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SPECIMEN LABEL
REDUCED TO 75%

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ESTERON* 245

HERBICIDE

FOR THE CONTROL OF TREES, BRUSH AND BROADLEAF WEEDS

Low-Volatile Brush and Weed Herbicide for Industrial Vegetation Control, Fencerows, and Rangeland

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS

Hazards to Humans and Domestic Animals

HARMFUL IF SWALLOWED • MAY CAUSE IRRITATION

Avoid Contact with Skin, Eyes, or Clothing

Wash Thoroughly After Handling

Statements of Practical Treatment

In case of contact, immediately flush eyes or skin with plenty of water. Get medical attention if irritation persists. If swallowed, induce vomiting by drinking two glasses of water and sticking finger down throat. Call a physician. Do not induce vomiting if anything by mouth to an unconscious person.

Physical or Chemical Hazards

Do Not Cut or Weld Container

Environmental Hazards

This product is toxic to fish. Keep out of lakes, streams or ponds. Do not apply where runoff is likely to occur. Do not contaminate water by cleaning of equipment or disposal of wastes. Do not contaminate irrigation ditches or water used for irrigation or domestic purposes.

In case of an emergency endangering life or property involving this product, call collect
517-636-4400

AGRICULTURAL CHEMICAL

Do Not Ship or Store with Foods, Feeds,
Drugs or Clothing

ACTIVE INGREDIENT

2,4,5-Trichlorophenoxyacetic Acid

Butoxyethyl ester 59.1%

INERT INGREDIENTS:

2,4,5-Trichlorophenoxyacetic Acid Equivalent — 42.5% 40.9%

1 Pounds per Gallon

E.P.A. Registration No. 464

E.P.A. Est. 464-MI-1

PRECAUCION AL USUARIO: Si usted no lee Ingles, no use este producto hasta que la etiqueta le haya sido explicada ampliamente.

TRANSLATION: (TO THE USER:) If you cannot read English, do not use this product until the label has been fully explained to you.

18.93 L / 5 gal

SPECIMEN LABEL
(BACK)
REDUCED TO 75%



ESTERON 245^{BE} HERBICIDE

Contains [Butoxyethyl] Ester]

of 2,4,5-T • Acid Equivalent: 4 Pounds per Gallon

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

ESTERON 245^{BE} herbicide is recommended for industrial vegetation control, fence rows, and rangeland. Do not use in forest lands, rights-of-way, or pastures.

This herbicide controls herbaceous and woody plants including such 2,4-D resistant species as - ash, black gum, brambles, groundcherry, hawthorn, horehound, maple, mesquite, oak, osageorange, palmetto, poison ivy, pricklypear cactus, redbay, salmonberry, sweetgum, wild blackberry, wild rose, and certain species of Ribes. Do not apply ESTERON 245^{BE} where spray drift may contact nearby 2,4,5-T susceptible crops or other desirable plants or may contaminate water intended for irrigation or domestic purposes. Read and follow all Use Precautions on this label.

PREPARING THE SPRAY

Use only diesel oil, No. 1 or No. 2 fuel oil or kerosene where oil is recommended in the spray mixture.

Oil Sprays: Add ESTERON 245^{BE} to the required amount of oil in the spray tank or mixing tank and mix thoroughly. This mixture can be made at any time before actual use and no separation will occur. Do not let any water, or oil water mixture sprays get into the ESTERON 245^{BE} or into the finished mixture, as it may form a gel.

Water Sprays: Fill the spray tank about half full with clean water, add the required amount of ESTERON 245^{BE} and complete filling the tank. Mix thoroughly and continue agitation while spraying. Caution: See NOTE in paragraph on Oil-Water Mixture Sprays.

Oil-Water Mixture Sprays: When vigorous agitation is used, 1 gallon of ESTERON 245^{BE} will emulsify up to 10 gallons of oil in 100 gallons of spray mixture. First, premix the ESTERON 245^{BE} and oil in a separate container. Do not allow any water or mixture containing water to get into the ESTERON 245^{BE} or the premix. Fill the spray tank about half full with water, then slowly add the premix with continuous agitation and complete filling the tank with water. If the premix is put in the tank without any water, the first water added may form a thick "inverted" water in oil emulsion which will be hard to break. As an alternate procedure, the oil may be added after the ESTERON 245^{BE} is mixed in the water; but highly vigorous mechanical agitation is required and a poor emulsion may be formed. The premix method is preferred.

NOTE: 1. ESTERON 245^{BE} in water or oil-water sprays forms an emulsion, not a solution, and separation may take place unless sprays are agitated continuously. Mechanical agitation is recommended.

HIGH VOLUME SPRAYS

Basal Bark Treatment: Brush and small trees can be controlled by spraying the basal parts of brush stems and tree trunks to a height of 12 to 18 inches from the ground line. Use a solution of 3 gallons of ESTERON 245^{BE} in 100 gallons (1 pint in 4 gallons) of oil. With certain resistant species, 4 gallons of ESTERON 245^{BE} in 100 gallons (1 pint in 3 gallons) of oil, is effective. As only the basal portions of the brush are treated on a spot basis, the total amount sprayed per acre would not be expected to exceed 100 gallons. Knap-sack or power equipment may be used, but complete wetting of the indicated area is necessary, particularly at the ground line. This means spraying until run-down or run-off to the ground line is noticeable. Old or rough bark requires more spray than young or smooth bark. Low pressures are desirable. Apply at any time, including the winter months, except when snow, ice or water prevent spraying to the ground line. Often delayed response and killing can be expected.

Dormant Brush: Treat any time after brush is dormant and most of the foliage has dropped. Spray should be concentrated at the base of stems and in addition, the upper parts of the stems should be broadcast sprayed enough to wet them. Under recontouring species such as sumac, persimmon, osageorange and locust, also spray the ground area to control small root suckers that may not be readily

visible. Mix 1 1/2 gallons of ESTERON 245^{BE} in 100 gallons of oil. Brush of average density and 4 to 6 feet high may take up to 150 gallons of spray mixture per acre. **Stump Treatment:** Where growth is more than 6 to 8 feet tall, cut it close to the ground and spray the freshly cut stumps and stubs with 3 gallons of ESTERON 245^{BE} in 100 gallons (1 pint in 4 gallons) of oil, mixed thoroughly. For more resistant species, use 4 gallons of ESTERON 245^{BE} in 100 gallons (1 pint in 3 gallons) of oil. Wet thoroughly all exposed bark, as well as cut surfaces. This means spraying until run down or run off to the ground line is noticeable. Old or rough bark requires more spray volume than young or smooth bark. Apply at any time, including winter months, except when ice, snow or water prevent spraying to the ground line. Best results are obtained on freshly cut stumps two inches across or larger. Adequate coverage normally requires from 10 to 100 gallons per acre depending on density of stumps and stubs.

"Fill" Treatment: For large trees, make a singlecheck girdle or "fill" of overlapping cuts completely around the tree as close to the ground as feasible. Spray the fill thoroughly using a mixture of 2 gallons of ESTERON 245^{BE} in 100 gallons (1 pint in 3 gallons) of oil.

Spot Foliage Treatment: Use 1/4 pint of ESTERON 245^{BE} in 3 gallons of water and spray to wet all foliage, shoots, stems and bark without runoff.

LOW VOLUME SPRAYS

Apply low volume sprays containing ESTERON 245^{BE} when foliage is well developed and plants are actively growing. For best results on woody species, soil moisture should be sufficient to promote foliar growth. Spraying during prolonged hot, dry weather or after leaves have lost their normal green color and vigor may not give satisfactory control. Apply low volume sprays by air or ground equipment only when spray drift will not be a problem - note use precautions.

Basal Treatment Using Powered Knap-sack Sprayer: Mix 1 1/2 to 2 gallons of ESTERON 245^{BE} with fuel oil or kerosene to make 20 gallons of total spray solution. Apply with a portable knap-sack mistblower to all sides of lower brush stems including the root collar. Good coverage of the root collar is essential for best results. Run mistblower at 1/4 to 1/3 throttle for best spray delivery and coverage. For maximum drift control use a basal nozzle attachment and do not raise nozzle above the horizontal position.

AIR APPLICATION FOR BRUSH CONTROL

Consult the Agricultural Experiment Station, your local Extension Service Weed or Range specialists for best time to treat and need for re-treatment in your area. Do not use from early boot to milk stage where grass seed production is desired. **Mosquito:** Use 1 pint ESTERON 245^{BE} plus 1/2 to 1 gallon of oil in enough water to make 4 gallons of total spray per acre. Apply 40 to 80 days after first leaves appear.

Sand Shinnery Oak: Use 1/2 to 1 quart of ESTERON 245^{BE} plus 1 gallon of oil in enough water to make 4 gallons of total spray per acre.

Post and Blackjack Oaks: Use 2 quarts of ESTERON 245^{BE} plus 1 gallon of oil in enough water to make 4 to 8 gallons of total spray per acre.

USE PRECAUTIONS

Note: Do not graze dairy animals on treated areas within 2 weeks after application. Do not graze meat animals on treated areas within 2 weeks of slaughter.

AVOID CONTACT WITH 2,4,5-T SUSCEPTIBLE CROPS AND OTHER DESIRABLE BROADLEAF PLANTS: ESTERON 245^{BE} herbicide is injurious to most broadleaf plants. Therefore, do not apply directly to or otherwise permit even minute amounts to contact cotton, grapes, tobacco, fruit trees, vegetables, flowers, ornamentals or other desirable plants susceptible to 2,4,5-T. Do not use in or near a greenhouse.

DO NOT APPLY IN THE VICINITY OF COTTON, GRAPES, TOBACCO, TOMATOES OR OTHER DESIRABLE 2,4,5-T SUSCEPTIBLE CROPS OR ORNAMENTAL PLANTS.

DO NOT SPRAY WHEN WIND IS BLOWING TOWARDS SUSCEPTIBLE CROPS OR ORNAMENTAL PLANTS

AVOID SPRAY DRIFT: Applications should be made only when there is no hazard from spray drift since very small quantities of spray which may not be visible, may severely injure susceptible crops during both growing and dormant periods. Use coarse sprays to minimize drift under adverse weather conditions, fine spray droplets may drift a mile or more. The spray thickening agent, NALCO TROL[®], may be used with this product to aid in reducing spray drift. If used follow all use recommendations and precautions on the product label.

NALCO TROL[®] - Trademark of NALCO Chemical Company

GROUND EQUIPMENT: With ground equipment, spray drift can be lessened by keeping the spray boom as low as possible, by applying 20 gallons or more of spray per acre, by using no more than 20 pounds spraying pressure with large droplet producing nozzle tips, by spraying when wind velocity is 8 miles per hour or less. Do not apply with hollow cone type nozzle or other nozzles that produce a fine droplet spray.

AERIAL APPLICATION: With aircraft, drift can be lessened by applying a coarse spray, by using no more than 20 pounds spray pressure at the nozzles, by using straight stream nozzles directed straight back, by using a spray boom no longer than 3/4 the wingspan of the aircraft, and by spraying only when wind velocity is less than 6 mph.

DO NOT APPLY BY AIRCRAFT WHEN AN AIR TEMPERATURE INVERSION EXISTS: Such a condition is characterized by little or no wind and with air temperature lower near the ground than at higher levels. The use of a continuous smoke column at or near site of application is suggested to indicate inversion and velocity of air movement, and to indicate a temperature inversion by layering of the smoke.

At high temperatures vapors from this product may injure susceptible plants growing nearby. Do not use in or near a greenhouse. Excessive amounts of this herbicide in the soil may temporarily inhibit seed germination or plant growth.

Do not use around the home, recreation areas or similar sites.

This product is toxic to fish. Keep out of lakes, streams, and ponds. Do not contaminate water by cleaning of equipment or disposal of wastes.

Do not contaminate irrigation ditches or water used for irrigation or domestic purposes. This product can be stored in an unheated building but if exposed to sub-freezing temperatures, should be warmed to at least 40°F and mixed thoroughly before using. Do not store near fertilizers, acids, insecticides or fungicides. Do not reuse containers. To avoid injury to desirable plants, do not store, handle or apply other agricultural chemicals with the same containers or equipment used with ESTERON 245^{BE} except as specified on this label.

Re-use, re-fill and containers and disposal of waste by burning in well-ventilated areas away from water supplies. Containers should be disposed of by puncturing holes in them and burying with waste or follow official recommendations for poisonous disposal.

Local conditions may affect the use of herbicides. Consult your State Agricultural Experiment Station or Extension Service weed specialist for advice in selecting treatments from this label to best fit local conditions. Be sure that use of the product conforms to all applicable regulations. Apply this product only as specified on this label.

NOTICE: Seller warrants that the product conforms to its chemical description and is reasonably fit for the purpose stated on the label when used in accordance with directions under normal conditions of use, but neither the warranty nor any other warranty of MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE is expressed or implied as to the use of the product contrary to label instructions, or under abnormal conditions, or under conditions not reasonably foreseeable to seller, and buyer assumed the risk of any such use.

STORAGE AND DISPOSAL

Do not re-use empty containers or dispose of waste by burning in well-ventilated areas away from water supplies. Containers should be disposed of by puncturing holes in them and burying with waste or follow official recommendations for poisonous disposal. Local conditions may affect the use of herbicides. Consult your State Agricultural Experiment Station or Extension Service weed specialist for advice in selecting treatments from this label to best fit local conditions. Be sure that use of the product conforms to all applicable regulations. Apply this product only as specified on this label.

THE DOW CHEMICAL COMPANY

AND SUBSIDIARIES

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To: Johann Petas
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Notes

The Journal of Heredity 73:224-226, 1982.

2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes

C. Korte and S. M. Jalal

ABSTRACT: Potential for genetic damage in future generations from such widely used herbicide as 2,4-D (2,4-dichlorophenoxyacetic acid) is of serious concern. Yet the data, particularly on mammalian systems, continue to be inadequate and inconclusive. An attempt was made in this study to determine the clastogenic and mutagenic potential of 2,4-D in cultured lymphocytes. Chromosome damage though statistically insignificant occurred at dosages as low as 0.2 µg/ml. Chromosome damage was increased at a statistically significant level whenever the concentration was 50 µg/ml or higher. Mutagenicity, based on rates of increase in sister chromatid exchanges, was significant at 10 µg/ml or higher concentrations. Statistical testing was based on analysis of variance, Dunnett's multiple comparison tests and linear regressions. It seems imperative therefore to avoid indiscriminate use of 2,4-D, and to test the compound for long-range low-level exposures.

AN ESTIMATED 400 synthetic chemicals have been used in agriculture since the 1930's to control weeds, insects, rodents, and pathogens. Until recently, the most widely used herbicides were 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid). DDT (dichloro-diphenyl-trichloroethene), a chlorinated hydrocarbon, was the most abundantly used insecticide until its ban in the 1970's. Although 2,4,5-T is prohibited from use in the United States (its use in limited amounts has been permitted), 2,4-D continues to be used on a large scale.

