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1970. *Human*  
EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

HEARINGS

BEFORE THE

SUBCOMMITTEE ON ENERGY, NATURAL  
RESOURCES, AND THE ENVIRONMENT

*U.S. Congress Senate* OF THE  
COMMITTEE ON COMMERCE

UNITED STATES SENATE

NINETY-FIRST CONGRESS

SECOND SESSION

ON

EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

APRIL 7 AND 15, 1970

Serial 91-60

Printed for the use of the Committee on Commerce



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*Dr. S. Epstein  
Children's Cancer Research  
Foundation, INC.  
Harvard Medical School.*

Parameters to be considered in test and concurrent control animals should include the incidence of abnormal litters, the incidence of abnormal fetuses per litter, the incidence of specific congenital abnormalities, the incidence of fetal mortality, maternal weight gains in pregnancy, and maternal and fetal organ/body weight ratios.

Additionally, some pregnant animals should be allowed to give birth in order to identify abnormalities that may otherwise manifest only in the perinatal period.

Agents and their known metabolites should be administered to two or more mammalian species under various nutritional conditions during active organogenesis and by a variety of routes reflecting possible human exposure.

Of interest in this connection is the lack of data in the available literature on teratogenicity testing by the respiratory route. Respiratory exposure is particularly important for pesticide aerosols and vapors.

Agents should be tested at higher dose levels than might be anticipated in humans following high-level accidental exposure, as well as following extensive low-level exposure. This is essential to attempt to reduce the insensitivity of conventional test systems based on very small numbers of animals compared with the millions of humans at presumptive risk.

To illustrate this further, let us assume that at actual human exposure levels, a pesticide induces teratogenic effects or cancer in as many as 1 out of 10,000 humans, then the chances of detecting this in test groups of less than 50 rats or mice exposed at these actual levels would be very low. Indeed, many more than 10,000 rats or mice, depending on their spontaneous incidence of teratogenic effects or cancer, would be required to demonstrate a statistically significant effect, if we assumed that rats and humans have similar sensitivity to the teratogen or carcinogen being studied.

For some teratogens, humans may be less or may be more sensitive than test animals. Meclizine—a drug used for morning sickness in pregnancy—for example, is teratogenic in the rat, but not apparently in a restricted number of humans studied (King, 1965; Yerushalamy and Milhovich, 1965).

With thalidomide conversely, the lowest effective human teratogenic dose is 0.5 mg. per kg. a day. Corresponding values for the mouse, rat, dog, and hamster are 30, 50, 100, and 350 mg. per kg. a day (Kalter, 1968).

Thus humans are 60 times more sensitive than mice, a hundred times more sensitive than rats, 200 times more sensitive than dogs, 700 times more sensitive than hamsters.

Clearly, attempts to determine a safe level for thalidomide, based on animal teratogenicity data, would clearly expose humans to significant teratogenic hazards. Accordingly, it is routine practice to test for teratogenicity and carcinogenicity at a range of concentrations, including those higher than human exposure levels, and extending to maximally tolerated doses (MTD).

*Even at MTD levels administered to mice from day 7 of life until sacrifice at 18 months, less than 10 percent of the 140 pesticides tested in the recent Bionetics study were shown to be carcinogenic.*

The report of the advisory panel on teratogenicity states unambiguously \* \* \*

Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed.

The report also emphasizes the insensitivity of standard test systems imposed by the relatively insufficient numbers of litters conventionally tested.

The report further states \* \* \*

Thus, compounds showing no increase (in birth defects) cannot be considered nonteratogenic.

Epidemiological surveys of human populations may provide post hoc information on geographical or temporal clusters of unusual types or frequencies of malformations following exposure to undetected or untested teratogens in the environment. However, logistic considerations, quite apart from inadequate current surveillance systems, limit the utility of this approach.

It should be emphasized that no major known human teratogen such as X-rays, German measles, mercury, or thalidomide, has been identified by retrospective epidemiological analyses, even in industrialized countries with highly evolved and sophisticated medical facilities.

Prospective epidemiologic surveys on agents previously shown or suspected to be teratogenic, by experimental studies or by retrospective population surveys, are clearly inappropriate.

C. Bionetics studies on teratogenicity of 2,4,5-T: Bionetics Research Laboratories, Inc., of Litton Industries, under a contract from the National Cancer Institute, tested 48 pesticides, including 2,4,5-T and related compounds, for teratogenic effects during 1965-68.

Although the bionetics studies were originally designed for purposes of large-scale screening 2,4,5-T was tested more extensively than any other pesticide. Thus the data on 2,4,5-T may be regarded as more definitive.

The Bionetics Research Laboratory report is included in the appendix (III). A revised and more detailed statistical analysis of these data is summarized in the report of the Advisory Panel on Teratogenicity of Pesticides (appendix II).

2,4,5-T was tested on repeated occasions from 1965-68 in three strains of mice and in one strain of rats by subcutaneous and/or oral administration over a dose range from 4.6 to 113 mg. per kg. The total numbers of litters tested at each dose level, by each route in all strains and species, excluding C<sub>3</sub>H mice in which only one litter was tested, were as follows, and I list these in a table enclosed.

As can be seen, the bulk of the data was obtained with BL6 mice. Due to control variability, the BL6 data have been considered for three time intervals—prior to September 1966, from September to November 1966, and from November 1966 to August 1968.

Data on AK mice were considered for two time intervals—prior to November 1966, and from November 1966 to August 1968.

Data for BL6 mice, AK mice, and Sprague Dawley rats, as derived from the bionetics report, are as follows:

BL6 mice: 2,4,5-T administered on days 6-14 or days 9-17, and mice sacrificed on day 18 of pregnancy.

BL6/AK/mice: 2,4,5-T administered on days 6-14, and mice sacrificed on day 18 of pregnancy.

AK mice: 2,4,5-T administered from days 6-15, and mice sacrificed on day 19 of pregnancy.

Sprague Dawley rats: 2,4,5-T administered from days 10-15 and rats sacrificed on day 20 of pregnancy.

In the 4 tables that follow dealing with a wide dose range, the results are expressed as percentages of abnormal fetuses. I would point out those occasions where statistically significant incidence of results was noted.

Major abnormalities in mice were cleft palates and cystic kidneys, and in rats, cystic kidneys and gastrointestinal hemorrhages. Increased fetal mortality was generally concomitant with these abnormalities. It is of particular interest that 39 percent abnormal embryos with cystic kidneys were seen in rats even at the lowest dose tested. Thus the no effect level was not reached even at 4.6 mg./kg.

Teratogenicity data on 2,4,5-T, as summarized in the bionetics report (appendix III) are quoted in extenso below. Some critical sentences are italicized:

This compound was given by the oral route to BL6 mice at dosages of 46.6 and 113 mg/kg and to AKR mice at 113 mg/kg. It was given by subcutaneous injection to BL6 mice at dosages of 21.5 and 113 mg/kg and to AKR mice and B6AK hybrids at 113 mg/kg. It was also given subcutaneously to C<sub>57</sub>H mice at 215 mg/kg, but there were too few of these to merit inclusion in the discussion which follows.

Administration was for eight days (6th through 14th) in most cases; for nine days (6th through 15th) in some; and for five days (10th through 14th) in one case—the details are indicated in the tabulated results. Subcutaneous administration used DMSO as a vehicle; oral used 50 percent honey.

With the single exception of the lowest dosage used (21.5 mg/kg to BL6 subcutaneously) all dosages, routes and strains resulted in increased incidence of abnormal fetuses. The incidence of cleft palate was high at the 113 mg/kg dosage, but not at lower levels. The incidence of cystic kidney was also high except in the AKR strain and in the BL6 mice which received 46.4 mg/kg orally. Fetal mortality was increased in all groups given 113 mg/kg for eight or nine days, but not in mice (BL6) given this dosage for only five days nor in the two groups of BL6 mice given lesser dosages (46.4 mg/kg orally and 21.5 mg/kg subcutaneously).

Most fetal and maternal measurements showed inconsistent changes from which no conclusions can be drawn. In contrast, there was a highly consistent decrease in maternal weight gain in BL6 mice given 113 mg/kg by either route. Lower dosages and the AKR strain showed either no change or a slight increase. All dosages, strains, and routes showed an increase in the maternal liver weight and this led to a further study discussed separately below.

*These results imply a hazard of teratogenesis in the use of this compound. The problems of extrapolation preclude definition of the hazard on the basis of these studies, but its existence seems clear.*

The observed influence of 2,4,5-T on maternal liver weight as mentioned above raised a question as to its effect on the fetal liver. This was answered by a study carried out in BL6 mice using subcutaneous injections of DMSO solutions at a dosage of 113 mg/kg only. The period of administration was lengthened to cover the period from the 9th through 17th day of gestation. Separate control groups were used concurrently. Except for the inclusion of fetal liver weight, measurements were made as previously described.

The fetal livers of the 2,4,5-T treated mice weighed significantly more than those of controls given DMSO only and the weights of the whole fetuses were

significantly less. Correspondingly, there was an increase in the fetal liver weight expressed as percent of body weight.

