; instead combined glc-mass spectroscopy was used to elucidate their structures. The mass spectra of impurities I, II, and III are virtually identical (Figure 3), suggesting that they are positional isomers. Furthermore, since it is known that production grade 2,4-D (Lot #092440) contains small amounts of 2,6-dichlorophenol as an impurity (Wiffen, 1970), it is reasonable to assume that compounds I and II might have structures

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OIL FROM 82 PEANUT GENOTYPES

and 6. Structures 5 and 6 were synthesized and shown to be identical to I and II, respectively, by comparison of glc retention time, on two columns and mass spectra (Table III).



Semiquantitative analysis of the commercial sample of 2,4-D used in this study indicated that compounds I, II, and III were present at levels of 1, 10, and 30 ppm, respectively. The toxicological significance of these three compounds as impurities in production grade 2,4-D is not known. However, from a study of the teratogenic effects of both production

grade 2,4-D and purified 2,4-D (Khera, 1972), it appears that compounds I, II, and III have no adverse effects at the levels administered in his investigation.

ACKNOWLEDGMENT

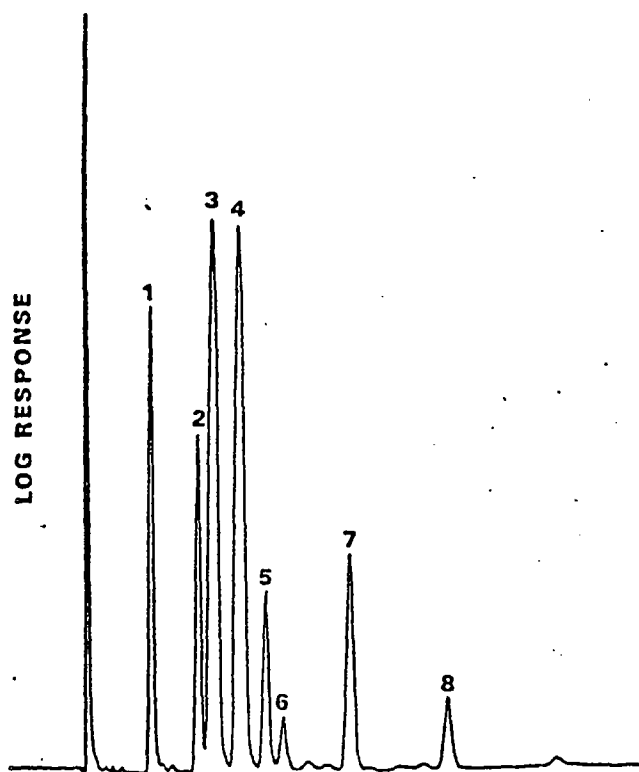
The author thanks Ray Ings for technical assistance and Walter Miles for obtaining the mass spectra.

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Received for review October 29, 1971. Accepted January 24, 1972

WORTHINGTON, HAMMONS, ALLISON



EXPERIMENTAL PROCEDURES

Genotypes. Selection of the 82 genotypes was based either upon prior knowledge of chemical composition (Holley and Hammons, 1968) or upon possible use in the development of new commercial varieties. Virginia (*A. hypogaea* subsp. *hypogaea*) and Spanish (subsp. *fastigiata*) botanical types, varying widely in fatty acid composition, seed size, growth habit, duration, and other characteristics, were included in the group, as were several representative commercial cultivars. All genotypes were grown at the Coastal Plain Station, Tifton, Georgia, in 1965, 1967, and 1968, employing standard cultural practices (McGill and Samples, 1969). At harvest the plants were dug, allowed to dry in the windrow for 24 to 48 hr, stacked, and cured in the stack for 6 to 8 weeks prior to picking.

Sample Preparation and Analysis. Samples were hand-shelled and stored at 4-5°C until time of analysis. Since fatty acid composition is influenced by seed maturity (Worthington, 1969) only those seeds that appeared to be fully mature, as indicated by seed size, absence of wrinkles, and by pigmentation of seed coat and interior of pericarp, were retained for analysis. Oil was expelled with a silver-plated Carver press, filtered through Whatman paper, and the autoxidation induction period measured by the method of Olcott and Einset (1958) as modified by Young and Holley (1965). Oil samples (0.2 ml in 10-ml beakers) were heated in



THE DOW CHEMICAL COMPANY

March 4, 1980

2,4,5-T AND SILVEX DEFENSE

Cancellation hearings for suspended and silvex are scheduled to begin on March 10, 1980, before the Federal District Court, Law Judge in Washington, D.C. This will be the first time in 10 years ago when Dow appealed EPA's proposed cancellation of 2,4,5-T for use in rice fields.

The core team handling the defense includes a scientist from the Product Registrations group, along with Dow attorneys and outside counsel. Dr. Marguerite L. Leng, Senior Registration Specialist, will serve as Science Advisor to the legal team headed by John Scriven of the Dow Legal Department. The day-to-day operations will be handled by Mark Tucker, also a Dow attorney. Outside counsel includes Edward W. Warren, L. Mark Wine, Richard L. McConnell, and John A. Zackrison of the Washington D.C. law firm of Kirkland & Ellis, and Rudolph H. Schroeter of the Los Angeles law firm of LaFollette, Johnson, Schroeter, and DeHaas.

Marguerite will provide a unique broad perspective on the science issues, including analytical methodology, environmental fate, metabolism, residues, and toxicology of 2,4,5-T, silvex and their TCDD contaminant. This in-depth knowledge was developed during 14 years in the registration group, mainly reviewing research reports and preparing interpretive summaries of data on Dow herbicides for submission to government agencies. Included were a total of 32 volumes on 2,4,5-T and silvex submitted in petitions for tolerances (safe residue limits) and in the Dow risk response to EPA's Rebuttable Presumption Against Registration (RPAR) for 2,4,5-T.

Her responsibilities will be to review witness statements and documents in the vast repository of exhibits supporting both sides of the issue. The critical period will be during the time EPA presents its risk case, estimated to take about 6 months after the hearings get underway. We appreciate the considerable personal sacrifice Marguerite will make in spending most of this time in Washington away from her family. However, as she says "I have devoted at least 6 years of my career at Dow on this project, and want to see it through to a favorable conclusion based on science instead of politics."

If you have any questions, please contact me or John Scriven.

John W. Weseloh
Manager, Product Registrations
Regulatory and Legislative Issues
Health and Environmental Sciences

gb

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J. H. Ashley, Washington
F. H. Ashley, 2030

cc: C. A. I. Goring, 9008
F. D. Hoerger, 2030
M. L. Leng, 9008
J. G. Scriven, 2030
M. D. Tucker, 2030
Registration Group

on

n. J. Disnburger, 9001
J. E. Donalds, 9008
B. C. Fischback, Pittsburg
A. C. Fischer, 9008
R. F. Flannery, 9008
D. R. Frayer, 2030
P. J. Gehring, 1603
M. E. Getzendaner, 9008
C. Goodman, 9001
J. H. Haines, 2030
R. G. Hanson, Walnut Creek
R. E. Hefner, 1776
J. C. Hansen, Washington
B. B. Holder, 2030
R. C. Hunter, 9008
O. K. Jantz, 9008
D. J. Jensen, 9008
J. E. Johnson, 2030
J. Jones, Pittsburg
E. E. Kenaga, 9008
R. J. Kociba, 1803
E. V. Luoma, 574
D. D. McCollister, 9008
K. R. McKennon, 2030
R. J. Moolenaar, 1702
R. D. Moss, 9008
W. W. Muelder, 9001
M. G. Norris, 9001
P. F. Orefice, 2030
D. W. Osborne, 9001
J. K. Priddy, 9008
M. E. Pruitt, 2020
D. L. Rooke, 2020
B. A. Schwetz, 1803
L. A. Shadoff, 574
T. D. Sinclair, 2030
J. M. Theis, 9008
J. Venable, 1603
S. R. Vranish, 9008
A. J. Watson, 9001
C. S. Williams, 9008
C. D. Woods, 2030

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The Mortality Experience of Workers Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident

Judith A. Zack, M.P.H., and Raymond R. Suskind, M.D.

A standardized mortality analysis was conducted on workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident at the Monsanto Company plant in Nitro, West Virginia. One hundred and twenty-one workers who developed chloracne resulting from this accident on March 8, 1949, were selected for study. Follow-up of this group was 100% complete. The standardized mortality ratio for all causes of death was shown to be 0.69, with 32 deaths observed and 46.41 expected. For the categories of malignant neoplasms and circulatory diseases, the standardized mortality ratios were 1.00 and 0.68, respectively. Because of the small size of the cohort and the relatively small number of deaths observed, the results of this study cannot be considered conclusive. However, it is important that no apparent excess in total mortality or in deaths from malignant neoplasms or diseases of the circulatory system was observed in a group of workers with a high peak exposure to tetrachlorodibenzodioxin who were followed over a period of nearly 30 years. The results of this study will be incorporated with those of a larger study which will include plant workers exposed in the course of 2,4,5-trichlorophenoxyacetic acid production during the period 1948 to 1969.

A wide variety of acute and sub-acute health effects has been reported in workers involved in the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) from 2,4,5-trichlorophenol (TCP). The most consistent clinical finding is chloracne, a skin disease characterized by com-

edones, cysts, pustules, and abscesses. Hepatic dysfunction, peripheral neuritis, disorders of fat metabolism, and porphyria cutanea tarda are other frequently reported findings in these workers.¹ Chloracne has been shown to be essentially due to 2,3,7,8-tetrachlorodibenzodioxin (TCDD),² a byproduct in the synthesis of 2,4,5-T. The subject of this paper is 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 at the Nitro, West Virginia, plant in 1949.

Production of trichlorophenol began in the fall of 1948 at the Nitro plant. In this process, the reactants 1,2,4,5-tetrachlorobenzene, sodium hydroxide, and methanol were all added to the autoclave. Heat was applied and, when the pressure reached the desired point, the autoclave was vented. On March 8, 1949, about six months after production start-up, a violent reaction and decomposition occurred when temperature and pressure within the autoclave became excessive. The relief valve opened and the fumes and tarry residues from the decomposed contents of the autoclave were discharged into the atmosphere and into the interior of the building.

Employees who worked in the area of TCP production or were involved in the clean-up began to develop symptoms immediately following exposure to the material which was discharged from the autoclave. Symptoms included eye and respiratory tract irritation, headache, dizziness and nausea, and a severe irritant reaction of the exposed skin. After these initial symptoms subsided, the chloracne and other symptoms became evident. Ashe and Suskind³⁻⁵ examined a total of 12 more severely affected workers on three occasions during the period of 1949 to 1953. Another 26 persons with chloracne, apparently not related to the accident, were also examined in 1953. The

From the Department of Medicine and Environmental Health, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63166 (Ms. Zack, Epidemiologist), and the Institute of Environmental Health, Kettering Laboratory, the University of Cincinnati Medical Center, Cincinnati, OH 45267 (Dr. Suskind, Director).

clinical symptoms, in order of relative frequency,⁴ included 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. On examination, all of the cases had chloracne. Several were severely hyperpigmented, especially on the face. Of the six workers examined in 1949 and 1950, four had liver enlargement and one had sensory loss in one foot. Liver impairment as indicated by hepatomegaly, tenderness and soreness in the right upper quadrant and epigastrium and a delayed prothrombin time, was observed.^{1,3,6}

In 1953, four of the six workers examined in 1949 and 1950 were re-examined and six additional workers involved in the accident were also examined. The findings in this later examination indicated a general regression of both the cutaneous and noncutaneous symptoms which had been present earlier. All of the workers showed a marked improvement in their skin lesions — there were residua of the acne and a few active lesions. In a few cases, workers continued to complain of aches and pains of the lower extremities and back, nervousness, excessive fatigue, and dyspnea. No clinical explanation for these complaints could be made based on the results of the physical examination.⁴

The findings of the examinations by Ashe and Suskind are consistent with those which have been reported in other industrial episodes which occurred subsequently.¹ The acute health effects of TCDD exposure are described in the literature,⁷ but little is known of the chronic effects.

Several reports describe the occurrence of cancer and other deaths in workers exposed to TCDD which suggests an association between exposure and the subsequent development of a variety of neoplasms.⁸⁻¹¹ These reports, however, are generally of small groups of workers with relatively short periods of follow-up and are considered to be preliminary in nature. In a 25-year follow-up study, 17 deaths were observed among a cohort of 75 German workers who had been involved in a 1953 TCP process accident.⁴ Of the 17 deaths observed (11-25 expected depending on the choice of a control population), six were from cancer (four or fewer expected), five from cardiovascular disease (as expected), two from suicide (fewer than one expected), one from liver cirrhosis, one from a urogenital tract disease, and two from external causes. Of the six cancer deaths, three were from stomach cancer in the age group 60-69, a number significantly higher than expected. Two other cancer deaths were from oat-cell carcinoma of the lung and one was from adenocarcinoma of the colon.

A similar accident occurred in the Netherlands in 1963 in a factory producing 2,4,5-T.⁴ Eight deaths have been observed among 93 exposed workers. Five or six of these deaths were from cardiovascular disease. The proportion of deaths due to myocardial infarction was noted to be high.

Jirasek et al.¹⁰ and Pazderova¹¹ followed 55 of the 78 Czechoslovakian workers who were affected by chloracne resulting from occupational exposure to 2,4,5-T and pentachlorophenol. In this study, five deaths were observed. These included two deaths from bronchiogenic carcinoma (less than one expected), one from cardiovascular disease, one from liver cirrhosis, and one from an

accidental industrial intoxication.

In 1976, a TCP process accident in Meda, Italy, resulted in the contamination of a large and densely populated area.⁴ A preliminary mortality study has been conducted in two of the 11 towns affected. The overall mortality rate did not differ from that expected, but increases in deaths from liver cirrhosis and leukemia were suggested.

The chronic toxicity of TCDD exposure to animals has been more extensively studied. TCDD toxicity has been thoroughly reviewed.⁷ Chronic toxicity to TCDD is manifested by liver necrosis, thymic atrophy, and depletion of the lymphoid organs. Two studies indicate that chronic administration of low levels of TCDD to rats is associated with an increased incidence of neoplasia. In one study, the oral administration of TCDD produced an increase in hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue.¹² In another study, TCDD fed to rats produced tumors in 38% of the test animals.¹³ Neoplastic nodules and cholangiocarcinomas of the liver were observed.

The study reported here will examine the mortality experience of a cohort of 121 employees involved in the 1949 trichlorophenol process accident, with special emphasis on cardiovascular disease and on neoplasms, particularly of the stomach, liver, lung, and skin.

Population and Methods

In this study, the development of chloracne, a hallmark of TCDD exposure, was used to identify employees for study. The study population consists of all persons with chloracne which could be attributed to the 1949 TCP process accident. One hundred and twenty-two employees who developed chloracne following this incident were identified from plant safety records dating to the time of the accident, and from workmen's compensation and plant medical records. One hundred and twenty-one white males were included in this study — one female who was living as of the endpoint of the study was not included in this mortality analysis. It is assumed that all of the skin disorders recorded in the plant records represent true cases of chloracne and not other types of occupational or nonoccupational dermatitis. An analysis of the chloracne cases and exposures not associated with this accident but rather with the normal TCP/2,4,5-T production processes will be the subject of a future paper.

The data were analyzed by the modified life-table method using the updated Monson program.¹⁴ In this method of analysis, the age-, race-, time- and cause-specific mortality rates for a standard population (in this case, the population of the United States) are applied to the person-years lived classified by age, race, and time. A standardized mortality ratio was calculated as the ratio of the observed deaths to the expected deaths for 22 selected causes of death. The statistical significance of differences between observed and expected numbers was based on the Poisson distribution and statistical significance was determined at the 5% level of significance.

For the purpose of analysis, each member of the study cohort was assumed to have entered the study on March 8, 1949, the date of the accident. The vital status of each member was determined using standard follow-up techniques and ascertained as of December 31, 1978. For each person found to be deceased, a death certificate was ob-

Table 1. — Observed and Expected Deaths Among 121 Males Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident.

Cause	ICD No. (Eighth Revision)	Observed	Expected	SMA
All causes of death		32	46.41	0.69*
All malignant neoplasms	140-209	9	9.04	1.00
Buccal cavity and pharynx	140-149	0	0.30	†
Digestive organs and peritoneum	150-159	0	2.59	†
Stomach	151	0	0.50	†
Liver	155-156	0	0.18	†
All other digestive organs	—	0	1.91	†
Respiratory system	160-163	5	3.02	1.66
Lung	162.163	5	2.85	1.75
All other respiratory organs	—	0	0.17	†
Skin	172.173	1	0.15	†
Genitourinary organs	185-189	0	1.16	†
Lymphatic and hematopoietic tissue	200-209	3	0.88	†
Other sites	—	0	0.94	†
Diseases of the nervous system and sense organs	320-389	0	0.36	†
Diseases of the circulatory system	390-458	17	25.01	0.68
Arteriosclerotic heart disease, including coronary heart disease	410-413	13	17.74	0.73
All other disease of the circulatory system	—	4	7.27	†
Diseases of the respiratory system	460-519	1	2.78	†
Diseases of the digestive system	520-577	0	2.26	†
All other diseases	—	2	3.18	†
External causes of death	800-998	3	3.78	†

*p < 0.05

†Less than 5 observed deaths

tained. The underlying cause of death was coded to the 8th Revision of the International Classification of Diseases, Adapted¹⁵ by an experienced nosologist.

Results

All of the 121 members of the study cohort were traced. Eighty-nine were verified living and 32 were verified deceased by death certificate.

The results of the standardized mortality analysis of the 121-member study cohort are shown in Table 1. The standardized mortality ratio for all deaths is shown to be 0.69, with 32 observed deaths and 46.41 expected. This is the only statistically significant difference shown in this table. There were nine deaths from malignant neoplasms with 9.04 expected. There were no deaths from stomach or liver cancer. There were five lung cancer deaths versus 3.02 expected and one skin cancer death with 0.15 expected. The malignant tumor was a fibrous histiocytoma presumably of dermal origin, which is rare. There were three deaths from neoplasms of lymphatic and

hematopoietic tissue with 0.88 expected.

There were 17 observed deaths from circulatory diseases with 25.01 expected. The standardized mortality ratio for circulatory diseases was low at 0.68.

Case summaries for the cancer deaths are given in Table 2.

Discussion

Because the study cohort was small and only 32 deaths were observed, the results cannot be considered conclusive. Nevertheless, the analysis of the mortality experience of these workers indicated no apparent excess of total mortality or of deaths due to malignant neoplasms or circulatory diseases.

The TCDD-exposed workers in the present study represent the largest group ever investigated after long-term follow-up. The criteria for inclusion (presence of the workers at the 1949 accident and the subsequent occurrence of chloracne) limit the group to those with a significant exposure at that time. The latency period of 29 years

Table 2. — Cancer Deaths Among a Cohort of 121 Males Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident.

Year of Birth	Year of Hire	Year of Death	Death Certificate Statement of Cause of Death	Smoking History*
1909	1943	1962	Lung cancer (162.1)	Cigarettes
1910	1927	1970	Pulmonary carcinoma (162.1)	Cigarettes
1911	1939	1964	Bronchiogenic carcinoma (162.1)	Cigarettes
1922	1945	1973	Bronchiogenic carcinoma (162.1)	Nonsmoker
1915	1939	1970	Lung cancer (162.1)	Cigarettes
1920	1946	1978	Malignant fibrous histiocytoma of soft tissue origin (173.9)	Cigarettes
1919	1943	1973	Hodgkin's disease (201.0)	Cigarettes
1907	1943	1971	Lymphatic leukemia (204.9)	Pipe
1910	1939	1978	Acute myelogenous leukemia (205.0)	Cigarettes

*Smoking history was obtained by interviews with former co-workers of the decedents

is longer than that of any previous study, and the follow-up is complete. Therefore, although the cohort is small, it represents the best opportunity so far to study the long-term effects of TCDD on mortality. By augmenting these data with the results of comparable mortality studies, the long-term effects of TCDD may be more definitely evaluated.

The authors wish to thank Mrs. Janet Yung, Mr. Randy Picolet, and Mrs. Phyllis Korte for their assistance with the data collection.

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15. ACUTE AND SUB-ACUTE ORAL TOXICITY OF
2,4,5-TRICHLOROPHENOXYACETIC ACID FOR DOGS

Victor A. Drill, Ph.D., M.D.
Professor of Pharmacology
Wayne University College of Medicine

1950

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ACUTE AND SUB-ACUTE ORAL TOXICITY OF
2,4,5-TRICHLOROPHENOXYACETIC ACID FOR DOGS

Victor A. Drill

Victor A. Drill, Ph.D., M.D.
Professor of Pharmacology
Wayne University College of
Medicine

1958

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Investigations have shown that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) can be used as a herbicide (1), in concentrations ranging from 0.1 to 0.2 per cent. Toxicological studies on the material are very limited.

In the present study the acute and chronic oral toxicity of 2,4,5-T was determined in dogs. In the present data histological changes are not included as this phase of the study is not yet 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 2,4,5-T used was commercial material with a purity of 100%.

In the acute studies the calculated dose of 2,4,5-T was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days at which time survivors were autopsied. In the chronic study the 2,4,5-T was administered orally in capsules, 5 days a week over a 13 week 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 (2). 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, ^{intestine} thyroid, and ovary or testes and fixed in 10% formalin.

RESULTS

I. ACUTE ORAL TOXICITY.

1. Symptoms produced. The survival at various dose levels of 2,4,5-T is listed in Table 1. The LD₅₀ is in the range of 100 mgm. per kilo or slightly higher.

The symptoms observed in the dogs were fairly mild and limited to a slight to moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses of 2,4,5-T. No symptoms were seen in the one dog that died following a single dose of 100 mgm. per kilo (Table 1).

2. Body weight. Doses of 100 mgm. per kilo or greater produced a slight fall in body weight during the test period. The results are somewhat variable with the 50 mgm. per kilo dose but indicate only a relatively slight effect of the drug on body weight (Table 1).

3. Gross pathology. The changes observed were limited to the gastro-intestinal tract and lungs. In dog 90 the duodenal mucosa was a slight diffuse red in color and in dog 74 small pin point hemorrhagic areas were observed in the mucosa together with some blood in the lumen. A small amount of blood was also found in the small intestine of dog 84. This same animal also presented a purple-black lower left lobe of the lung that had a solid blood consistency.

II. CHRONIC ORAL TOXICITY.

1. Mortality. Dogs that received doses of 2, 5 and 10 mgm. per kilo survived the 90 days. The dogs receiving 20 mgm. per kilo either in a single or divided dose died during the study.

2. General symptoms. No signs or symptoms were observed in the

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dogs that survived the study. In the dogs that died the prominent effects were weakness, slight stiffness in the hind legs, and difficulty in the swallowing of food, and bleeding from the gums in one dog.(Table 2).

3. Body weight. The 4 dogs that died showed a loss of over 2 kilos in body weight that started approximately 10 days before the death of the animal. The surviving animals do not show any significant change in body weight during this period, but tended to fluctuate about their original weight.

4. Blood count. The administration of 2,4,5-T did not have any significant effect on the hemoglobin or red cell count (Table 3). Dogs 103, 70, 57 and 105 showed some decrease in total white cell count during the study but the values did not fall lower than those seen in the control dogs. In the differential count taken before death, the per cent of lymphocytes fell to very low levels in dogs 57 and 105.

5. Gross pathology. Animal No. 93 showed a diffusely reddened duodenum and jejunum. Dog 105 autopsied on the 59th day showed a generalized jaundice as indicated by the sclera and the finding of yellow fat and yellow organs throughout the body. In dog 55 the renal capsule was stripped with difficulty.

6. Organ weights. The animals that survived the 90 day test period did not show any significant change in organ weights (Table 4). Various minor changes in organ weight can be noticed in some of the dogs fed the 20 mgm. per kilo dose who died during the study. These changes were limited to possible increase in heart, liver or kidney weight.

Discussion. Single large doses of 2,4,5-T can produce death in dogs. However, the central nervous system symptoms observed are quite mild, consisting chiefly of stiffness of the hind legs with some ataxia. These.

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symptoms seem to be definitely less than those obtained with comparable doses of 2,4-D. However, further studies with doses of 150 to 200 mg. per kilo might elicit a better series of signs and symptoms. The acutely intoxicated animals lost only a slight amount of weight. Gross pathological changes when observed were confined to the gastro-intestinal mucosa and in one dog to the lower left lobe of the lung. The decreased incidence of such changes as compared with 2,4-D paralleled the mild central nervous effects of the drug.

In the studies on chronic oral toxicity of 2,4,5-T, it was observed that the animals survived doses of 2, 5, and 10 mg. per kilo without grossly apparent ill effects, but succumbed to doses of 20 mg. per kilo. Thus the chronic toxic dose seems to be in the same range as that obtained with 2,4-D. In those animals receiving the highest dosage, 20 mg/kg of 2,4,5-T, evidence of toxic effects consistently included slight muscle spasm, difficulty in swallowing food, and loss in terminal body weight. A marked decrease in the percentage of lymphocytes in the peripheral blood was noted in two instances, while generalized icterus of the body organs and inflammation of duodenal mucosa were each observed once.

Summary. 1. The acute oral LD₅₀ for 2,4,5-T is in the range of 100 mg. per kilo or higher. Acutely poisoned dogs show mild spastic signs accompanied by a loss in body weight. Gross pathological changes were uncommon and were confined to the small intestines and lungs.

2. In chronic oral feeding of 2,4,5-T, doses of 2, 5 and 10 mg. per kilo allowed survival for 90 days and did not produce any symptoms or changes in body weight, organ weight or blood count. Dogs fed 20 mg. of

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2,4,5-T per kilo per day died during the study, showing mild spastic symptoms, a loss in body weight, and in 2 cases a decrease in the per cent of lymphocytes in the peripheral blood. Minor changes were present in the duodenum in one dog, and jaundice in another dog at autopsy.

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1. Hildebrand, E. M., Science, 103:465, 1946.
2. Evalyn, K. A., J. Biol. Chem., 115: 63, 1936.

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Table 1

Effect of single oral doses of 2,4,5-T in dogs

Dog No.	Dose mg/K	*Died day	Mortality	Wt loss Kg	Symptoms*
7M	400	2	1/1	-1.1	+
95M	250	3	1/1	-1.4	+
83F	100	S	1/4	-0.5	0
84F	100	7		-1.0	0
90F	100	S		-1.0	0
95F	100	S		-0.4	0
100M	50	S	0/4	+0.3	0
101M	50	S		-2.3	0
106F	50	S		+0.1	0
108M	50	S		-0.6	0

* S = survival for 14 day test period.

* column indicates general severity of symptoms as discussed in text. 0 = no change from normal.

Table 2

Body weight and survival of dogs fed 2,4,5 T for 90 days

Dog No.	Dose mg/K	Weight			Day Died
		Initial Kgm	Final Kgm	Diff. Kgm	
77F	none	7.3	7.7	+0.4	S
102M	none	12.1	14.5	+2.4	S
87F	2	11.3	12.7	+1.4	S
99M	2	8.9	10.0	+1.1	S
66F	5	8.6	8.4	-0.2	S
103M	5	12.3	12.5	+0.2	S
93F	10	12.3	11.5	-0.8	S
55M	10	13.0	12.2	-0.8	S
70F	10	9.8	9.2	-0.6	S
57M	*20	11.8	9.2	-2.6	49
69F	*20	10.5	7.9	-2.6	75
94F	20	14.9	12.7	-2.2	11
105M	20	10.9	8.2	-2.7	59

* = Daily dose divided and administered $\frac{1}{2}$ in A.M., and $\frac{1}{2}$ in P.M.

S = survived 90 day test period.

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Table 3

Effect of 2,4,5 T on hemoglobin, total blood and differential counts

Dog No.	Dose mg/K	Day	Hb gm. %	RBC mil.	WBC	Differential count					Death day
						Neut	Lymph	Mon	Eosin	Bas	
77F	none	0	9.60	5.03	16,100	67	26	1	0	6	
		30	10.75	5.63	13,300	56	16	2	0	3	
		90	12.74	6.82	14,700	81	18	1	0	0	
102M	none	0	12.29	5.53	14,500	55	39	2	0	4	
		30	13.31	5.78	14,900	63	26	4	0	7	
		90	13.95	6.47	6,400	48	51	1	0	0	
87F	2	0	11.96	5.82	12,450	70	24	2	0	4	
		30	14.49	6.13	16,700	62	23	12	0	3	
		90	14.52	6.53	25,900	80	18	2	0	0	
99M	2	0	10.75	5.28	17,100	75	22	0	0	3	
		30	13.55	5.56	14,800	56	34	9	0	1	
		90	14.46	6.85	17,200	67	25	6	0	2	
66F	5	0	11.90	6.21	12,600	48	47	2	0	3	
		30	12.93	6.17	17,400	46	48	5	0	1	
		90	13.82	7.03	13,300	55	44	1	0	0	
103M	5	0	10.94	5.30	18,100	47	20	3	0	30	
		30	12.67	5.79	16,600	54	21	8	0	17	
		90	12.95	6.24	7,350	71	29	0	0	0	
93F	10	0	11.90	6.50	16,150	69	24	4	0	3	
		30	12.74	6.72	13,000	66	28	3	0	3	
		90	12.67	6.43	18,400	77	15	3	0	5	
55M	10	0	11.58	5.91	13,250	56	15	6	0	23	
		30	12.54	5.49	18,650	56	24	4	0	16	
		90	11.84	6.23	15,850	70	27	3	0	0	
70F	10	0	12.65	6.37	15,400	78	12	6	0	4	
		30	14.46	6.28	16,400	54	28	8	0	10	
		90	14.34	7.29	8,300	67	33	0	0	0	
57M	20	0	12.54	5.35	16,400	72	12	6	0	10	
		30	8.64	3.93	16,450	77	13	3	0	7	
		49	8.98	3.07	6,900	94	4	2	0	0	49
69F	20	0	12.48	7.10	16,050	63	29	2	0	6	
		30	14.66	6.82	11,200	71	28	0	0	1	75
94F	20	0	12.44	5.73	13,300	63	32	4	0	1	11
105M	20	0	12.48	6.03	16,800	51	44	2	0	3	
		30	12.93	5.81	14,650	78	18	4	0	0	
		59	11.97	6.14	7,175	92	9	1	0	0	59

* = daily dose divided and administered $\frac{1}{2}$ in A.M. and $\frac{1}{2}$ in P.M.

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Table 4

Organ weights of dogs fed 2,4,5 T for 90 days
Based on final body weight

Dog No.	Dose mg/K	Kidney		Liver		Heart		Adrenal		Thyroid	
		wt g	g/kg	wt g	g/kg	wt g	g/kg	wt g	g/kg	wt g	g/kg
77F	none	33	4.94	268	34.8	61	6.10	1.0	0.130	0.4	0.052
102M	none	83	5.72	405	27.9	95	6.55	1.2	0.033	1.1	0.076
87F	2	58	4.56	557	43.1	78	6.15	1.1	0.037	1.3	0.102
99M	2	48	4.80	357	35.7	75	7.50	1.0	0.100	0.7	0.070
66F	5	35	4.17	341	40.6	63	7.50	0.8	0.095	0.5	0.050
103M	5	68	5.44	428	34.2	83	6.83	1.4	0.112	0.9	0.072
93F	10	48	4.17	382	33.2	74	6.44	1.2	0.104	---	---
55M	10	49	4.01	412	33.8	73	5.93	1.0	0.032	0.7	0.057
70F	10	37	4.02	292	31.7	70	7.60	1.2	0.130	---	---
57M	*20	51	5.55	335	36.5	65	7.07	1.4	0.152	---	---
69F	*20	62	7.85	390	49.4	67	8.49	1.5	0.190	---	---
94F	20	73	5.75	385	48.9	79	6.22	1.5	0.118	---	---
105M	20	68	8.30	264	32.2	67	8.17	1.1	0.134	0.8	0.098

* = daily dose divided and administered $\frac{1}{2}$ in A.M. and $\frac{1}{2}$ in P.M.

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14. THE ACUTE ORAL TOXICITY OF 2,4,5-TRICHLOROPHENOXYACETIC ACID
TO RATS, MICE, GUINEA PIGS AND CHICKS

V. K. Rowe, D. D. McCollister and H. C. Spencer
Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

18106

THE ACUTE ORAL TOXICITY OF 2,4,5-
TRICHLOROPHENOXYACETIC ACID TO RATS,
MICE, GUINEA PIGS AND CHICKS

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V. K. Rowe
D. D. McCollister
and
H. C. Spencer

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

1950

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Single oral doses of 2,4,5-trichlorophenoxyacetic acid as a 10 percent suspension in olive oil were administered by stomach tube to mixed sex groups of rats, mice and guinea pigs. Aliquots of a 3 percent solution in olive oil were administered to chicks. The white rats and the guinea pigs were selected from the stock colonies of this laboratory. White mice were obtained from the Carworth Farms and New Hampshire Red chicks were purchased from a commercial hatchery. Mature, young adult animals were used in each instance except for the chicks which were approximately three weeks of age.

Table I summarizes the concentrations and volumes of olive oil solutions containing 2,4,5-trichlorophenoxyacetic acid administered to each species.

TABLE I

SINGLE ORAL ADMINISTRATION OF
2,4,5-TRICHLOROPHENOXYACETIC ACID

<u>Species</u>	<u>Percent Material in olive oil</u>	<u>Volume Given by Stomach Tube</u>	
		<u>Minimum (cc.)</u>	<u>Maximum (cc.)</u>
Rats	10	0.7	2.9
Mice	10	0.03	0.3
Guinea Pigs	10	1.0	2.9
Chicks	3	0.3	5.8

Mortalities resulting from the various single oral dosages of 2,4,5-trichlorophenoxyacetic acid to the experimental animals are given in Table II. All surviving animals were observed until it was certain that they had fully recovered (usually about two weeks). Symptoms of poisoning, particularly with the higher doses, were ataxia and myotonia.

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TABLE II

MORTALITIES RESULTING FROM THE ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4,5-TRICHLOROPHENOXYACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS

Dosage (gm./kgm.)	Rats		Mice		Guinea Pigs		Chicks	
	No.fed	No.died	No.fed	No.died	No.fed	No.died	No.fed	No.died
0.1			3	0			6	0
0.2							6	0
0.3	10	0	6	2	5	0	6	4
0.4					5	4		
0.5	10	5	6	4	5	4	6	5
0.7	10	8	6	6	5	5	6	6
1.0	10	10	6	6			3	3

The acute oral LD₅₀ values with their 19/20 confidence limits were determined for each species by use of the method described by Litchfield and Wilcoxon¹. The results of the statistical treatment of the dosage-response data are presented in Table III.

TABLE III

ACUTE ORAL LD₅₀ VALUES FOR 2,4,5-TRICHLOROPHENOXYACETIC ACID WHEN ADMINISTERED TO RATS, MICE, GUINEA PIGS AND CHICKS

Species	LD ₅₀ (19/20 confidence limits) gm./kgm.	Slope Function*
Rats	0.500 (0.391 - 0.640)	1.49
Mice	0.389 (0.245 - 0.619)	1.80
Guinea Pigs	0.381 (0.307 - 0.472)	1.21
Chicks	0.310 (0.211 - 0.456)	1.40

* the fold change in dosage required to produce one unit standard deviation in response along the line.

1. Litchfield, J. T., Jr., and Wilcoxon, F.: J. Pharm. and Expt. Ther. 96: 99, 1949.

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The Litchfield and Wilcoxon tests for parallelism of two lines and estimate of relative potency revealed that the 2,4,5-tri-chlorophenoxyacetic acid was significantly (19/20 probability) more toxic to the chicks than to the rats. There were no significant differences in either parallelism or relative potency as a result of the following comparisons: rats versus mice, rats versus guinea pigs, mice versus guinea pigs, mice versus chicks and guinea pigs versus chicks.

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RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

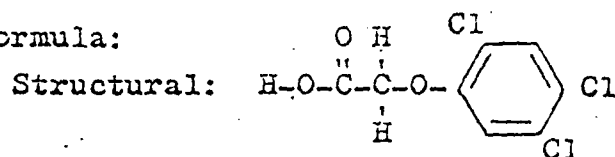
PROBLEM

The widespread handling of 2,4,5-trichlorophenoxyacetic acid made it desirable for us to have a significant amount of toxicological information. Data on the oral toxicity was necessary for presentation to the F.D.A. Hearing on residue tolerances.

MATERIAL

Name: 2,4,5-Trichlorophenoxyacetic acid

Formula:



Empirical: $\text{C}_8\text{H}_5\text{Cl}_3\text{O}_3$

EXPERIMENTAL RESULTS

The acute oral toxicity of 2,4,5-trichlorophenoxyacetic acid for rats, mice, guinea pigs and chicks is given in the attached summary which was presented in evidence at the F.D.A. Hearing. In concluding our testimony on this material we stated,

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13. RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

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RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

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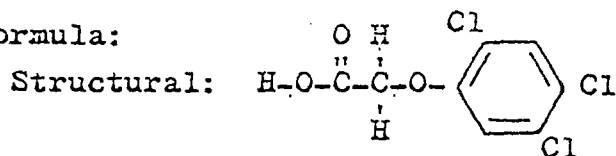
PROBLEM

The widespread handling of 2,4,5-trichlorophenoxyacetic acid made it desirable for us to have a significant amount of toxicological information. Data on the oral toxicity was necessary for presentation to the F.D.A. Hearing on residue tolerances.

MATERIAL

Name: 2,4,5-Trichlorophenoxyacetic acid

Formula:



Empirical: $\text{C}_8\text{H}_5\text{Cl}_3\text{O}_3$

EXPERIMENTAL RESULTS

The acute oral toxicity of 2,4,5-trichlorophenoxyacetic acid for rats, mice, guinea pigs and chicks is given in the attached summary which was presented in evidence at the F.D.A. Hearing. In concluding our testimony on this material we stated,

reference V

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"Because of the similarity in chemical structure and in toxicity and physiological effect between 2,4-D and 2,4,5-T, it seems that a residue tolerance for the latter on fresh fruits and vegetables should be the same as that established for 2,4-D; it would seem that a residue of 20 p.p.m. would provide a wide margin of safety".

Eye Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in propylene glycol was introduced into the eye of a rabbit, it produced marked pain and slight conjunctival irritation immediately. Five hours later, severe conjunctivitis and moderate corneal damage was apparent. This picture remained about the same for two days before healing began. Five days after the exposure mild conjunctivitis and corneal damage was still apparent.

Skin Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in butyl carbitol acetate was applied repeatedly to the rabbit ear and bandaged repeatedly to the shaven abdomen, a slight simple irritation developed in both instances.

SUMMARY

The toxicological properties of 2,4,5-trichlorophenoxyacetic acid may be summarized as follows:

1. The material is moderate in acute oral toxicity for the four species, rat, mouse, cavie, and chick. Of these species the rat seems to be the more resistant and the chick the most susceptible.
2. The material is markedly irritating to the eyes.
3. The material is slightly to moderately irritating to the skin upon prolonged exposure.

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HANDLING HAZARDS AND PRECAUTIONS FOR SAFE HANDLINGSwallowing

It does not seem likely that 2,4,5-trichlorophenoxyacetic acid can be swallowed accidentally in amounts dangerous to life, although this might be possible if concentrated solutions of the material were available to children. If substantial quantities of the material were to be swallowed by anyone, serious ill effects could be anticipated.

If the material should be swallowed, induce vomiting by giving a common emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Call a doctor.

Eyes

The 2,4,5-trichlorophenoxyacetic acid is capable of causing serious damage to the eyes. Precautions should be taken when handling this material to prevent possible eye contacts. It is recommended when handling strong solutions of 2,4,5-trichlorophenoxyacetic acid that goggles, face shield, or other similar device be worn to prevent accidental splashes in the eye. It is believed that the handling of the solid does not present a hazard simply because of its insolubility in aqueous medium.

If the material in any form should get into the eyes, they should be promptly washed with flowing water for at least 15 minutes and medical attention obtained.

Skin

Prolonged and repeated contact with the skin may result in irritation. Occasional contacts for short periods are not expected to cause irritation. Strong solutions are no doubt more likely to cause difficulty than the dry solid.

In case of contact wash the exposed area with soap and water. Do not wear clothing or shoes which have been contaminated with solutions of the material.

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Inhalation

No studies of toxicity upon inhalation of 2,4,5-trichloro-phenoxyacetic acid dust have been made. We believe, however, that in view of the similarity with this material to that of 2,4-dichloro-phenoxyacetic acid, that it is unlikely that toxic concentrations of the dust would be inhaled, primarily because of the irritation it will produce upon the upper respiratory passages.

DOW 2021434

Reference I

Reference II

Reference III

Reference IV

18116

IN THE UNITED STATES COURT OF APPEALS
FOR THE DISTRICT OF COLUMBIA CIRCUIT

No. 24,434

HARRISON WELLFORD, et al.,

Petitioners

v.

CLIFFORD M. HARDIN, Secretary of Agriculture,

Respondent

ON PETITION FOR REVIEW OF AN ORDER
OF THE SECRETARY OF AGRICULTURE

STATEMENT OF THE REASONS UNDERLYING THE
DECISION OF THE SECRETARY WITH RESPECT
TO THE REGISTRATIONS OF PRODUCTS CONTAINING 2,4,5-T

Pursuant to this Court's order of July 31, 1970, I have prepared, on behalf of the Secretary of the United States Department of Agriculture, (1) an explanation of the standard used to determine when a particular hazard is sufficiently imminent to warrant immediate suspension of the registration of an economic poison under the Federal Insecticide, Fungicide, and Rodenticide Act (hereafter FIFRA), as amended, 7 U.S.C. 135-135k; and (2) a description of the specific facts to which that standard has been applied in order to reach the conclusion that suspension is not

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warranted with respect to 2,4,5-T products for granular uses about the home, recreational areas and similar sites and for all uses on rights of way, food crops, hay, pasture, range lands and other farm uses.^{1/}

1. The FIFRA authorizes the Secretary to suspend the registration of an economic poison immediately "when he finds that such action is necessary to prevent an imminent hazard to the public." 7 U.S.C. 135b(c). Congress did not define the term "imminent hazard" in the 1964 amendment which created the suspension authority, and the legislative history of the amendment is silent with respect to definition of the term. While the Government does not agree with the result reached in Nor-Am Agricultural Products, Inc. v. Hardin (No. 18,478, decided July 15, 1970), where a divided Seventh Circuit panel concluded that there is judicial review in a district court of an order of the Secretary which suspends the registration of an economic poison, the Secretary does concur with the majority's exposition of the term "imminent hazard."^{2/} Specifically, the Seventh

Circuit majority said:

1/ On April 20, 1970, the Secretary suspended all uses of 2,4,5-T products in areas adjacent to lakes, ponds and ditch banks (the only uses near aquatic sites ever approved); and all liquid formulations about the home and recreational areas and similar sites (249). The introduction of 2,4,5-T into water sources could threaten humans and aquatic life, and the use of liquid formulations around the home, recreational areas and similar sites could involve direct exposure of pregnant women to undesirable concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin. It was not necessary to suspend granular uses about homes, recreational areas and similar sites because they contain small amounts of 2,4,5-T (as low as one percent) in fertilizer mixtures, do not have to be mixed by the applicator and thus do not present the direct exposure hazards that are present in connection with liquid formulations. On May 1, 1970, the Secretary issued notices of cancellation regarding all uses of 2,4,5-T products on food crops intended for human consumption and all granular uses about the home, and recreational areas and similar sites (250).

2/ I need not explore here the Government's disagreement with the Nor-Am majority which, since the Government's petition for rehearing in banc was granted on August 13, 1970, will now be considered by the full Court.

* * * The term [viz., "imminent hazard to the public"] appeared in federal legislation in "The Drug Amendments of 1962," an act amending the Federal Food, Drug and Cosmetic Act. Under that statute the "imminent hazard" finding is required for the summary suspension of a license to market drugs, if the suspension is to be effective prior to hearing. While the legislative history of the 1964 amendment to the Federal Insecticide, Fungicide, and Rodenticide Act, giving the Secretary of Agriculture similar summary authority, does not seem specifically to contain a discussion of the concept of "imminent hazard" it does refer to the fact that the new procedure is modeled after that contained in the Federal Food, Drug and Cosmetic Act. Viewing the similarity of the language utilized and the purposes for which it was intended, it seems clear that the language in both acts should be similarly interpreted.

The Senate report on the "Drug Amendments of 1962" reflected that an imminent hazard to the public health would exist when the evidence before the Secretary showed that a drug was so unsafe as to create a public health situation "which must be corrected immediately, and cannot be permitted to continue while a hearing is being held." The Committee contemplated that the power would be exercised only in the exceptional case of an emergency which did not permit the Secretary to correct it by other means. S. Rep. No. 1744 (pt. 2), 87th Cong., 2d Sess. 7 (1962). As Senator Eastland elaborated in his prepared report on the Senate floor:

"The committee believes that this authority, which could have grave effects upon a manufacturer and upon the confidence of the public in a drug which might later be found appropriate for continued availability to physicians, should only be exercised under the most extreme conditions and with the utmost care."
108 Cong. Rec. 17366 (1962).

Similarly the House of Representatives Report on the same provisions stated in part that it would be expected that in exercising the imminent hazard authority the Department would make every effort, within the limits of its public responsibility, to notify the applicant and allow him to advance arguments why summary suspension was not required, or after suspension has been invoked, why it might safely be withdrawn pending the hearing. H.R. Rep. No. 2464, 87th Cong. 2d Sess. 8-9 (1962).

While it is apparent that the Department has been concerned with the environmental impact of the use of mercury products, it is certainly much less clear that the data available to the Department, at least insofar as our record is concerned, indicated the necessity for emergency action as opposed to continued and in-depth analysis study. Slip op., pp. 17-19.

In a word, the Secretary agrees that an imminent hazard exists when an economic poison presents a public health situation which must be corrected immediately and cannot be permitted to continue while a hearing is being held and that the suspension authority must be exercised sparingly, i.e., only when a serious emergency exists.

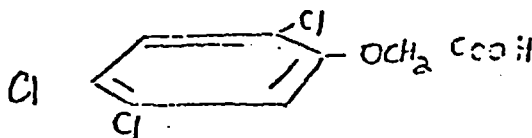
Effect and time, not time alone, are the determinatives of the Secretary's authority to suspend a registration, particularly in light of the fact that the FIFRA authorizes suspension "to prevent an imminent hazard to the public [emphasis supplied]." In this connection the Secretary concurs with Surgeon General Jesse Steinfeld's statement that "if an individual or an animal were exposed to a carcinogen, and developed a cancer 20 years later, this is nonetheless as far as I am concerned, an imminent hazard and we should remove it."

I consulted with seven Department scientists and we applied these criteria to the following facts regarding 2,4,5-T and, after consideration of all the scientists' recommendations and opinions, I conclude that only the use of 2,4,5-T products in areas adjacent to lakes, ponds and ditch banks, and the use of liquid formulations of 2,4,5-T around the home and recreational areas and similar sites present the threat of an imminent hazard to the public. I now turn to the scientific data supportive of my conclusion.

2a. Chemical Characteristics and Synthesis of 2,4,5-T

The herbicide, 2,4,5-T trichlorophenoxyacetic acid, commonly known as 2,4,5-T, consists of the atoms of chlorine, oxygen, hydrogen and carbon.

The 2,4,5-T acid has the following chemical formula:



Commercially, 2,4,5-T is synthesized through a complex series of chemical reactions, beginning with benzene and chlorine, under a variety of temperature and pressure conditions. Proper control of these conditions is essential to minimize side reactions that can result in impurities in the final product. Formulations containing acids, salts, esters and ester mixtures of 2,4,5-T, solvents and other ingredients, can be applied either as liquids or solids. 2,4,5-T, when applied at proper amounts, acts by disrupting normal metabolism in some plants, but not others. The great value of 2,4,5-T is that it acts selectively on plants, killing unwanted brush and weeds, but not significantly injuring other forms of plant life.

Considerable attention has been devoted to a trace impurity produced in step one of the reactive process. This is the formation of 2,3,7,8 tetrachlorodibenzo p-dioxin (TDD), a highly toxic substance. TDD is one of about 75 theoretically possible "dioxins." Only six to eight "dioxins" have been identified as trace contaminants in various chemical formulations, although TDD is one "dioxin" for which there is concrete evidence that it is a teratogen.

Consideration of the "dioxin" impurities originally arose from two independent sources: (1) the chick edema factor; and (2) chloracne poisonings of workers in chemical plants. In 1957, poultry farmers in the east and central United States suffered sporadic losses of their flocks (in some cases up to 95 percent of a single flock was afflicted). Afflicted poultry exhibited "hydropercardium (abnormal accumulation of serous fluid in the pericardial cavity), ascites (accumulation of serous fluid) [and] liver and kidney pathology" (181 at p. 8). It was ascertained almost immediately that the causative agent must be contained in fatty acids or animal feed fats of chlorinated aromatic contaminants (133-139, 146). However, it was not until 1957 that a scientist at the research laboratories of the Proctor and Gamble Company identified the "chick edema factor" to be of the polychlorodibenzo-p-dioxin family, specifically 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin, a chemical "relative" to TDD^{3/} (138).

Beginning in 1899, chemical workers were reported to be suffering a form of skin rash, called chloracne (248). German doctors who studied the affliction determined it could be eliminated by the reduction of "dioxin" impurities in the reaction processes (148, 162; see also, 189 at pp. 241 and 336). The first reports of 2,4,5-T chemical workers in Germany suffering chloracne were issued in 1957. The cause was identified as TDD.

^{3/} The data obtained in these investigations do not indicate that 2,4,5-T was involved with the chick edema problem.

In 1964, when the Dow Chemical Company in Midland, Michigan, responded to increased demand for 2,4,5-T for use in Viet Nam and attempted to speed up production by changing the reactive process, about 60 workers became afflicted with chloracne. Dow, a major producer of 2,4,5-T, immediately closed down its plant and undertook a study of the cause, which was identified as TDD.^{4/} Dow then changed the temperature and pressure conditions of the early reaction process to insure that not more than 1 ppm of TDD would be present in technical grade 2,4,5-T. Dow reports that all 2,4,5-T now being produced contains less than 1 ppm of TDD, in many cases less than 0.5 ppm, and some under 0.1 ppm. This has been confirmed by laboratory tests conducted by both the Departments of Agriculture and Health, Education, and Welfare.

b. Uses and Benefits of 2,4,5-T

Until relatively recent times the main weapon in weed control was physical removal (cultivation). Some chemicals like common salt, copper sulfate, sulfuric acid, arsenates, and heavy petroleum oils, kill all plant life and have been used for many years to produce temporary soil sterility. Fire has long been used to burn off weeds and other plants prior to replanting and along rights of way. Of these methods only cultivation [was] selective. Handler (ed.), Biology and the Future of Man, 824 (1970).

Although 2,4,5-T was first synthesized in the 1930's, it did not receive extensive research consideration until World War II, when, as with so many other technological possibilities, it was subjected to accelerated research for study of possible use in connection with the military effort in South Pacific jungles. However, the compound was never employed in connection with any military operation during World War II.

^{4/} Since 1941, Dow has monitored chemical effects at its plants by rabbit ear tests (189 at pp. 361 to 375).

2,4,5-T was first registered for domestic use pursuant to the FIFRA in 1948. In the intervening 22 years, it has found increasing use for the control of brush and weeds in areas utilized for agriculture, forestry (primarily to clear deciduous hardwoods from conifer stands), industry (for rights of way and powerlines) and home (for lawns and gardens). Between 1960 and 1968, production of 2,4,5-T in the United States expanded from 7.9 million pounds to 42.5 million pounds^{5/} (180 at p. 34).

In 1964, about 19 percent of domestic use was for agricultural purposes (approximately 7 percent on hay, pasture and range lands, and 12 percent for other farm uses), 7 percent was for lawn and turf treatment, 7 percent on Federal lands, approximately 10 percent on private nonfarm forests, 2 percent for margins of aquatic areas and 6 percent for other uses, including by state and local governments. Almost half of use, 49 percent, was on non-Federally controlled rights of way (179 at p. 14). These usages are, of course, subject to fluctuation.

Domestic use declined from 8.9 million pounds in 1964 to 4.6 million pounds in 1966, partly in response to shortage of supplies resulting from increased demand for military use abroad.

In a society dedicated to production of high quality foods available to all people at economic prices, all herbicides including 2,4,5-T play an important role by cutting losses to weeds that curtail food harvests. Similar considerations apply in the field of forestry. Much of the demand for herbicides has resulted from the out-migration of workers who formerly used hand cultivation methods in weed and brush control.

^{5/} A substantial percent of the increased production was for military use in Viet Nam and for general export.

The benefits stemming from the domestic use of 2,4,5-T have been summarized by Dr. Lee DuBridge, Science Advisor to the President and Director of the Office of Science and Technology in the Executive Office of the President:

We have here an example of a chemical substance intentionally placed in the environment by man for the betterment of his welfare. Where the aim has been to exchange capital for labor in land and waterway management, there can be no doubt that this herbicide has proved its worth (189 at p. 452, 454).

These benefits have been translated in economic terms by reports of the Agricultural Research Service and the Economic Research Service of the Department of Agriculture: "If 2,4,5-T was no longer used in this country it would cause an additional cost of 52 million dollars to achieve the same result, by use of alternative herbicides and manual methods. If alternative herbicides (primarily 2,4-D) were also banned, this cost would increase to 172 million dollars"^{6/} (179, 180).

c. Ecological Considerations and Persistence of Residues Involved in the Use of 2,4,5-T

2,4,5-T is not considered a persistent pesticide when compared with the chlorinated hydrocarbon insecticides. Degradation on plant tissue occurs rapidly and data available suggests that residues resulting from recommended rates of application are not of sufficient magnitude to justify concern as potential health hazards. Generally, 2,4,5-T persists in soils for not more than three months. It appears that within this

^{6/} Both these figures are based upon the assumption that we will maintain the present level of control.

period the residues are broken down by soil microorganisms that use chemicals as sources of carbon and energy (88-94, 96, 99, 100). Although wind and water might carry the residues to non-target areas during the three-month period, this does not appear to constitute a significant hazard. A potential danger to humans and aquatic life could exist if substantial treatments are carried on near water sources or by accidental spills into water; therefore, the Secretary has suspended all uses of 2,4,5-T around lakes, ponds, and ditch banks.

Residues in forage crops decline rapidly, and may remain at low levels for eight weeks on forage grasses. (97, 98) Care must be taken to defer grazing by dairy cattle soon after spraying. Analysis by the Food and Drug Administration of dairy products (discussed more fully, infra, at p. 14) reveals that not only is the finding of residues extremely rare, but, when found, residues are at very low levels. The available evidence suggests that animals eliminate residues even more quickly than plants. For instance, rats and pigs eliminate them very rapidly, one-half of the ingested dose being eliminated in 3 to 10 hours, mainly through the urine (33, 75). Dairy cattle also excrete 2,4,5-T through urine rapidly (255).

In terms of wildlife habitat, the application of 2,4,5-T can have either beneficial or adverse effects depending on the circumstances and the species involved (48). Thus, although an intense and unduly heavy application can adversely modify game habitat, judicious treatment of

selected areas of forests of low economic value can improve the habitat for the protection and production of wildlife such as game birds and deer. Although too high an application can reduce population of bees, proper applications on rights of way can create ecological "edge effects" very favorable as habitat for species such as grouse and wild turkeys (4).

d. Public Health Considerations Involved in the Use of 2,4,5-T

Recently there has evolved concern that 2,4,5-T may be a teratogen, i.e., an agent responsible for birth defects. However, the Secretary's orders of April 20, 1970, and May 1, 1970, suspending certain uses of 2,4,5-T, cancelling others, and not affecting other uses, were based on exhaustive examination of available scientific evidence, which was analyzed and interpreted by experts in the Departments of Agriculture; Health, Education and Welfare; Interior; and private sources.

In 1963, the National Cancer Institute entered into a contract with Bionetics Research Laboratories, Inc., of Litton Industries (Bethesda, Maryland). Bionetics was to undertake a study of the possible carcinogenic and teratogenic agents in a number of pesticides and related compounds. Eventually 86 compounds, including 2,4,5-T, were selected for study. After the study was completed by Bionetics in early 1969, the National Institute of Environmental Health Sciences began a statistical analysis

7/ See generally, Odum, Fundamentals of Ecology, 278-281 (2d ed., 1959); Hanson, Dictionary of Ecology, 123 (1964).

of the data accumulated by Bionetics. Because of the finding that 2,4,5-T appeared to be "causing significantly more deformities than expected," particular attention was paid to this compound. The raw data was also released to the Health, Education and Welfare Secretary's Commission on Pesticides and Their Relationship to Environmental Health (the Mark Commission).

While the data compiled by Bionetics did not reveal 2,4,5-T to be a carcinogen (see 43), it did indicate that the compound was teratogenic to strains of mice and rats injected doses suspended in DMSO, and fed dosages suspended in honey. Cleft palate and cystic kidney were the predominant teratogenic effects. However, the validity of the test results was almost immediately challenged. The Dow Chemical Company objected that the 2,4,5-T used in the study was not a representative sample of the 2,4,5-T presently being produced in this country. Dow pointed out that the 2,4,5-T used by Bionetics contained the "dioxin" contaminant TDD at the rate of 27±8 ppm, while the 2,4,5-T being produced commercially contained substantially less than 1 ppm.

Subsequent studies were undertaken by the Food and Drug Administration (FDA), the National Institutes of Environmental Health Sciences (NIEHS), the National Institute of Dental Research (on cleft palate defects), and by the Dow Chemical Company. Dow's preliminary studies indicated no teratogenic effects from relatively "pure" 2,4,5-T containing less than 1 ppm at dosage levels under 24 mg/kg per day.^{8/} The FDA and NIEHS studies

8/ It does not appear chemically possible commercially to formulate 2,4,5-T without traces of TDD, though such traces can be held as low as 0.1 ppm.

did show teratogenic effects when injected in DMSO at 100 mg/kg (though not at lower levels), even though relatively "pure" lots of 2,4,5-T were tested. Both the validity and the implications to human health of the latter two studies have also been subject to question. The NIENS study showed no teratogenic effect on rats, but did on mice.

FDA studies of the "dioxin" contaminants have used the chick embryo (see 189 at pp. 190-360). However, while the sensitivity of the chick embryo to many factors makes it ideal for experimental screening study, this very factor makes questionable its relevance to public health. For instance, an essential vitamin, vitamin A, is a teratogen under this test.

As the World Health Organization has stated:

The chick embryo contributes greatly to basic embryological knowledge. However, for the screening of drugs for teratogenicity, its use is not recommended. It is too sensitive to a wide range of agents and affords no parallel with the anatomical and physiological relationship existing between pregnant mammals and her [sic] conceptus (189 at p. 402).

2,4,5-T has been used in this country for more than two decades without one confirmed report of its having had a teratogenic effect on a human embryo or fetus, and the speculation that widespread use of 2,4,5-T has caused birth defects in Viet Nam has not been confirmed. Reports of alleged widespread damage caused by use of 2,4-D; 2,4,5-T and Silvex (a chemically-related compound to 2,4,5-T, but with a different selectivity) by the United States Forest Service near Globe, Arizona, "lost much of their significance upon medical surveillance for alleged illnesses in humans and malformations in animals"^{9/} (181 at p. 37). Not only is it

^{9/} Officials of this Department are currently preparing a definitive report with respect to these allegations.

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difficult to extrapolate results obtained from tests on animals to humans (and vice-versa: witness thalidomide, to which rat strains showed no adverse reaction, see 27), but further there is a tremendous difference between the massive dosage purposefully fed to test animals and the insignificant amounts of 2,4,5-T which reach the American people. The Food and Drug Administration has studied, inter alia, residues of 2,4,5-T on agricultural commodities between 1964 and 1968, and found that there were none in the vast majority of cases. A few cases of insignificant amounts were reported. I have previously mentioned that the FDA has set no tolerance for 2,4,5-T on agricultural commodities, and if a commodity bore a residue it would be subject to seizure. Of the 5,300 food samples studied by the FDA, only two showed detectable residues at 0.1 ppm limit of the detection methods.

It must be emphasized that teratogenicity can be induced in a wide variety of the highly sensitive embryonic or fetal organisms by many chemical agents, even vitamin A, if dosage is high enough. However, such a result on an animal does not allow the inference that a similar result would follow for a human embryo or fetus. Not only are there significant differences in metabolic sensitivity which can only be correlated by comparable metabolic study, but, more importantly, potential teratogenicity to humans and human embryos and fetuses must be gauged by analysis of the actual intake amounts that can reasonably be expected to enter the human body.

On the basis of the application of the standard I have described, supra, pp. 2 - 4, to the presently available scientific evidence heretofore summarized, I conclude that (1) the Secretary properly suspended all uses of 2,4,5-T in areas adjacent to lakes, ponds and ditch banks, and the use of all liquid formulations around the home, recreational areas and similar sites to prevent an imminent hazard to the public; (2) the Secretary appropriately issued notices of cancellation regarding all uses of 2,4,5-T products on food crops intended for human consumption and all granular uses about the home, recreational areas and similar sites since recent reports raise some question about the safe use of 2,4,5-T products in these respects; and (3) the other uses of 2,4,5-T products do not present any foreseeable hazard to the public.

Respectfully submitted,

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Ned D. Bayley
Director
Science and Education

DOW 2 008669

1135

Presence of chloracnegen and 2,3,7,8-tetrachlorodibenzo-p-dioxin
in 2,4,5-T from various manufacturers

	Compound	1965		1969	
		Animal Test *	Assay (GLC) **	Animal Test	Assay (GLC)
Hooker	TCP	neg.	<1 ppm		
Diamond	2,4,5-T	active (mod-severe)	S ₁ 5 ppm S ₂ 16 ppm		
	2,4,5-T	---	---	active	<1 ppm
Hercules	2,4,5-T	neg.	S ₁ -S ₆ <1 ppm S ₉ 3 ppm***		
Monsanto	2,4,5-T	active	S ₁ -S ₁₂ 6.5-11 ppm		
Dow	TCP	neg-active	'63 2 samples @ <1ppm		
			'64 6 samples, 1 @ 6 ppm 5 @ <1 ppm		<1 ppm
			'65 3 samples @ <1 ppm		

* chloracnegen test by rabbit ear bioassay.

** chemical assay for 2,3,7,8-tetrachlorodibenzo-p-dioxin

*** Dow was buying from Hercules in late 1965 and sample was from 3rd or 4th batch shipped and high dioxin thought due to "pushing" capacity of plant.

AG 00870 DOW 1 999743

REGISTRATION STATUS REPORT

1136

DESCRIPTION: Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

History of discussions and actions 1967-1968

Product KURON (silvex)

Date This Report _____

Date Filed
With Gov't. 12/29/67

No. _____
Estimated
Date of
Approval
Optimistic _____
Pessimistic _____

- ☐ VID (Veterinary Investigational Drug)-FDA
☐ IND (Human Investigational Drug)-FDA
☐ NDA (New Drug Application)-FDA
 ☐ Human ☐ Animal ☐ Original ☐ Supplement
☐ Antibiotic Form 5 ☐ or 6 ☐ - FDA
 ☐ Animal ☐ Human ☐ Original ☐ Amendment
☐ TEMPORARY PERMIT, PESTICIDE
☐ PESTICIDE REGISTRATION APPLICATION
 ☐ Original ☐ Revision
☐ PESTICIDE TOLERANCE PETITION
 ☐ Temporary ☐ Permanent
☐ FOOD ADDITIVE PETITION - FDA
☐ Other:

Agency: USDA

9-PP-1103

Country: USA

Dow Contact: (1) G. E. Lynn

(2) M. L. Leng

(3) D.D. McCollister

DATE	ACTION	Tot. Pa. El.
11/5/67	Meeting between C. L. Dunn of ITFPHT and G. C. Holsing of Hazleton Laboratories Inc., with J. Alpert and L. L. Ramsey of FDA to discuss tolerances for 2,4-D, 2,4,5-T, MCPA and silvex in range and/or pasture grass at the high levels that exist shortly after application. Mr. Alpert regarded 200 ppm as high and 700 ppm as unrealistic. Removal of animals from rangeland and adherence to pre-grazing intervals were considered to be questionable as restrictions in labeling. Exclusion of dairy animals was acknowledged to be practical. (FDA review of USDA petition for tolerances for 2,4-D in grain, PP 6F0459, concluded that 20 ppm in feed would result in less than 0.05 ppm in meat, but a two-week pregrazing interval was needed to avoid meat or milk contamination from animals grazing treated fields of small grain treated at 1.5 lb a.e./A)	
11/27/67	Request for extension of all currently registered uses of silvex on pasture and rangeland submitted by C. L. Dunn of Hercules on behalf of NACA ITFPHT. (Also 2,4-D, 2,4,5-T, MCPA)	
12/29/67	Extension granted by USDA until Jan. 1, 1969 (letter to C. L. Dunn). (Also 2,4-D, 2,4,5-T, MCPA)	
1/8/68	Notification of extension published by USDA as PR Notice 68-1 for uses of silvex on pastures and ranges listed on page 698 of the Summary of Registered Agricultural Chemical Uses. (Also 2,4-D, 2,4,5-T, MCPA)	
8/1/68	PR Notice 68-11 issued by USDA informing all registrants that further limited extensions will be considered based on a progress report received on or before November 1, 1968, or by submission of a petition for tolerances prior to January 1, 1969.	

18133

THE DOW CHEMICAL COMPANY

00198

REGISTRATION STATUS REPORT

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

9-PP-1103

Product SILVEX

Date This Report _____

DATE	ACTION	Total Days Elapsed
10/29/68	Request for further extension requested by C. L. Dunn on behalf of NACA ITFPHT using tabulation of residue data prepared by M. Leng.	
12/3/68	Extension granted until January 1, 1970 for all currently registered uses of silvex on pastures and ranges found on pages 698 of the USDA Summary (2nd edition). Assigned number 9-PP-1103. (Also 2,4-D, 2,4,5-T, MCPA)	
12/23/68	A letter from FDA reviewing PP 8F0675 for tolerances of silvex in various crops stipulated that if the present registration for silvex on pasture and rangeland grasses (extended) is to be retained, a tolerance for silvex on pasture and rangeland grasses should be proposed, and supporting residue data submitted. Depending upon the level needed, additional cattle feeding studies and proposals for meat and milk tolerances may be necessary. (Also others)	
1/10/69	PR Notice 69-1 lists extension of silvex at 0.75 and 4.0 lb/A in pastures and rangeland as shown on page 698 of the USDA Summary (Also all uses of 2,4-D, 2,4,5-T and MCPA in pasture and rangeland).	
1/15/69	Meeting in Washington of D. D. McCollister and C. L. Dunn of Industry Task Force with Harry Hays and Harold Alford of PRD and L. L. Danielson, D. L. Klingman and W. C. Shaw of ARS, USDA, to discuss Mr. Dunn's proposal of 10/29/68 to Dr. Hays that registration of the use of the four phenoxy herbicides on range and pasture grass be continued without the establishment of a tolerance on grass and that the regulatory tolerance be established only for the meat of animals feeding on treated grass. PRD concurred in the principle of exemption for grass <u>but pointed out that hay will require tolerances.</u>	
1/27/69	Meeting in Washington of D. D. McCollister and C. L. Dunn of Industry Task Force with Harry Hays, Harold Alford and David Bowen of PRD; L. L. Danielson, W. B. Ennis and D. L. Klingman of ARS; and L. L. Ramsey, F. J. McFarland, W. Stokes, J. G. Cummings, D. V. Reed, H. Blumenthal, G. E. Whitmore, H. R. Gittes and J. B. Lamb of FDA. It was agreed that ARS and the Task Force would prepare a protocol for feeding studies for meat and milk which should specifically support the principle of exemption for grass. The protocol would be circulated to FDA for their evaluation. All four compounds should be studied in one comprehensive project and feeding studies should be done only with the acids.	

Continuation Page 2

18134

DESCRIPTION: Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

Product SILVEX

Date This Report _____

9-PP-1103

THE DOW CHEMICAL COMPANY

DATE	ACTION	Total Days Elaps
2/26/69	Meeting of Task Force in which Mr. G. T. Scriba, NAC Director of Regulatory Services reported that FDA legally has no duty or power to issue a tolerance regulation or a tolerance exemption regulation in regard to range grass.	
2/27/69	Meeting of C. L. Dunn and M. E. Getzendaner with personnel of the Crop Protection Division and ADP of ARS USDA to discuss tentative protocol for feeding studies to justify the use of all four phenoxy herbicide compounds on range and pasture grass.	
4/30/69	Another meeting with ARS (ADP) of USDA to discuss protocol for feeding studies.	
6/19/69	Letter from C. L. Dunn of Task Force to Dr. Paul DeLay, Director of Animal Disease and Parasite Division of USDA, enclosing proposed protocol for the animal feeding studies. He proposed that registrations of phenoxy herbicides on ranges and pasture grass be continued without tolerances for range or pasture grass, but with tolerances to be set for meat at levels indicated by the data developed by this feeding study. Included were fixed level feedings and a declining-rate feeding study approximating actual range exposure as proposed by D. L. Klingman based on data obtained by Texas A&M on residues of 2,4-D and 2,4,5-T in range grass. The animal feeding, animal sacrifice, and taking of samples were to be conducted by USDA while the Task Force would be responsible for analyses of tissues and for submission of petitions, should tolerances be required.	199265
6/23/69	Letter from C. L. Dunn to L. L. Ramsey of FDA repeating proposal made 2/27/69 that registration for use of chlorophenoxy herbicides on range and pasture grass be continued without setting tolerances on the grass, provided that suitable tolerances for meat and milk are provided where needed. Enclosed was a copy of the proposed protocol for fixed level feeding for 4 weeks followed by immediate slaughter, for slaughter 7 days after discontinuing highest level, and for feeding at declining levels over an 8-week period. Arguments were presented that tolerances were not needed for grass (an opinion supported by representatives from PRD and the Crops Research Division and Crops Protection Research Branch of USDA), because grass does not move in interstate commerce and as a growing crop would represent a difficult policing problem. Although range grass is seldom cut for hay, appropriate label restrictions and precautionary	

REGISTRATION STATUS REPORT

DESCRIPTION: Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

Product SILVEX

Date This Report _____

9-PP-1103

THE DOW CHEMICAL COMPANY

DATE	ACTION	Total Days Elapsed
6/23/69 (cont'd)	statements would be required by PRD, USDA to prevent such harvest in the absence of a tolerance for hay. A separate project concerning milk is being undertaken (by Dow) and data from that study will be used in support of pasture grass registrations.	
7/7/69	Letter from L. L. Ramsey to C. L. Dunn stating that they (FDA) <u>do not agree</u> with his view that tolerances for these herbicides on range grass are not needed and that pasture grass is a separate situation. Reference was made to the Federal Register of 4/8/69 in which an order was published establishing a tolerance of 40 ppm for dicamba in or on grasses (pasture and rangeland) and grass hay. (See attached pages from EPA Summary for dicamba listing many restrictions)	
10/23/69	A request for extension submitted by C. L. Dunn on behalf of ITFPHT reported on progress in getting the necessary milk and meat residue data. Dairy cows are on hand in Midland and control pretreatment samples of milk are being collected. Feeding studies with 2,4-D and 2,4,5-T are scheduled to run concurrently at stepwise levels from 10 to 1000 ppm for 2 to 3 weeks at each level. Feeding studies with MCPA and silvex are scheduled to begin in February. The meat residue studies are to be conducted by USDA in Kerrville, Texas, in both cattle and sheep, at 300, 900 and 1800 ppm 2,4-D and 2,4,5-T and silvex, and at 250 and 500 ppm MCPA in the total diet for 4 weeks. The effect of a 7-day withdrawal and of declining levels in the diet will also be studied. Final details of the studies are still being discussed with USDA.	
10/29/69	Statement about 2,4,5-T issued by Dr. Lee A. DuBridge, Science Adviser to the President and Executive Secretary of the President's Environmental Quality Control, listing actions to be taken because of Bionetics report that this herbicide caused teratogenic effects in rats and mice.	
12/10/69	Dow initiated feeding study with 2,4,5-T in cattle in Texas at levels of 100, 300, 900, and 1800 ppm in the total diet for 4 weeks with slaughter 1 day and 7 days after the last feeding.	
12/29/69	Word was received by ITFPHT that USDA has granted extensions for the use of <u>2,4-D</u> , <u>MCPA</u> and <u>silvex</u> in pasture and range-land. No mention was made of 2,4,5-T although it was listed in the request for extensions submitted to USDA on 10/23/69.	

Continuation page 4

18136

REGISTRATION STATUS REPORT

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

 Product SILVEX

Date This Report _____

THE DOW CHEMICAL COMPANY

DATE	ACTION	Total Days Elaps
1/19/70	PR Notice 69-20 dated 12/22/69 received from USDA showing extensions granted for a number of pesticide registrations including 2,4-D on grass (hay crop), pasture (grass), and rangeland (range grass), rangeland clearance (ranges); MCPA on pastures (established); and silvex on pastures and ranges.	
3/4/70	USDA notified ITFPHT that extensions were being granted 2,4,5-T in pastures (grasses), rangeland clearance and in crops pending completion of studies in progress.	
3/19/70	Task Force notified that ARS was ready to proceed with the proposed cooperative meat residue study on 2,4-D, 2,4,5-T, silvex, and MCPA to be done at Kerrville, Texas.	
4/2/70	Meeting of Industry Task Force to discuss progress in getting residue data on 2,4-D, 2,4,5-T, silvex and MCPA in various food crops, meat, and milk. Cost estimates are around \$5,000 for most crops, \$75,000 for sugarcane (Dow), \$60,000 for milk (Dow) and \$38,000 for meat (Southwest Research Institute), plus cost of filing amendments and of filing petitions for tolerances to cover uses in range and pasture. Most companies are not willing to cost-share these large amounts.	
4/9/70	PR Notice 70-10 issued by USDA listing additional extensions including 2,4,5-T in crops and in pasture and rangeland. (Copy not received by regular channels; obtained by special request from USDA by M. Leng).	
4/15/70	USDA announced immediate suspension of 2,4,5-T for some uses, and cancellation for other uses. Range and pasture, forests, and rights-of-way are not affected.	
6/4/70	Letter from C. L. Dunn to R. D. Radeleff of USDA at Kerrville with revised protocol for feeding studies <u>only</u> at fixed levels of 2,4-D and silvex at 300, 1000 and 2000 ppm in the diet for cattle and at 2000 ppm for sheep; of MCPA at 250 and 500 ppm for cattle and 500 ppm for sheep; and of 2,4,5-T at 2000 ppm for sheep. (Dow had conducted feeding study with 2,4,5-T at 100, 300, 900, and 1800 ppm in diet of cattle)	
Continuation Page <u>5</u>		

18137

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT)..

Product SILVEX

Date This Report _____

THE DOW CHEMICAL COMPANY

DATE	ACTION	Total Days Elaps
6/17/70	Hearing before Senator Hart's Commerce Energy, Natural Resources and Environment subcommittee revealed that Ralph Nader's Center for the Study of Responsive Law, with some other organizations, has petitioned USDA to completely ban use of the herbicide <u>2,4,5-T</u> and the related herbicide <u>silvex</u> . The petition asked the suspension immediately of all esters and formulations of 2,4,5-T and silvex "around the home, recreation areas, lakes, ponds, and on food crops, whether or not they are relabeled." It also asked USDA to make public the names of all suspended products, and to issue notices of cancellation of the registrations of 2,4,5-T and Silvex in all formulations for all other uses not yet mentioned. FDA tests have shown that Silvex causes birth defects in chicks but tests in mammals are incomplete. <u>2,4-D</u> was also discussed at length. Dr. T. C. Byerly, USDA's Assistant Director for Science and Education said that "dichloro compounds such as 2,4-D are unlikely to result in the production of dioxins." DuBridge admitted that cases of substantial dangers have not been proven. Surgeon General Steinfeld said that the 2,4-D data was uncertain. As of now, data is not sufficient to say that 2,4-D is teratogenic to hamsters. H. Wellford of Nader's Raiders said that 2,4-D did not figure in their petition to USDA, but that it is "just as deserving of cancellation and probably suspension as Silvex," and that 2,4-D caused birth defects in chicks. The Surgeon General said that studies with 2,4-D in rats are underway.	8088661 197808
12/5/70	Notice published in Federal Register Vol. 35, No. 236, p. 18550, entitled Amendment of Statement for Implementation which specified that registration should be continued in those instances in which a petition for tolerance or exemption therefrom has been filed on or before December 31, 1970, pending decision on such petition.	
12/22/70	Dow submitted amendments to Task Force petitions (PP 8F0670 for 2,4-D, PP 8F0675 for silvex, PP 9F0761 for MCPA) and resubmitted 2,4,5-T petition (originally PP 8F0669, refi as PP 1F1102). Included data on residues in grass from application of 2,4-D, 2,4,5-T and silvex at industrial rates (6 to 12 lb/A), complete reports on residues in milk and preliminary reports of Dow analyses for residues in muscle of cattle. (Analyses were to have been conducted by Southwest Research Institute in San Antonio but they had been unable to develop a satisfactory method.) The tolerance requests were <u>not</u> changed from those listed in the original	

REGISTRATION STATUS REPORT

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT).

Product SILVEX

Date This Report _____

DATE	ACTION	Total Days Elaps
12/22/70 (Cont'd)	petitions submitted in December 1967. The 72-volume submission was hand-carried to Washington by E. R. Laning to assure filing prior to the 12/30/70 deadline.	
1/4/71	Letter dated 12/30/71 from Drew Baker of Pesticides Tolerances Division (formerly FDA) of the new EPA stating that tolerances <u>are</u> required for residues of phenoxy herbicides on pasture grass and rangeland grass. He referred to letter of 7/7/69 from L. L. Ramsey to C. L. Dunn on this subject.	
1/5/71	Telephone conversations between D. D. McCollister and Lowell E. Miller, C. L. Smith and Drew M. Baker, Jr. re need for tolerances in grass. Concluded that a definite, numerical tolerance figure <u>must</u> be proposed for rangeland or pasture grass to meet the requirements of the amendment published on 12/5/70.	1988661
1/6/71	Amendment to petitions submitted by D. D. McCollister modifying Section F to request 300 ppm tolerance for residues of 2,4-D, 2,4,5-T, silvex and MCPA in or on rangeland grasses or pasture grasses.	
2/5/71	Letter from EPA stating that additional fee of \$2100 needed for each of 2,4-D, silvex and MCPA, and \$1500 for 2,4,5-T.	
2/22/71	D. D. McCollister submitted additional fee of \$7800.	
3/11/71	Letter from EPA stating petition amended as of 2/26/71.	
5/3/71	PRD certified usefulness for silvex in pasture and rangeland (also for 2,4,5-T on 5/5/71, for 2,4-D on 5/14/71, and for MCPA on 4/21/71).	
7/12/71	Letter from PTD stating that certain animal residue studies crucial to their review have not been reported.	
7/21/71	Letter from Dow stating that residue studies had been completed and an amendment would be submitted in 30 to 60 days.	
7/29/71	PTD grants 60 days for receipt of data.	
10/13/71	Amendment to petition submitted including complete report on residues of silvex and 2,4,5-trichlorophenol in muscle, fat, liver and kidney of cattle and sheep. (Similar amendments submitted for 2,4,5-T on 9/29/71, for 2,4-D on 10/1/71, and for MCPA on 10/21/71.) Tolerance proposal modified to	

REGISTRATION STATUS REPORT

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force for Phenoxy Herbicide Tolerances (ITFPHT)."