Plant auxin analogs (2,4-D and 2,4,5-T) are hormone herbicides that at lower dosages act as growth regulators¹ much like the plant auxins. At higher dosages, however, they induce potent herbicidal effects. Agent Orange, an effective defoliant used in the Viet Nam conflict was composed of 50 percent 2,4-D and 50 percent 2,4,5-T^{2,3}. It is clear from an HEW report^{2,3} as well as the monograph of Epstein and Legator⁴ that the vast majority of testing for genetic damage has been conducted on microorganisms,

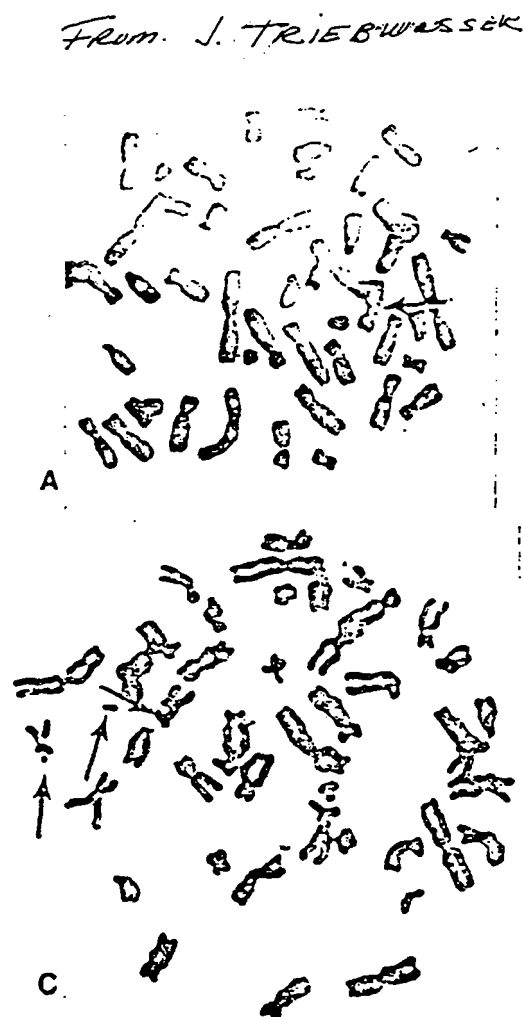


FIGURE 1 2,4-D treated human lymphocytes. A—chromatid gap. B—chromatid gap and a chromatid fragment. C—chromatid deletion, isochromatid fragment and chromatid gap. D—large isochromatid deletion.

insects, and higher plants. The trend seems to continue in recent years^{5,21}.

2,4-D must be potent biologically to effectively defoliate in a matter of a few days after surface contact. Although reports abound on its cytotoxic effects on a variety of organisms, assessment of its potential for genetic damage continues to be seriously inadequate. We wish to present our preliminary finding on chromosome damage and rates of sister chromatid exchanges upon exposure to 2,4-D in cultured human lymphocytes.

Materials and Methods

Lymphocyte culture from four male subjects was based on the standard whole blood culture technique¹¹. Solutions of 2,4-D in ethanol were prepared in eight concentrations (0.2, 2.0, 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 µg/ml) so that equal volumes of ethanol could be used for varying amounts of 2,4-D. Two controls were used, one with ethanol and the other without any treatment. After 20 hours of incubation of 0.2 ml of whole blood in 5 ml of the culture medium at 37°C, one µl of various dilutions of 2,4-D in ethanol was added to the culture. Incubation continued for another 48 hours. Air dried slides were prepared following hypotonic solution (0.075M KCL) and colcemid (0.04 µg/ml) treatments. The slides were stained in 6 percent Giemsa in phosphate buffer (pH 6.8-7.0). Data

were taken on chromosome aberrations based on 200 well spread cells for each treatment and for the controls. To eliminate bias, labels were disguised until the complete set was scored. A separation greater than the width of a chromatid was termed a deletion or a break, and a discontinuity less than the width of a chromatid was termed a gap. Mitotic indices were based on 1000 cells. The results are based on four separate replications, one each for each individual.

Mutagenicity of 2,4-D was tested on the basis of sister chromatid exchange (SCE) rates from various concentrations of 2,4-D in cultured human lymphocytes. The procedure adopted is a slight modification of Korenberg and Freedlander¹⁴ method. Lymphocytes were cultured in vials that had a wrapping of black cloth to protect photosensitive 5-bromodeoxyuridine (BrdU). Ten µg/ml of BrdU was added at the initiation of the culture. This insures BrdU uptake for two cell cycles since thymidine uptake is known not to occur in human lymphocytes in culture for about 24 hours. Harvesting of cells after 68-72 hours was by standard procedures. The air dried slides were left in open slide boxes for 2-3 days in artificial light to improve the resolution of SCE. The slides were then passed through 50 percent acetic acid at 37°C for two minutes and rinsed in distilled water. They were then gently immersed in phosphate buffer (pH 8.1) at 82-89°C for 12-15 minutes, allowed to

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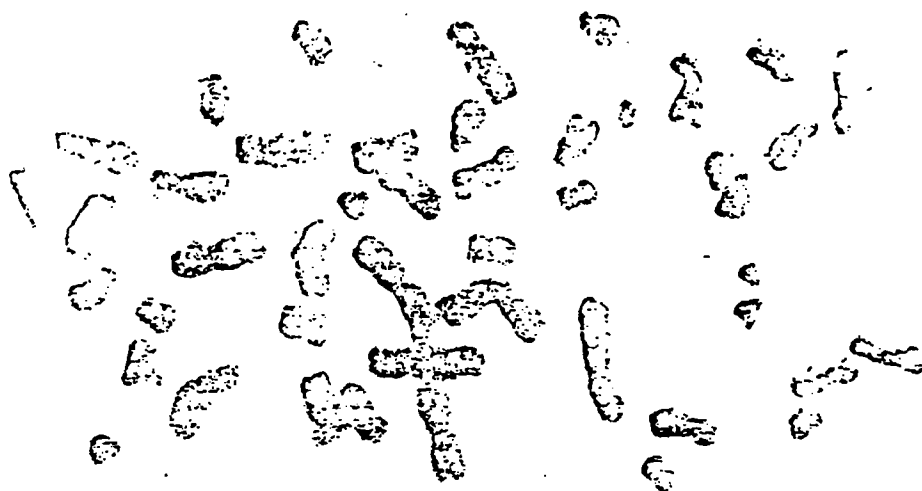


FIGURE 3 Five sister chromatid exchanges (SCE) induced in human lymphocyte cultures.

to the mutagenic effect of the compound. The proportion of SCE increased with the increased concentrations of 2,4-D. The Dunnett's multiple comparison test (Table II) indicates significance at each concentration level from 10 $\mu\text{g}/\text{ml}$ and higher, compared with the controls. There was no significant difference between alcohol treated and untreated controls. The average SCE rates of 6.14 in controls in this study is quite similar to those reported by others. The analysis of variance

between treatments and controls was highly significant. Nicholas et al.¹⁸ reported a significant increase of SCE in cultured human lymphocytes at 5 $\mu\text{g}/\text{ml}$ for an organophosphate, malathion (a pesticide). Our study clearly indicate that SCE rates are significantly higher at 10 $\mu\text{g}/\text{ml}$ or higher concentrations of 2,4-D.

At the lowest concentrations of 2,4-D (0.2 $\mu\text{g}/\text{ml}$) cross banding patterns similar to G bands were observed although the chromosome aberration levels were not significantly different from the controls. Mutagens at low concentrations are known to induce chromosome banding¹⁹. The induction of banding may occur by their interference with macroprotein biosynthesis in the G2 phase of the cell cycle¹.

Because of the potential for genetic damage, concerted efforts to carefully test and restrict the use of such compounds as 2,4-D is desirable and timely. 2,4-D is one of the most widely used herbicides and consequently it is present (0.001 mg to 0.004 mg) in dietary oils, fats and sugar products⁶. In a U.S. geological survey monitoring streams in the western United States from 1966-68, 2,4-D was to be the most frequently occurring herbicide¹⁶. Our results indicate statistically significant increases in sister chromatid exchange rates at 10 $\mu\text{g}/\text{ml}$ or higher concentrations. The chromosome damage reported here are conservative estimates since the data were taken after 48-52 hours of treatment by which time severely affected cells may not have survived. Even though a statistically significant increase in damage to genetic material occurs at relatively high dosages, the results clearly suggest caution from indiscriminate use of 2,4-D and indicate the need for further investigation, particularly on the consequences of long-term low-dosage exposure (such as to the nervous system).

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Table II. Rates of sister chromatid exchanges in human lymphocytes at various concentrations of 2,4-D

Concentr. ($\mu\text{g}/\text{ml}$) of 2,4-D	No. cells examined	Mean no. SCE per cell
0.2	9	6.44
10.0	17	9.59*
20.0	17	9.71*
30	13	10.23*
40	9	12.44*
50.0	11	12.73*
60.0	8	9.25*
EtOH	15	7.67
No inj	14	6.14
CD*		2.28
CD plus EtOH + no inj mean†		9.19
F value‡		19.78**

* Significant at $P 0.05$

** Significant at $P 0.01$

† Critical difference by which treatment group must exceed control group to be significantly greater according to Dunnett's test

‡ Minimum value that is significantly greater than control (obtained by adding critical difference to mean of two controls)

§ From the analysis of variance of SCE on 2,4-D concentration; with 7 and 90 df, significance at $P 0.01 = 2.84$

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J. ENVIRON. SCI. HEALTH, B17(4), 321-339 (1982)

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APPLICATOR EXPOSURE TO 2,4-D, DICAMBA, AND A DICAMBA LOOKER

Key Words: Dicamba, 2,4-D, Applicator Exposure, Formulation

W.M. Draper* and J.C. Street

Toxicology Program
Department of Animal, Dairy, and Veterinary Sciences
Utah State University
Logan, Utah 84322ABSTRACT

Potential respiratory and dermal exposure to applicators were estimated in a ground boom spray application of 2,4-D and dicamba. Time-weighted averages for airborne herbicide residues did not exceed 2.2 ug/cu-m. in the cabs of application vehicles allowing only minor respiratory exposure. Dermal exposure was important as relatively large amounts of 2,4-D (1.2 - 18 mg) and dicamba (0.32-6.6 mg) were rinsed from applicators' hands. Urine analysis showed that the maximum elimination of herbicides occurred between 16 and 40 h after terminating exposure. A dicamba

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isomer (20% of the active material in the commercial formulation) was excreted in higher concentrations than dicamba in applicators' urine suggesting different toxicokinetic properties for the two compounds.

INTRODUCTION

Mixtures of 2,4-D and dicamba are used widely for control of goatsrue, Canada thistle, prickly lettuce, and other noxious weeds in hay and grass pastures. Ground application is preferred as it improves accuracy, minimizes drift of herbicides, and requires less pesticide in areas of sparse weed infestation. The acreage thus treated for weed control is extremely large, particularly when related to the time-consuming application methods employed. For this reason, applicators may work throughout a season on one weed control project.

The objectives of this study were to determine to what extent applicators in different job functions were exposed to 2,4-D and dicamba in a "boomspray" herbicide application. Exposure was indexed by urine analyses and the importance of different exposure routes was estimated by measuring airborne residues within the application vehicles and residues on skin surfaces. The rate of urinary elimination of these compounds was of particular interest as preliminary studies on 2,4-D indicated a lengthy process.¹ The data obtained in the present study may be useful in evaluating how exposure to these commercially important pesticides can be minimized, a desirable goal in view of the

potential for chronic exposure over the year.

Herbicide Application

Mixed natural grass pastures were treated with a mixture of 2,4-D and dicamba (2,4-D and dicamba a.i./gal., Universal Chemical Co.) at rates of 2.5 and 1.0 gal/acre. The herbicide was applied by boom sprayer fitted with boom nozzles in swaths. Where necessary, the herbicide was applied in ditches or wet areas using hand guns.

Each truck was responsible for spraying. One crew was recently, but not working for several months. Approximate crew of the experience of this mixture.

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potential for chronic exposure of applicators at certain times of the year.

EXPERIMENTAL

Herbicide Application. The application site was a 42 acre, mixed natural grass pasture in Northern Utah being treated to eradicate goatsrue (Galega officinalis L.) on 15 July, 1980. 2,4-dichlorophenoxyacetic acid, (2,4-D, dimethylamine salt, 4 lbs a.i./gal., Universal Cooperatives) and 3,6-dichloro-2-methoxybenzoic acid (dicamba, dimethylamine salt, 4 lbs a.i./gal., Velsicol Chemical Co.) were mixed with water in tank trucks at the rate of 2.5 and 1.0 gal/200 gal., respectively. Two tank trucks, each fitted with boom jet nozzles, applied the herbicide mixture in 17 m swaths. Where the terrain was impassable (e.g., irrigation ditches or wet areas) workers applied the pesticides on foot using hand guns fed from the truck tanks.

Each truck was operated by a driver and a sprayer. Sprayers were responsible for all herbicide mixing, loading, and hand-spraying. One crew (operating vehicle A) had not been exposed recently, but the other crew (operating vehicle B) had been working for several weeks on a daily basis with this herbicide mixture. Approximately 5.5 h was required to complete the application. Crew A did not apply pesticides again during the course of the experiment while crew B continued regular daily spraying of this mixture.

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Sampling. High-volume air samplers were mounted in the cab of each truck near the applicator's breathing zone to continuously monitor airborne residues. Staplex Model TF1A samplers drew air through an adsorption train consisting of a glass fiber filter (outermost) and approximately 120 ml of preextracted XAD-4 macroreticular resin in a 1.8 cm bed. Particulates were trapped with the glass filter and vapors were sorbed in the resin. Flow rates were calibrated using instruments supplied by the manufacturer. At the completion of the herbicide application, dermal residues on the hands of workers were removed by thorough rinsing with 95% ethyl alcohol. First void urine samples were obtained on the morning prior to the experiment and on each morning for three days following the initial application. Urine specimens, hand rinse solutions, glass fiber filters, and XAD-4 resin were stored in glass jars with metal foil liners at -5°C until chemical analysis.

Analysis. Urine samples were analysed with a multiresidue procedure described in detail elsewhere²; samples were subjected to hydrolysis, acid/base partitioning, derivatization with ethereal diazomethane, and quantitated by electron capture gas chromatography (EC-GC). 2-(2,4-dichlorophenoxy)-propanoic acid (2,4-DP, 0.10 mg/L) was added initially to each sample as an internal standard. Calibration was by internal standard between the limit of detection (0.060 mg/L) and 0.2 mg/L and by absolute calibration for the more concentrated samples. The presence of the

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herbicide components in several of the urine samples was verified by GC-coupled mass spectrometry (GC-MS).² Urinary creatinine was determined colorimetrically.⁵

Glass fiber filters were extracted with acetone for 1 h in a Soxhlet apparatus and the extract was reduced just-to-dryness on a rotary evaporator. The residue was derivatized with ethereal diazomethane, concentrated under a stream of dry nitrogen, and diluted with hexane before EC-GC quantitation. XAD-4 resin was Soxhlet-extracted with acetone for 4 h and the residue of the concentrated extract was suspended in 50 ml of diethyl ether and extracted with 25 ml of 0.05 N sodium hydroxide and 2 x 30 ml of 4% sodium bicarbonate. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with 2 x 50 ml of diethyl ether. The ether layer was dried (sodium sulfate), concentrated, derivatized, and quantitated as above.

Ethanollic hand rinse solutions were concentrated, derivatized, and diluted with hexane before EC-GC analysis. Dicamba and 2,4-D were recovered in high yield after flash evaporation of the solvent.

Two dimensional thin-layer chromatography (TLC) was used to examine airborne residues. Methylated extracts were spotted on 20 x 20 cm fluorescent silica gel plates and developed in benzene on one axis and hexane-diethyl ether (9:1, v/v) on the second axis. Spots were visualized by quenching of UV light.

Dicamba formulation analysis. The dicamba formulation was fractionated as follows: 2 ml of the 49% amine salt were dissol-

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ved in 50 ml of 0.5% N sodium hydroxide, extracted with 3 x 40 ml of diethyl ether (organic layer discarded), acidified, extracted with 3 x 50 ml of methylene chloride, and the organic layer concentrated to give a yellow oil. The oil was esterified with 50 ml of ethereal diazomethane, and 3.53 g of the methylated material was separated on a 30 x 2.5 cm SilicAR (100-200 mesh) column with benzene as the eluant. The isolated components were characterized by mass spectrometry and proton magnetic resonance spectroscopy (PMR). PMR spectra were recorded on a Joel FX90Q, 90 MHz, Fourier transform instrument with 1% tetramethylsilane as the internal reference.