*Other observations were consistent with those reported above. The incidence of abnormal fetuses was unusually high as were those of cleft palate and cystic kidney.*

Because of the potential importance of the findings in mice, an additional study was carried out in rats of the Sprague-Dawley strain. Using dosages of 21.5 and 46.4 mg/kg suspended in 50 percent honey and given by the oral route on the 6th through 15th days of gestation, we observed excessive fetal mortality (almost 80 percent) and a high incidence of abnormalities in the survivors. When the beginning of administration was delayed until the 10th day, fetal mortality was somewhat less, but still quite high even when dosage was reduced to 4.6 mg/kg.

*The incidence of abnormal fetuses was threefold that in controls even with the smallest dosage and shortest period used. Fetal and maternal measurements showed only occasional instances of significant differences from controls except in the case of maternal liver weight which was consistently increased in all 2,4,5-T treated animals.*

*It seems inescapable that 2,4,5-T is teratogenic in this strain of rats when given orally at the dosage schedules used here. These findings lend emphasis to the hazard implied by the results of studies on mice.*

D. Recent reanalysis of the Bionetic data on teratogenicity of 2,4,5-T. More refined and more appropriate additional statistical analyses of these data were presented and discussed in the report of the Advisory Panel on Teratogenicity of Pesticides (appendix II). These are clearly confirmatory of the original conclusions of the Bionetics report on the teratogenicity of 2,4,5-T. Some relevant portions of the HEW panel report are quoted in extenso below:

Tested more extensively than other pesticides, 2,4,5-T was clearly teratogenic as evidenced by production of statistically increased proportions of litters affected, and increased proportions of abnormal fetuses within litters in both DMSO and honey for both C<sub>57</sub>BL/6 and AKR mice. In particular, cleft palate and cystic kidneys were significantly more prevalent. In addition, a hybrid strain resulting from a C<sub>57</sub>BL/6 female and AKR male showed significant increases in anomalies, in particular cystic kidney, when administered at 113 mg/kg of body weight in DMSA.

Additionally, 2,4,5-T was tested in Sprague-Dawley rats. When given orally at dosages of 4.6, 10.0, and 46.4 mg/kg on days 10 through 15 of gestation, an excessive fetal mortality, up to 60 percent at the highest dose, and high incidence of abnormalities in the survivors was obtained. *The incidence of fetuses with kidney anomalies was threefold that of the controls, even with the smallest dosage tested.*

E. Recent studies on teratogenicity testing of relatively pure 2,4,5-T. In view of the fact that the Bionetics study was conducted with a sample of 2,4,5-T which was subsequently shown to contain a relatively high concentration, 27 ppm, of a tetrachloro dioxin contaminant, testing has been recently repeated with relatively pure samples containing less than 1 ppm of this particular dioxin.

The results of these studies were presented by the FDA and NIEHS at a recent conference of February 24, 1970, at the FDA; the Dow Chemical Co. data were presented at the 9th annual meeting of the Society of Toxicology, Atlanta, March 17, 1970.

*As can be seen from the data summarized below, purified 2,4,5-T is teratogenic in three species—rats, mice and hamsters. These data should be regarded as preliminary. Confirmatory data on chick eggs are not presented here.*

1. Dow Chemical Co. studies (Emerson *et al.*, 1970). 2,4,5-T with 0.5 ppm dioxins, as a probable contaminant, was tested in pregnant

rats by repeated oral administration at doses of 1, 3, 6, 12, and 24 mg./kg.; the maximal dose tested was 24 mg./kg. No embryo deaths or weight losses were noted within the dose range tested. However, at 24 mg./kg. there was a sevenfold increase in the incidence of fetuses with defective ossification of the fifth sternbra; poor sternbra ossification was noted in four out of 103 control fetuses, and in 29 out of 103 fetuses of 2,4,5-T treated groups.

Defective sternbra ossification has been described in the rat as an expression of the teratogenic effects of drugs such as protamine zinc insulin and tolbutamide (Lichtenstein et al., 1951; Dawson, 1954).

2. NIEHS studies: Using the purest sample of 2,4,5-T, made available by Dow Chemical Co., teratogenic effects were induced in Swiss-Webster mice. Cleft palates were noted at dose levels of 150 mg./kg. and scattered abnormalities at 100 mg./kg.; the cleft palate incidence in control mice was essentially zero.

3. FDA studies: Hamsters were injected with five doses of 100 mg./kg./day of various batches of purified 2,4,5-T between days 6-10 of pregnancy. In one of these studies, there was a 66-percent incidence of mortality in 50 fetuses. Of the surviving fetuses, 17 percent had congenital abnormalities—crooked tail, missing limb, and defect in skull fusion. No data was presented on possible effects induced by doses less than 100 mg./kg.

Of additional interest was a report also presented at the same conference on purified 2,4-D, which produced a 22-percent incidence of congenital abnormalities in hamsters at a dose level of 100 mg./kg./day.

I should like now to address myself to the toxicity of dioxins.

Toxicity of dioxins: Rabbit ear skin is highly sensitive to dioxins, repeated application of which can produce chloracne, as a cumulative manifestation of local toxicity. Approximately 0.3 micrograms of the tetra isomer will produce a positive response; "more than 10 micrograms on a surface wipe sample indicates acute hazard" (to man) (Silverstein, 1970).

The acute oral LD-50 dose of tetra dioxin in male guinea pigs is 0.5-1.0 micrograms/kg., and in male and female rats, 22.5 and 45 microgram/kg., respectively. Feeding chicken edema factor diets, containing dioxins, produced cumulative toxicity in monkeys (Allen and Carstein, 1967). Storage of hexa, hepta and octa isomers, as identified by GLC, has been reported in chickens and rats fed chicken edema factor diets (FDA, unpublished). Chronic administration of 2,4,5-T or 2,4-D to dogs produces cumulative toxicity with gastrointestinal haemorrhage, suggestive of cumulative dioxin effects (Drill and Hiratzka, 1953).

#### TERATOGENICITY OF DIOXINS

1. FDA studies (FDA Conference, Feb. 24, 1970): A mixture of dioxins, 21 percent trichloro and 53 percent tetrachloro isomers, were injected in hamsters between days 6-10 of pregnancy over a dose range from 0.5 to 9.1 microgram/kg. per day. At the highest dose, the incidence of fetal mortality was 82 percent and the incidence of congenital abnormalities, 82 percent. At the 0.5 microgram/kg. dose, there was a 5 percent incidence of abnormalities. The no-effect level was thus not reached at 0.5 micrograms per kg.

2. Dow studies (Sparschu et al., 1970): The tetra dioxin isomer was fed to Sprague Dawley rats between days 6-15 of pregnancy, over a dose range from 0.03 to 8.0 micrograms/kg. per day. There was a marked increase in resorption sites at the 2 microgram level. Gastro-intestinal hemorrhages occurred over a range from 0.125 to 8 micrograms, dose-dependently. Additionally, at the 0.125 microgram/kg. level there was a decrease in male fetal weights.

*It should be emphasized that cystic kidneys were not seen at the 0.125 microgram/kg. dose of the tetra isomer or even higher levels. In the Bionetics study, 2,4,5-T at 4.6 mg./kg., containing 25 ppm of the tetra dioxin isomer equivalent to 0.124 microgram/kg., produced a 39 percent incidence of congenital abnormalities with cystic kidneys.*

There is thus a clear discrepancy between the teratogenic effects of 2,4,5-T containing 25 ppm of dioxin, and the effects of the equivalent concentration of the same dioxin. It is, however, conceivable that this discrepancy may reflect synergistic interactions between dioxin and 2,4,5-T.

#### SOME UNRESOLVED PROBLEMS RELATING TO 2,4,5-T AND DIOXINS

1. Chemical composition of 2,4,5-T formulations: Currently used 2,4,5-T formulations contain about 5 percent of known impurities, largely polychlorophenols. Analytic data on a sample of 2,4,5-T (Dow data, on production batch 120449) in the following table substantiates the approximate 5 percent of polychlorophenol impurities in 2,4,5-T formulations as currently used.

There are no available data on the presence and concentration of the more than 60 positional isomers of dioxin, other than the 2,3,7,8-tetrachloro dioxin isomer, in this batch of 2,4,5-T, or in other batches produced for food crop or other purposes in the United States and abroad.

In view of the relatively high concentration of polychlorophenol impurities in 2,4,5-T, it is likely that a wide range of dioxins are also present. *2,4-D and other phenoxy herbicides are similarly chemically uncharacterized.*

The higher positional dioxin isomers, hexa, hepta, and octa, have been identified in 2,4-dichlorophenol, a precursor of 2,4-D. Apart from the presence of dioxins in polychlorophenols, heating of polychlorophenols will produce additional and very high yields of dioxin.

Illustratively, heating 5 g. of pentachlorophenol at 300° C. for 12 hours yielded 1.5 g. of the octa-dioxin isomer (Cowan, 1970). There are no available data on the possible production of dioxins from combustion of 2,4,5-T or 2,4-D. While improved production techniques may well reduce the levels of polychlorophenols and the levels of the 2,3,7,8-dioxin isomer, apart from other isomers, in 2,4,5-T and other phenoxy herbicides, the degree to which this is practical does not yet appear to have been clearly defined.