Product SILVEX

Date This Report _____

DATE	ACTION	Total Days Elaps
10/13/71 cont'd.	request 300 ppm for <u>grass</u> , 0.5 ppm for rice straw and sugar-cane bagasse, 0.1 ppm (negligible residue) in or on apples, plums, rice and sugarcane, and in meat and meat byproducts of cattle, goats, and sheep; and 0.05 ppm (negligible residue) in <u>milk</u> .	
11/12/71	Receipt of amendment acknowledged by PTD and review begins.	
5/5/72	EPA proposed interim tolerances in Federal Register Vol. 37, No. 89, p. 9228-9229, including silvex at 0.1 ppm for apples, rice, sugarcane, and plums (prunes). No interim tolerances proposed for silvex in grass, milk, or meat. (Interim tolerances also proposed for 2,4-D and MCPA in crops but not for 2,4,5-T.)	
7/21/72	Letter from Pesticides Tolerances Division outlining deficiencies in petition for silvex tolerances, including need for data on bound residues in rice, sugarcane, grass, milk and meat. (Similar letters issued for 2,4,5-T on 5/4/72, for 2,4-D on 7/19/72 and for MCPA on 11/10/72.) Also stipulated that tolerance of 600 ppm silvex would be needed for grass (in contrast to need for 2000 ppm 2,4-D and 1000 ppm 2,4,5-T).	1978810
8/30/72	Interim tolerances finalized by EPA including 2,4-D, silvex and MCPA in the food crops covered by the petitions. (See Federal Register, Vol. 37, No. 169, p. 17554-17555).	
9/6/72	Dow requested 90 days to submit substantive amendments to the phenoxy petitions.	
9/13/72	Additional interim tolerances proposed by EPA including 2,4-D and MCPA at 300 ppm in grasses (pasture and rangeland) and grass hay, with statement that interim tolerances should <u>not</u> be set at that time for several pesticides including 2,4,5-T (in anything) and silvex (in grass and water). (See Federal Register Vol. 37, No. 178, p. 18565-18566)	
9/15/72	Letter from PTD stating that petitions would be held in abeyance until 12/6/72.	
9/29/72	Dow submitted comment to Hearing Clerk and requested that an interim tolerance of 300 ppm be established for silvex in grasses (pasture and rangeland) and grass hay, citing data in support and enclosing paper by M. Leng entitled "Residues in	

REGISTRATION STATUS REPORT

DESCRIPTION:

Use of silvex in pasture and rangeland. Request for extension of all registrations submitted by NACA Industry Task Force, for Phenoxy Herbicide Tolerances (ITFPHT).

Product SILVEX

Date This Report _____

DATE	ACTION	Total Days Elapsed
9/29/72 cont'd.	Milk and Meat and Safety to Livestock from the Use of Phenoxy Herbicides in Pasture and Rangeland" reprinted from DOWN TO EARTH Vol. 28, No. 1, p. 12-20, Summer 1972.	
10/13/72	Letter from Drew Baker advising us that the letter to the Hearing Clerk and the two attachments were being considered by chemists and toxicologists in PTD.	
12/2/72	Interim tolerances proposed by EPA on 9/13/72 were finalized, without mention of silvex or 2,4,5-T. (See Federal Register Vol. 37, No. 233, p. 25716-25717).	
12/7/72	Meeting of D. D. McCollister and M. L. Leng of Dow, and Lamar Brown of Rhodia with Drew Baker, now Acting Assistant Director of the new Registration Section of EPA, and Joe Cummings, Don Duffy, and Bob Quick of the chemistry review section, to discuss the status of work completed and underway at Dow to answer their questions of 5/14, 7/19, 7/21 and 11/10/72 on the phenoxy petitions. Mr. Baker stated that silvex was being <u>cancelled</u> for pasture and rangeland because the chemists could not agree on the tolerances needed, therefore the toxicologists could not determine that the levels proposed were safe. He also stated that "dioxin" was not the problem, although Joe Cummings indicated inadvertently that it was. Mr. Cummings also showed surprise that residues of the metabolite 2,4,5-trichlorophenol did <u>not</u> occur in significant amounts in tissues of animals fed high levels of <u>silvex</u> whereas residues of the trichlorophenol reached 4.7 ppm in liver and 5.8 ppm in kidney of cattle given 1800 ppm 2,4,5-T in the total diet for 4 weeks prior to slaughter, and were about 0.3 ppm in milk of cows ingesting 1000 ppm 2,4,5-T in their feed. Discussion of problems related to cancellations under the new pesticide law (FEPCA) did not deter Mr. Baker from his commitment to cancel, apparently under orders from above him in EPA. He believed that all we had to do was appeal and ask for review by an Advisory Committee, during which time we could furnish the additional data needed to set the tolerances. However, under FEPCA, the Administrator can either issue a notice of intent to cancel allowing any interested party to request a public hearing, or he can request a public hearing himself. In any case, the reviewers would have to be able to provide evidence supporting their view that interim tolerances could <u>not</u> be set for silvex as well as for 2,4-D and MCPA in grass in spite of the fact that considerable residue data was submitted on silvex in grass whereas no data was provided for MCPA in grass.	
Continuation Page <u>9</u>		18141

With a few exceptions, the modern herbicides are of a relatively low order of toxicity, thus limiting the hazard of their use.

A number of researchers have reported upon the toxicity of 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichloro-phenoxyacetic acid), and their esters (38,53, 63,77,107,108). The studies of Mitchell, et al, (1946) and of Grigsby and Farwell (1950), in which they grazed livestock on heavily treated pastures, indicate that the practical hazard with these materials is very low. Rowe and Hymas (1954) have studied 2,4,5-T by administering it to steers.

At Kerrville, we have studied several herbicides in sheep and cattle. (Table 4).

It is apparent that pentachlorophenol is one of the more toxic of these materials, producing poisoning in cattle and sheep in single doses of 25 mg./kg. Of this group, dinitro-o-sec-butyl phenol is the next most toxic, proving lethal for sheep after 4 daily doses of 25 mg./kg. Oddly, the alkanolamine salts of dinitro-o-sec-butyl phenol are much less toxic, one sheep having tolerated 35 daily doses of 25 mg./kg. each without showing symptoms.

In general, the phenoxy-acid group of herbicides was of very low toxicity for sheep. The most toxic of this group was 2-(2,4,5-trichlorophenoxy) propionic acid in the form of propylene glycol butyl ether esters, 11 daily doses of 100 mg./kg. each producing death. This would still class it as a relatively nontoxic compound.

The following materials were nontoxic when administered daily in 100 mg./kg. doses for 35 days: The alkanolamine salts of 2,4-dichlorophenoxyacetic acid and of 2-methyl-4-chlorophenoxyacetic acid, the propylene glycol butyl ether esters of 2,4-dichlorophenoxyacetic acid and of 2,4,5-trichlorophenoxyacetic acid, the triethylamine salts of 2,4,5-trichlorophenoxyacetic acid and the sodium salt of 2,2-dichloropropionic acid.

The absence of poisoning from these materials administered in such high dosages for 5 weeks indicates that there is little hazard to livestock to be expected from normal usage.

Dehydroabietylamine acetate (Delrad), an algacide based upon technical abietal-amines, is used at very low rates in reservoirs and irrigation canals. Cattle and sheep were unharmed by consuming water treated with 100 p.p.m. Delrad, which is several times the concentration usually employed. This compound is extremely distasteful to cattle and sheep and probably would never be consumed by them voluntarily even if the chemical were left exposed. Cattle were unharmed by doses of 200 mg./kg., sheep by doses of 250 mg./kg. Higher doses produced poisoning.

Because of the low toxicity and reduced hazard of herbicides, together with comparatively restricted usage, the number of publications is small. Much of the evaluation of toxicity of these materials has been done with laboratory animals rather than with livestock.

Reduction of Hazard by Control of Toxicity

While very little can be done to change the specific toxicity of a pesticide, several things can be done to control it.

There are certain compounds which may be administered simultaneously with the pesticide which antagonize its action directly or slow its action sufficiently to enable the organism to deal with it.

TABLE 4.--Summary of studies of the oral toxicity of herbicides to sheep and cattle at Kerrville, Tex.

Chemical	Animal	Dose	Frequency	Results
<i>Mg./kg.</i>				
Pentachlorophenol	Sheep	25	1 dose	mild toxicity
	Cattle	25	1 dose	mild toxicity
(Delrad) Dehydroabietylamine acetate	Sheep	250	1 dose	tolerated, higher doses toxic
	Cattle	200	1 dose	tolerated, higher doses toxic
Dinitro-o-sec-butyl phenol	Sheep	25	daily	death after 4 doses
Alkanolamine salts of Dinitro-o-sec-butyl phenol	Sheep	25	daily	tolerated 35 doses*
✓ 2-(2,4,5-Trichlorophenoxy) propionic acid, propylene glycol butyl ether esters	Sheep	100	daily	lethal after 11 doses
✓ 2,4-Dichlorophenoxyacetic acid, alkanolamine salts	Sheep	100	daily	tolerated 35 doses*
✓ 2,4,5-Trichlorophenoxyacetic acid, alkanolamine salts	Sheep	100	daily	tolerated 35 doses*
✓ 2,4-Dichlorophenoxyacetic acid, propylene glycol butyl ether esters	Sheep	100	daily	tolerated 35 doses*
✓ 2,4,5-Trichlorophenoxyacetic acid, propylene glycol butyl ether esters	Sheep	100	daily	tolerated 35 doses*
✓ 2,4,5-Trichlorophenoxyacetic acid, triethylamine salts	Sheep	100	daily	tolerated 35 doses*
2,2-Dichloropropionic acid, sodium salt	Sheep	100	daily	tolerated 35 doses*

*At time of preparation of this table, these tests were in progress.

Pesticides prepared in granular form for many uses accomplish their objective more effectively; yet, because of the weight of the granules, the amount remaining on foliage or drifting away from the area is greatly reduced.

Biological Cycles

To a certain extent we have biological cycles involved in the use of pesticides. For example, we may treat a body of water for insect control and accomplish the objective, but organisms not killed by the treatment may absorb and store the chemical. They, in turn, according to their biological position, may be consumed as food by other species, and these in turn by others, until there is ultimately a removal from that water of a species serving as food for mammals or birds, or man. The cycle is then continued outside the original environment.

The accumulation of pesticide may be attenuated or concentrated within this cycle, depending upon the part the animal or plant may provide of the total diet of the consum-

nervous tissue. Unfortunately, this indicator often fails because animals can die with high levels of the enzyme in the blood while others live quite normally with none in the blood.

Carbamates.--Insecticides of this group are cholinesterase inhibitors but are generally not so active in this regard. Symptoms of poisoning generally parallel those produced by the organic phosphorus compounds, with a few additions. Usually the ataxia is more pronounced. The poisoned animal generally attempts to move rapidly, but succeeds only in rolling end-over-end in a tumbling manner, recovers rapidly, and repeats the process. There is usually a great deal of trembling and shaking. The poisoning by carbamates follows the general course described for the organic phosphorus compounds.

Lesions of Poisoning

Chlorinated Hydrocarbons.--Acute poisoning by these compounds yields no pathognomonic lesions. The lesions found are those expected in violent deaths from other causes--cloudy swelling, blanched viscera, and hemorrhages on various organs. In addition, there may occasionally be congestion of the lungs. The heart is generally found to be in systole.

Subacute and chronic exposure lead to degenerative changes in the liver and kidneys.

Organic Phosphorus Compounds and Carbamates.--This group usually leaves the viscera unmarked. Certainly there are no diagnostic lesions. Occasionally the lungs are edematous or congested, and occasionally there may be congestion with hemorrhages in various viscera.

Herbicides.--The dinitro herbicides create severe damage in the kidneys and liver. The lungs are gray in color. Often there is a yellow discoloration of all tissues and frequently the odor of the compound is easily detected.

The 2, -(2,4,5-trichlorophenoxy) propionic acid salts produce degenerative changes in the liver and kidneys and rather spectacular hemorrhages on the surface of the heart.

The other phenoxy-acid compounds are reported to produce kidney and liver damage.

Diagnosis of Poisoning

Proper diagnosis of suspected poisoning of livestock has been a major concern of ARS. To aid in this problem, we have developed films describing and pointing out methods of arriving at correct diagnosis.

Differential diagnosis was not, and is not, easy in the case of chlorinated hydrocarbon pesticides. The symptoms mimic those of many diseases in which the central or peripheral nervous structures are afflicted. In many cases, a diagnosis can be reached only by eliminating all other possible conditions. Proper diagnosis are reached only after careful evaluation of the history of exposure, symptoms, and the lesions (the latter being non-pathognomonic in acute chlorinated hydrocarbon poisoning, but may suggest other disease processes).

Differential diagnosis of poisoning by other pesticides is not appreciably easier. All must be approached with caution and carefully studied.

Use of Cholinesterase Determinations in Diagnosis.--The organic phosphorus insecticides and the newer carbamates are recognized as inhibitors of cholinesterase, either as the original compound or as metabolites of the original.

Originally, we (92), and others, felt that blood cholinesterase activity would be an excellent indicator of exposure and of poisoning. For single exposures we feel the method

Some Salient Aspects of 2,4,5-T Toxicology,
Metabolism and Dissipation: Reaction of the Environment

Total effect on selected ecosystems is the basis on which society will reject or accept a pesticide in the future. Man has used the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and similar compounds to maintain synthetic ecosystems for the past 25 years. Ecological benefits from the use of herbicides are discussed in a recent article by Barrons (9). This review summarizes research data on the effects of the chlorinated phenoxy aliphatic acids on the world ecosystem.

Toxicological data are summarized by Rowe and Hymas (44), Palmer and Radeleff (41) and by Scifres, et al.^{1/} Acute toxicity is usually expressed as the amount of chemical (mg/kg body weight) required to kill half a population of laboratory animals. This amount, known as the LD₅₀, is from 300 to 1000 mg/kg for rats, mice, guinea pigs and rabbits for the commonly used herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-T and [(4-chloro-o-toyl)oxy] acetic acid (MCPA). These values correlate well with levels found to be toxic to cattle. Dogs are somewhat more sensitive and chickens less sensitive than laboratory animals. The acute toxicity of 2,4-D and 2,4,5-T is not considered an important biological property in terms of the net effect on natural ecosystems.

Compounds are administered in sublethal quantities over the entire life cycle to laboratory animals and its effects on tissues and organs

^{1/} Scifres, C. J., F. S. Davis and T. J. Allen. 1969. Unpublished compilation of scientific publications related to chlorophenoxyacetic acid herbicides with special reference to 2,4,5-T. Available on request from Range Science Department, Texas A&M University, College Station, Texas.

noted for the determination of chronic or subacute toxicity. These are so-called feeding studies. In general, the threshold for chronicity is about 10 to 25 percent of the LD₅₀ value (100 mg/kg) (44).

Carcinogenic and tumorigenic properties are of interest principally from the standpoint of human consumption of herbicide treated foods, either directly or indirectly. The "standard" procedure is to feed small laboratory animals (usually rats or mice) the maximal dosage which can be tolerated without fatalities by stomach tube or by mixing with the ration, over the life span of the animal, then to examine the animal thoroughly for tumors. In one recent study (26) 2,4-D and 2,4,5-T were among 118 compounds tested. Eighty-nine of these, including 2,4-D and 2,4,5-T, gave no significant indication of tumorigenicity after oral administration.

Chemicals causing malformations in the fetus, teratogens, have been publicized extensively in recent months. Research conducted by a commercial laboratory for the National Institute of Health^{2/} presented evidence that 2,4-D and 2,4,5-T, at oral dosages sufficient to cause death if fed over the entire life cycle, cause malformations of some fetuses when fed to pregnant mice and rats. There are three problems in interpreting findings of this type: First, there are undoubtedly species differences; that is some species or strains may be more susceptible to teratogenesis than others. Presently there is no way of knowing whether man is more or less susceptible than the mouse to induction of abnormal fetuses by

^{2/} Courtney, K. D., D. W. Gaylor, M. D. Hogan, H. L. Falk, R. R. Bates, and I. Mitchell. 1970. Teratogenic evaluation of 2,4,5-T. (Unpublished).

a given compound. Secondly, the dosage used in such studies is far in excess of that ever likely to be consumed by humans. Third, these laboratory experiments are designed to test a single chemical. Man's environment contains a complex mixture of chemicals. These mixtures may interact with each other or with integral parts of the ecosystem to either enhance or inhibit teratogenicity or other effects.

Although no specific data are available, 2,4,5-T has been used extensively on rangeland grazed by pregnant cows and sheep for more than 20 years with no reports of resultant fetal malformations. The conditions under which 2,4,5-T is used (up to one pound per acre, followed by deferment of grazing for at least 30 days) would seem to preclude any such effects.

Rates and pathways of metabolism of 2,4-D and closely related herbicides have been studied by many investigators since 1959 (5, 21, 25, 34, 42, 46) without clarifying certain details of metabolism. However, these compounds undergo a variety of reactions in plants from which the principal data have been obtained.

Decarboxylation is a common degradation route for 2,4-D and related chlorophenoxy compounds. The percentage degraded varies from as little as 1 to as much as 57 in various species (39, 48, 53).

Hydrolysis of the ester of 2,4,5-T (50) and probably the amide occurs readily in plants. Complexes with sugars, amino acids, proteins and other plant constituents also occur, but little quantitative data are available on this as a route of detoxification.

Although the preponderance of data suggests that metabolism of 2,4-D

and related compounds in plants is relatively rapid, no definitive data on the biological activity of metabolites are available. It is doubtful whether metabolism by plants is an important route of inactivation. However, it is possible that plants could store the herbicide in a slowly released form, resulting in long term residues at levels below 1 ppb, the present limit of resolution in water.

Persistence is a relative term relating the length of life of a pesticide or its first metabolites to that of other pesticides as measurable by our present analytical methods. The insecticide DDT is considered relatively persistent because it does not decompose rapidly and reappears later in other parts of the ecosystem. Even though 2,4-D is one of the most rapidly decomposed of all herbicides (4, 27), and is at the opposite end of the persistence spectrum, it cannot be considered absolutely non-persistent.

The principal routes of detoxication in soil were summarized by Bovey (12). Soil microorganisms rapidly decompose 2,4-D. The rate of decomposition of 2,4-D in the soil is therefore increased by conditions favoring rapid microbial growth. Klingman (29) states that if 0.5 to 3.0 lb/A of 2,4,5-T is applied to a moist, loam soil, it can be expected to persist for 2 to 5 weeks. Of prime importance is the lack of accumulation of 2,4-D in the soil from one year to the next at normal field rates (29).

The related herbicides 2,4,5-T, silvex, MCPA and 4(2,4-DB) are thought to be similar to 2,4-D in soil persistence properties (46). However, there are few data substantiating this belief.

Current research at Texas A&M with the herbicide 4-amino-3,5,6-trichloropicolinic acid (picloram^{3/}) may suggest the fate of phenoxyacid herbicides in soils. If the herbicide is applied to trees and grass when in full foliage, no more than 10 percent of the applied herbicide reaches the soil surface initially (). Depending on soil type, about half this amount (5 percent) is leached out of the root zone (into the subsoil or the ground water). Approximately 2 percent of picloram is adsorbed to soil colloids too tightly to be removed by plant roots. The remaining 3 percent comes into contact with the root system of the plant, and a small amount (less than 0.05 percent) is absorbed into the root system, and is retained or travels upward to aerial portions of the plant, some of which are eaten by animals. The remainder rejoins the herbicide not in contact with the root. Thus, approximately 7.5 percent or more of the original application moves into the subsoil or ground water. Thus, 0.018 kg/acre from an 0.5 lb/A initial application may move into the lower soil strata. Thus, leaching of picloram is the major route of dissipation from soil. The principal difference between picloram and the phenoxyacids in the soil is the microbial degradation of the latter group.

Aly (2) reported that 2,4-D persisted up to 120 days in lake waters aerobically incubated in the laboratory. It was decomposed biologically by lake muds, (83 percent within 24 hours) but only after extensive microbial adaptation techniques were used. The amounts of 2,4-D esters adsorbed on bentonite, illite and kaolinite ranged from 0.2 to 0.14 mg

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per gram. Whether photolysis represents a significant route for the environmental degradation of phenoxy herbicides is undetermined. Decomposition of 2,4-D to phenols occurred in artificial ponds under the influence of ultraviolet light (2) but was less extensive under natural conditions in the northeast (3).

No information for 2,4,5-T persistence in water is available. Considering the relatively large acreages on which 2,4,5-T is applied, this question seems to require additional study. Recent research has shown that some 2,4,5-T may be washed off treated areas. Trichell et al (51) found that a 2 lb/A application of 2,4,5-T caused 0.045 lb/A to appear in runoff water following 0.5 inches of simulated rainfall. The authors suggested that distance between the treated area and major water arteries is an important factor determining the amount of herbicide which will enter the water sources. When 4 lb/A of 2,4,5-T was sprayed directly over water to control riparian vegetation, maximum concentration in the stream was .04 ppm, and none could be detected downstream (43A).

Formulation affects susceptibility to washing and leaching. Hauser and White () found that as much as 27 percent of a foliage application of the ester of 2,4-D was washed away in the first 30 minutes of rainfall, while only 3 percent of the amine form was lost under the same conditions.

It is not certain whether usual herbicidal applications can result in appearance of 2,4,5-T in the ground water. The rationale for this uncertainty is discussed in the section on persistence in soil. One instance of contamination of well water by 2,4-D has been reported (42).

The persistence and mobility of chlorophenoxyacetic acids in air

has not been extensively studied. The short chain esters of 2,4-D and 2,4,5-T are considered volatile and can cause biological damage to organisms physically removed from the source (42). The question has never been subjected to quantitative analysis. Significant photolytic degradation of phenoxyacids may occur in air; Crosby and Ming-Yu Li recently outlined the technical difficulties inherent in such studies (16).

Although studies of pesticide persistence in plants are directly related to studies of plant metabolism, it seems appropriate to consider vegetation as a partitioning agent in the ecosystem as well as a metabolic agent. Most laboratory studies have demonstrated disappearance of 2,4-D in plants, with partial metabolism within a week (49). Hay and Thimann (24) found that a 50 µg application of 2,4-D to bean (Phaseolus vulgaris L.) seedlings eventually disappeared almost completely, and about half the absorbed compound was degraded in 24 hours. Morton (38) found that 80 percent of the 2,4,5-T applied to fully expanded honey mesquite (Prosopis juliflora (Swartz) DC var. glandulosa (Torr.) Cockr.) leaves was altered in 25 hours. The maximum rate of change occurred between 70 and 85°F. In this and many similar studies, however, 2,4,5-T may have been conjugated with sugars or proteins, and later released as parent molecule over time.

Morton et al (37) also studied persistence of 2,4-D and 2,4,5-T in and on range grasses in the field (Table 1). Rainfall was the most important factor influencing disappearance in these natural ecosystems. Esters were completely hydrolyzed after one week to the acid and unknown

metabolites. In 1963, after 8 weeks and 3.1 inches of rainfall, concentration of 2,4,5-T and unknown metabolites in and on green tissues of grasses such as Andropogon saccharoides Swartz (silver beardgrass), A. scoparius Michx (little bluestem) and Paspalum dilatatum Poir. (dallisgrass) was 25 and 7 ppm, respectively at 2.0 and 0.5 lb/A rates. Concentrations were 2.1 and 9.5 ppm, respectively in sideoats grama (Bouteloua curtipendula (Michx) Torr) 8 weeks with 3.0 inches of rainfall after similar applications in north central Texas. Residue from 2,4,5-T amine at 1 lb/A was 9 and 2 ppm at 8 and 16 weeks after application, respectively, from grasses treated in southeast Texas and was 19 and 5 ppm for the amine and acid formulations of 2,4,5-T respectively 8 and 16 weeks after treatment of grasses in north central Texas. Concentrations of the acid were 9.1 and 6 ppm, respectively, at 8 and 16 weeks after treatment in north central Texas. Baur, Bovey and Smith (10) applied 2 lb/A of 2,4,5-T ester and recovered 4 ppm from forage after a month, and 0.17 ppm after 6 months.

In summary, trends from data available suggest that 2,4,5-T residues available to grazing animals on rangeland would be on the order of 300 ppm after one day and 0.1 to 0.2 ppm after 6 months, depending upon rainfall.

Merkle and Fertig (35) found 1 to 3.5 ppm of 2,4,5-(trichloro)propionic acid (silvex) in trefoil 36 days after application of 1/2 lb/A. The residues were directly correlated to rate of application. These investigators showed that the amount of silvex residue remaining is more closely related to the time interval between applications and harvest

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than to rate of application. For example, 0.25 lb/A of silvex left a residue of .23 ppm in seedling trefoil 2 weeks after application, but only 0.19 ppm remained after 6 weeks even when the rate of application was increased to 0.75 lb/A. There was considerably less silvex in the plant regrowth than in the plant material originally sprayed. Nevertheless, silvex residues of 1.74 ppm were found in timothy regrowth originally treated with 3 lb/A. Silvex at 0.75 lb/A could be applied to trefoil in late October without leaving detectable residues in the plant growth the following spring and without serious damage to the trefoil stand.

Linscott (33) found in greenhouse studies that about 90 percent of C¹⁴ labelled 4(2,4-DB) applied to legumes disappeared within 30 days after treatment. Simulated rainfall accelerated herbicide removal. The herbicide was not found in legume regrowth after clipping.

Linscott, et al (32) studied the decomposition of 4(2,4-DB), 2,4-D and silvex in silage, and found that ensiling reduced but did not eliminate 4(2,4-DB) and silvex.

Cattle and sheep have been fed 2,4-D and 2,4,5-T on forages treated with these compounds, and pastured on 2,4-D treated fields. Elimination by excretion is apparently characteristic for the chlorophenoxyacetic acids (43). The following data are from studies in which 2,4-D or 2,4,5-T was fed directly to domestic livestock:

When a lactating cow was fed 5.5 g daily for 106 days, no 2,4-D was found in the milk, liver, kidneys or fatty tissue, or in the blood serum of a calf given the milk (36).

Gutenmann et al (22) fed 5 ppm 2,4-D to a Jersey cow and could not detect the herbicide by gas chromatography in her milk the following morning.

In sheep, about 96 percent of an oral dose of 2,4-DC¹⁴ was excreted unchanged in the urine within 72 hours (15). Slightly less than 1.4 percent was excreted in the feces. The 2,4-D in urine and feces was identified by electrophoresis and paper chromatography. The edible sheep tissue contained 0.05 ppm or less.

In another study, sheep given 2 grams of 2,4-D acid for ninety days were analyzed on the ninety first day and residues in all tissues were between 0.05 and 1 ppm (43).

Some data are also available for persistence in milk from animals fed 2,4-D treated forages. Klingman, et al (30) applied 2,4-D ester at 2 lb/A (twice the normal rate) to forage, then analyzed the milk from cows grazing the treated forages. After two days, milk contained 0.01 to 0.09 ppm. After 4 days, residues were below 0.01 ppm, the limit of sensitivity of the method.

The probability of 2,4,5-T being ingested by humans via the meat of domestic livestock seems remote, since most rangelands are sprayed in the spring, 4 to 6 months before the livestock are slaughtered. However, reports on 2,4,5-T persistence in meat of animals grazing 2,4,5-T treated pastures are not available.

No information is available on persistence in wildlife.

Mobility of herbicides in the environment is related to solubility in water, affinity for soil, and volatility. No comparative data exist

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for the phenoxyacetics. Some formulations are volatile, but the tendency in agricultural practice has been to curtail usage of these "high-volatile" formulations. The ester formulations are generally less water soluble than the amine for many herbicides, but can be carried in irrigation water.

If chemicals are ingested by successively higher links (animals, birds or fish) in the food chain, increases in tissue concentration may occur until injurious levels are reached. No instances of significant biological magnification of 2,4-D or its relatives have been recorded in some 25 years of extensive use. At present, no system of investigation which can evaluate this property in advance of large-scale usage in the environment has been developed.

Limited research findings are available indicating that 2,4-D and 2,4,5-T interact with other chemicals in the environment. When 2,4,5-T is mixed with picloram, mobility of the picloram and its concentration in the lower parts of the plant increase in mesquite, while in live oak (Quercus virginiana Mill.), 2,4,5-T mobility and concentration in the lower plant increase (10, 17).

Recent studies by Allen at Texas A&M suggest that the activity of 2,4-D can be increased and decreased by certain natural plant constituents.^{4/} Whenever biological activity of a herbicide is increased by interaction with other pesticides in the environment, the hazard from that herbicide may be increased, but the amount used may be reduced in view of the increased herbicidal activity. Very little specific data on the nature or frequency of pesticide interactions are available. This

^{4/} Allen, T. J. Personal communication.

question needs research attention.

Sheets and Danielson (46) indicated that when 2,4-D is present in soil the number of microorganisms capable of inactivating the compound apparently increase. Repeat applications of 2,4-D were less persistent in soil, and therefore less toxic than the initial application. Bacteria capable of inactivating 2,4-D have been isolated from soil and grown in pure culture (6, 8, 20, 27). In one study 4(2,4-DB) was converted to 2,4-D by microorganisms in the soil and a microflora capable of quickly inactivating both 2,4-D and 4-(2,4-DB) was present in soils which had received 4-(2,4-DB) previously (52).

Bollag, et al (11) reported that a phenoxyacetate-metabolizing Arthobacter spp. contained an enzyme that converts 2,4-dichlorophenol and other chlorophenols to catechol in the presence of NADPH and oxygen.

Persistence of many herbicides in the soil is related to halogenation of the benzene ring (18, 19, 47). Soil perfusion studies of Audus (4, 7) established that meta halogen substitution (2 or 5 position) renders a phenoxyacetic acid molecule more resistant to microbial degradation, but 4-substitution makes the molecule more labile. Alexander and co-workers (1, 13) indicated that resistance of the phenoxyacetics or their derivatives to microbial degradation was governed by the position of the halogen rather than the number of halogens on the ring, (meta substitution decreased rate of degradation) and the linkage and type of aliphatic side chain also influenced susceptibility to microbial breakdown.

The general conclusion is that the phenoxyacetics do not have permanent serious effects on soil microflora (46). However, in at least

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one study 2,4-D had a delayed lethal effect on 5 species of soil bacteria at concentrations which did not inhibit growth completely (31). High rates of 2,4-D are required for inhibition of nitrification and ammonification, with considerable variation in susceptibility of microorganisms to 2,4-D (28).

Few data specifically for 2,4,5-T are available.

The organisms which inhabit the top 3 to 5 inches of the ocean are extremely important to the well-being of man. Compounds which may influence the photosynthetic capability of these organisms, the phytoplankton, are potentially dangerous to the world oxygen supply. For 2,4-D and its relatives, some evidence as to effects on growth of phytoplankton exists. Butler (14) found slight inhibition of phytoplankton (mostly diatoms and dinoflagellates) growing in water containing 1 ppm of the salts or acids 2,4-D or 2,4,5-T. No measurements of effects on the photosynthetic capability are available.

Techniques have been for studying both acute and chronic toxicity of 2,4-D and some of the phenoxyacetics to fish have been studied extensively. The general conclusion is that 2,4-D is relatively non-toxic to fish at customarily used rates, but materials used in formulating the phenoxyacetics (e.g. xylene, petroleum derivatives) are sometimes toxic ().

Rowe and Hymas (44) found that ordinary field rates of 2,4-D and 2,4,5-T are not injurious to wildlife. Benefits in increase in forage availability from decreasing competition from woody, non-browse species through use of herbicides are summarized by Barrons (9). No data on

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residues of 2,4-D or 2,4,5-T in meat of game species are available.

Summary

Man has utilized herbicides such as 2,4-D and 2,4,5-T for the past 25 years to construct synthetic ecosystems which presumably serve his purposes better. When natural ecological processes are disrupted, as after the use of herbicides, the chemical tools might have to be used repeatedly in order to maintain the "stability" of the synthetic ecosystems. The chlorophenoxyacetic acid herbicides apparently serve man's purposes, ^{against} these beneficial effects. All possible ramifications of their use must be considered and weighed. They are not toxic to mammals at the dosages required for maintaining the synthetic ecosystems. The bulk of the evidence gathered to-date suggests that the chlorophenoxy herbicides dissipate rapidly from soils where they serve as a carbon source for the microbial population. Plants metabolize or complex the compounds readily. If domestic animals or wildlife ingest forage containing these herbicides, the chemicals are largely excreted via the urine. The possibilities of biological magnification seem remote. In general, the net effect on the ecosystem has been reflected in increased yields of desirable species for man's use.

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ACUTE AND SUB-ACUTE ORAL TOXICITY OF
2,4,5-TRICHLOROPHENOXYACETIC ACID FOR DOGS

DOW 2 007829


Victor A. Drill, Ph.D., M.D.
Professor of Pharmacology
Wayne University College of
Medicine

1958

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Investigations have shown that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) can be used as a herbicide (1), in concentrations ranging from 0.1 to 0.2 per cent. Toxicological studies on the material are very limited. In the present study the acute and chronic oral toxicity of 2,4,5-T was determined in dogs. In the present data histological changes are not included as this phase of the study is not yet 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 Croon vaccine. The dogs were fed a standard stock diet (Friskies) ad libitum. The 2,4,5-T used was commercial material with a purity of 100%.

In the acute studies the calculated dose of 2,4,5-T was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days at which time survivors were autopsied. In the chronic study the 2,4,5-T was administered orally in capsules, 5 days a week over a 13 week 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 (2). 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.

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RESULTS

I. ACUTE ORAL TOXICITY.

1. Symptoms produced. The survival at various dose levels of 2,4,5-T is listed in Table 1. The LD₅₀ is in the range of 100 mgm. per kilo or slightly higher.

The symptoms observed in the dogs were fairly mild and limited to a slight to moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses of 2,4,5-T. No symptoms were seen in the one dog that died following a single dose of 100 mgm. per kilo (Table 1).

2. Body weight. Doses of 100 mgm. per kilo or greater produced a slight fall in body weight during the test period. The results are somewhat variable with the 50 mgm. per kilo dose but indicate only a relatively slight effect of the drug on body weight (Table 1).

3. Gross pathology. The changes observed were limited to the gastro-intestinal tract and lungs. In dog 90 the duodenal mucosa was a slight diffuse red in color and in dog 74 small pin point hemorrhagic areas were observed in the mucosa together with some blood in the lumen. A small amount of blood was also found in the small intestine of dog 84. This same animal also presented a purple-black lower left lobe of the lung that had a solid blood consistency.

II. CHRONIC ORAL TOXICITY.

1. Mortality. Dogs that received doses of 2, 5 and 10 mgm. per kilo survived the 90 days. The dogs receiving 20 mgm. per kilo either in a single or divided dose died during the study.

2. General symptoms. No signs or symptoms were observed in the

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dogs that survived the study. In the dogs that died the prominent effects were weakness, slight stiffness in the hind legs, and difficulty in the swallowing of food, and bleeding from the gums in one dog.(Table 2).

3. Body weight. The 4 dogs that died showed a loss of over 2 kilos in body weight that started approximately 10 days before the death of the animal. The surviving animals do not show any significant change in body weight during this period, but tended to fluctuate about their original weight.

4. Blood count. The administration of 2,4,5-T did not have any significant effect on the hemoglobin or red cell count (Table 3). Dogs 103, 70, 57 and 105 showed some decrease in total white cell count during the study but the values did not fall lower than those seen in the control dogs. In the differential count taken before death, the per cent of lymphocytes fell to very low levels in dogs 57 and 105.

5. Gross pathology. Animal No. 93 showed a diffusely reddened duodenum and jejunum. Dog 105 autopsied on the 59th day showed a generalized jaundice as indicated by the sclera and the finding of yellow fat and yellow organs throughout the body. In dog 55 the renal capsule was stripped with difficulty.

6. Organ weights. The animals that survived the 90 day test period did not show any significant change in organ weights (Table 4). Various minor changes in organ weight can be noticed in some of the dogs fed the 20 mgm. per kilo dose who died during the study. These changes were limited to possible increase in heart, liver or kidney weight.

Discussion. Single large doses of 2,4,5-T can produce death in dogs. However, the central nervous system symptoms observed are quite mild, consisting chiefly of stiffness of the hind legs with some ataxia. These.

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symptoms seem to be definitely less than those obtained with comparable doses of 2,4-D. However, further studies with doses of 150 to 200 mg. per kilo might elicit a better series of signs and symptoms. The acutely intoxicated animals lost only a slight amount of weight. Gross pathological changes when observed were confined to the gastro-intestinal mucosa and in one dog to the lower left lobe of the lung. The decreased incidence of such changes as compared with 2,4-D paralleled the mild central nervous effects of the drug.

In the studies on chronic oral toxicity of 2,4,5-T, it was observed that the animals survived doses of 2, 5, and 10 mg. per kilo without grossly apparent ill effects, but succumbed to doses of 20 mg. per kilo. Thus the chronic toxic dose seems to be in the same range as that obtained with 2,4-D. In those animals receiving the highest dosage, 20 mg/kg of 2,4,5-T, evidence of toxic effects consistently included slight muscle spasm, difficulty in swallowing food, and loss in terminal body weight. A marked decrease in the percentage of lymphocytes in the peripheral blood was noted in two instances, while generalized icterus of the body organs and inflammation of duodenal mucosa were each observed once.

Summary. 1. The acute oral LD₅₀ for 2,4,5-T is in the range of 100 mg. per kilo or higher. Acutely poisoned dogs show mild spastic signs accompanied by a loss in body weight. Gross pathological changes were uncommon and were confined to the small intestines and lungs.

2. In chronic oral feeding of 2,4,5-T, doses of 2, 5 and 10 mg. per kilo allowed survival for 90 days and did not produce any symptoms or changes in body weight, organ weight or blood count. Dogs fed 20 mg. of

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2,4,5-T per kilo per day died during the study, showing mild spastic symptoms, a loss in body weight, and in 2 cases a decrease in the per cent of lymphocytes in the peripheral blood. Minor changes were present in the duodenum in one dog, and jaundice in another dog at autopsy.

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Table 1

Effect of single oral doses of 2,4,5-T in dogs

Dog No.	Dose mg/K	*Died day	Mortality	Wt loss Kg	Symptoms*
79M	400	2	1/1	-1.1	+
96M	250	3	1/1	-1.4	+
83F	100	S	1/4	-0.5	0
84F	100	7		-1.0	0
90F	100	S		-1.0	0
95F	100	S		-0.4	0
100M	50	S	0/4	+0.3	0
101M	50	S		-2.3	0
106F	50	S		+0.1	0
103M	50	S		-0.6	0

* S = survival for 14 day test period.

* column indicates general severity of symptoms as discussed in text. 0 = no change from normal.

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Table 2

Body weight and survival of dogs fed 2,4,5 T for 90 days

Dog No.	Dose mg/K	Weight			Day Died
		Initial Kgm	Final Kgm	Diff. Kgm	
77F	none	7.3	7.7	+0.4	S
102M	none	12.1	14.5	+2.4	S
87F	2	11.3	12.7	+1.4	S
99M	2	8.9	10.0	+1.1	S
66F	5	8.6	8.4	-0.2	S
103M	5	12.3	12.5	+0.2	S
93F	10	12.3	11.5	-0.8	S
55M	10	13.0	12.2	-0.8	S
70F	10	9.8	9.2	-0.6	S
57M	*20	11.8	9.2	-2.6	49
69F	*20	10.5	7.9	-2.6	75
94F	20	14.9	12.7	-2.2	11
105M	20	10.9	8.2	-2.7	59

* = daily dose divided and administered $\frac{1}{2}$ in A.M., and $\frac{1}{2}$ in P.M.

S = survived 90 day test period.

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Table 3

Effect of 2,4,5 T on hemoglobin, total blood and differential counts

Dog No.	Dose mg/kg	Day	Hb gm. %	Hct mil.	WBC	Differential count					Death day
						Neut	Lymph	Mon	Eos	Basin	
77F	none	0	9.60	5.33	16,100	67	25	1	0	6	
		30	10.75	5.63	13,300	56	16	2	0	3	
		90	12.74	5.82	14,700	51	13	1	0	0	
102M	none	0	12.29	5.53	14,500	55	39	2	0	4	
		30	13.31	5.73	14,900	63	25	4	0	7	
		90	12.95	6.47	6,400	48	51	1	0	0	
87F	2	0	11.95	5.82	12,450	70	24	2	0	4	
		30	14.49	6.13	16,700	62	23	12	0	3	
		90	14.52	6.53	14,900	50	13	2	0	0	
99M	2	0	10.75	5.28	17,100	75	22	0	0	3	
		30	13.55	5.56	14,800	56	34	9	0	1	
		90	14.46	6.55	17,200	67	25	6	0	2	
66F	5	0	11.90	6.21	12,600	43	47	2	0	3	
		30	12.93	6.17	17,400	46	43	5	0	1	
		90	13.92	7.03	13,300	55	44	1	0	0	
103M	5	0	10.94	5.30	13,100	47	20	3	0	30	
		30	12.67	5.79	16,600	54	21	8	0	17	
		90	12.95	6.24	7,250	71	29	0	0	0	
93F	10	0	11.90	6.50	16,150	69	24	4	0	3	
		30	12.74	6.72	13,800	66	29	3	0	3	
		90	12.67	6.43	13,600	77	15	3	0	5	
55M	10	0	11.58	5.91	13,250	56	15	6	0	23	
		30	12.54	5.49	13,650	56	24	4	0	16	
		90	11.84	6.23	15,350	70	27	3	0	0	
70F	10	0	12.65	6.37	15,400	73	12	6	0	4	
		30	14.46	6.28	16,400	54	23	8	0	10	
		90	14.34	7.29	8,500	67	33	0	0	0	
57M	*20	0	12.54	5.35	16,400	72	12	6	0	10	
		30	8.64	3.93	16,450	77	13	3	0	7	
		49	8.98	3.07	6,900	94	4	2	0	0	49
69F	*20	0	12.48	7.10	16,050	63	29	2	0	6	
		30	14.66	6.82	11,200	71	23	0	0	1	75
94F	20	0	12.44	5.73	13,300	63	22	4	0	1	11
105M	20	0	12.48	6.03	16,800	51	44	2	0	3	
		30	12.93	5.81	14,650	73	18	4	0	0	
		59	11.97	6.14	7,175	92	9	1	0	0	59

* = daily dose divided and administered $\frac{1}{2}$ in A.M. and $\frac{1}{2}$ in P.M.

18172

Table 4

Organ weights of dogs fed 2,4,5 T for 90 days
Based on final body weight

Dog No.	Dose mg/K	Kidney		Liver		Heart		Adrenal		Thyroid	
		wt g	g/kg	wt g	g/kg	wt g	g/kg	wt g	g/kg	wt g	g/kg
77F	none	38	4.94	268	34.8	61	6.10	1.0	0.130	0.4	0.052
102M	none	83	5.72	405	27.9	95	6.55	1.2	0.033	1.1	0.076
87F	2	58	4.56	557	43.1	78	6.15	1.1	0.037	1.3	0.102
99M	2	48	4.30	357	35.7	75	7.50	1.0	0.100	0.7	0.070
66F	5	35	4.17	341	40.6	63	7.50	0.8	0.095	0.5	0.050
103M	5	68	5.44	428	34.2	86	6.83	1.4	0.112	0.9	0.072
92F	10	48	4.17	382	33.2	74	6.44	1.2	0.104	---	---
55M	10	49	4.01	412	33.8	73	5.93	1.0	0.082	0.7	0.057
70F	10	37	4.02	292	31.7	70	7.60	1.2	0.130	---	---
57M	*20	51	5.55	335	36.5	65	7.07	1.4	0.152	---	---
69F	*20	62	7.85	390	49.4	67	8.49	1.5	0.190	---	---
94F	20	73	5.75	385	48.9	79	6.22	1.5	0.118	---	---
105M	20	68	8.30	264	32.2	67	8.17	1.1	0.134	0.8	0.098

* = daily dose divided and administered $\frac{1}{2}$ in A.M. and $\frac{1}{2}$ in P.M.

18173

DOW 2 007839



THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

1166

A COMPILATION OF TOXICOLOGICAL INFORMATION REGARDING
2,4,5-TRICHLOROPHENOXYACETIC ACID

CONTENTS

- Assembled July 1963 -

References

- I RESULTS OF TOXICOLOGICAL TESTS ON 2,4,5-TRICHLORO-PHENOXYACETIC ACID
- II ACUTE AND SUB-ACUTE ORAL TOXICITY OF 2,4,5-TRICHLORO-PHENOXYACETIC ACID FOR DOGS
- III THE ACUTE ORAL TOXICITY OF 2,4,5-TRICHLOROPHENOXY-ACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS
- IV SOME EFFECTS OF HERBICIDES ON PASTURE AND ON GRAZING LIVESTOCK
- V TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID
- VI 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

18174

RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

18175

RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

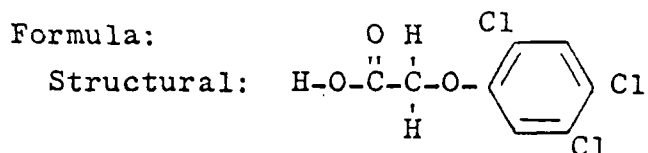
PROBLEM

The widespread handling of 2,4,5-trichlorophenoxyacetic acid made it desirable for us to have a significant amount of toxicological information. Data on the oral toxicity was necessary for presentation to the F.D.A. Hearing on residue tolerances.

MATERIAL

Name: 2,4,5-Trichlorophenoxyacetic acid

Formula:



Empirical: $\text{C}_8\text{H}_5\text{Cl}_3\text{O}_3$

EXPERIMENTAL RESULTS

The acute oral toxicity of 2,4,5-trichlorophenoxyacetic acid for rats, mice, guinea pigs and chicks is given in the attached summary which was presented in evidence at the F.D.A. Hearing. In concluding our testimony on this material we stated,

18176

DOW 2 007825

"Because of the similarity in chemical structure and in toxicity and physiological effect between 2,4-D and 2,4,5-T, it seems that a residue tolerance for the latter on fresh fruits and vegetable should be the same as that established for 2,4-D; it would seem that a residue of 20 p.p.m. would provide a wide margin of safety".

Eye Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in propylene glycol was introduced into the eye of a rabbit, it produced marked pain and slight conjunctival irritation immediately. Five hours later, severe conjunctivitis and moderate corneal damage was apparent. This picture remained about the same for two days before healing began. Five days after the exposure mild conjunctivitis and corneal damage was still apparent.

Skin Irritation

When 2,4,5-trichlorophenoxyacetic acid as a 10% solution in butyl carbitol acetate was applied repeatedly to the rabbit ear and bandaged repeatedly to the shaven abdomen, a slight simple irritation developed in both instances.

SUMMARY

The toxicological properties of 2,4,5-trichlorophenoxyacetic acid may be summarized as follows:

1. The material is moderate in acute oral toxicity for the four species, rat, mouse, cavie, and chick. Of these species the rat seems to be the more resistant and the chick the most susceptible.
2. The material is markedly irritating to the eyes.
3. The material is slightly to moderately irritating to the skin upon prolonged exposure.

18177

HANDLING HAZARDS AND PRECAUTIONS FOR SAFE HANDLING

Swallowing

It does not seem likely that 2,4,5-trichlorophenoxyacetic acid can be swallowed accidentally in amounts dangerous to life, although this might be possible if concentrated solutions of the material were available to children. If substantial quantities of the material were to be swallowed by anyone, serious ill effects could be anticipated.

If the material should be swallowed, induce vomiting by giving a common emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Call a doctor.

Eyes

The 2,4,5-trichlorophenoxyacetic acid is capable of causing serious damage to the eyes. Precautions should be taken when handling this material to prevent possible eye contacts. It is recommended when handling strong solutions of 2,4,5-trichlorophenoxyacetic acid that goggles, face shield, or other similar device be worn to prevent accidental splashes in the eye. It is believed that the handling of the solid does not present a hazard simply because of its insolubility in aqueous medium.

If the material in any form should get into the eyes, they should be promptly washed with flowing water for at least 15 minutes and medical attention obtained.

Skin

Prolonged and repeated contact with the skin may result in irritation. Occasional contacts for short periods are not expected to cause irritation. Strong solutions are no doubt more likely to cause difficulty than the dry solid.

In case of contact wash the exposed area with soap and water. Do not wear clothing or shoes which have been contaminated with solutions of the material.

DOW 2 007827

18178

Inhalation

No studies of toxicity upon inhalation of 2,4,5-trichloro-phenoxycetic acid dust have been made. We believe, however, that in view of the similarity with this material to that of 2,4-dichloro-phenoxycetic acid, that it is unlikely that toxic concentrations of the dust would be inhaled, primarily because of the irritation it will produce upon the upper respiratory passages.

DOW 2 007828

18179



THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

1166

A COMPILATION OF TOXICOLOGICAL INFORMATION REGARDING
2,4,5-TRICHLOROPHENOXYACETIC ACID

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- Assembled July 1963. -

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18180

RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

18181

Reference 1

RESULTS OF TOXICOLOGICAL TESTS
ON 2,4,5-TRICHLOROPHENOXYACETIC ACID

Biochemical Research Department
The Dow Chemical Company
Midland, Michigan

August, 1950

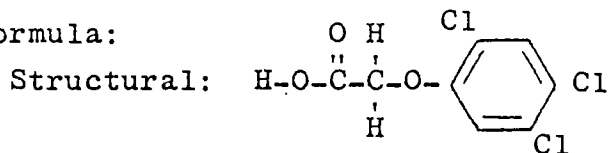
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18182

DOW 2 007825

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18183

HANDLING HAZARDS AND PRECAUTIONS FOR SAFE HANDLINGSwallowing

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DOW 2 007827

18134

Inhalation

No studies of toxicity upon inhalation of 2,4,5-trichloro- , phenoxyacetic acid dust have been made. We believe, however, that in view of the similarity with this material to that of 2,4-dichloro-phenoxyacetic acid, that it is unlikely that toxic concentrations of the dust would be inhaled, primarily because of the irritation it will produce upon the upper respiratory passages.

DOW 2 007828

1. Geographic Data FTT		1. Report No. NCI-DCCP-CG-1973-1-1		2. PB 223 153	
3. Title and Subtitle Evaluation of Carcinogenic, Teratogenic, & Mutagenic Activities of Selected Pesticides and Industrial Chemicals. Volume I: Carcinogenic Study				5. Report Date Completion-Aug. 1968	
7. Author(s)				6.	
7. Performing Organization Name and Address Bionetics Research Laboratories 7300 Pearl Street Bethesda, Maryland 20014				8. Performing Organization Rept. No.	
10. Project/Task/Work Unit No.				11. Contract/Grant No. PH 43-64-57 PH 43-67-735	
12. Sponsoring Organization Name and Address National Cancer Institute Division of Cancer Cause & Prevention Carcinogenesis Program Bethesda, Maryland 20014				13. Type of Report & Period Covered FINAL 1963 - Aug. 1968	
14.					
15. Supplementary Notes Concept - Dr. Paul Kotin and Dr. Hans Falk, NIH Initial Planning - Dr. A. J. Pallotta and Dr. E. Ross Hart, BRL					
16. Abstracts Vol. I evaluates the carcinogenic potential of 150 commercial pesticides which have been used in part as insecticides, herbicides, & fungicides. A total of over 20,000 mice were used in the experiment. Eighteen virgin males & 18 virgin females from hybrid strains (B6AKF1 and B6C3F1) were selected for a test on each compound for each route of administration used. An equal number of mice served as untreated controls for both routes & another group of 36 served as vehicle controls for each solvent. Two types of 18-month studies were carried out: 1) a single s.c. injection in 0.05 ml of suspension in nape of neck at 28th day of age; 2) daily oral stomach tube administration from 7th to 28th day of age, and thereafter mixed in feed. MTD calculated on average body weight per group was determined in a preliminary acute toxicity study. No doses above 1,000 mg/kg were studied, except N-Propyl isomer at 2,000 mg/kg. Urethane was studied at a total dose of 3 mg/mouse (cont.)					
17. Key Words and Document Analysis. 17a. Descriptors					
Bioassay		Reticulum Cell Sarcoma		DOW 2 007801	
Carcinogens		Pulmonary Neoplasms			
Pesticides		DDT			
Mice		Chlorohydrocarbons			
Toxicology		Thiocarbamates			
Liver Neoplasms		Environments			
Herbicides		Halohydrocarbons			
Fungicides					
17b. Identifiers/Open-Ended Terms B6AKF1 Hybrid Mice B6C3F1 Hybrid Mice Dual clean/dirty corridor system Pesticide analysis Pesticide Residue					
17c. COSATI Field/Group 06-Biological & Medical Sciences 07-Chemistry					
18. Availability Statement Release Unlimited		19. Security Class (This Report) UNCLASSIFIED		21. No. of Pages	
		20. Security Class (This Page) UNCLASSIFIED		22. Price	

TABLE IV

LIST OF CHEMICAL COMPOUNDS STUDIED WITH CODE NUMBER,
COMMON OR CHEMICAL NAME, DOSAGE AND VEHICLE OR SOLVENT

Code No.	Common or Chemical Name	Subcutaneous		Oral		
		Dosage mg/kg	Solvent	Dosage mg/kg	Vehicle*	ppm
024	Simazine	1000	0.5% gelatin	215	0.5% gelatin	603
025	Propazine	1000	0.5% gelatin	46.4	0.5% gelatin	102
026	Captan	1000	0.5% gelatin	215	0.5% gelatin	560
027	Piperonyl Butoxide	100	Corn oil	100	0.5% gelatin	300
028	Piperonyl Sulfoxide	46.4	Corn oil	46.4	0.5% gelatin	111
029	N-Propyl Isome	1000	DMSO	2000	0.5% gelatin	6000
030	2,4-D Isopropyl Ester	100	Corn oil	46.4	0.5% gelatin	111
031	2,4-D Butyl Ester	21.5	Corn oil	46.4	0.5% gelatin	149
032	2,4-D Isooctyl Ester	21.5	Corn oil	46.4	0.5% gelatin	130
034	Ethyl Carbamate	25	DMSO	158	0.5% gelatin	600
047	Sevin	100	DMSO	4.64	0.5% gelatin	14
048	IPC	215	DMSO	215	0.5% gelatin	560
049	SDDC	464	Dis. H ₂ O	215	Dis. H ₂ O	692
050	Dowcide-7	46.4	Corn oil	46.4	0.5% gelatin	130
051	Zineb	1000	0.5% gelatin	464	0.5% gelatin	1298
052	o,p'-DDD	464	DMSO	215	0.5% gelatin	560
053	Diuron	1000	0.5% gelatin	464	0.5% gelatin	1400
054	Dodine	1000	0.5% gelatin	21.5	0.5% gelatin	82
056	Maneb	100	0.5% gelatin	46.4	0.5% gelatin	158
057	Maleic Hydrazide	1000	0.5% gelatin	1000	0.5% gelatin	3000
058	Thiram	46.4	0.5% gelatin	10	0.5% gelatin	26
059	Monuron	100	0.5% gelatin	215	0.5% gelatin	517
060	PCNB	1000	0.5% gelatin	464	0.5% gelatin	1206
061	2,4,5-T	215	DMSO	21.5	0.5% gelatin	60
062	Ferbam	100	0.5% gelatin	10	0.5% gelatin	32
063	2,4-D	464	DMSO	100	0.5% gelatin	323
	2,4-D	215	DMSO	46.4	0.5% gelatin	149
065	p,p'-DDT	464	0.5% gelatin	46.4	0.5% gelatin	140
066	Atrazine	100	0.5% gelatin	21.5	0.5% gelatin	82
067	p,p-DDD	464	Corn oil	100	0.5% gelatin	300
068	Ethyl Tellurac	1000	0.5% gelatin	46.4	0.5% gelatin	149
069	Captax	215	0.5% gelatin	100	0.5% gelatin	323
070	Ethyl Zimate	464	0.5% gelatin	100	0.5% gelatin	260
071	Phenyl Isothiocyanate	100	Corn oil	46.4	0.5% gelatin	158
072	Perthane	215	DMSO	215	0.5% gelatin	815
073	Chloranil	464	0.5% gelatin	215	0.5% gelatin	646
074	Altax	1000	0.5% gelatin	464	0.5% gelatin	1577
075	Unads	100	DMSO	100	0.5% gelatin	377
076	Dichlone	21.5	0.5% gelatin	10	0.5% gelatin	30
077	Dicryl	1000	DMSO	21.5	0.5% gelatin	73
078	Ethylene imine	4.64	Dis. H ₂ O	4.64	0.5% gelatin	13
079	Nabam	10	Dis. H ₂ O	21.5	Dis. H ₂ O	73
080	Agerite DPPD	1000	DMSO	1000	DMSO	3385
081	Vancide PB	10	DMSO	46.4	DMSO	121
082	Bismate	1000	0.5% gelatin	10	0.5% gelatin	34

*Used during stomach tubing period only.

18136

TABLE VII

NUMBER OF EXPECTED TUMORS AND MINIMUM TUMOR INCIDENCE IN SUBCUTANEOUS
STUDY MICE THAT CAN BE REGARDED AS SIGNIFICANT AT P=.05 AND P=.01 LEVELS FOR
SPECIFIED NUMBERS OF MICE NECROPSIED IN THE STUDY GROUP*

No. of mice necropsied in study group	Expected total mice with tumor	P=.05 Level				P=.01 Level			
		Total mice with tumor (1)	Reti- culum cell sarcoma (2)	Pulm. Adenoma (3)	Hepa- toma (4)	Total mice with tumor (1)	Reti- culum cell sarcoma (2)	Pulm Adenoma (3)	Hepa- toma (4)
72	7	13	5	8	4	15	7	10	6
71	7	13	5	8	4	15	7	10	6
70	7	13	5	8	4	15	6	10	5
69	7	13	5	8	4	14	6	10	5
68	7	13	5	8	4	14	6	10	5
67	7	12	5	8	4	14	6	9	5
66	7	12	5	8	4	14	6	9	5
65	6	12	5	7	4	14	6	9	5
64	6	12	5	7	4	14	6	9	5
63	6	12	5	7	4	13	6	9	5
62	6	12	5	7	4	13	6	9	5
61 ✓	6	12	4	7	4	13	6	9	5
60	6	11	4	7	4	13	6	9	5
59	6	11	4	7	4	13	6	9	5
58	6	11	4	7	4	13	6	9	5
57	6	11	4	7	4	13	6	9	5
56	6	11	4	7	4	12	6	8	5
55	5	11	4	7	3	12	6	8	5
54	5	11	4	7	3	12	6	8	5
53	5	10	4	6	3	12	6	8	5
52	5	10	4	6	3	12	5	8	5
51	5	10	4	6	3	12	5	8	5
50	5	10	4	6	3	11	5	8	5

DOW 2 007803

Using Chi Square Method "Fourfold Table" - Yates Correction used for all but total tumors.

Based on 613 Mice Necropsied in the Total Negative Control Groups:

- (1) of which 61 developed tumors.
- (2) of which 14 developed reticulum cell sarcoma.
- (3) of which 30 developed pulmonary adenoma
- (4) of which 10 developed hepatoma.

18167

ATTACHMENT

PERTINENT DATA ABSTRACTED FROM TABLES XI AND XXV OF BIONETICS STUDY (1968)

Compound Name	Comp. No.	Strain (1)	No. of Mice At Start		Total Mice Necropsied		Pulmonary Adenoma	
			M	F	M	F	M	F
Untreated Control-OA Subcutaneous		A	18	18	16	17	1	1
		B	18	18	17	18	1	1
Untreated Control-OB Subcutaneous		A	18	18	17	18	2	0
		B	18	18	18	17	0	2
Untreated Control-OC Subcutaneous		A	18	18	17	17	2	0
		B	18	18	18	18	2	1
Untreated Control I Subcutaneous		A	18	18	18	18	1	0
		B	18	18	18	18	0	1
Untreated Control II Subcutaneous		A	12	18	12	18	1	1
		B	18	18	18	18	0	1
Untreated Control III Subcutaneous		A	—	4	—	4	—	0
		B	11	6	11	6	0	1
Gelatin Control Subcutaneous		A	18	18	16	18	1	0
		B	18	18	18	18	0	0
Corn Oil Control Subcutaneous		A	24	24	21	21	2	1
		B	24	24	19	20	2	1
DMSO Control Subcutaneous		A	24	24	24	23	0	1
		B	24	24	24	24	1	2
Total Negative Control Subcutaneous		A	150	160	141	154	10	4
		B	167	162	161	157	6	10
2,4,5-T Subcutaneous		A	18	18	17	18	0	0
		B	18	18	18	18	3	1

061

(1) Strain of mice: A-B6C3F1 (Strain X in CAG report)
 B-B6AKF1 (Strain Y in CAG report)

DOW 2 007804

18188

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

18.269

Subject TOXICITY OF 2,4,5-TRICHLOROPHENOXY ACETIC ACID.

File T21142-1
Chg. 1219A
Rec'd 3-15-45
Fin'd 8-8-45
Work By C. Geisel

*orig. work
done in March
April of 1945
- C.G.*

To Tech. Service & Develop Check
W. W. Allen
W. R. Veazey

*C. Williams
8-25-45*

Rept. By *T. K. Rame*
8-25-45

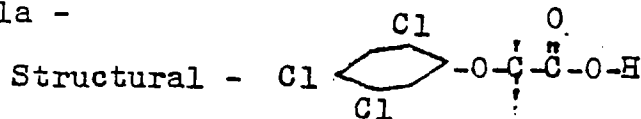
Problem -

What are the handling hazards to be associated with
2,4,5-trichlorophenoxy acetic acid?

Material -

Name - 2,4,5-trichlorophenoxy acetic acid - K4568-2

Formula -



Empirical - $C_8H_5O_3Cl_3$

Source - Organic Research Lab. - L. Begin

Ref. - #410-5-258

Experimental Results -

Acute Oral - Weighed doses of the acid were emulsified in
5-10% gum arabic solution and fed to cavies in single oral doses
by means of a stomach tube. The material as the sodium salt, ad-
justed to pH = 10, was also fed. The results are given in Table I

Table I

The Acute Oral Toxicity of 2,4,5-Trichlorophenoxy
Acetic acid and its Sodium Salt for Cavies.

<u>Material</u>	<u>100% Survival Dose g./kg.</u>	<u>100% Lethal Dose g./kg.</u>
Acid	0.3	1.0
Salt - pH = 10	0.3	0.5

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THE DOW CHEMICAL COMPANY

00963DOW 2000318

These figures indicate that the subject material is moderately toxic when given in single oral doses. No information upon the effect of repeated administrations was obtained.

It was noted in this acute oral work that the animals did not exhibit the nervous symptoms caused by 2,4-dichlorophenoxy acetic acid, sodium salt. The animals fed large doses of this latter material exhibited paralysis of the posterior portion of their bodies.

Skin Irritation - The acid as a 10% solution in butyl carbitol acetate was applied 10 times in 11 days to the ear and shaven belly of a rabbit. A very slight irritation developed on the ear and a slight to moderate irritation appeared on the belly.

A 1% aqueous solution of the sodium salt failed to produce any significant irritation on the ear even after 20 exposures in 27 days; similar exposure on the belly resulted in a very slight simple irritation.

Conclusions -

2,4,5-Trichlorophenoxy acetic acid has a moderate acute oral toxicity and is slightly irritating to the skin of rabbits.

We conclude that excessive exposure to the dust of either the free acid or the salt would be likely to cause skin irritation as would exposure to concentrated solutions for prolonged periods of time. Exposure to dilute solutions such as those used in practical application would not be expected to cause trouble except among those who persist in spraying themselves or who do not take the necessary precautions to prevent prolonged contact with the material.

Reasonable handling precautions which will prevent ingestion and prolonged skin contact with the material will suffice to prevent serious trouble.

jr-5 copies.

18190

BIOCHEMICAL RESEARCH LABORATORY

Test on

Acute oral toxicity. ~~Rat~~ Cavy

Prob. T 23.14-23-1

825

BIOCHEMICAL RESEARCH LABORATORY

Feed or Na Sal
pH adjusted to

Test on K4568-2 (Trichlorophenoxyacetic Acid) (Salt is K785)

Acute oral toxicity. Rat. Cavy 07

Prob. T 23.14-23-1

(1 cc = 0.01 gm) in H₂O.

Date	Animal #	Weight kg.	Dose gm/kg	Dose gm.	Dose cc	Remarks	1/3	1/6
330-45	224	510	0.1	.0510	5.10		528	550
	218	520	0.1	.0520	5.20	F.d.	580	595
	225	521	0.2	.1042	10.42	930 AM. 1045 6 cc. 442	590	599
	214	558	0.2	.1116	11.16	7 cc.	416	590604
	205	570	0.3	.1770	17.70	10 cc. 7.70	592	627
	204	614	0.3	.1842	18.42	10 cc. 842	645	652
	203	535	0.5	.2675	26.75	10 cc. 10.00 6.75 Dead	6.75	Dead
	207	516	0.5	.2580	25.80	10 cc. 10.00	5.80	Dead
	222	568	1.0	.568	56.8	10 cc. 10.00 Dead 3-30-45		
	215	571	1.0	.571	57.1	10 cc. 10.00 Dead 3-30-45		

Animals here did not seem to respond as did those fed the dichloro compound. No rigidity with hind quarters was apparent although the rectal temperatures were about 1° low. - 10
X signed & finished 4.20-45 C

Feed as Na S
pH adjusted to 10

Acute oral toxicity. ~~Ant.~~ Cavy 

Prob. T 23.14-23 -/

(1 cc = 0.01 mm in H_2O)

1219-43-5C-642

18193

1044 12.5.5

1.

No. of Applications up to 10

~~Asdemen~~

Rabbit # 9-17207 Cage # 9 Prob. T 23.14-23-1

DOV ZUUDKJ

1/0 en,
de Ha. Su

K4565-2

(Trichloroethenoxy Acetic Acid)

No. of Applications

upto 20

~~Abdomen~~

Apply 5 times a week to Elysean

Rabbit #

9-190 vt

Cage #

238

Prob. T 23.14-23-1

X U. K. Rame
4-26-45

10 in H_2O
as Na_2SO_4

Skin Irritation

No. of Applications 645 to 20

~~Quantitative~~

EST

Abdomen

Bandage on 5 times a week.

Rabbit # 9-19004 Cage # 38 Prob. T 23.14-23-1

000000



THE DOW CHEMICAL COMPANY

1173

MIDLAND DIVISION
April 25, 1967

K. E. Coulter
Midland Division Research & Development
566 Building

MN 117354

CHLORACNE RESEARCH PROGRAM

History of Chloracne Incidences at Dow:

Historically, Dow Chemical has been involved in chloracne incidences ever since Dow began the production of chlorophenols. At first, the commercial production of chlorophenols was conducted at 206 Building. During the period 1934-36 there was a severe outbreak among the employees which resulted in an unsuccessful lawsuit. The chloracne incidents were traced back to poor working conditions and the manufacture of Dowicide P. The manufacture of Dowicide P has since been terminated. New and improved working facilities for the Dowicide group were constructed at 265 Building in 1940.

In the late 1930's, Wes Stoesser in 20A lab got a serious chloracne attack from chlorinated diphenylene oxide. Drastic treatments were used to cure this incident and no further work was done on this series of compounds. It is suspected that many of the other incidences of chloracne are caused by chlorinated diphenylene oxides or analogues thereof.

In the next ten years, another unfortunate situation occurred in the chloracne situation. Some of the Dow customers complained about dermatitis and/or chloracne from the use of Dow's Dowicide 3. I understand financial adjustments were made and the production of Dowicide 3 terminated. A purified material of related structure is now sold as Dowicide 31 and 32.

During the period 1940-65, the product 6X (diphenyl oxide chlorinated to the hexa level) was manufactured at 206 Building and at least one severe case of chloracne occurred because of this product. The production of 6X has been terminated.

In the research lab at 172 Building, there were some cases of chloracne from research exposures. In one case, a severe case resulted from the recycling of residues from the manufacture of 2,4,5-trichlorophenol using glycol as a solvent. In another case, several mild cases occurred hydrolyzing polychlorobenzenes using aqueous caustic at high temperature. The Chemical Physics Lab had several incidences of chloracne from the recycling of caustic insolubles in the alcoholic caustic hydrolysis of tetrachlorobenzene.

DOW 1516484

18198

This doesn't tell the story as I know it.

In 1964, the 2,4,5-trichlorophenol hydrolysis step was modified for economy and safety reasons from 100% caustic to 23% caustic, but this change caused an unforeseen increase in the chloracnigens. This increase in chloracnigens was sufficient to cause many mild incidences of chloracne and two severe cases (lost timers). In 1966 a new process was put into operation using the Boehringer procedure involving a batch reaction at low temperatures; and, so far, has operated satisfactorily. - 7

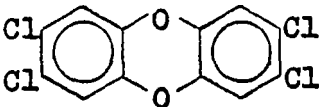
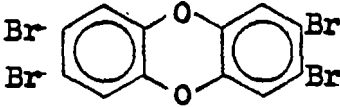
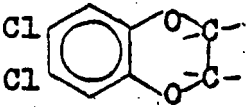
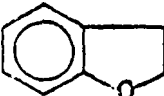
After they started up their Dowicide plant, the Canadians began to experience chloracne incidences in their employees. In Midland, more than half of the Dowicide employees have chloracne of varying intensity and it is impossible to say when or where these incidences occurred.

Research Program in Progress for Chloracne Reduction:

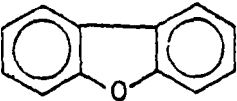
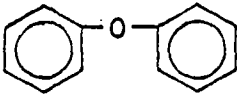
Because of the prevailing existence of chloracne in the Dowicide plants and a sincere desire to reduce or eliminate this, research has been initiated in 1966 on this problem. Progress has been slow due to the complexity of the problem. The problem involves isolating and determination of the identity of the chloracnigens. After being properly identified, work can progress on its reduction or elimination in the process.

In commenting on chloracne, we must keep in mind that chloracne is a cosmetic evidence of the attack and serious liver damage is an invisible effect of the attack. Rabbit ear tests are a positive sensitive method of determining the chloracne activity of chlorophenol impurities.

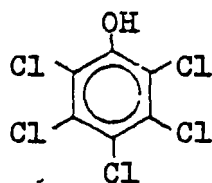
Present Knowledge of Chloracnigens:

<u>Compound</u>	<u>Activity</u>
	2,3,7,8 Tetrachloro - Very positive Cl ₁₋₃ - Not active Cl ₃₋₇ - Possibly some cpds. active Cl ₈ - Not active
	Very active
	Very active
	Cl _{1-X} - Unknown activity

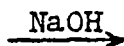
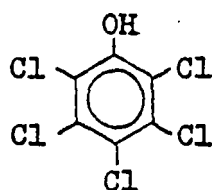
DOW 1516485

<u>Compound</u>	<u>Activity</u>
	Cl ₁₋₂ - Unknown activity Cl ₃₋₆ - Some very reactive
	Cl ₄₋₆ - Some activity?

Materials possessing unexplained chloracne activity:

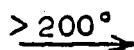
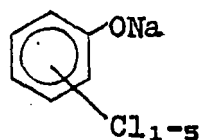


Some Midland batches are mildl
reactive
Many Canadian batches are mild
reactive

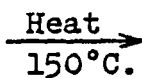
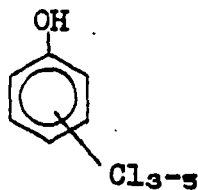


Dowicide G

All sludges are active



All decompositions are active



Some have activity

DOW 1516486

18200

Midland Research Program on Chloracne:

The following chloracne activity fractions are being examined for isolation, identification, and minimization of chloracne activity. Unfortunately, due to shortage of technical help the program is proceeding quite slowly.

1. Dichlorophenol still residue.
2. Pentachlorophenol process samples.
 - a. Dowicide 6 and 7 "active" batches.
 - b. Dowicide G sludge.
 - c. Dowicide G scrubber sludge.

The method of research is to first concentrate the sample (remove chlorophenols), then fractionate by chromatography, test fraction on rabbits, then further fractionate by chromatography, then test fraction on rabbits, etc., then determine structure by micro analysis, then determine method of analysis in original sample, then investigate process changes which will minimize the chloracnigens in the process.

The Benzene Research Lab and the Biochem Research Lab are collaborating in this project. The Benzene Research Lab does the chemical research and the Biochem Research Lab does the testing on the rabbits on their charge.

Alex Widiger

Alex Widiger
Benzene Research Laboratory
474 Building

je

cc: W. H. Haberstroh, 474 Bldg.
R. C. Sauers, 474 Bldg.
S. L. Bender, 172 Bldg.
E. C. Staehling, 258 Bldg.

cc [signature]

DOW 1516487

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

3 December 1966

MN 012332

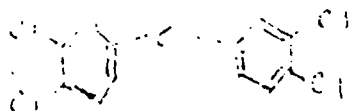
DOW 1568621

Mr.
 Dow Chemical Company
 Midland, Texas 79701

Dear Sir:

Reference is made to our telephone conversation on December 1st, 1966, regarding 2,4,5 trichlorophenoxyacetic acid. It is very kind of you to

propose the determination of the toxic compound present in the commercially available 2,4,5 trichlorophenoxyacetic acid which is the subject of our interest. As I mentioned, Kinsig published a paper in Environmental Health, Vol. 114-115, which gave evidence that the skin and liver damage was due to the 2,4,5 trichlorophenoxyacetic acid, but not to the by-product which is present in very small quantities. The compound is thought to be responsible is 2,6,7,8 tetrachloro-2,3,7,8-dioxin:



This compound can produce chloroma and liver damage in doses of micrograms per kg. body weight. There is no conclusive evidence that this or any other compound is responsible, since no one has yet separated the various compounds present in commercial 2,4,5 T.

We would like, therefore, are the following:

1. 10 grams of the purest preparation of 2,4,5 trichlorophenoxyacetic acid possible.

2. 10 grams of the commercial 2,4,5 trichlorophenoxyacetic acid, that is, as it is esterified, in powder form.

3. 10 grams of the commercial 2,4,5 trichlorophenoxyacetic acid iso-octyl ester, before the emulsifiers have been added.

Picloram (cont.)

³⁰
Mutagenicity

090 R

247 AU - YODER J ; WATSON M ; BENSON WM
TI - LYMPHOCYTE CHROMOSOME ANALYSIS OF AGRICULTURAL WORKERS DURING
EXTENSIVE OCCUPATIONAL EXPOSURE TO PESTICIDES
SI - EMIC/73/016741
SO - MUTAT RES; 21:335-340,1973
AB - EMIC/ORNH

human
mut

1 DOW 2157361

18204

Picloram (Cont.)

(161)
Mutagenicity

- 249 AU - ANDERSEN KJ ; LEIGHTY EG ; TAKAHASHI MT
TI - EVALUATION OF HERBICIDES FOR POSSIBLE MUTAGENIC PROPERTIES
SI - EMIC/72/013029
SO - J AGRIC FOOD CHEM; 20:649-656,1972
AB - EMIC/ORNH

105
PV

- 370 AU - ARNOLD WR ; SANTELMANN PW ; LYND JQ
AD - OKLAHOMA AGR. EXPT. STA., STILLWATER.
TI - PICLORAM AND 2,4-D EFFECTS WITH ASPERGILLUS NIGER PROLIFERATION
SI - CA/003/003379C
SO - WEEDS; VOL 14, ISS 1, 1966,89-90
AB - CBAC COPYRIGHT: CHEM ABS THE EFFECTS AND RELATIVE DEGRADATION OF (94-75-7) 2,4-DICHLOROPHOXYACETIC ACID 2,4-D AND (1918-02-1) 4-AMINO-3,5,6-TRICHLOROPICOLINIC ACID PICLORAM ON ASPERGILLUS NIGER, STRAIN ATC6275, WERE DETERMINED BY BIOASSAY WITH TOPCROP VARIETY OF SNAP BEANS. FUNGAL PROLIFERATION WAS REDUCED BY 2,4-D AT 10 AND 50 PPM., WHILE PICLORAM AT UP TO 50 PPM. DID NOT REDUCE FUNGAL GROWTH. ASPERGILLUS NIGER DEGRADED 2,4-D MORE EFFECTIVELY THAN PICLORAM. FUNGAL MYCELLIUM CONTAINED BOTH HERBICIDES AFTER EXPOSURE. BOTH HERBICIDES CAUSED PROFUSE MERISTEMATIC CELL PROLIFERATION WITH ABNORMAL INTERNODE ELONGATION AND SEVERE LEAF AND STEM CURLING IN THE SNAP BEANS.

104
DOM2157357

- 15 AU - Aulicino F ; Bignami M ; Carere A ; Conti G ; Morpurgo G
AU - Velcich A
TI - Mutational studies with some pesticides in Aspergillus nidulans.
SI - PESTAB/76/2257
SO - Mutat. Res. 38(2): 138; 1976.
AB - PESTAB. Induction of mutation and somatic recombination by the following pesticides has been studied utilizing Aspergillus nidulans as test organism: aminotriazole (amitrole), captan, dalapon, dichlorvos, dinobuton, dodine, ioxynil, mecoprop, neburon, picloram, and tordon (picloram as the triisopropanolamine salt). Point mutation has been studied as induction of 8-azaguanine resistance utilizing a spot test at three different pHs. Only captan turned out to be mutagenic. Induction of non-disjunction and mitotic crossing-over were studied with a diploid strain of the mold by: a) utilizing a spot test previously described at three different pHs, b) plating conidia of the mold on a medium supplemented with the maximum nonlethal dose of the pesticide and screening for the appearance of recombinant sectors in the colonies. Captan, aminotriazole,

103

and dichlorvos induce somatic recombination. Especially noteworthy is the action of dichlorvos in inducing crossing-over. (Author abstract by permission, abridged)

- 174 AU - Bignami M ; Aulicino F ; Velcich A ; Carere A ; Morpurgo G
AD - Ist. Superiore di Sanita, Rome, Italy
TI - Mutagenic and recombinogenic action of pesticides in Aspergillus nidulans.
SI - PESTAB/77/2780
SO - Mutat. Res. 46(6): 395-402 1977 (27 References)
AB - PESTAB. Thirteen pesticides, aminotriazole, benomyl, captafol, captan, dalapon-Na, dichlorvos, dinobuton, dodine, ioxynil,

102

mecoprop, neburon, picloram and tordon were tested for ability to induce (1) point mutations to 8-azaguanine resistance, (2) mitotic crossing-over, and (3) mitotic non-disjunction and haploidization in Aspergillus nidulans. Tests were performed at three different pHs, i.e. 4.5, 7, 8.2. Three of the pesticides, captan, captafol and dichlorvos induced point mutations; dichlorvos also induced a high frequency of mitotic crossing-over and non-disjunction; benomyl induced a very high frequency of non-disjunction whereas aminotriazole induced weakly both types of somatic segregation. (Author abstract by permission)

18205

Picloram (cont)

(15)
Livestock

PV

106

- 133 AU - Palmer JS ; Radeleff RD
TI - The toxicity of some organic herbicides to cattle, sheep and chickens.
SI - HAPAB/70/61212
SO - U.S. Dept. Agr. Prod. Res.; Rept. No. 106: 1-26, 1969; (REF:21)
AB - HAPAB The results of studies of the toxicity of 29 organic herbicides to cattle, sheep and chickens have been presented. These included chlorophenoxy compounds (2,4-D, 2,4,5-T, silvex and MCPA), amide compounds (diphenamid, CDAA and TCBC), phenyl

urea compounds (monuron, linuron, fenuron and diuron), thiocarbamate compounds (diallate and triallate), triazine compounds (atrazine, simazine, prometon, propazine, etc.), and benzoic acid compounds (dicamba and 2,3,6-TBA). Miscellaneous compounds investigated included bromacil, isocil, polychlorobicyclopentadiene isomers, propanil, dichlobenil, picloram and dalapon. A total of 126 yearling cattle, 190 1- to 2-year- old sheep and 700 6-week-old chickens were studied. Repeated doses of the herbicides were administered in gelation capsules or as water- diluted solutions by drench or in pipettes. The usual period of study was 10 days or until toxicologic effects appeared. However, in a number of instances, longer and shorter term studies were made. The signs of poisoning by most of the herbicides included anorexia and reduced weight gains. In many instances, idgestion in the rumen seemed to have been impaired. Macroscopic lesions were somewhat variable and nonspecific. The liver and the kidneys were most frequently involved. An arbitrary yield of forage and rate of consumption were selected to evaluate the hazard for cattle, sheep and chickens likely to exist under most severe conditions of use. The most common rates of application of many of these herbicides formulations are not a hazard to cattle, sheep or chickens. The maximum rates of application of some approach or surpass a hazardous level in one or more of the tests species. (Author abstract modified) Toxicology and Pharmacology 70/08/00, 360 1969

106W2157356

18266

Picloram (cont.)