Analytical reference standards of 2,4-D and dicamba were provided by the Quality Assurance Section, U.S. Environmental Protection Agency (Research Triangle Park, N.C.). Pesticide residue grade solvents were used and diazomethane was prepared by alkaline hydrolysis of N-methyl-N-nitroso-p-toluenesulfonamide in a distillation apparatus designed for this purpose (Aldrich Chemical Co.).

RESULTS AND DISCUSSION

Dicamba Formulation. The preparative-scale fractionation of the formulated dicamba product yielded two major components: methyl dicamba [MS(70eV) m/e 203 (base, $C_8H_5Cl_2O_2$, 234 (20%, K^+), 185 (30%, $C_7H_2Cl_2O_2$); PMR ($CDCl_3$) δ 7.36 (d, 1.02, phenyl, $J=8.79$ Hz), δ 7.10 (d, 0.978, phenyl, $J=8.79$ Hz), δ 3.96 (s, 2.99, $COOCH_3$).

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δ 3.91 (s, 3.0 eV) m/e 203 ($C_7H_2Cl_2O_2$) phenyl, $J=8.79$ (s, 2.88, $COOCH_3$) polar than dicamba) on both methyl showed major were further coupling con the contamin aromatic sub 3,5-dichloro- reported in 1 mination, the mer in a 5.0:

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d 3.91(s,3.01, OCH₃) and a methylated isomer of dicamba [KS(70 eV) m/e 203 (base, C₈H₅Cl₂O₂), 234 (30%, M+), 188 (30%, C₇H₂Cl₂O₂), 173 (20%), 201 (60%); NMR (CDCl₃) d 7.67 (d, 0.934, phenyl, J=2.64 Hz), d7.53 (d,0.887, phenyl, J=2.63 Hz), d3.923 (s,2.88, COOCH₃), d3.916 (s,3.30,OCH₃)]. The isomer was more polar than dicamba exhibiting a mobility of 0.8 (relative to dicamba) on TLC plates developed in benzene. Mass spectra of both methylated compounds were similar, but the NMR spectra showed major differences. The aromatic protons of the isomer were further downfield by 0.37 ppm and they exhibited meta spin coupling constants (J=2.6Hz). These spectral data indicate that the contaminant differed from dicamba only in the pattern of aromatic substitution. These findings are consistent with that of 3,5-dichloro-2-methoxybenzoic acid, a contaminant previously reported in formulated dicamba.⁴ Based on a gravimetric determination, the present formulation contained dicamba and its isomer in a 5.0:1 ratio.

Airborne Residues. Herbicides were detected in air samples taken inside the truck cabs. Total time-weighted average concentrations were 0.71-1.8 ug/cu.m., 0.18-0.65 ug/cu.m., and 1.2-2.2 ug/cu.m. for dicamba, dicamba isomer, and 2,4-D respectively. In each case the majority of the pesticide was trapped in the glass fiber filter indicating that aerosols and not true vapors predominated (Table 1).

The glass fiber filters used exhibit "good" trapping efficiency for particles in the range of 0.01 to 10 um (Staplex Co.

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TABLE 1
Airborne Herbicide Residues in Application Vehicles (ug/cu.m.)

Vehicle	Sampling Interval	Glass Fiber Filter			Resin		
		Dicamba	Isomer	2,4-D	Dicamba	Isomer	2,4-D
A(a)	40 min	0.65	0.24	1.3	0.24	(b)	0.03(c)
A	140	0.29	0.07	0.4	0.04	<0.01	<0.01
A	80	1.10	0.35	2.5	0.18	(b)	<0.02
B(d)	65	1.20	(b)	2.0	0.27	0.06	0.27
B	95	0.74	0.28	1.3	0.19	(b)	<0.03
B	30	5.8	1.5	5.5	0.33	0.10	0.33

- a. Sampler flow rate, 0.71 cu.m./min.
 b. Data not available.
 c. Not detected (i.e., below the estimated detection limit of 1.0 ug/resin sample).
 d. Sampler flow rate, 0.37 cu.m./min.

product literature). Dicamba appeared more volatile than 2,4-D as greater amounts were present in the XAD-4 resin. The amine salt formulations studied here are generally less volatile than low molecular weight ester formulations of these herbicides.

Airborne herbicides quantitated by EC-GC were confirmed by two-dimensional TLC followed by GC-MS analysis of the eluted bands (Figure 1). The air samples contained a large number of UV-quenching (aromatic) compounds as well; these compounds were probably of natural origin.

Dislodgeable Dermal Residue. Herbicides were detected at relatively high levels in the ethanolic hand rinse solutions

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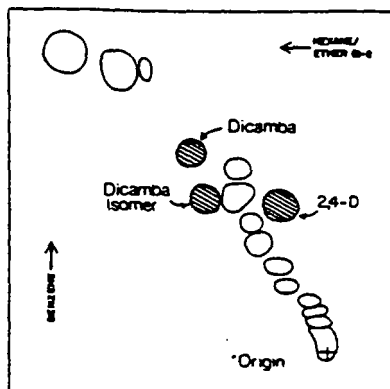


FIGURE 1. Airborne herbicides and naturally-occurring compounds in applicator's breathing zone.

(Table 2). On the average, 2.5 mg of dicamba, 7.5 mg of 2,4-D and 0.75 mg of dicamba isomer were dislodged. Dermal residues were approximately 5 times greater for the sprayers involved in mixing, loading, and spraying functions than for the drivers. Sprayer B exhibited the highest dermal residue, a quantity almost 3 times that of Sprayer A. Higher dislodgeable dermal residues were attributed to the use of the hand spray gun and not the number of tank mixings. Sprayer B used the gun for over 70 min during the study period while Sprayer A only logged 5 min.; Sprayer A stopped to mix and load the tank three times whereas Sprayer B only performed this function twice. The efficiency with which residues were washed from the skin with ethanol is not known, nor is the impact of this procedure upon the subsequent absorption of remaining residues. At no time during the study did applicators wear protective gloves. Field investigators

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Table 3.
Herbicide Concentrations in the Urine of Exposed Workers (mg/L).

Subject	Sample	Dicamba	Dicamba isomer	2,4-D
Driver A	Prespray	0.065	(a)	0.12
	24 h	0.50	0.47	1.0
	48 h	0.47	0.63	1.2
	72 h	0.37	0.39	1.1
Sprayer A	Prespray	(a)	(a)	(a)
	24 h	0.33	0.52	1.3
	48 h	1.4	1.7	3.6
	72 h	1.0	1.5	2.6
Driver E	Prespray	0.83	0.78	1.8
	24 h	1.7	2.2	4.1
	48 h	16.	15.	20.
	72 h	5.3	6.6	8.8
Sprayer E	Prespray	1.6	2.2	4.0
	24 h	1.0	1.3	2.6
	48 h	4.1	7.0	12.
	72 h	3.1	3.7	7.3

(a) Not detected (detection limit 0.060 mg/L).

was 1:2:1 (dicamba isomer:dicamba); which represents a nearly 6-fold magnification of the isomeric impurity in relation to dicamba. It is clear from the data that there is a major difference in the toxicokinetic properties of the two dicamba isomers. The ratio of dicamba to its isomer remained relatively constant in successive urine samples (Figure 2) suggesting that the rates of distribution (after uptake) and of renal elimination are similar for these isomers, but that dermal uptake rates may differ considerably. Differences in uptake rates for dicamba

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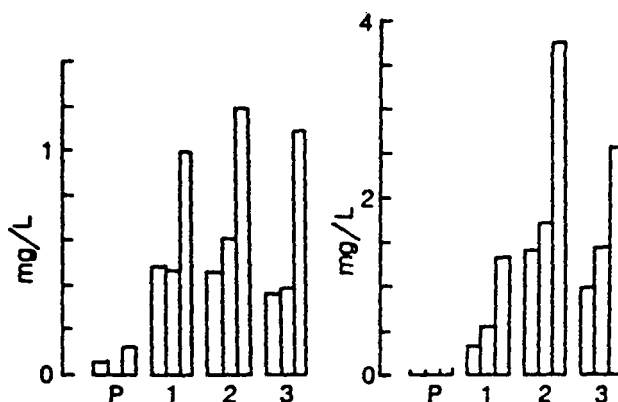


FIGURE 2. Urinary elimination of herbicides on three consecutive days after a single herbicide application; Driver A (left) and Crayer A (right). P represents prespray and numerals indicate 24, 48, and 72 h samples; consecutive bars represent dicamba, dicamba isomer, and 2,4-D, respectively.

isomers may be related to polarity differences noted above.

These preliminary findings indicate the need for more detailed toxicokinetic study of these compounds.

Several of the urine samples were subjected to GC-MS analysis. Computer reconstructed, single-ion chromatograms were generated for dicamba (m/e 234, 203, and 188) and 2,4-D (m/e 234, 199, 175, and 161) and in each case the retention times and relative intensities of the diagnostic ions were consistent.

Estimating Absorbed Dose. Chlorophenoxy acid herbicides are excreted largely unmetabolized in the urine of animals⁵ and the urinary levels are well correlated with the rates of exposure.^{6,7} For these and related compounds the total of residues eliminated in the urine is approximately equivalent to the absorbed dose.

APPLICATOR EXPOSURE

The creatinine in the analysis of the estimate of the dose is based upon the (creatinine elimination) method used permits the technique may variation in creatinine modeling of creatinine

The absorbed urinary elimination isomer, and 3.2 mg elimination in 72 h isomer, and 11.5 mg the absorbed dose, after 96 h. This is appropriate for applicator herbicide mixture of dicamba isomer is evidence some enrichment of 2 well, although the evidence 1.5 to 1.

Respiratory vs. Urinary

were utilized to estimate tidal volumes were 1

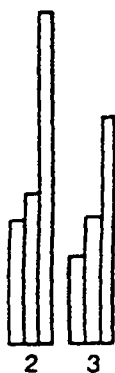
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The creatinine index method was used to extrapolate the results of the analysis of first void urines (Table 3) to obtain an estimate of the daily urinary elimination (Table 4). The method is based upon the assumption of an average creatinine index value (creatinine eliminated/kg/24 h).⁶ Although the creatinine index method used permitted only approximate urine volume estimates, the technique may be enhanced in accuracy by allowing for dietary variation in creatine ingestion and utilization of mathematic modeling of creatinine excretion.⁹

The absorbed dose received by Driver A (estimated from the urinary elimination) was 1.3 mg of dicamba, 1.4 mg of dicamba isomer, and 3.2 mg of 2,4-D. For sprayer A his total urinary elimination in 72 h was 3.9 mg of dicamba, 5.2 mg of dicamba isomer, and 11.5 mg of 2,4-D. These data somewhat underestimated the absorbed dose, as low-level urinary excretion continued even after 96 h. This approximation of dose absorbed was not appropriate for applicators in vehicle B as they continued to spray the herbicide mixture on subsequent days. The preferential uptake of dicamba isomer is evident from these data. There may have been some enrichment of 2,4-D relative to dicamba in those urines as well, although the effect was only on the order of 1.2-1.5 to 1.

Respiratory vs. Dermal Exposure. The following assumptions were utilized to estimate potential inhalation exposure: tidal volumes were 1.0 L/inspiration, breathing rates were 15

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TABLE 4
Estimated Urinary Excreted Herbicides (a)

Subject	Sample	Creatinine (mg/ml)	Estimated creatinine output (mg/24h)	Estimated urine output (ml/24h)	Estimated Herbicide Excretion		
					Dicamba (mg/24h)	Isomer (mg/24h)	2,4 - D (mg/24h)
Driver A	Prespr.	(b)	1700	960 (c)	0.062	<0.058	0.11
	24 h	1.6	1700	1000	0.50	0.47	1.0
	48 h	1.7	1700	990	0.46	0.62	1.2
	72 h	1.9	1700	880	0.33	0.34	0.97
Sprayer A	Prespr.	1.3	1500	1200	<0.072	<0.072	<0.072
	24 h	0.7	1500	2200	0.073	1.1	2.9
	48 h	(b)	1500	1500 (c)	2.1	2.5	5.7
	72 h	1.4	1500	1100	1.1	1.6	2.9
Driver B	Prespr.	1.0	1500	1500	1.2	1.2	2.7
	24 h	1.2	1500	1300	2.2	2.9	5.3
	48 h	1.4	1500	1100	18	16	22
	72 h	1.0	1500	1500	7.9	9.9	13
Sprayer B	Prespr.	(b)	1500	1400 (c)	2.2	3.1	5.6
	24 h	1.2	1500	1300	1.3	1.7	3.6
	48 h	1.1	1500	1400	5.7	9.8	17
	72 h	1.0	1500	1500	4.6	5.5	11

a. Estimations based upon an average creatinine coefficient of 21 mg/kg/24 h; subjects weighed 73 kg except Driver A who weighed 80 kg.

b. not determined.

c. Estimated from 3-day mean urine volume.

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	1.4	1.3	1.7	3.6
48 h	1.1	5.7	9.8	17
72 h	1.0	4.6	5.5	11

a. Estimations based upon an average creatinine coefficient of 21 mg/kg/24 h; subjects weighed 73 kg except Driver A who weighed 80 kg.
b. not determined.
c. Estimated from 3-day mean urine volume.

APPLICATOR EXPOSURE

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inspirations/min, and airborne residues (total of vapor and aerosol) were considered to undergo quantitative deposition in the upper respiratory tract and were thus equivalent to oral exposure. The breathing rate used in this approximation is typical of light to moderate exertion. For vehicle A, the estimated maximum inhaled doses during the 5.5 h application did not exceed 10 ug of either herbicide. The data in Table 5 represent exposures on a ug/kg basis.

Respiratory exposures were extremely small in comparison to the absorbed doses approximated by the overall 72 h elimination in urine. The dislodgeable dermal residues, however, were of the same magnitude as those eliminated in the urine, which emphasizes the importance of dermal exposure. Although the association between dermal exposure and the absorbed dose is unequivocal, it is difficult to interpret these data in detail. The high level of dicamba isomer excreted indicates that the total herbicide residue on the applicator's skin may have been much greater than that determined with the ethanol rinse procedure. The absorbed dose is frequently approximated as 10% of the applied dermal dose, although this relationship was developed with pesticides other than these water-soluble herbicides.¹⁰ However, data from our laboratory on the dermal absorption of 2,4-D from a mixture of formulated 2,4-D- and dicamba- (2.5:1) amine salts applied to guinea pig skin indicated approximately eighty-five percent uptake in 24 hours (based upon recovered chemical on skin swabs).¹

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TABLE 5

Potential Respiratory Exposures, Dermal Residues, and Estimated Absorbed Doses (based upon team in vehicle A).

Sample	Potential respiratory exposure (ug/kg)			Dislodgeable dermal residues (ug/kg)			Estimated absorbed dose (ug/kg) (a)		
	Dicamba	Isomer	2,4-D	Dicamba	Isomer	2,4-D	Dicamba	Isomer	2,4-D
Driver (b)	0.055	0.015	0.080	12	2.2	46	16	16	40
Sprayer (c)	0.060	0.016	0.088	31	9.2	100	53	71	160

a. Total residues eliminated in the urine in 72 h/subject weight.

b. 80 kg

c. 73 kg

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but in this study the use of hand spraying, mixing greater exposure was estimated to herbicide concentration of the majority of the urinary elimination occurred over the onset of exposure herbicides indicate would increase on single day's work dicamba and 160 ant in the formulation as its rate of elimination. Despite minor toxicity of these 2,4-D and dicamba ly. The impact level chronic or chemicals is poor

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CONCLUSION

Applicator exposure occurs during boom-spray applications, but in this study the majority of exposure was associated with the use of hand spraying equipment. Individuals involved in spraying, mixing, and loading were subjected to significantly greater exposures than the vehicle drivers. Respiratory exposure was estimated to be minor due to the relatively low airborne herbicide concentrations. Exposure of the skin accounted for the majority of the absorbed dose.

