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TOXICOLOGY RESEARCH LABORATORY

HEALTH AND ENVIRONMENTAL RESEARCH

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

TESTIMONY FOR 2,4,5-T HEARING: TERATOGENIC POTENTIAL OF 2,4,5-T AND CHLORINATED DIBENZO-P-DIOXINS

B. A. SCHWETZ

DRAFT FOR THE PURPOSE OF CIRCULATING FOR COMMENTS AND CRITICISM

2/25/74

INTRODUCTION FOR BERNARD A. SCHWETZ

Dr. Schwetz received his Doctor of Veterinary Medicine (D.V.M.) degree from the University of Minnesota in 1967. During part of his years as a student in the College of Veterinary Medicine, he was also a trainee financed by the National Institutes of Health doing research involving studies of antidotes for thallium poisoning. In June of 1967, Dr. Schwetz started a graduate program in the Dept. of Pharmacology in the College of Medicine of the University of Iowa, Iowa City, Iowa. He received his Masters of Science degree in August of 1968 following completion of a research project with Dr. Gabriel L. Plaa involving studies of the role of catecholamines in carbon tetrachloride induced hepatotoxicity. He received his Ph.D. degree from the same department in May of 1970. His major advisor was a teratologist, Dr. Bernard A. Becker. His research project for the Ph.D. degree was a study of the effect of inhalation anesthetics on the developing embryo and fetus of laboratory animals.

Since receiving his Ph.D. degree, Dr. Schwetz has worked as a toxicologist in what was originally called the Biochemical Research Laboratory of The Dow Chemical Company in Midland, Michigan. He was in charge of the unit that was responsible for teratology and reproduction studies. At the present time, Dr. Schwetz is in charge of teratology, reproduction and

mutagenesis studies, as well as chronic dietary feeding studies in the Toxicology Research Laboratory of Health and Environmental Research of Dow Chemical U.S.A., Midland, Michigan 48640.

Dr. Schwetz has been involved in a variety of professional and research activities. His research efforts have involved studies with heavy metals, catecholamines, hepatotoxins, toxicity studies in neonates, studies of the transfer of chemicals across the placenta, teratology studies with inhalation anesthetics, herbicides, chlorinated phenolic compounds, ion exchange resins, inhaled solvents and a variety of other compounds, as well as reproduction studies in rodents and Japanese Quail. He has authored or co-author over 15 publications on his research projects in toxicology. All of these have been published in recognized scientific journals in the areas of toxicology or pharmacology. He has presented 11 papers at meetings of professional societies—the Federation Meetings, the Society of Toxicology and the American Society for Pharmacology and Experimental Therapeutics. Dr. Schwetz is a member of the Society of Phi Zeta, the national honorary veterinary society, the American Veterinary Medical Association, and the Industrial Veterinarians Association, The Society of Toxicology, the Environmental Mutagen Society and the Society of Teratology.

At the present time, Dr. Schwetz is the Project Manager of a contract with the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. This project has been ongoing for over one year and is expected to continue for several years in the future. The awarding of this contact to Dr. Schwetz's group by the Governmental agency (NIEHS) from among a large number of universities, research institutes and industrial laboratories recognizes the expertise developed by Dow in this area.

Dr. Schwetz is listed or will be listed in the American Men and Women of Science and Who's Who in the Midwest.

2/25/74

TESTIMONY FOR THE HEARING ON 2,4,5-T: TERATOLOGY STUDIES

The purpose of this testimony is to review and interpret the various studies that have been conducted to evaluate the effect of 2,4,5-T and the chlorinated dibenzo-p-dioxins on the developing embryo and fetus. In order to facilitate the interpretation of these studies by unexperienced individuals, the first part of this testimony will be a discussion of the methods of conducting teratology studies and some of the problems that are involved in interpreting the results. Based on the criteria that I will present for the design, conduct and interpretation of teratology studies, my overall conclusion is that 2,4,5-T as currently manufactured does not represent a teratogenic hazard to human beings. High dose levels of 2,4,5-T have been associated with teratogenic responses in certain strains of mice, but this effect has not been observed among other animal species. The dose levels of 2,4,5-T required to cause any kind of embryotoxic effect are far in excess of those which may be incurred with normal manufacture and use of 2,4,5-T. With regard to the highly toxic contaminant in 2,4,5-T, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), untoward effects on the embryo and fetus associated with the presence of this in 2,4,5-T are discernible only when the level of contamination exceeds 1 ppm. Thus, the TCDD content of currently produced 2,4,5-T containing a maximum of 0.1 ppm does not represent a teratogenic hazard. Those conclusions are

based on the review and interpretation of the existing literature as presented in Part II of this testimony. As I indicated previously, Part I of this testimony is a general review of the methods of conducting teratology studies and interpreting results. This first part does not apply specifically to 2,4,5-T or TCDD, but instead applies to teratology studies on any chemical whether it be a drug, pesticide or other environmental contaminant.