(3)
Public Issues

- 126 AU - World Health Organization
TI - Health aspects of chemical and biological weapons; report of a WHO Group of Consultants.
SI - HAPAB/70/01829
SO - WHO, Geneva, 1960; 31 p., 1970
AB - HAPAB This report is an attempt to analyze the health effects of possible chemical and biological warfare on different civilian population groups-the purely military aspects are largely not considered; qualitative and quantitative estimates of the health effects of selected chemical and biological agents employed under specified hypothetical conditions are made. Among the agents discussed are the anti-plant agents 2,4-D, 2,4,5-T, picloram, cacodylic acid, monuron, bromacil, agent white, agent blue and agent purple, as well as tabun, sarin and soman. References are given at the ends of sections. GENERAL 70/11/00, 497 1970

133

- 138 AU - Galson AM
TI - Plants, people and politics.
SI - HAPAB/70/00844
SO - BioScience; 20(7): 405-10, 1970; (REF:17)
AB - HAPAB Contemporary man is confronted with many problems concerning social unrest, pollution and overpopulation. Some of the chemicals that man depends so much on to produce high levels of agricultural products are having deleterious effects on man and his environment. Chlorinated hydrocarbons like DDT, dieldrin and aldrin, once considered very useful, have now become manaces because of their persistence in the biosphere and their potential poisoning of various kinds of creatures. Present and future pesticides must be more thoroughly tested and then chosen and used with greater selectivity. Some of the more recently introduced herbicides have been shown to be potential herbicidal analogs of DDT. One of the suspected chemicals is 4-amino-3,5,6-trichloropicolinic acid (picloram or Tordon). Under optimum conditions in some soils, 20 to 50% of applied picloram disappeared after 467 days (Youngson et al., 1967). On other soils low in moisture and poor in inorganic matter and air, only 3.3% of the applied picloram disappeared in a similar period. It takes between 10,000 and 100,000 parts of exogenous carbon to oxidize one part of Tordon (Tschirley, 1969). The dangers of further use of this herbicide are evident. Another chemical, 2,4-D, can also become a menace under certain conditions by causing a massive increase in the nitrate content of pasture plants as to sicken animals eating these plants (Stabler and Whitehead, 1950) and must be used very carefully. Pesticides fabricated around heavy metals, such as lead and mercury, or other elements such as arsenic (trivalent) do not become completely detoxified once applied. The presence of rising quantities of lead and mercury in the environment has caused growing concern in public health. Science must not be misapplied. Thoughtless and indiscriminate use of chemicals such as 2,4-D, picloram, 2,4,5-T and arsenical cacodylic acid by industry, the military and other groups and organizations has and may continue to cause ecological havoc. The legal structure of our government as well as the influence of international committees and commissions offer many ways to promote rational use and limited control of our environment and must be employed before it is too late. GENERAL 70/07/00, 263 1970

132

DOM2157344

18207

Picloram (cont.)

Public Issue

(2)

PV

- 78 AU - ANON
TI - Degree of hazard when using herbicides.
SI - HAPAB/73/02233
SO - S. Afr. Sugar J.; 57(2): 71; 1973 ; (REF:1)
AB - HAPAB The most useful factor for assessing the potential hazard of a pesticide to humans and domestic animals, although not completely reliable, is the acute toxicity of the chemical. Oral and dermal toxicities according to the LD50 values, are quite high for paraquat, ioxynil, diquat, and some other herbicides while picloram, bromacil, asulam, simazine, dalapon, and diuron have relatively low toxicities. Inhalation toxicity of materials

is more difficult to assess than dermal or oral toxicity and is generally less important except in the case of specific fumigants and other volatile materials.

- 75 AU - Neillands JB
TI - Survey of chemical and related weapons of war.
SI - HAPAB /73/2870
SO - Naturwissenschaften60: 177-183; 1973(REF:29)
AB - HAPAB. The use of chemical pesticides in warfare probably began in 1961 when herbicides were employed to defoliate areas of S. Vietnam. Agent Orange, the primary defoliant, is a mixture of 2,4-D and 2,4,5-T and was used until 1970, when it was shown highly teratogenic. A 5-fold increase in the incidence of primary hepatoma in heavily defoliated areas of S. Vietnam was believed caused by residues of tetrachlorodioxin in the mixture. Agent White, a mixture of 2,4-D and picloram and substituted for Agent Orange, retains its phytotoxicity in soil for decades. Organophosphates have also been produced for warfare although their use has not often been documented. These compounds attack the active center of AChE, causing an accumulation of ACh and a consequent paralysis of voluntary and involuntary muscles; concentrations of as little as 0.01 mg per m(SUP)3 of air are almost instantly fatal. They are rapidly hydrolyzed in alkaline solution, and poisoning can be treated by simultaneous administration of atropine and pralidoxime.

- 85 AU - Westing A H
TI - Herbicides in war: current status and future doubt.
SI - HAPAB/73/01014
SO - Biol. Conserv.; 4(5): 322-327; 1972 ; (REF:13)
AB - HAPAB Approximately 26 million kg of 2,4-D, 22 million kg of 2,4,5-T, 1.5 million kg of picloram, and 3 million kg of dimethylarsinic acid were used by the U.S. to clear forests and damage crops in South Vietnam between 1961 and 1971. Single applications were made on approximately 15% of the forest area and multiple ones on four percent. Alterations included reduced diversity of plant and animal communities, loss of mineral nutrients, accelerated erosion, and reduced productivity. Flights over affected areas showed almost complete recovery of agricultural areas, but few signs of regeneration in mangrove and forest areas; it is estimated that one decade will be necessary to restore singly-sprayed areas and several decades for the multiply-sprayed areas. Timber losses probably totaled 47 million cubic meters, and rice destroyed would have fed about 894,000 people. Farmers and woodsmen have experienced mild temporary ailments attributed to herbicides. Death and illness of livestock reported by civilians were probably caused by the anti-personnel chemical 'CS' (ortho-chlorobenzalmalononitrile) or the malathion used in malaria control programs.

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DOM2157343

135

134

18208

HEW



NEWS

150

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
FOR RELEASE IN A.M. PAPERS
Tuesday, February 7, 1978

National Institutes of Health
Office of Cancer Communications
(301) 496-6641

Availability of a report on animal tests of picloram for cancer-causing activity (carcinogenicity) was announced by HEW's National Cancer Institute in today's Federal Register.

Technical-grade picloram, a systemic herbicide, was given in feed to rats and mice for 80 weeks. According to a summary of the report included in the announcement, under these test conditions the incidence of neoplastic nodules of the liver was suggestive of the ability of the compound to cause benign liver tumors in female Osborne-Mendel rats. In mice, picloram was not found to cause tumors.

The tests are part of the Institute's Carcinogenesis Testing Program. Copies of the report, Bioassay of Picloram for Possible Carcinogenicity, are available from the Office of Cancer Communications, National Cancer Institute, Bethesda, Maryland 20014.

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DOM2157250

Scandinavian Journal of Work, Environment & Health

VOLUME 3 NUMBER 2 MARCH 1982

10-01N-11-NC-45

— 146 —

100-443887-100

Validation of questionnaire information on cigarette smoking (continued)

4. Estimated delivery date 12/15/2010

Mortality of Zebra daphnophagous ctenopods	Zenith chlorophyll <i>a+b+c</i>
acid herbicide application during bloom	0.69 mg/L
spectrophotometric determination	0.78 mg/L

Exposure to solvents: I have been exposed to benzene, toluene, xylene, acetone, ethyl alcohol, and other organic solvents.

Application of the Richter and Gutenberg formulae to the above records:

1. Personnel 2. Equipment 3. Training 4. Logistics 5. Medical 6. Communications 7. Intelligence 8. Security 9. Weather 10. Other

Specialty Publications
 • Books • Journals • Reference • Periodicals • Audio • Video • Microfilm • Microfiche

Acute solvent-ethanol interactions with specific nerve tetrodotoxins

10-10-54

Immune response to WEHDNA. *Journal of Virology*, 52: 101-107, 1985. [W. H. Aronoff, J. M. Heath, and R. S. Baruch, Eds.]

100-443887-1

1. Explain the importance of the following factors in the development of a country's economy:
 a. Human Resources
 b. Capital Resources
 c. Technology
 d. Government Policy
 e. Infrastructure
 f. Trade and Investment
 g. Education and Health
 h. Environmental Sustainability
 i. Political Stability
 j. Legal System
 k. Financial System
 l. Energy Resources
 m. Land Resources
 n. Water Resources
 o. Climate Change
 p. Globalization
 q. Demographics
 r. Corruption
 s. Unemployment
 t. Inflation
 u. Interest Rates
 v. Exchange Rates
 w. Balance of Payments
 x. Foreign Debt
 y. Trade Balance
 z. Current Account
 aa. Capital Account
 ab. Financial Account
 ac. Reserve Account
 ad. Government Budget
 ae. Corporate Tax
 af. Personal Income Tax
 ag. Corporate Governance
 ah. Shareholder Rights
 ai. Board of Directors
 aj. Executive Compensation
 ak. Financial Reporting
 al. Internal Controls
 am. Risk Management
 an. Business Ethics
 ao. Corporate Social Responsibility
 ap. Environmental Management
 aq. Human Rights
 ar. Labour Rights
 as. Consumer Protection
 at. Product Safety
 au. Food Safety
 av. Medicine Safety
 aw. Transportation Safety
 ax. Building Safety
 ay. Fire Safety
 az. Disaster Preparedness
 ba. Emergency Response
 bb. Public Health
 bc. Vaccine Development
 bd. Antibiotic Resistance
 be. Non-Communicable Diseases
 bf. Infectious Diseases
 bg. Chronic Diseases
 bh. Acute Diseases
 bi. Preventive Care
 bj. Curative Care
 bk. Rehabilitation
 bl. End-of-Life Care
 bm. Palliative Care
 bn. Hospice Care
 bo. Long-Term Care
 bp. Respite Care
 bq. Home Care
 br. Community Care
 bs. Primary Care
 bt. Secondary Care
 bu. Tertiary Care
 bv. Quaternary Care
 bw. Specialty Care
 bx. General Practice
 by. Family Medicine
 bz. Internal Medicine
 ca. Cardiology
 cb. Neurology
 cc. Oncology
 cd. Pediatrics
 ce. Obstetrics and Gynecology
 cf. Orthopedics
 cg. Urology
 ch. Ophthalmology
 ci. Otolaryngology
 cj. Dermatology
 ck. Radiology
 cl. Pathology
 cm. Pharmacy
 cn. Nursing
 co. Physiotherapy
 cp. Occupational Therapy
 cq. Speech Therapy
 cr. Psychology
 cs. Psychiatry
 ct. Behavioral Science
 cu. Public Health
 cv. Epidemiology
 cw. Biostatistics
 cx. Health Economics
 cy. Health Law
 cz. Health Policy
 da. Healthcare Delivery
 db. Healthcare Financing
 dc. Healthcare Regulation
 dd. Healthcare Quality
 de. Healthcare Access
 df. Healthcare Equity
 dg. Healthcare Efficiency
 dh. Healthcare Innovation
 di. Healthcare Research
 dj. Healthcare Education
 dk. Healthcare Training
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 dn. Healthcare Administration
 do. Healthcare Information Systems
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 dr. Healthcare Robotics
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 dt. Healthcare Biotechnology
 du. Healthcare Materials Science
 dv. Healthcare Engineering
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 ec. Healthcare Adaptability
 ed. Healthcare Flexibility
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 ef. Healthcare Replicability
 eg. Healthcare Transferability
 eh. Healthcare Generalizability
 ei. Healthcare Applicability
 ej. Healthcare Feasibility
 ek. Healthcare Viability
 el. Healthcare Desirability
 em. Healthcare Acceptability
 en. Healthcare Appropriateness
 eo. Healthcare Effectiveness
 ep. Healthcare Efficiency
 eq. Healthcare Equity
 er. Healthcare Accessibility
 es. Healthcare Affordability
 et. Healthcare Quality
 eu. Healthcare Safety
 ev. Healthcare Reliability
 ew. Healthcare Consistency
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 ey. Healthcare Measurability
 ez. Healthcare Comparability
 fa. Healthcare Benchmarking
 fb. Healthcare Monitoring
 fc. Healthcare Evaluation
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① K-2372 - (ICC)
② K-4563 - (ICC)

Scand j work environ health 8 (1982) 37-42

^① Mortality of 2,4-dichlorophenoxyacetic acid and ^②2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland

First report of an ongoing prospective cohort study

by Vesa Riihimäki, MD, MSc, Sisko Asp, MPOlSc, Sven Hernberg, MD¹

RIIHIMÄKI V, ASP S, HERNBERG S. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland: First report of an ongoing prospective cohort study. *Scand j work environ health* 8 (1982) 37-42. Some recent epidemiologic studies have suggested that chlorinated phenoxy acid herbicides are human carcinogens. The mortality experience in a cohort of 1,926 men who had sprayed 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) during 1955-1971 has been followed prospectively from 1972 to 1980. The total phenoxy acid exposure was generally rather low because the duration of work had mostly been less than two months. In 1972-1976 mortality from all natural causes in the cohort was only 54 % of the expected value (based on age-specific rates for the general population), and in the succeeding 4-a period 81 % of the expected value. In the assessment of cancer, mortality allowance was made for 10- and 15-a periods of latency between the first exposure and the start of the recording of vital status during the follow-up. No increase in cancer mortality was detected, and the distribution of cancer types was unremarkable. No cases of death from lymphomas or soft tissue sarcomas were found. The study results must, however, be viewed with great caution owing to the small size of the cohort, the low past exposure, and the brief follow-up period.

Key terms: chlorinated phenoxy acids.

The herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been used in Finland since about 1955 to destroy brushwood vegetation. Their peak consumption occurred during the latter part of the 1960s when 50-70 t of active ingredients of both compounds were sprayed per year. Since 1972 the use of these chemicals has declined. Four governmental organizations or state-owned companies (the Finnish State Railways, the Highway Authority, the Forestry Authority and an electric power company) have been responsible for the majority of herbicidal use to control brushwood, and during the years of intensive appli-

cation about 500 workers, almost exclusively males, were employed annually in this work (20).

The chemical control of brushwood mainly involved the spraying of foliage in rights-of-way (railroads, highways, electric transmission lines) and in forests. The spraying season was restricted to about two to eight weeks during the summer. Spraying was, for the most part, performed with portable, manually operated or motor-driven knapsack rigs, with the exception of that done by the Highway Authority which mainly used moving vehicles. Throughout the 1950s and 1960s the methods of application and the work habits did not change appreciably. Because the workers were not informed of any hazards connected with the spraying, no special precautions were taken to avoid inhalation of the aerosols or skin contact.

¹ Institute of Occupational Health, Helsinki, Finland.

Reprint requests to: Dr V Riihimäki, Institute of Occupational Health, Haartmaninkatu 1, SF-00290 Helsinki 29, Finland.

0355-3140/82/010037-06USD2.50

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The most extensively used commercial herbicide preparations were 2:1 mixtures of emulsified esters of 2,4-D and 2,4,5-T dissolved in water. Even pure 2,4,5-T preparations were used. It is now well appreciated that the eventual hazards from the use of 2,4,5-T may be associated with a toxic dioxin impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It is not known with certainty how extensive the exposure to TCDD may have been in the early years of 2,4,5-T application. Analysis of some old herbicide preparations used in Finland in 1962-1967 suggested that the TCDD content ranged between 0.1 and 0.9 mg/kg; in one of the samples it was below the detection limit (18).

Public concern about alleged adverse health effects from brushwood-control herbicides precipitated an extensive survey of the situation in 1972 by an ad hoc working group (20), which, however, did not find material evidence to support such claims with regard to morbidity. However in a nationwide search for suspected phenoxy acid-related symptoms and signs, several reports of skin disorders were received. These mostly pointed to obvious toxic reactions, and, in at least one case, the skin disease, which was also later seen by a dermatologist, suggested a mild form of chloracne. A mortality study incorporated into the survey did not allow any conclusions to be drawn regarding mortality rates in general and cancer mortality rates in particular owing to the doubtful validity of the cohort and the short period of latency. It was intended to follow this cohort of herbicide sprayers in the years to come.

New suspicions of a possible carcinogenic effect of exposures to chlorinated phenoxy acids were raised in Sweden (1, 8), and they prompted us to begin a 5-a prospective mortality study in 1977. An interim report was prepared at the request of the International Agency for Research on Cancer (19), but it was not refined into a scientific report because we had reservations as to its power to provide significant evidence with respect to the problem of the cancer risks associated with phenoxy acid exposures.

More recently further epidemiologic studies (2, 5, 11, 12) and a case report (9) have shown or suggested an association

between exposure to chlorinated phenoxy acids and increased cancer risk. In response to the renewed public concern, Finnish health authorities established a new working group in 1979 to evaluate risks associated with the use of chlorinated phenoxy acids (and some other herbicides). The working group supplemented earlier mortality studies by assessing the cancer morbidity in the cohort in 1967-1974 through the files of the Finnish Cancer Register. The observed numbers of different types of cancer did not significantly differ from those expected on the basis of the cancer incidence in the general population, and no cases of soft tissue sarcoma were found (15).

Subsequently, a further 4-a prospective follow-up of mortality in the original cohort was instituted.

The debate on the hazards of chlorinated phenoxy acids still continues, and only a limited number of studies have been made available for the assessment of risks. Our observations, while not formally published, have been referenced in this context. We therefore felt it was important to present the results of this 9-a prospective follow-up on the mortality experience of the Finnish 2,4-D and 2,4,5-T herbicide applicators in detail although we acknowledge the preliminary nature of these results.

Material and methods

The cohort of herbicide applicators was collected in 1972 from the personnel records of the four main Finnish employers involved in chemical brushwood control. The cohort included 1,971 male workers who had been exposed for at least two weeks during 1955-1971 to 2,4-D and 2,4,5-T. The data were collected by office personnel in the respective companies from different sources including old payrolls, because the files did not always provide information on specific assignments. Therefore, in some cases the files of exposed workers were based on the memory of the foremen and clerks, and the reported exposure characteristics, eg, the duration of exposure, were likely to be imprecise. Special instructions were given to guarantee that pensioners and those

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who had changed jobs would not be omitted.

The duration of exposure to the herbicides had been quite brief. Three-fourths of the study population had a summed exposure duration of two to seven workweeks, and only one-fourth of eight weeks or more, as of 1971, divided over one or more summer seasons. For many persons in the cohort further exposure may have taken place after 1971. About two-thirds of the study population had had their first exposure rather recently, in 1966—1971.

At the onset of exposure one-third of the individuals in the cohort had been less than 25 a of age, and the study population is still relatively young. In the beginning of 1972 the median age of the Forestry Authority employees was 33.5 a, and the corresponding figures for the Highway Authority employees, power company employees and State Railways employees were 38.0, 38.3 and 47.8 a, respectively.

During 1955—1971, 45 individuals in the cohort of 1,971 persons died. Thus in the beginning of 1972 there were 1,926 persons alive who were followed through 1972—1980. The lists of names and identification numbers were checked against the population data register at the Social Insurance Institution, where every citizen is registered. In all, 15 individuals could not be traced, and they were removed from the cohort. For those who had died, the underlying causes of death were obtained from death certificates registered at the Central Statistical Office. The expected numbers of deaths from different causes were calculated by multiplying the age-

specific person-years of observation by the cause and age-specific national death rates for 1975. The fractional age group values were summed to yield corresponding figures for the whole cohort. The person-years and expected numbers of deaths were assessed in a conventional way, as well as by making allowance for the minimum of 10- and 15-a periods (of latency) between the first exposure and the start of the recording of vital status during the follow-up.

Results

During the 9-a prospective follow-up period the cohort accumulated 16,694 person-years of observation, and 105 persons had died from natural causes versus 155 expected. The follow-up was divided into two time periods, 1972—1976 and 1977—1980, and the observed, as well as expected, deaths are presented in table 1. In 1972—1976 the number of deaths from all natural causes was only 54 % of the expected value, whereas, during the succeeding 4-a period, the observed deficit in deaths was less than 20 % (observed/expected = 0.81). At variance to the previously published mortality data over 1972—1976 (19) the present investigation found one more death from cancer and six additional deaths from other natural causes. One case had been omitted due to a technical error. The loss of three cases may be attributed to a delay in reporting since the deaths had occurred at the end of 1976, whereas the inability to find the three others is unexplained. In the present investigation an error of this kind is un-

Table 1. Observed (O) and expected ^a (E) deaths among 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applicators during the prospective follow-up period of 1972—1980. In the calculations no allowance was made for latency period.

Follow-up period	Number of person-years	All deaths		All cancers		All natural causes of death	
		O	E	O	E	O	E
1972—1976	9,442	62	92.9	9	18.1	41	76.7
1977—1980	7,252	82	91.1	17	18.4	64	78.6
1972—1980	16,694	144	184.0	26	36.5	105	155.3

^a Based on age-specific national death rates for males in 1975.

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Table 2. Observed (O) and expected^a (E) deaths among 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applicators during the prospective follow-up period of 1972—1980. Allowance was made for 10- and 15-a latency periods in the calculations.

Cause of death	10-a period of latency		15-a period of latency	
	O	E	O	E
All causes	88	118.6	35	53.6
All cancers	20	24.3	5	11.3
Lung cancer	12	11.1	4	4.7
All natural causes	71	103.6	33	48.1

^a Based on age-specific national death rates for males in 1975.

Table 3. Localization of malignant tumors found among 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applicators, and expected values,^a during the prospective follow-up period 1972—1980. Allowance was made for a 10-a latency period in the calculations.

Site or type of tumor	Observed number of cases	Expected number of cases
Lung	12	11.1
Stomach + esophagus	4	3.7
Prostate	2	1.1
Multiple myeloma	1	0.2
Metastatic, unknown origin	1	0.3
Other sites and types	—	7.9
Total	20	24.3

^a Based on age-specific national death rates for males in 1975.

likely since the search for individuals in the cohort was performed by the register officials themselves.

When allowance was made for 10- and 15-a latency periods, the numbers of person-years were restricted to 8,445 and 2,888, respectively, and the numbers of deaths from natural causes were 71 and 33, respectively. Even in these populations there were considerably fewer natural deaths than expected (mortality rates 69 % of the expected values) (table 2). The total number of cancer deaths was also lower than that expected, while the occurrence of certain cancer types approximated the expected values. The distribution of different types of malignant tumor in the population with a 10-a latency period was rather unremarkable, and no cases of death from lymphomas [International Classification of Diseases (ICD) 200—202, 8th revision] or soft tissue sarcomas (ICD

171) were found (table 3). Follow-up for mortality in the four employer groups with the 10-a latency period included (table 4) showed a marked and unexplained undermortality among the Highway Authority employees.

Although there were uncertainties with regard to data on the actual duration of exposure, mortality figures were assessed separately for the reportedly more heavily exposed populations. Mortality figures (all natural causes and all cancers) for those exposed for six weeks or more or during 5 a and for those exposed for eight weeks or more or during 5 a (table 5) did not uncover any indications of an increased mortality in these subgroups.

Discussion

We have followed the mortality of a cohort of 1,971 men exposed over a period of at least two weeks to 2,4-D and 2,4,5-T herbicides during 1955—1971. Because of the methods used in collecting individuals for the cohort in 1972, selection bias could not be totally excluded, and thus the validity of the retrospective follow-up is questionable. For the prospective follow-up of all those alive in 1972 (totaling 1,926 men) similar problems do not exist.

The results of this investigation show that the general population was not an appropriate comparison group for the herbicide applicators, probably because of work requirements which selected able-bodied and healthy individuals. This undermortality seems to decrease with time, a phenomenon typical of the "healthy worker effect" (16), although even 10—15 a after the first exposure, at a relatively

young age, natural mortality was still slightly less than 70 % of that for the general population. With regard to cancer mortality the healthy worker effect is known to play a less significant role (16), and the present observations show the same tendency.

Chemically-induced cancer has a long induction-latency period, and therefore it was necessary to assess the mortality experience of the herbicide applicators after a relatively long time, at least 10 a, had elapsed since the first exposure. This was even more important because the estimated total exposure had been quite low and of short duration. When allowance was made for 10- and 15-a periods of latency, which of course restricted the small material even more, no increase of cancer mortality was uncovered. Furthermore, the distribution of the cancer types was unremarkable. No cases of death from lymphomas or from soft tissue sarcomas were found in the population with the 10-a latency period, the expected values being 0.8 for lymphomas and 0.1 for soft tissue

sarcomas. No information was available on the smoking habits of the individuals in the cohort.

The question arises of whether the present findings can be used in support of arguments that exposures to 2,4-D and 2,4,5-T, and to impurities in their commercial preparations, do not cause increased long-term risks of fatal diseases, particularly cancer. This investigation cannot be regarded as a conclusive negative study for a number of reasons: (i) the cohort was relatively small, (ii) the exposures to herbicides were low, particularly in terms of duration, and (iii) the follow-up period was still too brief to demonstrate in full the possible cancer risks from herbicide exposures. Obviously, further observations on the mortality of this cohort with the passage of time will permit the drawing of more valid conclusions.

Yet, it needs to be pointed out that the present results do not confirm the previously shown association between mixed herbicide exposures and increased overall cancer risks (2) as far as specific exposures

Table 4. Observed (O) and expected ^a (E) deaths among 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applicators of different employers during 1972-1980. Allowance was made for a 10-a latency period in the calculations.

Cause of death	State Railways (2,209 person-years)		Highway Authority (2,302 person-years)		Forestry Authority (3,343 person-years)		Power company (591 person-years)	
	O	E	O	E	O	E	O	E
All causes	39	49.0	12	27.4	31	35.1	6	7.1
All cancers	10	10.6	3	5.4	6	6.9	1	1.4
All natural causes	34	44.7	10	23.3	22	29.5	5	6.1

^a Based on age-specific national death rates for males in 1975.

Table 5. Observed (O) and expected ^a (E) deaths among the more heavily exposed 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applicators.

Cause of death	No latency period			10-a period of latency			15-a period of latency		
	Person-years	O	E	Person-years	O	E	Person-years	O	E
<i>Exposure for six weeks or more during 5 a</i>									
All natural causes	6,300	34	66.0	3,588	29	48.0	1,378	15	23.9
All cancers	6,300	8	15.4	3,588	8	11.3	1,378	1	5.7
<i>Exposure for eight weeks or more or during 5 a</i>									
All natural causes	4,338	23	50.0	2,717	21	40.1	1,151	13	21.5
All cancers	4,338	5	12.0	2,717	5	9.5	1,151	1	5.1

^a Based on age-specific national death rates for males in 1975.

to 2,4-D and 2,4,5-T are concerned. Neither did our observations support the finding that a markedly increased risk of lymphoma is associated with exposures to chlorinated phenoxy acids (11). The association between an increased risk of soft tissue sarcoma and exposures to chlorinated phenoxy acids, chlorinated phenols, and their chlorinated dibenzodioxin (TCDD in particular) and dibenzofuran impurities has been strengthened by recent epidemiologic (6, 10) and clinical (3, 13, 17) observations. Unfortunately, no certain conclusions on the risk of soft tissue sarcoma can be drawn in the present study because the material is small. TCDD is a recognized animal carcinogen, whereas the chlorinated phenoxy acids, including 2,4,5-T, are not (4, 7, 14). How great a potential risk of cancer for man is associated with exposures to 2,4,5-T containing small amounts of TCDD is not known. The results of this investigation can only suggest that minor exposures to 2,4,5-T do not pose great risks of cancer.

Acknowledgment

The authors wish to thank Dr L Teppo for his valuable comments on the manuscript.

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Received for publication: 16 December 1981



Volume 25 Number 1
August 1982

Toxicology and Applied Pharmacology

DOM2158409

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1982

	Name	
FILE	1. 2,4-D (2,4-dichlorophenoxyacetic acid)	K-2372-(100) 198
	2. 2,4-D, n-butyl ester	K-12935-(100) 198
	3. 2,4-D, isooctyl ester (Ded-Weed LV-69)	K-20054-(100) 198
	4. Dibenzofuran	K-2238-(100) 198
	5. 2,7-Dichlorodibenzo-p-dioxin (DCDD)	K-7453-(100) 198
	6. DMA-4 (2,4-D/dimethylamine salt)	K-8851-(100) 198
	7. 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)	K-4568-(100) 1984
	8. 2,4,5-T, n-butyl ester	K-12496-(100) 198
	9. 2,4,5-T, isobutyl ester	DR-0115-5471-(100) 198
	10. 2,4,5-T, isooctyl ester (Ded-Weed LV-4T)	K-20871-(100) 198
	11. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	K-66681-(100) 1984



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Mutagenicity Testing of Agent Orange Components and Related Chemicals

KRISTIEN MORTELMANS,* STEVE HAWORTH,† WILLIAM SPECK,‡ AND ERROL ZEIGER§¹

*Microbial Genetics Department, SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025;

†Genetic Toxicology Division, Microbiological Associates, 5221 River Road, Bethesda, Maryland 20816;

‡Department of Pediatrics, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106;

and §Cellular and Genetic Toxicology Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Received March 26, 1984; accepted May 18, 1984

Mutagenicity Testing of Agent Orange Components and Related Chemicals. MORTELMANS, K., HAWORTH, S., SPECK, W., AND ZEIGER, E. (1984). *Toxicol. Appl. Pharmacol.* 75, 137-146. Components of the herbicide Agent Orange—2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their esters, and the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)—and related chemicals were tested for mutagenicity using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. No mutagenic activity was observed for any of the chemicals tested.

The herbicide Agent Orange, used as a defoliant in Vietnam during the 1960s, was formulated as a 50:50 mixture of butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). It also contained free 2,4-D and 2,4,5-T, as well as a number of other esters, di- and trichlorophenol, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Hughes *et al.*, 1975; Young *et al.*, 1978). Dioxins, including TCDD, are produced during the manufacture of 2,4,5-T and other pentachlorophenols, and were present as contaminants of the commercially used products (IARC, 1977).

Although there is no new exposure to Agent Orange, 2,4-D is currently used by home gardeners, and both 2,4-D and 2,4,5-T are used for industrial control of rangeland, forest land, and in the cultivation of rice (R. Mountfort, EPA, January 1984, personal communica-

tion). Dioxins, specifically TCDD, are a by-product of 2,4,5-T manufacture (IARC, 1977), have been reported as possible products of combustion (Rawls, 1979), and are biologically available in contaminated soil (McConnell *et al.*, 1984). Additionally, a number of accidents have occurred that resulted in humans being exposed to polychlorinated dibenzodioxins and polychlorinated dibenzofurans (Huff *et al.*, 1980). Because of the widespread exposure of Vietnamese and American military personnel and civilians to Agent Orange in Vietnam, and the widespread exposure of farm workers and home gardeners to 2,4-D and 2,4,5-T preparations, these substances were tested for mutagenicity in the Ames *Salmonella* assay.

This report presents the *Salmonella* test results of 2,4-D, 2,4-D/dimethylamine salt, 2,4-D *n*-butyl ester, 2,4,5-T, 2,4,5-T *n*-butyl and isobutyl esters, TCDD, 2,7-dichlorobenzo-*p*-dioxin, and dibenzofuran, in addition to commercially available (1979) home gardening

¹ To whom all correspondence and requests for reprints should be addressed.

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TABLE I
AGENT ORANGE-RELATED CHEMICALS TESTED IN *Salmonella*

Name	CAS No.	Supplier*	Lot	Label purity (%)	Analyzed purity (%)
1. 2,4,-D (2,4-dichlorophenoxyacetic acid)	94-75-7	Dow	AGR171114	NS*	99
2. 2,4-D, <i>n</i> -butyl ester	94-80-4	Radian†	NS	NS	99.6
3. 2,4-D, isooctyl ester (Ded-Weed LV-69)	25168-26-7	Thompson-Hayward	F80553	67	—
4. Dibenzofuran	132-64-9	Fisher	B4X	Technical	—
5. 2,7-Dichlorodibenzo- <i>p</i> -dioxin (DCDD)	33857-26-0	IITRI	NS	NS	—
6. DMA-4 (2,4-D/dimethylamine salt)	2008-39-1	Dow	M924024	NS	—
7. 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)	93-76-5	Dow	AGR133711	NS	99+
8. 2,4,5-T, <i>n</i> -butyl ester	93-79-8	Radian	NS	NS	99.4
9. 2,4,5-T, isobutyl ester	4938-72-1	Radian	NS	NS	99.5
10. 2,4,5-T, isooctyl ester (Ded-Weed LV-4T)	25168-15-4	Thompson-Hayward	9057	64	—
11. 2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin (TCDD)	1746-01-6	IITRI	NS	NS	97.6

* Dow Chemical Co., 202 Dow Center, Midland, Mich. 48640; Radian Corp., P.O. Box 9948, Austin, Tex. 78766-0948; Thompson-Hayward Chemical Co., P.O. Box 2083, Kansas City, Kans. 66110; Fisher Scientific, P.O. Box 1307, Houston, Tex. 77251; Illinois Institute of Technology Research Institute, Chicago, Ill. 60616.

† Not specified.

‡ By Radian Corp.

ions of 2,4-D (i
ecyl ester) (Tab

METS

Materials were tested
of the Ames *Salmonella*
et al., 1975; Maron a
and are described else-
from overnight cultu-
rations TA1535, TA1538,
from Professor B. N. J.
with the 9000g superna-
tant (G9 fraction) from
the Dunley rat or male
rats were mixed with
0.1 sodium phosphate bu-
ffer, pH 7.0, and 50 or 100 µl of 10
of three tubes. This
was 20 min, after which
a trace amount
amount of histidine was
added onto minimal agar
at 48 hr, after which un-
colonies were counted.

It was the chemicals to
The testing laboratories
as coded aliquots. Solu-
tions, and safe handling
procedures.

Materials initially were re-
ceived as concentrations, separate
and anhydrous (DMSO) were
10 mg/plate unless lim-
ited. The high dose was self-
assessing range-finding
in the presence and absence
of TCDD available
prior to the first experiment.

Tests were repeated at least
in triplicate of the origi-
nal performed in triplicate
with control chemicals (TA1535
TA1535 and TA100, x-
anthene, 2-aminofluorene,
2-aminonaphthalene, and
2-aminanthracene) in the pres-
ence of histidine. Concurrent solv-
ent controls in all experiments
were as a reproducible, dose-
independent, dose-
independent, dose-
independent. According to
the data and the data were

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preparations of 2,4-D (isooctyl ester) and 2,4,5-T (isooctyl ester) (Table 1).

METHODS

All chemicals were tested in a preincubation modification of the Ames *Salmonella* plate incorporation assay (Ames *et al.*, 1975; Maron and Ames, 1983); details of the protocol are described elsewhere (Haworth *et al.*, 1983). Briefly, fresh overnight cultures of *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 (obtained from Professor B. N. Ames, Berkeley, Calif.) were used with the 9000g supernatant fraction of liver homogenates (S9 fraction) from Aroclor 1254-induced male Sprague-Dawley rats or male Syrian hamsters. About 1×10^8 bacteria were mixed with 0.5 ml of either 0.1 M (pH 7.4) sodium phosphate buffer or S9 mix (containing 10% S9), and 50 or 100 μ l of the test chemical or solvent, in each of three tubes. This mixture was incubated at 37°C for 20 min, after which time 2 ml of molten top agar containing a trace amount of biotin and a growth-limiting amount of histidine was added. The mixture was then poured onto minimal agar plates and incubated at 37°C for 48 hr, after which time histidine-revertant colonies were counted.

Table 1 lists the chemicals tested, source, purity, and grade. The testing laboratories received and tested all chemicals as coded aliquots. Solubility information, storage conditions, and safe handling procedures accompanied each chemical.

All chemicals initially were tested in triplicate plates at five dose concentrations, separated by half-log intervals. Dimethyl sulfoxide (DMSO) was the solvent. The high dose was 10 mg/plate unless limited by solubility and/or toxicity. The high dose was selected based on the results of a preliminary range-finding study conducted with TA100 in the presence and absence of S9. Because of the limited amount of TCDD available, it was tested up to 100 μ g/plate in the first experiment and 1000 μ g/plate in the second.

All tests were repeated at least once, at least 1 week following completion of the original test. The repeat test was also performed in triplicate. The following direct-acting positive control chemicals dissolved in DMSO were used: for TA1535 and TA100, sodium azide; for TA98, 4-nitro-*o*-phenylenediamine; and for TA1537, 9-aminoacridine. 2-Aminoanthracene was used as the positive control for all strains in the presence of the hamster and rat liver S9 mixes. Concurrent solvent and positive controls were included in all experiments. A mutagenic response was defined as a reproducible, dose-related increase in the number of histidine-independent colonies over the spontaneous incidence; there was no requirement for a specific magnitude of increase. Decoding took place after all testing was completed and the data were evaluated.

RESULTS

Nine chemicals were tested in only one laboratory each, while 2,4-D and 2,4,5-T were each tested in two laboratories. No mutagenic responses to the test chemicals were observed under any of the assay conditions either in the original or the repeat tests; however, varying degrees of toxicity were observed with the different chemicals. All positive controls gave the expected responses. Differences observed in levels of toxic responses by the different bacterial strains can be explained by the fact that the strains are not isogenic. There is always the possibility that weak mutagenic responses could be obscured by cell toxicity. While this possibility has been considered, there is no available evidence which demonstrates that some chemicals are mutagenic on the plate test only in the presence of cell toxicity, and that this toxicity is sufficient to mask the mutagenic response. Table 2 presents the test results for each chemical. Since each chemical was tested at least twice, with the dose range adjusted for the second test based on the results of the first test, Table 2 presents only the data from the final test of each chemical.

DISCUSSION

Epidemiological studies have been performed in an attempt to establish a correlation between exposure to chlorinated phenoxy herbicides and carcinogenesis in humans. Special attention was given to 2,4,5-T and 2,4-D, major constituents as *n*-butyl esters in Agent Orange, and contaminants of these products. TCDD, the most biologically active and most widely studied dioxin, is of particular interest because of its carcinogenicity. In general, the epidemiological data on the phenoxy herbicides are considered inconclusive because of small sample sizes, limited exposure levels, and/or design of the study (Coggon and Acheson, 1982).

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TABLE 2
TESTING RESULTS

Dose ^a	TA100			TA1535			TA1537			TA98		
	NA ^b	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
2,4-D (2,4-dichlorophenoxyacetic acid) [DMSO] ^c [CWR] ^d												
0.0	115 ± 14.3 ^e	136 ± 6.1	138 ± 6.9	7 ± 3.2	8 ± 0.0	9 ± 2.0	4 ± 0.9	6 ± 0.6	5 ± 1.5	16 ± 1.2	21 ± 3.2	21 ± 3.5
33.0	92 ± 8.8			6 ± 3.0	8 ± 1.7	11 ± 2.1	3 ± 0.3			15 ± 1.5		
100.0	107 ± 8.0	143 ± 6.7	129 ± 6.2	5 ± 0.9	7 ± 2.6	9 ± 0.7	4 ± 0.7	6 ± 2.0	6 ± 0.9	15 ± 2.7	17 ± 2.3	18 ± 2.6
333.0	102 ± 4.7	127 ± 3.2	124 ± 3.5	8 ± 3.9	7 ± 1.0	7 ± 0.9	3 ± 1.2	7 ± 1.7	6 ± 1.5	17 ± 2.1	21 ± 4.1	20 ± 1.9
1,000.0	113 ± 11.3	135 ± 4.1	117 ± 6.0	4 ± 1.5	7 ± 0.9	5 ± 1.2	1 ± 0.9	9 ± 2.2	6 ± 0.3	17 ± 1.5	21 ± 2.8	15 ± 1.9
3,333.0	79 ± 4.9	143 ± 6.5	115 ± 9.9	4 ± 2.0	1	3 ± 0.9	1 ± 0.7	6 ± 0.9	5 ± 0.9	13 ± 1.5	22 ± 3.0	13 ± 2.0
10,000.0		55 ± 14.0 ^p	48 ± 9.2 ^p					1 ± 1.0 ^p	1 ± 0.7 ^p		5 ± 2.5 ^p	5 ± 2.6 ^p
POS ^e	646 ± 13.4	1453 ± 277.3	1898 ± 61.8	1031 ± 18.5	158 ± 2.2	123 ± 6.1	1274 ± 254.2	130 ± 24.1	157 ± 23.1	331 ± 19.0	694 ± 84.6	2657 ± 268.2
2,4-D (2,4-dichlorophenoxyacetic acid) [DMSO] [SRI]												
0.0	107 ± 4.2	94 ± 3.7	78 ± 5.8	13 ± 3.0	7 ± 1.3	7 ± 2.7	3 ± 0.9	6 ± 1.5	5 ± 1.2	20 ± 2.7	30 ± 1.7	31 ± 0.9
100.0	88 ± 6.2	96 ± 9.6	78 ± 8.2	7 ± 2.6	6 ± 0.7	3 ± 0.9	3 ± 0.3	4 ± 0.0	7 ± 3.8	23 ± 3.1	29 ± 3.4	25 ± 4.0
333.3	78 ± 5.3	91 ± 4.1	71 ± 4.1	5 ± 1.5	6 ± 0.7	7 ± 0.6	4 ± 0.6	7 ± 0.3	5 ± 1.0	23 ± 4.6	28 ± 1.8	27 ± 0.9
1000.0	80 ± 5.2	81 ± 3.6	68 ± 2.3	9 ± 1.7	5 ± 0.7	6 ± 0.7	3 ± 0.3	7 ± 1.0	3 ± 0.3	21 ± 2.0	24 ± 3.7	22 ± 2.2
3,333.3	23 ± 3.8	91 ± 5.3	81 ± 5.0	3 ± 0.9	4 ± 0.0	5 ± 0.7	4 ± 1.5	5 ± 1.2	5 ± 0.6	8 ± 0.9	28 ± 4.6	28 ± 0.9
10,000.0	1 ± 0.5 _s	0 ± 0.0 _s	0 ± 0.0 _s	0 ± 0.0 _s	0 ± 0.0 _s	1 ± 0.7 _s	0 ± 0.0 _s	1 ± 0.7 _s	0 ± 0.0 _s	3 ± 2.5 _s	3 ± 3.0 _s	7 ± 1.7 _s
POS	350 ± 23.3	807 ± 30.1	1991 ± 73.8	353 ± 18.9	288 ± 21.2	413 ± 24.4	164 ± 42.6	189 ± 15.9	397 ± 37.6	784 ± 38.8	534 ± 14.2	1664 ± 90.1
2,4-D, n-butyl ester [DMSO] [EGG]												
0.0	141 ± 7.7	139 ± 1.2	152 ± 14.9	30 ± 2.1	11 ± 2.0	17 ± 0.3	7 ± 0.9	5 ± 2.2	11 ± 1.5	14 ± 1.9	32 ± 1.8	34 ± 1.7
0.3	138 ± 5.9			32 ± 1.7			5 ± 0.9			22 ± 2.0		
1.0	131 ± 1.5			33 ± 5.0			8 ± 2.3			17 ± 2.0		
3.3	143 ± 7.0			38 ± 3.3			6 ± 0.7			21 ± 2.9		
10.0	151 ± 8.9			35 ± 1.5			7 ± 1.2			16 ± 3.2		
33.0	128 ± 3.3 _s			23 ± 1.2 _s			7 ± 1.8 _s			16 ± 1.7		
100.0		133 ± 6.1	165 ± 5.8		14 ± 0.6	11 ± 2.3		5 ± 1.8	7 ± 2.2		30 ± 1.8	35 ± 2.5
333.0		132 ± 3.2	149 ± 13.1		15 ± 2.0	19 ± 1.3		7 ± 1.7	8 ± 1.8		37 ± 1.7	29 ± 1.0
1,000.0		153 ± 12.6	168 ± 6.4		13 ± 0.9	15 ± 2.9		8 ± 0.3	8 ± 0.9		29 ± 0.6	30 ± 3.2
3,333.0		156 ± 1.0	180 ± 2.1		16 ± 0.9	12 ± 1.5		6 ± 1.0	11 ± 2.6		28 ± 3.0	29 ± 3.8
10,000.0		150 ± 3.5	161 ± 2.9		11 ± 0.3	14 ± 4.0		4 ± 0.6	11 ± 3.2		26 ± 1.2	32 ± 2.7
POS	1259 ± 37.3	1032 ± 14.6	1001 ± 24.1	937 ± 31.8	65 ± 1.5	60 ± 3.8	127 ± 45.5	72 ± 7.3	79 ± 1.3	1567 ± 77.3	861 ± 24.8	890 ± 20.3

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DOI: 10.1584.13

2,4-D, isooctyl ester (Ded-weed, LV-69) [DMSO] [SRI]

0.0	92 ± 12.6	123 ± 4.9	98 ± 6.7	20 ± 4.4	8 ± 1.7	10 ± 1.7	5 ± 1.7	6 ± 1.2	8 ± 1.3	34 ± 4.5	43 ± 1.0	33 ± 2.3
10.0	103 ± 3.8		107 ± 11.0	16 ± 0.9								
33.3	80 ± 13.3	94 ± 11.3	86 ± 11.3	23 ± 4.4	17 ± 0.9	15 ± 1.5	5 ± 0.9		7 ± 1.2	33 ± 2.3		19 ± 2.9
100.0	90 ± 4.2	98 ± 2.8	81 ± 5.2	19 ± 4.2	12 ± 1.8	10 ± 2.2	4 ± 1.5	6 ± 0.3	6 ± 0.9	26 ± 2.0	43 ± 6.4	13 ± 0.3
333.3	82 ± 11.4	94 ± 5.0	83 ± 6.4	20 ± 3.2	13 ± 3.0	8 ± 0.0	5 ± 1.0	6 ± 1.5	7 ± 0.3	27 ± 6.4	44 ± 2.4	12 ± 0.3
1,000.0	61 ± 10.8 _s	80 ± 3.1	52 ± 6.2 _s	24 ± 3.7 _s	10 ± 1.4	11 ± 2.0		7 ± 1.2	6 ± 0.6	24 ± 6.3	38 ± 4.0	11 ± 2.1

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MUTAGENICITY OF AGENT ORANGE COMPONENTS

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2,4-D, isooctyl ester (Ded-wed, LV-60) [DMSO] [SR1]

POS	1259 ± 37.3	1032 ± 14.6	1001 ± 24.1	937 ± 31.8	65 ± 1.5	60 ± 3.8	127 ± 45.5	79 ± 1.3	1567 ± 77.3	RM1 ± 24.8	RM2 ± 20.3
0.0	92 ± 12.6	123 ± 4.9	98 ± 6.7	20 ± 4.4	8 ± 1.7	10 ± 1.7	5 ± 1.7	6 ± 1.2	8 ± 1.3	34 ± 4.5	43 ± 1.0
10.0	101 ± 3.8	16 ± 0.9	107 ± 11.0	16 ± 0.9	15 ± 1.5	15 ± 1.5	5 ± 0.9	7 ± 1.2	7 ± 1.2	31 ± 2.3	33 ± 2.3
33.3	30 ± 13.3	94 ± 11.3	86 ± 11.3	23 ± 4.4	17 ± 0.9	13 ± 0.3	6 ± 0.6	6 ± 0.3	6 ± 0.9	26 ± 2.0	43 ± 6.4
100.0	90 ± 4.2	98 ± 2.8	81 ± 5.2	19 ± 4.2	12 ± 1.8	10 ± 2.2	4 ± 1.5	6 ± 1.5	7 ± 0.3	27 ± 6.4	44 ± 2.4
333.3	82 ± 11.4	94 ± 5.0	83 ± 6.4	20 ± 3.2	13 ± 3.0	8 ± 0.0	5 ± 1.0	7 ± 1.2	6 ± 0.6	24 ± 6.3	38 ± 4.0
1,000.0	61 ± 10.86	80 ± 3.1	52 ± 6.26	24 ± 3.76	10 ± 1.5	14 ± 2.9	3 ± 0.3	4 ± 1.56	22 ± 2.76	31 ± 3.0	15 ± 0.9
3,333.3	56 ± 4.46	56 ± 4.46	7 ± 2.46	7 ± 2.46	7 ± 2.46	7 ± 2.46	0 ± 0.36	0 ± 0.36	0 ± 0.36	29 ± 4.6	29 ± 4.6

Dibenzofuran [DMSO] [CWR]

POS	416 ± 11.3	349 ± 71.3	2115 ± 14.6	346 ± 14.4	167 ± 4.9	266 ± 9.5	847 ± 54.3	219 ± 24.6	411 ± 10.3	671 ± 57.5	365 ± 22.9	1371 ± 3.8
0.00	116 ± 19.3	148 ± 20.5	152 ± 5.5	10 ± 1.2	11 ± 0.7	14 ± 2.0	8 ± 1.2	14 ± 1.3	18 ± 2.6	19 ± 2.1	27 ± 1.8	27 ± 0.5
0.10	91 ± 14.1	116 ± 13.6	141 ± 4.3	10 ± 2.6	11 ± 1.5	16 ± 2.2	11 ± 0.9	17 ± 2.6	15 ± 1.9	20 ± 6.0	28 ± 3.3	26 ± 0.3
0.33	121 ± 5.7	153 ± 12.9	141 ± 10.7	13 ± 3.8	12 ± 1.5	15 ± 3.2	4 ± 1.7	17 ± 2.0	17 ± 1.3	27 ± 4.5	31 ± 0.9	27 ± 1.8
1.00	126 ± 3.5	145 ± 9.5	136 ± 13.4	8 ± 1.2	15 ± 2.3	10 ± 0.9	8 ± 2.3	17 ± 0.7	15 ± 1.5	23 ± 3.1	26 ± 7.5	18 ± 5.5
3.30	116 ± 4.9	147 ± 7.8	142 ± 7.2	11 ± 2.8	11 ± 2.3	16 ± 4.5	10 ± 1.9	14 ± 1.2	16 ± 2.5	19 ± 3.7	29 ± 2.8	30 ± 3.2
10.00	94 ± 2.4	121 ± 6.4	146 ± 6.3	9 ± 2.9	8 ± 0.7	13 ± 3.2	9 ± 1.5	20 ± 0.6	16 ± 3.5	17 ± 4.3	29 ± 0.6	29 ± 5.0

2,7-Dichlorodibenzo-p-dioxin (DCDD) [DMSO] [SR1]

POS	867 ± 19.3	1963 ± 146.2	2180 ± 21.7	921 ± 25.5	228 ± 38.1	317 ± 11.5	682 ± 7.6	375 ± 10.3	393 ± 6.7	512 ± 60.2	1772 ± 14.5	1619 ± 44.2
0.0	103 ± 3.5	105 ± 4.7	106 ± 3.1	27 ± 3.2	10 ± 1.9	8 ± 1.9	7 ± 0.9	6 ± 1.7	5 ± 0.7	18 ± 0.7	27 ± 2.1	34 ± 3.7
10.0	117 ± 6.8	127 ± 10.7	106 ± 3.1	19 ± 1.7	4 ± 1.5	5 ± 1.9	5 ± 0.3	7 ± 0.7	7 ± 0.7	16 ± 1.2	27 ± 4.1	26 ± 4.4
33.0	106 ± 3.1	118 ± 10.0	118 ± 9.5	18 ± 1.2	5 ± 1.9	9 ± 2.4	4 ± 0.9	7 ± 1.5	6 ± 0.6	13 ± 2.5	24 ± 3.6	24 ± 3.8
100.0	110 ± 4.7p	103 ± 11.4	80 ± 6.7	21 ± 3.8p	3 ± 0.9	7 ± 1.2	7 ± 1.2p	13 ± 1.5	6 ± 0.9	16 ± 2.4	27 ± 6.9	24 ± 3.8
333.0	65 ± 13.46	66 ± 9.5p	36 ± 14.9p	19 ± 0.9p	3 ± 0.5p	2 ± 0.0p	5 ± 0.96	13 ± 2.7p	5 ± 1.7p	15 ± 1.9p	26 ± 3.7p	30 ± 5.9p
1,000.0	59 ± 7.06	34 ± 8.8p	34 ± 8.8p	3 ± 0.56	3 ± 0.56	3 ± 0.7p	12 ± 1.24	10 ± 1.74	4 ± 2.4p	14 ± 2.36	24 ± 3.36	17 ± 1.0p
3,333.0	55 ± 4.26	55 ± 4.26	55 ± 4.26	12 ± 1.24	12 ± 1.24	12 ± 1.24	12 ± 1.24	11 ± 5.16	11 ± 5.16	19 ± 2.84	19 ± 2.84	19 ± 2.84

DMA 4 (2,4-D)dimethylamine salt [DMSO] [SR1]

POS	260 ± 14.2	354 ± 31.1	1029 ± 74.8	274 ± 13.1	155 ± 9.3	489 ± 46.3	145 ± 22.0	159 ± 8.0	508 ± 24.3	1084 ± 5.0	403 ± 23.4	2031 ± 79.1
0.0	101 ± 5.2	115 ± 11.3	92 ± 5.2	14 ± 3.3	9 ± 2.0	7 ± 0.0	11 ± 2.3	11 ± 2.5	10 ± 1.8	21 ± 3.1	29 ± 4.9	24 ± 2.9
100.0	102 ± 2.8	102 ± 18.6	108 ± 11.0	12 ± 1.5	5 ± 1.7	6 ± 0.0	8 ± 2.3	14 ± 0.3	16 ± 1.9	20 ± 0.3	36 ± 3.2	27 ± 1.8
333.3	90 ± 4.7	107 ± 11.6	112 ± 7.5	14 ± 1.0	5 ± 0.6	7 ± 1.2	9 ± 2.1	19 ± 3.2	15 ± 1.5	19 ± 2.5	39 ± 5.0	28 ± 1.5
1,000.0	96 ± 3.3	104 ± 9.3	103 ± 0.9	11 ± 3.1	3 ± 0.6	9 ± 2.3	6 ± 0.9	15 ± 1.7	11 ± 2.1	17 ± 1.0	38 ± 0.3	27 ± 1.5
3,333.3	79 ± 6.4	104 ± 4.2	101 ± 14.7	9 ± 2.0	6 ± 1.8	5 ± 1.3	3 ± 1.5	9 ± 2.6	6 ± 2.1	17 ± 2.6	27 ± 1.8	33 ± 5.9
10,000.0	24 ± 1.56	67 ± 13.46	96 ± 5.0	2 ± 1.0	4 ± 1.2	6 ± 1.2	2 ± 0.3	1 ± 0.74	5 ± 1.5	8 ± 2.6	22 ± 7.0	23 ± 1.2

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TABLE 2—Continued

Dose*	TA100			TA1535			TA1537			TA98		
	NA*	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
2,4,5-T (2,4,5-Trichlorophenoxyacetic acid) [DMSO] [CWR]												
0.0	86 ± 17.6	91 ± 17.4	122 ± 15.9	6 ± 0.7	10 ± 1.5	15 ± 3.1	8 ± 2.3	11 ± 1.5	15 ± 1.5	21 ± 2.0	26 ± 2.6	26 ± 0.7
100.0	87 ± 2.5	126 ± 10.1	112 ± 8.5	5 ± 0.6	13 ± 2.1	12 ± 3.2	11 ± 0.7	14 ± 1.9	11 ± 0.7	18 ± 8.3	33 ± 3.1	
333.3	100 ± 1.5	116 ± 10.1	123 ± 6.8	9 ± 0.3	10 ± 0.6	8 ± 1.2	15 ± 0.9	11 ± 1.8	6 ± 0.3	25 ± 3.5	23 ± 2.4	
1000.0	86 ± 2.5	112 ± 8.2	107 ± 6.8	4 ± 0.3	14 ± 1.0	11 ± 1.3	7 ± 0.6	14 ± 0.6	12 ± 0.3	24 ± 0.3	29 ± 1.2	22 ± 0.9
3333.0	92 ± 3.4	111 ± 9.0	111 ± 9.8	4 ± 0.3	12 ± 1.8	13 ± 3.1	7 ± 0.3	16 ± 2.1	13 ± 1.8	24 ± 2.3	24 ± 0.6	25 ± 4.3
10000.0	70 ± 3.2	72 ± 5.4	1	2 ± 0.7	10 ± 1.0	7 ± 1.0	4 ± 0.9	12 ± 0.0	1	24 ± 2.0	19 ± 2.9	18 ± 2.3
POS	886 ± 44.0	1741 ± 146.1	1834 ± 79.6	417 ± 48.4	48 ± 5.6	72 ± 7.2	100 ± 31.1	147 ± 23.2	138 ± 31.5	361 ± 14.0	821 ± 62.6	1694 ± 50.8
2,4,5-T (2,4,5-Trichlorophenoxyacetic acid) [DMSO] [SRH]												
0.0	107 ± 4.2	94 ± 3.7	78 ± 5.8	13 ± 3.0	7 ± 1.3	7 ± 2.7	3 ± 0.9	6 ± 1.5	5 ± 1.2	20 ± 2.7	30 ± 1.7	31 ± 0.9
100.0	90 ± 6.2	92 ± 3.8	89 ± 4.9	4 ± 2.3	6 ± 1.3	5 ± 0.6	5 ± 0.3	7 ± 1.2	6 ± 0.0	31 ± 3.2	32 ± 2.5	32 ± 2.6
333.3	83 ± 9.4	95 ± 8.5	84 ± 2.8	6 ± 1.5	6 ± 1.2	6 ± 0.6	4 ± 0.3	4 ± 0.9	4 ± 0.7	26 ± 3.9	34 ± 6.6	37 ± 1.7
1000.0	92 ± 2.3	91 ± 4.0	71 ± 8.7	10 ± 3.0	5 ± 0.7	6 ± 1.2	3 ± 0.3	4 ± 0.3	4 ± 1.2	26 ± 0.7	28 ± 1.2	34 ± 2.1
3333.3	69 ± 10.9	55 ± 5.9	38 ± 2.9	9 ± 1.8	3 ± 0.0	4 ± 0.3	5 ± 1.2	3 ± 1.2	4 ± 1.0	19 ± 0.6	12 ± 1.8	26 ± 4.3
10000.0	0 ± 0.0	0 ± 0.0	2 ± 2.3	1 ± 0.7	1 ± 1.3	1 ± 0.7	1 ± 1.0	0 ± 0.0	0 ± 0.0	3 ± 1.7	3 ± 1.7	13 ± 7.8
POS	350 ± 23.3	807 ± 30.1	1791 ± 73.8	353 ± 18.9	288 ± 21.2	413 ± 24.4	164 ± 42.6	189 ± 15.9	197 ± 37.6	784 ± 38.8	584 ± 14.2	1664 ± 90.1
2,4,5-T, n-butyl ester [DMSO] [ECG]												
0.0	141 ± 6.8	148 ± 7.1	148 ± 8.2	38 ± 4.2	20 ± 1.3	18 ± 2.8	9 ± 2.6	12 ± 1.2	12 ± 2.9	18 ± 0.9	27 ± 1.7	31 ± 0.9
100.0	140 ± 3.5	140 ± 7.3	138 ± 4.6	31 ± 2.4	23 ± 0.3	18 ± 1.9	6 ± 0.9	9 ± 1.2	7 ± 2.0	21 ± 0.9	31 ± 2.7	34 ± 3.8
333.0	135 ± 5.0	118 ± 7.2	117 ± 10.6	29 ± 3.2	20 ± 3.2	17 ± 1.8	5 ± 0.3	10 ± 1.2	10 ± 0.0	18 ± 3.2	31 ± 2.3	31 ± 3.8
1000.0	142 ± 1.5	123 ± 4.1	130 ± 5.0	29 ± 4.0	16 ± 2.5	15 ± 0.9	4 ± 2.0	13 ± 2.5	9 ± 1.5	20 ± 4.0	35 ± 1.7	35 ± 2.6
3333.0	140 ± 4.7	112 ± 14.9	116 ± 7.4	33 ± 3.4	17 ± 3.1	16 ± 2.7	4 ± 1.5	11 ± 1.5	6 ± 0.7	16 ± 3.2	25 ± 3.5	27 ± 1.2
10000.0	141 ± 5.5	95 ± 3.8	94 ± 1.0	31 ± 3.2	12 ± 3.2	11 ± 1.2	6 ± 2.0	6 ± 1.8	6 ± 1.2	19 ± 3.2	18 ± 0.9	27 ± 2.9
POS	1168 ± 35.2	1296 ± 46.2	1264 ± 50.8	912 ± 38.7	82 ± 5.0	89 ± 4.7	408 ± 3.5	97 ± 10.8	119 ± 6.0	1385 ± 7.2	962 ± 22.6	1124 ± 33.1

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2,4,5-T, isobutyl ester [DMSO] [ECG]

0.0	165 ± 7.1	149 ± 7.8	175 ± 3.5	37 ± 2.1	17 ± 0.9	17 ± 2.0	6 ± 1.7	7 ± 1.7	8 ± 3.0	21 ± 3.2	34 ± 1.0	31 ± 5.2
100.0	125 ± 11.6	144 ± 7.1	164 ± 2.7	34 ± 1.3	16 ± 0.7	13 ± 3.1	5 ± 0.6	7 ± 0.9	8 ± 2.7	19 ± 2.3	27 ± 1.0	16 ± 1.7
333.0	134 ± 5.0	141 ± 2.6	162 ± 4.9	28 ± 1.7	11 ± 0.6	11 ± 3.1	5 ± 1.0	6 ± 1.1	4 ± 1.1			
1000.0	143 ± 11.9	107 ± 13.0	182 ± 6.0	11 ± 1.1								

2,4,5-T, isobutyl ester [DMSO] [EGG]

0.0	168 ± 7.1	149 ± 7.8	175 ± 3.5	37 ± 2.1	17 ± 0.9	17 ± 2.0	6 ± 1.7	7 ± 1.7	8 ± 3.0	21 ± 3.2	34 ± 1.0	31 ± 5.2
100.0	125 ± 11.6	144 ± 7.1	164 ± 2.7	34 ± 1.3	16 ± 0.7	13 ± 2.1	5 ± 0.6	7 ± 0.9	8 ± 2.7	19 ± 2.3	27 ± 4.9	38 ± 5.5
333.0	136 ± 5.0	141 ± 2.6	162 ± 4.9	28 ± 1.7	11 ± 2.6	12 ± 2.1	5 ± 0.0	6 ± 0.9	9 ± 0.3	19 ± 3.5	27 ± 1.9	40 ± 2.4
1,000.0	145 ± 11.9	107 ± 12.0	152 ± 6.0	31 ± 5.2	10 ± 0.9	11 ± 2.4	6 ± 1.0	6 ± 1.0	9 ± 1.8	17 ± 1.5	26 ± 2.0	34 ± 1.9
3,333.0	144 ± 5.7	100 ± 4.3	144 ± 10.7	31 ± 4.0	14 ± 1.9	14 ± 1.2	3 ± 0.7	8 ± 0.3	7 ± 2.0	21 ± 3.1	25 ± 0.9	25 ± 4.5
10,000.0	138 ± 9.0 ^a	102 ± 5.0 ^a	115 ± 9.7 ^a	27 ± 2.1	10 ± 0.6	10 ± 0.9 ^a	4 ± 0.6	3 ± 1.0	3 ± 1.8	18 ± 1.7	14 ± 0.0	25 ± 2.4
POS	1381 ± 17.2	1081 ± 28.9	1440 ± 101.1	966 ± 19.1	81 ± 1.3	90 ± 1.2	440 ± 98.8	94 ± 6.1	134 ± 1.2	1480 ± 28.6	1028 ± 15.0	1405 ± 54.9

2,4,5-T, isooctylester (Ded-weed LV-4T) [DMSO] [CWR]

0.0	88 ± 12.6	140 ± 7.8	154 ± 4.2	9 ± 1.2	10 ± 2.5	10 ± 1.0	5 ± 0.3	6 ± 1.0	11 ± 1.7	15 ± 1.2	24 ± 1.2	22 ± 1.5
3.3	93 ± 4.9	156 ± 8.0	130 ± 10.4	10 ± 2.0	13 ± 0.3	11 ± 1.9	6 ± 0.3	12 ± 0.9	8 ± 1.9	12 ± 1.5	21 ± 3.0	23 ± 3.5
10.0	114 ± 7.2	152 ± 14.0	134 ± 9.1	13 ± 1.2	14 ± 1.5	11 ± 2.0	5 ± 0.6	9 ± 2.2	10 ± 0.5	13 ± 0.9	21 ± 2.9	22 ± 3.5
33.0	90 ± 5.2	166 ± 6.5	128 ± 10.7	11 ± 1.5	15 ± 1.8	13 ± 3.2	5 ± 0.9	9 ± 1.3	10 ± 0.3	16 ± 3.2	23 ± 2.5	21 ± 3.8
100.0	100 ± 1.3	139 ± 11.1	122 ± 14.9	9 ± 1.2	15 ± 1.2	10 ± 0.7	6 ± 0.9	10 ± 2.1	10 ± 1.7	12 ± 1.7	24 ± 1.0	21 ± 1.5
333.0	102 ± 1.5	135 ± 14.2	158 ± 7.0	11 ± 1.2	15 ± 2.2	12 ± 2.0	5 ± 0.6	10 ± 2.3	8 ± 1.5	13 ± 0.9	23 ± 0.7	22 ± 2.3
POS	621 ± 46.4	714 ± 29.8	1093 ± 28.0	686 ± 93.4	92 ± 20.3	120 ± 9.3	137 ± 39.5	44 ± 0.3	90 ± 6.4	343 ± 32.6	572 ± 121.4	803 ± 107.0

TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin) [DMSO] [SRI]

0.0	141 ± 10.1	154 ± 9.0	155 ± 12.8	13 ± 1.3	8 ± 1.0	5 ± 1.0	5 ± 1.3	9 ± 1.7	5 ± 0.0	19 ± 3.5	24 ± 2.1	21 ± 5.2
10.0	170 ± 14.9	188 ± 7.3	117 ± 12.3	15 ± 3.5	11 ± 2.5	8 ± 0.7	5 ± 0.3	7 ± 0.9	5 ± 1.5	22 ± 3.3	27 ± 3.5	23 ± 3.6
33.0	178 ± 8.6	172 ± 2.5	117 ± 7.5	17 ± 0.6	11 ± 3.7	11 ± 1.2	6 ± 0.0	11 ± 1.0	4 ± 0.6	20 ± 4.6	29 ± 2.7	28 ± 2.0
100.0	162 ± 5.7	195 ± 5.0	111 ± 0.6	17 ± 0.7	8 ± 3.4	8 ± 3.4	7 ± 0.6	4 ± 1.2	6 ± 3.0	18 ± 3.7	26 ± 5.8	24 ± 3.5
333.0	176 ± 0.9	154 ± 5.5	124 ± 12.4	18 ± 0.6	7 ± 1.2	9 ± 3.2	5 ± 0.7	8 ± 1.0	8 ± 0.3	18 ± 3.1	21 ± 1.5	23 ± 2.2
1,000.0	149 ± 7.8	177 ± 6.8	116 ± 9.4	18 ± 3.9	8 ± 2.0	8 ± 2.3	7 ± 2.0	7 ± 1.2	7 ± 1.5	18 ± 3.1	26 ± 0.6	18 ± 2.8
POS	374 ± 21.2	853 ± 45.0	1527 ± 66.5	308 ± 17.0	121 ± 14.4	242 ± 12.2	122 ± 11.1	164 ± 4.2	413 ± 22.8	725 ± 32.2	384 ± 22.8	1204 ± 10.8

^a µg/plate.^b NA, not activated; RLI, Aroclor 1254-induced rat liver S9; HLI, Aroclor 1254-induced Syrian hamster liver S9.^c DMSO, dimethyl sulfoxide (solvent).^d CWR, Case Western Reserve University; EGG, EG&G Mason Research Institute; SRI, SRI International.^e His⁺ revertants: mean ± SEM (three plates).^f p, precipitate present on plates; s, slight clearing of background lawn; t, complete clearing of background lawn; colonies not counted.^g POS, positive control. TA100 and TA1535, NA: sodium azide (CWR: 3.2 µg/plate; EGG: 2.5 µg/plate; SRI: 1.0 µg/plate); TA98, NA: 4-nitro-*p*-phenylenediamine (CWR: 3.3 µg/plate; EGG: 12.0 µg/plate; SRI: 5.0 µg/plate); TA1537, NA: 9-aminoacridine (CWR: 33 µg/plate; EGG: 80 µg/plate; SRI: 50 µg/plate); TA100, TA1535, TA1537, and TA98 with S9: 2-aminoanthracene (CWR, RLI and HLI: 1.0, 2.0, 2.0, and 1.0 µg/plate; EGG, RLI: 1.5 µg/plate (all strains); EGG, HLI: 0.75 µg/plate (all strains); SRI, RLI and HLI: 1.0, 2.5, 2.5, and 1.0 µg/plate).

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TCDD was carcinogenic for male and female Osborne-Mendel rats and B6C3F1 mice following administration in the diet for 2 years at dose levels of 0.01, 0.05, and 0.5 $\mu\text{g/kg/week}$ for rats and male mice, and 0.04, 0.2, and 2.0 $\mu\text{g/kg/week}$ for female mice (National Toxicology Program, 1982a). These findings confirmed and extended an earlier 2-year chronic study which produced tumors in male and female Sprague-Dawley rats fed 0.01 and 0.1 $\mu\text{g TCDD/kg/day}$ for up to 2 years (Kociba *et al.*, 1978). TCDD also was carcinogenic for female, but not for male, mice in a skin-painting study (National Toxicology Program, 1982b). DCDD, however, was not carcinogenic for Osborne-Mendel rats of either sex, or female B6C3F1 mice, but produced marginal increases in a variety of tumor types in male mice, which was considered as "suggestive of a carcinogenic effect" (National Cancer Institute, 1979). TCDD has also been reported to act as a promoter of diethylnitrosamine carcinogenesis in the livers of female rats (Pitot *et al.*, 1980), and of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine carcinogenesis of the skin of female, hairless mice (Poland *et al.*, 1982).

There appears to be disagreement on the bacterial mutagenicity of TCDD. Hussain *et al.* (1972) and Seiler (1973) reported TCDD to be mutagenic for *Salmonella* strain TA1532 without metabolic activation. Gilbert *et al.* (1980), using *Salmonella* strains TA98, TA100, TA1530, TA1532, TA1535, TA1537, TA1538, TA1950, TA1975, TA1978, and G46, and Geiger and Neal (1981), using *Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538, reported TCDD non-mutagenic both with and without metabolic activation. The report by Hussain *et al.* (1972) is difficult to evaluate since the data are presented only as mutation frequency against survival, and the positive response by Seiler (1973) was obtained in a spot test, which requires that the test chemical diffuse into the agar. Although the dose and solvent are not indicated, the reported solubility of TCDD in water (0.2×10^{-7} g/100 ml; 0.2 ppb), and in

chloroform (0.037 g/100 ml) (Crummett and Stehl, 1973) makes it unlikely that TCDD would diffuse into the agar. The studies by Gilbert *et al.* (1980) did use TA1532, although Geiger and Neal (1981), as well as our study, did not use TA1532, but instead used TA1537 which is isogenic to TA1532 and contains the *rfa* mutation (Ames *et al.*, 1975), which renders the cells more able to take up large molecules. In all probability, the doses in our and all other reported studies were beyond the range of solubility of the TCDD. This lack of solubility may be the reason for the lack of toxicity at doses up to 1000 $\mu\text{g/plate}$ in our study (Table 2) and at the maximum doses tested (2000 and 20 $\mu\text{g/plate}$) in the other two negative studies; Hussain *et al.* (1972), however, reported a mutagenic response at survival rates between approximately 30 and 1%.

The lack of agreement between the bacterial mutagenicity and carcinogenicity of TCDD could be the result of a number of factors: the chemical was too insoluble to be taken up by the *Salmonella* (addressed above), the S9 preparations used were not capable of metabolizing TCDD to a DNA-binding moiety, DNA-bound TCDD does not induce mutation in these *Salmonella* strains, or TCDD cannot form DNA-binding moieties under the conditions of the *Salmonella* assay.

There is evidence that TCDD is metabolized by rodents, *in vivo* and *in vitro*, via an NADPH-dependent cytochrome P-450-dependent mixed-function oxygenase (Gasiewicz *et al.*, 1983), albeit at a slow rate, but no metabolites have been identified. This information suggests that either the S9 mix used in *Salmonella* mutagenicity experiments is not the correct composition to support TCDD metabolism, or is not used under the optimum conditions (i.e., TCDD concentration, liver enzyme activity, incubation time, etc.) for metabolizing TCDD. However, there is no evidence as to whether the metabolites of TCDD represent toxic or detoxified products (Gasiewicz *et al.*, 1983).

There are no studies that demonstrate the presence of TCDD in the environment. It probably induces indirect mechanisms to catalyze the induction of enzymes (Poland *et al.*, 1982) to mediate other effects.

The lack of data on *monella* with TCDD is consistent with the literature (Shirai *et al.*, 1977; Probst *et al.*, 1983; Schoeny, 1983). Published mutagenicity data for 2,4-D, 2,4,5-T, and 2,4,6-T.

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There are no studies reported which demonstrate the presence of DNA-bound TCDD. If TCDD cannot interact with DNA directly, it probably induces cancer in rodents by an indirect mechanism. TCDD receptors which catalyze the induction of mixed-function oxidases (Poland and Knutson, 1982) also may mediate other TCDD-related effects.

The lack of mutagenic responses in *Salmonella* with 2,4-D, 2,4,5-T, and dibenzofuran is consistent with the results reported in the literature (Shirasu *et al.*, 1976; Zetterberg *et al.*, 1977; Probst *et al.*, 1981; Moriya *et al.*, 1983; Schoeny, 1982). There are no prior published mutagenicity data on the esters of 2,4-D, 2,4,5-T, or the dimethylamine salt of 2,4-D.

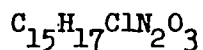
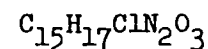
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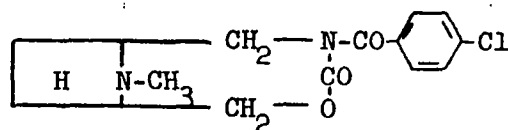
18225

3-OXA-5,10-DIAZABICYCLO(5.2.1)DECAN-4-ONE:
5-(p-CHLOROBENZOYL)-10-METHYL-,



K-92,666

3-OXA-5,10-DIAZABICYCLO(5.2.1)DECAN-4-ONE:
5-(p-CHLOROBENZOYL)-10-METHYL-,



ALC-LP 1791

L# 7247

Sensi DCR

DLSO

Chim. II

3/22/68

2 g. Midland

Lit. Ref. New MP: 95-96°C. White crystals
Est. Sol. <1% water; 25% ethanol

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H. 8-58

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NDI for Distribution
(100)

CHLOROPHENOXY HERBICIDES

Drinking Water Limits

0.1 mg/l

There is an urgent need to control aquatic weeds in certain lakes, streams, and irrigation ditches, especially in tropical or semitropical areas. Waters to which such applications are made generally are not used directly as sources of drinking water. However, there is a possibility that some herbicide will find its way to finished water and, therefore, a need for a standard.

This section deals with three closely related compounds, 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and 2,4,5-TP or silvex² (2,4,5-trichlorophenoxypropionic acid).

Each of these compounds is available in a variety of salts and esters that may have marked differences in their practical herbicidal properties. However, in the body, the salts or esters are rapidly hydrolyzed to the corresponding acid.

Studies of the acute toxicity of the chlorinated phenoxyalkyl acids are summarized in Table 1. There is considerable variation (up to about three-fold) between the various species studied with regard to oral toxicity. It appears that the acute oral toxicity of the three compounds is of about the same magnitude. That of silvex may be somewhat less.

The subacute toxicity of the chlorinated phenoxyalkyl acids to various species of experimental animals is summarized in Table 2. Dosages of 10 mg/kg/day for about 90 days frequently lead to mild injury.

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K2372
T23.14-11

K4568
T23.14-2

K3548
T23.14-15

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In a two-year feeding study of silvex in both male and female rats, the no-adverse-effect level was found to be 53 ppm (about 2.6 mg/kg/day) of the acid equivalent. In a similar two-year study in dogs, the no-adverse-effect level was 100 ppm (about 2.6 mg/kg/day) of the acid equivalent for female beagles and 30 ppm (about 0.9 mg/kg/day) for male beagles.

Available data on the toxicity of chlorinated phenoxy-acids to man is summarized in Table 3. The entries all relate to 2,4-D. A daily oral dosage of 500 mg (about 7 mg/kg) of 2,4-D produced no apparent ill effects in a volunteer over a 21-day period (Kraus, as cited by Mitchell et al., 1946). When 2,4-D was investigated as a possible treatment for disseminated coccidioidomycosis, the patient had no side effects from 18 intravenous doses during 33 days; each of the last 12 doses in this series was 800 mg (about 15 mg/kg) or more, the last being 2000 mg (about 37 mg/kg) (Seabury, 1963). A nineteenth and final dose of 3600 mg (67 mg/kg) produced mild symptoms.

In addition to these specific data, the favorable record of use experience of 2,4-D is also pertinent. Sixty-three million pounds of 2,4-D was produced in 1965. In association with this very large production and use of 2,4-D, there have been no confirmed cases of occupational poisoning and few instances of any illness even due to ingestion.

The data given in Table 3 indicate that the acute oral dose of 2,4-D required to produce symptoms in man is probably 3000 to 4000 mg (or about 45 to 60 mg/kg). A comparison of other toxicity values

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for 2,4-D, 2,4,5-T, and silvex as listed in Table 1 and 2 indicates that the toxicity of these three compounds is of the same order of magnitude. Therefore, in the absence of any specific toxicologic data for 2,4,5-T and silvex in man, it might be estimated that the acute oral dose of 2,4,5-T and silvex required to produce symptoms in man would also be about 3000 to 4000mg (or about 45 to 60 mg/kg).

Although the chlorinated phenoxy-acids act as growth hormones in plants, they have no hormonal action in animals. However, the mechanism of their toxic action in animals is poorly understood.

Parenteral and oral administration of toxic doses of 2,4-D to experimental animals brings about a characteristic complex of signs and symptoms which has been studied particularly in dogs (Dalgaard-Mikkelsen and Poulsen, 1962; Bucher, 1946; Drill and Hiratzka, 1953; and Hill and Carlisle, 1947). After a few hours the animals display a disinclination to move. This passiveness is gradually aggravated, and a picture of myotonia develops with rigidity of the skeletal muscles and ataxia. The condition may improve transitorily with movement. In severe cases the animals show progressive apathy, depression, and muscular weakness, especially of the hindlegs, with periodic, clonic spasms, and, finally, coma. Animals killed quickly by large doses of 2,4-D are thought to die of ventricular fibrillation. It may be significant that the myotonia which is so characteristic of 2,4-D poisoning in animals has not been seen in man. The muscular signs in animals are accompanied by marked anorexia; frequently, irritation of the nose and eyes is indicated by scratching reactions. Further, bleeding from the nose and mouth may occur, as well as diarrhea with blood-stained stools. Local irritation of the alimentary

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tract often causes vomiting. However, this sign may be absent, even after oral administration to dogs. Autopsy may reveal necrotic ulcers of the oral mucosa and signs of irritation with histologically demonstrable inflammatory changes and necrosis of the small intestinal mucosa, as well as focal necrosis in the liver and degeneration of the renal tubules. Cattle given repeated oral doses of silvex have developed swellings in the parotid or submaxillary region, apparently from a local irritating effect (Palmer et al., 1964).

When given by mouth to dogs, even in fatal cases, 2,4,5-T has produced only weak signs in the forms of ataxia and stiff movements of the hindlegs.

Nonspecific hyperemia of the lungs, liver, and brain was found in the case of a man who committed suicide by ingesting a large amount of 2,4-D.

Repeated doses in animals, which may or may not eventually lead to death, produce loss of appetite, loss of weight, vomiting, depression, roughness of coat, and general tenseness and muscular weakness.

The action of ingested phenoxy-acid herbicides on muscular function, including their production of myotonia and ventricular fibrillation, which is reminiscent of that following administration of halogenated acetic acid compounds, suggests an interference with carbohydrate metabolism (Dalgaard-Mikkelsen and Poulsen, 1962). Two cases of a transitory diabetiform condition observed in spraying personnel following work with chlorinated phenoxy-acid herbicides point in the same direction. However, hyperglycemia and glycosuria could not be reproduced with certainty in rabbits; only one out of five animals responded in this way in exploratory experiments, where

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the doses ranged from 125 to 500 mg/kg and the period of administration from 6 to 50 days (Lorenzen and Lyngsøe, 1957). No effect of silvex on blood sugar levels was noted in cattle fed quantities of the herbicide sufficient to cause significant symptoms in all test animals and death in one animal (Palmer et al., 1964).

Following feeding to dairy cows, silvex was almost quantitatively excreted in urine (St. John et al., 1964). No residue of this compound was detected in milk.

The current recommendation for control of alligatorweed by silvex is application of the propylene glycol butyl ether ester at the rate of 8 pounds acid-equivalent per acre. A second application is usually made during the same summer to kill plants missed in the first application.

Assuming that the water was 45 cm (about 1.5 ft) deep, application of silvex at the rate of 8 pounds per acre would result in a concentration of 2 ppm. Since the average depth of rivers, lakes, reservoirs, and other potable water supply sources is probably considerably greater than 45 cm, the average concentration would actually be less than 2 ppm. Furthermore, although it has been shown (Cochrane et al., 1965) that the free acid persists in water for many weeks, silvex applied to a stream would be carried along by the current and would be diluted as tributaries entered the stream. Thus, anyone who received his regular drinking water from a treated stream would receive the compound in a significant concentration only for a few hours in connection with any single application. Furthermore, since only two water treatments are planned per year, the total duration of dosage would be short.

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In studies of the persistence of silvex in closed artificial aquatic environment in the laboratory, mean concentrations ranged from 0.820 ppm immediately after application to 0.046 ppm after nineteen weeks (Cochrane et al., 1965). The ester of silvex was rapidly hydrolyzed to the free acid. The authors speculated that, in addition to loss through adsorption or absorption of hydrosol or aquatic vegetation, degradation also occurred.