2. Stability and persistence of dioxins: The extent of usage of 2,4,5-T and other phenoxy herbicides on food crops and for other purposes in the United States and abroad dictates the scale of resulting environmental contamination with 2,3,7,8-dioxin and other

rats by repeated oral administration at doses of 1, 3, 6, 12, and 24 mg./kg.; the maximal dose tested was 24 mg./kg. No embryo deaths or weight losses were noted within the dose range tested. However, at 24 mg./kg. there was a sevenfold increase in the incidence of fetuses with defective ossification of the fifth sternebra; poor sternebra ossification was noted in four out of 103 control fetuses, and in 29 out of 103 fetuses of 2,4,5-T treated groups.

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In view of the relatively high concentration of polychlorophenol impurities in 2,4,5-T, it is likely that a wide range of dioxins are also present. *2,4-D and other phenoxy herbicides are similarly chemically uncharacterized.*

The higher positional dioxin isomers, hexa, hepta, and octa, have been identified in 2,4-dichlorophenol, a precursor of 2,4-D. Apart from the presence of dioxins in polychlorophenols, heating of polychlorophenols will produce additional and very high yields of dioxin.

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The extreme inadequacy of toxicological data on dioxins clearly precludes consideration of potential human hazards due to dioxins in air, food or water, and consideration of possible safety margins following exposure to dioxins.

I would like to reemphasize the conclusions of the HEW panel on teratogenicity:

"The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposure. Such pesticides, in current use, include Captan; Carbaryl, the butyl, isopropyl, and isooctyl esters of 2,4-D Folpet; mercurials; PCNB; and 2,4,5-T. The teratogenicity of 2,4-D, the other salts and esters of both 2,4-D and 2,4,5-T, and that of IPC should be investigated further."

Finally and critically, available data on the toxicology of the dioxins, and more importantly on the lack of data on the toxicology—acute and chronic toxicity, carcinogenesis, mutagenesis, and teratogenesis—of the numerous positional isomers of dioxins, indicate an urgent need for restriction of human exposure to dioxins. Similar restrictions should extend to polychlorophenols, polychlorophenolic containing formulations, and their combustion products.

I thank you, sir.

(A list of the references to the statement follow:)

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Senator HART. Thank you, Doctor.

In your summary you refer to 2,4-D among other things, and earlier in your statement you said that 2,4-D produced birth deformi-

ties in the hamster. What is your opinion as to the teratogenicity of 2,4-D in comparison to 2,4,5-T?

Dr. ERSTEIN. The findings of the Bionetics study in relation to 2,4-D, were certainly not as conclusive as for 2,4,5-T. But, on the other hand, in the wide range of tests performed in the Bionetics study there were strong suggestions of teratogenic effects. That was as far as one could go. For these reasons the HEW Panel on Teratogenicity clearly recommended that further work should be done on 2,4-D and that a high degree of suspicion should be attached to it.

Moreover, the recent FDA studies on hamsters would seem to confirm the earlier suspicions of the Bionetics studies in mice and would indicate to say the least the need for a high degree of caution and minimally, indicate the need for restriction of further human exposure to 2,4-D pending clarification of these problems.

I think to await further definitive studies before taking action would not be appropriate in the light of the available data.

Senator HART. That gets us back, and we return here all the time, to this balancing of principle.

On the point that Mr. Bickwit was developing with Dr. Johnson, on the business of the cumulative effect of dioxin I take it you regard them as cumulative; is that correct?

Dr. ERSTEIN. On the basis of the available data in the literature, one could say there is a strong suggestion of cumulative effects. The rabbit ear skin test depends on cumulative toxicity and the chick edema effect is cumulative. One can demonstrate the pickup of dioxins in rat and chicken liver. So available data indicate they are persistent and cumulative, although I would regard these data as far from definitive.

Senator HART. What is your feeling as to the adequacy of the evidence? What is the extent of the evidence on the degradability of the dioxins in ultraviolet light?

Dr. ERSTEIN. I would submit that data on strong sunlight and shortwave UV is probably irrelevant. If you expose dioxins to strong sunlight you are exposing material to light which contains short wave ultraviolet in a manner not closely related to normal conditions.

I would like to see studies on conditions of photo-degradability of dioxins on conditions of shade and normal daylight. This is not available.

Senator HART. Well, we always seem to run into the lack of available data in pursuing answers to these questions. Now let's get to the availability of substitutes. What alternatives exist, or are you in position to know, for 2,4,5-T and 2,4-D?

Dr. ERSTEIN. I am obviously not an agricultural expert and we are now talking about crop use, I presume. Does your question relate to crop use of herbicides or total use?

Senator HART. I guess I should ask it totally and if there are areas where there is an alternative, identify that.

Dr. ERSTEIN. With the qualification that I am no expert in this field and not qualified to comment on the relative merits of various

herbicides, I would merely point out they fall into two categories, the pre-emergence and post. For the pre-emergence group, you have amiben, trifluralin, and atrazine, which all seem to be appropriate for use under a wide range of conditions.

As far as post-emergence herbicides, 2,4-D and 2,4,5-T are herbicides with which we are familiar, additionally there are others e.g., endothal, atrazines and dinoseb. Atrazines are pre-emergence and post-emergence herbicides. There is thus a wide range of herbicides for use on both pre- and post-emergence levels.

Senator HART. We have staff inquiry as to whether you have any knowledge as to the cost differences, if any, as between 2,4-D and 2,4,5-T and some of these other items.

Dr. EPSTEIN. I am not competent to answer that.

Senator HART. What tests have been made on the dioxins for genetic damage or for cancer?

Dr. EPSTEIN. There have been no tests made on any dioxin for genetic damage. There have been no tests made on any dioxin for carcinogenicity, either by oral administration or by injection or by inhalation. I must point out that we are talking about very small levels of highly potent compounds and we must remember certain carcinogens are active at less than a part-per-million level. We have no ball park data on this at all. We just don't know whether we are dealing with significant ranges or nonsignificant ranges.

Senator HART. Is the same true with respect to testing of the exposure by inhalation or respiration of the herbicides or dioxins? Early in your paper you remind us of this.

Dr. EPSTEIN. There are no data in the total literature of chronic carcinogenicity or mutagenicity of any pesticide whatever by inhalation. This is a point brought out quite forcefully in the Mraz Panel report. Inhalation is a very significant route of human exposure and the experimental test systems do not reflect recognition of this exposure.

Senator HART. What possible dangers may result from either 2,4,5-T or 2,4-D in noncrop use, and other herbicides in noncrop use?

Dr. EPSTEIN. Well, a wide range of possibilities exist and it is not possible to define many of these.

First of all, you get drifts of the actual 2,4,5-T and 2,4-D on the crop use and I have given data on the extent of this and the concentration of herbicides in Washington. Over and above that, material which is sprayed like timber, pasture, and shrubs may well be burned.

In fact it is quite common to burn shrubs after you spray and we already know if you heat 5 grams of pentachlorophenol at 300 degrees you get 1.5 grams of the octadioxin isomer. So you are putting into the environment material rich in polyphenols and which are likely to be combusted. We have no idea how long these dioxins will persist and these data are just not available.

But there are very real possibilities of formation of significant quantities of dioxins from phenoxy herbicide use. But I must emphasize that similar considerations also obtain to a rather wide range of polychlorophenols which we have not adequately discussed here.

Senator HART. If you had an opportunity to examine the announcement made and reported to us today, with respect to the suspension or cancellation of 2,4,5-T, given the possible danger of the noncrop use that you discussed, do you concur in the action taken? Do you think it goes too far or not far enough?

Dr. EPSTEIN. Do I have to answer that?

Senator HART. Sir?

Dr. EPSTEIN. Do I have to answer that?

Senator HART. Well, you don't have to, but it would be helpful. I am sure it would be accepted as from a person whose qualifications to make the judgment are excellent.

Dr. EPSTEIN. My view then, based on available data and also lack of data on the phenoxy herbicides, is that their use under conditions where humans are exposed is clearly not warranted.

Senator HART. Does that include the application on grazingland? Would you include that use when you say use to which humans would be exposed?

Dr. EPSTEIN. I would submit that phenoxy herbicides should, under no circumstances, be used on the crops. I would submit there is a strong presumption for suspension of their use under any circumstances in the environment.

Mr. BICKWIT. What possible dangers do you see resulting from use of chlorophenol?

Dr. EPSTEIN. Before responding to that, might I just list the categories of use of polychlorophenols? Again, this is an industrial area in which I am not competent to go into any detail. But as far as I can see polychlorophenols are used for a wide range of purposes.

First of all, for treatment of timber; then as slimicides and fungicides; for paints, varnishes, lacquer; laundry starch; for shampoos; paper and paper coatings, including food wrappings; for curing of hides; rendering of fats; production of feeds for animals and for man; and for manufacture of a wide range of pesticides.