Urinary elimination following a single spraying session occurred over several days and reached a maximum 48 h after the onset of exposure. The prolonged urinary elimination of these herbicides indicates that the body-burden of these compounds would increase over the span of a five day work week. Following a single day's work, the estimated absorbed dose was 53 ug/kg of dicamba and 160 ug/kg of 2,4-D. An isomer of dicamba, a contaminant in the formulation, was apparently absorbed preferentially as its rate of elimination in urine exceeded that of dicamba.

Despite minimal protective measures, the exposures experienced in this study were low compared to any known measure of toxicity of these herbicides. For example, oral LD₅₀ values for 2,4-D and dicamba in the rat are 375 and 1040 mg/kg, respectively.¹¹ The impact on the human health of weed spray crews of low-level chronic or sub-chronic pesticide exposure to such pesticide chemicals is poorly understood.

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These estimated exposure values are notably greater than those observed in similar studies of workers involved in aerial forest applications (both ground personnel and pilots)¹² and in ground and aerial applications to wheat fields.¹³ While those observations showed, as did ours, that the job task itself is a major factor affecting worker exposure, the maximum values were considerably lower than those reported here. Mixer-loaders in the forestry study had urinary residues totalling 0.038 to 0.056 mg/kg body weight; their counterpart workers in the wheat study were reported as having absorbed 0.020 mg/kg.^{12,13} In this study, sprayer A (with no previous exposure) excreted 0.16 mg/kg and sprayer B excreted 0.54 mg/kg. Use of impermeable gloves during handspraying and mixing/loading could have minimized the major factor contributing to the exposure we observed.

ACKNOWLEDGEMENTS

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Effects of Chronic Exposures to Pesticides on
Animal Systems, edited by James I. Chambers,
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Effects of Pesticides on the Kidney

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The mammalian kidney is a dynamic and complex organ. Excretion of wastes is a primary function, but the kidney also plays a significant role in the regulation of total body homeostasis. Regulation of extracellular volume and control of electrolyte and acid-base balance are important renal functions. The kidney is the major site of formation of hormones that influence systemic metabolic functions: erythropoietin is a potent stimulus to erythrocyte formation; the relatively inactive 25-hydroxy-vitamin D₃ is metabolically activated to the active 1,25-dihydroxy-vitamin D₃; renin, the trigger to the formation of angiotensin and aldosterone, is formed in the kidney; and recent evidence indicates that the kidney produces several vasoactive prostaglandins and kinins. A toxicological insult to the kidney from pesticides or any other chemical could affect any or all of these functions. The susceptibility of the kidney or any other organ to toxic insult as well as the methods used to assess any alteration in function of that organ are important factors in determining the degree of toxic effect, if any, caused by a chemical (27).

A great deal of descriptive data are available concerning the effect of pesticides on the kidneys. In this review, however, rather than provide an exhaustive list of pesticides and their effects on the kidney, we will discuss three specific chemicals that illustrate key points concerning nephrotoxicity and pesticides. These three com-

pounds—1,3-hexachlorobutadiene (HCB), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)—produce effects that describe the gamut of changes in renal function observed after pesticide exposure, i.e., direct tissue damage, physiological alteration with no tissue damage, and indirect biochemical effects with subtle morphological changes seen only with electron microscopy. We will also discuss the renal effects of another environmental pollutant, the polybrominated biphenyls (PBBs). Although not pesticides, PBBs are close chemical relatives and represent another type of potentially hazardous mixture.

UNUSUAL SUSCEPTIBILITY OF THE KIDNEY TO CHEMICALS

As a consequence of its normal function, the kidney is susceptible to the noxious effects of many chemicals in the blood (27). Renal blood flow is quite high; the two kidneys together receive about 25% of the cardiac output. Approximately one-third of the plasma water reaching the kidney is filtered; from this material approximately 98 to 99% of the salt and water is reabsorbed. Maintenance of normal function requires delivery of large amounts of metabolic substrates and oxygen to the kidney. Because of the high blood flow, any chemical in the circulation will be delivered in relatively high amounts to this organ. As salt and water are reabsorbed from the glomerular filtrate, the materials remaining (including the potential toxicant) in the urine may be concentrated in the tubular lumen. Thus, a nontoxic concentration of a chemical in plasma could become toxic in the kidney subsequent to concentration within the urine (Fig. 1). Furthermore, a chemical reaching the kidney might be concentrated in the cells by other mechanisms. If the material is actively secreted into the tubular urine, it will first be accumulated within the cells of the proximal tubule, thus exposing these cells to very high concentrations of the agent, which could produce toxicity. Similarly, a material that is reabsorbed (even by passive means) from the urine into the blood will pass through the cells of the nephron in a relatively high concentration. In addition, the kidney

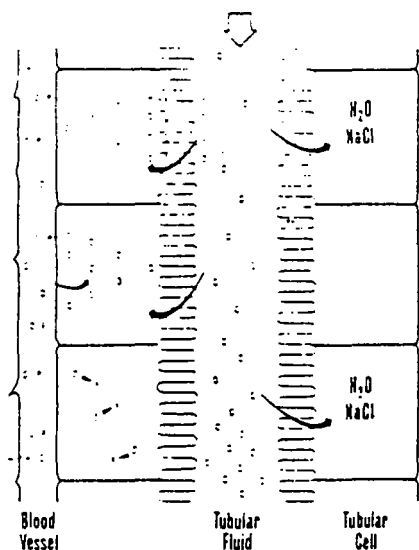


FIG. 1. Scheme of renal handling of a chemical (D). If material is filtered, its concentration in tubular fluid will rise as salt and water are reabsorbed. It may then diffuse down its concentration gradient into the blood. The chemical may be secreted into the tubular cell from the blood (may even be removed from protein binding sites) and into the tubular fluid. The chemical may enter the cell and be metabolically altered ($D \rightarrow X$).

is exposed to chemicals in another unique fashion. Binding of chemicals to plasma proteins may protect most cells of the body from the potential toxic action of these compounds. However, if a compound is actively secreted by the nephron, it can be removed from plasma binding sites and transported into renal tubular cells and into the tubular urine. Thus, although other cells of the body may be protected by protein binding, the renal cells might be exposed to these potentially toxic molecules or ions (Fig. 1).

The renal medulla is unique in relation to the nephrotoxicity of chemicals. Since only about 10% of total renal blood flow enters the renal medulla, relatively less drug or chemical might reach this region via the blood than would enter the cortex. However, any chemical in the tubular urine will pass through the loop of Henle and the medullary collection duct, exposing the cells of the medulla to high concentrations. In addition, the countercurrent mechanism within the medulla and the relatively low blood flow may trap

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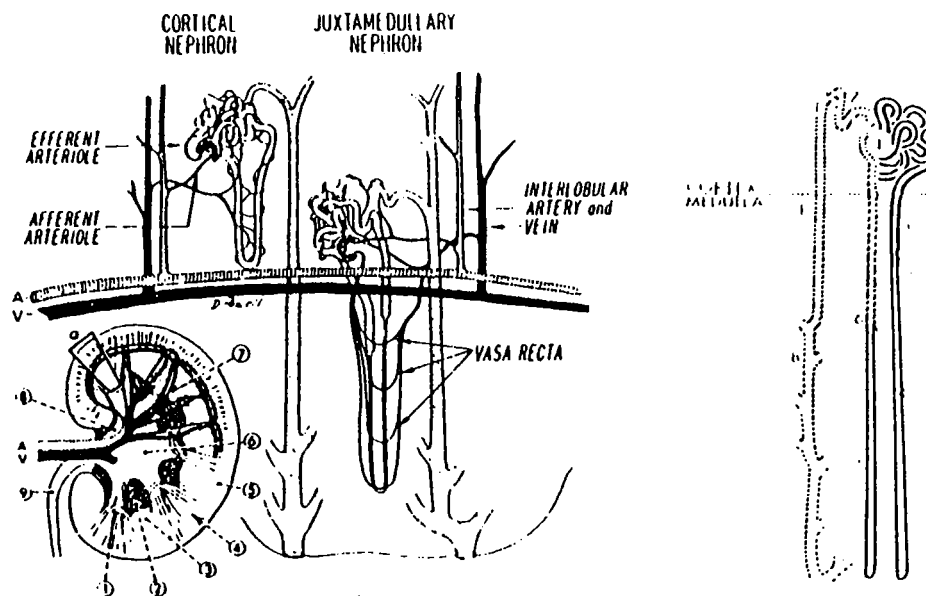


FIG. 2. Left: Sagittal section of a mammalian (human) kidney is illustrated in lower left. A, renal artery; V, renal vein; 1, minor calix; 2, fat in sinus; 3, renal column of Bertin; 4, medullary ray; 5, cortex; 6, pelvis; 7, interlobar artery; 8, major calix; 9, ureter. Insert (a) from the upper pole of the kidney is enlarged to illustrate the relationships between the nephrons and the vasculature. Right: Anatomy of a juxta-medullary nephron. Note the demarcation between cortex and medulla. 1, glomerulus; 2, proximal convoluted tubule; 3, proximal straight tubule (pars recta); 4, descending limb of the loop of Henle; 5, thin ascending limb of the loop of Henle; 6, thick ascending limb of the loop of Henle; 7, distal convoluted tubule; 8, collecting duct. (From Hook, ref 27, reprinted with permission of Macmillan Publishing Co.)

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compounds, further leading to the development of relatively high concentrations (14,67).

ASSESSMENT OF RENAL FUNCTION

The anatomy of the kidney is most appropriately based on the functional unit of the kidney, the nephron (Fig. 2). The nephron may be considered in three portions: the vascular element including afferent and efferent arterioles, the glomerulus, and the tubular element. All nephrons have their primary vascular elements and glomeruli in the cortex. The proximal convoluted tubule is localized in the cortex and sends the pars recta (straight portion) of the proximal tubule and loop of Henle deep into the substance of the kidney. Those glomeruli close to the medulla (juxtamedullary glomeruli) are associated with nephrons that send their loops of Henle deep into the medulla. Other glomeruli closer to the surface of the kidney often form nephrons whose loops of Henle are contained within the cortex. The relative proportion of nephrons with long versus short loops varies with species (67).

When attempting to evaluate the effect of chemicals on nephron function, it must be realized that the nephron operates with these three separate components functioning in series. The vascular component delivers blood to the nephron. The glomerular component is a membrane through which fluid and selective solutes are filtered. The tubular component reabsorbs the filtrate and secretes materials into the tubular urine. Since these elements are arranged in series, it is difficult to isolate an initial lesion when the kidney has been damaged. For instance, if tubular function is monitored by measuring urine volume and composition, increased volume and decreased osmolality might result from chemical exposure. Such an effect could be due to direct damage to tubular cells, altering their ability to reabsorb constituents of the tubular fluid. On the other hand, such an effect could be secondary to glomerular damage, causing an increased permeability, leading to excess filtrate entering the tubule exceeding the reabsorptive capacity (59). Alternatively, a constrictor effect in the vasculature could decrease delivery of

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nutrient to the tubule, limiting tubular function resulting in increased urine volume. In the same example, the initial event could be selective vasospasm leading to redistribution of blood within the kidney. An increase in medullary blood flow would dissipate the renal medullary hypertonicity leading to increased urine volume. Conversely, if the effect of a suspected nephrotoxicant were monitored by evaluating blood chemistry, an increase in blood urea nitrogen (BUN) and plasma creatinine might be observed. Such an effect could be due to the formation of crystals occluding lumens and preventing excretion of waste material. Alternatively, increased BUN could be due to selective damage to the glomerulus or vasospasm of renal blood vessels (2).

The toxic effects of chemicals may be evaluated *in vitro* by directly adding the agent to the preparation or following administration to the animal (2). This allows a distinction to be made between an effect on the kidney due to direct chemical insult and secondary effects such as those subsequent to metabolism or vascular effects. The renal cortical slice technique has been used extensively to evaluate the influence of nephrotoxicants on the secretory transport of organic anions such as *p*-aminohippurate (PAH) and organic cations such as *N*-methylnicotinamide (NMN) or tetraethylammonium (TEA) (1,31). Attempts have been made to evaluate transport in a reabsorptive direction using the nonmetabolized amino acid analog α -aminoisobutyric acid and the nonmetabolized sugar α -methyl-D-glucoside. In addition, the ability of the kidney to produce ammonia and glucose from added substrates can be quantified *in vitro* and can provide more specific information about metabolic alterations produced by a nephrotoxicant (31). Isolated tubular preparations may be employed to evaluate the influence of chemicals on selected areas on the nephron. Micropuncture and microperfusion techniques have also been utilized in attempts to identify specific loci and action of nephrotoxicants (59).

Histopathological examination of tissue can demonstrate structural changes that have occurred in response to chemicals and can often identify selected areas that have been affected. For instance, light microscopy can demonstrate the papillary necrosis produced

by nonnarcotic analgesics (58) and can isolate the proximal tubular damage induced by mercury and other heavy metals (65). Histopathology will often show that an injury has occurred even in a situation in which function is not noticeably altered. The use of standard light microscopy can also provide information concerning the appearance in the tubule of protein casts of sloughed brush border, and crystals or stones in the kidney and urine. Very elegant experiments have been conducted using microdissection techniques. Following nephrotoxic insult, entire tubules have been carefully dissected and specific areas of damage have been identified by light microscopy (4). Many histochemical techniques are also available to evaluate renal response to poisons. Electron microscopy provides information concerning subcellular localization of tubular injury. Changes in mitochondria can very easily be identified, as can alterations in other organelles. Electron microscopy has been extensively employed in efforts to understand the changes in glomerular structure that might account for changes in permeability following nephrotoxicant exposures (5).

HCBD

HCBD is an industrial liquid often found in association with hexachlorobenzene as an industrial by-product in the manufacture of perchlorethylene (39,44). It has been suggested to be potentially useful as an insecticide, a herbicide, a fumigant, a bactericide, and an algacide (3) and it has been used as a fungicide (17). Currently, it is thought to be widely dispersed in the environment, especially along the Mississippi River (43,44). The kidney appears to be the organ most sensitive to the toxic effects of HCBD (23,40,45,57).

HCBD has been shown to have direct nephrotoxic effects: it produces damage directly to the kidney and in high doses may produce acute renal failure. Renal toxicity in the form of an increase in the kidney-weight/body-weight ratio as well as renal tubular degeneration, necrosis, and regeneration has been observed in rats receiving 30, 65, and 100 mg/kg of HCBD for 30 days (40). Renal tubular damage in the rat was also observed following inhalation exposure

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(21) and after dermal application in rabbits (16). Histological examination of the kidneys 24 hr after a dose of 100 mg/kg of HCBP revealed a discrete band of damage to the straight portion of the proximal tubules in the outer stripe of the outer medulla (45). This localization of the lesion to the pars recta or straight portion of the proximal tubule is very characteristic of the renal damage produced by HCBP.

The functional characteristics of nephrotoxicity of HCBP in the rat are similar to those produced by several other well-known direct-acting nephrotoxicants such as mercury and chromium (21). Increases in urine volume, proteinuria, and an increase in excretion of *N*-acetyl- β -D-glucosaminidase and alkaline phosphatase have been observed at doses about 100 mg/kg HCBP in the rat (45). Only proteinuria was observed at doses less than 100 mg/kg in the rat (45). Ingestion of 2 mg/kg/day for up to 2 years caused lesser degrees of toxicity, i.e., increases in urinary excretion of coproporphyrin and renal tubular hyperplasia (41). Rats ingesting 0.2 mg/kg/day HCBP for up to 2 years apparently had no adverse effect from HCBP (40).