PART 1, 2,4,5-T TESTIMONY: TERATOLOGY STUDY METHODOLOGY AND PROBLEMS OF INTERPRETATION OF RESULTS

Teratology can be defined as the study of the toxic effect of physical, chemical and infectious agents on the developing embryo and fetus (Slide 1). Prior to the early 1960's investigations of the cause of fetal malformations were conducted almost exclusively by nutritionists and pediatricians. Experimental teratology as studied by toxicologists, pharmacologists, anatomists, pathologists and clinicians has developed since the early 1960's as a result of the thalidomide tragedy. While the initial concern was with exposure to drugs, that concern has expanded today to include all things to which pregnant females are exposed.

The ultimate teratology experiment (Slide 2) would of course be performed in humans. However, because of ethics, prospective studies cannot be performed in humans. Unfortunately, retrospective studies of case histories have not provided enough information to serve as a reliable predictive measure. There are several reasons for limitations of retrospective studies. For instance, it is often difficult to establish that the woman was actually exposed to a specific compound during the critical period of pregnancy. A survey reported in 1967 indicated that on an average, mothers took between 3 and 4 potential teratogens during pregnancy (15). A more recent survey, reported in 1973

(16), revealed that an average of 10 drugs were consumed by pregnant women in this country, including pain killers, antihistamines, diuretics, antibiotics, drugs to suppress nausea and appetite, vitamins. The data source is frequently limited to prescription records or an individual's memory, neither of which is sufficiently precise. Home remedies and household chemicals are most frequently ignored or completely overlooked. In addition, the number of case histories is far too small to draw precise conclusions. Thus, human studies either prospective or retrospective, are not the answer. We must be prepared to accept alternate test methods.

Prior to 1966, the effects of chemicals on the developing embryo and fetus were assessed indirectly by conducting multiple generation reproduction studies (Slide 3). In these studies, male and female mice or rats were fed the chemical through a series of generations and the offspring were examined for evidence of malformations (14). Primarily, the information obtained in these studies assessed the potential of the chemical to interfere with conception and lactation or to induce toxicity in the newborn pups. The test did not adequately assess the teratogenic potential of the compound under test because pups having morphological alterations sufficiently severe to be recognized either die or are killed in the mother and eaten. Although the information gleaned from reproduction studies is useful in assessing the safety of a chemical, definitive assessment of teratogenic potential is minimal.

Since 1966, various government agencies such as the Food and Drug Administration and the Environmental Protection Agency have provided guidelines for performing teratology studies which indicate the species to be studied, the number of animals to be used and the regimen of administration of the agent - dose, route and frequency of administration (Slide 4, ref. 19,20,21,22). Teratology studies should be done in at least two species of laboratory animals. Generally, rabbits are used in addition to either mice or rats. Enough animals should be used to allow for appropriate statistical evaluation; this requires at least 20 pregnant rodents per treatment group and at least 10 pregnant nonrodents per group. At least two dose levels should be used. The high dose level should be the highest that can be given to pregnant females without causing signs of toxicity (death, weight loss, lack of appetite, etc.). The low dose level should be some multiple of an expected use level. Frequently, it is desirable to establish the dose-response relationship as well as a level which causes no discernible untoward effects.

The pregnant females must be treated during the critical period of gestation; in the mouse and rat, this is on days 6-15 of gestation, in the rabbit on days 6-18. The route of administration should be the one of most likely intended use or exposure. This may be by inhalation, oral ingestion, application to the skin or by injection under the skin or in the abdominal cavity.

As will be pointed out in the review of the 2,4,5-T teratology studies, in many cases too few animals were used or the 2,4,5-T was given by an inappropriate route and the effect of treatment on the health-status of the pregnant mother was not indicated. The results of such studies are difficult to interpret.