Pentachlorophenol is used as a herbicide quite apart from being used on sugarcane, so there is a wide range of use for this in the environment. If you take 5 grams of pentachlorophenol and heat it at 300 degrees centigrade, you form 1.5 grams of the octadioxin.

We are not now talking about a billionth or trillionth part per gram; we are talking about 1.5 gram of the octadioxin isomer. I cannot tell you the degree of resulting hazard because nobody has tested it for carcinogenicity, mutagenicity, either by injection, skin application, or inhalation.

Therefore, your question is unanswerable except to say what little information we have on the dioxins indicate that these are highly toxic. That is as far as one can go.

Senator HART. Well, the data on the risk side, then, in so many of these areas, are a little skimpy, if not nil. So that when agencies have to make this judgment balancing the benefits which are identifiable against the possible health risk, how can they make that kind of judgment in the absence of adequate risk data?

Dr. EPSTEIN. I agree entirely. The concept of balancing risk against benefit is predicated on two assumptions. We usually get a good idea what the benefits are, and secondly we should know as exactly as possible what the risks are.

In this instance we do not know what the risks are, and, therefore, this equation cannot be evaluated here. We cannot discuss benefit if we have no information whatsoever on carcinogenicity, mutagenicity, and teratogenicity.

Senator HART. Well, without answering the hard question of whether you take a hard line and not permit anything on the market until you get the needed data or whether you use the lack of data as the reason for permitting it to go out until the data are available, the long and short of your testimony, and that of others we have heard today, is that it is extremely important to develop and intensify our efforts to get that data. Is not that the obvious message of today?

Dr. EPSTEIN. This is one of the messages, yes. But we have had information on dioxin for 20 years. We have known of the carcinogenicity of DDT since the late 1940's.

Senator HART. Well, if you are being critical, that is fair enough, but wouldn't you agree that whether we are slow in reacting or not, we had better react now?

Dr. EPSTEIN. Yes.

Senator HART. Doctor, thank you very much for this wonderful statement. I perhaps have indicated my own feelings by my interruption. I have had the same reaction on this subject today as we have on so many subjects. If we could stop the world for 7 days and get one part of one set of problems fixed, then crank the world up before stopping it again to move on the rest, in a lifetime we might get most of the things fixed. But you do have a feeling of wondering whether we will manage to move in time with sufficient intelligence to respond to all the problems that confront us.

(The appendices follow:)

## APPENDIX I

SAMUEL S. EPSTEIN

### Personal:

Born: April 13, 1926, Eildesborough, Yorkshire, England.  
Naturalized U. S. citizen - Married - Three children

Private address: 33 Powell Street  
Brookline, Massachusetts 02146, U.S.A.

Professional address: Children's Cancer Research Fdn., Inc.  
35 Binney Street  
Boston, Massachusetts 02115, U.S.A.

### Qualifications:

1947	B.Sc. (Physiology) London University, England.
1950	M.B.U.S. (Bachelor of Medicine, Bachelor of Surgery) (Double Honors) London University, England.
1952	D.T.M.H. (Diploma of Tropical Medicine and Hygiene, Bacteriology and Parasitology) London University.
1954	D.Path. (Diploma of Pathology) London University.
1958	M.U. (Doctorate of Medicine, Thesis in Pathology and Bacteriology) London University, England.
1963	Diplomate, in Public Health and Medical Laboratory Microbiology, of the American Board of Microbiology.

### Positions Held:

1950	Demonstrator, Morbid Anatomy, Guy's Hospital, London.
1951	House Physician, St. John's Hospital, London.
1952	Postgraduate Student in Tropical Medicine, Pathology ( Bacteriology and Parasitology, Royal Army Medical College, London.
1952 - 1955	Specialist in Pathology, Royal Army Medical Corps
1955 - 1958	Lecturer in Pathology and Bacteriology, Institute of Laryngology and Otolaryngology, University of London.
1958 - 1960	British Empire Cancer Campaign Research Fellow, in conjunction with the Chester Beatty Cancer Research Institute and Tumor Pathologist at the Hospital for Sick Children, Great Ormond Street, London.
1960	Consultant in Pathology, The Memorial Hospital, Peterborough, England.
1961 to date	Research Associate in Pathology and Microbiology, The Children's Hospital Medical Center and the Children's Cancer Research Foundation, Inc., Boston.
1961 to date	Chief, Laboratories of Carcinogenesis and Toxicology, Applied Microbiology and Histology, the Children's Cancer Research Foundation, Inc., Boston.
1962 to date	Senior Research Associate in Pathology, the Children's Cancer Research Foundation, Inc., Boston, and Research Associate in Pathology, Harvard Medical School, Boston.

Awards:

1. Military Awards in Royal Army Medical Corps, 1953
  - (a) Montefiore Gold Medal in Tropical Medicine
  - (b) Montefiore Prize in Tropical Hygiene
  - (c) Ronald Martin Prize in Military Surgery
2. Society of Toxicology, 1963 Achievement Award

Society Memberships:

British Medical Association  
 Society of Clinical Pathologists  
 Society for Pathology and Bacteriology  
 Society for General Microbiology  
 Society of Protozoologists  
 Air Pollution Control Association  
 American Association of Pathologists and Bacteriologists  
 American Society for Experimental Pathology  
 American Association for Cancer Research  
 American Board of Microbiology  
 Society of Toxicology  
 Environmental Mutagenesis Society

Committees:

- (a) Member, Committee on the Relation of Protozoology to Public Health. The Society of Protozoologists, 1962--1969.
- (b) Chairman, Committee on Biological Effects of Air Pollution. Air Pollution Control Association, 1963--1969.
- (c) Member, Technical Council of the Air Pollution Control Association, 1963--1969.
- (d) Executive Secretary, Environmental Mutagenesis Society, 1969.
- (e) Chairman, Committee on Cyclamates and Caffeine, Environmental Mutagen Society, 1969.
- (f) Chairman, Committee on Liaison, Environmental Mutagen Society, 1969.
- (g) Chairman, 1969, HEW Panel, Mutagenicity of Pesticides
- (h) Chairman, 1969, HEW Panel, Teratogenicity of Pesticides
- (i) Member, 1969, HEW Panel, Pesticide Interactions
- (j) Member, 1969, HEW Panel, Carcinogenicity of Pesticides
- (k) Chairman, NIMH Panel on Chronic Non-psychiatric Hazards of Drugs of Abuse, 1969.

Congressional Testimony:

On "Cancer and Mutation-Producing Chemicals in Polluted Urban Air", at Hearings before the *Subcommittee on Air and Water Pollution of the Committee on Public Works*. July 25-31, 1968, presided by Senator Edmund S. Muskie.

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## APPENDIX II

## SUMMARY AND CONCLUSIONS

Teratology deals with the etiology and development of congenital malformations. Congenital malformations are generally defined as gross structural abnormalities of prenatal origin, present at birth or manifesting shortly after, which kill or disable. In a broader sense, teratogenesis is considered to include histological, biochemical, and functional abnormalities of prenatal origin.

Congenital malformations present obvious personal, medical, and social stresses. Additionally, it has been recently estimated that the costs to society of one severely malformed child, in terms of medical and other care and deprivation of potential earnings, amount to several hundred thousand dollars.

There are now well over 400 substances that, in various forms and combinations, are currently used as pesticides. Pesticides may represent an important potential teratogenic hazard. Therefore any teratogenic pesticide to which the population is exposed should be promptly identified so that appropriate precautions can be taken to prevent risk of human exposure. It is feasible to test these substances for teratogenic effects in test animals so that potential hazards to human health can be evaluated.

For these and other reasons detailed in the report, we conclude that:

a. All currently used pesticides should be tested for teratogenicity in the near future in 2 or more mammalian species chosen on the basis of the closest metabolic and pharmacologic similarity to human beings possible. Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed. Test procedures should also reflect routes related to human exposure. Apart from the obvious route of ingestion, attention should be directed to other routes of exposure, including inhalation exposures from pesticide aerosols and vaporizing pesticide strips used domestically and exposures from skin absorption. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion.

b. The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately

restricted to prevent risk of human exposure. Such pesticides, in current use, include Captan; Carbaryl; the butyl, isopropyl, and isooctyl esters of 2,4-D Folpet; mercurials; PCNB; and 2,4,5-T. The teratogenicity of 2,4-D, the other salts and esters of both 2,4-D and 2,4,5-T, and that of IPC should be investigated further.

c. Pesticides found to be inactive after appropriate testing can be considered as provisionally safe, unless other evidence of teratogenicity develops.

d. No new pesticide should be registered until tested for teratogenicity by suitable procedures. Any pesticide found to be teratogenic should only be used in circumstances where risk of human exposure is minimal.

e. Efforts should be made to improve and standardize procedures for teratogenicity testing and population monitoring.

A scientific group or commission should be charged with responsibility for continued surveillance of the whole problem of pesticide teratogenesis.