A field study of silvex persistence was carried out in a creek having very little water movement that had been treated at a rate of 8 pounds acid equivalent per acre (Cochrane et al., 1965 and Nicholson, unpublished data). After the first application, concentrations ranged from 0.083 ppm one day after spraying to 0.0011 ppm after 21 days. Silvex was not detectable after 35 days. After the second application, concentrations ranged from 0.019 ppm one day after spraying to 0.048 ppm after 70 days. Following the third application, concentrations decreased from 73 ppb immediately after spraying to 2 ppb after 48 days, and to 0.1 ppb after 6 months. Silvex could not be detected one year after application. Another field test was carried out in a fast-flowing stream which provided maximal opportunity for dilution and interchange of water. Residues of silvex were not detected except during the first few hours following treatment at the rate of 4 pounds of silvex acid equivalent. The maximal level found in this study was 0.00005 ppm (Nicholson, unpublished data).

These studies indicate that the 2 ppm value cited above as a maximum for silvex concentration immediately after spraying is reasonable,

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but that the compound would persist in a given location for only a short period, particularly in flowing water sources.

As calculated above, the maximal concentration of silvex in the water supply following use for aquatic weed control would be approximately 2 ppm. Based on an ingestion of 2 liters of water, the maximal daily intake per person of silvex from this source would be 4 mg per treatment (0.06 mg/kg for a 70-kg man). The toxicity studies discussed above for silvex in experimental animals, and the much more extensive data in experimental animals and/or in man for the closely related compounds 2,4-D and 2,4,5-T, indicate that this level should be safe. However, there need be no situation in which a phenoxy herbicide is applied to water that is used immediately for drinking. Dilution and decomposition will account for significant loss. Therefore the maximal acceptable level may be set safely and practically at 0.1 mg/l.

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Table 1: The Acute Toxicity of Some Chlorinated Phenoxyalkyl Acids to Experimental Animals.

Compound	Species	Sex	Route	LD ₅₀ Value (mg/kg)	Reference
2,4-D	Guinea pig	-	Oral	1000	Hill and Carlisle, 1947
	Mouse	-	Oral	375	Hill and Carlisle, 1947
	Rabbit	-	Oral	800	Hill and Carlisle, 1947
	Rat	-	Oral	666	Hill and Carlisle, 1947
	Rat	-	Oral	500	Lehman, 1951
	Rat	M	Oral	375	Rowe and Hymas, 1954
	Dog	M & F	Oral	100	Drill and Hiratzka, 1953
2,4,5-T	Rat	M	Oral	500	Rowe and Hymas, 1954
	Rat	-	Oral	300	Lehman, 1951
	Dog	M & F	Oral	100	Drill and Hiratzka, 1953
Silvex	Chick	M & F	Oral	1190	Rowe and Hymas, 1954
	Guinea pig	M	Oral	1200	Rowe and Hymas, 1954
	Mouse	F	Oral	1410	Rowe and Hymas, 1954
	Rabbit	F	Oral	819	Rowe and Hymas, 1954
	Rat	M & F	Oral	650	Rowe and Hymas, 1954
	Rat	M & F	Oral	500	Dow Chemical Co., 1964

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Table 2: Toxicity of Repeated Doses of Chlorinated Phenoxyalkyl Acids to Experimental Animals

Compound	Species	Dosage	Duration	Effect	Reference
2,4-D	Dog	10 mg/kg/day	90 days	none detected	Drill & Hiratzka, 1953
	Dog	20 mg/kg/day	90 days	3/4 died 18th to 49th day	Drill & Hiratzka, 1953
	Rat	about 15 mg/kg/day (300 ppm in diet)	112 days	none detected	Hill & Carlisle, 1947
	Rat	about 50 mg/kg/day (1000 ppm in diet)	112 days	4/5 died	Hill & Carlisle, 1947
	Rat	about 150 mg/kg/day (3000 ppm in diet)	---	5/5 died in 12 days	Hill & Carlisle, 1947
	Sheep	100 mg/kg/day	481 days	none detected	Palmer & Radeleff, 1964
2,4,5-T	Cow	250 mg/kg/day	7 days	lethal, sick after 4 days	Palmer & Radeleff, 1964
	Dog	10 mg/kg/day	90 days	none detected	Drill & Hiratzka, 1953
	Dog	20 mg/kg/day	---	4 of 4 animals died 11th to 75th day	Drill & Hiratzka, 1953
	Sheep	100 mg/kg/day	369 days	lethal	Palmer & Radeleff, 1964
	Sheep	250 mg/kg/day	7 days	lethal, sick after 3 days	Palmer & Radeleff, 1964
Silvex	Cow (yearling)	25 mg/kg/day	73 days	anorexia, dyspnea, and parotid enlargement	Palmer & Radeleff, 1964
	Cow (yearling)	50 mg/kg/day	73 days	after 28th dose, all animals developed anorexia, dyspnea, and parotid enlargement - one animal died	Palmer & Radeleff, 1964
	Cow (yearling)	100 mg/kg/day	29 days	death	Palmer & Radeleff, 1964

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Table 2: (Continued)

Compound	Species	Dosage	Duration
Silvex	Dog (female)	0.9 mg/kg/day (30 ppm in diet)	2 years
	Dog (male)	2.6 mg/kg/day (100 ppm in diet)	2 years
	Dog	13 mg/kg/day (300 ppm in diet)	89 days
	Dog	40 mg/kg/day (1000 ppm in diet)	89 days
	Rat	about 2.6 mg/kg/day (53 ppm in diet)	2 years
	Rat	about 5 mg/kg/day (100 ppm in diet)	90 days
	Rat	10 mg/kg/day (about 200 ppm in diet)	90 days
	Rat	about 15 mg/kg/day (300 ppm in diet)	90 days
	Rat	30 mg/kg/day (about 600 ppm in diet)	90 days
	Rat	about 50 mg/kg/day (1000 ppm in diet)	90 days
	Rat	100 mg/kg/day (about 2000 ppm in diet)	90 days

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Effect	Reference
No effect	Dow Chemical Co., 1965
No effect	Dow Chemical Co., 1965
none detected	Palmer & Radeleff, 1964
growth depression and liver pathology, possible anemia and increased alkaline phosphatase	Dow Chemical Co., 1964
No effect	Dow Chemical Co., 1965
liver and kidney weights increased - no other evidence of pathology	Dow Chemical Co., 1964
none detected, although liver and kidney weights apparently not determined	Dow Chemical Co., 1964
slight growth depression - pathological changes in heart, liver, and kidney	Dow Chemical Co., 1964
liver weight increased	Dow Chemical Co., 1964
some growth depression - pathological changes in heart, liver, and kidney	Dow Chemical Co., 1964
liver weight increased	Dow Chemical Co., 1964

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Table 2: (Continued)

Compound	Species	Dosage	Duration
Silvex	Rat	about 150 mg/kg/day (3000 ppm in diet)	90 days
	Rat	300 mg/kg/day (about 6000 ppm in diet)	90 days
	Rat	about 500 mg/kg/day (10,000 ppm in diet)	90 days
	Rat	600 mg/kg/day (about 12,000 ppm in diet)	90 days
	Sheep	100 mg/kg	11 doses

1000
1000
1000
1000
1000

Effect	Reference
7/20 died before 90 days - growth retardation and pathological changes in heart, liver, and kidney	Dow Chemical Co., 1964
liver weight increased and growth retarded	Dow Chemical Co., 1964
8/20 dead at 32 days - anorexia and weight loss - pathological changes in heart, liver, kidney, and testes	Dow Chemical Co., 1964
13/20 animals died - liver weight increased and growth retarded in survivors	Dow Chemical Co., 1964
death	Palmer & Radeleff, 1964

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Table 3: Summary of Available Information on Toxicity of the Chlorinated Phenoxyalkyl Acid Herbicides to Man

Compound	No. of persons	Exposure	Route	Dosage	Symptomatology	Outcome	Reference
2,4-D	1	volunteer	oral	500 mg/day for 21 days	none	no illness	Kraus, as cited by Mitchell <i>et al.</i> , 1946
	3	occupational ^{1/}	dermal & resp.	very small	peripheral neuritis ^{1/}	illness persisted	Goldstein <i>et al.</i> , 1959
	1	therapeutic	I.V.	18 doses during 33 days; each of last 12 doses was 800 mg (about 15 mg/kg) or more; 18th was 2000 mg (about 37 mg/kg)	no side effects	-----	Seabury, 1963
	1	therapeutic	I.V.	19th and final dose of 3600 mg (67 mg/kg)	coma, muscle fibrillation, hyporeflexia, urinary incontinence, and profound muscle weakness	recovered	Seabury, 1963
	1	suicide attempt	oral	unknown	prolonged stupor	recovered	Hayes, 1963
	1	suicide	oral	>6500 mg	violent convulsions ^{2/}	death	Nielsen <i>et al.</i> , 1965

^{1/} These three cases of "peripheral neuritis" among men very recently exposed to 2,4-D were seen at one clinic in a single month. No valid toxicological or epidemiological evidence was given to support a causal relationship. The symptom picture was unlike that seen in experimental animals or in man in 2,4-D poisoning. Although very large amounts of 2,4-D and related compounds have been manufactured and used, no other similar cases have been reported. Thus, epidemiologic considerations and an inconsistent symptom picture suggest that other etiologies should be sought for these illnesses.

^{2/} Possibly of anoxic origin.

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Biochemical Pharmacology

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ISSN 0006-2952

BCPCA6 33(2) 169-342 (1984)

**Pergamon Press**

Oxford · New York · Frankfurt

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EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID ON PEROXISOMAL ENZYMES IN RAT LIVER

YOICHI KAWASHIMA,* HARUYO KATOH,* SETSUKO NAKAJIMA,* HIROSHI KOZUKA* and
MITSURU UCHIYAMA†

* Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University,
2630 Sugitani, Toyama 930-01, Japan; † National Institute of Hygienic Sciences,
1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

(Received 20 May 1983; accepted 18 August 1983)

Abstract—The effects of feeding 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on the level of peroxisomal enzymes in rat liver were studied. The concentration of triglyceride in serum was decreased and the activity of cyanide-insensitive palmitoyl-CoA oxidation, catalase and carnitine acetyltransferase increased. However, the extent of the increase in the activity of these enzymes by treatment with 2,4-D was less pronounced than that by 2,4,5-T treatment. The administration of 2,4-D or 2,4,5-T increased the concentration of polypeptide with a mol. wt of 80,000 in the light mitochondrial fractions of the liver from the rats.

Several hypolipidemic agents are known to cause proliferation of peroxisomes in rat liver [1-6]. Ethyl 4-chlorophenoxyisobutyrate (clofibrate) is one of the most common inducers of peroxisomes and is used clinically as a drug. Lazarow and de Duve [7] presented evidence suggesting that hypolipidemic action of clofibrate is attributable to the induction of cyanide-insensitive fatty acyl-CoA oxidizing activity and that the activity is localized in the peroxisomes. Recent studies on several peroxisome proliferators including clofibrate have shown that these compounds are carcinogenic in rats and mice [8-14]. It is important therefore to study the effects of other aryloxy acids on peroxisomal enzymes. 2,4-D and 2,4,5-T are well known as herbicides. In spite of their structural similarity to clofibrate, little information is available about their effects on lipid metabolism, particularly their effects on peroxisomal enzymes. The present study was undertaken to investigate the effects of 2,4-D and 2,4,5-T on the induction of peroxisomal enzymes involved in lipid metabolism.

MATERIALS AND METHODS

Chemicals. Palmitoyl-CoA, acetyl-CoA, L-carnitine, clofibrate and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO); NAD and CoA from Oriental Yeast Co. (Tokyo, Japan); and 2,4-D and 2,4,5-T from Tokyo Chemical Industry Co. (Tokyo, Japan). All other chemicals were of analytical grade.

Animals. Male rats of Wistar strain, weighing 150-200 g. were used. Rats were fed *ad lib.* a commercial diet or a ground diet containing 0.25% (w/w) 2,4-D, 2,4,5-T or clofibrate for 3, 7 or 14 days.

Subcellular fractionations. The rats were decapitated and the livers isolated. The livers were perfused

with ice-cold 0.9% NaCl and rinsed in cold 0.25 M sucrose. Part of the liver was homogenized with cold 0.25 M sucrose and fractionated into nuclear, heavy mitochondrial, light mitochondrial, microsomal and cytosolic fractions by the method of de Duve *et al.* [15]. The other part of the liver was frozen at -30° until the peroxisomal enzymes were assayed. The frozen livers were used within 1 month as described previously [16].

Enzyme assays. Cyanide-insensitive palmitoyl-CoA oxidation was assayed by the method of Lazarow and de Duve [7] with minor modification [16]. One unit of activity was defined as the amount of enzyme required to reduce 1 μ mole of NAD per min. Catalase activity was determined by the method of Aebi [17]. Carnitine acetyltransferase was assayed according to the method of Kahonen [18]. Urate oxidase and glutamate dehydrogenase were assayed according to Leighton *et al.* [19].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed essentially according to the method reported by Reddy and Kumar [20], except for the use of slab gel of 2 mm thickness. Light mitochondrial protein (20 μ g) was loaded onto a stacking gel. Electrophoresis was carried out at 25 mA for ca 5 hr until the marker dye reached the bottom of the gel. The proteins were fixed in the gel with 20% trichloroacetic acid for 30 min and then stained with 0.25% Coomassie brilliant blue in methanol-acetic acid-water (5:1:5, v/v) for 2 hr. Destaining was done by repeated washing in 20% methanol containing 7% acetic acid.

Other analytical procedures. The concentration of cholesterol in the serum was measured by the method of Zurkowski [21]. To measure the concentration of triglyceride in the serum, lipid was extracted from serum by the method of Bligh and Dyer [22] and triglyceride was isolated by TLC on silica gel G using

Table 1. Effects of 2,4-D and 2,4,5-T on the concentration of triglyceride and cholesterol in rat serum

Conditions		Triglyceride (μ moles/ml serum)	Cholesterol (μ moles/ml serum)
Control	(3)	1.15 $\pm$ 0.14	2.50 $\pm$ 0.25
2,4-D	(3)	0.37 $\pm$ 0.03	1.65 $\pm$ 0.11
2,4,5-T	(3)	0.36 $\pm$ 0.10	1.85 $\pm$ 0.54

Each value represents mean $\pm$ S.D. The amount of triglyceride and cholesterol was determined as described in the text. Numbers in parentheses are the numbers of animals used. The rats were fed a control diet or a diet containing 2,4-D or 2,4,5-T for 7 days.

petroleum ether-diethyl ether-acetic acid (80:30:1, v/v) as developing solvent. After adding penta-decanoic acid as internal standard, triglyceride was recovered from the silica gel by extraction with chloroform-methanol-water (2:2:0.5, v/v) according to Stern and Pullmann [23]. The amount of triglyceride was determined by measuring the amount of fatty acid in triglyceride by GLC. The conditions of gas chromatographic analysis have been reported previously [24]. The concentration of protein in the enzyme preparations was determined by the method of Lowry *et al.* [25], with bovine serum albumin as standard.

RESULTS

Table 1 shows the effects of 2,4-D and 2,4,5-T on the concentration of triglyceride and cholesterol in the serum. On administration of 2,4-D or 2,4,5-T for 7 days, the concentration of serum triglyceride decreased to approximately one-third of the control. The concentration of cholesterol was also decreased by this treatment, although the extent of the decrease was less considerable. The effects of 2,4-D, 2,4,5-T and clofibric acid on relative liver weight are compared in Fig. 1. Marked hepatomegaly was produced following the administration of clofibric acid to rats, in good agreement with earlier reports [16, 26]. The relative liver weight increased 1.6 times compared

to that of control rats after treatment with clofibric acid for 14 days. Significant hepatomegaly was produced by the administration of 2,4,5-T, although the effect was less marked than that observed with clofibric acid. 2,4-D did not cause hepatomegaly.

Figure 2 shows the time-course of changes in activity of cyanide-insensitive palmitoyl-CoA oxidation, catalase and carnitine acetyltransferase when rats were fed a diet containing 2,4-D, 2,4,5-T or clofibric acid. The activity of cyanide-insensitive palmitoyl-CoA oxidation was increased by the administration of 2,4,5-T and reached its maximum value 7 days after the initiation of the administration. The maximum activity was approximately 10 times that of the control and approximately 60% of that induced by clofibric acid, when compared on a g liver basis. However, the total activity of cyanide-insensitive palmitoyl-CoA oxidation in the whole liver was 34% of that induced by clofibric acid feeding owing to the less hepatomegaly effect of 2,4,5-T compared with that of clofibric acid. The administration of 2,4-D also increased the activity of cyanide-insensitive palmitoyl-CoA oxidation, although the extent of the increase was less pronounced than that obtained by treatment with 2,4,5-T. The activity of catalase per g liver was slightly increased by treatment with either 2,4,5-T or clofibric acid, whereas 2,4-D did not change total activity of catalase in the whole liver. 2,4-D did not affect catalase activity. The activity of carnitine acetyltransferase, as well as cyanide-insensitive palmitoyl-CoA oxidation, was induced by treatment with either 2,4,5-T or clofibric acid. Treatment with 2,4-D caused a slight, but significant, increase in the activity of carnitine acetyltransferase.

Figure 3 shows the data on subcellular distribution of cyanide-insensitive palmitoyl-CoA oxidizing activity and marker enzymes for both mitochondria and peroxisomes. The highest activity of cyanide-insensitive palmitoyl-CoA oxidation was localized in the light mitochondrial fraction. The distribution pattern of the activity is similar to that of urate oxidase, but not to that of glutamate dehydrogenase.

Figure 4 shows the electrophoretic profiles of protein from the light mitochondrial fractions from livers of rats which had been fed 2,4-D, 2,4,5-T or clofibric acid. In accordance with the findings reported by Reddy and Kumar [20], the concentration of polypeptide with a mol. wt of approximately 80,000 in light mitochondria was increased by feeding with clofibric acid. 2,4-D and 2,4,5-T also increased effec-

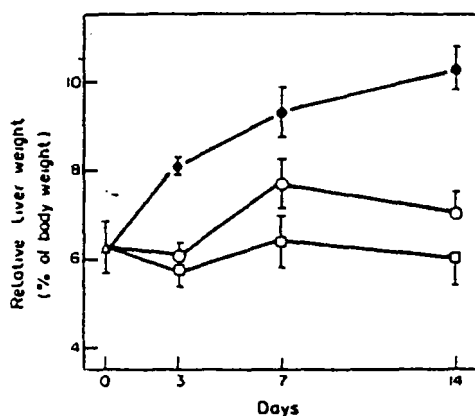


Fig. 1. Effects of 2,4-D and 2,4,5-T on relative liver weight. 2,4-D (□), 2,4,5-T (○) and clofibric acid (●). Each value was obtained from three to ten animals. Vertical bars represent the S.D.

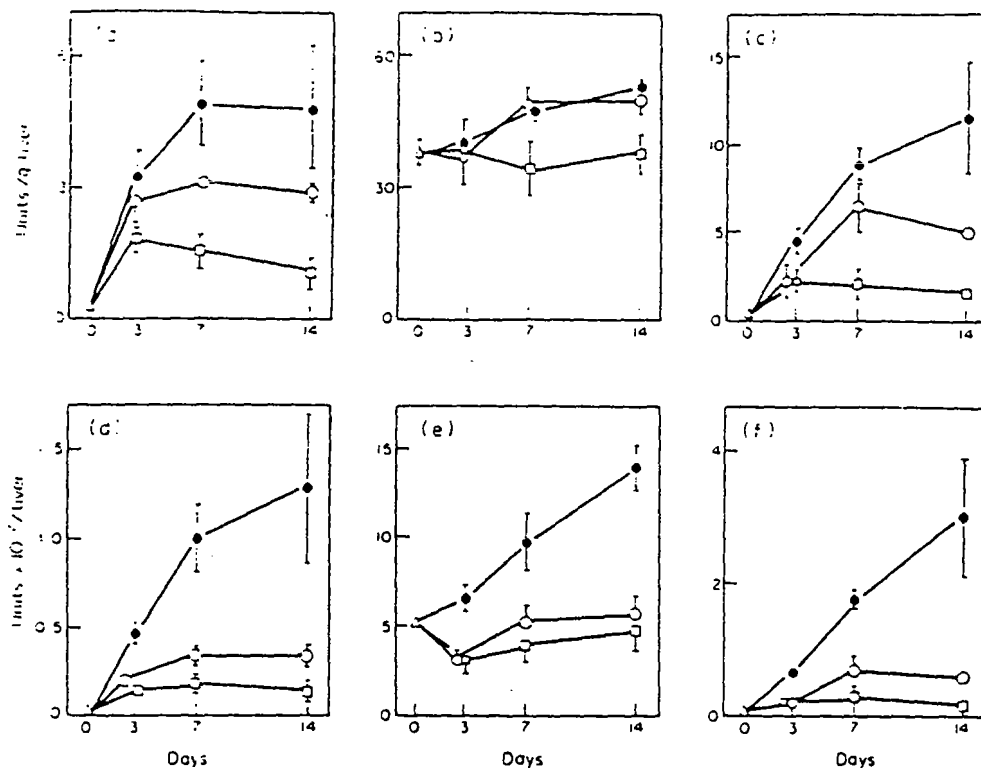


Fig. 2. Effects of 2,4-D and 2,4,5-T on peroxisomal enzymes. Rats were fed a diet containing 0.25% (w/w) 2,4-D, 2,4,5-T or clofibrate. Enzyme activity in the post-nuclear supernatant of the livers was assayed as described in the text. Values represent the mean $\pm$ S.D. from three or four experiments. A and D, cyanide-insensitive palmitoyl-CoA oxidizing activity; B and E, catalase; C and F, carnitine acetyltransferase. 2,4-D (□), 2,4,5-T (○) and clofibrate (●).

tively, but somewhat less markedly than clofibrate, the concentration of the polypeptide in the light mitochondrial fractions.

DISCUSSION

Clofibrate is a hypolipidemic drug and a potent inducer of peroxisomal β -oxidation [7]. Since many other peroxisome proliferators increase the activity of peroxisomal β -oxidation [27-29] and decrease the serum lipid level [4, 30], it is considered that the hypolipidemic action may be due to the increased activity of peroxisomal β -oxidation. The peroxisomal β -oxidation system is distinct from the mitochondrial one in its response to KCN [7].

In the present study, we showed that the administration of either 2,4-D or 2,4,5-T causes a decrease in the serum triglyceride concentration and an increase in the cyanide-insensitive palmitoyl-CoA oxidizing activity. Not only the oxidation activity, but also catalase and carnitine acetyltransferase were induced by 2,4,5-T. Catalase is a peroxisomal enzyme and, in rats, is induced slightly by treatment with clofibrate [7, 16]. Carnitine acetyltransferase has been reported to be localized in both mitochondria and peroxisomes [18] and to be greatly induced by treatment with peroxisome proliferators [6, 18].

Our subcellular fractionation studies showed that the distribution of cyanide-insensitive palmitoyl-CoA oxidizing activity induced by 2,4,5-T is consistent with that of urate oxidase, a marker enzyme for peroxisomes. These results suggest that the increase in the activity of palmitoyl-CoA oxidation in the liver is due to the increase in peroxisome activity. In spite of the fact that catalase is a peroxisomal enzyme, a large amount of catalase was distributed in the cytosolic fraction. This may result from leakage of the enzyme from the peroxisomes into cytosol, because it has been suggested that peroxisomes grow and then burst [31]. Similar leakage of catalase into cytosol was observed in the livers of rats which had been treated with other peroxisome proliferators [29, 30].

The present study showed that the concentration of polypeptide with a mol. wt of approximately 80,000 in the light mitochondrial fractions was effectively increased by either 2,4-D or 2,4,5-T. The polypeptide has been reported to be increased markedly in peroxisomes of livers from rats treated with peroxisome proliferators [20]. Osumi and Hashimoto [32] showed that a peroxisomal protein having activities of both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase corresponds to the peroxisome-specific polypeptide with a mol. wt of 80,000. Accordingly, our results show that both

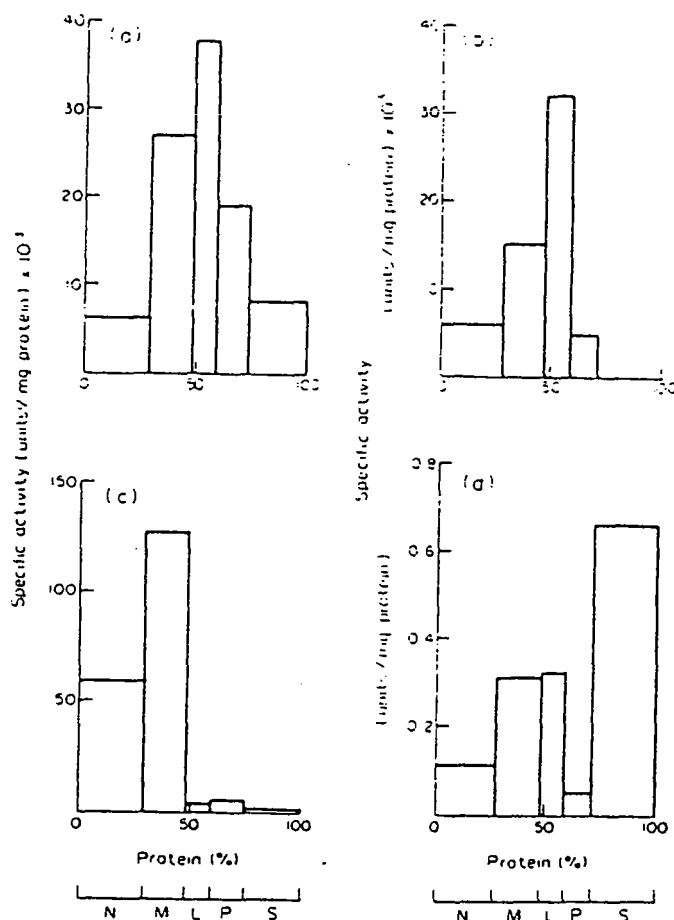


Fig. 3. Subcellular distribution of cyanide-insensitive palmitoyl-CoA oxidizing activity induced by 2,4,5-T. The livers were homogenized and fractionated into nuclear (N), heavy mitochondrial (M), light mitochondrial (L), microsomal (P) and cytosolic (S) fractions as described in the text. Enzyme activity was determined as described in the text. Each value represents the mean of three separate experiments. Rats were fed a diet containing 0.25% (w/w) 2,4,5-T for 7 days. A, Cyanide-insensitive palmitoyl-CoA oxidation; B, urate oxidase; C, glutamate dehydrogenase; D, catalase.

2,4-D and 2,4,5-T, like clofibrate, are inducers of peroxisomal β -oxidation enzymes.

The ability of 2,4,5-T to induce peroxisomal β -oxidation was less pronounced than that of clofibrate, whereas the effect of 2,4,5-T on peroxisomal enzymes was greater than that of 2,4-D. Most of the peroxisome proliferators are known to cause hepatomegaly and induction of catalase as well as of peroxisomal β -oxidation and carnitine acetyltransferase. In fact, 2,4,5-T produced hepatomegaly and induced catalase, peroxisomal β -oxidation and carnitine acetyltransferase. However, 2,4-D differed from 2,4,5-T in not causing hepatomegaly or catalase induction. Lazarow *et al.* [33] pointed out that an increase in activity of peroxisomal β -oxidation is not always accompanied by an increase in catalase activity or liver size. Our previous study also showed that the increase in peroxisomal β -oxidation activity by clofibrate is not always correlated to the production of hepatomegaly and an increase in catalase activity in rats of a hormonally altered state [16].

The potency of 2,4-D and 2,4,5-T to induce peroxisomal enzymes may be due to their structural similarity to clofibrate. However, it should be noted that phenoxyacetic acid, 2-chlorophenoxyacetic acid and 4-chlorophenoxyacetic acid hardly caused hepatomegaly or an increase in the activity of peroxisomal β -oxidation, catalase and carnitine acetyltransferase (data not shown).

Several inducers of peroxisome, including clofibrate and its derivatives [8-14], have been found to produce tumours when administered to rats for prolonged periods and a relatively low dose of clofibrate promotes hepatocarcinogenesis induced by dimethylnitrosamine [34]. A case-control study showed that exposure to 2,4-D and 2,4,5-T causes a 6 times increase in the risk of soft-tissue sarcomas [35]. It may therefore be necessary to take the potency of these herbicides to proliferate peroxisomes into consideration in the study of tumorigenesis of these aryloxy acids.

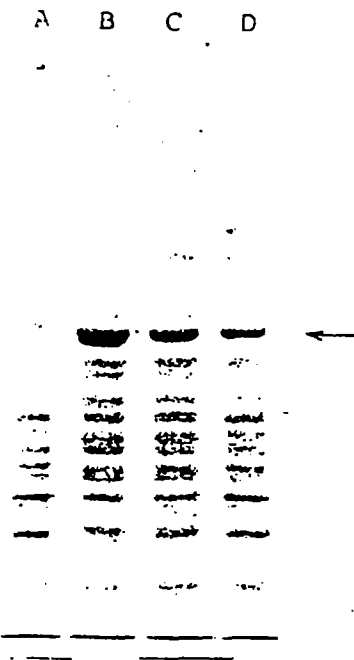


Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the light mitochondrial fractions of the livers. Rats were fed a diet containing 0.25% (w/w) clofibrate, 2,4-D or 2,4,5-T for 7 days. Approximately 20 μ g of light mitochondrial protein was analyzed by electrophoresis as described in the text. A. Control; B. clofibrate fed rats; C. 2,4,5-T fed rats; D. 2,4-D fed rats. The arrow indicates the position of the polypeptide with a mol. wt of 80,000.

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THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

REQUEST FOR PRELIMINARY TOXICOLOGICAL STUDIES - 1803 BLDG.

TOX. OR K NO.

K 2372

FILE

T23.14-11-(12)

MOLECULAR FORMULA	NAME
MOLECULAR WEIGHT	SYNONYMS
STRUCTURAL FORMULA - OR COMPOSITION	

Lot # 90530 T# 2602 Lot # 90970 T# 2605
 # 90540 # 2603 # 94949 T# 2606
 # 90670 # 2604

2,4-Dichlorophenoxyacetic Acid
2,4-D

PHYSICAL AND CHEMICAL PROPERTIES	BOILING POINT	EXPLOSIVE LIMIT (% BY VOL. IN AIR)	FLASH POINT	IGNITION TEMP.	MELTING POINT	VAPOR PRESSURE mmHg. 25°C
	°C	mmHg.	°F	°C	°C	
	CORROSIVENESS (To Common Metals)			PHYSICAL STATE	COLOR	
	CHEMICAL REACTIVITY			ODOR (Include Concentration in air)		
STABILITY (To pH Change, Heat, Light)						

solid white

ACCOUNT OR PROBLEM FOR TOXICOLOGY				SAMPLE REFERENCE AND SOURCE				ESTIMATED COST			
ACCOUNT NUMBER				PROBLEM INDEX NUMBER				REPORT TO (ADDRESS)			
LEDGER LOCATION GRP. SECTION SUFFIX				LAB PROBLEM				DATE			
FUNCTION SUB FUNC.				1990000905				V.K. Rowe			
SUPERVISOR (Signature)				DATE				BLDG.			
PRODUCT SAFETY COORDINATOR (Signature)				DATE				SUBMITTED BY (Signature)			
								DATE			
								BLDG. NO.			
								PHONE N			

See Above

1990000905

V.K. Rowe

2/12/70

DO NOT WRITE BELOW THIS LINE - COMPLETE INFORMATION ON REVERSE SIDE

SAMPLE RECEIVED BY TOXICOLOGY AND PROTOCOLS COMPLETED:						ESTIMATED COMPLETION DATE:					
2/13/70											
TEST	ANIMAL	SEX	SOLVENT	DOSE (g/kg.)	ANIMALS ON TEST	TESTS DONE	ADDITIONAL WORK	DONE	TOX. RESULTS	PHONED TO DATE	
ACUTE ORAL	RAT		CORN OIL	.063						18248	
	GUINEA PIG		WATER	.126							
	RABBIT		UNDILUTED	.252							
	CHICK			.50							
				1.0							
				2.0							
				3.98							
EYE CONTACT	RABBIT		WATER	100%						18248	
			PROPYLENE GLYCOL	10%							
				1%							
SKIN CONTACT REPEATED	2 RABBIT @		WATER	100%							
	Chloroform		CHCl ₃	10%							
				1%							
SKIN CONTACT SINGLE EXPOSURE											
SENSITIZATION	GUINEA PIG										
SKIN ABSORPTION	RABBIT			.126							
SINGLE EXPOSURE				.252							
				.50							
				1.0							
				2.0							
ACUTE INHALATION	RAT		SAT. VAPOR	☐							
			PpM								
			PRESSURIZED CAN								
			AEROSOL								

REPORT DRAFTED

REPORT ISSUED

SAMPLE RETURNED

SAMPLE TO K STORAGE

SAMPLE DESTROYED

REASON OR NEED FOR TOXICOLOGICAL TESTS:

STAGE OF DEVELOPMENT:

☐

RESEARCH

☐

BENCH

☐

PILOT PLANT

☐

SEMI PLANT

☐

PLANT

☐

REGISTRATION

PROPOSED AND POTENTIAL USE:

METHOD OF HANDLING:

AMOUNTS TO BE HANDLED:

HUMAN EXPERIENCE IN HANDLING:

NOTE:

DOM 2158372

18249

60 T23.14-11 1725

K2372

P



THE DOW CHEMICAL COMPANY

MIDLAND

April 13, 1970

G. E. Lynn
Ag. Products
9008 Building

RESULTS OF CHLORACNE STUDIES CONDUCTED ON ASSORTED 2,4-D,
2,4,5-T, SILVEX SAMPLES AND THEIR ESTERS; ORANGE AND
DIAMOND'S CROP RIDER AMINE 4T-2.

As per our telephone conversation on April 1, 1970 here is
a summary of results of all chloracne studies recently com-
pleted on assorted 2,4-D, 2,4,5-T, Silvex Samples and their
Esters, Orange and Diamond's Crop Rider Amine 4T-2.

Chloracne T-Number	Name	Reference Number	Chloracne Response
2578	2,4,5-T	120449	Negative
2586	2,4,5-T	120349	Negative
2587	2,4,5-T	120369	Negative
2588	2,4,5-T	120419	Negative
2589	2,4,5-T	120459	Negative
2590	2,4,5-T	120479	Negative
2601	2,4,5-T	120070	Negative
2609	2,4,5-T	Butyl Ester 60/40 PPI-93-3	Negative
2611	2,4,5-T	Iso Octyl Ester PPI-93-5	Negative
2612	2,4,5-T	Dowanol Ester (PiB) PPI-93-6 Lot # 130667	Negative
* 2602	2,4-D	Lot # 90530	Negative
* 2603	2,4-D	Lot # 90540	Negative
* 2604	2,4-D	Lot # 90670	Negative
* 2605	2,4-D	Lot # 90970	Negative
* 2606	2,4-D	Lot # 94847	Negative
2613	2,4-D	Butyl Ester 60/40 ASI	Negative
2669	2,4-D	Iso Octyl Ester AS-7 2/5/70	Negative
2670	2,4-D	Iso Octyl Ester DE 20 A 2/6/70	Negative

DOH2158369

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G. E. Lynn

-2-

April 13, 1970

<u>Chloracne T-Number</u>	<u>Name</u>	<u>Reference Number</u>	<u>Chloracne Response</u>
2671	2,4-D	Dowanol Ester (PiB) AS-2 2/5/70	Negative
2607	2,4-D	Dowanol Ester (PiB) PPI-93-1	Negative
2608	Silvex	Dowanol Ester (PiB) PPI-93-2	Negative
2610	Silvex	Iso Octyl Ester 60/40 PPI-93-4	Negative
2668	Orange	M-3151 Lot # IRS 7	Negative
2667	Crop Rider Amine	4T-2 Diamond Sample	Positive

Duplicates and triplicates were run on the majority of the above samples. Only Diamond's Crop Rider Amine 4T-2 exhibited a positive chloracne response.

Fred L. Dunn

Fred L. Dunn
Toxicologist
Biochemical Research Laboratory
1803 Building
Phone: 636-3165

wkw

cc: V. K. Rowe, 1803
J. M. Norris, 1803
J. Fernandez, 1710
File

100H2158370

18251

Todd, R. L., A Case of 2,4-D Intoxication. J. Iowa Med.
Soc. 52, 663-664 (1962)

IDOW2158368

Désherbants sélectifs et substances de croissance.

Aperçu technique.

Effet pathologique sur l'homme au cours de la fabrication de l'ester du 2,4 D. (1)

Par M.

M. ASSOULY

(Paris).

Le désir d'obtenir de belles récoltes a, de tous temps, été le souci permanent des agriculteurs. Pour y parvenir, ils ont essayé divers moyens : enrichissement du sol par des engrais; accroissement du rendement par l'emploi de substances de croissance; enfin, destruction des mauvaises herbes qui, en absorbant les engrais, l'eau et les matières minérales, entravent le développement des plantes utiles.

On est parvenu aujourd'hui, dans le domaine des produits de croissance et des herbicides, à des résultats tout-à-fait remarquables. Mais leur fabrication suscite des inquiétudes dont le *Conseil Supérieur d'Hygiène de France* s'est fait l'écho dans sa séance du 13 février dernier. On a d'abord pensé que ces produits pouvaient présenter une action cancérogène, et cette appréhension s'explique fort bien quand on voit la manière dont ces substances agissent sur les plantes, où elles provoquent des tumeurs, des hyperplasies. Mais finalement, cette action cancérogène a été écartée par le professeur Simonnet. Par contre, le *Conseil Supérieur d'Hygiène de France* a souligné le danger d'intoxication que pouvaient présenter ces produits pour les ouvriers qui les fabriquent.

Avant de nous attacher à l'étude de la toxicité des substances en cause, nous croyons utile de rappeler quelques notions techniques à leur sujet.

COMPOSITION ET ACTION DES HORMONES VÉGÉTALES

Nous avons employé les mots d'hormones végétales, parce que c'est ainsi que l'on désigne couramment ces produits destinés à accroître le rendement. Il conviendrait peut-être d'employer plutôt (comme le remarque Pierre Chouard) le nom de *substances de croissance* qui désigne, sans préjuger de leur nature intime, les agents chimiques de tous ordres, capables d'exercer, à de très petites doses, une stimulation puissante sur l'élongation des cellules végétales jeunes. Primitivement, ces substances ont été appelées *auxines* ou *hormones de croissance végétales* ou *phyto-hormones*.

En réalité, les *auxines* sont seulement les substances fabriquées par la plante elle-même et qui exercent ce phénomène de stimulation, d'élongation cellulaire par un mécanisme hormonal.

On s'est aperçu, ces 10 dernières années, qu'une multitude d'autres substances avaient le même pouvoir que les auxines; on les a dénommées *hormones végétales*.

Ces hormones ont une action qui varie suivant le moment d'utilisation et le but recherché : elles exaltent la croissance des plantes ou, au contraire, la ralentissent; elles accélèrent la germination des graines, excitent la formation de racines, de germes, de boutons, de fleurs; ou, au contraire, ralentissent ces fonctions; elles permettent la formation artificielle de fruits sans graines, sans fécondation par le pollen; elles aident

(1) COMMUNICATION à la Société de Médecine du Travail, le 20 novembre 1950.

à la réparation des dégâts causés par la gelée, facilitent la transplantation, le bouturage. C'est dire que leur champ d'application est vaste.

Que sont ces hormones ? — Ce sont des dérivés oxy-acétiques du noyau benzène et du noyau naphthalène; des acides phényl, naphtyl et indol-acétique, propionique et butyrique, ainsi que certains de leurs dérivés, comme par exemple des nitrides, des esters alkylés, des sels d'alcali et d'ammonium, etc... L'un des plus connus est l'acide 2-4 dichlorophénoxyacétique, qu'on appelle, par abréviation, le 2-4 D.

Chacun de ces produits a une action particulière et leur emploi varie donc suivant le résultat à obtenir : par exemple, pour le bouturage, on utilise l'acide indol-acétique, l'acide indol-butyrique ou l'acide naphthalène-acétique plutôt que le 2-4 D. qui a une action trop déformante dans ce cas; contre la chute des fruits, on se sert de l'acide naphthalène-acétique ou de ses sels, ou bien encore du 2-4 D. mais à grande dilution. Signalons aussi que le 2-4 D. appliqué en pulvérisations ou aérosols, stimule puissamment la maturation des fruits.

Un nombre considérable de corps ont été ainsi étudiés et mis au point et certains (comme le 2-4 D. dont nous parlons plus haut) se sont révélés être de l'ordre d'une centaine de fois plus actifs que l'auxine proprement dite; si bien qu'aux doses ordinaires ce n'est plus le pouvoir normal de croissance qui s'exerce, mais l'excès de ce pouvoir, aboutissant à la toxicité. De cette toxicité a dérivé un emploi nouveau : l'utilisation comme herbicide.

Cette utilisation des hormones comme herbicides sélectifs est venue aussi de la connaissance de leur action stimulante sur les diverses fonctions de la plante, y compris la formation de tumeurs. L'usage des hormones, en tant que substances de croissance, provoque souvent, après l'application, des hyperplasies, des cals cicatriciels qui se forment sur la surface de section d'un organe, tige ou pétiole en général. Les hormones, à doses herbicides, ont une action intensifiée; elles provoquent une exaltation folle des manifestations de croissance par hypertrophie et hyperplasie, une activité désordonnée qui entraîne l'apparition de monstruosité les plus diverses, la consommation intense des réserves et finalement la mort, au stade herbacé.

Il s'agit donc d'une destruction radicale, puisqu'il n'y a pas formation de graines.

Cette action est, en outre, sélective; les hormones s'attaquent aux mauvaises herbes mais respectent les plantes utiles. Et ceci s'explique facilement : la résistance des plantes varie suivant les espèces; celle des céréales, par exemple, est beaucoup plus forte que celle des ravenelles, coquelicots ou bleuets. C'est pourquoi le traitement est sans effet sur les céréales, à condition cependant qu'elles aient dépassé le stade 3 feuilles. Il devient aisé de composer un produit de telle manière que seules les mauvaises herbes soient touchées, tandis que les autres plantes resteront insensibles à son action.

Il convient, en outre, d'observer, lors de l'emploi, certaines précautions pour obtenir un bon résultat. En général, on opère à la fin du printemps, lorsque les tiges des plantes ont atteint leur plein développement et ont épuisé les réserves de leurs racines. On vaporise le produit, en solution, sur le feuillage; il adhère fortement, pénètre rapidement dans les feuilles; la sève le véhicule jusqu'aux racines; il provoque alors, en même temps qu'un arrêt de croissance au stade herbacé, une prolifération désordonnée des cellules qui aboutit à la mort, ainsi que nous l'avons vu. La destruction est obtenue en 15 jours ou 1 mois.

Ces produits présentent un avantage considérable sur les désherbants habituellement employés : chlorate de soude, qui détruit toute végétation sans distinction; acide sulfurique, qui acidifie le terrain, ou sulfate de cuivre, susceptible d'une légère sélectivité, mais dont la manipulation présente de graves dangers ou des inconvénients et dont l'action n'est pas aussi totale.

FABRICATION DE L'ESTER DU 2-4 D

La fabrication de ces hormones végétales s'effectue seulement pendant quelques mois, en général de février à mai, c'est-à-dire pendant la période où l'agriculteur a besoin de ces produits.

Il existe sur le marché deux sortes de produits désherbants; l'un est à base d'ester de l'acide 2-4 D. que l'on émulsionne dans de l'huile minérale avec un mouillant. L'utilisateur procède à une seconde émulsion du produit dans l'eau et le projette ensuite

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sur les surfaces à désherber au moyen d'un pulvérisateur. En cas de pluie, le produit, fixé par le mouillant, adhère néanmoins. Le second qui semble avoir la faveur des agriculteurs, ne contient ni émulsifiant, ni mouillant. A base de sel de soude du 2-4 D. Il est directement soluble dans l'eau. Mais il risque d'être lavé par la pluie, puisqu'il ne contient pas de mouillant.

De nouveaux produits à base d'ester sont actuellement en fabrication, comprenant une plus grande quantité d'émulsifiants. On pourra alors verser le produit directement dans l'eau. Le mélange se fera seul, sans qu'on ait même besoin de l'agiter.

Dans l'usine que nous avons visitée, on procédait seulement à la réaction d'estérification de l'acide 2-4 D. qui arrive tout prêt d'une usine de province.

Ouvrons une parenthèse pour signaler que dans cette usine qui fabrique l'acide lui-même, les ouvriers sont imprégnés d'une très forte odeur de phénol, caractéristique de ce produit. Cette odeur est extrêmement tenace; elle persiste, même lorsqu'ils quittent leurs vêtements de travail. Ce qui leur a occasionné quelques désagréments: on considère, par exemple, leur présence indésirable dans les lieux publics, cafés, tramways, etc.... Fermons ici notre parenthèse et revenons à notre usine parisienne.

La réaction d'estérification a lieu à partir du sel de soude de l'acide 2-4 D. sur lequel on fait agir de l'alcool éthylique à 95°, porté à une température de 70°. A ce mélange, on ajoute ensuite de l'acide sulfurique à 98°, ce qui entraîne une élévation considérable de température. La cuve est alors refroidie et maintenue à 70°. Cette opération ayant lieu en vase clos, l'alcool distille et retombe dans la cuve.

Le produit est ensuite lavé à l'eau, toujours en vase clos et on procède à une décantation. Il reste alors dans la cuve un liquide sirupeux qui contient environ 1 p. 100 d'alcool et qui dégage encore une forte odeur de phénol.

PATHOLOGIE

L'interrogatoire des ouvriers affectés à cette opération révèle qu'ils présentent les troubles suivants: somnolence avec sensation de jambes lourdes, irritation des voies aériennes supérieures, gastralgie avec perte d'appétit, goût sucré dans la bouche avec hypersalivation, sensation d'ébriété et d'hypersensibilité de l'ouïe, le moindre bruit faisant sursauter les ouvriers.

Les ouvriers employés au conditionnement se plaignent d'asthénie avec jambes lourdes, sensation d'ébriété et de tête vide.

A quoi pourraient être dus les symptômes signalés ci-dessus? On peut imaginer qu'ils sont causés par le produit fini lui-même ou par les émanations des différents constituants, au cours de la fabrication.

C'est ainsi que l'irritation des voies aériennes supérieures est bien connue dans l'intoxication par les phénols, qui produit des catarrhes (laryngites, trachéites).

D'autre part, il est signalé par les auteurs que l'intoxication chronique par le phénol — d'ailleurs discutée — peut se manifester entre autres par des troubles digestifs, anorexie, pyalisme, céphalées, vertiges. Or, les ouvriers nous ont signalé tous ces signes, parmi les troubles qu'ils rattachent à l'exercice de leur profession.

On pourrait rattacher également quelques-uns des symptômes relevés aux émanations probables des vapeurs d'alcool en cours de fabrication. Cette opinion n'est toutefois pas partagée par l'ingénieur que nous avons vu pendant notre visite, car, d'après lui, d'autres fabrications mettant en jeu des quantités importantes d'alcool ne présenteraient pas les inconvénients signalés par le personnel.

En résumé, on peut donc dire que la plupart des signes pathologiques peuvent être dus au dégagement de vapeurs de phénol. Toutefois, des signes plus particuliers, tels que le goût sucré, la sensation d'hypersensibilité de l'ouïe paraissent originaux et dus au produit fini lui-même.

Les troubles rapportés ci-dessus pourraient constituer un syndrome caractéristique du travail au contact des produits en question, si d'autres auteurs les avaient signalés. Nous avons procédé à des recherches bibliographiques, mais nous n'avons pu trouver de référence, du moins dans les sources que nous avons utilisées, concernant leur action sur le personnel employé à la fabrication.

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Par contre, diverses publications signalent les travaux effectués sur leur toxicologie. Il nous paraît intéressant de résumer ci-dessous les recherches effectuées dans les différents pays du monde.

TOXICOLOGIE

Le professeur R. Fabre, dans un rapport présenté aux Journées Médicales Internationales de Paris en 1937, signale les tumeurs qui se forment chez la plante autour d'une piqûre d'hétéro-auxine, acide indol 3-acétique. Il termine son rapport sur quelques questions pleines d'intérêt : « Les auxines végétales ont-elles une action sur le métabolisme ou la croissance d'un organisme animal ? Aucune expérience définitive n'a été tentée dans ce sens jusqu'ici. Mais il serait fort intéressant d'établir un parallèle entre l'action encore bien discutée des hormones sexuelles sur le développement des plantes et celle des auxines sur le développement des animaux. Et s'il y a action, sur quelle cellule de l'organisme porte-t-elle ? Autant de questions sans réponses, qui doivent tenter physiologistes et médecins. »

De même, cette question de la toxicité du 2-4 D. pour les humains fut posée lors de la deuxième réunion du North Central States Weed Control Conference. Quelques membres relatèrent alors des cas d'ingestions accidentelles de quantités variables, mais toujours sans altération apparente de la santé des accidentés. Kraus fit savoir à l'assemblée qu'il avait lui-même absorbé quotidiennement 1/2 g de 2-4 D. pur pendant 3 semaines, sans ressentir de désagrément.

Des expériences furent tentées à Beltsville, afin d'éprouver la toxicité du 2-4 D. sur les animaux. La première consista à faire paître, pendant 15 jours, des vaches et des moutons dans un pâturage préalablement traité avec l'herbicide. On avait même doublé la dose habituellement nécessaire à la destruction des mauvaises herbes. Les animaux furent ensuite examinés par des vétérinaires, et trouvés en excellente santé. Dans la seconde expérience, on fit absorber à une vache 5,5 g de 2-4 D. par jour, et ce pendant 3 mois. L'animal consomma facilement le grain d'alimentation avec lequel la substance chimique était mélangée. Sa production laitière ne subit aucune baisse et un veau, nourri entièrement de son lait pendant 1 mois, ne montra aucun symptôme de maladie. Une autopsie n'a pas révélé d'effet apparent du 2-4 D. sur les différents organes de cette vache.

M. K. Bjorn et H. T. Northen donnèrent pendant 4 semaines une dose quotidienne de 200 mg/kg à des poulets. Cela fut sans effet. Mais la dose de 765 mg/kg fut mortelle.

E. V. Hill, et H. Carlisle, entreprirent des expériences sur 450 souris, 150 rats blancs, 125 cobayes, 70 lapins et 3 singes. Le 2-4 D. fut administré avec les aliments, en injections intra-veineuses, à des doses croissantes pour atteindre et déterminer la dose mortelle. A ce stade, les animaux meurent soudainement de fibrillation ventriculaire aiguë. S'ils échappent à la mort instantanée, on voit apparaître la raideur des extrémités, avec de l'incoordination musculaire, la léthargie, la stupeur et finalement, le coma et la mort.

Ces symptômes sont constants, quelle que soit la façon d'administrer le 2-4 D. et quel que soit l'animal utilisé pour l'expérience.

L'investigation montre que le 2-4 D. est un composé relativement peu toxique dont la dose mortelle est de :

375	mg/kg	de souris,
1 000	—	de cobaye,
666	—	de rat,
800	—	de lapin.

quand ces doses sont administrées en solution aqueuse par la bouche.

En supposant que la tolérance pour l'homme soit similaire à celle des animaux, la plus forte dose que pourrait supporter un homme de 75 kg serait de 15 g.

Ces expériences ont été conduites avec du 2-4 D. utilisé comme herbicide, sans que sa toxicité soit augmentée par celle de ses solvants.

Aucune expérience n'a été entreprise pour mettre en évidence l'intoxication chronique avec les effets de l'absorption par la voie respiratoire.

De leur côté, King et Penfound firent des essais en aquarium sur la brème et le bar. Ils trouvèrent que dans ce milieu clos, le 2-4 D. à 1 p. 100 n'est pas toxique pour ces

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poisons, mais qu'il le devient légèrement à 100 p. 100, concentration qui ne peut être que temporaire et locale en eau courante.

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En vérité, on se trouve là devant un produit nouveau dont le mode d'action lui-même sur les végétaux n'est pas encore très défini en agronomie et reste encore un peu mystérieux.

En ce qui concerne l'organisme humain, le problème est entier, autant du moins que nos recherches nous permettent de l'affirmer.

RÉSUMÉ

On a donné le nom d'hormones végétales à certaines substances chimiques capables d'exercer, à de très petites doses, une stimulation puissante sur l'élongation des cellules végétales jeunes, action exercée habituellement par une substance élaborée par la plante elle-même. Ces hormones sont des dérivés oxy-acétiques du noyau benzène et du noyau naphthalène, des acides phényl, naphtyl et indol-acétique, propionique et butyrique, ainsi que certains de leurs dérivés : nitriles, esters alkylés, sels d'alcali, d'ammonium, etc... L'un des plus connus est l'acide 2-4 dichlorophénoxyacétique, appelé couramment 2-4 D.

L'action intensive de ces hormones les a fait utiliser comme herbicides sélectifs. Elles agissent en provoquant une exaltation folle des manifestations de croissance, une activité désordonnée qui entraîne l'apparition des monstruosité les plus diverses, la consommation intense des réserves, et finalement la mort au stade herbacé.

Les ouvriers employés à la fabrication de l'ester du 2-4 D. se plaignent de somnolence avec sensation de jambes lourdes, d'irritation des voies aériennes supérieures, de gastralgie avec perte d'appétit, de goût sucré dans la bouche avec hypersalivation, de sensation d'ébriété et d'hypersensibilité de l'ouïe, le moindre bruit les faisant sursauter.

Des expériences faites sur les animaux ont montré que le 2-4 D. absorbé avec les aliments ou inoculé en injections intra-veineuses, n'était toxique qu'à doses assez élevées.

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FILED 1983
K-4568-
K-2372-
1983

Chemosphere, Vol. 12, No. 4/5, pp 749-759, 1983
Printed in Great Britain

0045-6535/83/050749-11\$03.00/0
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A REVIEW OF ON-GOING EPIDEMIOLOGIC RESEARCH IN THE UNITED STATES ON THE PHENOXY
HERBICIDES AND CHLORINATED DIOXIN CONTAMINANTS

A.L. Young and B.M. Shepard
Office of Environmental Medicine, Veterans Administration
Washington, D.C. 20420, U.S.A.

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ABSTRACT

Few environmental or occupational health issues have received the sustained international attention that has been focused on the phenoxy herbicides and their associated chlorinated dibenzo-p-dioxins contaminants. The Veterans Administration in cooperation with other Federal agencies in the United States Government has initiated extensive health studies of veterans exposed to Agent Orange and its dioxin contaminant during the Vietnam Conflict. The basis for resolving phenoxy herbicides/dioxin controversies must in large measure stem from the results of scientific inquiry.

INTRODUCTION

The use of herbicides to control vegetation has been one of the most persistent controversial subjects arising from the Vietnam Conflict. The United States Air Force applied most of these herbicides in dense jungle areas to uncover and expose hidden enemy staging areas, clear vegetation from the vicinity of military bases and along lines of communication, and to destroy enemy crops. The objectives were to provide defoliated zones that would reduce ambushes and disrupt enemy attacks. The most commonly used "defoliant" was "Agent Orange," a mixture of the two commercial herbicides, 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), widely employed for many years for brush control in forestry programs throughout the United States and for weed control in agriculture.

During a 5-year period from 1965 to 1970, the U.S. Air Force applied more than 40 million liters of Agent Orange in South Vietnam. Some two million American military personnel served 1-year tours during the same period. Recently, many veterans of that era have reported medical problems that possibly stem from exposure to Agent Orange during their military assignments. Their complaints have ranged from tingling in the extremities to rare forms of cancer. Some veterans have fathered children with birth defects and have suggested that Agent Orange is the culprit. The basis for resolving the Agent Orange controversy must in large measure stem from the results of scientific inquiry.

Accordingly, the Veterans Administration (VA) in cooperation with other Federal agencies in the United States Government has initiated extensive health studies of veterans exposed to Agent Orange and its dioxin contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during the Vietnam Conflict. In addition, numerous studies of non-veteran populations exposed to the phenoxy herbicides and TCDD have been encouraged. This article will briefly review the various federally sponsored health studies.

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DISCUSSION

To conduct epidemiologic studies of human populations exposed to the phenox herbicides and the dioxin contaminants, it is necessary to first know what health outcomes should be selected for study. Secondly, one must be able to identify a study cohort(s) with a high likelihood of exposure and a control group with a low or no probability of exposure. Lastly, a sufficient number of individuals in each cohort must be located, recruited and examined in order to give statistical validity to the results. Unfortunately, these three requirements, so important for the conduct of valid studies, are in themselves not clearly defined with respect to either the chemicals used in Vietnam or the population at risk. It is apparent that health studies of the effects of phenox herbicides and/or the dioxin contaminant are very difficult under conditions of normal agricultural use situations, but become immensely more complex when conducted with cohorts briefly exposed a decade ago during the conditions of war in a tropical environment.

Brief Literature Review

Very little is reported in the scientific literature concerning the long-term adverse effects of 2,4-D, 2,4,5-T, and TCDD on humans. Acute and subacute effects are reported quite uniformly following accidental exposures, suicidal gestures, and industrial accidents, but there is a great deal of confusion concerning the nature of long-term effects if any. Much of the medical knowledge concerning the effects of 2,4-D and 2,4,5-T is derived from case reports. Since many of the patients described in these reports were exposed to multiple chemical agents, it is difficult to determine the chemicals that produced specific symptoms. Of the vast array of symptoms attributed to 2,4-D, the most consistently reported problems involve psychological behavior, the nervous system, the liver and the intestines.

Medical data associated with exposure to 2,4-D come primarily from spraying incidents, but data for 2,4,5-T and TCDD come from industrial exposures. Since the first commercial production of 2,4,5-T, numerous industrial episodes have involved exposure to trichlorophenol, 2,4,5-T and TCDD. Fifteen of the 23 episodes recorded in the literature were apparently the result of occupational exposures during industrial production of chlorinated phenols. But, on eight occasions, personnel were exposed during cleanup following explosions or to improperly decontaminated workshops (15). Unfortunately, the effects of 2,4,5-T in these episodes could not be clearly distinguished from the possible effects of TCDD. Symptoms attributable to 2,4,5-T and TCDD exposure include all of the symptoms of 2,4-D exposure, in addition to a skin disease, chloracne. Many scientists believe that chloracne is the "hallmark" of exposure to the dibenzo-p-dioxins, especially 2,3,7,8-TCDD. Chloracne is a skin reaction characterized by a acniform dermatitis with comedones (blackheads) and inclusion cysts or papules and frequently pustules so severe that they cause permanent scarring. Morphologically, it is similar to adolescent acne, but it is usually more severe, particularly on the upper face, ears, and neck. Active chloracne lesions have been reported many years after exposure to TCDD, but the condition usually clears up spontaneously in a few months. Premature aging of involved skin areas has been reported in some instances as well as the appearance of hyperpigmentation.

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Several case control epidemiological studies conducted by Swedish scientists have reported evidence of a statistical relationship between "soft tissue cancers" and exposure to the phenoxy herbicides and TCDD (4) while similar studies by Finnish (9) and New Zealand scientists (11) show no relationship to soft tissue sarcoma. The data by Tung et al. (13) from Vietnam has received widespread attention, but the scientific community has viewed these studies with caution. Except for angiosarcoma, a rare type of cancer, which is known to be caused by vinyl chloride exposure, there are virtually no cancers of connective or other "soft tissue" which have a known etiology related to chemical exposure.

The first reports of human birth defects attributed to Agent Orange appeared in Vietnamese newspapers in June 1969. As a result of the public and scientific furor caused by these reports, Cutting et al. (3) and Meselson et al. (7) conducted two independent surveys of South Vietnamese hospital records. Although neither report reached definite conclusions on the validity of the accusations, both reports acknowledged that searches of the records probably would have revealed any marked increase in birth defects or introduction of a striking defect, such as the defects produced by thalidomide. Subsequent reports by Tung et al. (13) in 1971 and Rose and Rose (10) in 1972 centered on clinical observations and interviews conducted in Hanoi with refugees who claimed that they were repeatedly sprayed with defoliants in South Vietnam. The authors reported unusually high rates of abortions and severe birth defects in both humans and domestic animals.

In announcing the results of studies conducted in South Vietnam in 1972 and 1973 (2), a committee appointed by the National Academy of Sciences reported that it could find no conclusive relationship between exposure to herbicides and birth defects in humans, but the committee recognized that available birth records were not adequate for definite conclusions. The committee also could not confirm or deny reports that some humans, especially the Montagnards, and domestic animals became ill or died after they were exposed to herbicide sprays or after they consumed treated plants or contaminated water. In the cover letter to the report, the president of the National Academy of Sciences stated: "On balance, the untoward effects of the herbicide program on the health of the South Vietnamese people appear to have been smaller than one might have feared".

In 1982, Townsend et al. (12) released a report on their study of the offspring of production workers exposed to 2,4,5-T and TCDD. The study was based on an interview questionnaire administered to 370 wives of men who had worked in areas where they could have been exposed to TCDD and to a control group of 345 wives of men in the same company who had never worked in such areas. The study found no statistically significant differences in the instances of miscarriages, stillbirths, infant deaths, or congenital malformations in the two groups.

Early Federal Efforts

The United States Environmental Protection Agency, the United States Department of Agriculture and the National Institute of Occupational Safety and Health have all conducted

limited studies on the potential associations of phenoxy herbicides with human spontaneous abortions and birth defects.

Two studies were conducted by the Environmental Protection Agency to evaluate the relationship between herbicides spraying and reproductive events. Johnson (6) reported on the data used in early 1979 by the Environmental Protection Agency in the decision to order the suspension of registrations of 2,4,5-T for uses in forest, rights-of-way, and pastures. He reported that the incidence of spontaneous abortions over a 6-year period in Alsea, Oregon, was found to be higher than the rates in two other regions of Oregon that had lower rates of 2,4,5-T usage. Although, a cyclic pattern in the incidence of abortions in the Alsea group correlated positive with the pattern of annual 2,4,5-T spray usage, the Agency acknowledged it was not proof of a cause and effect relationship. In another study sponsored by the EPA, however, the incidence of cleft palate over a period of 32 years in Arkansas was shown not to be related to the usage of 2,4,5-T. The incidence of cleft palate for regions with a low proportion of rice acreage (indicating low exposure to 2,4,5-T) and for regions with high 2,4,5-T exposure increased over the years studied. This trend was attributed to better detection of cleft palate and not to herbicide usage (8).

The United States Department of Agriculture sponsored a study designed to evaluate the relationship between human male exposure to 2,4-D and the incidence of spontaneous abortions in their wives. In this study reported by Carmelli et al. (1), questionnaires pertaining to these two factors were mailed to almost 15,000 people who were employed in occupations involving the manufacture or use of herbicides. From the respondents, a group of 134 cases of miscarriages were selected and compared to a control group of 311 cases of live births. The cases were analyzed to determine the incidence parental exposure to 2,4-D prior to conception, and the incidence of other relevant confounding factors, such as smoking illnesses, and drug use. The data were obtained or verified by telephone interviews. No positive association was established between 2,4-D exposure and abortions, for the entire study population. For a group of 21 cases of wives of young forest and commercial workers, an association was suggested, but was not found for any other subgroup. Further investigations were suggested to clarify whether this relationship resulted from biases in study design or reflected a real increase among this small subset.

In November 1979, the National Institute for Occupational Safety and Health received a request to determine whether an excess number of birth defects occurred among the children of the maintenance employees working for the Long Island Railroad, New York, and whether these defects may have been caused by exposure to 2,4,5-T. Honchar (5) investigated 170 live births observed from among the study population of approximately 1,400 employees. She reported that 42 of the live births had at least one noninfectious health problem during the first year of life. It was found that all major birth defects combined and inguinal hernia were underrepresented in the study population (3 observed versus 3.81 expected for major birth defects and 2 observed versus 2.3 expected for inguinal hernia). However,

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metatarsus adductus (8 observed versus 3.47 expected) and tear duct obstruction (2 observed versus 0.22 expected) were both significantly overrepresented in the study population. Bonchar concluded that these latter defects were probably overrepresented in the study population due to diagnostic bias.

Previous studies therefore suggest that there is very little epidemiologic data associating TCDD or the phenoxy herbicides with any long-term health effects in humans other than chloracne. However, as noted by Wolfe (14), neither is there strong evidence to validate the absence of such effects. Most studies have not included sufficient numbers of subjects to detect an increased risk of an uncommon condition. Also the period of observation in many studies has been inadequate to detect an illness with a long latency period between exposure and illness. There is currently no reliable evidence that links dioxin exposure to cancer or birth defects in humans.

Health Status of Vietnam Veterans

As of August 1982, more than 15,000 Vietnam veterans have submitted claims to the Veterans Administration for disabilities which they believe are the result of exposure to herbicides. These claims describe more than 130 different effects in five major categories: psychiatric, dermatological, reproductive, neurologic, and cancer. The scientific data validate specific links between exposure to Agent Orange and TCDD in the sense that symptoms reported by the veterans have also been documented in other cases of exposure to the herbicides or to TCDD. But most of these symptoms, e.g., peripheral neuropathy, fatigue, weight loss, and some psychological disturbances, are acute symptoms that manifest themselves shortly after exposure. Such symptoms arising years after the last known exposure are most likely not caused by 2,4-D and 2,4,5-T or TCDD. A large number of the veterans have claimed dermatological problems, and many believed they had chloracne. To date, however, no cases of chloracne have been confirmed by physical examination in this group of veterans.

Despite the lack of solid scientific evidence to support the veterans' allegations that Agent orange is the cause of their difficulties, one recognizes that some of the veterans have definitely experienced serious health problems. Therefore, the purpose of many of the government studies is to determine whether Agent Orange is responsible for these health problems. If Agent Orange is not the culprit, other factors associated with the Vietnam war may be responsible. Consequently, the goal of other research efforts is to determine if Vietnam veterans as a group are experiencing more or different health problems than their counterparts who did not serve in that part of the world. In such a complex situation no single study can provide all of the answers. Thus, there is a need for a number of different approaches to examining the health of the Vietnam veteran.

CURRENT INVESTIGATIONS

What has been the response of the Veterans Administration and other governmental agencies to the critical issues raised by Agent Orange? Early action by the VA included the development of a communication process for gathering scientific and medical information

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which would bear directly on the problem. In addition, early in 1978, the Veterans Administration established the Agent Orange Registry in order to accomplish four objectives:

- (1) To identify all Vietnam veterans expressing a concern about the possible adverse health effects of their exposure to Agent Orange.
- (2) To provide a mechanism for Vietnam veterans to voice their concerns to a physician, receive a physical examination and obtain responsible answers to some of their questions.
- (3) To serve as a mechanism for follow-up of these individuals if, at a later date, new information develops as a result of the various research efforts underway or being planned.
- (4) To obtain, as a by-product, some preliminary information on the current health status of the veterans who have participated in the registry.

The Agent Orange Registry provides us essentially with a listing of names and addresses, together with some background data on a self-reported group of veterans with service in Vietnam. A complete physical examination and a group of baseline laboratory tests are provided to each veteran. The information from this effort is currently being placed into a computer data bank. There are almost 100,000 Vietnam veterans who have been examined by the Veterans Administration. We are in the process of collating the data to determine whether or not there are any significant health trends in these individuals which might suggest areas for further investigation.

TCDD Assay of Human Adipose Tissue

Since TCDD is known to accumulate preferentially in the adipose tissue of certain species of laboratory animals, it was suggested, early in the history of the Agent Orange issue, that the analysis of human fat for TCDD might provide a way of determining Agent Orange exposure. In addition, if detectable levels of this substance were present, there might be some relationship to health problems. Although methods for TCDD analysis have improved in recent years, no such study had been carried out in humans with known exposure to herbicides containing this toxic contaminant. Consequently the VA embarked on a small feasibility study to test the methodology and see what conclusions might be drawn regarding the significance of the results. The study was carried out in three groups of adult males as follows:

- (1) Twenty Vietnam veterans who claimed health problems related to Agent Orange exposure and who volunteered for the fat biopsy.
- (2) Three U.S. Air Force officers with known heavy and relatively recent exposure in connection with herbicide disposal operations but who did not serve in Vietnam.
- (3) Ten veterans with no service in Vietnam and no known exposure to herbicides who were undergoing elective abdominal surgery and who volunteered to serve as controls.

The procedure called for the removal of 20-30 grams of subcutaneous adipose tissue from the abdominal wall and subsequent analysis by high resolution gas chromatography-mass

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spectrometry. The results of this very complex and technically difficult analysis indicated that very low levels of TCDD, believed to be 2,3,7,8-TCDD, could be detected in human adipose tissue in the range 3 to 99 parts-per-trillion. The levels, however, did not correlate well with known exposure and non-exposure, and there was no correlation with health status. The study results, however, indicated that the assay method was feasible, but would serve no clinically or administratively useful purpose until additional data were available on background levels of TCDD in the general United States population.

Accordingly, the Veterans Administration, in cooperation with the Environmental Protection Agency (EPA) has initiated an interagency agreement to study levels of 2,3,7,8-TCDD in adipose tissue from a selected group of U.S. males.

The Environmental Protection Agency has been demographically collecting adipose tissue from the U.S. general population (i.e., by region, age, sex and race). This National Adipose Tissue Bank was initiated in 1968 and now contains specimens from over 12,000 individuals. Represented within this bank is available adipose tissue from approximately 500 males born between 1937 and 1952. Many of these individuals will have served in the military during the Vietnam-era and it is reasonable to assume that some actually served in Vietnam during the period of Agent Orange use. A retrospective study of selected chlorinated dioxins and furans may establish data on background levels of 2,3,7,8-TCDD in the U.S. male population as well as whether service in the military and especially in Vietnam has had an effect on the levels of TCDD in adipose tissue.

The study will be conducted in three phases. Phase I will be to obtain the name and social security number for the approximately 500 males noted above. This information will be used to determine military service status. Phase II will be the development of analytic methods for the determination of selected dioxins (especially the 2,3,7,8-TCDD) and furans in human adipose tissue. The method will be subjected to rigorous interlaboratory validation by an independent Analytic Referee, e.g., the Association of Official Analytical Chemists. Phase III will be the analysis of the adipose tissue and the preparation of a final report. Phases I and II should be completed within fiscal year 1983, and the report from Phase III should be available in early 1985.

Vietnam Veteran Mortality Study

The VA has initiated two large mortality studies which will analyze and compare death rates and cause-of-death profiles between veterans with service in Vietnam and comparable veterans with no service in Vietnam. It is estimated that approximately 300,000 Vietnam and Vietnam-Era veterans have died since the start of the Vietnam Conflict. This number includes approximately 52,000 combat deaths. The studies will use existing computer records to identify a group of approximately 60,000 deceased veterans and determine cause of death and Vietnam service status. The studies also include independent validation of the computer data bases used in the studies. It is important to note that the mortality studies will not provide information specifically related to phenoxy herbicide exposure, but rather to service in Vietnam.

Vietnam Experience Twin Study

The Vietnam Experience Twin Study was initiated by a group of research staff members at the Veterans Administration Medical Center, Saint Louis, Missouri. The study will identify pairs of twin veterans where one twin served in Vietnam during the period of Agent Orange spraying and the other served in an area other than Southeast Asia. Approximately 600 pairs of twins will be examined using a series of psychologic, physiologic and biochemical tests.

The development of a protocol for the study and the establishment of a Twin Registry from which to solicit participants are now underway. The study will focus primarily on the total Vietnam experience rather than any single factor such as Agent Orange. If sufficient numbers of identical twin pairs are identified, there may be an opportunity to draw some conclusions regarding the effects of specific aspects of the Vietnam experience such as combat, herbicide exposure or use of insecticides.

Birth Defects and Military Service in Vietnam Study

In 1981 the Centers for Disease Control (CDC) in Atlanta, Georgia initiated a study designed to determine if Vietnam veterans are at an increased risk of having children with birth defects. Since 1968 CDC has maintained a registry of all babies born with defects in the greater metropolitan Atlanta area. Of the more than 15,000 children in this registry, approximately 7,500 had significant anatomical defects at birth. The investigators will attempt to locate and interview both parents of all 7,500 of the children in this group. In addition, the parents of 3,000 matched control normal babies born during the same time period will be interviewed. Since the major objective of this study will be to determine whether an unusually high proportion of fathers of babies born with defects served in Vietnam, information will be gathered about Vietnam service as well as other factors which may be associated with the occurrence of birth defects. If the study demonstrates that a Vietnam veteran has an increased risk of fathering a child with a defect it may be desirable to attempt to determine if the increase is associated with Agent Orange exposure or with some other factor(s). The study is scheduled to be completed by the end of 1983.

The Air Force Health Study

In 1979 the Air Force initiated the protocol for a comprehensive epidemiologic study of the RANCH HAND personnel, a group of approximately 1,250 men who actually conducted the aerial, fixed-wing herbicide spraying missions in Vietnam from 1962 through 1971. The study is designed around the question, "Have there been, are there now, or will there be in the reasonably foreseeable future, any adverse health effects among RANCH HAND personnel caused by repeated exposure to Herbicide Orange?"

The investigation is composed of three integrated elements — a mortality study of those individuals who have died since their exposure, a morbidity study to examine the current health status of the study subjects, and a follow-up study to look for delayed effects over the next 20 years. The mortality and morbidity study elements are being conducted simultaneously on the RANCH HAND personnel and a very carefully matched control group by

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the use of personnel tracking procedures coupled with an extensive review of military medical and personnel records; detailed face-to-face questionnaires to ascertain current and past health events as well as occupational and family data; and comprehensive physical examinations, psychological testing and diagnostic laboratory studies to determine exact health status. Additional questionnaires and physical examinations are to be administered periodically during the follow-up phase. Results from the mortality analysis are to be reported in early 1983 and the results of the initial questionnaire and current health status analysis are to be released in mid-1983.

Epidemiologic Study of Ground Troops Exposed to Agent Orange

Public Law 96-151 charged the Veterans Administration to "design a protocol for and conduct an epidemiological study of persons, who while serving in the Armed Forces of the United States during the period of the Vietnam conflict, were exposed to any of the class of chemicals known as 'the dioxins' produced during the manufacture of the various phenoxy herbicides (including the herbicide known as 'Agent Orange') to determine if there may be long-term adverse health effects in such persons from such exposure." Efforts are still underway to successfully complete a protocol and to identify suitable cohorts. This will be a huge research effort which will include interviews and examinations involving several thousands of veterans. A contract for the design of the study was awarded to the School of Public Health, University of California, Los Angeles. A major problem in the design of such a study related to the fact that there were very few records maintained that would link specific ground troops to herbicide exposure. Another problem encountered was that little is known concerning long-term or delayed human health effects resulting from exposure to the phenoxy herbicides or the contaminant, TCDD. Consequently it is difficult to design data collecting instruments and techniques when the outcome variables are so ill-defined. However, much design work has been accomplished, and it is hoped that the actual study will get underway early in 1983.

CONCLUSION

Neither the government nor the scientific community has resolved the numerous controversies (environmental, medical, or political) involving the use of Agent Orange in Vietnam from 1962 to 1970 nor the use of 2,4,5-T within the agricultural setting of the United States. A report by the National Academy of Sciences in 1974 documented some of the environmental impacts of Agent Orange, but unfortunately, the arrangements that terminated the conflict precluded additional scientific studies in that area. Such studies might have minimized current medical concerns about herbicide exposure. Moreover, to date Federal agency positions on environmental hazards associated with TCDD and 2,4,5-T are either not well defined or not uniformly accepted, thus perpetuating the controversy.

The scientific community must continue to conduct valid research on controversial environmental and health-related issues in order to provide a reliable basis for appropriate decisionmaking. The Veterans Administration stands firmly committed to working closely with other agencies of the Federal Government as well as with the private sector to

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obtain as many answers as quickly as possible consistent with sound scientific principles in order to resolve this perplexing issue. The resolution of the controversy, however, will be achieved only following the public's acceptance of the outcome of scientific investigations. To that end scientists must accept the responsibility for not only conducting quality research, but also for translating the results of their efforts to legislators, to the courts, to the media and ultimately to the public at large.

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Chemical(s) identified: TCDD

DR-65-0986-(100)
K-066681-(100)
[1981]

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Clark

DEM218886

Species studied: C57B1/6 male mice

Dose: Total doses 100 $\mu\text{g}/\text{kg}$; 0.4 $\mu\text{g}/\text{kg}$, 4 $\mu\text{g}/\text{kg}$, 40 $\mu\text{g}/\text{kg}$, 0.004 $\mu\text{g}/\text{kg}$

Health effects:

Abstract. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a toxic halogenated aromatic hydrocarbon, acts in the body as a cumulative poison. The chronic immunotoxic effects of TCDD were studied in C57B1/6 male mice. Total doses of 100 $\mu\text{g}/\text{kg}$ or greater produced cellular depletion in thymus, spleen, and lymph nodes, and the animals appeared sick. No cellular depletion was seen following 0.4 $\mu\text{g}/\text{kg}$, and only thymus was affected by 4 and 40 $\mu\text{g}/\text{kg}$. The antibody response to SRBC and TNP-*Brucella abortus* was impaired following 40 $\mu\text{g}/\text{kg}$ TCDD, the delayed hypersensitivity response to oxazalone was impaired by 4 $\mu\text{g}/\text{kg}$ and the generation of alloantigen-specific cytotoxic T cells (CTL) was sensitive to as little as 0.004 $\mu\text{g}/\text{kg}$ TCDD. *In vitro* analysis of the mechanism of suppression using limiting dilution techniques showed that TCDD did not deplete the precursors of CTL but generated cells capable of suppressing CTL generation *in vitro*.

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TABLE I. EFFECT OF TCDD TREATMENT ON DELAYED HYPERSENSITIVITY REACTIONS

Treatment given	Increment in footpad swelling 24 hr after oxazalone challenge ^a ($\times 10^{-2}$ mAf, mean $\pm$ SEM)	Increment in ear thickness 24 hr after oxazalone challenge ^b ($\times 10^{-2}$ mAf, mean $\pm$ SEM)	
		Immune recipient	Control recipient
Control (corn oil)	23.7 $\pm$ 5.2 (10)	10.9 $\pm$ 1.9 (8)	0.17 $\pm$ 0.87
0.4 μ g TCDD	24.5 $\pm$ 5.1 (10)	16.8 $\pm$ 4.1 (8)	-0.83 $\pm$ 1.35
4 μ g TCDD	34.5 $\pm$ 4.1 (10)	6.8 $\pm$ 1.4 (8) ^c	-0.83 $\pm$ 1.35
40 μ g TCDD	14.7 $\pm$ 4.9 (10)	7.4 $\pm$ 0.7 (8) ^c	0 $\pm$ 1.29

^a The mice were sensitized by intravenous injection of $1-2 \times 10^5$ SRBC and tested 5 days later by injection of 2×10^5 SRBC into the left hind footpad. A similar volume of saline was injected into the right hind footpad to control for the nonspecific effects of the fluid injection. Parentheses enclose the number of measurements.

^b The mice were sensitized to oxazalone in ethanol and ear swelling in sensitized and nonsensitized animals was measured 24 hr after application of oxazalone in olive oil.

^c Significantly lower than control by student's *t* and Mann-Whitney *U* tests. $P < 0.05$.

TABLE II. EFFECT OF TCDD ON THE FREQUENCY OF CTLp AND GENERATION OF CTL IN *IN VITRO* MARE MLC CULTURES

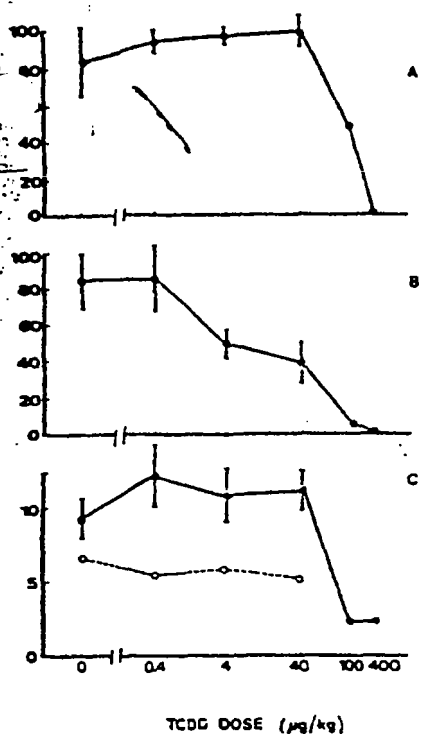
Treatment of mice	CTLp frequency ^a	Number of cells per culture ^b ($\times 10^4$)	Cytotoxic activity per culture ($\text{NAT} \times 10^3$)	Estimated CTLp per culture	Yield CTL/CTLp
1 Control PLN	1/292	1	4635 $\pm$ 394	3425	1.35
0.4 μ g PLN	1/211	1	2505 $\pm$ 317	4739	0.53
4 μ g PLN	1/255	1	2617 $\pm$ 233	3922	0.67
40 μ g PLN	1/150	1	4590 $\pm$ 197	6667	0.69
400 μ g PLN	NC	NC	NC	NC	—
Control spleen	1/1660	2	4156 $\pm$ 351	1205	3.45
0.4 μ g spleen	1/1561	2	1408 $\pm$ 97	1282	1.10
4 μ g spleen	1/1469	2	2347 $\pm$ 170	1361	1.72
40 μ g spleen	1/1607	2	1745 $\pm$ 104	1245	1.40
400 μ g spleen	1/206	2	2007 $\pm$ 100	9709	0.21
2 Control PLN	1/1463	3	326 $\pm$ 18	2051	0.159
4 μ g PLN	1/1006	3	184 $\pm$ 20	2982	0.062
4 μ g PLN	1/1296	3	116 $\pm$ 17	2315	0.050
0.4 μ g PLN	1/1229	3	130 $\pm$ 43	2441	0.053
Mixture					
Control PLN + 0.4 μ g PLN	—	1.5 $\pm$ 1.5	131 $\pm$ 21	2246	0.058

^a Frequency of precursors of CTL was determined using limiting dilution microcultures as described under Materials and Methods. NC = insufficient cells to perform culture.