HCBP has been shown to alter the renal transport of organic ions (3,45). In the rat, administration of HCBP markedly inhibits the transport of the organic anion, PAH, by renal cortical slices (45). Transport of organic cations, as measured by tetraethylammonium uptake into slices, is not inhibited. Lock and Ishmael (45) compared this inhibition of the ion transport systems to the effect produced by mercuric chloride and found that mercuric chloride inhibited both the organic anion and organic cation transport systems. As both organic ion transport systems are located in the proximal tubule, this has been interpreted to suggest that the site-specific lesion of HCBP is well localized in the rat to the major site or sites of the organic anion transport system (3,45). Interestingly, in mice the effect of HCBP on the two transport systems is the same, i.e., both are inhibited and to the same degree (Hook and Lock, *unpublished observations*). This suggests that the separation of the organic anion and organic cation transport systems in the proximal tubule and/or the effect of HCBP on one or both of these processes is species-specific.

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The biochemical mechanisms of the nephrotoxic effect of HCBd is unknown. Recent studies by Lock and Ishmael (46) suggest that the compound might be metabolically activated to some reactive intermediate prior to inducing nephrotoxicity. These conclusions were based on the observation that the increase in BUN produced by HCBd was magnified after the administration of Aroclor 1254, a known inducer of several hepatic and renal drug metabolizing enzyme systems. However, the nephrotoxicity of HCBd was not blocked by SKF-525A, an inhibitor of hepatic mixed-function oxidase enzyme systems. Thus, the evidence is far from clear concerning the necessity of metabolic activation prior to the nephrotoxicity of HCBd. In fact, Hook and Lock (*unpublished observations*) used a wide variety of enzyme inducers and inhibitors and failed to find evidence for metabolic transformation of HCBd prior to the nephrotoxicity.

Along this same line, HCBd has been found to produce depletion of nonprotein sulfhydryl (NP-SH) concentration (mainly in the form of glutathione) in the liver but not in the kidney (46). In view of the role of glutathione in the protection of hepatocytes and renal tubular cells from electrophilic attack by alkylating metabolites and the sensitivity of the kidney cells to HCBd, it would have been expected that the NP-SH would have been depleted in the kidney rather than in the liver. Kluwe and Hook (*unpublished observations*) found glutathione depletion in the kidney of mice following HCBd administration. The conflicting data suggest that the lack of effect on rat kidney may reflect the site specificity of the lesion rather than an inability to reduce glutathione concentrations. It is also possible that in the rat, a glutathione-HCBd conjugate is formed in the liver, which is taken up into the bloodstream and produces toxicity in the kidney.

2,4,5-T

2,4,5-T is an organic acid that possesses the property of regulating plant growth at low dosages and killing plants at high dosages. It has been widely used, since the early 1940s, for such things as inducing coloration in fruit, as a fruit set and antidrop agent, for

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brush control, and for control of aquatic and herbaceous land plants (18). 2,4,5-T is prepared by reacting the sodium salts of trichlorophenol and monochloroacetic acid. One of the side products of the reaction is a tetrachlorodibenzo-*p*-dioxin, which is a highly toxic substance. Present manufacturing procedures are designed to remove this impurity.

In direct contrast to HCBd, most evidence suggests that 2,4,5-T is not highly toxic in animals. This low toxicity is apparently due, in part, to its rapid excretion via the kidneys. Essentially all of the 2,4,5-T is absorbed into the body and excreted, unchanged, in the urine of humans (22) and rats and dogs (60). 2,4,5-T is more toxic to dogs than to rats. Piper et al. (60) demonstrated that the rate of renal excretion of 2,4,5-T was greater in rats than in dogs and suggested that this difference could account for the differences in toxicity. Hook et al. (28) used an *in vitro* technique to demonstrate that 2,4,5-T is actively transported by renal cortical tissue of the rat and dog. They suggested the primary route of elimination of 2,4,5-T to be active secretion of the compound. The greater ability of adult rat tissue, as compared to dog tissue, to transport 2,4,5-T was offered as the explanation for the shorter biological half-life of 2,4,5-T in the rat. However, the prolonged plasma half-life of 2,4,5-T in dogs (77 hr) indicated that factors other than secretion into the urine are important determinants of elimination in the dog. In a subsequent study, Hook et al. (29) observed extraordinarily low renal clearances of 2,4,5-T, less than 1% of the clearance of inulin. Although clearance was increased somewhat by alkalinizing the urine (therefore indicating some reabsorption of 2,4,5-T) the data, nevertheless, suggested that most of the 2,4,5-T in urine remained in the urine and was not reabsorbed. Since 2,4,5-T is highly bound to plasma protein, little would enter the urine by filtration and the low clearance suggested that, in contrast to transport *in vitro*, little 2,4,5-T was secreted *in vivo*. Subsequently they demonstrated a correlation between plasma protein binding and the rate of secretion (29). Classic renal physiology suggests that a compound may be secreted even if it is bound to plasma proteins. Between 80 to 90% of PAH, for instance, is bound to plasma proteins and yet it can be

completely extracted from the plasma perfusing the cortex (56). Thus, the affinity of the kidney for PAH and for other anions is greater than that of the binding sites of the plasma protein. In this study, however, the addition of plasma to renal cortical slices significantly enhanced accumulation of PAH and simultaneously depressed the transport of 2,4,5-T. This strongly suggests that 2,4,5-T is indeed more tightly bound to plasma proteins than to kidney and thus provides a plausible explanation for the low clearance of 2,4,5-T in the dog (29).

The acute effect of 2,4,5-T on renal function is minimal. Although Courtney et al. (7) reported a high incidence of cystic kidney, enlarged renal pelvis, intestinal hemorrhage, and lethality in rat fetuses maternally treated with up to 46.4 mg/kg of 2,4,5-T, subsequent studies (8) suggested that these nephrotoxic effects were caused by the presence of the impurity 2,3,7,8-TCDD and not by 2,4,5-T.

Hook et al. (28) demonstrated that 2,4,5-T inhibits the transport of an organic anion like PAH but does not influence the transport of an organic cation. The mechanism of this inhibition of the organic anion secretory process by 2,4,5-T is thought to be competitive inhibition. In general, active transport in biological systems may be regarded as substrate penetration through a membrane against an electrochemical gradient caused by a process that requires cellular metabolic energy. Interaction of the transported substrate with a cellular receptor molecule(s) is implicit in the concept of active transport (12). The interaction of substrates with finite numbers of receptor molecules confers characteristics on active transport systems that may be used, in part, to define substrate movement as active. Thus, active transport systems would be expected to exhibit saturation kinetics, substrate specificity, and competition of substrates for transport (11,66). It follows that inhibition of PAH secretion by 2,4,5-T is competition between two similar substrates for the same transport mechanism. Hook et al. (28) used kinetic analysis of *in vitro* transport data to confirm this competitive interaction.

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This explanation of the mechanism of the 2,4,5-T effect on the kidney may be oversimplistic. Koschier and Berndt (42) found that following 2,4,5-T administration, inhibition of 2,4,5-T and 2,4,D uptake was less than inhibition of PAH uptake. They concluded from this difference in susceptibility that 2,4,5-T and 2,4,D are transported by a system separate from the classic organic anion transport system. This, however, does not explain all the data. In these studies, Koschier and Berndt (42) also found that organic cation transport was inhibited by 2,4,5-T. If 2,4,5-T does inhibit both anion and cation transport, we are left with the conclusion that the decreased uptake of PAH in 2,4,5-T treated animals may be a form of toxicity and not simple competitive inhibition.

Stroo et al. (64) considered the possibility that prolonged exposure to low levels of 2,4,5-T in rats would produce a cumulative toxic injury to the nephron or accumulation of the chemical to cytotoxic concentrations. They found that acute administration of 100 mg/kg 2,4,5-T to rats decreased PAH and 2,4,5-T accumulation into renal cortical slices by 58 and 34%, respectively. Daily administration of 100 mg/kg 2,4,5-T for 2 weeks did not, however, enhance the depression in PAH or 2,4,5-T accumulation produced by a single dose. Acute administration of 20 mg/kg 2,4,5-T decreased PAH clearance *in vivo* 24% and PAH accumulation into renal cortical slices 51% when evaluated 4 hr after treatment. An equal dose was without detectable effect on PAH accumulation or clearance when evaluated 24 hr after the last treatment. Thus, only when 2,4,5-T was present in renal tissue (4 hr after administration) was an effect seen. These results demonstrate that chronic administration of low doses of 2,4,5-T is not likely to produce cumulative inhibition of renal organic anion transport and it appears unlikely that chronic low level exposure to 2,4,5-T would lead to progressive or cumulative renal damage as a result of accumulation within renal tissue.

TCDD

TCDD is produced as an unwanted contaminant of crude preparations of 2,4,5-trichlorophenoxyacetic acid and other biphenyl

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compounds. TCDD, with a half-life in soil of about 1 year (32), is a very stable compound. It is also one of the most toxic compounds known: the oral LD_{50} for many animal species is in the microgram range. In animals, death after a single dose of TCDD may be delayed for as long as 40 days and is preceded by progressive debilitation (24,25). Fetal toxicity and malformations, gastrointestinal hemorrhage with necrosis and ulcerations of the stomach mucosa, cerebrovascular hemorrhage, hepatotoxicity, and atrophy of the lymphatic system are toxic effects produced by TCDD (34).

TCDD is an extremely potent inducer of hepatic and renal microsomal drug-metabolizing enzymes (19,26,47). It is 3×10^4 times more potent than 3-methylcholanthrene as an inducer of hepatic aryl hydrocarbon hydroxylase, with the same maximal response (61). In conjunction with enzyme induction, the smooth endoplasmic reticulum (SER) content of hepatic and renal cells is increased by TCDD (19,20). This increase in SER is the predominant morphological change produced by TCDD in the kidney, and is confined to cells of the pars recta of the proximal tubule.

The high toxicity of TCDD and the morphological changes induced in the SER of the proximal tubule cells suggest the possibility that excretory functions of the proximal tubule may also be affected by TCDD. This, however, is not the case. As described above, one of the most sensitive indices of nephrotoxicity is the ability of renal cortical tissue to transport organic compounds actively. Using these parameters, adult rats treated with 10, 25, and 50 $\mu\text{g}/\text{kg}$ TCDD were evaluated (30): 10 $\mu\text{g}/\text{kg}$ TCDD was given and 3 or 7 days after treatment PAH uptake by renal cortical slices was unaffected. *N*-Methylnicotinamide (NMN) accumulation was slightly decreased following treatment. A higher dose of TCDD (25 $\mu\text{g}/\text{kg}$) decreased the capacity of renal tissues to transport both PAH and NMN 7 days after exposure. Glomerular filtration rate and effective renal plasma flow were decreased in rats after 25 or 50 $\mu\text{g}/\text{kg}$ TCDD. Volume expansion did not alter this relationship. Fractional sodium excretion was less than 1% in both control and TCDD-treated animals. Thus, sodium reabsorption, the major work function of the kidney, was not affected by the dioxin (30).

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Although TCDD is an extremely toxic compound and specific ultrastructural and biochemical alterations in renal cortical tissue appear to be produced, the effects of the compound on measurable parameters of kidney function most probably can be attributed to a decline in general health of the animal at the time of experimentation (30). Even though TCDD has marked effects on the cells of the proximal tubule, the treatment regimens used did not produce specific changes in physiological functions of these cells.

Thus, TCDD is capable of causing serious systemic disorders but apparently does not affect renal function to any measurable extent. This does not mean that TCDD is without functionally significant effects on the kidney. Either inappropriate functions were measured or the changes in renal function may have been too subtle to be recognized.

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In an effort to characterize further the effect of TCDD on renal function, the ability of TCDD to induce renal and hepatic enzymes was investigated. TCDD has been found to be an extraordinarily potent inducer of two hepatic enzymes— δ -aminolevulinic acid synthetase, the initial and rate-limiting enzyme in heme synthesis, and aryl hydrocarbon hydroxylase, a cytochrome P-450-mediated microsomal monooxygenase (62). It is also an inducer of other renal mixed-function oxidases (20). The work done with polybrominated biphenyls (PBBs), which are also capable of inducing hepatic and renal mixed-function oxidases (9,51), suggested that a stimulator of renal drug metabolizing capabilities, such as TCDD, might alter the susceptibility of the kidney to toxicity caused by a nephrotoxic agent.

An example of a compound that is metabolically activated to a more toxic moiety by renal and hepatic enzymes systems is chloroform. In animals pretreated with PBBs, renal drug-metabolizing enzymes were stimulated and the nephrotoxicity of chloroform was enhanced (35). It is indeed surprising, therefore, that in animals treated with TCDD, the nephrotoxicity of chloroform was inhibited (Table 1). This inhibition of nephrotoxicity was seen as a reduction of the effect of chloroform on kidney-weight/body-weight ratio, BUN, and organic anion transport. Interestingly, the effect of TCDD

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TABLE 1. *Effect of TCDD and PCBs on chloroform nephrotoxicity in mice*

Pretreatment	Kidney-weight/body-weight $\times 100$	
	Control	CHCl ₃
None	1.49	2.09 ^a
TCDD	1.64	1.56
PCBs	1.51	1.54

^aSignificantly different than control.
From Kluwe et al. (38).

was similar to that of another chlorinated hydrocarbon, the polychlorinated biphenyl (PCB), Aroclor 1254. Both of the halogenated hydrocarbons markedly attenuated the nephrotoxicity of CHCl₃ (Table 1), with an effect similar to that found with 3-methylcholanthrene, a well-recognized enzyme inducer (38).

PBBs

The interaction between PCBs and chloroform (the attenuation of chloroform nephrotoxicity by PCBs) stimulated further investigations into the effect of polyhalogenated biphenyl compounds that are known environmental pollutants. A compound of considerable interest to the state of Michigan is the mixture Firemaster BP-6; a mixture of brominated biphenyls of which the major component is hexabromobiphenyl.

PBBs are fire retardants that were added accidentally to livestock and poultry feed in Michigan (15). Brilliant et al. (6) estimated that by 1976 over 85% of Michigan residents had detectable body burdens of PBBs. Although no human health effects have been attributed definitively to PBBs, a variety of alterations have been produced in other mammalian species. Body weight gain has been retarded and components of the immune and endocrine systems have been modified in several species following exposure to PBBs (13,33). Morphological and functional changes have been detected in livers and kidneys from animals fed PBBs (54,55,63).

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PBBs, administered acutely or subchronically, increase the activity of hepatic drug-metabolizing enzymes in rats (9,10). The stimulation of hepatic microsomal enzymes following PBBs is similar to that observed after treatment with phenobarbital and 3-methylcholanthrene (9), two compounds representing the classic inducers of cytochrome P450 and P₁450, respectively. Treatment with PBBs in adult rats only retarded weight gain after 90 days exposure (52). McCormack et al. (52) tested the effect of PBBs on renal function *in vivo* and found that PBBs had no effect on BUN, the clearance of inulin, PAH, or fractional sodium excretion. The *in vitro* accumulation of PAH and NMN by thin cortical slices as well as ammoniogenesis and gluconeogenesis were also not affected by PBBs. Thus, treatment with PBBs, just as with TCDD and PCBs, resulted in alteration of the activity of renal microsomal enzyme systems but had no detectable effect on renal function.