#### METHODOLOGIES FOR TERATOGENICITY TESTING

##### *Introduction*

Prior to 1963, the Food and Drug Administration did not require evaluation of teratogenicity. As a result of the thalidomide disaster, the need for data on teratogenicity became evident. In 1963, the President's Science Advisory Committee on "Use of Pesticides" recommended that toxicity studies on pesticides include effects on reproduction through at least 2 generations in at least 2 species of warmblooded animals. Observations to be included were effects on fertility, size and weight of litters, fetal mortality, teratogenicity, and growth and development of sucklings and weanlings. Such toxicity studies including the three-generation procedure were not designed primarily to detect teratogenicity and thus may not be appropriate.

The potential teratogenicity of chemicals may be detected by two complementary approaches. First, chemicals or other agents may be administered to experimental animals to determine whether they induce prenatal damage. Secondly, and on a *post hoc* basis, human populations may be epidemiologically surveyed to detect geographical or temporal clusters of unusual types or frequencies of congenital malformations. Combinations of these approaches are likely to ensure early detection and identification of teratogenic hazards.

Experimentally, a complex of factors are needed to elicit teratogenic effects. These relate to gestation period, genotype of the pregnant animals, dosage, mode of administration and metabolic transformation of teratogen. For example, teratogens may be effective only at a certain dose range, whether high or low, narrow or wide, below which develop-

ment is apparently undisturbed, and above which death *in utero* results.

Most agents are teratogenic only in the developmentally labile early period of gestation, during which active organogenesis occurs. In humans, this sensitive period extends approximately from the end of the first week of pregnancy to the 12th week. Other circumstances may also influence the effectiveness of human teratogens, such as maternal nutritional, demographic, socioeconomic, and cultural factors, physiological states, and temporal and seasonal situations. Thus a potential teratogen may manifest its effect only when particular conditions conjoin.

The relationship between human exposure to a teratogen and subsequent induction of congenital abnormalities is generally not obvious. Any one teratogen may produce a multiplicity of effects and any specific effect may be produced by various teratogens. In test animals, the teratogenetic response may differ from species to species. In humans, differences in genetic, metabolic, and environmental influences may contribute to a variety of specific effects from exposure to a particular teratogenic agent. Induced and spontaneous effects may be difficult to distinguish. The teratogenicity of thalidomide might have been missed had it not produced malformations rarely encountered; additionally, only a fraction of the pregnant women who took thalidomide had defective children.

Consequently, further data on the possible teratogenic effects of pesticides in experimental animals are urgently needed to provide a basis for evaluating potential hazards to human health.

##### *Ancillary methods*

Preliminary screening can be accomplished by the use of nonmammalian species, particularly the chick embryo. These tests may give useful ancillary data prior to further testing in mammals. However, negative results in these systems alone should not be considered proof of safety.

##### *Use of lower mammalian species*

a. Purity, composition, stability, and source of compounds under test should be determined.

b. At least two mammalian species should be tested. These should be chosen on the basis of metabolic and pharmacokinetic similarity to humans. If possible, commercially available inbred strains should be used; if not, intra-species variability must be recognized. Species commonly used include mice, rats, hamsters, rabbits, dogs, cats; sheep and swine have also been used.

c. Preliminary mammalian experiments should determine the amounts of the compound and its appropriate metabolites necessary

to produce serum levels comparable to ranges likely to be found in humans after high level accidental exposure as well as potential exposures assuming extensive use of that pesticide. Multiples of these dosages, up to the mammalian maternal LD<sub>50</sub> should be administered to determine the lowest dosage causing a significant increase in fetal death, or resorption. Dosage in this critical range should be tested for teratogenic effects with care to distinguish these effects from other embryotoxicity and to determine dose-response relationships.

d. Compounds should be administered, by appropriate routes, within the critical dose range determined by preliminary tests. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion. Compounds should first be tested by single administrations of a range of doses at various times during the phases of active organogenesis. The substance should be administered at discrete times throughout the period of organogenesis as various organs are developing, since some substances have specific effects on the development of particular organs. By this technique, the possibility of inducing hepatic microsomal or other enzymes facilitating metabolic detoxification or activation of the substance is also minimized. If no teratogenic effects are detected by this technique, subsequent testing should be based on repeated administrations of the substance at daily intervals or if feasible, intervals of less than 24 hours during the entire period of organogenesis.

e. When appropriate, metabolites should also be tested for teratogenic effects.

f. Additional investigations should include—

i. Determination of appropriate plasma and fetal levels of compounds;

ii. Determination of the biological half-life of the compound in test animals;

iii. Metabolic studies to identify mechanisms of detoxification or activation of compounds when appropriate; and

iv. Determination, when appropriate, of the possible potentiating effects of protein deprivation or concomitant exposure to other pesticides or other environmental agents.

g. All procedures, including those relating to animal breeding, housing, handling, feeding, husbandry, methods for examining fetuses for congenital malformations, defining the onset of pregnancy, and classifying congenital malformations should be rigorously standardized. Numbers of pregnant animals and offspring must be adequate for statistical significance. All tests must be replicated on independent occasions and with contemporaneous controls.

### *Nonhuman primates*

Results from lower mammalian species may warrant subsequent testing in nonhuman primates. The following considerations should be noted:

a. Records of menstrual cycles are essential. Primates whose reproductive history is known and have previously delivered normal young should be selected for testing. Timing of ovulation, and therefore gestation, should be accurately determined by allowing the males and the females to be together for no more than 3 consecutive days. Vaginal smearing, to determine the presence of spermatozoa should be avoided; the use of Tullner's method for determining chronic gonadotropin levels and rectal palpation is preferable.

b. Compounds should be carefully administered in controlled dosages.

c. Pregnant animals should be handled only minimally.

d. Compounds should be administered during the various phases of organogenesis. Embryos can be obtained by laparotomy any time after the first 100 days of gestation; the mother may be subsequently used for other experimental procedures. Additionally, some young should be allowed to go to term to identify possible teratogenic effects detectable only in the neonatal period.

### *Population monitoring*

It has been shown (see Literature Review) that some pesticides induce congenital malformations in experimental animals providing a critical dose is appropriately administered at critical times. When animal experiments indicate that a pesticide is teratogenic, human effects should be retrospectively evaluated, when possible, by study of pregnancies during which the mothers were inadvertently exposed to the pesticide, such as a result of farm work, accidental ingestion, or industrial exposure. Prospective epidemiologic approaches may involve follow-up of large numbers of people over long periods of time, and be slow, tedious, expensive, or difficult to implement. It is not appropriate to conduct prospective epidemiological studies on human populations with pesticides previously shown to be teratogenic by experimental animal studies or retrospective human data. Human exposure to such compounds must be minimized by appropriate regulatory preventive action.

Prospective epidemiological approaches for pesticides in current use may provide important information, however, it should be realized that no major teratogen has yet been recognized in this way. The malformations induced by X-ray, German measles, thalidomide, and mercury—Minamata disease, were each recognized by an alert medical

practitioner who observed a cluster of cases and then traced the cause to its source.

What can be done to enhance prompt recognition of such clusters should they occur from previously unsuspected teratogens in the future? A variety of existing data resources can be used for this purpose. In each, the occurrence of congenital malformations in substantial segments of the population is being recorded in a standard fashion. The best of these resources are local, rather than statewide or national. The prepaid medical program of the Kaiser-Permanente Hospitals and Clinics in the San Francisco Bay Area are of particular interest. A detailed study there of the occurrence of malformations among 16,000 births represents a good model for additional investigations. A similar study has been made by the Health Insurance Plan of Greater New York, but its 30 or more cooperating clinics are less easily coordinated than the Kaiser system.

A citywide surveillance, known as the Metropolitan Atlanta Congenital Defects Program (jointly directed by Emory University School of Medicine, the Georgia Department of Public Health, and the National Communicable Disease Center, USPHS), involves reports on all children with congenital malformations born to residents of the five-county Atlanta area. As yet, no cluster of cases has suggested an environmental influence since the program began in October, 1967.

In a substantial number of States, birth certificates contain an item concerning congenital malformations. The completeness and accuracy of such reporting varies considerably and depends on the physician's interest and diligence and on the conspicuousness of the abnormality. Birth-certificate data on malformations in New York State are more extensive than those of many other States and have been effectively used in several research studies. Nationally, however, no attempt has been made to collect and evaluate all data on malformations that are available on birth certificates.

A select committee convened by the National Center for Health Statistics (NCHS), has recommended, in an excellent but little known report, that efforts be made to improve and use information on congenital malformations recorded on birth certificates (Vital and Health Statistics, Documents and Committee Reports, NCHS Series 4, Number 7, March 1968). Implementation of this recommendation would be of great value, for monitoring to detect the teratogenic effects of newly introduced or geographically localized environmental chemicals or other agents.

To enhance our ability to recognize significant changes in congenital malformation rates, a systematic collection of data from concentration points should be established. Specifically, a surveil-

lance should be made of claims submitted to private, State, or local agencies for the medical care of children with birth defects. Because the Children's Bureau, DHEW, has so much experience with these agencies, its assistance should be sought in planning the surveillance network.

Data from foreign countries should also be evaluated as part of a national effort to study possible relationships between pesticides and congenital malfunctions.