^b The test lymph node or spleen cells from the treated mice were cultured with irradiated F₁ stimulator cells in MLC tube cultures and the resulting cytotoxic activity was measured after 5 days incubation. The number of initial CTLp per culture was calculated by multiplying cell number by the CTLp frequency.

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TCDD DOSE (µg/kg)

FIG. 1. Effect of TCDD on cellular content of lymphoid organs. Data are given as mean $\pm$ SEM for spleen (A), thymus (B), and peripheral lymph nodes (C) studies in four to six independent experiments. 4 µg/kg represents a dose of approximately 4 parts per billion. In three experiments done 1–6 weeks after the first dose of TCDD, the proportion of thy 1.2-bearing cells was determined in PLN by complement-dependent lysis (two experiments) or by immunofluorescent staining (one experiment) as described under Materials and Methods. The PLN from control mice contained 70.4–74.7% thy 1.2-positive cells whereas PLN from mice given 0.4 µg/kg TCDD contained 43–46%, from mice given 4 µg/kg TCDD 40–60%, and from mice given 40 µg/kg TCDD 35–55%. The dotted line in C shows the estimated number of thy 1.2-positive cells in lymph nodes.

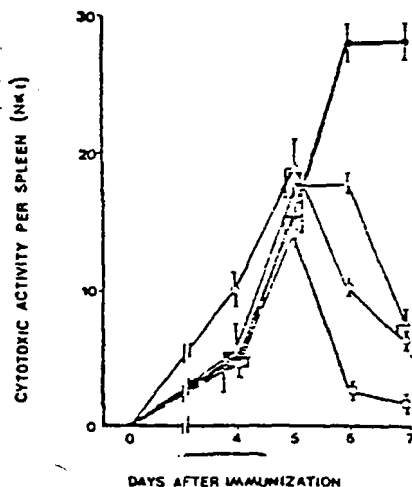


FIG. 2. Effect of TCDD treatment on the generation of CTL *in vivo* following alloantigen challenge. Control mice (●) or mice given 0.4 (Δ), 4 (○), or 40 (□) µg/kg TCDD are immunized with irradiated allogeneic P-815 melanoma cells as described under Materials and Methods. CTL activity was measured in spleen cell suspensions from groups of three to four mice at the times indicated.

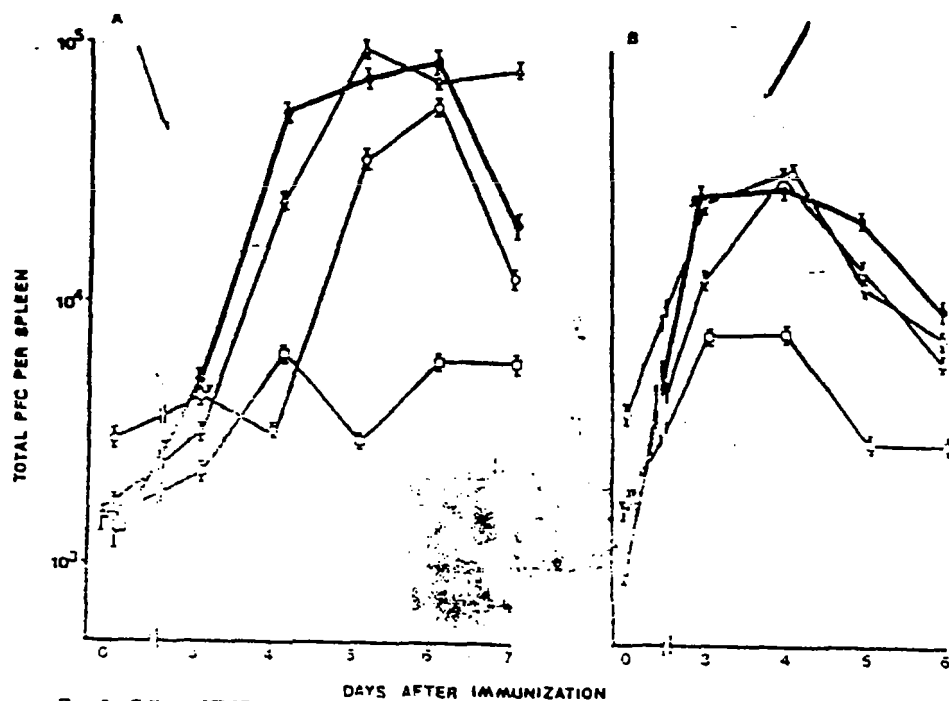


FIG. 3. Effect of TCDD treatment on the antibody response. Control mice (●) or mice treated with a total of 0.4 (Δ), 4 (○), or 40 (□) µg/kg were given an intraperitoneal immunization with SRBC (A) or TNP-BA (B). The spleens were removed at the times indicated and the number of PFC was measured as described under Materials and Methods. The total IgM + IgG + IgA PFC number $\pm$ SEM is shown for groups containing three to four pooled mouse spleens.

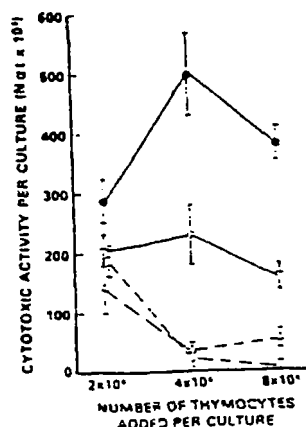


FIG. 4. Thymocytes from TCDD-treated mice suppress CTL generation *in vitro*. The effect of different numbers of thymocytes from control mice (●) or from mice given 0.004 (▽), 0.04 (○), or 0.4 (Δ) µg/kg TCDD on the generation of CTL from 2 × 10⁴ normal PLN is shown. Five to six thymus donors were used per group.

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PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND
MEDICINE, V168, N2, P290-299, 1981
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PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 290-299 (1981)

Enhanced Suppressor Cell Activity as a Mechanism of Immunosuppression by
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin¹ (41275)

DAVID A. CLARK, JACK GAULDIE, MYRON R. SZEWCZUK, AND
GEORGE SWEENEY

Departments of Medicine and Pathology, Host Resistance Program, McMaster University,
1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

Abstract. TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), a toxic halogenated aromatic hydrocarbon, acts in the body as a cumulative poison. The chronic immunotoxic effects of TCDD were studied in C57BL/6 male mice. Total doses of 100 $\mu\text{g/kg}$ or greater produced cellular depletion in thymus, spleen, and lymph nodes, and the animals appeared sick. No cellular depletion was seen following 0.4 $\mu\text{g/kg}$, and only thymus was affected by 4 and 40 $\mu\text{g/kg}$. The antibody response to SRBC and TNP-*Brucella abortus* was impaired following 40 $\mu\text{g/kg}$ TCDD, the delayed hypersensitivity response to oxazalone was impaired by 4 $\mu\text{g/kg}$ and the generation of alloantigen-specific cytotoxic T cells (CTL) was sensitive to as little as 0.004 $\mu\text{g/kg}$ TCDD. *In vitro* analysis of the mechanism of suppression using limiting dilution techniques showed that TCDD did not deplete the precursors of CTL but generated cells capable of suppressing CTL generation *in vitro*.

Halogenated aromatic hydrocarbons (HAHs)² such as polychlorinated biphenyls (PCBs)², polychlorinated naphthalenes, polybrominated biphenyls (PBBs), polychlorinated dibenzofurans, pentachlorophenols (PCP), and polychlorinated dibenzo-*p*-dioxins, pose a serious man-made environmental threat to health (1-9). They are fat soluble, poorly metabolized, and tend to accumulate in the environment and in the body where they act as persistent poisons (1). Polychlorinated dibenzo-*p*-dioxins such as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) formed industrially by pyrolysis of chlorophenols, a particularly toxic subgroup of the halogenated aromatic hydrocarbons, provide a good model system for studying HAH toxicity. Although species vary in the types of

lesions developing after TCDD exposure (8, 10, 11), a consistent observation in all animal species which have been studied is atrophy of the thymus. TCDD-treated rodents show impairment of delayed hypersensitivity responses, reduced ability to mount a graft-versus-host reaction and reduced ability to reject allografts, and show an increased susceptibility to certain infectious agents (8, 10, 12-15). Thus, one of the potentially important aspects of toxicity of TCDD and related HAHs is the effect on the immune system. In order to better understand the mechanism of TCDD immunotoxicity, we have analyzed the effects of different doses of TCDD on several types of immune function of C57BL/6J male mice. Particular attention has been directed to the chronic effects of TCDD since chronicity typifies human exposure. We find that the generation of cytotoxic T lymphocytes (CTL) following alloantigen challenge is particularly susceptible to impairment by low doses of TCDD, and this impairment is caused by a disturbance in the cells which regulate the CTL response. Measurement and characterization of this disturbance may provide a sensitive test for presence of biologically significant amounts of TCDD in the body.

Materials and Methods. *Mice.* C57BL/6

¹ Presented in part at the 23rd Annual Meeting of the Canadian Federation of Biological Societies, June 11, 1980, and published in abstract form Vol 23, abstract 295.

² Abbreviations used: HAHs, halogenated aromatic hydrocarbons; PBBs, polybrominated biphenyls; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; SRBC, sheep red blood cells; TNP-BA, trinitrophenylated *Brucella abortus* organisms; PLN, peripheral lymph nodes (axillary, brachial, inguinal); CTL, cytotoxic T lymphocyte; CTLp, precursor of CTL; PFC, plaque-forming cell; oxazalone, 4-ethoxymethylene-2-phenyl.

male mice age 6–8 weeks were purchased from the Jackson Laboratory, Bar Harbor, Maine, and housed six per cage. Teklad chow (6% fat) and water were provided *ad lib*. DBA/2J mice were similarly obtained and were used for maintenance of P-815-X2 (H-2^d) mastocytoma cells by serial ascities transfer. C3H/HeJ × DBA/2J F₁ and C57Bl/6J × DBA/2J F₁ hybrids were produced in our own colony. RNC nude mice were purchased from the Ontario Veterinary College, Guelph, Ontario.

TCDD treatments. TCDD Lot No. 851-144-2 purity >99% was obtained from Dow Chemical, Sarnia, Ontario, Canada. Two hundred and fifty micrograms was dissolved in *p*-dioxane and diluted in corn oil. Mice were injected ip weekly with 100 μ l of corn oil containing specified amounts of TCDD and studied 1 week after the fourth dose.

Cell preparations. The TCDD-treated and control mice were killed by cervical dislocation. The spleen, thymus, and peripheral lymph nodes (axillary, brachial, and inguinal) were aseptically removed, and a single-cell suspension was obtained by pressing minced tissue through a 60-mesh stainless-steel screen into cold medium. Culture medium consisted of α -MEM supplemented with 10% v/v fetal bovine serum (Grand Island Biological Co., Grand Island, N.Y.), 100 units penicillin G and 100 μ g streptomycin/ml and 20 mM Hepes buffer. 2-Mercaptoethanol was added at a concentration of 5×10^{-5} M for cell culture experiments.

Anti-Thy 1.2 treatment. AKR anti-C3H serum was purchased from Cedarlane Laboratories, Hornby, Ontario (Lot 5101). The proportion of thy 1.2-bearing cells in peripheral lymph node (PLN) cell suspensions was determined in two ways. PLN suspension initially prepared in Dulbecco's phosphate-buffered saline with 0.2% Fraction V bovine serum albumin was labeled by incubation with Na₂⁵¹CrO₄ (New England Nuclear) and washed. Labeled cells at 10⁷/ml were incubated with antiserum at a 1/20 dilution at 4° for 45 min and resuspended in a 1/12 dilution of Low-Tox rabbit complement (Cedarlane Laboratories). After 90 min of incubation at 37°, the degree

of ⁵¹Cr release was calculated and percentage lysis by anti-thy 1.2 plus complement calculated by subtraction of the complement control. Since TCDD treatment might have altered the sensitivity of the cells to complement, a second method was used. PLN were incubated in a 1/4 dilution of anti-thy 1.2 serum for 45 min at 4°, washed, and then incubated in a 40- μ g/ml solution of fluorescein-labeled protein A (Pharmacia). After washing, the proportion of cells showing membrane immunofluorescent staining was determined by counting 200–400 cells under indirect ultraviolet illumination (Leitz).

Antibody response. The antibody response to two different antigens was measured. Mice were injected intraperitoneally with either 5×10^7 washed sheep red blood cells (SRBC) or, 0.1 ml of an 8% suspension of TNP-conjugated *Brucella abortus* (TNP-BA). Spleens were removed from SRBC- or TNP-BA-challenged mice 3–7 days later and tested for antibody-forming cells against SRBC or TNP-SRBC, respectively, using a plaque assay as modified for slides by Dresser and Greaves (16). Freshly frozen guinea pig serum absorbed with 50% packed SRBC was thawed and added at a final dilution of 1/30 as a source of complement to detect "direct" plaque-forming cells. To detect IgG and IgA PFC, direct IgM PFC were first blocked by adding a 1/200 dilution of goat anti-mouse μ during the initial incubation of target and spleen cells. Indirect IgG PFC were then developed by adding rabbit anti-mouse IgG at a predetermined optimal 1/200 dilution, and IgA PFC were developed using a 1/100 dilution of rabbit anti-mouse IgA. The IgG-developing serum was prepared by immunizing rabbits with purified mouse IgG (Miles, Lot 51), and showed only a single line against mouse serum and IgG on Ochterlony plates. The IgA-developing serum was prepared in rabbits using pure mouse myeloma MOPC 315 (IgA λ 2) and was depleted of anti-light-chain activity by passage through a murine IgG–Seph-rose-immunoabsorbent column. Pure mouse IgA or IgG added to the PFC assay inhibited only IgA or IgG PFC, respectively, when the developing sera were used.

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Delayed hypersensitivity response. In the first method used, TCDD-treated and control mice were sensitized by injecting $1-2 \times 10^5$ SRBC iv and 5 days later the mice were tested by injecting 2×10^4 SRBC in 20 μ l into one of the hind footpads (17). Saline (20 μ l) was injected into the other footpad and the degree of swelling was measured after 24 hr. The net amount of swelling observed following SRBC challenge was corrected for swelling produced by medium alone. In a second method, TCDD treated or control mice were sensitized by painting both ears with 3% oxazalone (4-ethoxymethylene-2-phenyl, BDH Chemicals, Poole, England) dissolved in 95% ethanol. Nine to sixteen days later, sensitized and unsensitized control animals were challenged with 1% oxazalone in olive oil (18). Ear thickness was measured prior to challenge and at 24 hr using a constant-pressure micrometer.

Generation and assay of cytotoxic T cells. CTL were generated against the H-2^d alloantigen *in vivo* and *in vitro*. The *in vivo* response to TCDD-treated mice was determined following injection of 2×10^7 P-815 tumor cells (H-2^d) that had been treated with 5000 rad ⁶⁰Co to prevent proliferation. Spleens were removed from the recipients 3-6 days later and the CTL activity was assayed in a 6-hr ⁵¹Cr-release assay against ⁵¹Cr-labeled P-815 targets as described in detail elsewhere (19). The titration curve (percentage specific ⁵¹Cr release vs concentration of sensitized spleen cells) was analyzed by computer for the parameter N_{50} which is proportional to the number of CTL lytic units in the cell suspension. $693 N_{50} \times 10^3$ units is equivalent to 1 lytic unit (the number of cells required to produce 50% lysis in the absence of interfering bystanders (19)).

CTL were generated *in vitro* in two different ways. First, mixed lymphocyte culture (MLC) was performed in 17 $\times$ 100-mm polystyrene tubes (Falcon Plastics, No. 2057) in 3 ml culture medium. Briefly, $1-3 \times 10^6$ spleen or PLN cells from the C57Bl/6J mice were mixed with 10^6 C3D2F₁ or B6D2F₁ spleen cells that had been inactivated with 1500 rad. CTL content of the culture was determined after a 5-days incu-

bation period at 37° in a 4-hr ⁵¹Cr-release assay using 2×10^4 P-815 target cells. In some experiments, thymocytes from treated or control mice were added to the cultures. Second, the frequency of precursors of CTL (CTLp) was measured using the limiting dilution microculture method of Teh *et al.* (20). Briefly, small numbers of C57Bl/6J spleen or PLN cells were cultured in V-bottom microwells (Linbro 96-MVC-TC) in 0.2 ml culture medium with 2×10^5 irradiated F₁ spleen and 2×10^5 RNC nude spleen cells. Here, the nude spleen cells provide "help" which allows the development of CTL from single precursor cells (20). After 6 days incubation at 37°, 2000 ⁵¹Cr-labeled P-815 target cells were added to each well and the amount of lysis was determined after 5 hr. Those wells showing release exceeding the 95% confidence interval of spontaneous release from negative control wells containing F₁ stimulators and nude spleen alone were scored positive. Under limiting dilution conditions, the number of lymphoid cells yielding 37% negative wells defines the cell number containing, on the average, 1 CTLp according to the Poisson distribution: $P(0) = e^{-u}$, where $P(0)$ represents the fraction of negative wells and u the mean frequency of CTLp per well. The value of u was calculated using a computer program derived from Porter *et al.* (21). The value of u allowed an estimate of the number of CTLp present in the tube MLC cultures and thus, a determination of the yield of CTL per CTLp ($N_{50} \times 10^3$ per 1 CTLp).

Results. Effect of different doses of TCDD on cellularity of lymphoid organs. Figure 1 shows the effects of different doses of TCDD on the cellularity of different organs of the mouse immune system. The reported LD₅₀ in mice for TCDD is approximately 100 μ g/kg (8), although the time of death is quite variable and often delayed. When a total dose exceeding 40 μ g/kg was administered, cellularity of the spleen, thymus, and peripheral lymph nodes was markedly reduced and the animals appeared ill. In contrast, doses of 0.4-40 μ g did not reduce cell numbers in the spleen and peripheral lymph nodes and the animals appeared healthy. Nevertheless, cellular

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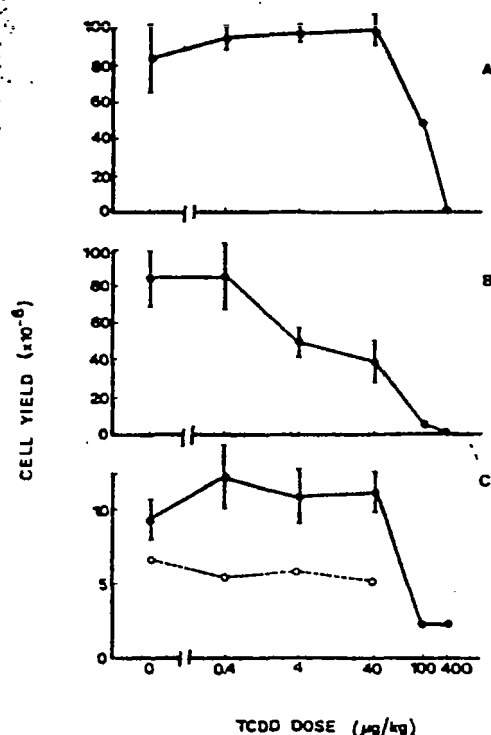


Fig. 1. Effect of TCDD on cellular content of mouse lymphoid organs. Data are given as mean $\pm$ 1 SEM for spleen (A), thymus (B), and peripheral lymph nodes (C) studies in four to six independent experiments. 4 $\mu\text{g/kg}$ represents a dose of approximately 4 parts per billion. In three experiments done 1–6 weeks after the last dose of TCDD, the proportion of thy 1.2-bearing cells was determined in PLN by complement-dependent lysis (two experiments) or by immunofluorescent staining (one experiment) as described under Materials and Methods. The PLN from control mice contained 70.4–74% thy 1.2-positive cells whereas PLN from mice given 0.4 $\mu\text{g/kg}$ TCDD was 43–46%, from mice given 4 $\mu\text{g/kg}$ TCDD 43–60%, and from mice given 40 $\mu\text{g/kg}$ TCDD, 40–55%. The dotted line in C shows the estimated number of thy 1.2-positive cells in lymph nodes.

depletion was already apparent at the 4 $\mu\text{g/kg}$ dose when the thymus was examined. Since these data suggested that the thymus was particularly sensitive to the effects of TCDD compared to other lymphoid organs, we tested whether TCDD affected the peripheral T-cell population, by determining the proportion of thy 1.2-positive cells in PLN suspensions from TCDD-treated and control animals. Three

experiments showed that the percentage thy 1.2-positive cells decreased following TCDD treatment at all doses studied (see Fig. 1). The dotted line in Fig. 1C shows that the estimated number of thy 1.2-positive cells in mouse lymph nodes also decreased after TCDD treatment. A decrease in the absolute number of thy 1.2-positive cells was also seen in spleen in one experiment where this organ was studied.

Effect of TCDD on immune responses.

Figure 2A shows a representative experiment illustrating the effect of TCDD on the humoral immune response to SRBC. The number of background PFC in the spleens of unimmunized mice was consistently increased by TCDD treatment and the greatest increase was observed at 4 $\mu\text{g/kg}$ dose (Fig. 2, Day 0). Following immunization with SRBC, PFC number increased in control (corn-oil-injected) mice reaching a peak on Day 5. TCDD at a dose of 40 $\mu\text{g/kg}$ of TCDD produced a marked suppression of the PFC response. This suppression was reproducible and all three isotypes (IgG, IgM, IgA) were affected (data not shown). The result shown in Fig. 2A could have resulted from an effect of TCDD on the T-dependent component of the anti-SRBC response (22). Therefore, the response to a relatively T-independent antigen (23), TNP-B. *abortus* was tested. Similar to the result obtained using SRBC as antigens (Fig. 2A), Fig. 2B shows that the 0.4 and 4 $\mu\text{g/kg}$ doses of TCDD had little effect on the anti-TNP-BA response and the 40 $\mu\text{g/kg}$ dose suppressed the response. We concluded that the dramatic suppression of the antibody response to SRBC by 40 $\mu\text{g/kg}$ TCDD could not be explained by elimination of T helper cells.

The effect of various doses of TCDD on the delayed hypersensitivity response is shown in Table I. Footpad swelling elicited by SRBC was only slightly reduced by the 40 $\mu\text{g/kg}$ dose of TCDD but this reduction was not statistically significant. The ear swelling response to oxazalone appeared to be more sensitive. It can be seen that the 4 and 40 $\mu\text{g/kg}$ doses impaired the ear swelling response whereas little effect occurred at the 0.4 $\mu\text{g/kg}$ dose.

The effect of TCDD on the generation of CTL following alloantigen challenge *in vivo*

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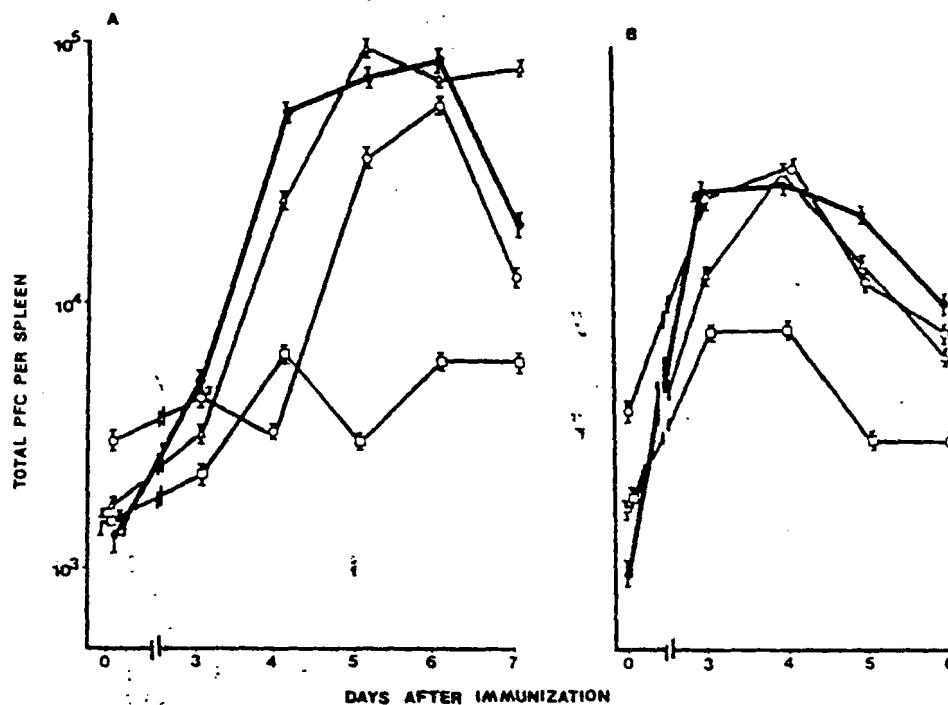


FIG. 2. Effect of TCDD treatment on the antibody response. Control mice (●) or mice treated with a total of 0.4 (Δ), 4 (○), or 40 (□) µg/kg were given an intraperitoneal immunization with SRBC (A) or TNP-BA (B). The spleens were removed at the times indicated and the number of PFC was measured as described under Materials and Methods. The total IgM + IgG + IgA PFC number $\pm$ SEM is shown, for groups containing three to four pooled mouse spleens.

is shown in Fig. 3. Even the 0.4 µg/kg dose of TCDD impaired the ability to generate a maximal response on Days 6 and 7. We concluded that the CTL response was particularly sensitive to functional impairment

by TCDD. We therefore decided to direct our subsequent investigations toward elucidation of the mechanism of impairment of CTL generation by TCDD.

In vitro analysis of effect of TCDD on

TABLE I. EFFECT OF TCDD TREATMENT ON DELAYED HYPERSENSITIVITY REACTIONS

Treatment given	Increment in footpad swelling 24 hr after oxazalone challenge ^a ($\times 10^{-2}$ mM, mean $\pm$ SEM)	Increment in ear thickness 24 hr after oxazalone challenge ^b ($\times 10^{-2}$ mM, mean $\pm$ SEM)	
		Immune recipient	Control recipient
Control (corn oil)	23.7 $\pm$ 5.2 (10)	10.9 $\pm$ 1.9 (8)	0.17 $\pm$ 0.87
0.4 µg TCDD	24.5 $\pm$ 5.1 (10)	16.8 $\pm$ 4.1 (8)	-0.83 $\pm$ 1.35
4 µg TCDD	34.5 $\pm$ 4.1 (10)	6.8 $\pm$ 1.4 (8) ^c	-0.83 $\pm$ 1.35
40 µg TCDD	14.7 $\pm$ 4.9 (10)	7.4 $\pm$ 0.7 (8) ^c	0 $\pm$ 1.29

^a The mice were sensitized by intravenous injection of $1-2 \times 10^5$ SRBC and tested 5 days later by injection of 2×10^5 SRBC into the left hind footpad. A similar volume of saline was injected into the right hind footpad to control for the nonspecific effects of the fluid injection. Parentheses enclose the number of measurements.

^b The mice were sensitized to oxazalone in ethanol and ear swelling in sensitized and nonsensitized animals was measured 24 hr after application of oxazalone in olive oil.

^c Significantly lower than control by student's *t* and Mann-Whitney *U* tests. $P < 0.05$.

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CYTOTOXIC ACTIVITY PER SPLEEN

FIG. 3. Effect of TCDD treatment on the CTL response. Control mice (●) or mice treated with a total of 0.4 (Δ), 4 (○), or 40 (□) µg/kg were given an intraperitoneal immunization with SRBC. The spleens were removed at the times indicated and the number of CTL was measured as described under Materials and Methods. The total CTL number $\pm$ SEM is shown, for groups containing three to four pooled mouse spleens.

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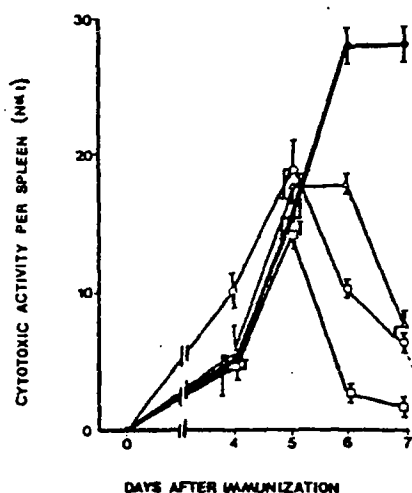


FIG. 3. Effect of TCDD treatment on the generation of CTL *in vivo* following alloantigen challenge. Control mice (●) or mice given 0.4 (Δ), 4 (○), or 40 (□) $\mu\text{g/kg}$ were immunized with irradiated allogeneic P-815 tumor cells as described under Materials and Methods. CTL activity was measured in spleen cell suspensions from groups of three to four mice at the times indicated.

CTL generation. The suppressive effect of TCDD treatment on the generation of CTL was analyzed *in vitro* using the same methods that have been employed to characterize the mechanism of immunosuppression in pregnant mice (24). Experiment 1 in Table II shows that TCDD treatment impaired the ability of spleen and PLN cells from TCDD-treated mice to generate CTL against the H-2^d alloantigen *in vitro* in tube cultures. The frequency of CTLp, however, was normal or slightly increased in TCDD-treated animals as determined by limiting dilution microcultures. This observation indicated that TCDD did not have a direct toxic effect on CTLp. In agreement with this conclusion was the marked increase in CTLp frequency seen in the spleens of animals given a 400 $\mu\text{g/kg}$ dose of TCDD that produced gross cellular depletion in this organ. It can also be appreciated from the experiment that the yield of cytotoxic activity per unit precursor was usually subnormal when lymphoid cells from TCDD-treated animals were studied. Experiment 2 shows that CTL generation was affected by doses of TCDD as low as

0.004 $\mu\text{g/kg}$. Furthermore, mixing cells from TCDD-treated mice with those of normal mice appeared to suppress the CTL response of the untreated cells.

Demonstration of suppressor cells in the thymus of TCDD-treated mice. The above observations suggest that TCDD may enhance suppressor cell activity. We examined this possibility by adding thymocytes from TCDD-treated or control mice to MLC cultures containing normal PLN cells and quantitated the number of CTL generated *in vitro*. The effect of different numbers of thymocytes on the generation of CTL from normal PLN is shown in Fig. 4. Dramatic suppression of CTL generation was observed when $4-8 \times 10^6$ thymocytes from mice given 0.4 $\mu\text{g/kg}$ and 0.04 $\mu\text{g/kg}$ TCDD were tested. Less suppression was seen when thymocytes from mice given 0.004 $\mu\text{g/kg}$ were used. Direct addition of TCDD up to doses of 0.025 μg to the cultures produced no suppression (data not shown). If the entire dose of TCDD given to a mouse was concentrated in the thymus (containing on the average 8×10^7 cells) then 4×10^6 thymocytes from a mouse given 0.4 $\mu\text{g/kg}$ would add 0.00050 μg TCDD to the culture. These data suggest that the suppression of CTL generation in TCDD-treated mice represented suppressor cell activity and was not a direct toxic effect of TCDD.

Discussion. The data in this paper show that the type of immunotoxicity produced by chronic exposure to TCDD depends on the dose. In agreement with the results of Faith *et al.* (25), we found reduced delayed hypersensitivity reactions as assessed by ear swelling at a 4 $\mu\text{g/kg}$ dose of TCDD that had little effect on the antibody response. The most interesting finding, however, was the exquisite sensitivity of the CTL response to TCDD. CTL generation was impaired *in vivo* at the 0.4 $\mu\text{g/kg}$ TCDD dose which produced no thymic atrophy or detectable impairment of antibody or delayed hypersensitivity responses. Impairment of CTL generation could be demonstrated *in vitro* with TCDD doses as low as 0.004 $\mu\text{g/kg}$ when washed spleen or lymph node cells from TCDD-treated animals were stimulated in tube cultures with alloantigen.

Some insight into the cellular basis for

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TABLE II. EFFECT OF TCDD ON THE FREQUENCY OF CTLp AND GENERATION OF CTL IN *IN VITRO* MASS MLC CULTURES

Treatment of mice	CTLp frequency ^a	Number of cells per culture ^b ($\times 10^4$)	Cytotoxic activity per culture ($\text{Nat} \times 10^3$)	Estimated CTLp per culture	Yield CTL/CTLp
1 Control PLN	1/292	1	4635 $\pm$ 394	3425	1.35
0.4 μg PLN	1/211	1	2505 $\pm$ 317	4739	0.53
4 μg PLN	1/255	1	2617 $\pm$ 233	3922	0.67
40 μg PLN	1/150	1	4590 $\pm$ 197	6667	0.69
400 μg PLN	NC	NC	NC	NC	—
Control spleen	1/1660	2	4156 $\pm$ 351	1205	3.45
0.4 μg spleen	1/1561	2	1408 $\pm$ 97	1282	1.10
4 μg spleen	1/1469	2	2347 $\pm$ 170	1361	1.72
40 μg spleen	1/1607	2	1745 $\pm$ 104	1245	1.40
400 μg spleen	1/206	2	2007 $\pm$ 100	9709	0.21
2 Control PLN	1/1463	3	326 $\pm$ 18	2051	0.159
0.004 μg PLN	1/1006	3	184 $\pm$ 20	2982	0.062
0.04 μg PLN	1/1296	3	116 $\pm$ 17	2315	0.050
0.4 μg PLN	1/1229	3	130 $\pm$ 43	2441	0.053
Mixture					
Control PLN + 0.4 μg PLN	—	1.5 + 1.5	131 $\pm$ 21	2246	0.058

^a Frequency of precursors of CTL was determined using limiting dilution microcultures as described under Materials and Methods. NC = insufficient cells to perform culture.

^b The test lymph node or spleen cells from the treated mice were cultured with irradiated F_1 stimulator cells in MLC tube cultures and the resulting cytotoxic activity was measured after 5 days incubation. The number of initial CTLp per culture was calculated by multiplying cell number by the CTLp frequency.

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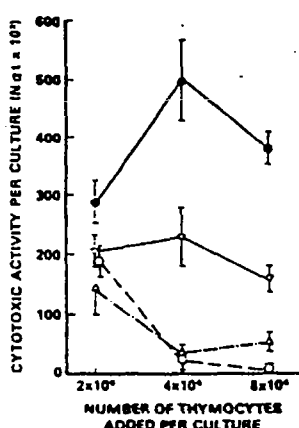


Fig. 4. Thymocytes from TCDD-treated mice suppress CTL generation *in vitro*. The effect of different numbers of thymocytes from control mice (●) or from mice given 0.004 (▽), 0.04 (○), or 0.4 (△) $\mu\text{g/kg}$ on the generation of CTL from 2×10^4 normal PLN is shown. Five to six thymus donors were used per group.

this impairment was obtained by measuring the frequency of CTLp in limiting dilution microcultures. It was found that the frequency of CTLp was not reduced by treatment with TCDD. Thus, the impaired CTL generation *in vivo* and in tube MLC cultures represented a defect in the development of CTLp into CTL. This observation suggested that TCDD treatment affects those cell types which regulate the generation of CTL in tube MLC cultures. For example, TCDD could either reduce the amount of "help" available from T helper cells and accessory cells, or increase suppressor T-cell activity that can be detected under *in vitro* conditions allowing sufficient cell-cell interaction (24). The reduction in thy 1.2-positive cells in the PLN of TCDD-treated mice (Fig. 1) is consistent with a reduction in T helper subpopulations. On the other hand, several observations have suggested that suppressor cells may be generated. Figure 3 shows that the generation of CTL *in vivo* to allogeneic P-815 tumor cells that lack Ia antigens needed to activate T helper cells (26, 27) was normal in TCDD-treated animals at early times but seemed to "shut off" as the

response continued. Secondly, mixing lymph node cells from TCDD-treated animals with those from normal mice produced suppression (experiment 2, Table II). Thirdly, thymocytes obtained from mice treated with low doses of TCDD (0.004-0.4 $\mu\text{g/kg}$) suppressed the generation of CTL *in vitro*. This suppression could not be explained by the amount of TCDD that would be present in the thymocytes. Additional experiments have shown that the presence of thymic suppressor activity parallels the impairment of CTL generation in PLN and that both alterations ultimately disappear as the dose of TCDD is progressively reduced to 0.00004 $\mu\text{g/kg}$. ((31), manuscript in preparation). Taken together, these data indicate that TCDD may impair the generation of CTL through the generation of suppressor T cells.

The mechanism by which TCDD alters the thymocyte population to produce suppression is unclear. It is unlikely that the effects of low dose TCDD on thymus can be attributed to malnutrition or to an increase in cortisone levels in the body. Malnutrition tends to deplete cells in the murine spleen and thymus and to increase the cellular and humoral immune response (28). Our mice showed normal antibody responses and DTH but impaired CTL generation at TCDD doses that caused neither lymphoid organ atrophy (Fig. 1) nor significant weight loss (data not shown). Serum cortisol levels in TCDD-treated animals have been found to be normal by Vos *et al.* (10). TCDD is known to interact with a cytoplasmic binding protein or "receptor" and this "receptor" is present in the thymus as well as liver (29, 30). Indeed, in the rat, the highest concentration of "receptor" is found in thymus (30). Thus, it is possible that TCDD may act directly on thymus to alter T-cell differentiation or perhaps the life span of certain T-cell subpopulations thereby promoting the generation of suppressor T cells. Alternatively, the alteration in thymus could be mediated indirectly through effects of TCDD on hepatic enzyme activity (5, 31).

It can be seen from Table I that there was a slight (but not statistically significant) increase in oxazolone-induced ear swelling

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after 0.4 $\mu\text{g/kg}$ TCDD and a similar effect on footpad swelling after 4 $\mu\text{g/kg}$ TCDD. The stimulatory effect of 0.4 and 4 $\mu\text{g/kg}$ TCDD on the background level of PFC has already been noted. We also found that these doses of TCDD increased the number of spleen colony-forming cells in bone marrow by 25–39% (data not shown) and thus it is possible that the 0.4 and 4 $\mu\text{g/kg}$ doses of TCDD have a small nonspecific stimulatory effect on a variety of different cell types in addition to suppressor cells that influence the immune system.

Our data showing that low doses of TCDD in mice impairs the generation of CTL may have some relevance to the effects of exposure to other HAHs. We have recently found that PCBs also impair CTL generation in mice ((31), manuscript preparation). Bekesi *et al.* (32) have reported that Michigan residents exposed to PBBs in their diet show a reduced number of T cells in blood and impaired mitogen and MLC responses *in vitro*. The effects of TCDD on the murine immune system may therefore have some generality.

The biologic significance of the immunotoxicity detected *in vitro* following exposure to low doses of TCDD and HAHs is not yet known. Our data show that a sufficiently high dose of TCDD produces impairment of the antibody response to SRBC in mice. Cattle heavily exposed to PBBs develop mastitis and furunculosis due to bacterial infections (33), and TCDD-treated mice are reported to be more susceptible to *Salmonella* infection (14). However, we and others (25) find that lower doses of TCDD impair primarily the cellular arm of the immune response, and as shown in this paper, the generation of CTL appears to be particularly sensitive. One would predict from these observations that low doses of TCDD and related HAHs should increase susceptibility to only certain types of infectious agents. For example, CTL appear to play an important role in the defense against certain types of virus infection (34, 35). Susceptibility to duck hepatitis virus (15) and susceptibility to herpesvirus infection in mice (36) is increased by PCB exposure. Experiments are currently in progress

in our laboratory to determine if the increased thymic suppressor cell activity produced by very low TCDD doses in mice confers an increased susceptibility to lethal viral infections.

We thank Ann Robson, Angela Mulvihill, Dalgeet Banwat, Fred Krestynsk, Dr. Mark McDermott, and Dr. David Basford for expert technical assistance and Dr. W. E. Rawls for helpful comments during preparation of the manuscript. We thank Roberta Axler for typing the manuscript and Rita Campbell for preparing the figures. Grant support from the Ontario Ministries of Environment and Labour is acknowledged. David A. Clark and Myron R. Szwedczuk hold scholarships from the Medical Research Council of Canada.

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Received April 16, 1981. P.S.E.B.M. 1981, Vol. 168.

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Chlorinated Dibenzodioxins and Dibenzofurans*

by James Edward Huff[†] and John S. Wassom[‡]

BOW 2160612

The problems and universal concern about chlorinated dibenzodioxin and dibenzofuran compounds were brought to the forefront by the scientific community during the National Institute of Environmental Health Sciences' (NIEHS) Conference on this subject which was held at Research Triangle Park, North Carolina, April 2 and 3, 1973. This idea for this literature collection emanated from that scientific gathering and resulted in this annotated bibliography of 242 references. These references are categorized by year and arranged alphabetically by author. The number of references per year are: 56/1973; 67/1972; 66/1971; 24/1970; 29/1969-1934. Sources searched are summarized in Table 1. Due to the time available to complete this collection, some errors and omissions were inevitable; we apologize for these and hope those using this literature survey will supply us with past, present, and future topical

reprints or citation information. We plan to maintain and up-date this file continually.

Most of the nomenclature or terms searched are listed in Table 2. Each particular author, journal, secondary abstracting service, and news copy unfortunately utilizes separate and distinct terminology when reporting on the chlorinated dibenzodioxins and dibenzofurans. As can be seen from Table 2, it is vitally important, therefore, to become thoroughly familiar with the sources being utilized before mounting a massive effort to collate all that is written or reported about a particular compound, series, or class of compounds, or subject. The magnitude of the search effort for this report is self-evident when noting all the necessary terms used.

The original papers were annotated whenever possible; some were gleaned from abstract journals. We did not alter author's remarks or conclusions; the facts are presented in these annotations as they appeared in the literature. As many salient points as possible, due to space limitations, were taken from each paper or report. Many papers and reports were referred to, after the fact, by more recent authors as having dealt intimately with the chlorinated dibenzodioxins and dibenzofurans; these were not included in this bibliography unless the dioxins or furans were mentioned specifically: most of the

* Work supported by Toxicology Information Program, National Library of Medicine; National Institute of Environmental Health Sciences; and the National Cancer Institute under contract with the Union Carbide Corporation.

† Biomedical Studies and Toxicology Information Response Center.

‡ Environmental Mutagen Information Center, P.O. Box Y, Bldg. 9224, Environmental Information System Office, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

articles were consulted and read however to determine if these compounds were present. For example, numerous articles reporting on the adverse effects of 2,4,5-T were screen-

ed but not included because direct mention of TCDD and other dioxin derivatives was missing.

Table 1. Sources and time periods searched.

Source	Period
Multidisciplinary Information Resources	
<i>Bibliography of Agriculture</i>	Vol. 21 (1957) to Vol. 37(1) (1973)
<i>Biological Abstracts</i>	Vol. 31 (1957) to Vol. 55(8) (1973)
<i>Biological and Agricultural Index</i>	Vol. 19 (1964) to Vol. 24 (1970)
<i>Bioresearch Index</i>	Vol. 1 (1965) to Vol. 9(4) (1973)
<i>Chemical Abstracts</i>	Vol. 1 (1907) to Vol. 78(20) (1973)
<i>Chemical-Biological Activities</i>	1965 to 1971
<i>Food Chemical News</i>	Vol. 13 (42) 1972 to Vol. 15(9) (1973)
<i>Health Aspects of Pesticides Abstract Bulletin</i>	Vol. 1 (1968) to Vol. 6(4) (1973)
<i>Health Effects of Environmental Pollutants Index Medicus</i>	Vol. 1 (1972) to Vol. 2(3) (1973)
<i>Pesticide Chemical News</i>	Vol. 60 (1966) to Vol. 14(5) (1973)
<i>Science Citation Index</i>	Vol. 1 (1-24) (1973)
<i>Teratology Lookout</i>	Vol. 1 (1961) to Vol. 6 (1965); 1966 to 1972
<i>Toxicology Bibliography</i>	Vol. 3 (1972)
	Vol. 1 (1968) to Vol. 6(1) (1973)
Specialized Information Centers and Libraries	
Environmental Mutagen Information Center (EMIC)	
Environmental Information System Office (EISO)	
Oak Ridge National Laboratory (ORNL)	
Toxicology Information Response Center (TIRC)	
On-Line Computer Data Bases	
MEDLine	
TOXLine	
Journals	
<i>Ambio</i>	Vol. 1 (1972) to Vol. 2 (1973)
<i>Archives of Environmental Health</i>	Vol. 7 (1963) to Vol. 26(2) (1973)
<i>Bulletin of Environmental Contamination and Toxicology</i>	Vol. 1 (1966) to Vol. 7 (1973)
<i>Clinical Toxicology</i>	Vol. 1(1) (1971) to Vol. 6(1) (1973)
<i>Environment</i>	Vol. 12 (1970) to Vol. 15(3) (1973)
<i>Environmental Health Perspectives</i>	No. 1 (1972) to No. 5 (1973)
<i>Federation Proceedings</i>	Vol. 16(1) (1957) to Vol. 32(4) (1973)
<i>Food and Cosmetic Toxicology</i>	Vol. 1 (1969) to Vol. 10(6) (1972)
<i>Journal of Agriculture and Food Chemistry</i>	Vol. 1 (1953) to Vol. 21(2) (1973)
<i>Journal of the Association of Official Analytical Chemists</i>	Vol. 40 (1957) to Vol. 56 (1973)
<i>Journal of Chromatography</i>	Vol. 1 (1958) to Vol. 73 (1973)
<i>Mutation Research</i>	Vol. 1 (1964) to Vol. 10(6) (1972)
<i>Residue Reviews</i>	Vol. 1 (1962) to Vol. 41 (1972)
<i>Science</i>	Vol. 157 (1967) to Vol. 179 (1972)
<i>Teratology</i>	Vol. 1 (1968) to Vol. 6(2) (1972)
<i>Toxicology and Applied Pharmacology</i>	Vol. 1 (1959) to Vol. 24(3) (1973)

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ANONYMOUS. New look may be taken at trichlorophenol compounds. *Pest. Chem. News* 1(21): 9-10 April 25, 1973).

Work showing "surprisingly high" levels of dioxin (ppt) in fish caught in Vietnam has placed doubt on earlier government consideration about dioxin residues. It had been previously thought that the dioxin content of 2,4,5-T was so low that there was little opportunity of residues appearing. In addition to 2,4,5-T and silver, any compound using trichlorophenol intermediates "may be suspect".

ANONYMOUS. Correction. *Pest. Chem. News* 1(22): 2 (May 2, 1973).

The National Academy of Science's Advisory Committee on 2,4,5-T recommended that the registration of 2,4,5-T be restored with the following exceptions: (a) a permissible residue of not more than 0.1 ppm 2,4,5-T on edible parts of food products and in water for human consumption and (b) a limit of 0.5 ppm of contamination with TCDD, except that in all formulations to be used around the home and recreational areas, TCDD contamination should be limited to 0.1 ppm.

ANONYMOUS. Recall program, another look at trichlorophenols urged on EPA. *Pest Chem. News* 1(23): 3-5 (May 9, 1973).

The General Accounting Office urged the Environmental Protection Agency (EPA) to implement full-scale recall procedures for suspended pesticides and raised questions on the dioxin content of trichlorophenol herbicides. GAO stated that "because silvex, ronnel, erbon, and hexachlorophene can contain the same level of dioxin as 2,4,5-T and because a safe level of dioxin has not been determined, we believe EPA should establish a standard for dioxin content and prohibit the use of all pesticides containing dioxin in excess of the established standard."

BAUGHMAN, R. W., and MESELSON, M. S. An analytical method for detecting TCDD (dioxin): levels of TCDD in samples from Vietnam. *Environ. Health Perspect.* (No. 5): 27 (1973).

An analytic procedure involving extensive clean-up and mass spectroscopy detects approximately one picogram of TCDD. The method separates TCDD from DDE, PCB's, and other chlorinated hydrocarbon residues.

BOWES, G. W., SIMONEIT, B. R., BURLINGAME, A. L., DE LAPPE, B. W., PEAKALL, D. B., and RISEBROUGH, R. W. The search for chlorinated dibenzofurans and chlorinated dibenzodioxins in wildlife populations showing elevated levels of

embryonic death. *Environ. Health Perspect.* (No. 5): 191 (1973).

Embryonic deaths have been recorded in the laboratory among birds treated with PCB. These deaths have been attributed to chlorinated dibenzofuran contaminants. Also, birth defects in wild populations of birds and sea lions are believed caused by chlorinated dibenzodioxins. High-resolution mass spectrometry was used to examine prepared from aborted sea lions and dead embryos of the herring gull and osprey.

CROSBY, D. G., MOILANEN, K. W., and WONG, A. S. Environmental generation and degradation of dibenzodioxins and dibenzofurans. *Environ. Health Perspect.* (No. 5): 259 (1973).

Both the chlorinated dibenzodioxins and dibenzofurans are unstable to light in the presence of organic substrates. Even if generated under environmental conditions, light provides a mechanism for rapid destruction.

CRUMMETT, W. B., and STEHL, R. H. Determination of chlorinated dibenzodioxins and dibenzofurans in various materials. *Environ. Health Perspect.* (No. 5): 15 (1973).

Chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans can be determined in chlorinated phenols, chlorinated phenoxy herbicides, ronnel, fat, and combustion products by such analytical techniques as gas chromatography, liquid chromatography, thin-layer chromatography, and gas chromatography-mass spectrometry.

DOUGHERTY, W. H.; COULSTON, F.; GOLBERG, L. Non-teratogenicity of 2,4,5-trichlorophenoxyacetic acid in monkeys (*Macaca mulatta*). Twelfth Annual Meeting, Society of Toxicology, New York, NY (March 18-22, 1973), Abstract 9, p. 7.

Technical grade 2,4,5-T which contained less than 0.05 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin was administered orally to forty pregnant Rhesus monkeys daily from day 22 through day 38 of gestation. Dose levels used in the experiment were 0.05, 1.0, and 10.0 mg/kg. Hematology, clinical chemistry, and urinalysis data were recorded for all females before and at various times following treatment until parturition; no toxicity was observed. Examination of live born infants revealed no terata.

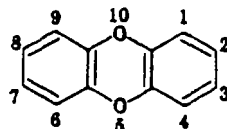
ENVIRONMENTAL PROTECTION AGENCY, PUBLICATIONS AND INFORMATION SECTION. Toxicology and pharmacology of 2,4,5-T

Environmental Health Perspectives

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Table 2. Terms searched.

Nomenclature	Chemical Abstracts registry number	Chlorinated
		Chlorinated dibenzofuran(s)
		1-322 Chlorodibenzo-
		K-322 benzo
		K-322 Dibenzo-
		K-322 dibenzofuran
		K-1476 Furan
		K-466 Halogenated dibenzofuran(s)
		K-466 Polychlorinated dibenzofurans



10 Dibenzo-p-dioxin (diphenylene dioxide) (phenodioxin)	262-12-4
K-717 2,3-Dichloro-	29446-15-9
K-7453 2,7-Dichloro-	33857-26-0
X 2,3,7-Trichloro-	33857-28-2
DD-528 1,2,3,4-Tetrachloro-	30748-58-8
DD-6099-2 2,3,6,8-Tetrachloro-	33423-92-8
X 2,3,6,7-Tetrachloro-	
K-6466 2,3,7,8-Tetrachloro	1746-01-6
X Pentachloro-	36088-22-9
K-316 Hexachloro-	34465-46-8
K-3476 2,3,7,8,9-Hexachloro	19408-74-3
X Heptachloro-	
K-5176 1,2,3,4,6,7,8,9-Octachloro-	3268-87-9
X Benzo-	
X Biphenyl-	
X Chlorinated	
X Chlorinated dibenzodioxin(s)	
X Chlorinated dibenzo-p-dioxin(s)	
X Dibenzo-	
X Dibenzo	
X Chlorodibenzo dioxin(s)	
DD-528 Chlorodibenzo-p-dioxin(s)	
DD-528 Dibenzodioxin(s) K-7453	
X Dibenzo-p-dioxins	
X Dibenzo-p-dioxins, tetra-chloro-	
X Dibenzo-p-dioxin, other chloro-	
K-6305 Dioxin	
X Halogenated dibenzo dioxin(s)	
X Halogenated dibenzo-p-dioxin(s)	
X OCDD	
X Polychlorinated dibenzodioxin	
K-666 Polychlorodibenzodioxin	
K-666 TCDD	
Dibenzofuran (biphenylene oxide)	132-64-9
K-304 3-Chloro-	25074-67-3
K-7453 2,4-Dichloro-	24478-74-8
K-304 2,4-Trichloro-	24478-73-7
K-304 Tetrachloro-	23076-57-6
K-304 2,3,4-Tetrachloro-	24478-72-8
K-304 Pentachloro-	23076-58-7
X Benzo-	
X Biphenyl-	

1973

ANONYMOUS. Herbicides better for birds than bacteria. *Food Cosmet. Toxicol.* 11(1): 149-150 (1973).

Teratogenic effects of the 2,4,5-T contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), are well known and the effect of this compound on liver enzymes resembles that of certain carcinogens. Dioxin has also been associated with mutagenic properties through its possible intercalation with DNA; the observed effects resemble those of acridine.

ANONYMOUS. TCDD residue disappears. *Down To Earth* 28(4): 18 (Spring 1973).

TCDD was not detected (<1 ppm) in 3-ft soil core samples in a sandy area where a total of 947 lb of 2,4,5-T per acre was applied over a 3-yr period. No TCDD residues were found (<0.05 ppm) in bald eagle tissue gathered from 15 states.

ANONYMOUS. 2,4,5-T comes to public attention again. *Pest. Chem. News* 1(19): 6-8 April 11, 1973).

EPA was asked to suspend the remaining uses of 2,4,5-T until the extent, if any, of TCDD-contaminated food chains is determined. TCDD was labeled a cumulative poison and one of the most potent agents of birth defects in animals ever discovered.

ANONYMOUS. Health hazard of dioxins still uncertain. *Chem. Eng. News* 51(16): 12 (April 16, 1973).

A selected summary report of the National Institute of Environmental Health Sciences meeting on chlorinated dibenzodioxins and dibenzofurans held at Research Triangle Park in North Carolina on April 2-3, 1973. The conference as a whole, however, seemed to raise as many questions as it answered. From the data discussed there is no doubt that these contaminants are highly toxic and teratogenic, but there is still some doubt as to how much of an actual hazard they represent to human health.

September 1973

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(includes dioxins). Bibliography Number 73-04 (February 1973).

A bibliographic listing of 62 references pertaining to 2,4,5-T and dioxins.

ENVIRONMENTAL PROTECTION AGENCY, PUBLICATIONS AND INFORMATION SECTION. Chemistry and residues of 2,4,5-T (includes dioxins). Bibliography Number 73-05 (February 1973).

A bibliographic collection of 58 references on 2,4,5-T containing a limited number of citations on dioxins.

ENVIRONMENTAL PROTECTION AGENCY AND INSTITUTE OF RURAL ENVIRONMENTAL HEALTH. Environmental chemicals: human and animal health. Fort Collins, CO (July 23-27, 1973).

The Institute of Rural Environmental Health, Colorado State University, and the Office of Pesticide Programs, U. S. Environmental Protection Agency, conducted a one-week course on environmental problems, contaminants, toxicants, and chemicals (including dioxins); and human and animal health problems; and poisoning.

FIRESTONE, D. Etiology of chick edema disease. *Environ. Health Perspect.* (No. 5): 59 (1973).

Early work indicated that chick edema factors (CEF) were chlorinated aromatic compounds; later, the compounds were shown to belong to a family of chlorodibenzo-p-dioxins. Further investigation showed that (a) chlorophenols were precursors of the chlorodioxins and (b) chlorodioxins and related compounds are commonly present as minor components in commercial chlorophenols. Characteristic chick edema disease symptoms include excessive fluid in the heart sac and abdominal cavity followed by high mortality starting in the third week.

FOWLER, B., LUCIER, G., BROWN, H., and McDANIEL, O. Ultrastructural changes in rat liver cells following a single injection of TCDD. *Environ. Health Perspect.* (No. 5): 141 (1973).

Ultrastructure changes in rat liver microsomes and mitochondria were examined at various intervals from 1 to 30 days following a single TCDD injection of 0, 5, or 25 ug/kg. No histologic difference was noted between groups. Observed changes included: proliferation of smooth endoplasmic reticulum (SER), mild increase in rough endoplasmic reticulum (RER), moderate swelling of mitochondria; at 7 days, large aggregates of SER, massive

amounts of RER, and small numbers of moderately swollen mitochondria were seen from the 25 mg/kg dosed rats.

GREIG, J. B. Biochemical toxicity of TCDD in rat liver. *Environ. Health Perspect.* (No. 5): 211 (1973).

The persistent toxic effect of 2,3,7,8-tetrachlorodibenzodioxin in rats is evidenced by death as long as 15 weeks after a single oral dose. Alterations, however, in liver constitution (microsomes and cytochrome P-450) and drug metabolism (zoaxazolamine and hexobarbital) occurred within 24 hr after dosing.

GUPTA, B., VOS, J., MOORE, J., ZINK, J., and BULLOCK, B. C. Pathologic effects of TCDD in laboratory animals. *Environ. Health Perspect.* (No. 5): 125 (1973).

Gross and microscopic examinations were performed on rats, guinea pigs, and mice treated with TCDD. A spectrum of dose ranges and schedules were used. Lymphoid organs (thymus, spleen, and lymph nodes) were affected consistently. Thymus atrophy (dose related decrease in weight) was found to be a sensitive index of TCDD exposure. The most severe hepatic effects were seen in rats that received a lethal dose of TCDD. The magnitude of the degenerative and necrotic liver changes were diminished in guinea pigs and mice.

HARRIS, M., MOORE, J., and VOS, J. General biological effects of TCDD in laboratory animals. *Environ. Health Perspect.* (No. 5): 101 (1973).

Albino rats were grouped and treated with single oral doses of 0, 5, 25, 50, or 100 μ g/kg TCDD in an acetone-corn oil mixture. Animals that eventually died continued to lose weight until death while survivors exhibited a depressed weight gain. Ruffled hair coat, hunched posture, inactivity, and jaundice were the overt signs seen in the high dose group. Daily oral administration of 10 μ g/kg caused death in 15/16 rats with a mean time of 21.8 days. Death resulted in 9/10 female guinea pigs after receiving an oral dose of 3 μ g/kg; the mean survival time was 18.1 days. A single oral dose of 1, 10, or 50 μ g/kg to adult mice had no effect on appearance or body weight.

HUTZINGER, O., SAFE, S., WENTZELL, B. R., and ZITKO, V. Photochemical degradation of di- and octachlorodibenzofurans. *Environ. Health Perspect.* (No. 5): 267 (1973).

Irradiation of 2,8-dichlorodibenzofuran (low chlorine content) and octachlorodibenzofuran (high chlorine content) in hexane and methanol caused

decomposition to compounds which are formed by reductive dechlorination as well as polar substances.

HWANG, S. W. Effect of TCDD on biliary excretion of indocyanine green. *Environ. Health Perspect.* (No. 5): 227 (1973).

Bile flow and biliary excretion of indocyanine green (ICG) in male rats 1, 7, and 16 days after receiving a single oral dose of 5 or 25 $\mu\text{g/kg}$ TCDD. Bile flow rate increased at day 1 through day 16. During a 20-min. collection period, both the concentration and total ICG excreted in bile decreased. ICG disappearance rate decreased with time. These effects were dose related. In contrast, the 5 $\mu\text{g/kg}$ dosed rats accumulated more ICG in liver.

JENSEN, S., and RENBERG, L. Various chlorinated dimers present in several technical chlorophenols used as fungicides. *Environ. Health Perspect.* (No. 5): 37 (1973).

The presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 2,4,5-trichlorophenoxy acid esters originates from 2,4,5-trichlorophenol during the manufacturing process. Dimerization occurs when the phenol is produced by the action of alkali on tetrachlorobenzene. All products originating from alkali-treated chlorinated benzenes logically may contain chlorinated dibenzo-*p*-dioxins. Dimers in pentachlorophenol and in 2,4,6-tri- and 2,3,4,6-tetrachlorophenols from direct chlorination of phenol were presented.

JOHNSON, R. L., GEHRING, P. J., and KOTIBA, R. J. Chlorinated dibenzodioxins and pentachlorophenol. *Environ. Health Perspect.* (No. 5): 171 (1973).

Pentachlorophenol enjoys widespread use as a wood preservative. Commercial grades have been found to contain up to 2500 ppm chlorinated dibenzo-*p*-dioxins. The predominant dioxin is octachlorodibenzo-*p*-dioxin, one of the least toxic members. Evaluating pentachlorophenol toxicity in animals revealed that some untoward effects (chloracne, chick edema disease, and histopathologic alterations) were caused by chlorinated dibenzo-*p*-dioxin content. Purified pentachlorophenol did not produce these effects. A new procedure was capable of producing pentachlorophenol containing lowered concentrations of chlorinated dibenzo-*p*-dioxin and devoid of dioxin-like toxic effects.

KEARNEY, P. C., WOOLSON, E. A. ISENSEE, A. R., and HELLING, C. S. Tetrachlorodibenzo-*p*-dioxin in the environment: sources, fate, and decontamination. *Environ. Health Perspect.* (No. 5): 273 (1973).

TCDD does not leach in soils, does not reside in

the economic portion of plants growing in contaminated soil, degrades to about 50% after 1 yr in soils, and does not result from microbial or chemical condensation of 2,4,5-trichlorophenol in soil.

KENDE, A. S., and WADE, J. J., Synthesis of new steric and electronic analogs of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ. Health Perspect.* (No. 5): 49 (1973).

Structural-activity relationships for a series of TCDD analogs were accomplished emphasizing chemical studies in an attempt to distinguish steric from electronic requirements for toxicity. Catechol condensation was used to explore the scope and limitations of polyhalobenzene electrophiles.

KING, M. E., and SHEFNER, A. M., Carcinogenesis bioassay of chlorinated dibenzodioxins and related chemicals. *Environ. Health Perspect.* (No. 5): 163 (1973).

Chlorinated dibenzodioxins were dissolved in acetone and applied to the backs of mice three times a week to assess the activity of dioxins as complete carcinogens and/or promoting agents. Octachlorodioxin caused skin tumor formation in only one female mouse. No other dioxin produced papillomas.

LANGER, H. G. Formation of dibenzodioxins and other condensation products from chlorinated phenols and derivatives. *Environ. Health Perspect.* (No. 5): 3 (1973).

Chlorodioxins are formed in a two-step condensation reaction from ortho-substituted halophenoxy radicals or anions. Reaction of chlorine with pentachlorophenol at elevated temperature proceeds by radicals; anionic condensation products result from strongly exothermic reactions of alkali metal salts of chlorinated phenols above 300° C. Reaction product distribution depends on the total number of halogen substituents, the crystal lattice arrangement of the molecule, steric effects, and an electronic effect. Dioxin formation was the major condensation product only for sodium pentachlorophenolate.

LUCIER, G. W., MCDANIEL, O. S., FOWLER, B. A., FAEDER, E., HOOK, G., and SONAWANE, B. R. Studies on TCDD-induced changes in rat liver microsomal and mitochondrial enzymes. *Environ. Health Perspect.* (No. 5): 199 (1973).

A single oral dose of 5 or 25 $\mu\text{g/kg}$ was administered to male rats and time-course measurements were made on some hepatic microsomal and mitochondrial enzymes. Cytochrome P-450 and b-5 contents were increased, hydroxylation of aniline

was induced, microsomal protein contents were increased, aminopyrine demethylation rates were decreased, and most strikingly UDP glucuronyltransferase was increased about 8-fold.

MARTIN, R. L., PORTER, M. L., POMERANTZ, I. H. Studies on the formation potential and presence of chlorinated dibenzofurans in chlorinated biphenyls. NIEHS conference, April 2-3, 1973.

Chlorinated dibenzofurans may arise by photochemical alteration of chlorinated biphenyls under appropriate conditions; this did not occur with pentachlorobiphenyl or 2,2'-dichlorobiphenyl.

MATSUMURA, F., and BENEZET, H. J. Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environ. Health Perspect.* (No. 5): 253 (1973).

Most organisms capable of degrading other chlorinated hydrocarbons showed no ability to metabolize TCDD; a few exhibited a limited degree of TCDD-metabolizing activity. TCDD leached from sand to organic soil much less than did DDT. With pesticide-coated sand in aquaria containing various organisms, TCDD had the lowest biologic accumulation and affinity.

MESELSON, S. Vietnam dioxin contamination. Center for Short-Lived Phenomena, Event 51-73, No. 1611, Smithsonian Institution, Cambridge, Mass. (April 19, 1973).

Various fish and shellfish collected in 1970 from the Dong Nai and Saigon Rivers and along the Can Gio Coast contained dioxin; catfish had the highest concentration.

MESELSON, S. Vietnam dioxin contamination. Center for Short-Lived Phenomena, Event 51-73, No. 1627, Smithsonian Institution, Cambridge, Mass. (8 May 1973).

Samples of fish and crustaceans caught in Vietnam in September 1970 were analyzed for dioxin content by using mass spectrometry. Dioxin concentrations ranged from 18 to 814 ppt; Dong Nai river carp averaged 540 ppt dioxin.

MILLER, R. A., NORRIS, L. A., and HAWKES, C. L. Acute and chronic toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) in aquatic organisms. *Environ. Health Perspect.* (No. 5): 177 (1973).

Guppies and coho salmon fingerlings were exposed to dioxin concentrations ranging from 0.056 ppt to more than 0.2 ppb for 24, 48, and 96 hrs. The initial

concentration was found to be more important in causing death than duration of exposure. The coho salmon fingerlings threshold response level for all exposure periods was between 0.056 and 0.56 ppt dioxin. Mosquito larvae, oligochaete worms, and pulmonator snails were maintained in water initially dosed with 0.2 ppb dioxin. These aquatic organisms were less sensitive than fish.

MOORE, J.; GUPTA, B.; VOS, J.; ZINKL, J. Postnatal effects of maternal exposure to TCDD. *Environ. Health Perspect.* (No. 5): 81 (1973).

Maternal exposure of C5781/6 mice to TCDD caused dose-related variations in fetal kidney maturation and development. Thymuses were reduced in size; cystic kidneys developed. Mean body weights and thymus and spleen weights (absolute and relative) were reduced in litters whose mothers received 10 µg/kg TCDD; the 3 µg/kg dose group exhibited no weight deviations. Kidney effects were seen at both dose levels.

NEUBERT, D., ZENS, P., and ROTHENWALLNER, A. Survey of the teratogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian species. *Environ. Health Perspect.* (No. 5): 67 (1973).

The frequency of cleft palate induction was used as a criterion of the teratogenic effects of TCDD. Dose-response relationships and potentiating effects of TCDD with other agents were presented.

NIEHS Conference on Chlorinated Dibenzodioxins and Dibenzofurans. National Institute of Environmental Health Sciences, Research Triangle Park, N.C. (April 2-3, 1973).

The two-day conference reviewed critically and summarized the world literature and research activities on the chlorinated derivatives of dibenzodioxin and dibenzofuran. Approximately 35 papers were presented on all aspects ranging from chemical nature to ultimate biologic effects. More than 110 scientists were in attendance. The proceedings are given in full in *Environmental Health Perspectives*, Experimental No. 5, (1973). (this issue).

NORBACK, D. H., and ENGBLOM, J. F. Chlorinated dibenzo-p-dioxin distribution within rat tissues and subfractions of the liver. *Fed. Proc.* 32(3): 236 (1973) Abstract 138.

Radioactivity from orally intubated ¹⁴C-labeled octachlorodibenzo-p-dioxin in rats was confined to the liver, adipose tissue, and skin after 7 weeks on a control diet; concentrations were about 20% that in tissues of rats after 21 days.

NORBACK, D. H., ENGBLOM, J. F., and ALLEN, J. R. Chlorinated dibenzo-*p*-dioxin distribution within rat tissues and subfractions of the liver. *Environ. Health Perspect.* (No. 5): 233 (1973).

Male rats received daily for 21 days 100 μ g 14 C-labeled octachlorodibenzo-*p*-dioxin by gastric intubation. Feces contained 95% of the total dose and urine 4%. Significant levels were found in the kidneys, heart, and serum. The liver contained the highest concentration per unit weight; adipose tissue had 1/3 that of the liver. Microsomes (rough and smooth fractions) had 95% of the liver radioactivity. Urine radioactivity resided in the lipid fraction.

NOVICK, S. Dioxin. *Environment* 15(4): 23-24 (May 1973).

A news item reports the detection of dioxin in fish and shellfish used for food in Vietnam.

PLIMMER, J. R., RUTH, J. M., and WOOLSON, E. A. Mass spectrometric identification of the hepta- and octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans in technical pentachlorophenol. *J. Agr. Food Chem.* 21(1): 90-93 (1973).

The presence of contaminant dioxins and dibenzofurans in some samples of technical pentachlorophenol were confirmed by using mass spectrometry. Three samples collected in 1970 contained hexachlorodibenzo-*p*-dioxin (0.5 to 37 ppmw) and heptachlorodibenzo-*p*-dioxin (90 to 135 ppmw). It was reemphasized that high-resolution spectra do not provide chlorine orientation information.

PLIMMER, J. R. Technical pentachlorophenols—origin and analysis of base-insoluble contaminants. *Environ. Health Perspect.* (No. 5): 41 (1973).

Fats used as feed additives from hides preserved with technical pentachlorophenol have been implicated as a source of chick edema factor. Polychlorinated dibenzodioxins and dibenzofurans have been identified in the neutral fractions of pentachlorophenol by gas chromatography, mass spectrometry, and a combination of these two.

POHLAND, A. E., YANG, G. C., and BROWN, N. Analytical and confirmative techniques for dibenzo-*p*-dioxins based upon their cation radicals. *Environ. Health Perspect.* (No. 5): 9 (1973).

Chlorinated dibenzo-*p*-dioxins form cation radicals when dissolved in strong acids such as trifluoromethane sulfonic acid, in the presence of ultraviolet light, or an oxidizing agent like potassium nitrate.

These cation radicals are quantified by using electron spin resonance and visible spectroscopic techniques. A general bathochromic shift was observed with increasing chlorine content. These shifts were dependent upon the position of the chlorine atoms.

POLAND, A. P., and GLOVER, E. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: A potent inducer of δ -aminolevulinic acid synthetase. *Science* 179(4072): 476-477 (Feb. 2, 1973).

As little as 4.66×10^{-8} mole (1.5 ng) of TCDD per egg induced hepatic-aminolevulinic acid synthetase (ALAS) activity in the chick embryo. Enzyme induction was dose-related and prolonged in time: 70% of the maximum induced activity was present 5 days after a single 150 ng dose. TCDD was linked to an outbreak of porphyria cutanea tarda where 2,4,5-T was synthesized and manufactured. At least three of the 2,3,7, and 8 positions on the ring must be occupied to induce ALAS.

POLAND, A. P., and GLOVER, E. Studies on the mechanism of action of the halogenated dibenzo-*p*-dioxins. *Environ. Health Perspect.* (No. 5): 245 (1973).

Aminolevulinic acid synthetase (ALAS) was stimulated by TCDD in the chick embryo liver; 4.66×10^{-8} mole/egg (1.5 ng) caused doubling of ALAS activity and 1.55×10^{-8} mole/egg (0.5 μ g) caused a 35-fold stimulation of enzyme activity. A single dose of TCDD stimulates hepatic aryl hydrocarbon hydroxylase (AHH) and cytochrome P-450 for 35 days and more in the rat. AHH activity was induced in chick embryo liver. It is suggested that the chemically inert parent compound is not the toxic moiety, but that a highly reactive intermediate causes cell damage.

SCHWETZ, B. A., NORRIS, J. M., SPARSCHU, G. L., ROWE, V. K., GEHRING, P. J., EMERSON, J. L.; GERBIG, C. G. Chlorodibenzo-*p*-dioxin toxicology. *Environ. Health Perspect.* (No. 5): 87 (1973).

2,7-Dichlorodibenzo-(DCDD), 2,3,7,8-tetrachlorodibenzo-(TCDD), hexachlorodibenzo-(HCDD), and octachlorodibenzo-*p*-dioxin-(OCDD) were evaluated toxicology. TCDD and HCDD were acrogenic, embryo toxic (TCDD markedly so), teratogenic, and positive for chick edema factor (CEF). DCDD and OCDD were negative for acrogenicity, teratogenicity, and CEF; OCDD was embryotoxic, while DCDD was not. The lethal dose range for DCDD, TCDD, HCDD, and OCDD was g/kg, μ g/kg, mg/kg, and g/kg, respectively.

SHAPLEY, D. Herbicides: AAAS study finds dioxin in Vietnamese fish. *Science* 180 (4083): 285-286 (April 20, 1973).

Fish and shellfish from areas of South Vietnam that were heavily sprayed during the U. S. defoliation campaign contained significant quantities of dioxin. This is a news report on the data R. Baughman and M. Meselson presented to the NIEHS Conference on chlorinated dibenzodioxins and dibenzodifurans.

SHAPLEY, D. Herbicides: Agent Orange stockpile may go to the South Americans. *Science* 180(4081): 43-45 (April 6, 1973).

The U. S. Air Force has a surplus stockpile of 2,338,900 gal of Agent Orange (50% 2,4,5-T and 50% 2,4-D); some of these mixtures contain as much as 28 times the maximum acceptable safety limit of dioxin. Presently, dioxin concentrations permissible for new herbicides are 0.1 ppm and 0.5 ppm for stocks already manufactured.

VOS, J. G., MOORE, J. A., and ZINKL, J. Effect of TCDD on the immune system of laboratory animals. *Environ. Health Perspect.* (No. 5): 149 (1973).

TCDD at sublethal dose levels caused atrophy of the thymus, suppressed the cell-mediated immunity in both guinea pigs and mice, but did not affect the humoral immunity in guinea pigs.

WALDBOTT, G. L. *Effects of Environmental Pollutants*. C. V. Mosby Co., St. Louis, Mo., 1973.

Dioxin compounds are listed with other environmental pollutants as examples of airborne pollutants. These substances are contaminants of the popular weed killer, 2,4,5-T. Dioxins caused death and gastrointestinal hemorrhage in rat fetuses when mothers were treated with doses of 0.125 to 8 µg. The mutagenic effects are due to its intercalation with DNA. Dibenzofurans were implicated as being responsible for some of the toxic effects attributed to PCBs.

WEBBER, T. J. N., and BOX, D. G. The examination of tetrachlorvinphos and its formulations for the presence of tetrachlorodibenzo-p-dioxins by a gas-liquid chromatographic method. *Analyst* 98: 181-189 (1973).

A gas-liquid chromatographic analytical method was developed for use in detecting tetrachlorodibenzo-p-dioxin impurities in samples of the insecticide tetrachlorvinphos and its formulations. Tetrachlorvinphos is the Z- or trans-styrene isomer of 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate. Sequential use of silica gel and aluminum oxide column chromatography followed by concentrated sulfuric acid treatment of the resultant

eluate made it possible to detect TCDD levels down to a limit of 0.025 ppm with electron-capture detection. Tetrachlorodibenzo-p-dioxins were not detected in any of the 21 tetrachlorvinphos samples or any of its formulations selected for analysis either before or after accelerated storage at 55°C for 2 weeks.

WEISSBERG, J., and ZINKL, J. Effects of TCDD upon hemostasis and hematologic function in the rat. *Environ. Health Perspect.* (No. 5): 119 (1973).

Daily oral doses of 10 µg/kg TCDD to female rats for 10 and 14 days caused nonspecific alterations of hematopoietic function, thrombocytopenia, thrombocytoasthenia, and derangements in blood coagulation. Platelet aggregation, bleeding time, and platelet factor III activity were normal, but clot retraction was abnormal.

WILSON, J. G. Teratological potential of 2,4,5-T. *Down to Earth* 28(4): 14-17 (Spring 1973).

A chronology of the hazards of 2,4,5-T is presented. The report issued by the President's Science Advisory Committee appointed to study the 2,4,5-T question served as the basis for this review. The dioxin contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-p-dioxin, is discussed briefly as a toxicogen.

WOODS, J. S. Studies of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on mammalian hepatic δ-aminolevulinic acid synthetase. *Environ. Health Perspect.* (No. 5): 221 (1973).

No differences in hepatic δ-aminolevulinic acid synthetase activity were seen in control rats and those receiving orally 5, 25, or 100 µg/kg TCDD for up to 30 days. Mice and guinea pigs were also non-reactive. Thus, TCDD is not porphyrogenic in mammals, even at several times the LD₅₀ dose levels.

ZINKL, J., MOORE, J. A., VOS, J. G., and GUPTA, B. N. Hematologic and clinical chemical effects of 2,3,7,8-tetrachlorodibenzo-dioxin in laboratory animals. *Environ. Health Perspect.* (No. 5): 111 (1973).

TCDD-induced changes observed in female rats after 10 days treatment with 10 µg/kg and 17 days at 1 µg/kg were increases in serum glutamic oxaloacetic transaminase. After 13 days treatment with 10 µg/kg serum glutamic pyruvate transaminase was increased. Platelet depression was observed after 10 days at all dose levels.

ZITKO, V., WILDISH, D. J.; HUTZINGER, O., and CHOI, P. M. K. Acute and chronic oral

toxicity of chlorinated dibenzofurans to salmonid fishes. *Environ. Health Perspect.* (No. 5): 187 (1973).

Dry fish food was fed to juvenile Atlantic salmon contaminated with a mixture of 2.7 µg/g di-, 5,7 tri-, 2,8 tetra-, and 9,1 octachlorodibenzofuran. Median mortality was 12 ± 30 days. Only octachlorodibenzofuran was found in tissues of dead fish (0.03 µg/g in muscle and 0.2 µg/g in the gut). Fish surviving 140 days feeding contained corresponding values of 0.01 and 0.02 µg/g.

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ABELSON, P. H. Pollution by organic chemicals. *Our Chemical Environment*, J. C. Giddings and M. D. Monroe, Eds., Canfield Press, San Francisco, 1972, Chap. 28, pp. 183-184.

The most toxic chlorine-containing compound known is 2,3,7,8-tetrachlorodibenzodioxine (C₁₂H₄O₂Cl₄), often called dioxin. The acute oral LD₅₀ dose in male guinea pigs is about 10⁻⁴ g/kg. In spite of its toxicity, the behavior of dioxin in the food chain has not been worked out.

ANONYMOUS. TCDD residues disappear. *Agr. Res.* 21(4): 6 (1972).

The 2,4,5-T contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), can be formed during synthesis of some chlorinated phenols if high temperatures are used.

ANONYMOUS. Research heightens concern over PCB's. *Chem. Eng. News* 50(10): 27-28 (1972).

Certain polychlorinated biphenyls may contain traces of tetrachlorodibenzofuran. A contaminant found in PCB's, not conclusively identified, has the same mass spectrum as tetrachloro-p-dibenzofuran.

ANONYMOUS. Dioxin with a bang! *Food Cosmet. Toxicol.* 10(1): 110-111 (1972).

During a manufacturing plant explosion, dioxin was formed by the interaction of sodium 2,4,5-trichlorophenolate molecules under the influence of the exothermic decomposition of sodium 2-hydroxyethoxide.

ANONYMOUS. . . . But 2,4,5-T is in the dock again. *Food Cosmet. Toxicol.* 10(5): 722 (1972).

Negative teratogenic results have been reported for rats and rabbits treated with 2,4,5-T samples with very minute levels of dioxin impurity. Teratogenic effects have been detected, however, in three mouse strains treated with 2,4,5-T containing as little as 0.05 or 0.5 ppm dioxin.

ANONYMOUS. 2,4,5-T and dioxins accused of teratogenicity. *Food Chem. News* 13(43): 24-27 (Jan. 17, 1972).

Responding to challenges on 2,4,5-T cancellation, EPA concentrated its replies on lack of proof that 2,4,5-T and contaminants are not teratogens. The dose-response curves for 2,4,5-T and dioxin (TCDD) have not been determined, and the possibility of no effect levels is only a matter of conjecture.