The most striking effect of PBBs, however, is the marked increase in nephrotoxicity to chloroform (38). Kluwe et al. (38) fed male mice diets that contained 0, 1, 25, or 100 ppm of PBBs for 14 days prior to challenge with a single injection of chloroform. Dietary PBBs potentiated the chloroform-induced depression of PAH accumulation by renal cortical slices and the chloroform-induced rise in BUN concentration in a PBBs intake related manner. The kidney-weight/body-weight ratio was elevated in PBB-treated mice but not in mice consuming the control diet following administration of chloroform. Thus, although doses as high as 100 ppm for 2 weeks in the diet had no effect on BUN or on organic anion transport systems, the effect of chloroform was markedly enhanced. In a similar study, Kluwe et al. (37) found the effect of carbon tetrachloride on organic anion transport in rats to be markedly potentiated by PBBs.

The difference between the effect of PBBs and PCBs on chloroform toxicity is reflected in measurements of the nonprotein sulphydryl concentrations (mainly glutathione) in the liver and the kidney. One of the effects of chloroform is to deplete renal and hepatic cell content of reduced glutathione. This effect on glutathione concentration is altered by pretreatment with PBBs and PCBs. Mice were fed a control diet or a similar diet formulated to contain

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200 ppm PCBs or 100 ppm PBBs for 20 days prior to a single intraperitoneal injection of chloroform. Chloroform decreased the concentration of reduced sulphydryl groups in the kidney and liver of control mice (Table 2). In the liver, the chloroform-induced glutathione depletion was enhanced by both PBBs and PCBs as would be expected if chloroform is metabolized to an electrophilic metabolite by cytochrome P-450-dependent enzymes. In the kidney, PBBs similarly enhanced glutathione depletion produced by chloroform; PCBs protected (Table 2).

The differences between the effects of PBBs and PCBs on chloroform toxicity suggest that these agents may have different effects on the overall metabolism of chloroform. That is, PBBs and PCBs, although they share many physical and chemical characteristics, may have qualitatively different effects on xenobiotic metabolism. PCBs stimulated hepatic xenobiotic metabolism as well as renal metabolism; the serum half-life of chloroform, however, was significantly less in PCB-treated mice than in control mice (Kluwe and Hook, *unpublished observations*). Thus, the protective effects of PCBs on chloroform nephrotoxicity may have been a consequence of increased hepatic chloroform metabolism resulting in decreased delivery of chloroform to the kidney.

The question arises as to the duration of effect of the interaction between the halogenated aromatic hydrocarbons and such nephrotoxic agents as chloroform. These compounds are highly lipophilic, are only slightly metabolized, and thus are retained in the body for extended periods of time (48,49). McCormack et al. (53) demon-

TABLE 2. Effect of PCBs and PBBs on chloroform-induced depletion of reduced non-protein sulphydryl groups (NP-SH)

Pretreatment	Hepatic NP-SH ($\mu\text{g g}$)		Renal NP-SH ($\mu\text{g g}$)	
	Control	CHCl_3	Control	CHCl_3
None	2.010	1.250	880	550
PCBs	1.990	1.020	850	760
PBBs	2.120	400	830	290

From Kluwe and Hook, *unpublished observation*.

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strated the effects of PBBs on renal drug metabolism in the kidneys of rats that had been exposed prenatally and up to 28 days of age through the maternal diet and were then weaned onto a control diet. Marked enhancement of renal enzyme activity produced by PBBs seen at 28 days were still seen 300 days later. Furthermore, induction of drug-metabolizing enzymes in the kidney may be carried through milk to subsequent generations (53). Female rats received 10 or 100 ppm of PBBs in their diet; there was a marked induction of enzyme activity in the kidneys and livers of their pups at 28 days of age. Some of these animals were then allowed to mature and were fed only a control diet. They were then bred and their pups were evaluated at 28 days of age. Even in this next generation, there was still marked enzyme induction in the kidneys. Only in the third generation was the effect eliminated (53). Thus, these compounds have a profound effect for extended periods of time. It is assumed that the enhanced toxicity of chloroform seen in a single generation is correlated with the enzyme activities measured even though those specific activities are not responsible for activation of chloroform. Nevertheless, it is clear that the interaction described may extend far beyond the time of direct exposure and increase the risk of enhanced toxicity even to subsequent generations.

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U.S. ENVIRONMENTAL PROTECTION AGENCY OFFICE OF PESTICIDE PROGRAMS (WH-567) WASHINGTON, D.C. 20460		1. COMPANY/REGISTRATION NO.	2. USE ONLY
LABEL TECHNICAL DATA (See INSTRUCTIONS on back of last part)		464	
F		3. PRODUCT NAME ESTERON 245 BE	
4. APPLICATION SITES (Check all that apply)		5. PEST TYPE (Check all that apply)	7. USER TYPE (Check all that apply)
☐ 01 CROPS (Fruit)	☐ 01 ALGAE	☒ 01 UNSPECIFIED GENERAL USE	
☐ 02 CROPS (Vegetable)	☐ 02 AMPHIBIAN/REPTILE	☐ 02 UNSPECIFIED RESTRICTED USE	
☐ 03 CROPS (Field)	☐ 03 BACTERIA	☐ 03 HOMEOWNER USE	
☐ 04 CROPS (Spice)	☐ 04 BIRDS	☐ 04 JANITORIAL USE	
☐ 05 CROPS (Nut)	☐ 05 FISH	☐ 05 PEST CONTROL OPERATOR USE	
☐ 06 CROPS (Other)	☐ 06 FOULING ORGANISMS	☐ 06 COMMERCIAL APPLICATOR USE	
☐ 10 SOIL TREATMENT (No crop specified)	☐ 07 FUNGI	☐ 07 FARMER USE	
☐ 20 FOREST	☐ 08 INSECTS AND MITES	☐ 08 MEDICAL USE	
☐ 30 ORNAMENTALS	☐ 09 MAMMALS	☐ 09 VETERINARY USE	
☐ 40 TURF	☐ 10 NEMATODES	☐ 10 GOVERNMENT AGENCY USE	
☐ 50 STORED PRODUCTS TREATMENT	☒ 11 PLANTS	☐ 11 MANUFACTURING USE	
☐ 61 ANIMALS (Livestock)	☐ 12 RODENTS	8. FORMULATION (Check one only)	
☐ 62 ANIMALS (Dairy)	☐ 13 SLIME	☐ 01 TECHNICAL CHEMICAL	
☐ 63 ANIMALS (Pet)	☐ 14 SLUGS AND SNAILS	☐ 02 FORMULATION INTERMEDIATE	
☐ 64 ANIMALS (Laboratory)	☐ 15 VIRUS	☐ 03 DUST	
☐ 65 ANIMALS (Other)	☐ 16 OTHER (Specify)	☐ 04 GRANULAR	
☐ 71 OUTDOOR (Nocrop Agricultural)		☐ 05 PELLETTED/TABLETTED	
☐ 72 OUTDOOR (Resident/Commercial)		☐ 06 WETTABLE POWDER	
☒ 73 OUTDOOR (Non agricultural)		☐ 07 WETTABLE POWDER/DUST	
☐ 81 BUILDINGS (Agricultural)	9. MODE OF ACTION (Check all that apply)	☐ 08 CRYSTALLINE	
☐ 82 BUILDINGS (Commercial)	☐ 01 ATTRACTANT	☐ 09 MICROENCAPSULATED	
☐ 83 BUILDINGS (Food Processing)	☐ 02 BIOLOGICAL CONTROL	☐ 10 IMPREGNATED MATERIALS	
☐ 84 BUILDINGS (Medical)	☐ 03 CHEMOSTERILANT	☐ 11 SELF-GENERATING SMOKE	
☐ 85 BUILDINGS (Residential)	☐ 04 DEFOLIANT	☒ 12 EMULSIFIABLE CONCENTRATE	
☐ 91 EQUIPMENT (Commercial)	☐ 05 DESICCANT	☐ 13 INVERT EMULSION	
☐ 92 EQUIPMENT (Food)	☐ 06 FEEDING DEPRESSANT	☐ 14 FLOWABLE CONCENTRATE	
☐ 93 EQUIPMENT (Agricultural)	☐ 07 GROWTH INHIBITOR	☐ 15 SOLUBLE CONCENTRATE	
☐ 94 EQUIPMENT (Medical)	☐ 08 GROWTH REGULATOR	☐ 16 SOLUTION (Ready to Use)	
☐ 95 EQUIPMENT (Transportation)	☐ 09 POISON (Single dose)	☐ 17 OILS (No added pesticide)	
☐ 96 LAUNDRY AND DRY CLEANING	☐ 10 POISON (Multiple Dose)	☐ 18 PRESSURIZED (Gas)	
☐ 97 INDUSTRIAL PRESERVATIVES	☐ 11 PRESERVATIVE	☐ 19 PRESSURIZED (Liquid)	
☐ 98 PESTICIDE (Manufacturing only)	☐ 12 REPELLENT	☐ 20 PRESSURIZED (Dust)	
☐ 99 OTHER (Specify)	☐ 13 OTHER (Specify)	☐ 21 OTHER (Specify)	
REMARKS			

INSTRUCTIONS

GENERAL

This form must be submitted with all applications for new registration or registration renewal.

In addition, it must be submitted with any amendment or resubmission that changes any item previously reported. When submitting this form to report changes in the registration record, all information on the form should be completed rather than just the changes.

SPECIFIC

ITEM

1. Company/registration number. Put the registration number if registered, or the file symbol or company number if not registered.
2. For EPA use only.
3. Product name. Enter the product name exactly as stated on the basic application.
4. Application sites. Check all the sites that apply to the uses claimed on your label.
5. Pest type. Check all the pest types that are claimed on your label. For pesticide manufacturing use labels, leave blank.
6. Mode of action. Select the terms that apply to how the proposed product works.
7. User type. Check all user types to whom the marketing of this product will be directed.
8. Formulation. Check the single box that describes the product as packaged.

DOU 21158741A

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DOW CHEMICAL U.S.A.

9001 Building
February 21, 1983

MIDLAND, MICHIGAN 48640

M N 300822

Ralph Gantz
9008 Building

cc: Art Schober, 9008 Building
John Tichon, 489 Building

RE: USE DIRECTIONS FOR ESTERON* 245 BE (EARLY 1982 PRODUCTION)

Ralph:

As requested in your letter of February 16, 1983, here is a reasonable set of directions for one possible approach to using up ESTERON 245 BE that has turned into "oatmeal" upon storage. I leave the final wording to your discretion and Art's inputs.

"Bring the one-gallon can of ESTERON 245 BE to room temperature, at least 65°F. Shake the can well and pour the entire contents into a clean, dry five gallon can or other appropriate container.

Fill the ESTERON 245 BE can with one gallon of ESTERON 99 C and shake well. Pour the ESTERON 99 C liquid into the five-gallon can containing the ESTERON 245 BE.

Vigorously shake or stir the ESTERON 245 BE + ESTERON 99 C mixture until the solids have dissolved. The solids should dissolve in less than 10 minutes at 65°F.

Use the mixture as you would any emulsifiable concentrate formulation: (1) fill the tank half full with water (2) then add the required amount of herbicide mixture, with agitation (3) finally, add the rest of the water. Note: the mixture of ESTERON 245 BE and ESTERON 99 C in water forms an emulsion which tends to separate unless the mixture is kept agitated."

Ralph, I feel that the key to any instructions passed along to the sales offices is to stress that absolutely no ESTERON 245 BE is to be returned to Midland. This includes cans and drums and smaller samples. The salesman should make every reasonable accomodation to keep material from coming back to Midland.

Jim Havel
Application Systems
Agricultural Products Department

ls

*Trademark of the Dow Chemical Company
AN OPERATING UNIT OF THE DOW CHEMICAL COMPANY

DOW 2113072

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D-4770

0008295



DOW CHEMICAL U.S.A.

9008 Building
February 16, 1983

MIDLAND, MICHIGAN 48640

MN500822

J. J. Havel
9001 Building

cc: S. J. Howell, 9008
E. R. Laning, Jr., 9008
A. E. Schober, 9008
J. M. Tichon, 489
G. L. Ytzen, 9008

FIELD SOLUTION TO PRESCRIBE FOR ESTERON 245 BE HERBICIDE QUALITY COMPLAINTS

Jerry Ytzen suggests there is likely to be some ESTERON 245BE on dealer shelves somewhere that was not found in the 1982 product return campaign. In the user's hands, this material will likely generate complaints. Jerry suggests we may want to have a field mixing solution prescription ready that the Dow field sellers can use as a basis for servicing such inquiries.

Please prepare a brief written summary that identifies the procedure to follow if the user is willing to correct his problem by blending 1 gallon ESTERON 99 Concentrate with 1 gallon ESTERON 245BE herbicide. The user may then end up using the "2+2" blend as though it were straight 2,4,5-T product, but he would use twice the recommended volume rates shown on the ESTERON 245BE label.

The chance that the user may have some ESTERON 99 Concentrate on hand that is PGBE rather than butoxyethyl ester is possible, but considered remote. We will not concern ourselves with that aspect at this time.

I understand that the procedure you prepare will only be circulated through DSMs to field sellers. The field seller would have it as a reference to verbally handle phone calls, but not pass it on to distribution or end use customers as printed Dow information.

Your early attention to this request is appreciated.

R. L. Gantz
Ag Products Department

lmd

DOW 2113073

4675

D-4771



AGRICULTURAL PRODUCTS DEPT.
9008 Building

11-5-81

Jean Townsend
Quality Assurance
2030 Dow Center

Plan: Attached is proposed MSDS
for new MSDS similar to existing
EST. 245 (but changed AI from
PGBE ester to Butoxyethyl ester). It
will have a new product code.
We will continue to have old
EST. 245 in commerce until
inventories gone. Ple review
& initiate for approval. Hope to
later have missing info later if needed.
FR [redacted] gone for reciting
6-0256 chemicals should review
Not sure all this still appropriate. - RLB

MINIATURE

DOW 2113074

4676

D-4772

0008297

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260

4677

D-4773

CUSTOMER REQUEST

MIDLAND ANALYTICAL LABORATORIES

DO NOT CONFIDENTIAL INFORMATION

(1) AL NUMBER 83-00196
(2) DATE FINISHED 4-22-83
RETURN SAMPLE ☒ DISCARD SAMPLE ☒

LAB. NO. PROBLEM NUMBER ACCT. NO. SUB. FUNCT. DATE RECEIVED DATE NEEDED

REQUEST DETERMINATION OF:

FOR PROJECT:

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

SAMPLE IDENTIFICATION NUMBER:

SAMPLE OF (CHEMICAL NAME, BULK COMPOSITION):

NONE DATED 4-14-83

ESTERON 2,4,5

SAFETY HAZARDS

☒ SKIN IRRITANT

☐ LOW FLASH PT.

☐ RADIOACTIVE

☐ OTHER (

☐ NON-HAZARDOUS

☐ LACHRYMATOR

☐ TOXIC

☐ EXPLOSIVE

☐ UNKNOWN

REPORTS TO:

NAME

J. Tichen

DEPARTMENT

BLDG.

DOOR

PHONE

M. Seidel

489

834

RELATIVE ERROR SUFFICIENT:

%

APPROXIMATE LEVEL:

LOWER LIMIT:

REASON FOR REQUEST:

QUALITY CONTROL

(3) REPORT TITLE:

THE DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN ESTERON 245 BRUSH AND WEED KILLER.

(4) ADDITIONAL CHEMICAL, COMMON, OR TRADE NAMES TO INDEX:

(5) AUTHOR

D. GIRARDIN

(6) TECHNIQUE CODE NUMBER(S):

14

(7) CROSS REFERENCES:

DR-0065-0986

METHOD NO.

ML-AL 87-563 AND ML-AM-82-45

DATA BOOK REFERENCE

AL 1998 p 76

RESULTS:

2,3,7,8-TCDD = 0.018 ppm

D-4274

4678

ANALYST SIGNATURE

D. Girardin

PHONE

372-574

BUILDING

HOURS

RESULTS

PHONED

✓

REVIEWED BY:

J. Seidel



DOW CHEMICAL U.S.A.