In studying the possible relationships between exposure to pesticides and the occurrence of diseases, statistical associations, if present, will provide important information. However, when possible it is important to secure additional information concerning the following:

- a. Dose-response relationships.
- b. Absence of alternative explanations.
- c. Biological plausibility.
- d. Consistency with other knowledge from clinical, laboratory, and epidemiologic research.
- e. Disappearance of the effect when the presumed cause is removed.

In particular, as clusters of specific anomalies are recognized, through whatever resources that presently exist or may be developed, any possible relationships to pesticides would be clarified by the use of laboratory techniques to measure the maternal, fetal, or neonatal body burden of suspect chemicals.

There are national units engaged in teratologic research, but each is following a set method. There is a critical and immediate need to establish a national or international center to study congenital malformations in man not by a single method but by whatever techniques are most appropriate for testing or generating hypotheses. The center should be diversified and fast moving, ready to use local, national, or international resources in order to determine the significance of laboratory or clinical data.

#### LITERATURE REVIEW

##### *Animal studies*

For convenience, detailed results of the Bionetics study are presented in a subsequent section.

Much of the total available literature and data reviewed by this Panel were methodologically inadequate to support definitive conclusions. Additionally, the authors of many reports tended to confuse or equate embryotoxicity and other adverse effects on reproduction with teratogenicity. It is also apparent from the literature that insufficient attention has been directed towards problems of interactions in testing for teratogenesis.

The Panel considered the following information to be of significance:

*a. Captan and Folpet.*—These pesticides have been shown to be teratogenic in chicken embryos (Verrett et al., 1969). Captan was also shown to be teratogenic in rabbits (McLaughlin, 1969), although other rabbit studies yielded negative results (Kennedy et al., 1968; Fabro et al., 1965). The enhancement by protein deprivation of the acute toxicity of captan to rats (Boyd, 1968), was noted with particular interest. The teratogenicity of captan and Folpet in mice was demonstrated in Bionetics studies. Unpublished data on captan in monkeys were evaluated and found inadequate; in these studies, the duration of organogenesis was not entirely covered and controls were not appropriate. However, the 3/7 abortions observed at the highest dosage given, 25 mg./kg., may be indicative of an embryotoxic hazard due to captan.

*b. Carbaryl.*—This was tested at 66.7 and 200 p.p.m. in the diet of pregnant mice (FAO/WHO, 1967). In two litters at the 200 p.p.m. level, a total of seven instances of skeletal malalignment, nonfusion, incomplete ossification, and one case of cleft palate and gross facial malformation were noted, as opposed to no malformations in the low-level group and two cases of cleft palate in controls. Teratogenic findings for carbaryl are also reported in the Bionetics study. In a study in beagle dogs fed carbaryl during gestational periods at levels of 50, 25, 12.5, 6.25, and 3.125 mg./kg. body weight daily, teratogenic effects were found at all but the lowest dose level (Smalley, 1968).

*c. Mercurials.*—Organomercury compounds: Various mercury containing pesticides were evaluated under the heading "phenylmercury acetate (and other organomercury compounds)" by the 1966 Joint Meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues (FAO/WHO, 1967). The results of additional experimental work have been reported in the 1967 Evaluations of Some Pesticide Residues in Food. Additional information on "Methylmercury" was published by the Ecological Research Committee, the Swedish Natural Science Research Council (1969) Bulletin no. 4. by Goran Lofroth, where embryotoxic effects in mice (reported by Frölen and Ramel) were discussed along with other data. When given subcutaneously, in doses of 0.11 mg. on day 7 of gestation, phenylmercuric acetate was reported to cause fetal malformations in mice. Eye, tail, and central nervous system defects were noted (Murakami et al., 1956).

*d. Organochlorine.*—Embryotoxicity in rats and dogs has been reported for organochlorines including dieldrin, chlordane, and kepone. In the absence of convincing data, kelthane has been claimed

to be teratogenic in mice (An Der Lan, 1964); see also Bionetics studies.

*e. Organophosphates.*—The cholinesterase-inhibiting organophosphate insecticides, guthion, parathion, diazinon, Bidrin, Trithion, and EPN, have been shown to be teratogenic when injected directly in the yolk sac of chick embryos. The malformations were nonspecific or common to all organophosphates (Fish, 1966). It was also claimed that these compounds are teratogenic in mice. The data reported, however, suggested that organophosphates, like the organochlorines, act by reducing litter size and producing embryotoxicity rather than by producing specific teratogenic effects. See also Bionetics studies.

*f. Thiram.*—Thiram was reported to be teratogenic in hamsters at 250 mg./kg. (Robens, 1969). In the Bionetics study it was not found to be teratogenic. In a study of three generations of rats, no toxicological effects were observed at a dietary level of 48 p.p.m. (FAO/WHO, 1967). However, Thiram should be further investigated for possible teratogenic effects.

*g. Miscellaneous reproductive effects.*—Placental transfer of dieldrin and incidence of stillbirths have been studied in cows (Braund, 1968); increased stillbirth rates have been claimed in cows fed with DDT (Labon, 1965). The estrogenic activity of o,p'-DDT has been related to reproductive effects in chicken, quail, and rats (Bateman, 1968; Wurster, 1968; Porter and Weimeyer, 1969). Diminished population size and reproductive failure have been produced in sparrow hawks by DDT and dieldrin (Porter and Weimeyer, 1969). These resulted from a decreased eggshell thickness, increased breakage of eggs, and increased egg eating by parent birds. Other studies of interest include the following: Finnegan, 1949; Tauber, 1950; Fisher, 1952; Narpozzi, 1956; Swann, 1958; Cottrell, 1959; Marliac, 1964; Backstrom, 1965; Hathaway, 1967; Ware, 1967; Weiike, 1967; Carlton, 1968; Keplinger, 1968; Khera, 1968; Verrett, 1969; Legator, 1969.

#### *Bionetics animal studies*

Bionetics Research Laboratories of Litton Industries, during 1965-68 under a contract for the National Cancer Institute (NCI Contracts PH 43-64-57 and PH 43-67-735), tested various pesticides and related compounds for teratogenic effects. These studies were designed as large-scale screening tests. The Bionetics data were re-analyzed statistically to account for litter effects. The results of this statistical re-evaluation are presented in this section. More detailed material on these pesticides will be published in the future.

*a. Summary of findings from Bionetic animal studies.*—Tested more extensively than other pesticides, 2,4,5-T was clearly teratogenic as evidenced by production of statistically increased proportions of

litters affected, and increased proportions of abnormal fetuses within litters in both DMSO and Honey for both C57BL/6 and AKR mice. In particular, cleft palate and cystic kidneys were significantly more prevalent. In addition, a hybrid strain resulting from a C57BL/6 female and AKR male showed significant increases in anomalies, in particular cystic kidney, when administered at 113 mg./kg. of body weight in DMSO.

Additionally, 2,4,5-T was tested in Sprague-Dawley rats. When given orally at dosages of 4.6, 10.0 and 46.4 mg./kg. on days 10 through 15 of gestation, an excessive fetal mortality, up to 60 percent at the highest dose, and high incidence of abnormalities in the survivors was obtained. The incidence of fetuses with kidney anomalies was three-fold that of the controls, even with the smallest dosage tested.

PCNB produced an increase in renal agenesis between litters, and within litters, when administered orally from days 6-14 or days 6-10 of pregnancy. However, renal agenesis was not produced when PCNB was administered only from days 10-14 of pregnancy. These effects were produced in only the C57BL/6 strain of mice.

Other pesticides producing a statistically significant increase in the proportion of litters containing abnormal fetuses and in the increased incidence of abnormal fetuses within litters were: Captan, Folpet, 2,4-D isooctyl ester, 2,4-D butyl ester, 2,4-D isopropyl ester, carbaryl (Sevin), and IPC. These pesticides produced elevated incidence in one solvent only. The results for carbaryl and for IPC were less consistent than for other compounds. (The pesticides 2,4,5-T, PCNB, captan, Folpet, carbaryl, IPC, and the butyl and isopropyl esters of 2,4-D were statistically significant at the .01 level, for one or more tests. This criterion is similar to that adopted by the Technical Panel on Carcinogenesis, Chapter 5, to identify "positive" compounds. The isooctyl ester of 2,4-D was significant at the 0.05 level.)

Compounds inducing only an increase in the proportion of abnormal fetuses within litters were: *a*-naphthol, and 2,4-D methyl ester. The statistical significance of these results was relatively weak; further study is required before any conclusions can be reached. Similarly, 2,4-D produced only an increase in the proportion of abnormal litters during 1965 in AKR mice. Due to the teratogenic activity of certain of its esters, 2,4-D should be studied further.

Carbaryl plus piperonyl butoxide did not show an overall increase in nonspecific anomalies, but resulted in significantly more cystic kidneys for doses above 10 mg./kg. carbaryl plus 100  $\mu$ l./kg. piperonyl butoxide.

It must be emphasized that failure to detect statistically significant increases of anomalies may be due to insensitivity resulting

from experimental variation and small numbers of litters tested. In addition, higher fetal mortality among some of the "negative" compounds may be selectively eliminating abnormal fetuses.