ANONYMOUS. FDA annual report shows increase in enforcement. *Food Chem. News* 13(47): 20 (Feb. 14, 1972).

In the herbicide section of its 1971 annual report, FDA said the toxicity of 2,4,5-T is chiefly due to dioxins.

ANONYMOUS. Senate settlement expected on pesticides bill. *Food Chem. News* 14(27): 53-54 (Sept. 25, 1972).

A wide range of teratogenic dioxins can be produced both in the manufacture of 2,4,5-T and during pyrolysis (incomplete combustion).

BOER, F. P., NEUMAN, M. A., and ANILINE, O. 2,8-Dichlorodibenzo-p-dioxin. *Acta Crystallogr.* B28(9): 2878-2880 (1972).

Crystals of 2,8-dichlorodibenzo-p-dioxin are orthorhombic; molecules are slightly nonplanar with an unusual packing arrangement.

BOER, F. P., and NORTH, P. P. Crystal and molecular structure of 2,7-dichlorodibenzo-p-dioxin. *Acta Crystallogr.* B28(5): 1613-1616 (1972).

Three-dimensional single-crystal x-ray diffraction data revealed the crystal and molecular structure of 2,7-dichlorodibenzo-p-dioxin. The C-Cl bond distance is 1.742 Å, the C-O distances are 1.380 and 1.382, and the 6 C-C distances range between 1.370 and 1.397. The C-O-C angle in the heterocyclic ring is 116.3°.

BOER, F. P., VAN REMOORTERE, F. P., and MUELDER, W. W. Preparation and structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,7-dichlorodibenzo-p-dioxin. *J. Amer. Chem. Soc.* 94(3): 1006-1007 (1972).

The preparation, isolation, and isometric structures of 2,3,7,8-tetrachloro and 2,7-dichlorodibenzo-p-dioxin are described.

BOER, F. P., VAN REMOORTERE, F. P., NORTH, P. P., and NEUMAN, M. A. Crystal and molecular structure of 2,3,7,8-tetrachloro-

lorodibenzo-p-dioxin. *Acta Crystallogr.* B28(4): 1023-1029 (1972).

2,3,7,8-Tetrachlorodibenzo-p-dioxin was studied by using three-dimensional single-crystal x-ray diffraction. The four unique C-Cl distances range from 1.726 to 1.730 Å, the 4 C-O distances from 1.377 to 1.379, and the 12 C-C bonds are all between 1.374 and 1.388. The C-O-C angles are 115.6° and 115.8°.

BRENNER, K. S., MULLER, K., and SATTEL, P. Detection and determination of 2,3,7,8-tetrachloro dibenzo-p-dioxin in chloro-substituted phenoxyalkane acids. *J. Chromatogr.* 64: 39-48 (1972).

Dioxin separation from the herbicide phenoxyalkane acids was accomplished by extractive distillation of the potassium salts with n-hexane in the Bleidner apparatus. Quantitative determination of the hexane extracts for dioxin was done by gas chromatography. Sensitivity was about 0.1 ppm.

BROOKS, G. T. Pesticides in Britain. In: *Environmental Toxicology of Pesticides*, F. Matsumura, G. M. Boush, and T. Misato, Eds., Academic Press, New York, 1972, pp. 61-114.

The reported teratogenic effects of 2,4,5-T in mammals and the isolation of the highly toxic contaminant tetrachlorodibenzo-p-dioxin are cautionary, but 2,4,5-T has been used in Europe for nearly 15 years without evidence of ill effects. More studies are needed in comparative detoxication between mammals, birds, fishes, and insects.

BROWNRIGG, J. T., EASTWOOD, D., and HORNIG, A. W. Identification of polychlorinated biphenyls in the presence of DDT-type compounds. Office of Research and Monitoring, U.S. Environmental Protection Agency, Washington, DC (Oct. 1972), EPA-R2-72-004.

Low temperature (77°K) luminescence techniques could in principle be applied to a wide variety of compounds including the highly toxic chlorinated dibenzofurans and dibenzo-p-dioxins.

BUU-HOI, N. P., CHANH, P.-H., SESQUE, G., AZUM-GELADE, M. C., and SAINT-RUF, G. Enzymatic functions as targets of the toxicity of "dioxin" (2,3,7,8-tetrachlorodibenzo-p-dioxin). *Naturwiss.* 59(4): 173-174 (1972).

Following a single I.P dose of 10 mg/kg dioxin to rats, deep perturbations in several enzymatic systems were observed, i.e., a decrease in cholinesterase or an increase in serum glutamic oxaloacetic acid

transaminase. Effects in homeostasis indicate the liver is one of the main targets for dioxin intoxication.

BUU-HOI, N. P., CHANH, P.-H., SESQUE, G., AZUM-GELADE, M. C.; SAINT-RUF, G. Organs as targets of "dioxin" (2,3,7,8-tetrachlorodibenzo-p-dioxin). *Naturwiss.* 59(4): 174-175 (1972).

Organ damage was found along with weight loss and hematologic effects following IP injection of 1 and 10 mg/kg dioxin to rats: Ten days after treatment, damage was observed to the liver, thymus, heart. Less damage was observed in the lungs and blood cells.

BUU-HOI, N. P., SAINT-RUF, G., and MANGANE, M. Fragmentation of dibenzo-p-dioxin and its derivatives under electron impact. *J. Heterocycl. Chem.* 9(3): 691-693 (1972).

Mass spectra were reported and discussed on five chloro derivatives of dibenzo-p-dioxin: 2,7-dichloro-, 1,6-dichloro-, 1,3,6-trichloro-, 2,3,7,8-tetrachloro-, and 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin.

CHEN, J. T. Infrared studies of chlorinated dibenzo-p-dioxins and structurally related compounds. Division of Pesticide Chemistry, 164th National Meeting of the American Chemical Society, New York, Aug. 27-Sept. 1, 1972, Abstract No. 12.

Data are reported on the reference infrared spectra of 24 chlorinated dibenzo-p-dioxins and the observed characteristic frequencies are tabulated.

COURTNEY, K. D. The teratogenic evaluation of the herbicide 2,4,5-T and dioxin. Item 4.0785, Page 1-632. Part 4. Pesticides, Environmental Protection Research Catalog, Research Information Division, U.S. EPA, Washington, D.C., Jan. 1972.

Phenoxyacetic acid herbicides and dioxins are being evaluated for teratogenic and perinatal toxic effects.

CROSBY, D. G., MOILANEN, K. W., NAKAGAWA, M., and WONG, A. S. Photoreactive reactions of pesticides. *Environmental Toxicology of Pesticides*, F. Matsumura, G. M. Boush, and T. Misato, Eds., Academic Press, New York, 1972, pp. 423-433.

Photoreactive displacement of chloride by chlorophenoxide ion in o-chlorophenols introduced

the possibility of the photochemical generation of chlorinated dibenzo-*p*-dioxins. Sunlight wavelengths exposure to sodium pentachlorophenolate yielded 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin; no 2,3,7,8-TCDD resulted from irradiated 2,4,5-T or 2,4,5-trichlorophenol.

CUNNINGHAM, H. M., and WILLIAMS, D. T. Effect of tetrachlorodibenzo-*p*-dioxin on growth rate and the synthesis of lipids and proteins in rats. *Bull. Environ. Contam. Toxicol.* 7(1): 45-51 (1972).

Protein concentration in the rat liver was slightly reduced 3 days after dioxin treatment. This decrease was accompanied by a significant increase in ¹⁴C-leucine incorporation into liver proteins. The lowest single dose of dioxin that caused an increase in rat liver weight was 0.1 µg/kg.

CURLEY, A., BURSE, V. W., and JENNINGS, R. Metabolite or contaminant of Aroclor 1254® found in rat urine. Division of Pesticide Chemistry, 163rd National Meeting of the American Chemical Society, Boston, April 9-14, 1972, Abstract No. 5.

Mass spectra of urine collected from rats on dietary levels of 100 or 500 ppm Aroclor 1254 for intervals up to eight months indicated the presence of a molecular ion at *m/e* 304 and the characteristic isotopic cluster of 4 chlorine atoms. Others have shown the presence of tetra and pentadibenzofurans (mass numbers 304 and 338) as contaminants in the foreign products.

ENVIRONMENTAL PROTECTION AGENCY, PUBLICATIONS AND INFORMATION SECTION. Chemistry and residues of 2,4,5-T (includes dioxins). Bibliography Number 72-59 (1972).

A reference list of 43 references is given.

ENVIRONMENTAL PROTECTION AGENCY, PUBLICATIONS AND INFORMATION SECTION. Toxicology and pharmacology of 2,4,5-T (includes dioxins). Bibliography Number 72-58 (1972).

A list of 75 references is given.

EPSTEIN, S. S. Environmental pathology. A review. *Amer. J. Pathol.* 66(2): 352-373 (1972).

Toxicity testing must not be confined to the test agent *per se*, but should be extended to its chemical and metabolic derivatives, its pyrolytic and degradation products and its contaminants and reaction products, especially when various derivatives or de-

gradation products are of toxicologic or environmental consequence. Dioxin pyrolytic products in phenoxy herbicides are illustrative.

FIRESTONE, D., RESS, J., BROWN, N. L., BARON, R. P., and DAMICO, J. N. Determination of polychlorodibenzo-*p*-dioxins and related compounds in commercial chlorophenols. *J. Assoc. Offic. Anal. Chem.* 55(1): 85-92 (1972).

Twenty-one commercial chlorophenols were dissolved separately in aqueous alkali, extracted with petroleum ether, fractionated on an alumina column, and examined by electron capture gas chromatography and combined gas chromatography-mass spectrometry. The 2,3,7,8-tetrachlorodioxin was found in 3 of 6 samples of 2,4,5-trichlorophenol but not in any of the 11 samples of tetra- and pentachlorophenol. Hexachlorodioxin, present in all 8 pentachlorophenols tested, ranged from 0.17 to 39 ppm. Hexa-, hepta-, and octachlorodioxins and chlorofurans were present in most of the tetra- and pentachlorophenols.

FISHBEIN, L. Human directed aspects of PCBs. In: *Polychlorinated Biphenyls and the Environment*, Report No. ITF-PCB-72-1, (COM-72-10419), Interdepartmental Task Force on PCBs, Washington, D.C., May, 1972, pp. 122-151.

Work reported previously in the literature was summarized. Emphasis was placed on the tetra- and pentachlorodibenzofuran impurities in commercial PCB products; the tri- and tetrachlorodibenzofuran single oral liver necrotic dose (0.5 to 1.0 mg/kg) in rabbits; and the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contaminant in 2,4,5-T and 2,4,5-trichlorophenol caused lethal liver necrosis and chloracne in rabbits at a dose range of 0.05 to 0.1 mg/kg.

FISHBEIN, L. Chromatographic and biological aspects of polychlorinated biphenyls. *J. Chromatog.* 68(1): 345-426 (1972).

Chromatographic methods of analysis for chlorinated dibenzofurans were reviewed as part of a more extensive review of polychlorinated biphenyls.

FISHBEIN, L., and FLAMM, W. G. Potential environmental chemical hazards. Part II. Feed additives and pesticides. *Sci. Total Environ.* 1: 31-64 (1972).

The action of alkali on 1,2,4,5-tetrachlorobenzene, a by-product from lindane synthesis, produces 2,4,5-trichlorophenol which, when interacted with sodium monochloracetate, yields 2,4,5-T. The chloracnegen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, is an impurity produced in the manufacture of 2,4,5-T: 1,2,4,5-tetra-

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chlorobenzene under the influence of high pressure and temperature, methanol, and sodium hydroxide, is converted to sodium trichlorophenolate which reacts with another molecule of sodium trichlorophenolate which reacts with another molecule of sodium trichlorophenolate with high temperatures to form TCDD; or two molecules of trichlorophenol combine to form TCDD.

GOLDMANN, P. J. Severe acute chlorine acne caused by trichlorophenol decomposition products: A contribution to the perna problem. *Arbeitsmed. Sozialmed. Arbeitshyg.* 7(1): 12-18 (1972) (Ger.).

Occupational and case histories are reported describing 2,3,6,7-tetrachlorodibenzodioxin as the causative agent in 42 cases of serious skin changes, 14 cases of internal organ damage, and 7 cases of nervous system disturbances.

GREIG, J. B. Effect of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin on drug metabolism in the rat. *Biochem. Pharmacol.* 21(23): 3196-3198 (1972).

Rats given a single oral 200 µg/kg dose of dioxin exhibited a decreased duration of soxazolamine (100 mg/kg IP) induced paralysis by 54%. After 200 µg/kg oral dioxin, sleeping time induced by hexobarbital, 150 mg/kg (male rats) or 75 mg/kg (female rats), was prolonged—more than double at 3 days. These results indicate that dioxin has simultaneous stimulatory and inhibitory effects on different pathways of oxidative drug metabolism in the rat liver.

HAMMOND, A. L. Chemical pollution: polychlorinated biphenyls. *Science* 175 (4018): 155-156 (Jan. 14, 1972).

Some commercial PCB mixtures, especially those manufactured in Europe or Japan, may contain trace amounts of dibenzofurans or other toxic impurities. These may be the cause of some toxic effects of the PCBs.

HUSSAIN, S., EHRENBURG, L., LOFROTH, G., and GEJVALL, T. Mutagenic effects of TCDD on bacterial systems. *Ambio* 1(1): 32-33 (1972).

Results from three distinct bacterial assay systems showed TCDD to be mutagenic: (1) reversion to streptomycin independency in *E. coli* SD-4, (2) reversion to histidine prototrophy in *Salmonella typhimurium* strains, and (3) prophage induction in *E. coli* K-39. The results indicated that an acridine-like behavior of DNA intercalation may have caused these genetic effects.

HUSTON, B. L. Identification of three neutral contaminants in production grade 2,4-D.

J. Agr. Food Chem. 20(3): 724-727 (1972).

Three chemical impurities of 2,4-D are profiled. These contaminants interfere with the gas-liquid chromatographic analysis of 2,4-D for 2,3,7,8-tetrachlorodibenzo-p-dioxin.

JACKSON, W. T. Regulation of mitosis. III. Cytological effects of 2,4,5-trichlorophenoxyacetic acid and of dioxin contaminants in 2,4,5-T formulations. *J. Cell Sci.* 10: 15-25 (1972).

Inhibition of mitosis and development of cytological abnormalities observed in dividing endosperm cells of the African blood lily were believed caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin, a contaminant of 2,4,5-T, rather than the herbicide itself. In contrast to 2,4,5-T, which has no effect, dramatic inhibition of mitosis was observed in cells subjected to 0.2 to 1.0 µg/l dioxin, 0.2 µg/l dioxin plus 10⁻⁴M 2,4,5-T, or 10⁻⁴M 2,4,5-T containing dioxin as a contaminant. These preparations also induced formation of dicentric bridges and chromatin fusion with formation of multinuclei or a single large nucleus.

JENSEN, S. The PCB story. *Ambio* 1(4): 123-131 (1972).

The work of Vos et. al. is reported. Tetra- and pentachlorodibenzofuran impurities in commercial PCBs may have been formed from a phenolic contaminant in PCB in similar manner as 2,3,7,8-tetrachlorodibenzo-p-dioxin is formed from 2,4,5-T.

JENSEN, S., and RENBERG, L. Contaminants in pentachlorophenol: chlorinated dioxins and predioxins (chlorinated hydroxydiphenylethers). *Ambio* 1(2): 62-65 (1972).

Octachlorodioxin and 2-hydroxynonachlorodiphenyl ether (labeled predioxin) were identified in technical pentachlorophenolate using ion exchange, diazomethane treatment, gas chromatography-mass spectrometry, and thinlayer chromatography. Predioxins have not been discovered or detected previously because: (1) clean-up procedures remove the substance, e.g., alumina column chromatography or concentrated sulfuric acid treatment of extract, (2) predioxin spontaneously forms dioxin in a gas chromatograph.

KEARNEY, P. C., WOOLSON, E. A., and EL-LINGTON, C. P. Persistence and metabolism of chlorodioxins in soils. *Environ. Sci. Technol.* 6(12): 1017-1019 (1972).

Persistence of 1, 10, or 100 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was determined by electron-capture gas chromatography in soils after 20,

40, 80, 160, and 350 days. After 1 yr, 56-68% TCDD was recovered. Neither 2,7-dichlorodibenzo-p-dioxin (DCDD) nor TCDD was detected in soils receiving 10, 100, or 1000 ppm 2,4-D or 2,4,5-T after 70 days. TCDD is degraded slowly in soils. TCDD and DCDD are not biosynthesized by microbial condensation reactions.

KHERA, K. S., and MCKINLEY, W. P. Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats. *Toxicol. Appl. Pharmacol.* 22: 14-28 (1972).

2,4,5-T (containing less than 0.5 mg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin) induced fetopathy and skeletal anomalies in progeny from females treated with a single daily oral dose of 100 to 150 mg/kg on gestation days 6 to 15. Number of conceptions and numbers of viable and dead fetuses per litter gave no indication that *in utero* treatment of offspring with up to 100 mg/kg 2,4,5-T had impaired fertility.

KIMBROUGH, R. D. Toxicity of chlorinated hydrocarbons and related compounds. A review including chlorinated dibenzodioxins and chlorinated dibenzofurans. *Arch. Environ. Health* 25(2): 125-131 (1972).

Trace amounts of chlorinated dibenzofurans and dibenzodioxins were identified as contaminants in many chlorinated technical compounds, e.g., 2,4,5-trichlorophenol, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and European chlorinated biphenyls (Phenoclor DP6 and Clophen A60). Toxic fat containing chick edema factor also contained chlorinated dibenzodioxins.

KINS, N., and BARANDY, J. Short method for the detection of chick edema factor in fats, oils, and fatty acids by electron capture gas chromatography. *J. Amer. Oil Chem. Soc.* 49(2): 115-117 (1972).

A modification of the official electron capture-gas chromatographic AOAC assay for chick edema factor, e.g., hexa-, hepta-, and octachlorodibenzo-p-dioxins, was presented to shorten the assay time and eliminate problems in the alumina column fractionation. In place of the alumina fractionation following 2,2,4-trimethylpentane extraction, a second sulfuric acid treatment and a caustic wash were done before the final sulfuric acid wash.

KRAYBILL, H. F. Chemical and physical properties of PCBs. In: Polychlorinated Biphenyls and the Environment, Report No. ITF-PCB-72-1, (COM-72-10419), Interdepartmental Task Force on PCBs,

Washington, D.C. (May 1972). pp. 22-40.

Tetrachlorodibenzofuran (mass number 304) and pentachlorodibenzofuran (mass number 338) were identified from a fractionated PCB sample.

MAIER-BODE, H. Contribution to 2,4,5-T question. *Anz. Schaedlingskd. Pflanzenschutz* 45(1): 2-6 (1972) (Ger.).

The teratogenic effect of 2,4,5-T in an earlier U.S. experiment is attributable to the content (30 ppm) of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the test sample.

MARTIN, H. (Ed.). Pesticide Manual. Basic Information on the Chemicals used as Active Components of Pesticides, 3rd. ed. British Crop Protection Council, Worcester, England (1972).

A contaminant of 2,4,5-T (2,3,7,8-tetrachlorodibenzo-p-dioxin) caused serious acne in man and produced fetal death in hamsters at 9.1 µg/kg. Modern methods of synthesis now limit the dioxin concentration in 2,4,5-T to less than 0.5 ppm.

MATSUMURA, F. Biological effects of toxic pesticidal contaminants and terminal residues. In: Environmental Toxicology of Pesticides, F. Matsumura, G. M. Boush, and T. Misato, Eds., Academic Press, New York, 1972, pp. 525-548.

Chlorinated dibenzo-p-dioxins have been classified as terminal residues-chemicals which accumulate in biologic material in the environment as a result of pesticide introduction. The presence of terminal residues in the environment is due to stable pesticides, conversion products, and chemical impurities (dioxins) that remain in the environment longer than the principal pesticides. The importance of such terminal residues in relation to the final magnitude of environmental hazardousness needs further definition.

NEUBERT, D., and DILLMANN, I. Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 272: 243-264 (1972).

Oral doses of 1 µg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in NMRI mice on days 6 to 15 of gestation produced cleft palate; embryo mortality was significant for doses of 4.5 µg/kg or more. Cleft palate without pronounced embryo lethality was produced in mice with high single doses (20-50 µg/kg) TCDD given between days 7 and 13 of pregnancy. A maximum teratogenic effect was

observed on day 11 of gestation, but another peak in cleft palate frequency occurred when TCDBD was given on day 8 of pregnancy.

NEUMAN, M. A., NORTH, P. P., and BOER, F. P. Crystal and molecular structure of octachlorodibenzo-*p*-dioxin. *Acta Crystallogr.* B28(8): 2313-2317 (1972).

Three-dimensional single-crystal x-ray diffraction was used to elucidate the crystal and molecular structure of octachlorodibenzo-*p*-dioxin. The four unique C-Cl distances ranged from 1.714 to 1.718 Å, the two C-O distances were 1.373 and 1.374, and the six C-C bonds ranged between 1.382 and 1.396. The C-O-C angle was 115.8°.

NORBACK, D. H., and ALLEN, J. R. Chlorinated aromatic hydrocarbon induced modifications of the hepatic endoplasmic reticulum: concentric membrane arrays. *Environ. Health Perspect.* 1: 137-143 (1972).

Separate groups of rats were fed diets containing 1% highly chlorinated triphenyls (PCTs, Aroclor 5460), 0.02% polychlorinated biphenyls (PCBs, Aroclor 1254), and 0.002% chlorinated diphenyl-*p*-dioxin. The dioxin and PCT groups ate readily and attained 80% of control weight in 3 weeks. Liver hypertrophy varied from moderate enlargement (dioxin group) to an increase in relative liver weight of 8/100 body weight for the PCB and PCT groups.

POHLAND, A. E., and YANG, G. C. Preparation and characterization of chlorinated dibenzo-*p*-dioxins. *J. Agr. Food Chem.* 20(6): 1093-1099 (1972).

A series of 13 chlorinated dibenzo-*p*-dioxins (nine of these were new and previously unreported) was prepared containing from 1 to 8 chlorine atoms to provide pure standards of the various chlorinated derivatives for use in methods development and toxicology studies. Synthesis, utility, yield, purity, and physical and chemical properties were presented and discussed. Stability, color reactions, and infrared, ultraviolet, nuclear magnetic resonance, and phosphorescence spectra were also reported. Phosphorescence and triplet state lifetime wavelengths were dependent on the number of chlorine atoms and their positions on the dibenzo-*p*-dioxin nucleus.

RAPPE, C., and NILSSON, C.-A. An artifact in the gas chromatographic determination of impurities in pentachlorophenol. *J. Chromatogr.* 67: 247-253 (1972).

The main impurity of commercial samples of pentachlorophenol was 3,4,5,6-tetrachloro-2-(2,3,4,5-pentachlorophenoxy)phenol; this compound underwent ring closure during gas chromatography to 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin.

SAINT-RUF, G. Formation of "dioxin" in the pyrolysis of sodium α -(2,4,5-trichlorophenoxy)-propionate. *Naturwiss.* 59 (12): 648 (1972).

A residue of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin resulted from the pyrolysis (500°C for 5 hr) of sodium- α -(2,4,5-trichlorophenoxy)-propionate, a compound used in the synthesis of Sylvest. The dioxin impurity yield was much less than that observed during 2,4,5-T synthesis.

TASK FORCE. Polychlorinated biphenyls and the environment. Report No. ITF-PCB-72-1, (COM-72-10419), Interdepartmental Task Force on PCBs, Washington, DC (May, 1972).

This report emanated from a six-month review on the polychlorinated biphenyls by five Federal agencies. The main emphasis was PCBs; however, numerous statements and discussions centered on chlorinated dibenzodioxins and dibenzofurans. For example, fractionated samples of some PCBs of foreign manufacture contained as contaminants tetra- and pentachlorodibenzofurans. The task force recommended: (1) toxicological evaluation of a select number of representative, purified PCB isomers as well as purified trace contaminants such as the chlorinated dibenzofurans; (2) definitive mammalian elaboration of the kinetics, absorption, distribution, metabolism, and excretion of the technical PCBs as well as a number of key isomers and the chlorinated dibenzofurans; and (3) elaboration of the subcellular and intracellular actions of the technical PCBs as well as a number of representative isomers and chlorinated dibenzofurans.

VASILIU, G., and BACIU, I. Acetylation of some 2,7-dihalodibenzo-*p*-dioxins. *Rev. Chim. (Bucharest)* 23(9): 523-525 (1972) (Rom.).

Reaction of 2,7-dichlorodibenzo-*p*-dioxin with chloroacetic acid in the presence of aluminum chloride and carbon disulfide gave the corresponding 3-monoacetyl derivative. Confirmation was by infrared spectra.

VOS, J. G. Toxicology of PCB's for mammals and for birds. *Environ. Health Perspect.* 1: 105-117 (1972).

Polychlorinated dibenzofurans or other toxic impurities in crude PCB preparations caused difficulty in interpreting toxicity studies.

VOS, J. G. Toxicology of polychlorinated biphenyls (PCB's) and impurities. *Tijdschr. Diergeneesk.* 97(22): 1378-1385 (1972) (Neth.).

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The most toxic impurities in polychlorinated biphenyls were found to be chlorinated benzofurans.

VOS, J. G., and NOTENBOOM-RAM, E. Comparative toxicity study of 2,4,5,2',4',5',-hexachlorobiphenyl and a polychlorinated biphenyl mixture in rabbits. *Toxicol. Appl. Pharmacol.* 23: 563-578 (1972).

The major acenegenic action of crude PCB mixtures originates from chlorinated dibenzofurans. The probable contribution of chlorinated dibenzofuran and pure PCB to the toxicity of technical PCB preparations in rabbits is discussed: chloracne (-furan ++, PCB +), edema formation (+++, -), liver damage (+++, +), and hepatic porphyria (-, ++).

WARMBRUNN, K. Considerations regarding the ban or limitation on the use of some pesticides imposed July 23, 1971. *Gesunde Pflanz.* 24(1): 6-8 (1972) (Ger).

The dioxin content of 2,4,5-T produced in Germany is approximately 1 ppm, a level at which malformation hazards are practically eliminated.

WILLIAMS, C. S. The current status of phenoxy herbicides. *Down to Earth* 27(4): 20-24 (Spring 1972).

A chronology of events from April 13, 1968 to December 8, 1971 pertaining to phenoxy herbicides is presented, with particular attention devoted to TCDD.

WILLIAMS, D. T., and BLANCHFIELD, B. J. Screening method for the detection of chlorodibenzo-p-dioxins in the presence of chlorobiphenyls, chloronaphthalenes, and chlorodibenzofurans. *J. Assoc. Offic. Anal. Chem.* 55(1): 93-95 (1972).

The chlorodibenzo-p-dioxins were chlorinated to octachlorodibenzo-p-dioxin and identified by electron capture-gas-liquid chromatography. 2,3,7,8-Tetrachlorodibenzo-p-dioxin was determined at the 1 ppm level in corn oil. This method should be considered as a screen for dioxins, since it does not distinguish among the large number of isomers.

WILLIAMS, D. T., CUNNINGHAM, H. M., and BLANCHFIELD, B. J. Distribution and excretion studies of octachlorodibenzo-p-dioxin in the rat. *Bull. Environ. Contam. Toxicol.* 7(1): 57-62 (1972).

Absorption, distribution, and excretion of octachlorodibenzo-p-dioxin in the rat were examined. The AOAC method was used to analyze organs and tissues for dioxin content. The only gross pathology observed was congestion of the liver. Octachlorodi-

benzo-p-dioxin was absorbed by the rat mainly in the liver with small amounts in the adipose tissue and bile.

WILLIAMS, D. T., and BLANCHFIELD, B. J. Improved screening method for chlorodibenzo-p-dioxins. *J. Assoc. Offic. Anal. Chem.* 55(6): 1358-1359 (1972).

Conversion of 2,3,7,8-tetrachlorodibenzo-p-dioxin to octachlorodibenzo-p-dioxin allowed detection by electron capture-gas-liquid chromatography of as low as 0.05 ppm in corn oil. The author's previous method was modified to include different chlorination conditions and a later alumina column cleanup.

WILSON, J. G. Teratological potential of 2,4,5-T. Proceedings Twenty-Fifth Annual Meeting Southern Weed Science Society, Dallas, TX, Jan. 18-20, 1972.

A chronology of the hazards of 2,4,5-T was presented. The report issued by the President's Science Advisory Committee appointed to study the 2,4,5-T question served as the basis for this review. The dioxin contaminant of 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-p-dioxin, was discussed briefly as a toxicogen.

WOOLSON, E. A., THOMAS, R. F., and ENSOR, P. D. J. Survey of polychlorodibenzo-p-dioxin content in selected pesticides. *J. Agr. Food. Chem.* 20(2): 351-354 (1972).

Electron capture-gas chromatography was used to examine 129 samples of 17 different pesticides derived from chlorophenols for polychlorinated dibenzo-p-dioxins. Clean-up entailed concentrated sulfuric acid extraction of impurities from hexane and mild nitration of the chlorophenol extracts. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was detected in the samples analyzed: 76% contained less than 0.1 µg/g, 7% between 0.1 to 1.0 µg/g, and 9% had greater than 10 µg/g. The 20 tri-, tetra-, and pentachlorophenols examined contained no TCDD. Phenoxy herbicides from current production had less than 0.5 µg/g TCDD.

YANG, G. C., and POHLAND, A. E. Electron spin resonance studies of cation radicals in trifluoromethane sulfonic acid. *J. Phys. Chem.* 76(10): 1504-1505 (1972).

Electron spin resonance spectra were obtained for chlorinated dibenzo-p-dioxins in trifluoromethane sulfonic acid (MSA); no oxidizing agents were used to produce the spectra in sulfuric acid. An oxidizing agent or ultraviolet light was needed for octachlorodibenzo-p-dioxin to yield a spectrum in MSA; no spectrum was shown in sulfuric acid.

ZITKO, V. Absence of chlorinated dibenzodi-

oxins and dibenzofurans from aquatic animals. *Bull. Environ. Contam. Toxicol.* 7(2/3): 105-110 (1972).

Chlorinated dibenzodioxin and dibenzofuran residues were not detected in any of the aquatic samples analyzed.

ZITKO, V., HUTZINGER, O., and CHOI, P. M. K. Contamination of the Bay of Fundy-Gulf of Maine area with polychlorinated biphenyls, polychlorinated terphenyls, chlorinated dibenzodioxins, and dibenzofurans. *Environ. Health Perspect.* 1: 47-50 (1972).

No residues of chlorinated dibenzodioxins and dibenzofurans were found in the samples analyzed: muscle and liver of white shark, eggs of double-crested cormorants and herring gulls, commercial herring oil, and ground fish herring meal. Detection limits ranged from 0.01 $\mu\text{g/g}$ octachlorodibenzofuran wet tissue to 0.04 $\mu\text{g/TCDD/g}$ wet tissue.

1971

ANONYMOUS. A close look at TCDD. *Agr. Res.* 20(4): 8-10 (1971).

The toxicological properties of tetrachlorodibenzo-p-dioxin were investigated following the discovery that the mutagenic and teratogenic potential of 2,4,5-T may be due to this contaminant.

ANONYMOUS. Herbicides: More research on 2,4,5-T. *Chem. Eng. News* 49(20): 11 (1971).

The President's Science Advisory Committee's report on 2,4,5-T and its dioxin impurity is reviewed. The panel made a number of recommendations, including one asking for a mechanism that would temporarily restrict the use of certain registered pesticides on the basis of information that implicates the chemical as a possible health hazard, pending the collection of more conclusive information.

ANONYMOUS. Herbicides: Secret 2,4,5-T report. *Chem. Eng. News* 49(29): 15 (1971).

An advisory committee's report submitted to the Environmental Protection Agency concerning 2,4,5-T and its dioxin contaminant was criticized by a dissenting advisory committee member and the Committee for Environmental Information. Specifically stated was that a level at which TCDD is not teratogenic has not been established and that the report did not consider the consequences of the fate of TCDD in food chains and animal tissue.

ANONYMOUS. A taste of honey (flavoured

with 2,4,5-T). *Food Cosmet. Toxicol.* 9: 152 (1971).

This is a commentary on the teratogenic evaluation of 2,4,5-T by K. D. Courtney, et al.; a note added in proof indicate the 2,4,5-T contained 30 ppm dioxin. Thus, conclusions from the paper labeling 2,4,5-T teratogenic must be considered tentative.

ANONYMOUS. The PCB story unfolds. *Food Cosmet. Toxicol.* 9(4): 568-571 (1971).

Lesions resembling those produced by chick edema factor were traced to a PCB contaminant, chlorinated dibenzofurans which is present in some commercial PCBs.

ANONYMOUS. Working with 2,4,5-T. *Food Cosmet. Toxicol.* 9: 908-909 (1971).

Chloracne, characterized by inclusion cysts, comedones, and pustules, was found in 13 workers, and was correlated in severity with the presence of scarring, hyperpigmentation, hirsutism, and complaints of eye irritation. A. P. Poland et al. commented that chloracne was not correlated with occupations within plants manufacturing 2,4,5-T, duration of employment, or coproporphyrin excretion.

ANONYMOUS. Tetrachlorodibenzodioxin—indications of carcinogenicity? *Food Cosmet. Toxicol.* 9: 909 (1971).

Although such effects as described by Buu-Hoi, et al. of the carcinomimetic activity of TCDD exist, the relationship is by no means exclusive. For example, stimulation of hydroxylating enzymes can be induced by many compounds, including BHT, which has been shown not to be carcinogenic in long-term feeding studies.

ANONYMOUS. PSAC hiccoughs over 2,4,5-T. *Nature* 231(5300): 210-211 (1971).

The President's Science Advisory Committee (PSAC) report on 2,4,5-T is questioned: justification by the PSAC concerning permissible levels of dioxin in 2,4,5-T was not based on scientific review; also, the need for establishing a dose-response curve for the teratogenicity of 2,4,5-T should have been cited.

ANONYMOUS. 2,4,5-T report attacked. *Nature* 232(5308): 218 (1971).

The response of the Committee for Environmental Information (CEI) to the scientific advisory committee's recommendation that 2,4,5-T be restored was one of strong opposition. With respect to dioxins, the CEI indicated that dioxins may accumulate in the soil and that present analytical techniques were not sensitive enough to detect them. Furthermore, lack of concrete proof that the use of 2,4,5-T was correlated with the occurrence of birth defects in Vietnam did not mean that no correlation existed.

BAUGHMAN, R. W., and MESELSON, M. An improved analysis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDBD). Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971, Abstract No. 89.

Current analytical methods for the detection of potentially hazardous levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are inadequate. A combined gas chromatography-mass spectroscopy method was utilized with modifications in extraction concentration and detection.

BEVENUE, A., and KAWANO, Y. Pesticides, pesticide residues, tolerances, and the law (U.S.A.). *Residue Rev.* 35: 103-149 (1971).

Politics and science surround the 2,4,5-T and hence dioxin controversy. Contents include: Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended through 1969; the DDT episode; the 2,4,5-T episode; Federal Food, Drug, and Cosmetic Act (FDCA); and food imports and international pesticide control. Mention is made of the USDA's investigation of possible excessive contamination of 17 polychlorophenolic pesticides by tetrachlorodibenzo-*p*-dioxin.

BOER, F. P., NEUMAN, M. A., VAN REMOORTERE, F. P., NORTH, P. P., and RINN, H. W. X-ray diffraction studies of chlorinated dibenzo-*p*-dioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 82.

The crystal structures of four chlorinated dibenzo-*p*-dioxin compounds (2,7-dichloro; 2,8-dichloro; 2,3,7,8-tetrachloro; and octachloro) were determined by x-ray diffraction. The structures were elucidated in order to provide absolute standards for isomeric structure assignment.

BUU-HOI, N. P., HIEN, D. P., SAINT-RUF, G., and SERVOIN-SIDOINE, J. Canceromimetic properties of tetrachloro-2,3,7,8 dibenzo-*p*-dioxin ("dioxin"). *C. R. Acad. Sci. (Paris)* D272(10): 1447-1450 (1971) (Fr).

Tetrachloro-2,3,7,8-dibenzo-*p*-dioxin in rats caused induction of xoxazolamine hydroxylase, marked reduction in phenobarbital sedative effect, and a decrease in hepatic arginase. Dioxin therefore, has a pronounced inhibitory effect on the enzymatic sys-

tems as do the carcinogens benzo [a]-pyrene and *p*-dimethylaminoazobenzene.

BUU-HOI, N. P., SAINT-RUF, G., BIGOT, P., and MANGANE, M. Preparation, properties and identification of "dioxin" in pyrolysates of defoliants containing 2,4,5-trichlorophenoxyacetic acid, their esters, and contaminated plants. *C. R. Acad. Sci. (Paris)* D273(7): 708-711 (1971) (Fr).

Synthesis and physico-chemical properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its less toxic isomer 1,3,6,8-tetrachlorodibenzo-*p*-dioxin are described. Caution was urged about the burning of wooded areas treated with 2,4,5-T or its butyl ester because of possible dioxin formation.

CAMPBELL, A. D., and FIRESTONE, D. Chick edema factor—toxic dioxins. In: International Symposium on Identification and Measurement of Environmental Pollutants, June 14-17, 1971, Ottawa, Ontario, Canada, B. Wesley, Ed., National Research Council, Canada, 1971, pp. 195-198.

The formation, occurrence, toxicity, tissue distribution in the chick, and analytical methods for the detection of polychlorodibenzo-*p*-dioxins are described. Dioxins were implicated in chick edema disease. Dioxins belong to one of the most toxic classes of chlorinated compounds known, being almost 1000 times as toxic as most pesticides.

COLLINS, T. F. X., WILLIAMS, C. H., and GRAY, G. C. Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. *Bull. Environ. Contam. Toxicol.* 6(6): 559-567 (1971).

2,4,5-T commercial samples given on days 6 to 10 of organogenesis were fetotoxic and teratogenic in the golden Syrian hamster. The incidence of the observed effects increased with an increasing content of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Dioxin impurity caused edema and hemorrhages in newborn animals.

COMMITTEE FOR ENVIRONMENTAL INFORMATION. Critique of the report of the advisory committee on 2,4,5-T. *Environment* 13(7): 24, 29 (1971).

Criticism by the Committee for Environmental Information levied against the Advisory Committee's report on 2,4,5-T centered on the following areas: no effect levels; environmental accumulation, transmission and degradation; human hazards from

dioxins; 2,4,5-T teratogenicity; and the benefits vs. risks of 2,4,5-T usage.

COURTNEY, C. D., and MOORE, J. A. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Appl. Pharmacol.* 20(3): 396-403 (1971).

2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were teratogenic in three strains of mice; combinations of these compounds did not potentiate observed results. Both produced cleft palates and kidney malformations. Studies with rats gave negative results with 2,4,5-T; TCDD induced kidney anomalies.

CROSBY, D. G., WONG, A. S., PLIMMER, J. R., and WOOLSON, E. A. Photodecomposition of chlorinated dibenzo-p-dioxins. *Science* 173(3998): 748-749 (1971).

2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin homologs photodecompose rapidly in an alcohol solution exposed to artificial light and natural sunlight. The decomposition rate was correlated with the degree of chlorination. Negligible photodecomposition was observed in aqueous suspensions and on wet or dry soil.

DAVRING, L., and SUNNER, M. Cytogenetic effects of 2,4,5-trichlorophenoxyacetic acid on oogenesis and early embryogenesis in *Drosophila melanogaster*. *Hereditas* 68(1): 115-122 (1971).

2,4,5-T with dioxin contaminants less than 0.1 ppm was evaluated in a wild-type *Drosophila* population, Canton-S 109. Adult flies were exposed to 250 ppm 2,4,5-T in their food within or later than 24 hr of eclosion. Results indicated that this 2,4,5-T formulation effected early oogenesis and caused chromosome disturbances which could result in sterility.

ELVIDGE, D. A. The gas-chromatographic determination of 2,3,7,8-tetrachloro-p-dioxin in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-T ethyl-hexyl ester, formulations of 2,4,5-T esters and 2,4,5-trichlorophenol. *Analyst* 96(1147): 721-727 (1971).

A gas chromatographic method is described for the detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin impurities in herbicides. Dioxin recoveries ranged from 89 to 98%; limit of detection was 0.05 ppm.

EMERSON, J. L., THOMPSON, D. J., STREIBING, R. J., GERBIG, C. G., and ROBINSON, V. G. Teratogenic studies of 2,4,5-trichlorophenoxyacetic acid in the rat and rab-

bit. *Food Cosmet. Toxicol.* 9(3): 395-404 (1971).

Treatment of rabbits and rats with 2,4,5-T containing 0.5 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin did not cause any teratogenic or embryotoxic effects. Rats received 1, 3, 6, 12, or 24 mg 2,4,5-T/kg on days 6 to 15 of pregnancy. Rabbits received 0, 10, 20, or 40 mg 2,4,5-T/kg on days 6 to 18 of pregnancy.

FIRESTONE, D. Determination of polychlorodibenzo-p-dioxins (dioxins) in chlorophenols and lipids. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 80.

High resolution mass spectrometry was reported to offer a sensitive method for detecting specific dioxins after adequate sample cleanup, e.g., chromatography and gas chromatography.

FIRESTONE, D. FLICK, D. F., RESS, J., and HIGGINBOTHAM, G. R. Distribution of chick edema factors in chick tissues. *J. Assoc. Offic. Anal. Chem.* 54(6): 1293-1298 (1971).

Chickens fed toxic animal fat containing 9.9 ppm chlorinated dibenzo-p-dioxins excreted over 90% the ingested hexa-, hepta-, and octachlorodioxins. Decreased chlorination resulted in greater body retention. Residues were found in many tissues but was most prominent in the liver.

HANSEN, W. H., QUAIFFE, M. L., HABERMANN, R. T., and FITZHUGH, O. G. Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. *Toxicol. Appl. Pharmacol.* 20: 122-129 (1971).

2,4-D preparations, having undetectable levels of 2,7-dichlorodibenzo-p-dioxin or 2,3,7,8-tetrachlorodibenzo-p-dioxin, were tested for chronic toxicity in rats and dogs. No significant effect on growth, survival, organ weight, or hematologic values were noted.

HAYS, H., and RISEBROUGH, R. W. The early warning of the terms. *Natural History* 80(9): 39-46 (Nov. 1971).

Possible health implications to humans and wildlife from environmental pollution with chlorinated dibenzofurans are reviewed.

HELLING, C. S. Pesticide mobility in soils. II. Applications of soil thin-layer chromatography. *Soil Sci. Soc. Amer. Proc.* 35(5): 737-743 (1971).

Relative mobility by thin-layer chromatography and diffusion in moist and air-dry soils were used to test pesticide movements. The two chlorinated dibenzo-*p*-dioxins were immobile.

HOLDEN, C. Critics weigh EPA herbicide report, find it wanting. *Science* 173(3994): 312 (1971).

The EPA's Advisory Committee on 2,4,5-T recommended that the ban on 2,4,5-T be lifted with certain qualifications, i.e., no more than 0.1 ppm 2,4,5-T in drinking water and formulations containing no more than 0.1 ppm dioxin. The Committee for Environmental Information voiced five major objections.

ISENSEE, A. R., and JONES, G. E. Absorption and translocation of root and foliage applied 2,4-dichlorophenol, 2,7-dichlorodibenzo-*p*-dioxin, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Agr. Food Chem.* 19(6): 1210-1214 (1971).

Measured uptake of ¹⁴C-labeled 2,7-dichloro- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from nutrient solution, soil, and foliage by oats and soybeans indicated that accumulation of TCDD in plants via the soil is unlikely.

JOHNSON, J. E. The public health implications of widespread use of the phenoxy herbicides and picloram. *Bioscience* 21(17): 899-905 (Sept. 1, 1971).

A review presenting the myriad historical aspects of the dioxin-2,4,5-T (and other herbicides) picture. The potential adverse health effects from phenoxy herbicides were documented. One conclusion reported was that impurities—particularly the chlorodibenzo-*p*-dioxins—can be an important factor, but these can be controlled by proper manufacturing techniques.

JOHNSON, J. E. Safety in the development of herbicides. *Proc. Ann. Calif. Weed Conf.* 23: 43-67 (1971).

Experiments using 2,4,5-T alone and in combination with dioxin allowed the conclusion that commercially produced 2,4,5-T containing less than 1 ppm dioxin does not present a hazard to health.

JOHNSON, J. E. Safety in the development of herbicides. *Down to Earth* 27(1): 1-7 (1971).

After the discovery of highly toxic dioxin residues in the herbicides 2,4,5-T, precautionary control measures were instituted to insure greater quality control in pesticide production. Pregnant rats were treated orally with 2,4,5-T at doses of up to 100 mg/kg/day. No symptoms of teratology were pro-

duced in the offspring. Dioxin was toxic to embryos at 0.125 µg/kg/day, whereas 0.03 µg/kg/day was below the "no-effect" level. Some aspects of production and costs are discussed.

KEARNEY, P. C., and WOOLSON, E. A. Persistence and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in soils. Division Pesticide Chemistry, 161st National Meeting of the American Chemical Society, Los Angeles, March 28-April 2, 1971; Abstract No. 21.

TCDD applied 1, 10, and 100 ppm to two soils held at 30°C remained relatively unchanged (80-85% of original samples were recovered) after 20, 40, 80, and 160 days. Incubation of 10, 100, and 1000 ppm, 2,4-dichlorophenol, and 2,4,5-trichlorophenol in soils for 70 days produced no detectable di- or trichlorodioxins.

KEARNEY, P. C., ISENSEE, A., HELLING, C. S., WOOLSON, E. A., and PLIMMER, J. R. Environmental significance of chlorodioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 90.

Studies on TCDD under controlled environmental conditions revealed that TCDD was persistent and immobile in soil, not readily absorbed by plants, subject to photodecomposition, and slowly degraded to polar metabolites. Other studies revealed that the environmental contamination is small and not detectable in biological samples.

KHERA, K. S., and RUDDICK, J. A. Perinatal effects of dibenzodioxins in Wistar rats. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C. Sept. 12-17, 1971; Abstract No. 87.

Oral administration of 16 to 0.125 µg/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to pregnant rats on days 6-15 of gestation caused a reduction in viable litter size and a high incidence of resorptions. No effect was observed at the 0.125 level. Histological examination of fetuses revealed brain and intestinal hemorrhages and edema of subcutaneous tissue.

KHERA, K. S., HUSTON, B. L., and MCKINNEY, W. P. Pre- and postnatal studies on 2,4,5-T, 2,4-D, and derivatives in Wistar rats. *Toxicol. Appl. Pharmacol.* 19:369-370 (1971).

Results from teratogenic studies with rats treated with 2,4,5-T, 2,4-D, and several derivatives were

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either negative or inconclusive. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in these preparations were not known in all cases, but most were suspected to be less than 0.5 ppm.

LANGER, H. G., BRADY, T. P., DALTON, L. A., SHANNON, T. W., and BRIGGS, P. R. Thermal chemistry of chlorinated phenols. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 83.

At the decomposition temperatures of chlorinated phenols and derivatives, the following conditions are ideal for dioxin formation: (a) high concentration of the phenol, (b) high degree of chlorination of the phenol, and (c) presence of base.

LOEFROTH, G. Environmental poisons, man and the physician. *Laekartidningen* 68 (18): 2113-2133 (1971) (Swed.).

Possible adverse health effects from environmental poisons demands caution. Tetrachlorobenzo-*p*-dioxin was mentioned for its adverse effects of fetal development.

MILNES, M. H. Formation of 2,3,7,8-tetrachlorodibenzodioxin by thermal decomposition of sodium 2,4,5-trichlorophenolate. *Nature* (London) 232(5810): 395-396 (1971).

Hydrolysis of 1,2,4,5-tetrachlorobenzene in methyl alcohol with sodium hydroxide at high pressures or in ethylene glycol at 1 atm formed sodium monochlorophenolate. This allowed exothermic decomposition of 2,4,5-trichlorophenolate sodium to 2,3,7,8-tetrachlorodibenzodioxin.

MUELDER, W. W., and SHADOFF, L. The preparation of uniformly labeled ¹⁴C-2,7-dichlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 78.

2,7-Dichlorodibenzo-*p*-dioxin was prepared from isotopic potassium 2,4-dichlorophenolate uniformly labeled with ¹⁴C. Chlorination of the dioxin in a chloroform solution containing FeCl₃ and I₂ gave a mixture of tri-, tetra-, and pentachlorosubstitution products. Purification by digestion in boiling chloroform, fractional sublimation, and recrystallization from anisole yielded a product containing 92% 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

PIPER, W. N., and ROSE, J. Q. The excretion and tissue distribution of 2,3,7,8-tetra-

chlorodibenzo-*p*-dioxin in the rat. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 88.

TCDD was administered to male rats in a single oral dose of 50 µg/kg. The primary excretion route was via the feces and the highest tissue concentration was in the liver.

PLIMMER, J. R., CROSBY, D. G., WONG, A. S., and KLINGEBIEL, U. I. Photochemistry of dibenzo-*p*-dioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 85.

Ring closure and photochemical reaction of *o*-chlorophenols yield dioxin. Dibenzo-*p*-dioxins were decomposed in solution by light or sunlight. Absorption of dioxin on soil reduced its rate of photodecomposition.

PLIMMER, J. R., and KLINGEBIEL, U. I. Riboflavin photosensitized oxidation of 2,4-dichlorophenol: assessment of possible chlorinated dioxin formation. *Science* 174(4007): 407-408 (1971).

Chlorophenols in aqueous solution were exposed to the action of light in wavelengths greater than 280 nm. Products were characterized by gas chromatography and mass spectrometry. Possible chlorinated dibenzo-*p*-dioxins from ring closure of a 2-phenoxyphenol derivative were not detected in the photolysis products. Under environmental conditions, dioxins are unlikely products of lower chlorinated phenols or phenoxy-alkanoic acids.

POHLAND, A. E., YANG, G. C., and HANSEN, E. A. The preparation and characterization of chlorinated dibenzo-*p*-dioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 77.

Twelve chlorinated dibenzo-*p*-dioxin standard compounds containing from one to eight chlorine atoms were prepared as standards to develop analytical techniques and to use in toxicity studies. The wavelength associated with the observed phosphorescence and the triplet state lifetime were dependent upon the number of chlorine atoms and their positions on the dibenzo-*p*-dioxin nucleus.

POLAND, A. P., SMITH, D., METTER, G., and POSSICK, P. A health survey of workers

in a 2,4-D and 2,4,5-T plant. *Arch. Environ. Health* 22:316-327 (1971).

A study and review of employee health in a plant producing 2,4,5-T and 2,4-D. Chloracne was found in 13/73 male workers and was believed caused by chlorinated dioxins. The plant has begun several programs to reduce dioxin levels in 2,4,5-trichlorophenol; the contaminant concentration has dropped from 10 to 25 ppm to 1 ppm.

PORTER, M. L., and BURKE, J. A. Separation of three chlorodibenzo-*p*-dioxins from some polychlorinated biphenyls by chromatography on an aluminum oxide column.

Chlorodioxins (2,3-di-, 2,3,7-tri-, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) were separated from polychlorinated biphenyls by column chromatography on aluminum oxide. Recoveries of both dioxins and the polychlorinated biphenyls were approximately 100%.

RISEBROUGH, R. W. Determination of polychlorinated biphenyls in environmental samples. In International Symposium on Identification and Measurement of Environmental Pollutants, Ottawa, Ontario, Canada, June 14-17, 1971, B. Westley, Ed., National Research Council, Canada, 1971, pp. 147-153.

Even though it is not known whether dibenzofurans can be formed from environmental residues of polychlorinated biphenyls (PCB), they should be considered as a part of the PCB problem. Dibenzofurans have been found in commercial PCB mixtures sampled from France, Germany, and the United States in concentrations ranging from 5 to 20 ppm. The European samples had higher levels than the U.S. sample.

ROLL, R. Teratogenic effect of 2,4,5-T [2,4,5-trichlorophenoxyacetic acid] in mice. *Food Cosmet. Toxicol.* 9(5): 671-676 (1971) (Ger).

2,4,5-T having a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin content of less than 0.1 ppm caused embryotoxic effects and a significant increase in the incidence of cleft palate in mice. Concentrations used in the study ranged from 35 to 130 mg/kg 2,4,5-T/dioxin which were given orally on days 8-15 of pregnancy. The teratogenic "no-effect" level of 2,4,5-T/dioxin was found to be 20 mg/kg.

ROWE, V. K., NORRIS, J. M., SPARSCHU, G. L., SCHWETZ, B. A., and GEHRING, P. J. Toxicology of chlorinated dibenzo-*p*-dioxins. Division Pesticide Chemistry,

162nd National Meeting of the American Chemical Division, Washington, D.C., Sept. 12-17, 1971, Abstract No. 86.

The toxicology of several chlorinated dibenzo-*p*-dioxins were investigated in rats, guinea pigs, and rabbits. The compounds studied were 2,7-dichlorodibenzo-*p*-dioxin (DCDPD); 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); hexachlorodibenzo-*p*-dioxin (HCDPD), and octachlorodibenzo-*p*-dioxin (OCDDP). TCDD was found to be the most toxic of the compounds investigated. DCDPD and OCDDP were the least toxic. For instance, little if any fetotoxic effects were observed with these two compounds; doses of 2 to 4 g/kg were not lethal to rats. On the other hand, TCDD and HCDPD induced chloracne, fetotoxicity, and teratogenic effects.

SCHWETZ, B. A., SPARSCHU, G. L., and GEHRING, P. J. The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. *Food Cosmet. Toxicol.* 9:801-817 (1971).

The teratogenicity of 2,4-D was investigated in the rat. Formation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the manufacture of 2,4-D is not theoretically possible. The 2,4-D sample used was analyzed for the TCDD contaminant with a method having a 0.2 ppm sensitivity and none was found. Doses of 12.5, 25, 50, 75, and 87.5 mg/kg 2,4-D were administered orally on days 6-15 of gestation. High dose levels caused signs of embryotoxicity and fetotoxicity.

SOMERS, E., and SMITH, D. M. Source and occurrence of environmental contaminants. *Food Cosmet. Toxicol.* 9(2): 185-193 (1971).

The sources, occurrences, and some of the toxicological effects of polychlorinated biphenyls and chlorodioxins were discussed. Chlorophenols exposed to high temperatures may form chlorinated derivatives of dibenzo-*p*-dioxin. Inadequate methodology prevents analysis for these contaminants in foods.

SPARSCHU, G. L., DUNN, F. L., LISOWE, R. W., and ROWE, V. K. Study on the effects of high levels of 2,4,5-trichlorophenoxyacetic acid on foetal development in the rat. *Food Cosmet. Toxicol.* 9(4): 527-530 (1971).

Commercial-grade 2,4,5-T containing 0.5 ppm 2,3,7,8-tetrachlorodibenzo-*p*-dioxin did not induce a teratogenic response when administered orally to rats on days 6 to 15 of gestation at a dosage level of 50 mg/kg/day.

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SPARSCHU, G. L., DUNN, F. L., and ROWE, V. K. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Teratology* 4: 247 (Abstract) (1971).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was administered orally to pregnant rats during days 6 to 15 of gestation in doses of 0, 0.03, 0.125, 0.5, 2.0, and 8.0 g/kg/day [sic]. No effect was observed on the fetus or mother at the 0.03 dose level. However, at the 0.125 level and above, fetal mortality, early and late resorptions, and fetal intestinal hemorrhage occurred. The effects became more pronounced with the increase in dose. Maternal toxicity was noted beginning at the 0.5 level which also increased with the dose.

SPARSCHU, G. L., DUNN, F. L., and ROWE, V. K. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Food Cosmet. Toxicol.* 9(3): 405-412 (1971).

Investigations of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats revealed that this compound is capable of inducing a high degree of adverse effects in the fetus and mother. Doses of 0, 0.03, 0.125, 0.5, 2.0, and 8.0 µg/kg/day were given orally to pregnant rats during days 6 to 15 of gestation. Effects such as fetal mortality, intestinal hemorrhage, and early and late resorptions began appearing at the 0.125 level and increased with dose. It is suggested that the teratogenic effects of 2,4,5-T observed in other studies may have been due to this contaminant (30 ppm).

STEHL, R. H., PAPENFUSS, R. R., BREDWEG, R. A., and ROBERTS, R. W. The stability of pentachlorophenol and chlorinated dioxins to sunlight, heat, and combustion. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971; Abstract No. 92.

Degradation of pentachlorophenol in the environment could theoretically lead to the formation of chlorinated dibenzo-*p*-dioxins. Several simulated environmental conditions were checked for possible dioxin formation. Pentachlorophenol-treated wood and paper indicated no increase in dioxins when burned; likewise photolysis of sodium pentachlorophenol yielded only a trace of the compound. 2,7-Di- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were rapidly decomposed under artificial sunlight, whereas octachlorodibenzo-*p*-dioxin was not.

STEHL, R. H., WILKE, E., PAPENFUSS, R. R., and MATALON, R. Determination of non-

phenolic impurities in chlorinated phenols and related compounds. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Division, Washington, D.C., Sept. 12-17, Abstract No. 81.

Analytical techniques for the detection of neutral impurities (chlorinated dibenzo-*p*-dioxins) in chlorinated phenols, were ion-exchange chromatography for removal of the matrix, liquid-liquid chromatography for separation of the various neutral components, followed by gas chromatographic examination of the separated components using electron capture detection.

STORHERR, R. W., WATTS, R. R., GARDNER, A. M., and OSGOOD, T. Steam distillation technique for the analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in technical 2,4,5-T. *J. Assoc. Offic. Anal. Chem.* 54(1): 218-219 (1971).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was separated from 2,4,5-T by steam distillation and analyzed by microcoulometric gas-liquid chromatography.

THOMPSON, D. J., EMERSON, J. L., and SPARSCHU, G. L. Study of the effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on rat and rabbit fetal development. *Teratology* 4:243 (Abstract) (1971).

2,4,5-T was investigated for its effects on fetal development in the rat and rabbit. Doses ranging from 1 to 50 mg/kg were administered during days 6 to 15 of gestation. No clinical or gross pathology was observed. Detailed examination of the visceral and skeleton revealed no teratogenic effects. However, rats receiving 100 mg/kg during day 6 to 10 of gestation did produce maternal toxicity and death, early fetal resorptions, fetal toxicity, but no malformations.

CURRIER, W. F., GRAHAM, C., GRATKOWSKI, H., and NORRIS, L. A., U.S. Forestry Service. Report on background information for the phenoxy herbicides 2,4-D—2,4,5-T—2,4,5-TP. U.S. Forestry Service Report, pp. 1-165 (1971).

A thorough review is given on the phenoxy herbicides, 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and 2,4,5-TP [2-(2,4,5-trichlorophenoxy)propionic acid], and their potential contaminant, e.g., dioxin.

VINOPAL, J. H., YAMAMOTO, I., and CASIDA, J. E. Preparation of tritium-labeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structure-activity investigations of

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TCDD and other related dibenzo-*p*-dioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971, Abstract No. 79.

Preparation of tritium-labeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was investigated. One approach used was the tritiation of 2,4-dichlorophenol or 2,4,5-trichlorophenol, lithiummetalation and subsequent aqueous hydrolysis of a chlorinated dibenzo-*p*-dioxin, and the catalytic reduction of a chlorinated dibenzo-*p*-dioxin.

WESTING, A. H. Herbicides as agents of chemical warfare: Their impact in relation to the Geneva Protocol of 1925. *Environ. Affairs* 1(3): 578-586 (1971).

The use of herbicides as chemical warfare agents is reviewed with respect to the Geneva Protocol of 1925. The total impact of these agents on the environment and man is not usually known or considered before widespread use. For example, 47 million lb of 2,4,5-T was used in Indochina over an 8-yr period before it was discovered that about 1000 lb dioxin was also being applied as an unsuspected contaminant.

WILLIAMS, D. T., and BLANCHFIELD, B. J. Thin layer chromatographic separation of two chlorodibenzo-*p*-dioxins from some polychlorinated biphenyls and organochlorine pesticides. *J. Assoc. Offic. Anal. Chem.* 54(6): 1429-1431 (1971).

Thin-layer chromatography was used for the isolation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin from polychlorinated biphenyls and organochlorine pesticides. After isolation, these dioxins were analyzed by the electron capture-gas chromatography.

WILSON, J. G., BOUTWELL, R. K., DAVIS, D. E., DOST, F. N., HAYES, W. J., KALTER, H., LOOMIS, T. A., SCHULERT, A., STERLING, T. D., and BOWEN, D. L. Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency. (Submitted May 7, 1971).

The 2,4,5-T Scientific Advisory Committee's report to the U.S. Environmental Protection Agency was stimulated after experimental results and environmental indicators pointed to a potential health threat to humans. This nine-member committee was selected from a list of names supplied by the National Aca-

demy of Science. The Committee recommended by an 8 to 1 vote to restore registration of 2,4,5-T with certain conditions; those applicable to TCDD were: (1) a limit of 0.5 ppm TCDD on existing 2,4,5-T inventories and 0.1 ppm on future 2,4,5-T production, and (2) conduct specific research on TCDD for potential soil accumulation and food chain magnification.

WILLIAMS, C. S. The status of 2,4-D, 2,4,5-T, Silvex and MCPA herbicides. *Down to Earth* 26(4): 12-15 (1971).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the causative agent in experiments reporting teratogenic effects from 2,4,5-T. TCDD was found in 2,4,5-T formulations but not in 2,4-D samples. The precursor for 2,4,5-T is 2,4,5-trichlorophenol which is prepared from 1,2,4,5-tetrachlorobenzene; the high temperature and alkaline conditions necessary to produce this compound also favor dioxin formation. Since 2,4-D is formed by direct chlorination of 2,4-dichlorophenol and not by alkaline hydrolysis of 1,2,4-trichlorobenzene, no dioxin impurities are theoretically possible.

WOOLSON, E. A., REICHEL, W. L., and YOUNG, A. L. Dioxin residues in lakeland sand and eagle samples. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971. Abstract No. 91.

Between 1962 and 1969 a lakeland region in Florida was treated with 912 lb 2,4,5-T per acre. To assess the ecological importance of chlorinated dioxins in the environment, samples of soil to a depth of 3 ft. and tissues from eagles were analyzed for dioxins by electron capture-gas chromatography. No dioxins were detected at a minimum detection limit of 50 ppb for the eagle tissue samples and 5 ppb for the soil samples. Small residues of about 20 ppb 2,4,5-T were found in soil samples.

YANG, G. C., and POHLAND, A. E. Cation radicals from chlorinated dibenzo-*p*-dioxins. Division Pesticide Chemistry, 162nd National Meeting of the American Chemical Society, Washington, D.C., Sept. 12-17, 1971. Abstract No. 84.

Characteristic blue coloration and electron spin resonance (ESR) spectra of cation radicals were obtained following dissolution of chlorinated dibenzo-*p*-dioxins in trifluoromethanesulfonic acid. The mono- and dichlorodibenzo-*p*-dioxins showed well resolved ESR by hyperfine lines; the tetrachloro analogs exhibited single broad ESR lines.

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ABELSON, P. H. Pollution by organic chemicals. *Science* 170(3957): 496 (30 Oct. 1970).

When manufacture of 2,4,5-T is controlled carefully, dioxin contamination is less than 1 ppm. Dioxin was identified in 1962 as the culprit of damage and death in 1957 of uncounted numbers of chicks. Dioxin produces neurological disturbances and is teratogenic. The acute LD₅₀ for male guinea pigs is 0.000001 g/kg.

ANONYMOUS. HEW, USDA hold firm; 2,4,5-T ruling postponed. *Chem. Eng. News* 48(7): 11-12 (1970).

Because early studies indicated that most of the adverse toxicological effects of 2,4,5-T were due to high dioxin contamination (27 ppm) of test samples, the USDA did not feel obligated to cancel registration of currently manufactured 2,4,5-T containing about 1 ppm dioxin. Preliminary tests have shown that 2,4,5-T containing 1 ppm dioxin is not teratogenic. However, problems may still exist, since excessive heat exposure of the tri- or pentachlorophenols, used as intermediates in 2,4,5-T production, liberates dioxins.

ANONYMOUS. Government steps up pressure on pesticides; recent joint action restricting use of 2,4,5-T herbicide portends changes in pesticide regulations and testing. *Chem. Eng. News* 48(18): 60-61 (1970).

Evidence from Congressional hearings catalyzed the government's eventual suspension and cancellation of consumer and food crop usage of 2,4,5-T and curtailment of its use in Vietnam. HEW and the USDA issued a joint statement declaring that both 2,4,5-T and its dioxin contaminant may cause birth defects. The pesticide industry reacted adversely to these actions and statements.

ANONYMOUS. Pesticide policies scored at Ag meeting. *Chem. Eng. News* 48(42): 7 (1971).

The politics and pressures brought about by the 2,4,5-T/dioxin controversy between government and industry were reviewed. The Chairman of the Secretary's Pesticide Advisory Committee (SPAC) castigated both the federal government and the pesticides industry for failure to avoid the panicky crises which brought about an "era of chemical McCarthyism."

ANONYMOUS. The strange case of the government vs. 2,4,5-T. II. *Farm Chem.* 133(3): 26 (1970).

A review of the government involvement in the

2,4,5-T controversy which began after preliminary research reports indicated 2,4,5-T was teratogenic. These government studies were instituted in an attempt to determine whether 2,4,5-T or its dioxin contaminant was the causative agent of these effects.

ANONYMOUS. The 2,4,5-T identification parade. *Food Cosmet. Toxicol.* 8: 596-597 (1970).

Inconsistent test data on the teratogenicity of 2,4,5-T were thought resultant of the level of its chief contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Levels of 27 ppm have been found in some 2,4,5-T samples.

ANONYMOUS. Defoliants, deformities: What risk? *Med. World News* 11(9): 15-17 (1970).

Fertilized chicken eggs were injected with 2.5 pg (2.5 x 10⁻⁴g) (50 ppt) of 2,3,6,7-tetrachlorodibenzo-p-dioxin. The dioxin caused leg deformities, cleft palates, and beak defects in 11 of 15 survivors from 25 eggs. Unhatched birds showed tissue edema, necrotic livers, and deformities similar to hatched birds.

ANONYMOUS. Another herbicide on the blacklist. *Nature* 226(5243): 309-311 (1970).

A commentary was presented to clarify the controversy of whether 2,4,5-T or 2,3,7,8-tetrachlorodibenzo-p-dioxin was the causative teratogenic and mutagenic agent. Hearings with government and industrial representatives were held before the Subcommittee on Energy, Natural Resources and the Environment of the Senate Committee on Commerce.

ANONYMOUS. The tangled tale of 2,4,5-T. *PANS* 16(3): 421-422 (1970).

Use of 2,4,5-T has been restricted where foodstuffs for human consumption might be contaminated. The possibility exists that dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) may be the contaminant in 2,4,5-T formulations or that there may be a synergistic effect between 2,4,5-T and dioxin. Hypothetically, if dioxin contaminated the 2,4,5-T reported in food in the U.S. to the extent of 1 ppm, it would take 100 years for a normal person to accumulate a dose 600 times less than that which produced toxic effects during the pregnancy of a rat.

COURTNEY, K. D., GAYLOR, D. W., HOGAN, M. D., FALK, H. L., BATES, R. R., MITCHELL, I. Teratogenic evaluation of 2,4,5-T. *Science* 168(3933): 864-866 (May 15, 1970).

2,4,5-T samples were evaluated in rats and mice for teratogenicity. Results indicated that 2,4,5-T s.c. or orally administered was teratogenic and fetotoxic

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to the mouse and orally to the rat. Cleft palate and cystic kidneys were among the effects observed. A note added in proof indicated that the 2,4,5-T samples contained 30 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin.

DARSOW, G., and SCHNELL, H. Chlorodibenzo-p-dioxins. Ger. Patent 1,930,259, Appl. 13 June 1969, Granted 17 Dec. 1970, 10 pp (1970).

Chlorodibenzo-p-dioxins were prepared from tetra- or hexachlorobenzene. The compounds were useful for flameproofing polymers, e.g. polyesters.

DAY, B. E. 2,4,5-T and government decisions. *Proc. West. Soc. Weed Sci.* 23: 4-6 (1970).

Unpublished material which influenced the 2,4,5-T ban was held as questionable and suspect since preparations tested contained 27 ppm dioxin. It was stated that 2,4,5-T containing 1 ppm dioxin is not teratogenic; therefore, the alarm about the adverse effects of 2,4,5-T was unjustified.

EMERSON, J. L., THOMPSON, D. J., GERBIG, C. G., ROBINSON, V. B. Teratogenic study of 2,4,5-trichlorophenoxyacetic acid in the rat. *Toxicol. Appl. Pharmacol.* 17: 317 (1970).

The teratogenicity of 2,4,5-T containing 1 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin was evaluated in rats. No teratogenic effects from 1,3,6,12, or 24 mg/kg/day 2,4,5-T or other pathological signs were observed. These results do not substantiate the adverse effects observed with 2,4,5-T containing 27 ppm dioxin.

EPSTEIN, S. S. A family likeness. *Environment* 12(6): 16-25 (July/August, 1970).

The human and environmental implications of the widespread chemical exposure problem have been accentuated by the increased use of herbicides during the past ten years. For example, although recently dioxin impurities in 2,4,5-T have been reduced, dioxin is still present; even minute concentrations are potential hazards. The analysis, formation, and teratogenic and other toxicological consequences of dioxins were reviewed.

EPSTEIN, S. S. Testimony on teratogenic effects of 2,4,5-T formulations. U.S. Senate Hearing before the Subcommittee of Energy, Natural Resources and the Environment of the Committee on Commerce (April 15, 1970).

Polychlorophenol contaminants and their dioxin pyrolytic products in phenoxy herbicides are of toxicologic and environmental consequence. Some dioxins are highly toxic and teratogenic at the microgram per kilogram level; most dioxins are, however, toxicologically uncharacterized.

collogic and environmental consequence. Some dioxins are highly toxic and teratogenic at the microgram per kilogram level; most dioxins are, however, toxicologically uncharacterized.

GALSTON, A. W. Herbicide usage. *Science* 168 (3939): 1607 (1970).

The phenoxyacetic acid herbicides should not be used until all questions regarding the teratogenicity of 2,4,5-T have been resolved. A tetrachlorodibenzo-dioxin impurity found in some commercial preparations of 2,4,5-T was the teratogenic agent in 2,4,5-T samples tested for teratogenicity by the Bionetics Research Laboratories. The possibility that phenoxyacetic acids may be degraded in plants and soil or by fire or bright sunlight into dioxin-like teratogens should be studied. Extensive research should be accomplished on all aspects of the biomedical and environmental consequences of dioxins.

Hearings Before the Subcommittee on Energy, Natural Resources and the Environment of the Committee on Commerce, U. S. Senate, Serial 91-60, Governmental Printing Office, Washington, D.C. (1970).

An impurity in 2,4,5-trichlorophenol was found to be 2,3,7,8-tetrachlorodibenzo-p-dioxin, a compound with a high mammalian toxicity and teratogenic effect.

HORWITZ, W. (Ed.). Official Methods of Analysis, 11th Ed. Association of Official Analytical Chemists, Washington, D.C., 1970, p. 468 (28.109).

The official first action gas chromatographic method for chick edema factor is presented in Section 28 on oils and fats. Fat, oil, fatty acid, or lipid is treated with H₂SO₄, and extracted with petroleum ether. The extract is purified on an Al₂O₃ column, further treated with H₂SO₄, and examined by electron capture-GLC. Peaks with retention times relative to aldrin (R_i) between 8 and 45 indicate presence of chick edema factors (hexa-, hepta-, and octachlorodibenzo-p-dioxins).

KEARNEY, P. C. Paper presented before a joint (United Kingdom, Canada, and United States) meeting on Pesticides, Washington, D.C., (November 5, 1970).

The Agricultural Research Service, USDA, began a program to assess the significance of chlorinated dioxin impurities in currently registered pesticides. Theoretically, any pesticide with a chlorinated phenoxy nucleus or which is derived from a chlorinated phenol precursor could contain chlorinated dioxin contaminants.

RESS, J., HIGGINBOTHAM, G. R., and FIRESTONE, D. Methodology for chlorinated aromatics in fats, oils, and fatty acids. *J. Assoc. Offic. Anal. Chem.* 53(3): 628-634 (1970).

Following review of the current status of chemical and biological methods for chlorophenols and chlorinated dibenzo-*p*-dioxins in fats, oils, and fatty acids, the modified electron capture-gas liquid chromatography method for the detection of chick edema factors was recommended for adoption as official first action to replace all existing gas-liquid chromatography methods.

ROBSON, J. M. Testing drugs for teratogenicity and their effects on fertility: The present position. *Brit. Med. Bull.* 26(3): 212-216 (1970).

The problems of interpreting test results involving teratogenic compounds were outlined using data gathered on the teratogenicity of 2,4,5-T and its dioxin contaminant.

SPARSCHU, G. L., DUNN, F. L., and ROWE, V. K. Teratogenic study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Toxicol. Appl. Pharmacol.* 17(1): 317-318 (1970).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was studied to determine whether this 2,4,5-T impurity could account for fetal abnormalities in test animals. Rats received 0, 0.03, 0.125, 0.5, 2.0, and 8.0 $\mu\text{g/kg}$ body weight/day on days 6 to 15 of gestation. At the 0.5 level, the number of fetuses was reduced, and the number of resorptions and fetal deaths was increased.

VOS, J. G., and KOEMAN, J. H. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicol. Appl. Pharmacol.* 17: 656-668 (1970).

Briefly stated were the concerns and evidence pertaining to dioxins, particularly to the chick edema factor and whether PCBs and/or the dioxins were the culprit(s).

VOS, J. G., KOEMAN, J. H., VAN DER MAAS, H. L., TEN NOEVER DE BRAUW, M. C., and DE VOS, R. H. Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated

biphenyls. *Food Cosmet. Toxicol.* 8(6): 625-633 (1970).

Three commercial polychlorinated biphenyl compounds (Phenoclor DP6, Clophen, and Aroclor 1260) were analyzed for impurities. In two of these (Phenoclor DP6 and Clophen A60) pentachlorodibenzofuran and tetrachlorodibenzofuran were among the polar compounds found.

1969

CANTRELL, J. S., WEBB, N. C., and MABIS, A. J. Identification and crystal structure of a hydropericardium-producing factor: 1,2,3,7,8,9-hexachloro-dibenzo-*p*-dioxin. *Chem. Abstr. Acta. Crystallogr.* B25(1): 150-156 (1969); 70: 51305u (0000).

Chemical identity of hexachlorodibenzo-*p*-dioxin, one of the toxic substances capable of producing the chick edema disease, was established by x-ray crystallography of a sample of edema-inducing lipid material.

RIED, W., and ENG, J. T. S. Reactions with diazocarbonyl compounds. *Ann. Chem.* 727: 219-221 (1969) (Ger.); *Chem. Abstr.* 72: 12458e (0000).

o-Quinone diazides added benzene with nitrogen elimination to yield chloro substituted dibenzofurans: 1,2,3,4-tetrachloro-, 1,2,3-trichloro-, and 1,3-dichloro-.

1968

HIGGINBOTHAM, G. R., HUANG, A., FIRESTONE, D., VERRETT, J., RESS, J., and CAMPBELL, A. D. Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-*p*-dioxin. *Nature* 220(5168): 702-703 (1968); *Chem. Abstr.* 70:18509c (0000).

Commercial chlorophenols including 2,4-dichlorophenol, 2,4,5-trichlorophenol, and 2,3,4,6-tetrachlorophenol, were pyrolyzed to chlorinated dibenzo-*p*-dioxins. These were examined by electron capture-gas liquid chromatography and tested biologically by the chick embryo assay. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was prepared by direct chlorination of dibenzo-*p*-dioxin. These dioxins proved to be chick edema factors (hydropericardium factors) and toxic.

1967

ANONYMOUS. Search for chick edema factor. *Chem. Eng. News* 45(5): 10 (Jan. 30, 1967).

Chicks afflicted with edema, or hydropericardium, suffer an accumulation of fluid in the heart sac and gross kidney and liver damage; as little as 5 µg can kill a chick. X-Ray crystallographers have determined the molecular structure of one of the toxic compounds known as the chick edema factor. Single-crystal structural analysis showed that the structure is 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. Two crystals, each about 0.2×0.1×0.1 mm and weighing about 3µg were recrystallized from a benzene - hexane solution. The final data showed that the 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin molecules are nearly planar, and are packed in the 044 crystallographic planes, with an interplanar separation of about 3.3 Å. No unusual bond lengths or angles were apparent.

1965

COX, J. M., WRIGHT, B. A., and WRIGHT, W. W. Thermal degradation of poly(phenylene oxides). *J. Appl. Polymer Sci.* 9(2): 513-522 (1965).

The thermal degradation in vacuum of various phenylene oxide polymers and copolymers was studied by a weight-loss method. Thermal stability decreased with increasing substitution in the aromatic nuclei. This may be due to the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin, formed as an alternate reaction product. Chloro derivatives were more stable than the corresponding bromo derivatives, except for the tetra-substituted phenylene oxides.

KULKA, M., Perhalodibenzo-p-dioxins. Can. Patent 702,144, Appl. May 3, 1961; Granted Jan. 19, 1965, 13 pp. (1965)

Octahalodibenzo-p-dioxins, useful as chemical intermediates, biocides, and flame retardants, were prepared by using a pentahalophenol in the presence of a catalytic amount of halogen or a halogenated cyclohexadienone at 200-400°C. An 83% yield was attained for octachlorodibenzo-p-dioxin.

1962

JONES, E. L., and KRIZEK, H. A technique for testing acnegenic potency in rabbits, applied to the potent acnegen 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Invest. Dermatol.* 39: 511-518 (1962).

Hyperkeratinization of the inner surface of the rabbit ear was induced by topical application of 2,3,7,8-tetrachlorodibenzo-p-dioxin in acetone. The weight of keratin served as a criterion of acnegenic activity.

KULKA, M., Octahalodibenzo-p-dioxins. Belg. Patent 616,197, Appl. May 2, 1961; Granted July 31, 1962, 9 pp. (1962)

Octahalodibenzo-p-dioxins were prepared by heating pentahalophenols in the presence of catalytic amounts of halogen or halocyclohexadiene. For example, 40 g pentachlorophenol and 2 g 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone in 120 ml 1,2,4-trichlorobenzene was refluxed at 213°C to give 30 g of octachlorodibenzo-p-dioxin.

1961

BAUER, H., SCHULZ, K. H., and SPEIGELBERG, U. Occupational intoxications in manufacturing chlorophenol compounds. *Arch. Gewerbepath. Gewerbehyg.* 18: 538-555 (1961).

Thirty-one workers in an industry manufacturing 2,4,5-trichlorophenol by sodium hydroxide saponification of 1,2,4,5-tetrachlorobenzene exhibited acne and symptoms of psychopathologic disturbances. Solutions of 0.01 to 0.05% of 2,3,6,7-tetrachlorodibenzo-p-dioxin painted on rabbit ears produced acne; oral administration of 0.05-0.1 mg/kg caused serious liver damage.

KULKA, M. Octahalodibenzo-p-dioxins. *Can. J. Chem.* 39: 1973-1976 (1961).

Nearly 100% conversion from pentahalophenols to 1,2,3,4,6,7,8,9-octahalo-dibenzo-p-dioxins was accomplished by using reaction initiators such as halogens or halogen-generating compounds.

1960

DENIVELLE, L., FORT, R., and VAN HAI, P. Octachloro- and octabromodibenzo-p-dioxins and decachloro- and decabromodiphenyl ether. *Bull. Soc. Chim. France* 1960. 1538-1543 (1960).

Pentachlorophenol was pyrolyzed at 300°C extracted with carbon tetrachloride and aqueous alkali, recrystallized from phenyl nitrite, and yielded "perchlorophenylene oxide" (melting point of 326°C). Steric considerations dictated ring fusion in the 1,2-position leaving octachlorodibenzo-p-dioxin the only acceptable structure for perchlorophenylene oxide. Sodium 2,4-dichlorophenate gave dichlorodibenzo-p-dioxin.

KAUPP, J., and KLUG, H. Perchlorodiphenylene dioxide. Ger. Patent 1,092,480, Appl. June 27, 1956, Granted Nov. 10, 1960 (1960).

Octachlorodibenzo-p-dioxin was prepared by heating pentachlorophenol and 2,3,4,4,5,6-hexachloro-2,5-cyclohexadien-1-one; the yield was 84%.

1959

TOMITA, M., UEDA, S., and NARISADA, M. Dibenzo-*p*-dioxin derivatives. XXVII. Synthesis of polyhalodibenzo-*p*-dioxin. *Yakugaku Zasshi* 79: 186-192 (1959).