May 19, 1982

MIDLAND, MICHIGAN 48640

MN067843

Dave Buzzelli
834 Bldg.

cc: ~~S. R. Gledhill - 474 Bldg.~~
E. R. Laning - 9008 Bldg.
C. H. Goodman - 2030 Bldg.

R. E. Woodward - 834 Bldg.
E. S. Conyers - 8008 Bldg.

ESTERON 2,4,5-BE, TCDD ANALYSIS

The attached letter from EPA is self-explanatory. Do we have the analytical data they want? If so, would you please send me 3 copies so I can send it to EPA.

If we have disposed of the drummed material by selling it as technical material under EPA Reg. No. 464-518 let me know so I can advise EPA. If we are going to use it in our formulation, we need to advise EPA of the TCDD content.

Thanks for your assistance.

Arthur E. Schober
Product Registration Manager
Agricultural Products Department

nbe
Attach:

DOW 2113482

D-4775



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

RECEIVED
MAY 17 1982
REGISTRATION

MAY 10 1982

Mr. Arthur E. Schober
Dow Chemical Company
P.O. Box 1706
Midland, MI 48640

Dear Mr. Schober:

Subject: TCDD Analyses
Esteron 245 EE
EPA Registration Number 464-574
Followup to our Registration Notice of March 29, 1982

We have completed review of your additional data on this subject submitted March 24, 1982. You are reporting TCDD concentrations within the maximum (0.008 ppm) stipulated in your formula statement of March 18. Since you have described this method as unvalidated, however, submit a method description and recovery data (including chromatograms) of TCDD-spiked samples. We note that you also plan to submit further analyses of the 16,500 lb of material packaged in 55-gallon drums as they become available. We appreciate your cooperation in this matter.

Sincerely Yours,

Richard F. Mountfort
Richard F. Mountfort
Product Manager (23)
Fungicide-Herbicide Branch
Registration Division (TS-767)

DOM 2113483

D-4776

4680008302

DISTRIBUTION EMERGENCY

August 5, 1981
10:28 A.M.

W. Heitzig, Manager
Security
1100 Building

ESTERON 245 - ROBINSON, ILLINOIS

Mr. M. Hayes, of Robinson, Illinois telephone 618-544-8502, called and related the following: Approximately two weeks ago he sprayed the fence line of his pasture with Esteron 245 plus a little Roundup he had left and wanted to dispose of. Mr. Hayes wanted to know how long does he have to wait before he can put his cattle back in to graze, and also what will happen if the cattle eat any of the grass killed by the Esteron 245? I informed Mr. Hayes a technical person would be notified to return his call. Mr. Hayes requested the call back to be around 12:30 P.M., their time, as he would be in for lunch.

Mr. L. Downs, of Ag Research, was called and informed. Mr. Downs stated he would call Mr. Hayes at 1:30 P.M. our time.

J. M. Weed, Dispatcher
Security
1100 Building
jrd

DISTRIBUTION: B. Caldwell, B. Lee, R. Zweigle, G. Shafer, Public Relations,
Industrial Hygiene, L. Downs.

DOW 2113531

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DOW KNOWLEDGEABLE CONTACT: Follow up letter describing incident cause, corrective action and material disposition must be reported within 48 hours.

Copies: 1. Persons contacted above
2. Plant Protection, 1100 Building
3. Office Emergency Response, 690 Building

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CARCINOGENICITY AND TOXICITY OF 2,4-DICHLOROPHENOXY- ACETIC ACID

MELVIN DWAIN REUBER

11014 Swansfield Road, Columbia, MD 21044 (U.S.A.)

(Received February 9th, 1983; accepted May 4th, 1983)

DOW CHEMICAL

JAN 18 1984

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ABSTRACT

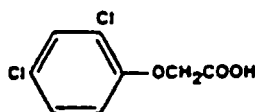
2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

INTRODUCTION

The auxinlike, plant growth-promoting chemical, 2,4-dichlorophenoxyacetic acid (2,4-D), has been used for many years as a plant herbicide [1]. The acid, its salts, and its esters are used to kill dicotyledonous weeds and certain other plant species. The chemical is readily absorbed from the digestive tract of animals and excreted by the kidney. Acute and subacute toxicity tests have shown that 2,4-D can cause poisoning in animals.

2,4-D is widely used as a herbicide to control certain types of vegetation. It is sprayed from airplanes on many millions of acres to kill shrub and broad-leaved plant life on specialized farms and range and pasture lands used for grazing. 2,4-D is used to kill vegetation adjacent to railroads, highways and other roads, pipe lines, power lines, and in and around lakes, ponds and irrigation ditches. It is used in aerial spraying operations of forest lands to kill broad-leaved vegetation and encourage soft wooded trees such as spruce and pine. It is present in preparations sold at retail for home gardeners and is widely used on home lawns and grass covered areas used for recreational purposes. 2,4-D is also used on cereal crops such as rice, on sugar cane and to control ripening of bananas and citrus fruits, to delay preharvest dropping of some fruits, and for the control of rot when lemons are stored. 2,4-D



2, 4-D

was extensively sprayed together with 2,4,5-trichlorophenoxyacetic acid on millions of acres as a defoliant in Vietnam.

With the widespread use of this herbicide, possible toxic effects are important. 2,4-D finds its way to humans through direct contact either occupationally or by spraying humans purposely or accidentally. Drift after spraying can contaminate gardens, plant nurseries, water and other areas. Residues may inadvertently be encountered in food, vegetables, meat and water.

The production and use of 2,4-D can only be estimated because the data often is considered as trade secret by the manufacturers. The latest data available are for the year 1975. An estimated 27 million kg were used in the U.S.A. The Federal Republic of Germany and the U.K. are the major producing countries of western Europe where annual production was estimated to be 3-30 million kg. In eastern Europe it was estimated to be less than 10 million kg. In Japan production amounted to 511 thousand kg.

This review includes, to the best of our knowledge, every study on the carcinogenicity of 2,4-D, or its esters, and a metabolite, 2,4-dichlorophenol in animals. The raw data and histological sections were reviewed for the FDA 2,4-D Rat Study and FDA 2,4-D Dog Study [1-3] and the results are based on this examination. The Innes et al. Mouse Studies for 2,4-D, or its esters [4], the Archipov and Kozlova 2,4-D Mouse and Rat Studies [5], and the Boutwell and Bosch 2,4-Dichlorophenol Mouse Study [6] are also included.

Statistical tests of significance were *p* (probability) values obtained with Fisher's exact test and tests for positive linear trend and departure from linear trend.

FDA 2,4-D RAT STUDY

Osborne-Mendel rats, three weeks of age, ingested either 0, 5, 25, 125, 625, or 1250 ppm of 2,4-D in the diet for 104 weeks [1, 3]: There were 25 males and 25 females at each dose. The rats were individually caged and were weighed weekly. Hematologic studies were done periodically.

Survivors were killed after 104 weeks, and organ weights were taken for heart, liver, spleen, kidneys, and testes. Organs were also weighed from moribund rats killed after 52 weeks; however, no organ weights were included from rats that died.

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TABLE 1
NUMBER OF MALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS
(FDA RAT STUDY)

Dose (ppm)	Carcinomas	p	Sarcomas	p	Both ^a	p
0	0/25 (0%)		1/25 (4%)		1/25 (4%)	
5	2/25 (8%)		2/25 (8%)		3/25 (12%)	
25	6/25 (24%)	0.011	4/25 (16%)		9/25 (36%)	0.0053
125	6/25 (24%)	0.011	6/25 (24%)	0.049	9/25 (36%)	0.0053
625	5/24 (21%)	0.022	4/24 (17%)		6/24 (25%)	0.043
1250	3/23 (13%)		7/23 (30%)	0.018	9/23 (39%)	0.0033
5-1250	22/122 (18%)	0.012	23/122 (19%)	0.052	36/122 (30%)	0.0038
				0.021 ^b		0.039 ^b
		0.057 ^c				0.034 ^c

^a Some rats had both carcinoma and sarcoma and are counted only once.

^b Test for positive trend.

^c Departure from linear trend.

Detailed histologic examination was made of tissues from six male rats and six female rats from the 1250 ppm 2,4-D groups and the control groups. Liver, kidney, spleen, and ovary or testes, as well as tumors and other gross lesions, were sectioned histologically from the rats on all other doses of 2,4-D.

At the end of 104 weeks the following rats were alive: 12 males, 6 females (0 ppm); 8 males, 4 females (5 ppm); 9 males, 9 females (25 ppm); 9 males, 8 females (125 ppm); 10 males, 9 females (625 ppm); and 5 males, 11 females (1250 ppm).

Body weights and the organ-body weight ratios for liver, kidney, heart and testes were reported as showing no differences among the various groups. These results have little meaning, however, since the rats that died with the most chance of being abnormal were not included. The spleen-body weight ratio was slightly elevated in rats given 625 ppm or 125 ppm 2,4-D ($p < 0.05$).

Terminally, the red blood cells of rats given 5 ppm, 625 ppm, or 1250 ppm 2,4-D showed macrocytosis, slight polychromasia, and slight to moderate hypochromasia. These changes were not present, or were of a minor degree in control rats.

Malignant neoplasms at all sites in male rats

There were increased incidences of malignant neoplasms in male rats given all doses of 2,4-D (Table 1). Nine of 25 rats (36%) given 25 or 125 ppm ($p = 0.0053$) and 9 of 23 (39%) given 1250 ppm of 2,4-D ($p = 0.0033$) developed carcinomas and sarcomas. The incidences of these neoplasms were dose related ($p = 0.039$). For some unexplained reason, the rats receiving 625 ppm, 6 of 24 (25%), had fewer malignant neoplasms than male

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TABLE 2

NUMBER OF MALE RATS INGESTING 2,4-D WITH LYMPHOSARCOMAS AND NEUROSARCOMAS (FDA RAT STUDY)

Dose (ppm)	Lymphosarcoma	<i>p</i>	Subcutaneous neurosarcomas
0	0/25 (0%)		0/25 (0%)
5	2/25 (8%)		0/25 (0%)
25	4/25 (16%)	0.055	0/25 (0%)
125	5/25 (20%)	0.025	1/25 (4%)
625	3/24 (13%)		1/24 (4%)
1250	6/23 (26%)	0.0082	1/23 (4%)
5-1250	20/122 (16%)	0.018	3/122 (2%)
		0.034 ^a	

^a Test for positive trend.

rats in other groups ($p = 0.043$). Thirty-six of 122 (30%) 2,4-D-treated male rats had carcinomas and sarcomas ($p = 0.0038$).

Sarcomas in male rats

The sarcomas in 2,4-D-treated male rats were mainly lymphosarcomas, however, there were a small number of subcutaneous neurosarcomas (Table 2). Sarcomas were present in 4 of 25 rats (16%) ingesting 25 ppm, 6 of 25 rats (24%) ingesting 125 ppm ($p = 0.049$), and 7 of 23 (30%) receiving 1,250 ppm of 2,4-D ($p = 0.018$) (Table 1). Twenty-three of 122 (19%) male rats given 2,4-D had sarcomas ($p = 0.052$).

Carcinomas in male rats

Carcinomas in male rats treated with 2,4-D were seen in the endocrine system. Six of 25 male rats (24%) ingesting 25 or 125 ppm ($p = 0.011$) and 5 of 24 rats (21%) given 625 ppm ($p = 0.022$) of 2,4-D developed carcinomas (Table 1). Twenty-two of 122 (18%) male rats given 2,4-D had carcinomas ($p = 0.012$).

Malignant neoplasms at all sites in female rats

Eight of 20 (40%) female rats given 5 ppm ($p = 0.019$), 11 of 22 (50%) given 25 ppm ($p = 0.058$), 13 of 23 (57%) on 125 ppm ($p = 0.022$), 18 of 24 (75%) receiving 625 ppm ($p = 0.00047$), and 17 of 25 (68%) female rats ingesting 1,250 ppm ($p = 0.0022$) of 2,4-D developed malignant neoplasms at all sites (Table 3). Altogether, malignant neoplasms were observed in 67 of 114 female rats (59%) ingesting 2,4-D ($p = 0.0018$).

Lesions of the lymphoreticular system in female rats

The highest incidence of lymphosarcomas was 12 of 24 (50%) female rats given 625 ppm of 2,4-D ($p = 0.00007$) (Table 4). Female rats in the other

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TABLE 3
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH CARCINOMAS AND SARCOMAS (FDA RAT STUDY)

Dose (ppm)	Carcinomas	<i>p</i>	Sarcomas	<i>p</i>	Both ^a	<i>p</i>
0	4/22 (18%)		1/22 (5%)		5/22 (23%)	
5	8/20 (40%)		5/20 (25%)		8/20 (40%)	
25	7/22 (32%)		8/22 (36%)	0.011	11/22 (50%)	0.058
125	10/23 (43%)	0.065	8/23 (35%)	0.013	13/23 (57%)	0.022
625	14/24 (58%)	0.0059	12/24 (50%)	0.00007	18/24 (75%)	0.00047
1250	13/25 (52%)	0.017	10/25 (40%)	0.0044	17/25 (68%)	0.0022
5-1250	52/114 (46%)	0.013	43/114 (36%)	0.0017	67/114 (59%)	0.0018
		0.017 ^b		0.024 ^b		0.0022 ^b
				0.089 ^c		0.090 ^c

^a Some rats had both carcinomas and sarcomas and are counted only once.

^b Test for positive trend.

^c Departure from linear trend.

2,4-D treatment groups had 24-27% lymphosarcomas ($p = 0.011-0.018$). Thirty-one percent of all females ($p = 0.00073$) developed lymphosarcomas. The findings are even more significant if hyperplasia of the lymphoreticular system is considered along with lymphosarcomas, i.e., 39% ($p = 0.00005$) of female rats ingesting 2,4-D. Female rats also had sarcomas of the uterus.

Carcinomas at all sites in female rats

Carcinomas at all sites were seen in 43% (10 of 23) ($p = 0.065$) of female rats on 125 ppm, 58% (14 of 24) ($p = 0.0059$) given 625 ppm, 52% (13 of 25) ($p = 0.017$) receiving 1250 ppm and 46% (52/114) ($p = 0.013$) of female rats given 2,4-D (Table 3). Carcinomas were found in the reproductive system, particularly the mammary gland, and occasionally in endocrine organs.

Neoplasms of the mammary gland in female rats

The incidence of mammary gland tumors was higher in treated female rats, particularly on gross examination, which is generally quite reliable. The number of rats with such tumors was less in all but one group after histologic examination (Table 5).

On gross examination, 18 of 21 female rats (86%) ingesting 25 ppm ($p = 0.0064$), 20 of 24 rats (83%) ingesting 625 ppm ($p = 0.0080$) and 16 of 23 rats (70%) given 1250 ppm of 2,4-D had neoplasms of the mammary gland, compared to 10 of 22 (45%) control female rats. The findings are quite different for the numbers of rats with histological sections examined by pathologists at FDA and sections available for this examination.

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TABLE 4
NUMBER OF FEMALE RATS INGESTING 2,4-D WITH LESIONS OF THE LYMPHORETICULAR SYSTEM (FDA RAT STUDY)

Dose (ppm)	Hyperplasia	<i>p</i>	Lymphosarcoma	<i>p</i>	Hyperplasia and lymphosarcoma	<i>p</i>
0	0/22 (0%)		0/22 (0%)		0/22 (0%)	
5	0/22 (0%)		5/20 (25%)	0.018	5/20 (25%)	0.018
25	2/22 (9%)		6/22 (27%)	0.011	8/22 (36%)	0.0018
125	2/23 (9%)		6/23 (26%)	0.012	8/23 (35%)	0.0023
625	4/24 (17%)	0.065	12/24 (50%)	0.00007	16/24 (67%)	< 0.00001
1250	2/25 (8%)		6/25 (24%)	0.016	8/25 (32%)	0.0034
5-1250	10/114 (9%)		35/114 (31%)	0.00073	45/114 (39%)	0.00005
						0.039 ^a
						0.0004 ^b

^a Test for positive trend.