*b. Methods.*—Four strains of mice were used: C57BL/6, AKR, C3H, and A/Ha. Most of the studies were performed with the C57BL/6 strain. A hybrid fetus resulting from mating a C57BL/6 female with an AKR male was used to study a few compounds. More restricted studies were also made on Sprague Dawley rats; results of these with reference to 2,4,5-T are considered separately.

Most compounds were administered subcutaneously in 0.1 ml. solutions of dimethylsulfoxide (DMSO). Water soluble compounds were administered in saline, and some times also in DMSO. Compounds administered orally were given by gavage in 0.1 ml. in a 50-percent honey solution. Groups of positive controls and untreated controls were included, as well as controls receiving only DMSO, saline, or honey. While controls were run periodically throughout the duration of the study, compounds and controls were not matched with respect to either route or date of administration.

Virgin females were used in these studies. The onset of pregnancy was determined by detection of vaginal plugs. Compounds were administered daily from the sixth to the 14th day of pregnancy (15th day for AKR mice). Mice were sacrificed on the 18th day (19th day for AKR mice) of gestation. On sacrifice, fetuses were examined for anomalies. Approximately two-thirds of the fetuses were then stored in Bouin's solution until necropsy. Remaining fetuses were stained with alizarin red S after proper processing. Numbers of resorption sites and dead fetuses were also scored.

*c. Statistical analysis.*—All analyses were performed on a *per* litter basis rather than a *per* fetus basis, since initial investigations indicated that the occurrences of anomalies among fetuses within litters were correlated. The large litter-to-litter variation may reflect some maternal effect, an indication of the effective dose level of the compound actually reaching the fetuses, experimental variation, or, as is most likely, some combination of the three factors.

While there were no statistically significant time trends within the various control groups in terms of the onset of fetal anomalies in the C57BL/6 mice, the incidence of fetal mortality was certainly time-dependent in this strain, with 1965 being characterized by a low incidence of prenatal deaths. Furthermore, there was a period of approximately 6 months, extending from the latter part of 1965 into early 1966, during which no control animals were tested. During this period a change in the substrain of C57BL/6 mice used in the study took place. Finally, among abnormal litters, as defined by litters con-

taining at least one abnormal fetus, there was some suggestion that the distribution of abnormal fetuses *per* litter was stochastically larger in the DMSO controls than it was in the untreated controls. Thus, the possibility exists of a time/strain/solvent interaction that is undetectable in the controls because the level of background teratologic activity is relatively low. This potential interaction effect could either enhance or dissipate the effect of any given compound, depending on the conditions under which it was administered. Thus, the data were necessarily separated by both time period and solvent for the purposes of analysis. Similarly, an increase in fetal anomalies in the DMSO controls of the AKR mice was noted after November 1966. Thus, the AKR data were analyzed separately in two time periods.

It should be noted that not all compounds were administered on more than one occasion or in more than one solvent or strain. Thus, in general the compounds in the study cannot be compared for teratogenic potential, since those that were tested extensively were more likely to show some adverse effect and, perhaps, less likely to appear consistent over time, solvent, and/or strain.

As noted, approximately two-thirds of the fetuses were stored in Bouin's solution until necropsied; the remainder being stained with alizarin red. However, in many instances the proportion of necropsied fetuses was slightly higher for the compound under investigation than for the corresponding controls. It is doubtful if this discrepancy could have any appreciable effect on the conclusions since the incidence of anomalies detectable only by necropsy among control animals was relatively low. Furthermore, if all of the control and test mice had been necropsied, the significance of the differences observed in this study would be intensified. Thus, no effort was made to correct for inequalities in the necropsy/stain ratio in the present analysis. Additionally, no attempt was made to correct for differences in litter sizes or sex-ratios within litters, since both of these factors may, at least in part, reflect effects of the compound under test.

*d. Results.*—Data for pesticides yielding a statistically increased level of anomalies in C57BL/6 and AKR mice are listed in tables 1 and 2, respectively. The proportion of abnormal litters gives the proportion of litters containing one or more abnormal fetuses, as a measure of the prevalence of anomalies across litters. The proportion of abnormal fetuses *per* litter gives a measure of the prevalence of anomalies within litters. The proportion of abnormal fetuses *per* litter for litters containing abnormal fetuses gives a measure of the prevalence of anomalies within effected litters. A significant increase of dead fetuses and resorptions is also listed. Some tests were conducted on only one par-

ticular day or on adjacent days as listed. Eye anomalies, mainly microphthalmia and anophthalmia, accounted for approximately 50 percent of the individual anomalies in C57BL/6 mice. To a large extent, results in table 1 reflect changes in the incidence of eye anomalies. Yet, when the data were analyzed excluding fetuses with microphthalmia only, there were no striking changes in the results. In the last column of table 1, statistically significant increase in various types of anomalies other than eye anomalies are listed. The positive controls, trypan blue and ethyleneimine, table 1, and 6-aminonicotinamide, table 2, showed elevated levels of anomalies, although the latter control did not yield consistent results over all dose levels.

Only those test conditions which resulted in statistically elevated incidences of anomalies are listed in tables 1 and 2. Some compounds gave no increase in anomalies (based on the overall incidence if tested in both time periods) when tested in other solvents, strains, or dose levels (table 3). It must be emphasized that failure to detect a statistically significant increase in anomalies may only be a reflection of experimental insensitivity due to experimental and biological variation and insufficient number of litters. Thus, compounds showing no increases cannot be considered nonteratogenic. For example, trypan blue in DMSO at the highest dose level tested, 37.5 mg/kg., did not show an increase in anomalies, possibly due to higher fetal mortality. Standard corrected  $2 \times 2$  chi-square tests (1) were used to compare the proportion of abnormal litters for the compound with the controls in the same solvent. In the cases where tests were conducted in two time periods, the results from the two chi-squares were combined (1). The levels of statistical significance for the combined tests are listed under the total column for proportion of abnormal litters.

The distribution of the proportion of abnormal fetuses *per* litter (tables 1 and 2) for compounds were compared with the appropriate control distribution by use of the nonparametric Mann-Whitney U-test (2). This test requires that the proportion of abnormal fetuses *per* litter is independent from litter to litter, but requires no assumption about the frequency distribution of these proportions. Again, where litters were run in both time periods, the significance level for the combined tests is given under the total column. Bracketed data include groups which were combined before statistical tests were conducted.

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TABLE 1.—Tests which displayed significant increases of anomalies (C57BL/8 mice)

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters			Proportion of abnormal fetuses per litter			Proportion of abnormal fetuses per litter in abnormal litters			Increased mortality	Tests repeated over time	No. of live litters		Increased anomalies other than eye	
			1965	1966-68	Total	1965	1966-68	Total	1965	1966-68	Total			1965	1966-68		
<b>Negative controls:</b>																	
Untreated	None		.42	.39	.40	.06	.11	.10	.18	.28	.25			26	60		
Controls	DMSO		.63	.41	.46	.16	.12	.13	.33	.26	.29			70	112		
Do.	Saline		.62	.37	.43	.13	.10	.11	.24	.28	.26			31	46		
Do.	Honey			.47	.47		.15	.15		.32	.32				32		
<b>Positive controls:</b>																	
Trypan blue	DMSO	5.0 mg	.60		.60	.32		.32	.64		.54	Yes		5		Hydrocephaly.	
Do.	DMSO	12.5 mg	.86		.86	.44***		.44***	.52**		.62**	Yes		7			
Do.	DMSO	37.5 mg	.60		.60	.36		.36	.60		.60	Yes		5			
Do.	Saline	5.0 mg	1.00		1.00	.61***		.61***	.61**		.61**			5		Hydrocephaly.	
Do.	do.	12.5 mg	.71		.71	.49**		.49**	.69***		.69***	Yes		7			
Do.	do.	37.5 mg	.71		.71	.33*		.33*	.46**		.46**	Yes		7			
Ethyleneimine	do.	4.64 µl	1.00*		1.00*	.49***		.49***	.49***		.49***	Yes	No	7			
<b>Experimental:</b>																	
2,4,5-T	DMSO	113 mg		.79**	.79**		.66***	.66***		.71***	.71***	Yes		14		Cleft palate cystic kidney.	
2,4,6-T	Honey	46.4 mg		1.00*	1.00*		.87**	.87**		.87	.87		No	6			
2,4,6-T	do.	113 mg		1.00**	1.00**		.70***	.70***		.70***	.70***	Yes		9		Cleft palate cystic kidney.	
PCNB (days 6-14)	do.	215 mg		.88*	.88*		.25**	.25**		.29	.29		No	8			
PCNB (days 6-14)	do.	464 mg		.67**	.67**		.25	.25		.38	.38			12		Renal agenesis.	
PCNB (days 6-10)	do.	464 mg		1.00	1.00		.38	.38		.37	.37		No	10			
Captan	DMSO	100 mg	1.00*	.61	.71***		.66***	.27		.35***	.68**	.44	.49**	6	18		
Folpet	DMSO	100 mg		.77**	.77**		.29***	.29***		.20***	.38*			6	13		
2,4-Isocetyl ester	DMSO	48 µl	1.00*		1.00*		.24	.24		.24	.24			6			
2,4-D Isocetyl ester	DMSO	130 µl		.67	.67		.26**	.26**		.41*	.41*				15		
2,4-D Butyl ester	DMSO	100 µl		.76**	.76**		.25***	.25***		.34	.34				20	Agnathia.	
2,4-D Isopropyl ester	DMSO	94 µl		.70**	.70**		.26***	.26***		.37*	.37*				20		
Carbaryl	DMSO	100 mg	1.00*	.54	.71**		.46***	.16		.26**	.46*	.29	.37		6	11	Hydrocephaly, skeletal
IPC	DMSO	850 mg	1.00**	.43	.71*		.46***	.09		.27**	.46*			7	7		
α-Naphthol	DMSO	10 mg		.86	.86		.33*	.39*		.38	.38			7			
2,4-D Methyl ester	DMSO	106 mg		.83	.83		.30*	.30*		.36	.36				6		
Carbaryl+Piperonyl Butoxide	DMSO	10 mg + 100 µl		.60	.60		.13	.12		.26	.26		No	6		Cystic kidney	
Do.	DMSO	46.4 mg + 464 µl		.60	.60		.10	.10		.21	.21			12			