Pentachlorophenol was heated at 300°C for 12 hr. Recrystallization of the product from benzene, yielded octachlorodibenzo-*p*-dioxin needles.

1958

SANDERMANN, W., CASTEN, R., KRASTING, W., and PIEPER, J. Studies on wood-protection chemistry. IX. Investigations concerning oily blue stain protection agents. *Holzforsch. Holsverwert.* 10(4): 57-66 (1958).

Compounds formed by heat treatment of pentachlorophenol such as octachlorodiphenylene oxide were inactive for preventing blue stain. Tetrachlorodiphenylene oxide was especially toxic to man and beast.

1957

GILMAN, H., and DIETRICH, J. J. Halogen derivatives of dibenzo-*p*-dioxin. *J. Amer. Chem. Soc.* 79: 1439-1441 (1957).

Dibenzo-*p*-dioxin was synthesized and reacted with chlorine gas to form 2-chlorodibenzo-*p*-dioxin; a similar run coupled with ultraviolet light gave 2,7-dichlorodibenzo-*p*-dioxin.

KIMMIG, J., and SCHULZ, K. H. Chlorinated aromatic cyclic ethers as a cause of the so-called chloracne. *Naturwiss.* 44: 337-338 (1957) (Ger.).

Trichlorodibenzo furan, tetrachlorodibenzofuran, and 2,3,6,7-tetrachlorodibenzodioxin were especially effective acne-inducing agents on rabbit ears. 2,3,6,7-Tetrachlorodibenzodioxin proved most toxic; the characteristic changes were achieved with concentrations of 0.01 to 0.002%. Higher concentrations caused liver necrosis and death. Single oral administration of 0.05 to 0.1 mg/kg body weight caused death in 1-2 weeks. Autopsy revealed necrosis and fatty degeneration of the liver. Pure 2,4,5-trichlorophenol and pentachlorophenol did not cause chloracne.

KIMMIG, J., and SCHULZ, K. H. Occupational acne (so-called chloracne) due to chlorinated aromatic cyclic ethers. *Dermatologia* 115 (4): 540-546 (1957) (Ger.).

The agent causing occupational chloracne in 31 employees of chlorophenol producing factories was found to be 2,3,6,7-tetrachlorodibenzodioxin. The toxic action in animals was due to the alkaline hydro-

lysis product of 1,2,4,5-tetrachlorobenzene. Tetrachlorodibenzo-*p*-dioxin and tri- and tetrachlorodibenzofuran were active skin irritants. 2,3,6,7-Tetrachlorodibenzodioxin formation from sodium trichlorophenolacetate was established as was isolation from the by-products.

SANDERMANN, H. S., CASTEN, R., and STOCKMANN, H. Pyrolysis of pentachlorophenol. *Chem. Ber.* 90: 690-692 (1957).

Octachlorodiphenylene dioxide was formed after pentachlorophenol was heated at 300°C for 24 hr, distilled at 320°C, and crystallized from benzene or extracted with benzene and fractional crystallization. 2,3,7,8-Tetrachlorodiphenylene dioxide was active against insects and wood-destroying fungi; octachlorodiphenylene dioxide was inactive.

SCHULZ, K. H. Clinical and experimental studies on the etiology of chloracne. *Arch. Klin. Exp. Derm.* 206: 589-596 (1957).

The acnegenic properties occurring as a refractive dermatologic condition in factory workers involved in the production of chlorinated aromatic compounds were ascribed to TCDD, an unwanted side product in the synthesis of 2,4,5-T.

1955

OITA, K., JOHNSON, R. G., and GILMAN, H. The chlorination of dibenzofuran and some of its derivatives. *J. Org. Chem.* 20: 657-667 (1955).

Step-by-step reaction procedures were presented for the chemical synthesis of chlorinated dibenzofurans, i.e., 2-chloro-, 2,8-dichloro-, 3-chloro-, and a chlorodibenzofuran.

1954

CERNIANI, A., PASSERINI, R., and RIGHI, G. Ultraviolet spectra of some dibenzofurans. *Boll. Sci. Fac. Chim. Ind.* 12: 75-79 (1954).

2-Chloro- and 3-chloro-dibenzofurans were characterized by ultraviolet spectrometry. These derivatives had little effect on spectra of the parent compound.

1953

JULIA, M., and BAILLARGE, M. Growth factors in plants. III. (1-Carboxymethyl-2-naphthoxy)acetic acid and (2-carboxymethyl-4-chlorophenoxy)acetic acid. *Bull. Soc. Chim. France.* 640-643 (1953).

In the preparation of 2,4-dichlorophenol, a portion of the mixture was insoluble in benzene-water; the substance was extracted with boiling ligroine and recrystallized to yield 2,7-dichlorodibenzo-*p*-dioxin.

1952

SHIBATA, I. S., NATORI, S., and SUMI, Y. Antibacterial effects of lichen substances and their related compounds. *J. Pharm. Soc. Japan* 72: 1335-1336 (1952).

The chlorinated dibenzofurans synthesized and tested for antibacterial activity included: 2-chloro-, 3-chloro-, 2,8-dichloro-, and 3,8-dichloro-. The chlorine atom, when in the 3-position of the dibenzofuran ring, increased its antibacterial effect.

1951

TOMITA, M., and WATANABE, W. Antibacterial activity of some organic compounds in vitro. II. Antibacterial activity of dibenzo-*p*-dioxin, phenoxathiin, and 1,4-benzodioxan derivatives on *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Escherichia coli*. *J. Pharm. Soc. Japan* 71: 1204-1206 (1951).

2,7-dichlorodibenzo-*p*-dioxin was tested for antibacterial activity and found to be inactive.

1941

HIGASI, K. The molecular structures of diphenylene dioxide and phenoxathiin as revealed by dipole-moment data. *Sci. Papers Inst. Phys. Chem. Research* 38: 331-340 (1941).

2,8-Dichlorodiphenylene dioxide exhibited a dipole moment of 0.62 Debyes. This indicated a folded molecule and no optical isomers.

HIGASI, K., and UYEO, S. Polarity and molecular structure of diphenylene dioxide. *J. Chem. Soc. Japan* 62: 396-399 (1941).

2,6-Dichlorodiphenylene dioxide was found to have a small but finite dipole movement. This molecule and the parent probably fold along the line joining the two oxygen atoms.

UEO, S. 2,6-Dichlorodiphenylene dioxide. *Bull. Chem. Soc. Japan* 16: 177-179 (1941).

Synthesis of 2,8-dichlorodiphenylene dioxide, a light yellow compound with a melting point of 207°C and a dipole moment of 0.62 was described. The dipole movement of 2,8-dichlorodiphenylene dioxide was somewhat less than the dipole moment of diphenylene dioxide which was 0.64.

1939

British Thompson-Houston Co. Ltd. Electric insulation. Brit. Pat. 506,560 (May 31, 1939).

Direct chlorination of diphenylene oxide in the presence of a catalyst produced solid, waxlike products of high dielectric constants; i.e., mixture of chlorinated diphenylene oxide and pentachlorobiphenyl.

CLARK, F. M. Chlorinated diphenylene oxide composition for treating paper in capacitors, U.S. Patent 2,198,473 (April 23, 1939).

Chlorinated diphenylene oxide containing 1 to 4 atoms of chlorine per molecule imparts a constant electrical capacity when used to impregnate paper in capacitors.

1936

BELL, F. Pyrolysis of chlorophenols. *J. Chem. Soc.* 1936: 1244.

o-Chlorophenol was pyrolyzed at 180-340°C; diphenylene oxide and 3,8-dichlorodiphenyl oxide were isolated from the distillate.

1934

GILMAN, H., BROWN, G. E., BYWATER, W. G., and KIRKPATRICK, W. H. Dibenzofuran. III. Nuclear substitutions. *J. Amer. Chem. Soc.* 56: 2473-2477 (1934).

2-Chloro- and 2,8-dichlorodibenzofuran were synthesized. Dibenzofuran and chlorine in carbon tetrachloride gave 38% of the 2,8-dichloro derivative which had a melting point of 185°C. Dibenzofuran in alcohol at 60°C gave the 2-chloro derivative which had a melting point of 102.5°C.

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Appearing monthly - Volume 46 No 3 - September 15th 1983
Completing Volume 46

ISSN 0009-2797
CBINA 46 (3) 283-388

Chemico-Biological Interactions

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COMPOUNDS IN URBAN AIR COMPETE WITH 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN FOR BINDING TO THE RECEPTOR PROTEIN

RUNE TOFTGÅRD*, GÖRAN LÖFROTH*, JAN CARLSTEDT-DUKE*, RABINDER KURL* and JAN-ÅKE GUSTAFSSON*

*Department of Medical Nutrition, Karolinska Institutet, Huddinge University Hospital F69, S-141 86 Huddinge and *Nordic School of Public Health, S-413 46 Gothenburg and Department of Radiobiology, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

(Received November 29th, 1982)

(Revision received April 7th, 1983)

(Accepted April 8th, 1983)

SUMMARY

Acetone extracts of filter-collected urban airborne particulate matter contain compounds which can competitively inhibit 2,3,7,8-[1,6-³H]tetrachlorodibenzo-*p*-dioxin (TCDD) binding to the rat liver TCDD-receptor protein. The concentration of conventional polycyclic aromatic hydrocarbons (PAHs) or chlorinated dioxins and dibenzofurans cannot account for more than 1-30% of the observed competition for [³H]TCDD binding to the receptor protein. The difference in potency between samples collected in urban areas during different periods of the year and a background sample is 25-400-fold. Collecting samples in the presence of increased concentrations of nitrogen dioxide, nitrous acid, nitric acid or ozone did not increase the amount of compounds with receptor affinity. However, with nitrogen dioxide and especially with nitric acid, a substantial increase of the mutagenic effects in the Ames Salmonella assay in the absence of mammalian activation as well as a degradation of several PAHs were noted. Affinity for the TCDD-receptor protein, mutagenicity in the absence of mammalian metabolic activation in the Ames Salmonella assay and PAH-content are characteristics of urban particulate matter showing the presence of compounds, that represent potential health risks. The compounds with affinity for the receptor may constitute a group of substances different from both conventional PAHs and direct-acting mutagens.

Abbreviations: AHH, aryl hydrocarbon hydroxylase; B[a]P, benzo[a]pyrene; DMSO, dimethyl-sulfoxide; PAHs, polycyclic aromatic hydrocarbons; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDBF, 2,3,7,8-tetrachlorodibenzofuran.

0009-2797/83/\$03.00

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Printed and Published in Ireland

Key words: Air pollution—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin—Receptor binding—Mutagenicity—Polycyclic aromatic hydrocarbons

INTRODUCTION

Urban airborne particulate matter contains a complex mixture of organic compounds. The mutagenicity of extracts of such particulate matter in the Ames Salmonella assay in the absence of rat liver metabolic activation [1-4] indicates the presence of compounds different from conventional polycyclic aromatic hydrocarbons. Presently much interest is focused on nitroaromatics. Several compounds belonging to this class, e.g. nitropyrenes and dinitropyrenes, have been shown to be extremely powerful 'direct-acting' mutagens [5,6] and are believed to represent a major fraction of the 'direct-acting' mutagens in particulate emissions from diesel engines [7-11]. Furthermore, experiments with simulated atmospheres indicate that nitroaromatics can be formed when certain PAHs are exposed to nitrogen dioxide [12].

With respect to chemical carcinogens and mutagens, e.g. PAHs, requiring metabolic activation, the level and substrate specificity of the specific cytochrome *P*-450 isozymes that are present in the target tissue are of great importance. The induction of isozymes capable of activating PAHs, generally known as aryl hydrocarbon hydroxylase (AHH), is thought to be mediated by the interaction of the inducer with a highly specific intracellular receptor protein [13,14]. This receptor, often called the TCDD-receptor or Ah-receptor, is present in several tissues including lung [15]. It is also known that certain PAHs as well as cigarette smoke induce AHH in the lung [16].

The present study was undertaken to investigate the possible presence in urban particulate matter of compounds with an affinity for the TCDD-receptor protein and to monitor the effect of adding nitrogen dioxide, nitrous acid, nitric acid and ozone during environmental sampling of particulate matter on mutagenicity and levels of compounds having an affinity for the receptor.

METHODS

Particulate matter was collected on glass fiber filters by high volume sampling at roof top levels for 24 h in central Stockholm and a suburban site. Collection, Soxhlet extraction with acetone, extract preparation in dimethylsulfoxide (DMSO) and mutagenicity testing of the extracts in the Salmonella assay have been described earlier [3,4]. The Salmonella strains TA98 and TA100 were obtained from Dr. B.N. Ames, Berkeley, CA and the nitroreductase deficient strains TA98NR, TA98/1, 8 DNP, and TA100NR were obtained from Dr. H.S. Rosenkranz, Cleveland, OH.

For the addition of nitrogen dioxide, nitric acid, nitrous acid and ozone, one high volume sampler equipped with a dosing system was run in parallel with

a conventional sampler. Details of the dosing system, concentration measurements and filter extraction are given elsewhere [17,18]. These samples were collected in central Gothenburg. A background sampler was collected at Rörvik located on the west coast of Sweden.

Affinity of compounds present in particulate extracts for the rat liver TCDD-receptor

Liver cytosol was prepared as described [19] and diluted to 3.5 A₂₈₀₋₃₁₀/ml (2-2.5 mg protein/ml). Each experiment consisted of a series of ten incubations of 1 ml of cytosol with 1.5 nM [³H]TCDD. [1,6-³H]TCDD (spec. act. 36.6 Ci/mmol) was a kind gift from Dr. A. Poland (McArdle Laboratory for Cancer Research, WI). Two incubations were carried out in the presence of 20 μl of DMSO (total binding). Two incubations contained 150 nM radioinert 2,3,7,8-tetrachlorodibenzofuran (TCDBF) (non-specific binding). The remaining six incubations contained varying amounts of the extract of the particulate fraction of an air sample. A quantity of the extract (2.5-20 μl), dissolved in DMSO, was added to the incubation mixture and the volume of DMSO was then adjusted to 20 μl/incubation. After incubation at 0°C for 60 min, the incubations were treated with dextran-coated charcoal and the amount of [³H]TCDD bound to the receptor in each of the ten incubations was measured by isoelectric focusing carried out in polyacrylamide gel as described previously [19].

The non-specific binding of [³H]TCDD was obtained from the two incubations in the presence of 150 nM radioinert TCDBF and was subtracted from the total binding in each of the other incubations (specific binding). The specific binding of [³H]TCDD in the presence of extracts of the air samples was expressed as a percentage of the specific binding in the control incubation ([³H]TCDD + DMSO). The relative binding affinity for the individual air samples was calculated from log-logit plots of the competition for TCDD-binding where $\text{logit } b = \ln(b/1-b)$. The log-logit plots were calculated using linear regression analysis. Each extract was analysed 3-4 times at a suitable dilution. An unused glass fiber filter was extracted in the same manner as the other samples and tested for competition for receptor binding. Benzo[a]pyrene (B[a]P) and TCDBF were dissolved in dioxane and the competition for binding to the receptor measured.

Chemical analysis

Some samples (790410, 790705 and 800204) have been analyzed for TCDD, TCDBF and PAHs by the Swedish Water and Air Pollution Research Institute, Gothenburg [20].

Other samples reported in Table II were analyzed for PAHs by three laboratories (Swedish Water and Air Pollution Research Institute, Gothenburg, Central Institute for Industrial Research, Oslo and Department of Analytical Chemistry, University of Stockholm). The results are presented as the average concentration reported by the different laboratories.

RESULTS AND DISCUSSION

The results shown in Fig. 1 and Table I describe the mutagenic effects in *Salmonella* strains TA98 and TA100 in the absence of mammalian metabolic activation and the relative binding affinities expressed as the concentration of air sample extract that competes for 50% of the specific TCDD-binding to

TABLE I

MUTAGENICITY AND COMPETITION FOR RECEPTOR BINDING BY EXTRACTS OF URBAN PARTICULATE MATTER

nd, not determined

Sample		Receptor affinity ^a EC ₅₀ (m ³ air/ml cytosol)	Mutagenic response ^b (revertants/m ³)	
			TA98	TA100
790410	Inner city	0.05 ± 0.01	19	11
790410	Suburban	0.04 ± 0.02	14	8
790705	Inner city	0.14 ± 0.07	3	nd
790705	Suburban	0.30 ± 0.15	1	nd
800204	Inner city	0.02 ± 0.01	59	79
800205	Inner city	nd	92	102
800206	Inner city	0.04 ± 0.01	30	20
800207	Inner city	0.05 ± 0.03	9	7
800108-11	Inner city ^c	0.05 ± 0.03	80	74
800310-14	Inner city ^c	0.06 ± 0.04	48	50
800414-18	Inner city ^c	0.08 ± 0.01	30	52
800414-18	Inner city ^d	0.14 ± 0.09	31	53
800414-18	Suburban ^c	0.19 ± 0.10	19	32
800609-13	Inner city ^c	0.20 ± 0.12	12	17
810408-14	Background ^e	7.8 ± 2.4	< 0.5	< 0.5
TCDBF		2.7 ± 1.9 nM		
B[a]P		18.2 ± 9.9 nM		

^aSamples were collected at roof top levels for 24 h starting about 6 a.m. in central Stockholm and at a suburban site 22 km NNW Stockholm. The EC₅₀ is the concentration of competitor that competes for 50% of the specific binding of [³H]TCDD. The EC₅₀ (in nM) for TCDBF and B[a]P are given for comparison. The values represent the means ± S.D., n = 3-4.

^bThe mutagenic effect was determined with 1 plate/dose level at 3 or more dose levels in at least 3 independent tests and the response was calculated from the linear or almost linear part of the dose-response curve. The mutagenic response is given in the absence of mammalian metabolic activation.

^cThree or four 24-h samples were pooled.

^dSample collected with a lower air flow (34 m³/h) than normal (68 m³/h).

^eSample collected with an electrostatic precipitation sampler at Rörvik on the west coast of Sweden.

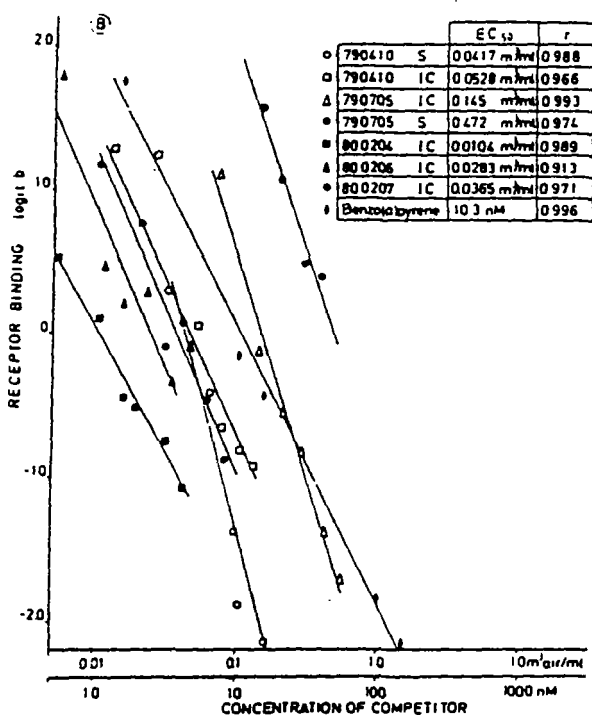
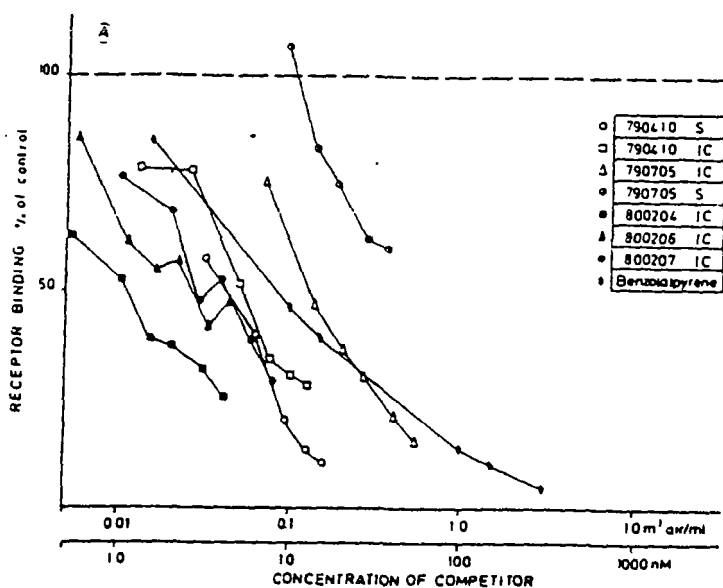


Fig. 1A: Competition for the binding of [3 H]TCDD to the TCDD-receptor protein in rat liver cytosol by extracts of filter-collected urban particulate matter. For description of the different samples see Table I. Typical values for total and non-specific binding in the assay are 1100–1200 and 200–300 cpm, respectively. Designations are: IC, inner city; S, suburban. B: log-logit plots of the data from (A). The relative affinity is expressed as the concentration of extracts that competes for 50% of the specific TCDD-binding (EC_{50} , logit $b = 0$). The values given in the upper right corner are the results of the 9 individual experiments shown in this figure.

the TCDD-receptor protein. These results demonstrate the presence of both 'direct-acting' mutagens and compounds competing with TCDD for receptor binding in extracts of particulate air samples collected in central Stockholm and at a suburban site. Control experiments with unused filters carried through the entire extraction procedure showed that the measured effects were due to components extracted from the particulates. In Fig. 2 the competitive nature of the inhibition of TCDD-binding to the receptor protein is shown by the use of Lineweaver-Burke plots. The absence of significant differences in the slope of the log-logit plots for the different air samples indicate that they compete for the same binding site. There seems to be a gross correlation between the binding affinity and the pollution level as measured by mutagenic effects.

Samples collected during the winter have lower EC_{50} -values than samples collected during the summer and late spring as illustrated by a maximal 15-fold variation. The very marked difference (25–400-fold) between a background sample collected on the west coast of Sweden and the samples collected in greater Stockholm indicate that local emissions may be of major importance with regard to receptor binding components. Samples collected with the electrostatic precipitation sampler used for collection of the background sample have in separate experiments been shown to give mutagenic effects and binding affinities comparable to samples collected with the conventional high-volume sampler (unpublished observations).

Several compounds are known to bind to the TCDD-receptor protein including many PAHs [13,21,22] present in urban particulate matter. Some of the particulate samples have been analyzed for PAHs (Table II). Assuming that all analyzed PAHs present at concentrations equal to or above 0.1 ng/m³ have the same binding affinity for the receptor protein as B(a)P, between 1%

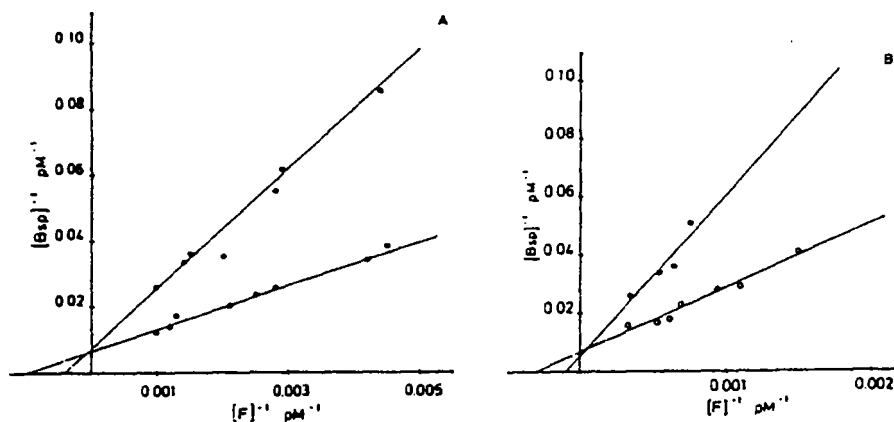


Fig. 2. Lineweaver-Burke plots of data obtained when incubating different concentrations of [³H]TCDD in the absence and presence of a constant concentration of particulate air sample extract. The samples used in these experiments were collected in central Gothenburg. *F* and *B_{sp}* represent the concentration of free and specifically bound [³H]TCDD, respectively.

TABLE II

CONTENTS OF PAHs IN EXTRACTS OF FILTER COLLECTED URBAN PARTICULATE MATTER^{a,b}

PAH content (ng/m ³)	Sample								
	790410 suburban	790705 inner city	800204 inner city	800108-11 inner city	800310-14 inner city	800414-18 inner city	800414-18 inner city ^d	800414-18 suburban	800609-13 inner city
Phenanthrene	0.5		5.6	2.2	2.2	0.8	0.7	0.4	0.1
1-Methylphenanthrene			4.8	0.5	0.2	0.2	-	0.1	
2-Methylphenanthrene	-	-	-	-	0.2	0.1	-	0.1	
3-Methylphenanthrene	-	-	-	-	0.2	0.1	-	0.1	
4-/9-Methylphenanthrenes	-	-	-	-	0.2	0.1	-	0.1	
Fluorene			1.7	0.7			-		
Dibenzothiophene			0.8	0.4	0.2	0.3	-		
Pyrene	0.5	0.2	1.2	3.2	2.6	1.0	1.2	0.4	0.2
1,2-Dihydropyrene			3.8	0.4	0.4	-	-	-	
1-Methylpyrene				0.2	0.1		-		
4-Methylpyrene	-	-	-	-	0.1		-		
Benz[a]anthracene	0.5		7.3	2.1	0.9	0.3	0.3	0.1	0.3
Chrysene	0.5	0.1	4.6	2.8	2.0	0.8	1.5	0.5	0.4
Triphenylene									
Fluoranthene	0.6	0.1	9.8	3.2	3.1	0.9	1.6	0.6	0.2
Benzo[a]fluorene			1.1	1.4	0.4	0.2	-		
Benzo[b]fluorene			1.4	0.5	0.2		-		
Benzo[c]pyrene			2.3	1.6	1.1	0.5	0.5	0.3	0.3
Benzo[a]pyrene			1.7	1.0	0.6	0.4	0.3	0.2	0.2
Benzo[b/k]fluoranthene	0.2		5.4	4.4	2.8	1.3	1.6	0.8	0.8
Benzo[j]fluoranthene	-	-	-	-	1.3	0.4	-	0.3	0.2
Benzo[g,h,i]fluoranthene	-	-	-	-	1.2	0.3	-	0.1	0.1
Benzo[c]phenanthrene									
Indeno[1,2,3-cd]pyrene			0.6	1.2	1.1	0.5	0.5	0.3	0.3
Benzo[g,h,i]perylene			3.8	2.1	1.5	0.9	0.8	0.5	0.5
Coronene	-	-	-	0.8	0.9	0.3	0.3	0.2	-
Total ^c	2.8	0.3	68.5	28.6	23.6	9.5	9.2	5.2	3.9

^aAll PAHs present at a concentration equal to or above 0.1 ng/m³ are included.^b-, not determined in the actual sample.^cTotal PAH content calculated by adding the amounts of each analyzed compound present at a concentration above 0.1 ng/m³.^dSample collected with a lower air flow (34 m³/h) than normal (68 m³/h).

and 30% of the observed binding can be accounted for. The above assumption is a conservative one based on the fact that when 16 of the PAHs in Table II were tested for receptor affinity only two showed a higher binding affinity than B[a]P (our unpublished observations). This indicates that other types of compounds might be more active.

TCDD and TCDBF have the highest binding affinities for the receptor protein among investigated compounds [23]. Two samples (790410 inner city, 790705 suburban) have been analyzed for TCDD and TCDBF and the concentrations were below the detection levels of 2 pg/m³. This is equivalent to a maximal concentration of 2 pM in an incubation where the specific [³H]TCDD binding is reduced by 50%. Pure TCDBF has an EC₅₀-value of 2.7 nM (see Table I). Consequently these compounds cannot account for the binding. At present we cannot, however, exclude the presence of other halogenated hydrocarbons with receptor affinity.

The experimental series with increased concentrations of reactive gases present during sampling was performed with the aim to (1) investigate the possible role of chemical transformation for the appearance of both mutagenic components and receptor binding compounds and (2) investigate the importance of artifact formation due to the sampling methods. From previous studies it is known that certain nitro-PAHs have a high affinity for the TCDD-receptor protein [24]. However, the results from the present study (Table III) failed to demonstrate an increased competition with TCDD for receptor binding in extracts of samples collected with increased concentrations of nitrogen dioxide, nitrous acid, nitric acid or ozone. Chemical analysis, details of which will be published elsewhere [17,18], showed that an extensive degradation of PAHs and formation of mono-nitro-PAHs took place in the experiment with nitric acid and to a smaller extent with nitrogen dioxide whereas nitrous acid and ozone were without effects. Thus, it seems that the nitro-aromatics are not the major receptor binding species in extracts of particulate air samples and that the artifact formation during sampling is negligible in this respect.

The mutagenic response of the samples in the Salmonella assay in the absence of exogenous metabolic activation were in the range found earlier, Tables I and III [3,4]. The presence of mammalian activation in the form of liver S9-mix from Aroclor 1254-induced male rats mostly, but not always, decreased the response (data not shown).

The experiments with nitrogen oxides show that nitrogen dioxide and nitric acid, but not nitrous acid, when present in the air can increase the mutagenic response in the absence of mammalian activation. This increase is probably due to formation of a variety of nitro compounds. The types of mutagenic compounds formed in the samples exposed to elevated concentrations of nitrogen dioxide and nitric acid are probably also similar to those present in the ambient samples as judged by the relative response in nitro-reductase proficient and deficient strains (Table III).

The increase of the mutagenic activity at about 1 ppm of added nitrogen dioxide is on the average fourfold with a range between 2- and 7-fold.

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TABLE III

EFFECT OF NITROGEN DIOXIDE, NITROUS ACID, NITRIC ACID AND OZONE PRESENT DURING SAMPLING OF URBAN PARTICULATE MATTER ON MUTAGENIC RESPONSE AND RECEPTOR AFFINITY*

Sample	Mutagenic response (revertants/m ³) ^b			TA100	TA100 NR	Receptor affinity ^c EC ₅₀ (m ³ /ml cytosol)
	TA98	TA98 NR	TA98/1,8 DNP ₄			
800225 Ambient	62	37	28	76	36	nd
950 ppb NO ₂	87	48	32	142	62	nd
800226 Ambient	14	8	3.2	14	5	0.17 ± 0.04
950 ppb NO ₂	37	24	6.5	41	20	0.09 ± 0.04
800228 Ambient	11	6	5	20	5	0.07 ± 0.02
950 ppb NO ₂	76	35	20	110	41	0.10 ± 0.03
800326 Ambient	4.3	nd	nd	2.9	nd	nd
200 ppb O ₃	4.6	nd	nd	3.3	nd	nd
800327 Ambient	28	11	4.5	21	5	0.08 ± 0.02
200 ppb O ₃	31	12	4.4	25	7	0.06 ± 0.04
800331 Ambient	10	nd	nd	8.4	nd	nd
200 ppb O ₃	16	nd	nd	14	nd	nd
810108-22 Ambient ^d	10	nd	nd	10	nd	50*
100 ppb HNO ₂	9	nd	nd	10	nd	53*
810126-0206 Ambient ^d	8	nd	nd	7	nd	70*
1000 ppb NO ₂	26	nd	nd	35	nd	68*
810224-0312 Ambient ^d	11	6.4	4.7	11	5.5	54*
120 ppb HNO ₂	40	29	20	70	32	54*

*Samples were collected at roof top levels in central Gothenburg during 24 h periods with two simultaneously operating high volume samplers.

One of the samplers was equipped with a dosing system enhancing the concentrations of nitrogen dioxide, nitrous acid, nitric acid or ozone.

^bThe particulate extracts were assayed for mutagenicity on at least three different occasions by the Salmonella plate incorporation method with bacterial cultures fully grown overnight. The tests were performed in the absence of mammalian metabolic activation. The response was calculated from the linear or almost linear part of the dose-response curve.

^cThe values represent the means ± S.D., n = 3-4.

^dEight 24-h samples were pooled.

^eThe data represent the remaining percentage specifically bound [³H]TCDD compared to the specific binding obtained in incubations without added particulate extract. Particulate extracts equivalent to 0.075, 0.060 and 0.065 m³ air were added in the experiments with HNO₂, NO₂ and HNO₃, respectively. The results are the mean of 2 separate experiments.

Assuming that the formation of mutagenic components during sampling has a first-order dependence on the concentration of nitrogen dioxide, it can be calculated that only exceptionally high ambient concentrations of nitrogen dioxide, i.e. >200 ppb over 24 h, would cause a pronounced increase of the mutagenic response.

Nitric acid seems to be more effective than nitrogen dioxide in increasing the mutagenic response. It is, however, possible that the effects observed from nitrogen dioxide are in fact caused either by nitric acid and nitrogen dioxide in combination or by nitric acid alone as the latter compound also would have been present in the nitrogen dioxide preparation. Ambient concentrations of nitric acid are generally much lower than the concentration used in the experiment suggesting that only exceptionally high ambient concentrations would cause a pronounced increase of the mutagenic response. With the exception of one sample, addition of ozone during sampling did not change the mutagenic response (Table III). The exceptional sample, 800331, was, however, collected during a period with a high nitric oxide concentration and an episode with an influx of a long-range transported air mass normally containing an acid sulphate aerosol [17]. This may have increased the ozone oxidation of nitrogen oxide to higher oxides and enhanced the nitration reactions.

The affinity of a compound for the TCDD-receptor is well correlated to the magnitude of aryl hydrocarbon hydroxylase induction caused by that compound [23]. This is especially true for halogenated aromatic hydrocarbons. Strains of mice with high AHH inducibility are more susceptible to pulmonary cancers caused by 3-methylcholanthrene than strains with low inducibility indicating a link between AHH inducibility and incidence of pulmonary tumors [25]. The inducibility of AHH in cultured explants of human bronchus [26,27] indicates the presence of the receptor protein also in this tissue. Animal experiments suggest that also other responses such as thymic atrophy and incidence of cleft palate correlate to receptor affinity [28]. Although TCDD has been shown to be a potent carcinogen in chronic feeding studies [29] it is apparent that TCDD shows no or very little mutagenic activity in *in vitro* test systems such as the Ames Salmonella test [30-32] and a very low covalent binding to DNA *in vivo* [33,34]. In two recent studies, however, it has been shown that TCDD is a potent tumor promotor in the rat liver and mouse skin two-stage models of carcinogenesis [35,36]. If this effect of TCDD is mediated via binding to the receptor is not yet known.

Affinity to the TCDD-receptor protein, mutagenicity in the absence of mammalian metabolic activation in the Ames Salmonella assay and PAH-content are different characteristics of urban particulate matter showing the presence of compounds, which represent potential health risks. The compounds with affinity for the receptor may constitute a group of substances different from both conventional PAHs and the direct acting mutagens.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Swedish Environ-

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ment Protection Board; Project: Air Pollution in Urban Areas, and the Swedish Tobacco Company. J.C.-D. is a recipient of a research fellowship from the Swedish Cancer Society. We thank Birgitta Fernström, Kerstin Svensson and Ann Ehnborg for skillful technical assistance.

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Appearing monthly - Volume 45 No. 3 - August 1st 1983
Completing Volume 45

ISSN 0009-2797
CBINA 45 13 283-419

190
K-66681-(100)
[1983]

Chemico-Biological Interactions

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K-66681-(100)
[1483]

Chem.-Biol. Interactions, 45 (1983) 393-399
Elsevier Scientific Publishers Ireland Ltd.

393

Short Communication

THE BINDING OF 2,3,7,8-TETRACHLORODIBENZODIOXIN TO
PLASMA LIPOPROTEINS MAY DELAY TOXICITY IN EXPERIMENTAL
HYPERLIPIDEMIA

MARINA MARINOVICH, CESARE R. SIRTORI, CORRADO L. GALLI and
RODOLFO PAOLETTI

*Institute of Pharmacology and Pharmacognosy, via Andrea Del Sarto 21, University
of Milano, 20129 Milan (Italy)*

(Received November 17th, 1982)

(Revision received February 2nd, 1983)

(Accepted February 6th, 1983)

Key words: 2, 3, 7, 8-Tetrachlorodibenzodioxin binding — Lipoproteins —
Experimental hyperlipidemia — LD₅₀

Introduction

The association between reduced blood lipid levels and increased incidence of tumors, particularly of the gastrointestinal tract, has been recently described by many authors [1-3]. The apparent increase of risk, particularly in hypocholesterolemic subjects, has been linked to: reduced plasma transport of carotene [4], loss of cell membrane cholesterol, with consequent increased cell vulnerability [5] and to a deficiency of immunoregulatory lipoproteins [6]. Lipophilic xenobiotic compounds, including most of the known carcinogens, are carried by plasma lipoproteins [7]; such is the case of chloroethylnitrosoureas [8], methylcholanthrene [9], benzopyrene [10], dimethylaminoazobenzene [11], etc. The toxicity of compounds of this type may be, therefore, modified, in conditions of hyper- or hypo-lipidemia.

2,3,7,8-Tetrachlorodibenzodioxin (TCDD) intoxication has raised considerable interest in recent years, particularly in view of numerous industrial accidents [12], with contamination of many thousands of individuals. The acute administration of non-lethal doses of TCDD has been associated, in studies by us and by other groups [13,14], with hyperlipidemia and increased high density lipoprotein cholesterol levels in rodent models. Since

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VDHL, very high density lipoproteins; VLDL, very low density lipoproteins; TCDD, 2,3,7,8-tetrachlorodibenzodioxin.

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the mode of TCDD transport in plasma has not been completely elucidated, we examined the binding pattern of this compound in normal and hyperlipidemic humans and rodents, also attempting to verify changes in toxicity in this latter experimental model.

Materials and Methods

TCDD was acquired from C. Erba (Milan, Italy) and dissolved in corn oil/acetone (9:1, v/v) prior to administration. [^{14}C]TCDD was from Eco.-Control, Inc., Cambridge, MA, U.S.A.

For the *in vitro* experiments, plasma was obtained from human subjects, either normal or with hyperlipoproteinemias types IIA and IV [15]. Rat plasma was from normal Sprague-Dawley animals (CD-1 Albino, Charles River, Calco, Italy) or from rats of the same strain treated for one week with a cholesterol-cholic acid diet [16] (Altromin Rieper, Vandoies, Bolzano, Italy).

Samples from human or rat plasmas (3.0 ml) were incubated with 0.5 μCi [^{14}C]TCDD (spec. act., 47.5 $\mu\text{Ci}/\text{mmol}$) for 90 min at 37°C. The TCDD-plasma solution was then eluted on a Bio Gel A-15 m agarose column (2.5 x 100 cm) with 0.01 Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl, 0.01% EDTA- Na_2 and 0.02% NaN_3 . The collected fractions were monitored for absorbance at 280 nm and for radioactivity in a liquid scintillator (Packard Tri-Carb 3255).

For the comparative analysis of multiple plasma samples, and to allow a complete radioactivity recovery, plasmas from hyperlipidemic patients were also separated by sequential ultracentrifugation [17]. Lipoprotein mass, calculated by the chemical compositional data, and radioactivity were determined for each separated lipoprotein.

To evaluate any changes in TCDD toxicity in the presence of hyperlipidemia, CD-1 Albino rats (C. River), body wt. 200–250 g, were injected *i.p.* with 30, 45, 67.5, 101, 152 $\mu\text{g}/\text{kg}$ of TCDD. At least 8 animals were used for each dose level. Rats were kept on either the standard or the hyperlipidemic [16] regimen for 1 week prior to the administration of TCDD and kept on the same diet thereafter. Mortality data were collected for 34 days. The method of probits was employed for the calculation of the LD_{50} at different time intervals during the toxicity study.

Results and Discussion

Incubation of [^{14}C]TCDD with plasma and with the lipoprotein ($d = 1.225$) fraction from normo- and hyperlipidemic individuals, resulted in a negligible binding to albumin, most of the radioactivity being associated with very low and low density lipoproteins (VLDL and LDL) (Fig. 1). To evaluate the different binding patterns in normo- and hyperlipidemia, representative sera from 3 normolipidemic individuals, 3 subjects with type IIA hyperlipidemia and 3 with type IV hyperlipidemia, *i.e.* characterized, respectively, by increased LDL and VLDL in plasma [15], were analyzed for the binding of labelled TCDD. As shown in Table I, both in normals

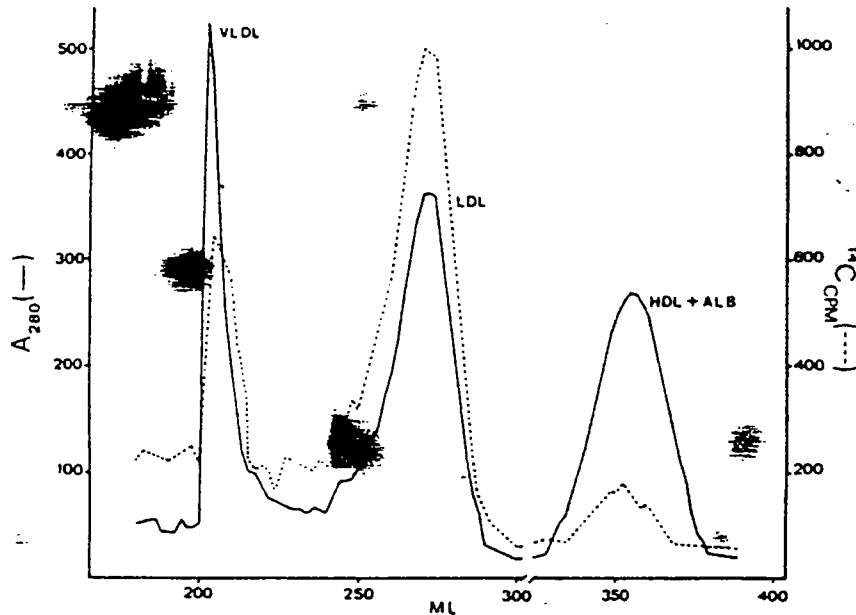


Fig. 1. Elution profile of plasma lipoproteins ($d > 1.225$ g/ml), isolated from a normolipidemic subject by ultracentrifugation and incubated with [^{14}C]TCDD. The separation was carried out on a Bio Gel A-15 m agarose column (2.5×100 cm). A 3.0 ml sample was eluted with 0.01 M Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl, 0.01% EDTA Na, and 0.02% NaN_3 . (—) A_{280} ; (---) radioactivity (cpm/fraction), associated with VLDL, LDL and HDL. Similar findings were obtained with whole plasma, radioactivity associated with albumin being less than 10% of the total.

TABLE I

PERCENTAGE DISTRIBUTION OF [^{14}C]TCDD AMONG HUMAN PLASMA LIPOPROTEINS SEPARATED BY ULTRACENTRIFUGATION

Values represents the percentages of radioactivity associated to the different lipoprotein fractions. Values in brackets indicate the molar ratio TCDD/lipoprotein. Duplicate samples from 3 normolipidemic subjects, 3 type IIA and 4 type IV patients ($\bar{x} \pm \text{S.E.M.}$). * $P < 0.01$ vs. LDL N; ** $P < 0.05$ vs. VLDL N; *** $P < 0.01$ vs. VLDL N.

	VLDL ($d < 1.006$)	LDL ($d 1.006-1.063$)	HDL ($d 1.065-1.21$)
Normolipidemic subjects	17.0 ± 1.7 (5.4 ± 1.3)	55.1 ± 2.6 (0.6 ± 0.03)	15.1 ± 2.3 (0.02 ± 0.003)
Type IIA patients	$5.2 \pm 1.9^{**}$ (2.4 ± 0.3)	$69.1 \pm 0.6^*$ (0.3 ± 0.05)	14.4 ± 2.3 (0.01 ± 0.001)
Type IV patients	$41.5 \pm 2.4^{***}$ (4.2 ± 0.5)	$36.8 \pm 2.7^*$ (0.3 ± 0.04)	10.4 ± 1.5 (0.02 ± 0.01)

and type IIA subjects, LDL are the major binding fraction. In contrast, VLDL are the quantitatively most important carrier in type IV patients. Relatively minimal amounts are bound to high density lipoproteins (HDL) and to very high density lipoproteins (VHDL). The binding of TCDD was also calculated per mole of lipoprotein. Molar lipoprotein concentrations are derived from chemical compositional data and published molecular weights [18]. From these data (Table I), it appears that the maximal binding capacity is exerted by VLDL, followed by LDL and HDL, thus confirming recent findings for benzopyrene in normolipoproteinemic male donors [10]. A similar evaluation of the lipoprotein binding of TCDD in normal and cholesterol-cholic acid fed rats, clearly showed that also in this species the toxic chemical is preferentially bound to plasma lipoproteins, a higher percentage of binding being observed in hyperlipidemic animals (Table II).

Based on these data, the acute toxicity of TCDD was evaluated in Sprague-Dawley rats, both normolipidemic and after dietary induction of hyperlipidemia (plasma cholesterol levels: 89 ± 8.1 mg/dl for controls and 1586 ± 249 mg/dl for hypercholesterolemics; plasma triglycerides: 118 ± 11.1 mg/dl for controls and 478 ± 121 mg/dl for diet treated rats at the end of the experiment). The lipoprotein distribution in this experimental model of hyperlipidemia is characterized by a marked increase, particularly of VLDL cholesterol and triglycerides [19].

The LD_{50} for rats, calculated after 3 weeks from the administration of TCDD, was significantly higher in hyperlipidemic animals as compared to controls on a normal diet (Fig. 2a). At a later interval, however, the LD_{50} tended to approach that observed in normal animals (Fig. 2b). The possible relationship between changes in LD_{50} and metabolic turnover of lipoproteins in hyperlipidemic rodents [20], resulting in the release of the bound toxic chemical, needs further investigation.

The reported findings indicate that most of circulating TCDD is bound to plasma lipoproteins; LDL carry the highest percentage of the compound; however, with elevations of VLDL, as in type IV plasmas, also this latter lipoprotein may carry a significant amount of the chemical. In normal rodents, which have markedly lower plasma lipids, compared to humans, TCDD is distributed almost equally among lipoproteins and a relatively

TABLE II

PERCENTAGE DISTRIBUTION OF [^{14}C]TCDD AMONG RAT LIPOPROTEINS ISOLATED BY SEQUENTIAL ULTRACENTRIFUGATION

Three duplicate samples from each group of animals ($\bar{x} \pm S.E.M.$) * $P < 0.01$ vs. controls.

	VLDL	LDL	HDL	VHDL
Normolipidemic animals	20.5 $\pm$ 2.2	17.8 $\pm$ 1.4	19.4 $\pm$ 1.8	41.9 $\pm$ 5.4
Hyperlipidemic animals	39.9 $\pm$ 1.3*	45.4 $\pm$ 1.6*	5.6 $\pm$ 0.09*	8.9 $\pm$ 0.3*

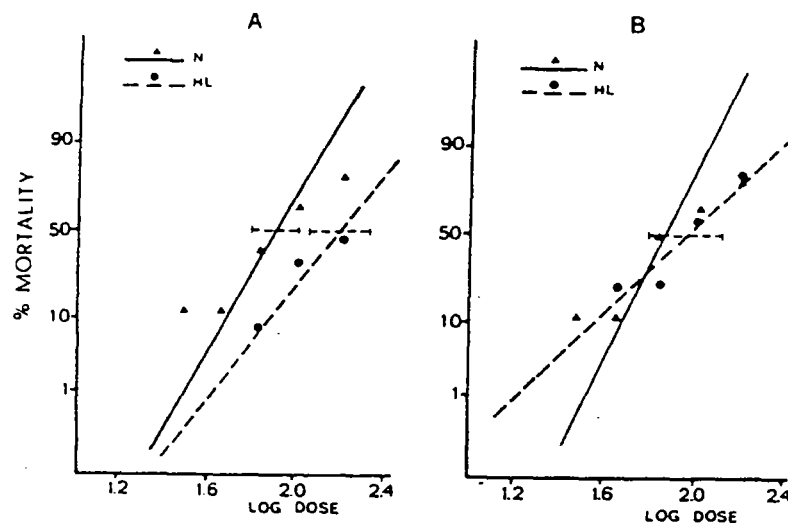


Fig. 2. LD_{50} -values for normo (—▲—) and hyperlipidemic (---●---) rats calculated at two different time intervals. A: 23 days; B: 34 days. The LD_{50} -values for normo- and hyperlipidemic animals, i.e. 76.9 (58.6–100.9 c.i.) and 151.2 (105–217.2) $\mu\text{g/kg}$, respectively, at 23 days, differ significantly ($P < 0.01$); at 34 days LD_{50} -values were 73.7 (58.5–92.8) and 90.9 (63.1–128.8) $\mu\text{g/kg}$, respectively (P , n.s.).

high percentage is also carried by VLDL. In this animal species, almost equal amounts are carried by VLDL and LDL after the experimental diet.

The possibility that binding to plasma lipoproteins may modify the long term toxic effects of lipophilic chemicals, is of compelling interest; if a chemical, in fact, is slowly released from the lipoprotein fraction to which it is bound, plasma and tissue concentrations might not reach sufficient levels to induce damage. Species differences in TCDD toxicity are well established: rats, which have significantly lower plasma lipid levels, compared to hamsters show a higher sensitivity to the toxic effects of TCDD [21]. The experimental induction of hyperlipidemia in rats, in our experiments, delayed the development of overt toxicity. The protocol of the in vivo studies did not allow examination of the binding of TCDD to lipoproteins; on the other hand, it is likely that the release of the bound chemical may occur after an interval of time, related to the metabolic turnover of lipoproteins. In hyperlipidemic rodents, turnover of VLDL and LDL is significantly delayed [20] compared to normolipidemic animals.

If plasma lipoprotein binding may modify toxicity, humans, which have the highest baseline lipids among known animal species, should, for this reason, be less sensitive to the development of parenchymal lesions. The general human toxicity from TCDD is still the object of debate; the only available long term report after an acute intoxication does not show increases in tumor and, indeed, cardiovascular accidents are reduced in contaminated individuals [22]. The deposition of lipophilic chemicals in tissues

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does not, apparently, follow the lipoprotein receptor pathway, being similar in receptor-positive and negative cells [23].

If lipoprotein binding and consequent recirculation of chemicals is an important factor in the induction of toxicity, then delayed and possibly reduced toxicity may be at hand in hyperlipidemic individuals. The converse, i.e. that hypolipidemic animals or individuals may be at higher risk of tissue damage remains, of course, to be established.

Experiments were carried out in the Special Research Laboratory of the Regione Lombardia in Seveso. The generous cooperation and support of the Regione Lombardia is gratefully acknowledged.

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Appearing monthly - Volume 45 No. 3 - August 1st 1983
Completing Volume 45

ISSN 0009-2797
CBINA 45 (3) 283-419

K-66681-(100)
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K-66681-(100)
[1983]

Chem.-Biol. Interactions, 45 (1983) 393-399
Elsevier Scientific Publishers Ireland Ltd.

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Short Communication

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MARINA MARINOVICH, CESARE R. SIRTORI, CORRADO L. GALLI and
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0009-2797/83/\$03.00

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Printed and Published in Ireland

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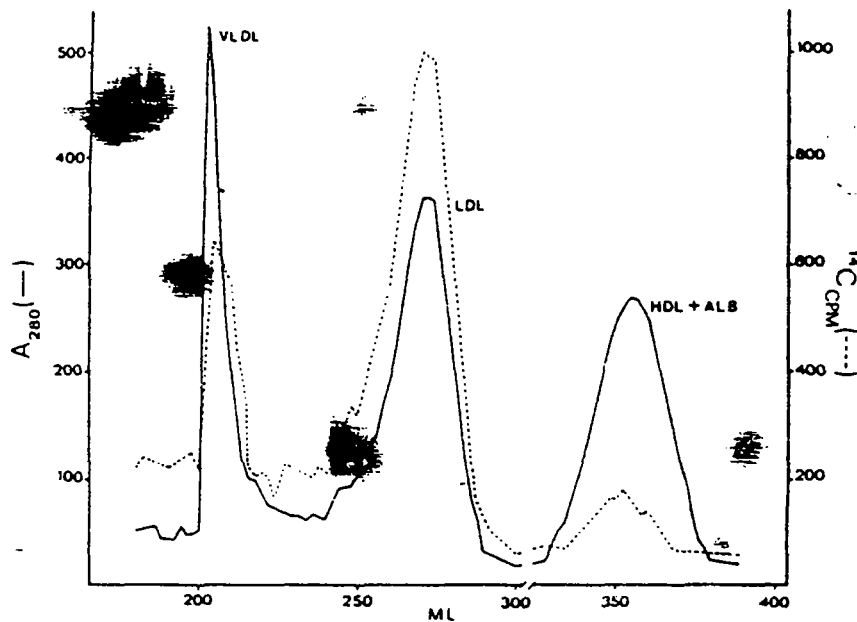


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TABLE II

PERCENTAGE DISTRIBUTION OF [14 C]TCDD AMONG RAT LIPOPROTEINS ISOLATED BY SEQUENTIAL ULTRACENTRIFUGATION

Three duplicate samples from each group of animals ($\bar{x} \pm$ S.E.M.) * $P < 0.01$ vs. controls.

	VLDL	LDL	HDL	VHDL
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Hyperlipidemic animals	$39.9 \pm 1.3^*$	$45.4 \pm 1.6^*$	$5.6 \pm 0.09^*$	$8.9 \pm 0.3^*$

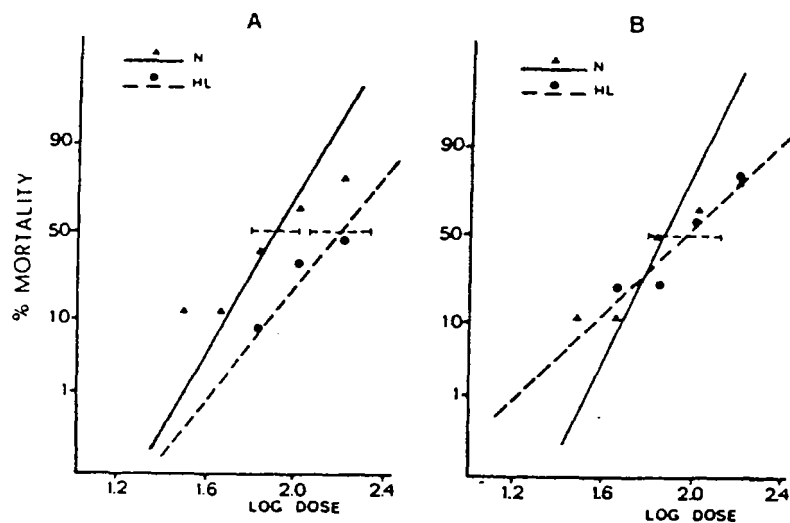


Fig. 2. LD₅₀-values for normo (—▲—) and hyperlipidemic (---●---) rats calculated at two different time intervals. A: 23 days; B: 34 days. The LD₅₀-values for normo- and hyperlipidemic animals, i.e. 76.9 (58.6–100.9 c.i.) and 151.2 (105–217.2) μ g/kg, respectively, at 23 days, differ significantly ($P < 0.01$); at 34 days LD₅₀-values were 73.7 (58.5–92.8) and 90.9 (63.1–128.8) μ g/kg, respectively (P , n.s.).

high percentage is also carried by VHDL. In this animal species, almost equal amounts are carried by VLDL and LDL after the experimental diet.

The possibility that binding to plasma lipoproteins may modify the long term toxic effects of lipophilic chemicals, is of compelling interest; if a chemical, in fact, is slowly released from the lipoprotein fraction to which it is bound, plasma and tissue concentrations might not reach sufficient levels to induce damage. Species differences in TCDD toxicity are well established: rats, which have significantly lower plasma lipid levels, compared to hamsters show a higher sensitivity to the toxic effects of TCDD [21]. The experimental induction of hyperlipidemia in rats, in our experiments, delayed the development of overt toxicity. The protocol of the in vivo studies did not allow examination of the binding of TCDD to lipoproteins; on the other hand, it is likely that the release of the bound chemical may occur after an interval of time, related to the metabolic turnover of lipoproteins. In hyperlipidemic rodents, turnover of VLDL and LDL is significantly delayed [20] compared to normolipidemic animals.

If plasma lipoprotein binding may modify toxicity, humans, which have the highest baseline lipids among known animal species, should, for this reason, be less sensitive to the development of parenchymal lesions. The general human toxicity from TCDD is still the object of debate; the only available long term report after an acute intoxication does not show increases in tumor and, indeed, cardiovascular accidents are reduced in contaminated individuals [22]. The deposition of lipophilic chemicals in tissues

does not, apparently, follow the lipoprotein receptor pathway, being similar in receptor-positive and negative cells [23].

If lipoprotein binding and consequent recirculation of chemicals is an important factor in the induction of toxicity, then delayed and possibly reduced toxicity may be at hand in hyperlipidemic individuals. The converse, i.e. that hypolipidemic animals or individuals may be at higher risk of tissue damage remains, of course, to be established.

Experiments were carried out in the Special Research Laboratory of the Regione Lombardia in Seveso. The generous cooperation and support of the Regione Lombardia is gratefully acknowledged.

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Volgens rapport RIV:

Dioxine niet volledig kankerverwekkend

De aanvaardbare dagelijkse opname van 2,3,7,8-TCDD (Dioxine) door de mens (ADI) bedraagt 0,004 nanogram (10⁻⁹) per kilogram lichaamsgewicht. Dioxine moet, zo stelt het Rijksinstituut voor de Volksgezondheid (RIV) worden beschouwd als een niet volledig kankerverwekkende stof, maar als een zogenaamde kankerbevorderende stof.



Voortijdig afgestorven bij het water langs de Volgermeerpolder waar grote hoeveelheden dioxine waren aangekomen uit de fabriek

Photo: Dijkstra

Staatscommissie

9 feb.

Prof. H.G. Verschuuren
for your info

Dat staat in een evaluatierapport dat het RIV op basis van de beschikbare toxiciteitsgegevens heeft gemaakt nadat in de Tweede Kamer op 26 augustus 1981 vragen waren gesteld over de gezondheidsrisico's voor de omwonenden van de Volgermeerpolder. In het antwoord op deze vragen dat al op 29 september 1981 werd gegeven, is de conclusie van het onderzoek reeds weergegeven.

Het nu verschenen rapport vormt de wetenschappelijke onderbouwing van de beoordeling van de kankerverwekkende en mutagene eigenschappen van 2,3,7,8-TCDD (Dioxine) op zijn effecten voor de mens. Het rapport is aan de Tweede Kamer gezonden.

In overeenstemming met het rapport van de Gezondheidsraad 'Advies inzake carcinogeniteit van chemische stoffen' van 10 november 1978, werd een ad hoc groep voor deskundigen geraadpleegd. Deze ad hoc groep bestaande uit prof. dr. P. Emmelot, prof. dr. J. H. van Genderen, prof. dr. J. H. Kueman, dr. R. Kroes en prof. dr. F. H. Sobels heeft zich verenigd met de in het rapport gestelde conclusie dat TCDD niet als volledig kankerverwekkende stof, maar als een zogenaamde kankerbevorderende stof moet worden beschouwd. Dit betekent dat voor Dioxine een voor de mens veilige dosis kan worden vastgesteld.

Staatscommissie

Staatscommissie

Dr. K. Boun

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Bloemenveiling

Hare Majesteit de Koningin zal op vrijdag 5 maart a.s. te Mierwijk het nieuwbouwcumplex van de Coöperatieve Bloemenveiling 'Beekel en Omstreken' W.A. officieel in gebruik stellen.

Haags Dierencentrum

H. M. de Koningin zal op dinsdag 23 maart a.s. de officiële opening verrichten van het nieuwe paviljoen van de Stichting Haags Dierencentrum.

Madurodam

H. M. de Koningin zal op woensdag 7 april a.s. de officiële heropening verrichten van 'Madurodam', ter gelegenheid van het 30-jarig bestaan van de miniatuurstad

Lichte daling ziekteverzuim

Het Centraal Bureau voor de Statistiek en het Nederlands Instituut voor Praeventieve Gezondheidszorg TNO stelden vast dat het ziekteverzuim in het vierde kwartaal 1981 voor mannen 8,6 en voor vrouwen 11,4 bedroeg.

In vergelijking met het vierde kwartaal van 1980 geven de cijfers een daling te zien van 0,5% voor mannen en 0,2% voor vrouwen. De ziektepercentages in het vierde kwartaal 1981 waren 49,2 per 100 mannen en 79,3 per 100 vrouwen tegen resp. 49,7 voor mannen en 78,6 voor vrouwen in het vierde kwartaal 1980.

De gemiddelde verzuimduren in kalenderdagen in het vierde kwartaal 1981 was voor mannen 15,9 en 13,1 voor vrouwen. In vergelijking met het vierde kwartaal 1980 betekent dit voor mannen en voor vrouwen een daling van resp. 0,6 en 0,3 dagen.

Inhoud

Europese Commissie: 2,3
Nederlandsche Zaken: 11

Europese Commissie:

Geen bezwaar tegen verhoging staatsdeelneming in Volvo

De Europese Commissie maakt geen bezwaar tegen de verhoging

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Nederlandse Regering en VCC/2000

Haagsche Squash Rackets Club

Z. K. H. Prins Claus der Nederlanden zal op vrijdag 19 maart a.s. de uitbreiding van het complex van de Haagsche Squash Rackets Club openen

Zeepost

Met de volgende schepen kan zeepost worden verzonden: De Data, waarop u correspondentie uiterlijk ter post moet zijn bezorgd, staan achter de namen

FROM: Staatscourant

9 Feb 82

REPORT OF R.I.V. STATES:

DIOXINE NOT A COMPLETE CARCINOGEN

The daily acceptable intake of 2,3,7,8-TCDD (dioxine) for humans (ADI) is 0.004 nanogram (10^{-9}) per kilogram body weight. Dioxine should, according to the National Institute for Public Health (RIV), not be considered to be a complete carcinogen, but is a so-called cancer promotor.

This is the essence of a report issued by RIV based on the evaluation of available toxicity data at a request of the parliament (2nd Chamber) of 26 August 1981, in view of the health risks of the population living around the Volgermeerpolder.

This recent report is the basis of the scientific evaluation of the carcinogenic and mutagenic properties of 2,3,7,8-TCDD and the implication for humans. It has been sent to the parliament.

In agreement with the document of the Health Council "Advice in Case of Carcinogenic Chemicals" dated 10 November 1978, an 'ad hoc' group of specialists was consulted. This group of specialists, consisting of Prof. Dr. P. Emmelot, Prof. Dr. J. H. van Genderen, Prof. Dr. J. H. Koeman, Dr. R. Kroes, and Prof. Dr. F. H. Sobels, is in agreement with the conclusion stated in the report that TCDD should not be considered as a 'complete' carcinogen, but as a so-called cancer promotor. This implies that safe exposure levels of dioxine for humans can be established.

Staatscourant: Daily paper which published the proceedings of prime minister and cabinet, and parliament, new laws and regulations. (cf. Federal Register.)

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British Journal of Industrial Medicine 1983;40:116

Notes and miscellanea

Tetrachlorodibenzodioxin

I would like to take issue with some of the conclusions in May's paper, "Tetrachlorodibenzodioxin, a survey of subjects ten years after exposure" (May 1982). Since 90 people were originally exposed sufficiently to develop chloracne but only 46 remained employed 10 years later, of whom 41 were studied, it is probably misleading to conclude that "there has been no death from neoplasm in the relevant population". Since the "relevant population" constitutes active (surviving) workers and about half were lost to follow-up, lack of deaths should not be surprising, even ignoring 10 years as an inadequate latent period for most tumours to become clinically apparent.

The scattergrams which show greater gamma-glutamyltransferase in the exposed groups are not accompanied by any calculations of statistical significance, but judging from the scatter, the difference may be significant. Further, the author assumes that this must be an artifact and that changes induced by dioxin must be transient once exposure was ceased. No evidence in support of this conclusion is given, merely an analogy with phenobarbital enzyme induction. His conclusion may or may not be correct: the evidence is not convincing.

The differences in fetal outcomes also have no calculations of statistical significance, although they may well be borderline. Again, they are dismissed without adequate evidence.

In short, the study is not designed to evaluate carcinogenesis, the numbers are probably inadequate to evaluate fetal outcomes, and there is a difference in enzyme induction between exposed and unexposed groups, which may be but is not necessarily related to exposure.

GRACE ZIEM

School of Hygiene and Public Health,
Johns Hopkins University,
Baltimore, Maryland 21205, USA

Dr May replies.

In answer to Ziem's first paragraph I must point out that the text of my article includes the phrase "the whereabouts of all living cases (89 out of 90) being known" and means exactly what it says. Though I was precluded from incorporating in my study those employees who had left the company, I was given every assistance in locating their current whereabouts. The death which did occur was due to cardiac failure in a retiree. I am at a loss to understand, therefore, how it can be misleading to say there has been no death from neoplasm.

The comment about the scattergrams does not dispute, indeed, appears to support, my own findings. It would have been easy to say, "there are significant variations in some of the biochemical results, and I attribute these to dioxin exposure albeit that exposure was ten years ago" (14 years now). Such an assumption would probably not have been disputed and for years to come reference would have been made to it by other authors. A case in point is the fact that a passing reference to cholesterol concentrations in my 1973¹ article is quoted widely and regularly and out of context. I believe, however, that the biochemical variations are unlikely to be due to dioxin exposure. I have therefore considered it necessary to offer an alternate and more acceptable proposition, not a statement of fact.

I do not see how, with such very small numbers and in the absence of national statistics, it would be possible to make significant calculations on fetal outcomes.

I accept Ziem's last paragraph. The study was undertaken for the benefit of the employees as a purely internal matter without thought of publication. The results were considered to be so interesting, however, that wider distribution was urged from the highest levels. I make no claims to be either an epidemiologist or a statistician but have endeavoured to acquaint the medical community with my findings, being careful to avoid deductions which the small sample was incapable of sustaining. The continued re-assuring passage of time reinforces the tenor of my article and increasingly defies the overreaction with which some greeted the original measurements.

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Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice

STRICT environmental contamination is a hazard in the use of derivatives of 2,4,5-trichlorophenol (TCP) as weedkillers¹ or defoliants² and in accidents during the manufacture of TCP³ (as in Seveso, Italy in 1976^{4,5}). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD or dioxin) is an inevitable toxic⁶, teratogenic⁷ and weakly mutagenic^{8,9} by-product which is always present as a contaminant. When TCP derivatives including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), were tested for carcinogenicity in C57BL/6 × CHH (Aurife) and C57BL/6 × AKR/J mice (experiments lasting 18 months) none increased tumour incidence¹⁰, while in another study there was a significant increase in the incidence of tumours in CHH mice treated with 2,4,5-T¹¹. During a chronic toxic study with TCDD (a potent inducer of microsomal enzymes¹²) in Sprague-Dawley rats some tumours were observed¹³. In another 2-yr study TCDD ingested at 0.1 µg per kg body weight per day caused an increased incidence of hepatocellular and squamous carcinomas in

Sprague-Dawley rats, but reduced the incidence of squamous tumours. Smaller doses of dioxin did not affect tumour incidence¹⁴. We report here that both 2,4,5-trichlorophenoxyethanol (TCP-E) a derivative of TCP and dioxin enhance liver tumours in male mice in a dose dependent manner. Amyloidosis is a long term complication often associated with severe skin lesions induced by TCDD¹⁵.

Because TCP-E is almost insoluble in water it was suspended in a salt solution containing 0.5% carboxymethylcellulose. Dioxin was dissolved in sunflower oil and determined by gas chromatography. Various doses of a combination of TCP-E and dioxin, of pure dioxin and of vehicle alone were administered (Table 1). In group 1, TCP-E contained 0.3% of contaminants. Ten week old, outbred Swiss H Rm mice (25-30g) were used in groups of 100 males plus 10 females, except for groups administered pure dioxin and sunflower oil alone, each of which consisted of 45 males only. Animals were kept in plastic cages at 25 ± 2 °C. Standard diet and water were provided *ad lib*. TCP-E contaminated with dioxin, pure dioxin or vehicle alone were administered by gastric tube once a week for 1 yr.

Animals were followed up for the rest of their lives. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. For light microscopy, sections were stained with haematoxylin and eosin. Amyloid deposits were identified with Congo red stain and polarised light¹⁶ and by electron microscopy. Pathological findings were assessed and analysed statistically. The effective number of animals was the number of survivors at the time the first tumour was observed. Significance was calculated by the χ^2 test. A difference in tumour frequency between groups was considered significant only when P was less than 1%. The carcinogenesis study was carried out in three phases: groups 1-3, 4-8 and 9-12 and comparisons were made with the respective control groups.

The LD₅₀ of an acute, peroral dose of TCP-E containing 0.1 ppm dioxin is 1,320 mg/kg¹⁷. The largest dose of TCP-E that induced no noticeable tissue lesions in the organs of the mice during 6 months of administration was 70 mg per kg. That was therefore taken as the maximum tolerated dose.

Table 1. Cumulative data on tumour incidence.

Groups	Treatment			Sex	Effective no. of mice	No. of tumour bearing mice	Animals with tumours (%)				Average lifespan (d)
	TCP-E (mg per kg)	Dioxin (µg per kg)	Vehicle* (mg per kg)				Liver	Adipose	Lymph nodes	Other organs	
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42	46	5	16	595
				F	83	61	7	16	2	25	552
2	70.0	0.167 (0.1 ppm)	50	M	98	78	57	58	24	11	571
				F	96	59	9	19	10	23	562
3	Control	Control	50	M	93	63	2	26	44	1	577
				F	84	57	4	15	41	23	539
4	7.0	0.67 (10 ppm)	50	M	93	79	15	27	34	16	641
				F	95	60	10	16	38	19	589
5	7.0	0.67 (10 ppm)	50	M	94	77	23	24	31	26	575
				F	93	71	8	9	42	30	560
6	0.7	0.0067 (0.1 ppm)	50	M	97	78	24	25	31	17	545
				F	94	64	5	5	36	22	566
7	—	—	50	M	96	74	11	13	44	14	575
				F	84	66	4	5	36	18	565
8	Control	Control	50	M	96	78	12	13	38	22	571
				F	92	67	4	4	31	24	549
9	—	7.0	10	M	43	27	13	31	1	7	474
10	—	0.7	10	M	44	36	17	48	18	4	643
11	—	0.0067	10	M	44	36	15	29	27	1	545
12	—	—	10	M	46	29	5	12	18	7	552

* Carboxymethylcellulose in groups 1-3, sunflower oil in groups 4-12.
¹ $P < 1\%$.
² $P < 1\%$.

Not Available Copy

Table 2. Occurrence of amyloidosis with skin lesions induced in mice by dioxin

Groups	Treatment		Effective dose (µg)	Skin lesions (no.)	Skin lesions + amyloidosis (no.)
	Dioxin (µg per kg body weight)	Vehicle (µg per kg)			
9	10	100	43	28	17
10	0.7	100	44	13	10
11	0.007	100	44	5	5
12		100	46	0	0

The cumulative data in Table 1 show that only the incidence of liver tumours of the males significantly differed between groups. Subsequent comparisons therefore relate to the males. The incidence of liver tumours in groups 1, 2 and 10 was twice that in the respective controls (groups 3 and 12). The smaller doses of TCPE and dioxin given to groups 5 and 6 (1, 10 and 1, 100 of maximum tolerated dose) caused less frequent tumours, there were no significant differences from groups 7 and 8. There were no significant differences in the frequency of liver tumours between groups 1 and 2 (given dioxin at 0.112 and 0.007 µg per kg) or groups 4 and 5. The latter two groups received equal amounts of TCPE, but group 4 received 100 times more dioxin than did group 5. Thus, dioxin in this dose range did not influence tumour frequency.

Groups 11 and 2 received equal amounts of dioxin (0.007 µg per kg) but there was no significant difference in liver tumour incidence between group 11 and its control, group 12. Thus dioxin has no liver tumour enhancing effect at this dose. The increased incidence of liver tumours in group 2 was caused by TCPE, and not by dioxin. In group 10, which received 100 times more dioxin than did group 11 (0.7 µg per kg), liver tumour incidence was twice that in the control, group 12. Thus dioxin has a liver tumour enhancing effect at a higher dose. Dioxin contamination in group 4 was less than 0.7 µg per kg—the threshold dose of TCDD in our experiment—and its hepatocarcinogenic effect could not be observed.

Spontaneous and induced liver tumours were not histologically different. The ratio of benign hepatomas to hepatocellular carcinomas in the control groups was not affected by treatment, only the absolute number of liver tumours increased. No cirrhosis was seen concurrently with tumours.

Average life span decreased considerably in group 9 (Table 1) which received the largest dose of TCDD. TCDD caused severe chronic, ulcerous skin lesions (probably similar to chloracne in humans) followed by generalised lethal amyloidosis (Table 2), which can be regarded as a process secondary to chronic skin lesion. This group was not evaluated in the carcinogenic bioassay for early death.

Until more is known about the people who have been exposed to them, the carcinogenicity of 2,4,5-T and structurally related chemicals in humans cannot be evaluated^{19, 21}. But it is clear that not only dioxin contamination, but also exposure to TCPE during its production or use should be controlled and reduced. Amyloidosis should be considered a possible later complication of the most severe forms of human chloracne induced by TCDD.

A preliminary report of this study was presented at the International Conference on Ecological Perspectives on Carcinogens and Cancer Control in Cremona in 1977²². This work was initiated by the late Professor B. Kellner. We thank Mrs Rose Gial Szabo for technical assistance.

K. TOTH
SUSAN SOMEFAL-REITE
J. SUGAR
JUDIT HINCEI

Research Institute of Oncopathology,
Ruth György 7-9,
H-1122 Budapest, Hungary

Received 17 May 1977; accepted 17 February 1979

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British Journal of
Industrial Medicine

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Medical and morbidity surveillance findings among employees potentially exposed to TCDD

G G BOND,¹ M G OTT,¹ F E BRENNER,¹ AND R R COOK¹

From US Area Medical¹ and Michigan Division Medical,² Dow Chemical USA, Midland, Michigan 48640, USA

ABSTRACT Available medical and morbidity surveillance findings from 1976 to 1978 for two employee cohorts potentially exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were compared with those of matched unexposed employees. The medical surveillance findings were derived from a screening programme offered to all active employees and included an analysis of various medical history questions and blood chemistry results. Group medical insurance claims served as the source of morbidity surveillance data and the period prevalence of selected diseases was analysed. Few significant differences between the exposed and unexposed were detected. Among the cohort of employees potentially exposed during the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a significantly greater frequency of x-ray proved ulcer was reported and significantly more members of this group had diseases of the digestive system diagnosed. Such findings were absent in the more highly TCDD-exposed cohort engaged in 2,4,5-trichlorophenol production, making it unlikely that dioxin was a cause.

The trace impurity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is formed as a byproduct during the manufacture of 2,4,5-trichlorophenol (TCP) under alkaline conditions at raised temperatures and high pressures. Since TCP is a precursor for the production of the herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), low level contamination of the herbicide by TCDD is expected. Thus the potential for occupational exposure to TCDD may arise among employees making either TCP or 2,4,5-T.

Recent evidence suggests that TCDD may also be formed during the routine combustion of carbonaceous fuels.¹ Chlorinated dioxins have been found in trace amounts in samples gathered from incinerator stacks as well as from chemical tar burners and a fossil fuelled power plant, vehicle silencers, fireplaces, chimneys, cigarette smoke, and portions of charcoal broiled steaks.

Mortality studies and case summaries of occupational cohorts presumably exposed to high levels of TCDD during the production of TCP, or exposed to lower levels during the synthesis of 2,4,5-T, have been published²⁻⁹ (J A Zack, unpublished observations). Reports have appeared of soft tissue sarcoma among employees with exposure severe enough to

cause chloracne²⁻⁷; however, no consistent unique mortality patterns have been noted among those with lower level exposure⁹ (J A Zack). A survey of reproductive outcomes among wives of employees exposed to dioxins was also recently completed and indicated no biologically important associations.¹⁰ Available evidence suggests that occupational cohorts exposed to lower levels of TCDD in the manufacture of 2,4,5-T experience no important long term health risks.

Investigations, including the evaluation of health examination findings and morbidity data for TCDD-exposed individuals with long latency, have only recently been started.¹¹ Clinical follow up 10 years later of 41 out of 79 individuals who developed chloracne in a 1968 TCP production accident, showed that other than minor persistent chloracne, there was no evidence of adverse health effects.¹² While relatively little data are available from sound epidemiological follow up of other exposed cohorts, reported systemic health effects after exposure to large amounts of TCDD include impaired liver function, nephropathy, gastrointestinal irritation, myopathy, and neuropathy, including depression and irritation of the central nervous system. These symptoms have not been progressive and have been reported to disappear with time. Vietnam war veterans, presumably exposed to lower levels of

Received 11 August 1982
Accepted 30 September 1982

TCDD and for shorter periods than industrial cohorts, have alleged a number of medical complaints—for instance, weight loss, liver damage, recurrent rashes, deformed offspring, stillbirths, cancer, sterility, personality changes, and "other" illnesses—which they attribute to their exposure to Agent Orange.¹¹ To date, there have been no well controlled epidemiological studies to substantiate the allegations that these complaints are attributable to exposure to the trace amounts of TCDD in Agent Orange.

Medical follow up of inhabitants of Seveso exposed to TCDD as a result of an explosion of a trichlorophenol reactor has continued to document a lack of severe lasting health effects. While the findings are still regarded as preliminary, no organs or body functions, except the skin, have been found to be impaired; no excessive derangement of gestation, fetal lethality and loss, gross malformations, growth retardation at term or cytogenetic abnormalities have yet occurred.¹²

The present study reports the cross sectional medical and morbidity surveillance findings from 1976 to 1978 among employees at Dow Chemical, Michigan, who were considered potentially exposed to TCDD and for whom mortality surveys have been reported.¹³ The objective of the present investigation was to determine whether patterns of selected health examination findings from the routinely administered medical surveillance examination, and indices of morbidity as reflected in medical insurance claims for employees potentially exposed to TCDD, differed from those of employees not exposed.

Methods and materials

The study population was assembled from two previously identified cohorts: (1) a group of employees who had been engaged in the manufacture of 2,4,5-T for at least one month between 1950 and 1971 and (2) a cohort of employees involved in a 1964 chloracne incident in the area of TCP production.

The methods for the selection of these cohorts have been described previously.¹³ Briefly, the 204 white men comprising the 2,4,5-T cohort were identified from annual department census lists and work history records as having worked one month or longer between 1950 and 1971 on one of four job assignments (reactor operator, salt wheel operator, acid wheel operator, or dryer operator) in the 2,4,5-T process. The TCP cohort of 61 white male employees was identified from monthly department census lists and lists of maintenance personnel who were known to have worked in the process area during a 1964 chloracne incident. The two cohorts have

been considered separately in the analysis to allow for differences in their potential for TCDD exposure, and for differences in their exposures to other agents.

Data of interest were derived from two separate sources: health examination findings from the routinely administered medical surveillance programme and morbidity surveillance as reflected in diagnoses from external medical service providers reported for payment of fee to the group insurance department.

The medical surveillance examination has been offered to all employees since 1967. The time required for the programme to cycle through the entire manufacturing complex is about two years. Only cohort members who had participated in a medical surveillance examination between 1976 and 1978 were eligible for consideration for this portion of the study.

Controls for the medical surveillance analyses were selected from among other white men employed at this location who had also participated in such an examination between 1976 and 1978. Employees considered at high risk because of a history of a job assignment as a pipe coverer, in the production or packaging of arsenical pesticides, considered as potentially exposed to high levels of vinyl chloride, or potentially exposed to dioxins were excluded from consideration as potential controls. Four controls were then matched from those eligible to each exposed on year of birth $\pm$ five years, whether hourly or salaried, smoking habit (never smoker, ex-smoker, current smoker) and, when possible, month and year of the most recent medical surveillance examination taken. When more than four controls were eligible for matching, the four with the date of hire nearest that of the exposed were selected.

Based on a review of relevant reports on animal and human exposure to TCDD, selected medical history questions and blood chemistry results were considered for analyses. The matching was retained throughout. For the dichotomous questionnaire responses (yes or no), the analysis was that which has been proposed for a fixed R:1 matching ratio with $R = 4$.¹⁴ Exact confidence limits were calculated with $\alpha = 0.10$ using a packaged set of programmes for a programmable calculator.¹⁵ Blood chemistry values for each exposed subject were contrasted with the mean for the four matched controls, and a paired *t*-test was used to test the hypothesis that the mean difference between the exposed and matched controls was zero. Linear regression was done to test the correlation between $\log_{10}$ (days of employment in 2,4,5-T production) and the difference in blood chemistry values.