^b Departure from linear trend.

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TABLE 5

NUMBER OF FEMALE RATS INGESTING 2,4-D WITH NEOPLASMS OF THE MAMMARY GLAND AND HISTOLOGIC EXAMINATION^a (FDA RAT STUDY)

Dose (ppm)	Gross ^b	<i>p</i>	Histologic ^c	<i>p</i>	Histologic ^d	<i>p</i>
0	10/22 (45%)		10/22 (45%)		8/22 (36%)	
5	8/20 (40%)		7/20 (35%)		7/20 (35%)	
25	18/21 (86%)	0.0064	11/21 (52%)		11/21 (50%)	
125	8/23 (35%)		10/23 (43%)		9/23 (39%)	
625	20/24 (83%)	0.0080	14/24 (58%)		15/24 (63%)	0.070
1250	16/23 (70%)	0.091	15/23 (65%)		11/23 (45%)	
5-1250	70/111 (63%)		57/111 (52%)		53/111 (47%)	
		0.0233 ^e		0.0334 ^e		
		0.0008 ^f				

^a Corrected for survival time, i.e., time of appearance of first neoplasms of the mammary gland.^b Gross necropsy at FDA.^c Histological examination by FDA.^d Histological examination by Reuber.^e Test for positive trend.^f Departure from trend.

Comments

It is worth noting that histological examination of tissues from this study was inadequate. Microscopic neoplasms would have been overlooked at the 5 to 625 ppm doses of 2,4-D (only gross neoplasms were sectioned histologically) and also even at the highest dose because only six rats of each sex were examined in detail. Also histological sections were not available for 19 mammary gland neoplasms described at the time of necropsy, and no explanation was given for this discrepancy.

Despite the shortcomings of this study, it must be considered as an acceptable study.

The largest numbers of rats alive were in the 25 to 1250 ppm doses; and the smallest number of rats alive was in the lowest dose, 5 ppm. These results suggest that rats in at least some of the 25 to 1250 ppm groups were not ingesting their diets because of toxicity. The differences in the incidences of neoplasms, therefore, would not be great.

Tumors were analyzed, on the basis of 25 rats per group, and the following conclusions were made by the authors: "There is a statistically significant ($p < 0.05$) linear relationship between the proportion of female rats with tumors and the level of the log dose, but not the arithmetic dose, indicating that there is a tendency for the proportion of female rats with tumors to increase with the log dosage. There is also a statistically significant ($p < 0.05$) linear relationship between the proportion of male rats with malignant tumors and the level of arithmetic and log dose. A comparison was made of the control group with each treatment group. Statistically significant ($p < 0.05$) differences were found only between the control group and the 1250 ppm dose level with respect to male rats with malignant tumors."

The authors also believed that the raw data and the pathologic interpretation did not support the statistical analyses and that "a carcinogenic effect of 2,4-D has not been shown". They also stated that "additional support for this interpretation has been given by the long-term study in mice".

Summary

Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats.

Conclusions

2,4-D is carcinogenic for male and female rats.

FDA 2,4-D DOG STUDY

Beagle dogs, 6 to 8 months old (3 males and 3 females per group) ingested 0, 10, 50, 100, or 500 ppm 2,4-D in the diet for 104 weeks [1, 2]. Organ weights were taken for brain, heart, liver, kidneys, spleen, thyroid, adrenals, and testes. Tissues from all dogs were studied grossly and microscopically.

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Many of the dogs ingesting 2,4-D lost weight. There were scattered lesions such as atrophy of the testes and prostate, interstitial nephritis, hemangioma of the adrenal, atrophic or cystic pituitary, atrophy of the thyroid, and hypoplasia of the bone marrow. Most of the lesions were seen in the endocrine organs. Control dogs generally did not have lesions.

Comments

Previous experience at FDA has shown that long-term chronic dog studies should be carried out for six years or longer in order for neoplasms to develop. Since they occurred predominantly in 2,4-D treated dogs, lesions may have progressed to neoplasms had the dogs been treated for a longer period of time.

Summary

A two-year feeding study in dogs cannot be considered a carcinogenicity study.

INNES et al. 2,4-D, OR ITS ESTERS, ORAL MOUSE STUDIES

1. 2,4-D

The maximum tolerated dose of 2,4-D was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)_{F1}, designated as "strain A" and (C57BL/6 x AKR)_{F1}, designated as "strain B" mice [4, 7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. 46.4 mg/kg was given in 0.5% gelatin daily by stomach tube beginning at 7 days of age. After the mice were weaned at 28 days of age, 149 ppm of 2,4-D was mixed directly in the diet and provided ad libitum. "Strain B" male and female mice were also given 100 mg/kg, followed by 323 ppm. Treatment was continued approximately 18 months.

Postmortem included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. Thyroid glands were not examined.

There was no increase in neoplasms in the 2,4-D-treated mice.

2. 2,4-D isopropyl ester

"Strain A" and "strain B" male and female mice were given 46.6 mg/kg of 2,4-dichlorophenoxyacetic acid, isopropyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 111 ppm in the diet for approximately 18 months.

There were neoplasms in the lungs of 4 of 18 "strain A" male mice (22%) ingesting 2,4-D isopropyl ester compared to 2 of 17 matched controls (12%) and 5 of 79 pooled male control mice (6%) ($p = 0.0584$) (Table 6).

In this study, "strain A" male mice receiving 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung.

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TABLE 6

NUMBER OF MALE AND FEMALE MICE INGESTING 2,4-D ISOPROPYL ESTER WITH NEOPLASMS OF THE LUNG (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female
"A" Matched	0	2/17 (12%)	0.0584	1/18 (6%)
"A" Pooled	0	5/79 (6%)		3/87 (3%)
"A"	111	4/18 (22%)		1/18 (6%)
"B" Matched	0	2/18 (11%)		0/17 (0%)
"B" Pooled	0	9/90 (10%)		3/82 (4%)
"B"	111	2/18 (11%)		0/17 (0%)

3. 2,4-D butyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, butyl ester in 0.5% gelatin daily by stomach tube. At 28 days of age the mice received 149 ppm in the diet for approximately 18 months.

Three of 18 "strain A" female mice (17%) and 4 of 87 pooled controls (5%) had reticulum cell sarcomas ($p = 0.0955$) (Table 7).

In this study, there was an increased incidence of reticulum cell sarcoma in 2,4-D butyl ester-treated "strain A" female mice.

4. 2,4-D isooctyl ester

"Strain A" and "strain B" male and female mice received 46.4 mg/kg of 2,4-dichlorophenoxyacetic acid, isooctyl ester in 0.5% gelatin by stomach tube. After 28 days they ingested 130 ppm in the diet for approximately 18 months.

Neoplasms in treated mice were found mainly in the liver and lung. One "strain A" treated male mouse had an "angioma" of the liver and another an "angioma" of the spleen.

In this study, neoplasms of the liver were slightly increased in 2,4-D

TABLE 7

NUMBERS OF MALE AND FEMALE MICE INGESTING 2,4-D BUTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Strain	Dose (ppm)	Male	p	Female	p
"A" Matched	0	1/17 (6%)	0.0714	2/18 (11%)	0.0955
"A" Pooled	0	5/79 (6%)		4/87 (5%)	
"A"	149	0/18 (0%)		3/18 (17%)	
"B" Matched	0	1/18 (6%)		2/17 (12%)	
"B" Pooled	0	1/90 (1%)		3/82 (4%)	
"B"	149	2/18 (11%)		0/16 (0%)	

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isooctyl ester-treated "strain A" male mice and tumors of the lung in "strain A" female mice. Two male mice had rare tumors; "angiomas" of the liver and spleen.

INNES et al. 2,4-D, OR ITS ESTERS, SUBCUTANEOUS MOUSE STUDY

A single subcutaneous injection of 2,4-D, or its esters, was given in the nape of the neck to two hybrid strains of mice, (C57BL/6 x C3H/Anf)_F₁ designated as "strain A" and (C57BL/6 x AKR)_F₁ designated as "strain B" at approximately the 28th day of age [7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. They were killed after approximately 18 months.

Mice received the following doses: 215 mg/kg or 464 mg/kg of 2,4-D in DMSO; 100 mg/kg of 2,4-D isopropyl ester, 25.5 mg/kg of 2,4-D butyl ester, or 21.5 mg/kg of 2,4-D isooctyl ester in corn oil.

Necropsy included thorough external examination and internal examination of the neck glands and the thoracic and abdominal cavities. Histological examination was done on all major organs and of all grossly visible lesions.

Reticulum cell sarcomas in "strain B" female mice

Five of 17 "strain B" female mice (29%) given 2,4-D isooctyl ester had reticulum cell sarcomas, compared to 5 of 157 control female mice (3%) ($p = 0.0009$) (Table 8).

Summary

2,4-D isooctyl ester was carcinogenic for the lymphoreticular system (reticulum cell sarcomas) in "strain B" female mice.

ARCHIPOV AND KOZLOVA MOUSE AND RAT STUDIES

Three groups, 100 each, of CBA x C57/BL hybrid mice were used [5]. Two groups of mice received one drop of a 0.5% solution of 3-methylcholanthrene

TABLE 8

NUMBER OF "STRAIN B" MALE AND FEMALE MICE GIVEN A SINGLE SUBCUTANEOUS INJECTION OF 2,4-D ISOCTYL ESTER WITH RETICULUM CELL SARCOMAS (INNES et al. [4, 7], MOUSE STUDY)

Dose (mg/kg)	Males	Females	<i>p</i>
0	0/161 (0%)	5/157 (3%)	
1	0/18 (0%)	5/17 (29%)	0.0009

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in benzene on the skin for 3 weeks. Later the skin of one group of mice was painted with a 10% solution of the amine salt of 2,4-D in acetone. The skin of the third group of mice was painted with a 10% solution of the herbicide. Mice were observed for 20 months.

Neoplasms of the skin

Papillomas developed on the skin of 17.7% of the mice treated with 3-methylcholanthrene followed by 2,4-D, and none for the mice receiving 3-methylcholanthrene only or 2,4-D only.

Comments

Groups of rats also ingested 1/10th the LD₅₀ of the amine salt of 2,4-D [5]. The dose was not given.

Summary

2,4-D is a promoter of neoplasms of the skin in mice.

BOUTWELL AND BOSCH 2,4-DICHLOROPHENOL MOUSE SKIN STUDY

Female Sutter mice, 2-3 months of age, were used [6]. Mice were painted on the skin with a single initiating dose of 0.3% dimethylbenzanthracene in acetone followed by 20% 2,4-dichlorophenol in benzene, benzene only, or no treatment. 2,4-Dichlorophenol was applied twice weekly. One group of mice was observed for 15 weeks and a second for 24 weeks.

Neoplasms of the skin

Mice treated with both chemicals developed an increased incidence of neoplasms of the skin. Thirteen of 27 treated mice (48%) ($p = 0.00607$) had papillomas and 3 of 27 treated mice (11%) had carcinomas of the skin, compared to 1 of 15 untreated mice (7%) with papillomas and 0 of 15 untreated mice (0%) after 15 weeks. Papillomas of the skin were observed in 12 of 16 treated mice (75%) ($p = 0.00004$) and carcinomas of the skin in 10 of 16 treated mice (62%) ($p < 0.00001$); whereas, papillomas were seen in 3 of 27 mice (11%) and carcinomas in 0 of 27 mice (0%) receiving benzene only after 24 weeks.

Conclusion

2,4-Dichlorophenol promoted the incidence of neoplasms of the skin in mice when administered after a single initiating dose of dimethylbenzanthracene.

DISCUSSION

2,4-D was found to be carcinogenic for rats in the original publication by

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Hansen et al. [1]. It was also concluded that "2,4-dichlorophenoxyacetic acid is carcinogenic in rats" in a preliminary review of the raw data, but not the histological sections, requested by Senator Edward M. Kennedy's Subcommittee on Administrative Practice and Procedure of the Committee on the Judiciary, United States Senate [8]. The examination and diagnoses of the histological sections, which was done for this review further strengthened the findings and conclusion that 2,4-D is carcinogenic in rats.

Experience has shown that it is necessary to review the raw data and histological sections of old chronic toxicity and carcinogenicity studies in animals [9, 10, 11]. The data from studies often is not adequately analyzed and the conclusions are suspect. It also is not unusual for results of such studies, which are statistically significant, to be ignored because they are described as not biologically significant. The pathology report for the FDA 2,4-D study in rats was completed in 1964; however, the results were not published until 1971 [1, 3].

2,4-D is mutagenic and teratogenic as well as carcinogenic [12]. 2,4-D and its derivatives are teratogenic in mice, rats and hamsters. 2,4-D induces skeletal malformations, cleft palats, eye malformations, subcutaneous edema and hemorrhages in the snout, abdominal cavity, liver and soft tissues in rats when administered during early pregnancy [7, 13-17]. 2,4-D is embryotoxic [18]. 2,4-D penetrates the placenta and the fetuses [19]. 2,4-D caused point mutations in animal cells without liver activation, damages DNA in a manner similar to ionizing radiation and stimulates mitosis [20-22]. Endothel-treated fruit flies developed an increase of recessive lethal mutations [23].

2,4-Dichlorophenol is a major product of the breakdown of 2,4-D by microorganisms [24]. The compound is also teratogenic in rats and carcinogenic in mice. [6, 14].

2,4-D is rapidly absorbed from the human gastrointestinal tract and distributed widely throughout the body in animals and in human beings [25-28]. It is fat soluble and rapidly absorbed through the skin and lungs [29]. Acute effects of 2,4-D poisoning in humans include headache, weakness, dizziness, nausea and vomiting, sore throat, irritation of nasal mucosa, impaired sense of taste and smell, substernal chest pain and loss of consciousness [30]. Peripheral neuropathy, which begins with tingling and numbness in the extremities, has been reported in humans hours or days after exposure to 2,4-D on the skin. The symptoms in some people increased through several weeks until pain, paresthesia, and paralysis were severe. Disability was protracted and recovery was incomplete even after a lapse of years [31, 32]. These symptoms resemble those of multiple sclerosis, and this has been confirmed by the finding of plaques of acute demyelination in all parts of the brain of a man who died after ingestion of 2,4-D [33].

The formation and occurrence of tumors in man and other mammals, such as rats and mice, is quite similar [11]. In tests undertaken to date, it has been demonstrated that virtually every chemical which has been found to be carcinogenic in man is also carcinogenic in one or more

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mammalian test animals. It has been shown that if these compounds will produce tumors in one species, they will very likely produce tumors in more than one; thus adding weight to any finding of carcinogenicity in tests using any mammalian species.

Sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that a finding of carcinogenicity in one mammalian species should be deemed to have relevance for other mammalian species — including man. This threat may not be manifested for up to 30 or 40 years, given the long latent period of many known human carcinogens. Given the state of carcinogenicity testing and knowledge, there can be only one safe tolerance for a carcinogen — an absolute zero tolerance — and the only way a substance shown to be carcinogenic in test animals will not be a threat to human health is simply to prevent that substance from entering the environment. To the extent that it does enter man's environment, it will constitute a very real threat to his health.

Recent reports have described an increase of malignant neoplasms in human beings exposed to phenoxyacetic acids. Humans exposed to phenoxyacetic acids or chlorophenols showed an increased risk for the development of soft-tissue sarcomas [34, 35]. Results also have indicated that humans in contact with phenoxy acids may also develop malignant lymphomas [36].

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