Significance level: \*(.10). \*\*(.05). \*\*\*(.01).

TABLE 2.—Tests which displayed significant increases of anomalies (AKR mice)

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters		Proportion of abnormal fetuses per litter		Increased mortality	Tests repeated over time	No. of live litters	Special anomalies	
			11/66	12/66††	Total	11/66					12/66††
Negative controls:											
Control	DMSO		.06	.37	.21	.01	.06	.03	.11	.15	
Do.	Honey		.00	.00	.00	.00	.00	.00	.00	.00	37 35 12
Positive controls:											
6-amino-nicotina-mide.	DMSO	.34 mg	.56 ***	.56 ***	.56 ***	.31 **	.31 **	.31 **	.55	.55	9
6-amino-nicotina-mide (I).	DMSO	.68 mg	.00	.00	.00	.00	.00	.00	.00	.00	7
Experimental:											
2,4,5-T	DMSO	113 mg	.50 ***	1.00 **	.71 ***	.20 **	.40 ***	.29 ***	.40 *	.40 *	8
Do.	Honey	113 mg	1.00 ***	1.00 ***	1.00 ***	.54 ***	.54 ***	.54 ***	.94	.94	yes
2,4,5-T	DMSO	98 mg	.43 **	.29	.36 *	.12	.06	.08	.28	.16	7
2,4-D	DMSO										7

\*Significance Level .10. \*\*Significance Level .05. \*\*\*Significance Level .01.  
††Through 11/66 †††After 11/66  
Note: (I) With the .68 mg/kg dose, as compared to the .34 mg/kg dose, fewer implants-  
tions and a higher fetal mortality were encountered, resulting in fewer live fetuses per litter.

TABLE 3.—Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
2,4,5-T	C57	DMSO	21.5 mg.	-----	6
PCNB (days 10-14)	C57	Honey	464 mg.	-----	9
PCNB	AKR	Honey	464 mg.	-----	9
Captan	C57	Honey	100 mg.	-----	12
Do.	AKR	DMSO	100 mg.	-----	13
Folpet	C57	Honey	100 mg.	-----	5
Do.	AKR	DMSO	100 mg.	-----	13
2,4-D Isooctyl ester	C3H	DMSO	48 µl.	-----	6
Do.	A/Ha	DMSO	24 µl.	-----	5
Do.	AKR	DMSO	130 µl.	-----	8
2,4-D Butyl Ester	C57	DMSO	46 µl.	-----	6
Do.	AKR	DMSO	100 µl.	-----	10
2,4-D Isopropyl Ester	C57	DMSO	46 µl.	-----	6
Do.	AKR	DMSO	94 µl.	-----	6
Carbaryl	C3H	DMSO	100 mg.	-----	8
Do.	C57 × AKR	DMSO	100 mg.	-----	6
Do.	AKR	DMSO	464 mg.	-----	13
IPC	C3H	DMSO	850 mg.	-----	11
IPC	AKR	DMSO	850 mg.	-----	13
2,4-D Methyl Ester	AKR	DMSO	106 mg.	-----	7
Do.	C57 × AKR	DMSO	106 mg.	-----	5
o,p'-DDD	C57	DMSO	100 mg.	-----	13
Do.	AKR	DMSO	100 mg.	Yes	12
2,4-D	C57	DMSO	100 mg.	-----	16
Do.	C57	Honey	100 mg.	-----	12
Do.	C3H	DMSO	100 mg.	-----	6
Do.	C57 × AKR	DMSO	98 mg.	-----	11
Zectran	C57	DMSO	10 mg.	-----	7
Do.	AKR	DMSO	10 mg.	-----	7
Thiram	C57	DMSO	10 mg.	-----	8
Do.	AKR	DMSO	115 mg.	-----	7
Ferbam	C3H	DMSO	4.64 mg.	-----	6
Do.	C57	DMSO	4.64 mg.	-----	6
Monuron	C3H	DMSO	215 mg.	-----	7
Do.	C57	DMSO	215 mg.	-----	13
Do.	C57	Honey	215 mg.	-----	9
Do.	AKR	DMSO	215 mg.	-----	13
Diuron	C3H	DMSO	215 mg.	-----	6
Do.	C57	DMSO	215 mg.	-----	6
2,4-D Ethyl Ester	C57	DMSO	215 mg.	-----	6
Do.	AKR	DMSO	86 µl.	-----	7
Atrazine	C3H	DMSO	86 µl.	-----	7
Do.	C57	DMSO	46.4 mg.	-----	6
Do.	C57	DMSO	46.4 mg.	-----	13
Do.	AKR	DMSO	46.4 mg.	-----	15

TABLE 3.—Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)—Continued

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
Piperonyl Butoxide	C3H	DMSO	1000 $\mu$ l	-----	6
Do.	C57	DMSO	1000 $\mu$ l	-----	6
Do.	C57	DMSO	21.5 $\mu$ l	-----	6
p,p'-DDD	C57	DMSO	46.4 mg.	-----	6
p,p'-DDT	C57	DMSO	46.4 mg.	-----	6
Carbaryl + Nicotinamide	C57	DMSO	100+61 mg.	-----	10
Nicotinamide	C57	DMSO	61 mg.	Yes	6
CIPC	C57	DMSO	1000 mg.	-----	6
Nabam	C3H	DMSO	21.5 mg.	-----	6
Do.	C57	DMSO	46.4 mg.	-----	6
Do.	C57	Saline	46.4 mg.	-----	14
Do.	AKR	DMSO	46.4 mg.	-----	5
Do.	AKR	Saline	46.4 mg.	-----	14
Propazine	C3H	DMSO	464 mg.	-----	6
Dieryl	C57	DMSO	21.5 mg.	-----	6
Perthane	C57	DMSO	100 mg.	-----	6
Ovex	AKR	DMSO	185 mg.	-----	7
Tedion	AKR	DMSO	217 mg.	-----	6
Amitrol	C57	Saline	464 mg.	-----	13
Do.	C57	Honey	215 mg.	Yes	8
Do.	AKR	Saline	464 mg.	-----	14

#### Human studies

Epidemiologic data on possible effects of pesticides on human reproduction and teratology are grossly inadequate. Prospective studies on this subject are difficult to design and almost nonexistent, except for the community pesticide program of the Food and Drug Administration.

**Chlorinated hydrocarbons.**—In a recent review (Khera and Clegg, 1969), no adverse human reproductive effects were attributed to DDT and other chlorinated hydrocarbons. Studies on 240 pregnant women indicated that 21 percent had significant first trimester pesticide exposure, and that 52 percent were exposed during their entire pregnancy. No statistical difference in numbers of patients with anomalies existed between these exposed groups (Nora et al., 1967). Low values of DDT residues have been found in a small number of human placentas (Rappolt et al., 1969). Sharply reduced tissue levels were also found in 68 newborn infants (Zavon, 1969). Pesticide levels in human milk have not shown any relation to perinatal toxicity (Laug et al., 1951; Lofroth, 1969; Curley and Kimbrough, 1969). Studies on 152

mothers showed transplacental passage of DDT and DDE (O'Leary, 1969). Low placental and high vernix levels were noted; fetal blood levels were one-half maternal levels. In a similar study on premature infants (O'Leary, 1969), high fetal levels were noted; no relationship between maternal blood levels of DDE and DDT and the incidence of first trimester spontaneous abortion were found, although the number of pregnant women reported on was inadequate for firm conclusions.

**Organophosphates.**—Evidence of teratogenic potential of organophosphates in humans has been reviewed and found inconclusive (Khera and Clegg, 1969).

**Mercurials.**—Consumption by Japanese pregnant women of fish and shellfish contaminated by methylmercury produced a high incidence of infantile cerebral palsy (Matsumoto et al., 1965). This condition has been termed fetal Minamata disease.

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