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Group medical insurance claims filed by employees for medical expenses incurred between 1 January 1976 and 31 December 1978 served as the source of morbidity data. Members of the original study cohorts who were eligible for benefits on 31 December 1978 from the Medical Care Programme administered by the group insurance department were included for study. This time restriction ensured that morbid events reported were independent of mortality as reported in earlier studies of these cohorts.¹³ Controls for morbidity comparisons were selected from among all white male employees eligible for Medical Care Programme benefits as of 31 December 1978. Employees with a history of certain job assignments were excluded from consideration as controls, as has been described above. From among the pool of eligible controls, four were matched to each exposed on the basis of year of birth $\pm$ five years, and whether hourly or salaried. When more than four controls were eligible, the four with the date of hire nearest that of the exposed were selected.

Diagnoses and expense dates were abstracted from Medical Care Programme claims by a registered nurse, who was unaware of the exposure status of the subjects. Only diagnoses for claims for hospital inpatient care, hospital outpatient care, surgery, diagnostic expenses, or emergency first aid were considered. This excluded diagnoses from physician office visits, unless diagnostic expenses were incurred or surgery was done, and information from drug prescriptions. No validation of diagnoses with any external source was attempted.

Coding of diagnoses to the international classification of diseases—ninth revision—clinical modification (ICD-9-CM) was done by trained coders without knowledge of the exposure status of subjects. Diagnostic categories of interest were derived based on the relevant animal and human toxicology data.

An individual could contribute, at most, only one observation to each diagnostic category. Period prevalence of all cancer, various site specific malignancies, diseases of porphyrin metabolism, digestive system diseases, hepatic disorders, renal disorders, and diseases of skin and subcutaneous tissue was compared, using an analysis that maintained the matching.¹⁴

Results

DEMOGRAPHIC CHARACTERISTICS

Table 1 presents the vital and employment status distributions for the two cohorts, together with eligibility for the medical surveillance and morbidity surveillance portions of the study. Two years of

Table 1 Vital and employment status of exposed cohorts as of 31 December 1978. (Percentage of original cohorts in parentheses)

Status	TCP cohort	2,4,5-T cohort
Original	61	204
Employed	40	116
Retired	11	21
Deceased	4	11
Left other than through retirement:	6	56
Deceased	0	2
Known alive	6	2
Unknown	0	52
Participated in medical surveillance exam	27(44)	87(43)
Eligible for medical care programme and morbidity surveillance	48(79)	135(66)

additional follow up with company records of the 2,4,5-T cohort identified two deaths not previously reported—one from cerebrovascular disease and the other from cardiovascular disease. Some 44% of the original 61 members of the TCP cohort and 43% of the 204 members of the 2,4,5-T cohort participated in medical surveillance examination between 1976 and 1978. Of those who were actively employed and thus eligible to participate, 68% of the TCP cohort and 72% of the 2,4,5-T cohort did so. These participation rates are significantly lower than the participation rate of 80% among all other white men employed at this location ($p = 0.04$ and $p = 0.02$, respectively). Of the 49 individuals in the original TCP cohort who had evidence of acne like lesions in 1964, 22 participated in a medical surveillance examination. Nearly all members of both cohorts (94% of TCP and 97% of 2,4,5-T), who were active or retired, were eligible for medical care programme benefits and thus could be surveyed for morbidity in this study.

Table 2 presents a comparison of demographic data for the subsets of each original cohort eligible for the medical surveillance examination and for morbidity surveillance and their matched controls. The exposed cohorts and their matched controls compared well with respect to all variables considered. In the analysis, potential confounding by cigarette smoking, age, year of hire, and socioeconomic status was controlled by matching and stratification on these variables or correlates.

MEDICAL SURVEILLANCE FINDINGS

Table 3 presents a comparison of the frequency of positive screens with selected questions from the medical surveillance examination for the two exposed cohorts and their matched controls. The frequency of positive screens was low for nearly all the questions, and the exposed and controls responded comparably. The only statistically

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Table 2 Comparison of exposed cohorts and their matched controls on selected demographic variables

Demographic variable	TCP cohort	Controls	2,4,5-T cohort	Controls
Medical surveillance:				
No eligible for study	27	108	87	348
Mean age $\pm$ SD	45.4 $\pm$ 7.6	45.4 $\pm$ 7.8	42.8 $\pm$ 8.2	42.8 $\pm$ 8.3
Year of hire	1953.6 $\pm$ 7.9	1953.3 $\pm$ 9.2	1957.1 $\pm$ 8.5	1956.6 $\pm$ 10.0
Salaried	5	20	14	56
Paid by the hour	22	88	73	292
Current smoker	6	24	25	100
Ex-smoker	10	40	37	148
Non-smoker	11	44	25	100
Morbidity surveillance:				
No eligible for study	48	192	135	540
Mean age $\pm$ SD	49.2 $\pm$ 9.5	49.2 $\pm$ 9.6	45.3 $\pm$ 10.2	45.3 $\pm$ 10.2
Year of hire	1951.4 $\pm$ 8.0	1951.7 $\pm$ 10.5	1956.7 $\pm$ 7.9	1955.0 $\pm$ 10.6
Salaried	5	20	17	68
Paid by the hour	43	172	118	472

Table 3 Frequency of positive screens to selected medical surveillance examination questions among TCP and 2,4,5-T cohorts and matched controls, most recent exam 1976-8

Question	TCP cohort		2,4,5-T cohort	
	N_E (n = 27)	N_C (n = 108)	N_E (n = 87)	N_C (n = 348)
Since your last Dow physical exam or health inventory, have you had any of the following?				
Skin trouble	2	5	6	14
Yellow jaundice	0	0	0	0
Problems with urination	0	0	1	5
Albumin or sugar in urine	0	0	0	3
Blood in urine	0	1	0	2
Frequent indigestion or heartburn	0	5	4	19
Intestinal trouble	0	0	1	2
Loss of weight	0	4	3	6
Coughing up of blood	0	0	0	1
Change in bowel habits	0	0	0	0
Blood in stools	0	1	0	2
Tarry stools	0	1	0	1
Do you have or have you had any of the following?				
High blood pressure	1	7	6	24
Liver trouble (hepatitis)	1	4	2	12
Kidney or bladder trouble	2	7	5	19
Cancer or malignant growth	0	0	0	3
x-ray proved ulcer	2	7	9*	18

* $R_{ML} = 2.11$; $X_{adj} = 1.78$, 90% CI = 1.06-4.20. N_E = Number of exposed responding "Yes." N_C = Number of controls responding "Yes."

significant difference was the greater frequency of positive screens for x-ray proved ulcer among members of the 2,4,5-T cohort ($R_{ML} = 2.11$, 90% CI = 1.06-4.20), based on nine of those exposed having reported such a history. As asked, the question of x-ray proved ulcer does not discriminate between incidence or prevalence, so the temporal relation between exposure and disease is not known.

Table 4 presents a comparison of results for nine selected blood chemistry tests performed as a part of the medical surveillance examination. Mean total bilirubin was slightly lower in both exposed cohorts and mean serum aspartate transaminase was lower in the 2,4,5-T cohort than in their respective controls; and while these differences were statistically significant at $\alpha = 0.10$, two tailed, they were not

regarded as clinically significant. Given the number of comparisons done and the level of accepting a type I error, some statistically significant differences were to be expected.

When the mean difference between blood chemistry values for the exposed and their matched controls was regressed against $\log_{10}$ (days of employment in 2,4,5-T), there was a lack of significant association for all laboratory tests except for a significant positive slope for blood urea nitrogen ($p = 0.022$); however, the independent variable explained only 6% of the variability in the dependent variable, suggesting that duration of employment in 2,4,5-T was not an important determinant.

Analyses of medical surveillance findings among only those members of the TCP cohort who had

Table 4 Comparison of exposed cohorts with matched controls for nine selected laboratory tests, most recent medical surveillance examinations 1976-8

Test procedure	Trichlorophenol cohort M $\pm$ SD	Trichlorophenol cohort less controls d $\pm$ 90% CI	2,4,5-T cohort M $\pm$ SD	2,4,5-T cohort less controls d $\pm$ 90% CI
Total bilirubin (mg/100 ml)	0.51 $\pm$ 0.17 (n = 26)	-0.08 $\pm$ 0.07	0.50 $\pm$ 0.21 (n = 84)	-0.06 $\pm$ 0.04
Total protein (g/100 ml)	7.17 $\pm$ 0.45 (n = 26)	0.04 $\pm$ 0.16	7.10 $\pm$ 0.33 (n = 84)	-0.01 $\pm$ 0.07
Protein albumin (g/100 ml)	4.16 $\pm$ 0.43 (n = 26)	0.002 $\pm$ 0.12	4.11 $\pm$ 0.35 (n = 84)	-0.05 $\pm$ 0.06
Blood urea nitrogen	16.08 $\pm$ 3.79 (n = 26)	0.37 $\pm$ 1.34	16.07 $\pm$ 3.64 (n = 84)	0.61 $\pm$ 0.65
Lactic dehydrogenase (mi-u/ml)	114.7 $\pm$ 30.4 (n = 26)	4.08 $\pm$ 6.75	108.3 $\pm$ 23.9 (n = 82)	-3.67 $\pm$ 4.49
Serum aspartate transaminase (u/ml)	27.69 $\pm$ 8.84 (n = 26)	5.32 $\pm$ 6.39	29.99 $\pm$ 7.72 (n = 85)	-2.53 $\pm$ 1.83
Serum alanine transaminase (u/ml)	32.26 $\pm$ 13.34 (n = 23)	1.21 $\pm$ 5.20	33.24 $\pm$ 14.66 (n = 79)	0.59 $\pm$ 2.95
Alkaline phosphatase	70.88 $\pm$ 21.52 (n = 26)	-0.36 $\pm$ 6.18	71.05 $\pm$ 24.13 (n = 84)	0.21 $\pm$ 4.54
Albumin-globulin ratio	1.38 $\pm$ 0.23 (n = 23)	-0.008 $\pm$ 0.08	1.40 $\pm$ 0.32 (n = 78)	-0.003 $\pm$ 0.06

(n = Number of matched quintuplets)

Table 5 Comparison of number of prevalent cases of selected disease conditions by exposure group: diagnoses from Medical Care Programme, 1976-8

Disease category	No of prevalent cases			
	TCP cohort		2,4,5-T cohort	
	N _E (n = 48)	N _C (n = 192)	N _E (n = 135)	N _C (n = 540)
Malignant neoplasms (140-209)	1	4	4	9
Mn of liver (155)	0	0	0	1
Mn of trachea, bronchus, and lung (162)	1*	0	1	1
Mn of connective and other soft tissue (171)	0	1	0	0
Mn of skin (172-173)	0	1	0	2
Malignant lymphoma (200-202)	1*	0	0	0
Diseases of porphyria metabolism (277-1)	0	0	0	0
Diseases of the digestive system excluding liver disease (530-535, 555-558, 564-1)	3	10	17†	27
Disorders of the liver (570-573)	0	5	2	5
Glomerulonephritis (580-583)	0	0	0	0
Renal failure (584-586)	0	0	0	1
Infections of kidney (590)	0	0	0	0
Diseases of skin and subcutaneous tissues (680-686, 692, 695)	0	2	2	11

*Refers to a single individual.

†R_{ML} = 2.51 X_{ML} = 2.957, 90% CI = 1.50-4.18.N_E = Number of prevalent cases among exposed cohort.N_C = Number of prevalent cases among controls.

exhibited acne like lesions in 1964 produced similar results to the analysis of the total eligible cohort.

MORBIDITY SURVEILLANCE FINDINGS

Of the 915 members of the exposed cohorts and the matched control groups eligible for Medical Care Programme benefits, 656 (71.7%) filed at least one claim for medical services during the study period 1976-8. A total of 6539 separate diagnostic entries were abstracted and coded and constituted the total morbidity data base from which the analyses of conditions of interest were carried out. The diagnostic

entries were distributed fairly evenly across the study years with 2126 entries in 1976, 2245 in 1977, and 2168 in 1978. Most of the diagnoses were on claims for physician's services (58.4%) and claims for hospital services (35.3%), with few diagnoses provided solely from laboratories (4.7%) or other sources (1.5%). About one quarter of all diagnoses provided were preceded to some disease categorisation scheme by the service provider.

Table 5 presents a comparison of the number of prevalent cases of disease conditions of interest for each of the exposed cohorts and their matched con-

Table 6 Detailed breakdown of diseases of digestive system among 2,4,5-T cohort and matched controls. Diagnoses from Medical Care Programme, 1976-8

Disease category	No of prevalent cases	
	N _E	N _C
Diseases of oesophagus (530)	2	1
Gastric ulcer (531)	2	3
Duodenal ulcer (532)	3	3
Peptic ulcer, site unspecified (533)	2	7
Gastrojejunal ulcer (534)	0	0
Gastritis and duodenitis (535)	3	4
Regional enteritis (555)	0	1
Other non-infectious gastroenteritis and colitis (558)	6	10
Irritable colon (564.1)	4	5

Note: An individual may have more than one condition.
N_E = Number of prevalent cases among 2,4,5-T cohort.
N_C = Number of prevalent cases among matched controls.

trol groups. The period prevalence of most conditions was low and was comparable between the exposed and their controls. An exception was the significantly greater prevalence of diseases of the digestive system excluding liver disease among the 2,4,5-T cohort ($R_{ML} = 2.51$, 90% CI = 1.50-4.18) based on 17 prevalent cases among the exposed. Table 6 gives a detailed breakdown of the distribution of prevalent cases among the 2,4,5-T cohort and their controls by disease within this category. The difference in prevalence cannot be explained on the basis of a preponderance of any one specific disease entity; instead, the 2,4,5-T cohort had relatively more cases of each condition.

Discussion

Results from this cross sectional examination of medical and morbidity surveillance findings among two cohorts who were potentially exposed to TCDD at some point in the past and their matched unexposed controls showed few differences. Those employees who were engaged in 2,4,5-T production at some point, between 1950 and 1971, reported the prevalence of x-ray proved ulcer significantly more often than their matched controls, and recorded significantly more diagnoses of diseases of the digestive system. The lack of a similar finding among the more highly TCDD-exposed in the TCP cohort makes it unlikely that the dioxin is associated with this finding. The departmental unit under which the 2,4,5-T process was first organised was also responsible for manufacturing various other products. Thus many of the individuals in the 2,4,5-T cohort were potentially exposed to numerous other substances during their employment with this unit.

The 2,4,5-T dust concentrations found in the plant were often high enough to be noticeably

irritating, and were believed to have resulted primarily from finishing operations where the end product was dried and fed through a hammer mill. One could postulate that ingested dust could lead to a greater frequency of diseases of the digestive system. The 2,4,5-T exposures, however, were incurred by subjects in this study a minimum of five years before the indication of disease prevalence, thus making latency an important issue.

A limitation of cross sectional studies is the uncertainty surrounding the temporal sequence in the exposure disease relationship. As was the case in this study, it is usually not possible to discriminate between disease incidence and prevalence, so that one is uncertain whether or not the exposure preceded the disease and could possibly be a cause.

Data on health outcome from medical and morbidity surveillance of the two cohorts in the present study were limited to the active or recently retired workforce. Members of the two cohorts who left the company for reasons other than retirement could not be studied. Inferences from the present study concerning the health status of former employees must be drawn carefully owing to possible retirement of former employees on the basis of health.

The study was further limited to medical and morbidity surveillance for a three year period (1976-8). Owing to the large amount of time required to locate and code archived medical claims, a larger sample was deemed unfeasible.

With the completion of this study, these employees have now been surveyed for morbidity, mortality, and reproductive outcomes. To date, the available data do not indicate serious long term health risks associated with exposures to TCDD at the levels encountered by these two industrial cohorts. Consideration is being given to continued medical and morbidity surveillance of these cohorts, and we recommend that other cohorts with similar exposures also be surveyed.

We gratefully acknowledge the contributions of Mr Bernard Lasich, Mr Stewart Gudmundsen, Mrs Janice Cartmill, Mrs Jean Townsend, Mrs Elsie McLaren, Dr Robert Kolesar, and Mrs Angie Grzegorzczuk of Dow Chemical, USA, and of Mr Larry Silverstein, formerly of Dow Chemical, USA, and now with the Dow Corning Corporation.

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**Studies on the effect of
2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on
vitamin A:**

a new aspect concerning the mechanism of toxicity

by
Tuula Thunberg



STOCKHOLM 1983

BOM2160817

STUDIES ON THE EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON VITAMIN A:
A NEW ASPECT CONCERNING THE MECHANISM OF TOXICITY

by Tuula Thunberg, Dr.Med.Sci., Department of Toxicology, Karolinska
Institute, S-104 01 Stockholm, Sweden

ABSTRACT

Chlorinated dibenzo-p-dioxins have been claimed to be ubiquitous in the environment. The most toxic of totally 75 possible isomers is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is formed during the industrial preparation of 2,4,5-tetrachlorophenol (TCP), which is used as a starting material for the manufacture of the herbicide 2,4,5-T and the germicide hexachlorophene. The presence of TCDD has also been demonstrated in fly ash from municipal incinerators. This thesis summarizes studies on the effect of TCDD on vitamin A. TCDD causes a dose-related reduction of hepatic vitamin A in the rat. The potency of TCDD in reducing the vitamin A level in the liver is more than 4×10^5 times that of phenobarbital (PB), and 3×10^6 times that of 3-methylcholanthrene (3-MC), two well-known enzyme inducers. However, no correlation between the UDPGT or AHH-induction and the reduction of hepatic vitamin A can be found. Dietary vitamin A can partly compensate for the TCDD-induced reduction of vitamin A in the liver, suggesting that TCDD does not cause a reduction in the hepatic storage capacity of vitamin A. The level of vitamin A in serum is not decreased by TCDD-treatment, indicating that the synthesis of the specific transport protein for vitamin A in the liver is not impaired by TCDD. Studies in guinea pig, rabbit, hamster, Sprague-Dawley and Gunn rats reveal a correlation between the TCDD-induced vitamin A reduction and its toxicity in these animals. Further evidence for TCDD-induced vitamin A deficiency was obtained by the finding that TCDD-treatment produces a similar effect on renal vitamin A level as does a vitamin A-deficient diet. The discovery of the TCDD-induced vitamin A deficiency may explain some of the signs in TCDD-intoxication, and may also prove to be important in evaluating the carcinogenic properties of TCDD.

Key words: TCDD, Vitamin A, Dioxin, Toxicity, Liver, Kidney, Serum, Enzyme induction

ISBN 91-7222-588-2

DOM2160518

STUDIES ON THE EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN ON VITAMIN A:

A NEW ASPECT CONCERNING THE MECHANISM OF TOXICITY

Akademisk avhandling

som för avläggande av doktorsexamen
i medicinsk vetenskap vid Karolinska Institutet
offentligen försvaras i Rättsmedicinska Institutionens
föreläsningssal, Doktorsringen 16, Karolinska Institutet
tisdagen den 24 maj 1983 kl 9

av

Tuula Thunberg

nat.kand.

STOCKHOLM 1983

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British Journal of
Industrial Medicine

Notes and miscellanea

Tetrachlorodibenzodioxin

I would like to take issue with some of the conclusions in May's paper, "Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure" (May 1982). Since 90 people were originally exposed sufficiently to develop chloracne but only 46 remained employed 10 years later, of whom 41 were studied, it is probably misleading to conclude that "there has been no death from neoplasm in the relevant population." Since the "relevant population" constitutes active (surviving) workers and about half were lost to follow-up, lack of deaths should not be surprising, even ignoring 10 years as an inadequate latent period for most tumours to become clinically apparent.

The scattergrams which show greater gamma-glutamyltransferase in the exposed groups are not accompanied by any calculations of statistical significance, but judging from the scatter, the difference may be significant. Further, the author assumes that this must be an artifact and that changes induced by dioxin must be transient once exposure was ceased. No evidence in support of this conclusion is given, merely an analogy with phenobarbital enzyme induction. His conclusion may or may not be correct: the evidence is not convincing.

The differences in fetal outcomes also have no calculations of statistical significance, although they may well be borderline. Again, they are dismissed without adequate evidence.

In short, the study is not designed to evaluate carcinogenesis, the numbers are probably inadequate to evaluate fetal outcomes, and there is a difference in enzyme induction between exposed and unexposed groups, which may be but is not necessarily related to exposure.

Dr May replies:

In answer to Ziem's first paragraph I must point out that the text of my article includes the phrase "the whereabouts of all living cases (89 out of 90) being known" and means exactly what it says. Though I was precluded from incorporating in my study those employees who had left the company, I was given every assistance in locating their current whereabouts. The death which did occur was due to cardiac failure in a retiree. I am at a loss to understand, therefore, how it can be misleading to say there has been no death from neoplasm.

The comment about the scattergrams does not dispute, indeed, appears to support, my own findings. It would have been easy to say, "there are significant variations in some of the biochemical results, and I attribute these to dioxin exposure albeit that exposure was ten years ago" (14 years now). Such an assumption would probably not have been disputed and for years to come reference would have been made to it by other authors. A case in point is the fact that a passing reference to cholesterol concentrations in my 1973¹ article is quoted widely and regularly and out of context. I believe, however, that the biochemical variations are unlikely to be due to dioxin exposure. I have therefore considered it necessary to offer an alternate and more acceptable proposition, not a statement of fact.

I do not see how, with such very small numbers and in the absence of national statistics, it would be possible to make significant calculations on fetal outcomes.

I accept Ziem's last paragraph. The study was undertaken for the benefit of the employees as a purely internal matter without thought of publication. The results were considered to be so interesting, however, that wider distribution was urged from the highest levels. I make no claims to be either an epidemiologist or a statistician but have endeavoured to acquaint the medical community with my findings, being careful to avoid deductions which the small sample was incapable of sustaining. The continued re-assuring passage of time reinforces the tenor of my article and increasingly defies the overreaction with which some greeted the original measurements.

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- ¹ May G. Chloracne from the accidental production of tetrachlorodibenzodioxin. *Br J Ind Med* 1973;30:276-83.

GRACE ZIEM
School of Hygiene and Public Health,
Johns Hopkins University,
Baltimore, Maryland 21205, USA

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[1984]

Journal Environ Pathology Toxicology and Oncology

*Official Organ of the International
Society for Environmental Toxicology
and Cancer*

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**Volume 5, Number 4/5
JULY, 1984**

ISSN 0731-8898

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IMMUNOTOXICOLOGIC EFFECTS OF 2,3,7,8 -TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN RABBITS

R. P. Sharma

Toxicology Program, Utah State University, Logan, UT 84322

R. J. Kociba and P. J. Gehring

Toxicology Research Laboratory, Dow Chemical USA, Mid-
land, MI 48640

Male white New Zealand rabbits were exposed orally to 0, 0.01, 0.1, 1 and 10 $\mu\text{g/kg/wk}$ of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for a period of 8 weeks. After 4 and 6 weeks of first TCDD administration, the rabbits were inoculated with a mixture of tetanus toxoid and Freund's adjuvant. TCDD exposure reduced the serum antitoxin titers, skin sensitivity to tuberculin, and the number of antibody producing cells in popliteal lymph nodes. At the end of the treatment period serum IgG levels were increased at the lowest dose of TCDD treatment while a marked depression was noticed at the highest dose level. An increase in the thymidine uptake by splenic lymphocytes in culture was noted at all levels of TCDD treatment whereas the response of these cells to phytoimitogens was decreased at high levels of TCDD exposure. All different immunologic effects were not altered at the lowest TCDD treatment but both humoral and cell-mediated immune responses were depressed at the highest level of TCDD exposure.

INTRODUCTION

The depression of immunologic responses by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been described in a number of mammalian species. One of the sensitive measures of TCDD toxicity is a dose-related atrophy of thymus (Buo - Hoi et al., 1972; Vos et al., 1973, 1978), although it is questionable whether this effect is related to the mortality induced by this chemical. Considerable species and age-related variation has been reported regarding the immuno-suppressive effects of TCDD. For example, TCDD depressed the delayed type tuberculin skin reactivity in young guinea pigs but not in rats (Vos et al., 1973). Vos and Moore (1974) showed the suppression of cellular immunity in rats and mice after maternal and post-natal exposures of offspring to TCDD. Thigpen et al. (1975) reported that TCDD at sublethal doses in-

Please send requests for reprints to: Dr. R. P. Sharma, Department of Animal, Dairy and Veterinary Sciences,
Utah State University, UMC 56, Logan, UT 84322

JEPTO 5-4/5:321-328

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creased mortality in mice when challenged by *Salmonella* infection and also shortened the incubation time of the infecting organisms. On the other hand, no effect on the mortality of pseudorabies virus (PRV) infected mice was demonstrated.

Vos *et al.* (1978) recently demonstrated that the increased sensitivity of TCDD-treated mice to *Salmonella* was because of endotoxin hypersensitivity, rather than the altered phagocytic action of macrophages. The thymic atrophy by TCDD, which resembles the neonatal thymectomy, was not prevented by the simultaneous thymosin treatment. Our recent studies with adult mice indicated that a continued treatment with TCDD may depress the immune response but the effects were less pronounced after an 8-week exposure, as compared to the early periods (Sharma and Gehring, 1979). This suggested a possible adaptation to the TCDD effects. Low doses of TCDD also produced an enhancement of splenic lymphocyte responsiveness in culture. The present study deals with the dose-related immunologic effects of TCDD in mature rabbits when the animals were repeatedly treated with this toxic chemical for a period of 8 weeks.

MATERIALS AND METHODS

Male white New Zealand rabbits (Langshaw Rabbitry, Carson City, MI), weighing approximately 3 kg each, were housed individually and provided free access to feed and water. After equilibration to the surroundings for one week, the animals were randomly divided into 5 groups of 5 animals each and were administered 0.0, 0.01, 0.10, and 10 μ g/kg of TCDD in corn oil by oral gavage, once a week for 8 weeks. The animals were kept in environment-controlled rooms with a 12-hour light/dark cycle throughout the experiment.

TCDD solutions for dosing these animals were prepared by dissolving 5.0 mg of the chemical (>99% purity) initially into 10 ml acetone (glass distilled) and mixing this solution with 190 ml of corn oil (Nutritional Biochemicals, Cleveland, OH). This stock solution containing 25 μ g TCDD/ml was diluted with corn oil containing 5% acetone to provide appropriate dosing solutions. The control group was dosed with 5% acetone in corn oil. Concentrations of all dosing solutions were adjusted to provide 0.4 ml/kg. The animals were weighed once a week.

After 4 and 6 weeks of the first dose the animals were injected with a 1:1 mixture of tetanus toxoid (Jensen-Salsbury Laboratories, Kansas City, MO) and Freund's complete adjuvant (GIBCO, Grand Islands, NY), given 0.3 ml/animal in their foot pad. Blood was collected from all animals by puncturing their ear vein before the first TCDD dose and just before the antigen inoculations. After an 8-week treatment period the animals were sacrificed by decapitation (under a CO₂ anesthesia) and their blood and visceral organs collected.

Twelve days after the first and second antigen challenge, i.e., two days prior to second antigen and sacrifice, respectively, the animals were tested for skin reaction against tuberculin. Tuberculin (Jensen-Salsbury Laboratory, Kansas City, MO) was injected intradermally, 0.1 ml, in a shaven flank area and the diameter of skin reaction measured 24 and 48 hr after this injection.

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Complete necropsy was performed on animals after sacrifice and their selected organs weighed. The organs (thymus, liver, kidney, spleen and popliteal lymph node) were stored in formalin for histological examinations. The tissues were routinely processed and examined microscopically. Part of the spleen was saved in sterilized saline for splenic cultures as described below. One popliteal lymph node was frozen for evaluating the antibody secreting cells.

The blood at all sampling times was used for total erythrocyte and total and differential leukocyte counts. Serum separated from blood was analyzed for serum IgG by quantitative immunoelectrophoresis using the method modified from Bjerrum *et al.* (1973), details of which have been described elsewhere (Sharma and Gehring, 1979). In serum samples obtained after the antigen inoculation, the antitoxin titers were determined by sensitizing sheep erythrocytes (SRBC) with a tetanus toxoid vaccine (1:200) and using these cells as indicator for a hemagglutination reaction. The highest dilution of serum causing complete hemagglutination in microtitration plates (V bottom, Cook Laboratories, Alexandria, VA) was considered an end point.

The splenic lymphocytes were cultured in triplicate either without or with the presence of an optimal amount of either phytohemagglutinin (PHA) or pokeweed mitogen (PWM). The culture technique has been described previously (Sharma and Gehring, 1979). The incorporation of thymidine per million lymphocytes was determined for each animal and the stimulation index for each mitogen calculated by dividing the disintegrations per minute (dpm) of cultures in the presence of the respective mitogen by the dpm of an identical culture from the same spleen but without any mitogen.

Frozen sections of popliteal lymph nodes, 6-8 μ m thick, were evaluated for the number of globulin secreting cells as described before (Street and Sharma, 1975). The number of fluorescent cells were counted in 10 random fields and averaged to determine a fluorescence score for each animal.

All values are presented as means and their standard deviations or standard error of means. The parameters of treatment groups were compared with the control group using a t-test. The body weight and organ weights were compared using a Dunnett's t (Steel and Torrie, 1960).

RESULTS

TCDD produced toxicologic manifestations in rabbits at the highest dose, i.e., 10 μ g/kg/wk. During the experiment two animals died in this group with clinical signs attributable to TCDD toxicity. These animals had accumulations of ascitic fluid and a gradual wasting syndrome leading to death. The deaths of five other animals (one receiving 10 μ g/kg/wk and 2 each from groups receiving 1 and 0.2 μ g/kg/wk) were caused by the deposition of dosing materials into their lungs. Table 1 illustrates the body and organ weights of animals surviving the 8-week experimental period. Whereas no effect of TCDD treatment is apparent on the body weights of surviving animals, the size of their liver was increased in animals receiving 1 or 10 μ g TCDD/kg/wk. A significant reduction in the size of thymus, adrenals and popliteal lymph nodes was also seen in the animals exposed to 10 μ g TCDD/kg/wk.

TABLE 1. Body and Organ Weights of Rabbits Treated with TCDD for 8 Weeks

	Weekly Dose of TCDD, $\mu\text{g/kg}$				
	0 (Control)	0.01	0.1	1	10
Body Weight (kg)	3.170 ± 0.193^1	3.388 ± 0.369	3.455 ± 0.152	2.787 ± 0.387	3.158 ± 0.106
No. of Animals					
Surviving	5	5	3	3	2
Organ Weight, g. (organ/body wt. g/100g) for					
Liver	89.64 ± 19.99 (2.82 ± 0.51)	78.38 ± 10.13 (2.31 ± 0.13)	87.14 ± 5.14 (2.53 ± 0.25)	111.98 ± 16.87 (4.01 ± 0.11) ²	152.04 ± 8.35 ² (4.82 ± 0.43) ²
Kidneys	16.82 ± 1.71 (0.53 ± 0.03)	15.64 ± 2.43 (0.46 ± 0.04)	16.51 ± 1.10 (0.48 ± 0.01)	16.61 ± 2.11 (0.60 ± 0.04)	16.52 ± 2.74 (0.52 ± 0.07)
Brain	9.17 ± 0.42 (0.29 ± 0.02)	9.52 ± 0.26 (0.28 ± 0.03)	9.20 ± 0.40 (0.27 ± 0.02)	8.86 ± 0.30 (0.32 ± 0.05)	9.87 ± 1.20 (0.31 ± 0.03)
Heart	6.10 ± 0.86 (0.19 ± 0.04)	6.87 ± 0.55 (0.20 ± 0.01)	6.51 ± 0.86 (0.19 ± 0.02)	4.83 ± 0.87 (0.17 ± 0.01)	5.30 ± 0.25 (0.17 ± 0.00)
Thymus	4.51 ± 1.83 (0.14 ± 0.05)	5.11 ± 1.28 (0.15 ± 0.03)	6.47 ± 1.37 (0.19 ± 0.03)	3.80 ± 0.60 (0.14 ± 0.02)	1.77 ± 0.36 ² (0.06 ± 0.01) ²
Spleen	1.38 ± 0.34 (0.04 ± 0.01)	1.91 ± 0.85 (0.06 ± 0.03)	1.89 ± 0.50 (0.05 ± 0.01)	1.81 ± 0.94 (0.06 ± 0.03)	1.16 ± 0.44 (0.04 ± 0.01)
Adrenals	0.57 ± 0.14 (0.02 ± 0.01)	0.68 ± 0.10 (0.02 ± 0.00)	0.70 ± 0.25 (0.02 ± 0.01)	0.40 ± 0.07 (0.01 ± 0.00)	0.25 ± 0.17 ² (0.01 ± 0.01)
Popliteal Lymph Nodes	0.58 ± 0.11 (0.02 ± 0.00)	0.64 ± 0.12 (0.02 ± 0.00)	0.69 ± 0.13 (0.02 ± 0.00)	0.52 ± 0.10 (0.02 ± 0.00)	0.41 ± 0.02 ² (0.01 ± 0.00) ²
Testes	5.86 ± 0.55 (0.19 ± 0.02)	5.99 ± 0.58 (0.18 ± 0.01)	7.30 ± 0.81 (0.21 ± 0.03)	4.76 ± 0.98 (0.17 ± 0.03)	5.18 ± 2.16 (0.16 ± 0.06)

¹Mean $\pm$ SD. The number of animals surviving is not related to TCDD induced deaths.²Indicate significant deviation from control at $p < .05$ (Dunnett's test).

Histological evaluation of selected organs did not show any specific TCDD related lesions, except the atrophy of thymic cortex. The extensive hepatic lesions, usually noticed in rodents after the TCDD treatment were not observed in rabbits. The livers showed occasional mild to moderate degeneration, presence of multinucleated or pigmented hepatocytes, and occasional fibrosis and leukocytic perivascular infiltration. Dilated sinusoidal spaces were also observed but all the lesions were neither consistent nor dose-related.

A slight reduction in the total erythrocyte and leukocyte counts was observed in animals exposed to the highest dose of TCDD, although this change was statistically significant only at 6 weeks of exposure (data not shown). Differential leukocyte counts revealed no consistent changes.

Rabbits injected with tuberculin after sensitization with Freund's adjuvant showed a dose-dependent decrease in the skin reactivity in two highest TCDD levels (Table 2). This effect was observed both after the primary and secondary

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TABLE 2. Skin Reactivity in Rabbits Exposed to Different Amounts of TCDD and Sensitized with Freund's Adjuvant

Exposure Level of TCDD $\mu\text{g/kg/wk}$	Primary Skin Response ¹		Secondary Skin Response	
	24 hr	48 hr	24 hr	48 hr
0	17.6 $\pm$ 2.7	23.2 $\pm$ 2.1	23.4 $\pm$ 1.3	17.2 $\pm$ 2.6
0.01	16.6 $\pm$ 1.6	18.4 $\pm$ 0.8 ²	18.2 $\pm$ 1.9 ²	13.6 $\pm$ 2.3
0.1	19.3 $\pm$ 0.9	23.3 $\pm$ 1.8	19.3 $\pm$ 2.3	16.7 $\pm$ 2.6
1	16.7 $\pm$ 0.7	14.3 $\pm$ 2.6 ²	14.3 $\pm$ 1.5 ²	12.3 $\pm$ 6.4
10	14.6 $\pm$ 2.4	4.3 $\pm$ 3.4 ²	10.0 $\pm$ 5.0 ²	10.5 $\pm$ 5.5

¹Primary skin response was measured 10 days after the first antigen injection whereas the secondary one was measured 10 days after the second antigen challenge. The values are millimeters of diameter of the skin reaction expressed as mean and its standard error.

²Indicate a significant difference from control at $p < .05$.

sensitization, although a significant effect was noted only 48 hr after the first and 24 hr after the second tuberculin testing, respectively. In both instances a decrease at the 0.01 μg TCDD/kg/wk dose but no effect in the second high dose, i.e., 0.1 $\mu\text{g/kg/wk}$, was observed.

Table 3 presents the serum antitetanus titers in control and TCDD treated rabbits. A consistent decrease was seen only when the weekly dose of TCDD was 1 $\mu\text{g/kg}$ or more. The fluorescence scores of popliteal lymph nodes in different groups of rabbits are also indicated in Table 3. An effect observed at the TCDD dose levels of 1 and 10 $\mu\text{g/kg/wk}$ corresponds well with the reduction in antitoxin titers in the serum.

Serum IgG levels at different periods of TCDD treatment are given in Table 4. At the end of the experimental period, the group receiving 0.01 μg TCDD/kg/wk had significantly higher IgG levels, whereas depressed IgG val-

TABLE 3. Serum Antitetanus Hemagglutination Titers in Rabbits and the Evaluation of Plasma Cells in Rabbit Lymph Node in Relation to TCDD Exposure

Exposure Level of TCDD $\mu\text{g/kg/wk}$	Serum Antitetanus Titers ¹		Popliteal Lymph Node Fluorescence Score ²
	Primary	Secondary	
0	5.6 $\pm$ 1.0	11.2 $\pm$ 2.0	15.0 $\pm$ 1.1
0.01	4.8 $\pm$ 0.8	11.2 $\pm$ 2.0	12.8 $\pm$ 2.5
0.1	2.7 $\pm$ 0.7 ³	8.0 $\pm$ 0.0	12.0 $\pm$ 3.2
1	2.0 $\pm$ 0.0 ³	5.3 $\pm$ 1.3 ³	9.0 $\pm$ 1.2 ³
10	2.0 $\pm$ 0.0 ³	4.0 $\pm$ 0.0 ³	10.0 $\pm$ 1.0 ³

¹The primary and secondary titers refer to the serum collected 2 weeks after the first and second antigen injection, respectively.

²Blind scoring of 10 random fields in each lymph node under a microscope. See Methods section for details.

³Significantly different from the respective control at $p < .05$.

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TABLE 4. Rabbit Serum IgG at Different Periods of TCDD Exposure

Exposure Level TCDD $\mu\text{g/kg/wk}$	Preexposure ¹	4 Weeks	IgG, mg/ml, at	
			6 Weeks ²	8 Weeks ³
0	14.2 $\pm$ 3.2	16.0 $\pm$ 2.0	15.4 $\pm$ 1.9	13.1 $\pm$ 2.4
0.01	17.1 $\pm$ 2.7	17.5 $\pm$ 1.9	16.5 $\pm$ 1.3	25.2 $\pm$ 1.9 ³
0.1	15.1 $\pm$ 1.8	16.5 $\pm$ 3.8	13.4 $\pm$ 2.3	24.6 $\pm$ 6.4
1	13.2 $\pm$ 2.6	16.7 $\pm$ 2.7	14.2 $\pm$ 3.9	12.6 $\pm$ 5.0
10	15.2 $\pm$ 2.9	13.0 $\pm$ 4.5	8.7 $\pm$ 2.0 ³	6.2 $\pm$ 2.1 ³

¹Values are means $\pm$ SE of 5 observations/group, except when the animals died during the experiment.

²Two and 4 weeks after inoculation with a mixture of tetanus toxoid and Freund's adjuvant.

³Significantly different from control at $p < .05$.

ues were observed in the group receiving 1 and 10 μg of TCDD/kg/wk for 6 and 8 weeks, respectively.

The incorporation of thymidine in splenic lymphocyte cultures from the rabbits is given in Table 5. A relatively high degree of thymidine uptake, consistent with the challenge of animals with two doses of antigen mixture, was apparent in the control animals. Treatment of rabbits with TCDD enhanced this spontaneous blast formation in these cultures, these became statistically significant in group receiving 1 μg TCDD/kg/wk, in spite of the inherent large variation in different animals within a group. The splenic lymphocyte cultures were responsive to the mitogen induced blast formation in TCDD-treated groups. The reduction of stimulation indices (to nearly one-half) indicated in Table 5 is partly because of a nearly three-fold increase in the spontaneous blast formation in group receiving the highest dose of TCDD.

TABLE 5. Incorporation of ³H-Thymidine in Lymphocytes and Mitogen Stimulation Indices in Rabbit Splenic Cultures from Animals Challenged with an Antigen Mixture and Given Different Amounts of TCDD¹

TCDD Exposure $\mu\text{g/kg/wk}$	DPM/10 ⁶ Cells without any Mitogen	Stimulation Index ² with	
		PHA	PWM
0	35,885 $\pm$ 6,528	7.99 $\pm$ 1.36	5.05 $\pm$ 1.81
0.01	50,261 $\pm$ 11,153	6.01 $\pm$ 1.95	6.95 $\pm$ 1.37
0.1	53,257 $\pm$ 17,134	7.09 $\pm$ 2.95	5.63 $\pm$ 2.43
1	85,392 $\pm$ 14,023 ³	4.33 $\pm$ 1.77	2.59 $\pm$ 0.53
10	90,276 $\pm$ 50,081	4.07 $\pm$ 1.32 ³	2.70 $\pm$ 1.23

¹The animals were given a mixture of tetanus toxoid and Freund's complete adjuvant, 2 and 4 weeks prior to their sacrifice. The values in the Table are Mean $\pm$ SE.

²Stimulation index = dpm of cultures with respective mitogen divided by the dpm of same cultures without a mitogen.

³Indicate a significant difference at $p < .05$.

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In conclusion it may be stated that relatively high doses of TCDD (in the order of 1 $\mu\text{g/kg/wk}$ or more) produced a consistent immunosuppression in adult rabbits. The T-dependent responses were influenced generally at a lower dose but an effect was also seen later on the humoral responses, which may have been caused by the interference with helper T-cells. Splenic lymphocytes from TCDD treated immunized rabbits showed a higher frequency of blast formation in culture, the exact implication of this finding is not clear.

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REGULATION OF EPIDERMAL GROWTH FACTOR BINDING AND UPTAKE IN CULTURED HUMAN EPIDERMAL CELLS BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). L.G. Hudson^{*}, W.A. Toscano, Jr.^{*}, and W.F. Greenlee^{*} (SPON: R.A. Hartline). Harvard Sch. Pub. Hlth., Boston, MA 02115 and CIIT, Research Triangle Park, NC 27709.

The proliferation of epidermal cells is regulated by several biochemical mediators including cyclic nucleotides and epidermal growth factor (EGF). We have examined the actions of TCDD, an agent that produces epidermal hyperplasia in humans and hairless mice on cell associated [¹²⁵I]-EGF in a human squamous cell carcinoma line (SCC 12F). Cell associated [¹²⁵I]-EGF was determined by measuring the specific binding and uptake of [¹²⁵I]-EGF at 37° after preincubating cells with TCDD. Treatment of cells with TCDD resulted in a concentration dependent decrease in cell associated [¹²⁵I]-EGF to a value of 35% of control with an apparent EC₅₀ = 1 nM. A decrease was detectable by 2 h and maximal after 72 h. The response of cells treated with 2,3,7,8-tetrachlorodibenzofuran, an isostereomer of TCDD, was equal to that of TCDD. There was no significant alteration in cell associated [¹²⁵I]-EGF in cells exposed to the nonisostereomeric analog, 2,7-dichlorodibenzo-p-dioxin. The observed potency (EC₅₀ = 1 nM) and stereospecificity suggest that the decrease in cell associated [¹²⁵I]-EGF produced by TCDD is mediated by the dioxin receptor. Sustained refractoriness of SCC 12F cells to EGF may be an important mechanism by which TCDD alters regulated cell proliferation. Supported in part by NIEHS Grant ES-08266; CIIT Predoctoral Fellowship to LGH.

---Federal Proceedings, V. 42, N 7, 1988, 1983.

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TCDD DIOXIN FOUND IN CHICKEN LIVER AND FAT

2,4,5-T charged from a number of the samples which had been tested in the Environmental Protection Agency's dioxin study (See Dec. 10, Page 3) became less likely as a candidate for cancellation. The investigators reported that 90 percent of the samples in a beef fat monitoring program showed no TCDD (dioxin) at current detection levels of 10 parts per trillion.

Data on the remaining 10 percent of samples, however, were incomplete and unconfirmed.

In a summary prepared of the chemists' conclusions in the Dec. 10 meeting, it was noted that although analytical methodology for determining dioxin content is satisfactory "in general," the data are not complete enough at present "to draw specific conclusions" concerning the overall procedure.

The panel also noted: "At absolute sensitivities (with which we are working) there are intrinsic uncertainties with the analytical method."

ADMINISTRATION STILL BACKS TOXIC SUBSTANCES LAW, TRAIN AFFIRMS

The Administration hasn't backed out from toxic substances legislation, Environmental Protection Agency Administrator Blair H. Tamm affirmed this week at his press conference on PCBs (see separate story), but he delivered a pessimistic estimate of when legislation could be passed.

Noting that Congress had adjourned for the year, he said the House Commerce Committee would probably not act on reauthorization of a subcommittee bill (See Dec. 10, Page 4) until February or "early spring." Pointing out, also, that the bill is not yet to the full Commerce Committee in the Senate, he said, "Obviously we have a long way to go."

EPA backs the McCollister bill in the house, "with amendments," he said, stating that the agency's suggested amendments were ready to go to Capitol Hill once Congress comes back from its recess.

Just before Congress left, Sen. Tunney (D-Calif.) said that "irreconcilable differences on key provisions" of the legislation "have prevented its enactment into law."

Tunney said, "A strong premarket screening provision is an absolute necessity to protect the public from environmental pollutants at the time when the costs of control are the lowest." Stating these costs are lowest at the premarket stage because jobs have not yet been committed and expensive equipment not yet purchased, Tunney continued:

"But even more importantly, control at this stage avoids the pain, misery, and in some cases, even death, that

Reversible Inhibition of *in Vitro* Epithelial Cell Proliferation by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin¹

JOHN F. GIERTHY AND DEBORAH CRANE

Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201

Received November 11, 1983; accepted January 12, 1984

Reversible Inhibition of *in Vitro* Epithelial Cell Proliferation by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. GIERTHY, J. F., AND CRANE, D. (1984). *Toxicol. Appl. Pharmacol.* 74, 91-98. Subconfluent cultures of a mouse epithelial cell line, which after prolonged subculturing exhibited an elevated saturation density as compared to the original cell line, were treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Cultures of cells with or without TCDD grew at equal rates until confluency was reached. At confluency, cultures treated with as little as 10^{-11} M TCDD showed a decline in cell proliferation relative to controls as demonstrated by cell enumeration and supported by reduced [³H]thymidine incorporation (both by liquid scintillation spectrometry of whole culture and autoradiography of individual cells). After 14 days of exposure, the saturation density of the treated culture was about 50% of the control culture. This TCDD-induced, increased sensitivity to density-dependent inhibition of replication (DDIR) was accompanied by a change from a fusiform morphology in the high-saturation-density control cells to a flat cobblestone appearance in the treated low-saturation-density cells. The nondividing cultures treated for 14 days with 10^{-11} M TCDD had the same viability as control cultures. Upon trypsin suspension and reseeding, these formerly quiescent cultures were again capable of growing to high cell density and of again showing susceptibility to TCDD-induced changes in cell growth and morphology. Evidence is presented to suggest that this reversible increase in sensitivity to DDIR and the morphological change are not a consequence of cell growth inhibition. This system may provide the basis for an *in vitro* model to study the effect of TCDD on the control of replication of these cells.

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic synthetic compound known (Poland and Kende, 1976; Poland and Knutson, 1982). Animal studies have demonstrated the pleotypic nature of this toxicity, which is characterized mainly by a prolonged wasting syndrome leading to death but also includes embryotoxicity and/or teratogenicity, thymic and lymphoid involution, hyperkeratoses, edema, hyperplasia of the epithelium of the stomach, intestine, urinary bladder, and bile duct, and hepatocellular damage (Kimbrough,

1974; Huff *et al.*, 1980). The development of acneform lesions is one of the most common toxic manifestations of TCDD exposure seen in, but not confined to, humans (Kimbrough, 1974; Huff *et al.*, 1980). The development of this chloracne appears to result from hyperkeratoses of the sebaceous glands leading to a cystic response (Kimbrough, 1974; Knutson and Poland, 1980b). The specific pathology and sensitivity to TCDD vary greatly among animal species. The LD50 for the species tested ranges from 1 µg/kg body weight in the guinea pig to 5000 µg/kg in the hamster (Schwetz *et al.*, 1973; Henck *et al.*, 1981). The specific cause of death due to TCDD intoxication is unknown.

¹ Presented in part at the 186th National Meeting of the American Chemical Society, Washington, D.C., August 28 to September 2, 1983.

Extensive studies of TCDD in animal and in *in vitro* systems over the past decade have suggested that the toxic response elicited by TCDD isomers and congeners is associated with their binding to a specific cytosolic receptor (Poland and Glover, 1975; Kende *et al.*, 1974). The affinity of these compounds for this receptor corresponds in rank order to their *in vivo* toxicity, as well as to the induction of a battery of enzymes which include the cytochrome P_1 -450-mediated microsomal monooxygenase aryl hydrocarbon hydroxylase (AHH) (Poland and Glover, 1977; Poland and Kende, 1976; Kende *et al.*, 1974). Additional evidence suggests that the induction of AHH is dependent upon the translocation of the TCDD-receptor complex from the cytoplasm into the nucleus (Greenlee and Poland, 1979; Okey *et al.*, 1979, 1980), where evidence suggests it induces genetic activation resulting in the production of cytochrome P_1 -450 mRNA (Tukey *et al.*, 1982).

Many cultured cell lines respond to TCDD exposure by inducing AHH. Despite this enzyme induction and the toxicity of TCDD in animals, extensive studies have not demonstrated significant *in vitro* toxicity in cultured cell lines exposed to TCDD. No change in growth or morphology was seen in 23 cell lines exposed to TCDD (Knutson and Poland, 1980a). It was reported, however, that *in vitro* keratinization could be induced in the cloned mouse-teratoma cell line, XB, by exposure to very low levels of TCDD (Knutson and Poland, 1980b). These results suggested the use of this system as an *in vitro* model for the *in vivo* keratinization response. These experiments also showed that this phenotype could change under conditions of cell passage. We have also observed a change in phenotype and determined that, under our conditions of cell culture, late-passage XB cells no longer keratinize upon exposure to TCDD although they continue to proliferate and to grow to a higher density than the early-passage XB cells. The late-passage XB cells, which we designate XBF, have been found to respond to very low

levels of TCDD by an increased sensitivity to density-dependent inhibition of replication (DDIR) and the appearance of a flat epithelial morphology, as compared to the fusiform morphology of high-density nonexposed replicate cultures. We also present evidence that this TCDD-induced decrease in saturation density observed in these cells does not result from a general toxic response and is reversible. The TCDD-induced DDIR seen in this cell line may provide the basis for an *in vitro* system to study the effect of this compound on the control of cell replication in these cells.

METHODS

TCDD was obtained from Dow Chemical (Midland, Mich.). Purity was determined by mass spectrometry to be >99%. The XB teratoma-derived mouse epithelial cells and the 3T3 fibroblast feeder cells were the generous gift of Dr. Howard Green (Harvard University). Analytical grade dimethyl sulfoxide (DMSO) was obtained from the Aldrich Chemical Co. (Milwaukee, Wis.).

Cell culture. XB cell stocks were grown in Costar plastic tissue culture flasks (75 cm²) in Dulbeccos Modified Eagle Medium (DMEM; Gibco) supplemented with 20% fetal bovine serum (Flow, Rockville, Md.), 100 U of penicillin/ml, and 100 µg of streptomycin/ml. This medium had been conditioned by exposure to a confluent culture of 3T3 feeder cells for 24 hr (25 ml of medium/75 cm² flask) and sterilized by filtration. The 3T3 feeder cell stocks were grown in DMEM supplemented with 10% calf serum (Flow).

The XB cells were routinely propagated every 1 to 2 weeks, when confluency was reached, by trypsinization (0.25%) and replating at a concentration of 2×10^4 cells per cm². The culture medium was renewed once a week between passages. The confluent density of these early XB cultures was about 4×10^4 cells per cm² under these conditions. These cells, when seeded with lethally irradiated 3T3 cells, were found to exhibit the TCDD-induced keratinization response described by Knutson and Poland (1980b). The magnitude of this keratinization response to TCDD exposure gradually declined after passage seven, and by passage 15 this response was greatly reduced compared to the early passage XB cells, although the sensitivity of this reduced response to TCDD was similar to that of the early passage cells. At this time the XB stock cultures, which were originally flat and polygonal, now had become more fusiform in morphology and reached confluency earlier than the low passage XB cultures. This alteration in growth and morphology at high density progressed dur-

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ing the next five to seven passages as the keratinization response to TCDD exposure disappeared. The confluent density of the cells at this time was about 15×10^4 cells per cm^2 . At this time it was discovered that medium conditioned by exposure to 3T3 cells was no longer required for the growth of these cells, which were now designated XBF to indicate their divergence from the XB line. Subsequently, stock cultures of XBF cells were routinely grown in DMEM supplemented with 20% fetal bovine serum (Flow), 100 U of penicillin/ml, and 100 μg of streptomycin/ml. The cultures were propagated by suspension in trypsin (0.25%), replated at a density of 3×10^4 cells per cm^2 once or twice a week as confluency was reached, and incubated at 37°C in a humidified atmosphere of 5% CO_2 .

Exposure of cells to TCDD. XBF cells were suspended by trypsinization and seeded into 24-well plates (16-mm-diameter wells, 5×10^4 cells per ml per well) in conditioned medium with 3T3 cells (5×10^5 per ml per well) which had been irradiated with 6000 rads from a cesium source. The cultures were incubated overnight and refed with a series comprising 10-fold dilutions of a stock solution of TCDD in DMSO or with DMSO alone in DMEM supplemented with 20% fetal bovine serum. This refeeding was repeated every 3 or 4 days for the duration of the experiment. The highest cumulative DMSO concentration was 0.1%.

At the end of incubation the cultures were washed with phosphate-buffered saline (PBS), fixed with formalin in PBS, and stained with Giemsa. Alternatively, the cultures were suspended by trypsinization at varying times after the initial exposure to TCDD. After thorough disaggregation of the cells by trituration, the number of cells per culture was determined with a Coulter Particle Counter (Coulter Instrument, Hialeah, Fla.). Complete disaggregation was confirmed by microscopic examination of the cell suspension immediately before counting. [^3H]Thymidine (New England Nuclear; specific activity = $0.67 \mu\text{Ci}/\text{mmol}$) incorporation at various times after exposure to TCDD was determined by pulse-labeling the cultures for the indicated times and counting the acid-precipitable radioactivity in a Packard Tricarb liquid scintillation spectrometer (Ellem and Gierthy, 1977). Autoradiography was performed as described elsewhere (Ellem and Gierthy, 1977).

Cell viability was determined by measuring the percentage of trypsin-suspended cells capable of excluding 0.1% trypan blue dye. Cloning efficiency was determined by seeding 200 cells in 5 ml of medium into a 60-mm plastic tissue-culture Petri dish (Falcon) and incubating for 2 weeks. Cultures were then washed in PBS, fixed in 10% formalin, and stained with Giemsa, and colonies were counted. Absolute cloning efficiency was defined as the percentage of the cells seeded capable of forming colonies.

Safety considerations. Safe handling, containment, and disposal of the substances used in this study conformed to the general principles and standards of biologic safety proposed by the Office of Biohazards and Environmental Control, National Cancer Institute, for class I hazards and to the guidelines of the Laboratory Chemical Carcinogen Safety Standards Subcommittee of the Department of Health and Human Services Committee to coordinate toxicology and related programs.

RESULTS

Induction of Morphological Change in XBF Cells by Exposure to TCDD

The XBF cells, cocultured with irradiated 3T3 cells for 14 days, grew to very dense cultures characterized by apparent multilayering and the appearance of parallel growth patterns indicative of a spindle-like morphology. Treatment with 0.1% DMSO had no effect on this morphology (Fig. 1a). Cultures grown in the presence of 10^{-9} M TCDD grew to confluency, and after 7 to 10 days began to develop into a cobblestone-like monolayer comprised of flat, evenly distributed cells very apparent by Day 14 (Fig. 1b). Cultures of irradiated 3T3 feeder-layer cells, seeded from the same stock used for these experiments, did not show any indication of growth or TCDD-induced morphological change. By Day 14 these cultures had essentially disintegrated.

This change in growth and morphology was induced by TCDD concentrations ranging from 10^{-11} to 10^{-6} M, the highest concentration tested. This dose-response effect was apparent in unmagnified, stained preparations (Fig. 2), as well as in the number of cells in each culture after 14 days of exposure (Fig. 3). Solvent control cultures treated with DMSO at concentrations corresponding to those in the dilutions did not show this effect. The presence of 0.1% DMSO did not affect the TCDD dose-response. The ability of TCDD to induce these changes in morphology and growth in XBF cells has remained stable for over a year of cell passage as described under Methods. Representative experiments are presented in the figures.

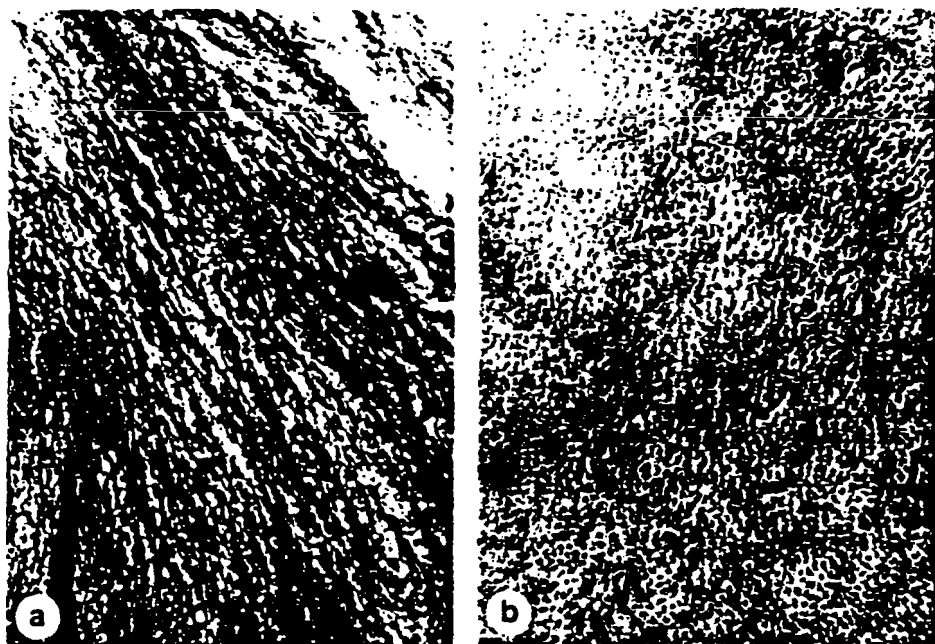


FIG. 1. Induction of the flat-cell response by TCDD: (a) The dense packing and multilayering of control XBF/3T3 cultures. (b) The apparent induction of density-dependent inhibition of cell proliferation and flat-cell morphology characteristic of XBF/3T3 cultures after 14 days of exposure to 10^{-9} M TCDD. Giemsa stain and methods, as described in text. 100X magnification.

Reduction in Cell Growth during Exposure to 2,3,7,8-TCDD

Investigation of the kinetics of cell growth in exposed cultures revealed that the decline in cell proliferation after TCDD exposure was not immediate. Cultures were seeded below confluency, as described under Methods, and after 24 hr were treated with 10^{-9} M TCDD and DMSO (0.1%) or with DMSO alone. Figure 4 shows that both treated and untreated cultures accumulated cells at equal rates until Day 7, at about which time confluency was reached. After this time the solvent control cultures continued to increase in cell number, while the 2,3,7,8-TCDD cultures did not. This cessation of cell proliferation is apparently TCDD-induced, as reflected in the TCDD-concentration-dependent decline in [3 H]thymidine incorporation into the acid-insoluble

fraction of treated cultures (Fig. 5). This experiment was done after 7 days of exposure to TCDD, at which time the cultures had reached confluency, and the cell numbers were the same per culture. The range of TCDD concentrations capable of diminishing [3 H]thymidine incorporation corresponds to that which caused the morphological change and is supportive of the decline in DNA synthesis which would be expected to occur with the observed inhibition of cell growth. Further evidence that the lack of increase in cell number was due to a cessation of cell division, rather than to the TCDD-treated cells simply sloughing off resulting in a steady-state, was a decrease in the number of cells incorporating [3 H]thymidine into DNA, as shown by autoradiography. A 6-hr pulse with [3 H]thymidine at Day 6, when the treated and untreated cultures showed equal growth rates,

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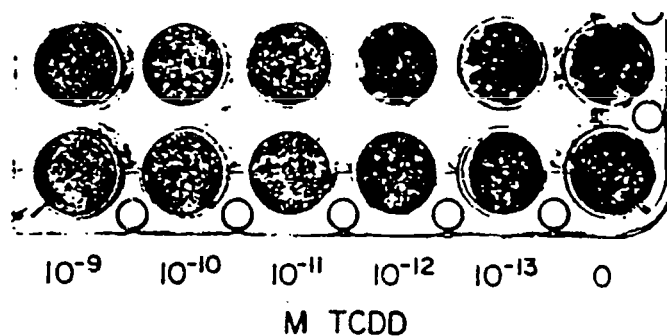


FIG. 2. Dose-response relation of flat-cell induction by TCDD. XBF/3T3 cultures were exposed to various concentrations of TCDD as described in text. After 14 days the cultures were fixed and stained with Giemsa stain. The less intensely stained low-density cultures (10^{-9} to 10^{-11} M) are similar to that shown in Fig. 1b, while the intensely staining high-density cultures (10^{-12} , 10^{-13} , and 0 M) are similar to that seen in Fig. 1a. The flat-cell induction is first evident with 10^{-11} M TCDD.

resulted in $45 \pm 4.4\%$ of the cells being labeled. By Day 13 of treatment the cultures exposed to 10^{-9} M TCDD showed a reduction in labeling to $1.6 \pm 1.2\%$.

The viability of cells from these treated cultures was tested by trypan blue exclusion, which indicated a high viability of $87 \pm 2.5\%$ in the 10^{-8} M TCDD-treated cultures, $85 \pm 4.2\%$ in control cultures (0.1% DMSO), and

$88 \pm 4.1\%$ in untreated cultures. Cells from these cultures gave plating efficiencies of $33 \pm 3.6\%$ for 10^{-8} M TCDD-treated cultures, $37 \pm 6.3\%$ for control cultures, and $34 \pm 4.5\%$ for untreated cultures.

When cells treated with 10^{-9} M TCDD for 14 days were suspended with trypsin, res seeded, and allowed to grow with or without added TCDD, again no difference in proliferation was seen until confluency was reached. At

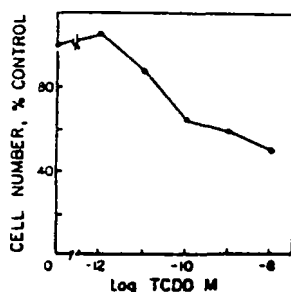


FIG. 3. Effect of TCDD on cell growth in XBF cultures. XBF cells were seeded with irradiated 3T3 feeder cells and incubated as described under Methods. Medium was replaced 24 hr after seeding and every 3 or 4 days thereafter with medium containing the indicated concentrations of TCDD in DMSO or DMSO alone. The cultures were trypsinized after 14 days, and cell number per culture well was determined. The data points represent the percentage of cells in the TCDD-treated cultures compared to control cultures and are the mean of four replicate cultures.

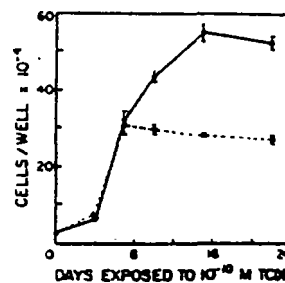


FIG. 4. Effect of TCDD on XBF cell proliferation kinetics. XBF cells were seeded and incubated with irradiated 3T3 feeder cells as described under Methods. Medium was replaced after 24 hr and every 3 or 4 days thereafter with medium containing TCDD with DMSO or DMSO alone. At the indicated times of exposure, the cultures were trypsinized, and cell number per culture well was determined. $\bullet$ — $\bullet$, XBF/3T3, 0.01% DMSO; $\bullet$ --- $\bullet$, XBF/3T3, 10^{-10} M TCDD, 0.01% DMSO; data points represent the mean of four replicates $\pm$ SE.

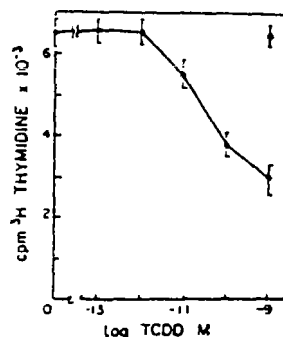


FIG. 5. Effect of TCDD on incorporation of [³H]thymidine into XBF cells. XBF cells were seeded with irradiated 3T3 cells and incubated as described under Methods. Medium was replaced after 24 hr and after 4 days with medium containing the indicated concentration of TCDD. On Day 7 all cultures had reached confluency and had the same number of cells. At this time the cultures were pulse-labeled with [³H]thymidine (0.5 μ Ci/ml, 6.7 Ci/mmol) for 1 hr, fixed, and counted for radioactivity as described under Methods. A DMSO concentration of 0.1% was the highest used in this experiment (O). Data points represent counts per minute $\times 10^{-3}$ per 16 mm culture well and are the mean of four replicates $\pm$ SE.

about this time the TCDD-treated cells showed a decline in growth, while the untreated cells continued to proliferate to a higher saturation density (Fig. 6). The reduction in cell growth of these cultures of pretreated cells had the same sensitivity to TCDD (10^{-11} M) as cells which were not pretreated as seen by microscopic flat-cell evaluation and in the gross observation of fixed and stained cultures (Fig. 2).

Inhibition of Cell Division and the Morphological Change

Subconfluent XBF cells were treated with other inhibitors of proliferation, which have various mechanisms, to determine if the flat-cell morphology was a consequence of a general toxic effect related to inhibition of specific macromolecular synthesis. Inhibitors of DNA (hydroxyurea), RNA (actinomycin D), and protein (cycloheximide) synthesis, as well as

the mitotic inhibitor colchicine, were tested in 11 10-fold dilutions starting with a concentration of 100 μ g/ml. The effects ranged from obvious toxicity, characterized by cell disintegration at the high concentration through inhibition of cell division, to no observable effects at the low ranges. The induction of a flat-cell morphology similar to that induced by TCDD in replicate XBF/3T3 cultures was not seen at any concentration of these agents.

DISCUSSION

The XBF cells used in this study evolve after serial passage, from the cloned XB epithelial cell line derived from a mouse teratoma. The XBF cells no longer have the requirement for a fibroblast feeder layer or for medium conditioned by a fibroblast culture for growth, as did the original XB line. The loss of the ability of the XBF line to be induced

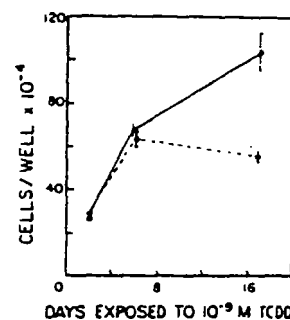


FIG. 6. Effect of TCDD on cell growth in XBF culture of cells previously treated with TCDD. XBF cells, which had been exposed in the presence of irradiated 3T3 cells to 10^{-9} M TCDD for 14 days and exhibited growth inhibition and morphological changes, were seeded with fresh, non-TCDD-treated irradiated 3T3 feeder cells and incubated as described under Methods. Medium was replaced 24 hr after seeding and every 3 or 4 days thereafter with medium containing either 10^{-9} M TCDD in 0.01% DMSO or 0.01% DMSO. The cultures were trypsinized at the indicated times and the cell number per culture well was determined. $\bullet$ — $\bullet$, XBF/3T3, 0.01% DMSO; $\circ$ — — $\circ$, XBF/3T3, 10^{-9} M TCDD, 0.01% DMSO. Data points represent the mean of four replicates $\pm$ SE.

by TCDD to keratinize is consistent with reports of others describing a change in this phenotypic expression in some XB cells after serial culture (Knutson and Poland, 1980b; Rheinwald and Green, 1975). We have found that continued culturing of XBF cells resulted in an increased cell density at saturation and acquisition of a fusiform morphology at high density, in contrast to the cobblestonelike appearance of the original XB parent cell line. This change in saturation density and morphology may be the result of a spontaneous *in vitro* transformation and has been stable for over a year of twice weekly subculturing.

The XBF cells exposed to TCDD have stopped replicating based on the absence of [^3H]thymidine-labeled cells and the lack of increase in cell number. This is supported by the decline in [^3H]thymidine incorporation into acid-insoluble products. These effects were not immediate, as would have been expected if a toxic response of the type seen with previously discussed inhibitors of macromolecular synthesis had occurred. The XBF cells were seeded at low density and divided in the presence of TCDD at a rate no different from that of control cultures, until confluency was reached. The cessation of cell division at that time, unlike that seen in the keratinized stratum corneum of the epithelium was probably not a consequence of terminal differentiation which results in cell death. This conclusion is based on the observation that the quiescent XBF cells, when trypsinized and reseeded at low density, grew to high density unless treated with TCDD, in which case they again grew to a lower saturation density than the untreated cultures. The population of cells that regrew was probably representative of the entire culture rather than of a selected resistant population, since trypan blue exclusion and cloning efficiency indicate the same degree of viability in high-density control cultures, low-density TCDD-treated cultures, and untreated stock cultures of XBF cells. These results demonstrate the reversibility of this effect in response to TCDD.

The fusiform nature of epithelial cells at high density was probably a consequence of the high saturation density of the XBF cells. The change from the morphology and growth characteristics of the XB to the XBF cells may reflect a progression to a less-differentiated progeny, with loss of regulatory control of growth or a decreased sensitivity to contact inhibition of proliferation, as seen in other cell cultures as a spontaneous *in vitro* transformation. Exposure to TCDD may reestablish this regulation, resulting in a return to the parent-cell morphology and saturation density characteristics.

The effect of TCDD on other cells in culture has been described previously and, to a lesser extent, in this report. In one of these studies (Knutson and Poland, 1980a) involving suspension cultures and anchorage-dependent cells grown on a substrate, no toxic effects (altered morphology, decreased viability, or diminished growth rate) were seen in 23 cultured cell types. Sparse cultures of the 16 anchorage-dependent cell types grown on a substrate were unaffected by exposure to 10^{-7} M TCDD in regard to the previously mentioned end points from the time of seeding to confluency, at which time they were trypsinized and evaluated for viability and density. Our results for preconfluence growth with the XBF line agree with these findings. However, the observed change in cell growth rates and morphology in TCDD-treated XBF cells was apparent only after confluency was reached, as the untreated cultures continued to proliferate resulting in the observed high density. This effect was not due simply to inhibition of cell growth since inhibitors of macromolecular synthesis and mitosis did not induce this effect. TCDD is extremely potent for inducing certain gene products and keratinization. Further studies may indicate the usefulness of TCDD for inducing a specific gene product associated with the regulation of cell proliferation in the XBF system.

The abilities of TCDD to induce keratinization in the parent XB cell line and mor-

phological and proliferative change in the XBF cells may be related. The existence of a high-affinity cytosolic receptor for TCDD in the XB cells has been demonstrated by others (Knutson and Poland, 1980b), and its interaction with TCDD has been postulated to be obligatory for a number of TCDD-induced responses (AHH induction, keratinization, etc.) (Poland and Knutson, 1982). Further studies of the XBF cell response to TCDD may determine if this receptor is part of the mechanism of the induction of the flat-cell effect. The reduction of saturation density and morphological change seen *in vitro* may be analogous to the *in vivo* epithelial change from proliferating basal cells to nondividing cells of the stratum spinosum. Studies using ultrastructural and biochemical analyses for specific markers of this differentiation may answer this question. The sensitivity of this effect suggests the use of this system as an assay for TCDD if specificity for this compound can be demonstrated.

ACKNOWLEDGMENTS

The authors thank Ms. Victoria Lamberton for preparation of the typescript. This work was supported in part by National Institute of Occupational Safety and Health Grant OHO 1533.

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Volume 15 Number 1
January 1982

Toxicology and Applied Pharmacology

Official Journal of the American Society of Toxicology

Editor

WILLIAM A. JONES



Published by the American Society of Toxicology

231

DOM2160237

18381

K-66681-(100)

1984

TOXICOLOGY AND APPLIED PHARMACOLOGY 73, 42-47 (1984)

Increased Epidermal Transglutaminase Activity following 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: *In Vivo* and *in Vitro* Studies with Mouse Skin

S. M. PUHVEL,¹ D. C. ERTL, AND C. A. LYNBERG

Division of Dermatology, Department of Medicine, School of Medicine, Center for Health Sciences,
University of California, Los Angeles, Los Angeles, California 90024

Received June 18, 1983; accepted October 25, 1983

Increased Epidermal Transglutaminase Activity following 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: *In Vivo* and *in Vitro* Studies with Mouse Skin. PUHVEL, S. M., ERTL, D. C., AND LYNBERG, C. A. (1984). *Toxicol. Appl. Pharmacol.* 73, 42-47. In previous studies it has been shown that topical treatment of hairless mice with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) induces hyperproliferation and hyperkeratinization in the epidermis of hairless mice. The present investigation demonstrated that such TCDD-induced morphological changes in skin *in vivo* are accompanied by increased levels in activity of epidermal transglutaminase (ETG), the enzyme associated with terminal epidermal differentiation. Exposure of mouse epidermal cells in tissue culture to 10^{-9} M TCDD also resulted in a significant increase in ETG activity, despite the fact that morphologically these cultures (grown at 0.07 mM ionic calcium concentrations) exhibited no signs of terminal differentiation. Thus one mechanism of action of TCDD in inducing cutaneous changes appears to relate to the stimulation of increased ETG levels.

In humans one of the first signs of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the development of a skin condition known as chloracne (Moore, 1978). This syndrome involves hyperkeratinization of ductal epithelium of cutaneous sebaceous follicles, resulting in comedo formation which may or may not proceed to pustular and cystic chloracne. Experimental chloracne has been induced in skin of hairless mice by topical application of TCDD (Puhvel *et al.*, 1982; Knutson and Poland, 1982). Even though follicular involvement in mouse skin is insignificant, the hyperproliferative, hyperkeratinizing changes induced in interfollicular epidermis are sufficiently well defined to permit the use of this animal model to monitor TCDD-induced enzymatic changes in skin.

In this report we investigated the effect of TCDD on epidermal transglutaminase (ETG)

activity first in skin of hairless mice *in vivo* following topical application of TCDD. Then, to verify our observations in a more defined system, changes in ETG activity of mouse epidermal cells in tissue cultures were monitored following addition of TCDD to culture media.

METHODS

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was obtained from KOR, Incorporated (Boston, Mass.), and putrescine dihydrochloride, 2,3-³H(N) (sp. act. 38.9 Ci/mmol) was obtained from New England Nuclear (Boston, Mass). Tissue culture Medium 199, calcium depleted Medium 199, fetal bovine serum (FBS), and antibiotic-antimycotic mixture were obtained from Grand Island Biological Co. (Grand Island, N.Y.); putrescine dihydrochloride, casein, dithiothreitol (DTT), and Trizma base were from Sigma Chemicals (St. Louis, Mo.); dimethylcasein was from Accurate Chemical and Scientific (Hicksville, N.Y.).

In Vivo Studies

Animals. HRS/J strain of hairless female mice were obtained from the Jackson Laboratories (Bar Harbor,

¹ To whom requests for reprints should be addressed.

Maine). Animals were 10 to 11 weeks old at the beginning of the experiment. They were fed Wayne Lablox (Universal Feeds, Inc., Colton, Calif.) laboratory chow *ad libitum* and were housed four to a cage in disposable plastic cages inside a Biohazard safety containment box (Class III Glove box) for the duration of the experiments.

Treatment. Three times a week for 4 weeks animals were treated topically with 0.1 μ g of TCDD dissolved in 0.1 ml of acetone. Control animals received 0.1 ml of acetone alone. Groups of four mice were killed by cervical dislocation at 7, 14, 21, and 28 days after the beginning of treatment. Skins were removed by dissection, and the epidermis was separated from dermis by first immersing the skins in a 55°C waterbath, followed by immersion in ice water. Following this treatment, the epidermis was scraped from the dermis with a scalpel, while maintaining the skins at 4°C. Epidermal samples were homogenized with a Polytron homogenizer, and the tissue cytosol was separated by centrifugation at 30,000g for 30 min. Supernatant fractions were used for the transglutaminase assays.

Transglutaminase assay of epidermal homogenates. The methods described by De Young and Ballaron (1982) were used. Briefly, 0.1 ml of tissue supernatant fractions was added to 0.5 ml of assay mixture containing 0.05 M Tris buffer (pH 8.1), 0.01 M CaCl_2 , 0.05 M DTT, 0.6 mg dimethylcasein, and 0.37 μ Ci of [^3H]putrescine. Mixtures were incubated at 37°C for 60 min, and the reaction was stopped by adding 0.6 ml of 10% trichloroacetic acid (TCA) and 5 ml of 5% TCA containing 0.1% unlabeled putrescine. After 30 min at room temperature, incubation mixtures were filtered on 25-mm Whatman GF/A filter discs. Discs were washed once with 10 ml 5% TCA containing 0.1% unlabeled putrescine and twice with 10 ml 100% ethanol. After drying, the discs were placed in Aquasol II (New England Nuclear) and the radioactivity was counted. Soluble proteins in the supernatant fractions were quantified by the Bio-Rad protein assay with bovine serum albumin as standard (Bradford, 1976).

In Vitro Studies

Tissue culture. Basal keratinocyte cultures from neonatal BALB/c mice were established following the methods described by Marcelo *et al.* (1978). Basal keratinocytes were separated by discontinuous Ficoll gradient centrifugation, and 1.5×10^6 cells were inoculated per 30-mm-diameter culture dish. Cultures were grown in parallel in the following media: one-third of the cells were plated in Medium 199 supplemented with antibiotics and 10% FBS; the remainder were plated in calcium-depleted Medium 199 supplemented with antibiotics and 10% FBS (5% chelated, 5% normal). The final ionic concentrations of calcium in these media were 1.2 and 0.07 mM, respectively.

TCDD treatment. On the third day of culture, TCDD dissolved in dimethylsulfoxide (DMSO) was added in a final concentration of 10^{-9} M to half the low calcium cultures. The remainder of the low calcium cultures re-

ceived DMSO alone in a final concentration of 0.1%. The medium was changed every other day and fresh TCDD and DMSO were added at every medium change.

Cells were harvested from four dishes of each of the three media on Days 3, 5, 7, 10, and 12 by scraping the plates with rubber spatula and collecting all cells (i.e., attached as well as free floating), by centrifugation. Cell pellets were washed twice in buffer and stored frozen at -60°C until used for the transglutaminase assay.

Transglutaminase assay of cell pellets. Cell lysates obtained by thawing and freezing cell pellets three to four times were used for the transglutaminase assay recommended by S. H. Yuspa and U. Licht (personal communication). This procedure was an adaptation of the method described by Ogawa and Goldsmith (1976). Cells were pelleted in a buffer containing 50 mM Tris (pH 7.5), 2.5 mM DTT, 0.13 M NaCl, 0.83 mM EDTA, and 8.3 mM CaCl_2 . The reaction mixture consisted of 100 μ l of cell lysate, 20 μ l of casein (20 mg/ml), 10 μ l of [^3H]putrescine (5 μ Ci), and 20 μ l of buffer. The mixture was incubated at 37°C for 10 min. Following incubation 50 μ l of the mixtures was spotted on filter paper discs (Whatman 3MM) and immediately precipitated by immersion in 10% ice cold trichloroacetic acid (TCA) plus 0.1% putrescine. The precipitates were washed in four separate 30-min washes of 5% TCA plus 0.1% putrescine, then immersed in 100% ethanol, and dried. The radioactivity of the precipitates on the filter paper discs was counted in a Beckman LS-7500 liquid scintillation counter. Results were corrected for background radioactivity found in lysate-free parallel assays. Soluble proteins in the cell lysates were determined by the Bio-Rad protein assay.

To monitor the effect of 10^{-9} M TCDD on epidermal cell proliferation, cell counts were performed at regular intervals in cultures grown in low calcium medium, with or without the presence of TCDD in the medium. The effect of TCDD on terminal differentiation of epidermal cells in culture was investigated by transferring cultures grown in low calcium medium (with and without the presence of 10^{-9} M TCDD) to high calcium medium on the seventh day of culture, and monitoring the morphology of the cultures and the rate of cornified cell envelope formation at 24 hr to 5 days after the change to the 1.2 mM Ca^{2+} medium. Cornified envelope counts were performed according to the methods described by Yuspa *et al.* (1982) with insolubility in 2% SDS and 20 mM DTT at 90°C for 5 min as the criterion for defining cornified envelopes.

RESULTS

In Vivo Studies

Hairless HRS/J mice treated topically with TCDD developed the same pattern of well-defined cutaneous changes which have been described in detail elsewhere (Puhvel *et al.*,

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