In the typical teratology study, groups of pregnant females are treated with the test material during the critical period of gestation (Slide 5). One day prior to normal delivery, one-half of the group is delivered by cesarean section and the fetuses are examined for sex, body measurements and external conformation and are examined internally for soft tissue anomalies. The skeletons are then preserved, stained and examined for malformations. The remaining pregnant females are allowed to deliver their young naturally and they are observed until weaning for evidence of abnormalities of development or survival.

Early teratology studies in our laboratory, as well as the results of teratology studies reported by others, demonstrated the need for definitions of the terms embryotoxicity, fetotoxicity and teratogenicity (Slide 6, ref. 3). Embryotoxicity is defined as the toxic effect on an embryo caused by treating pregnant females during the period of tissue differentiation and organogenesis. Fetotoxicity is the toxic or degenerative effect on already formed fetal tissues and organs. Embryo- and

fetotoxicity include a range of responses which extends from no functional or morphological significance to death of the embryo or fetus. Teratogenicity is that degree of embryotoxicity which interferes with normal morphological or functional development or survival of the offspring. The use of these definitions allows one to distinguish a serious degree of embryotoxicity, a teratogenic response, from minor forms of embryotoxicity which are of little or no consequence. Examples of responses which are considered teratogenicity (Slide 7) include cleft palate, missing lens, missing eyes or kidneys, malpositioned heart, failure of the abdominal wall to hold in the intestines, missing lower jaw or missing ribs or vertebrae, uncovered brain or spinal cord and malformed or missing limbs.

Responses which are considered embryotoxicity but not teratogenicity include fetal deaths, decreased body measurements, accumulation of fluid beneath the skin, delayed development of normal bones, the presence of extra or wavy ribs and delayed development of the sternum (2,4). In some cases, delayed development of bones could be a teratogenic response if it were serious enough in extent or location to interfere with development or survival.

Some embryotoxic responses may or may not be considered terata, depending upon the degree of involvement. Examples of these include smaller than normal eyes, dilated ventricles of the

brain (hydrocephalus or water head), hemorrhage in any one of various places in the body, larger than normal space within the kidney tissue or dilated ureters (the tubes from the kidney to the bladder), failure of the testicles to descend from the abdominal cavity, shorter or longer than normal lower jaw, missing or extra parts in the sternum or breast bone, fused ribs or fused bones in the sternum. It is emphasized that untoward effects not deemed teratogenic effects are still important in assessing the safety of a chemical. Although effects not deemed teratogenic are mild and reversible, exposure to levels of materials which induce such effects should not be incurred unnecessarily. A carefully and adequately conducted teratology study should include documentation of all discernible effects on both the mother and fetuses.

The usual dose-response relationship between embryotoxicity, teratogenicity and lethality is indicated on Slide 8. On the next three slides, I have indicated the percent of litters or the fetal population showing a given response - toxicity, teratogenicity or lethality - at various dose levels administered to pregnant females. At some dose level, a toxic response is usually observed in the fetal population and as the dose level increases, the percent of the fetal population involved also increases until many pups show some toxic response (5). At some higher dose level of many substances, a teratogenic response is observed. Likewise, as the dose level increases, the

proportion of litters or fetuses which show a teratogenic response also increases. At still higher dose levels, fetal death is observed until at some high dose level all of the embryos or fetuses are killed. Whether or not a compound is a serious teratogen is determined by the width of the dose range over which a teratogenic response is observed and how close the teratogenic dose level is to the dose level which causes a minor toxic response. A compound which would not be considered a serious hazard because of it teratogenic potential is depicted on Slide 9. The dose range is narrow over which a teratogenic response is observed. In addition, teratogenic effects were observed at dose levels which were considerably higher than those which caused other forms of toxicity, in fact at dose levels higher than required to cause death of some of the embryos or fetuses. In contrast, a compound which would be considered a potentially serious teratogen is depicted on Slide 10. The dose level which is sufficient to cause the teratogenic response is just slightly higher than one which would cause a minor toxic response and is somewhat lower than that required to cause death of the embryo or fetus. A compound with such a dose response relationship would be considered a potentially serious teratogen particularly if the fetus was more sensitive to the toxicity than the mother. Whether or not the compound represented a real teratogenic hazard would depend upon the likelihood of human exposure to high enough dose levels.

Certain problems are inherent in teratology studies and in some cases have made it difficult to interpret the results. Examples of this are species differences in susceptibility and the problem of false positives. First of all, regarding species differences in susceptibility (Slide 11), it must be remembered that almost all teratogens manifest their influence through a susceptible genetic locus (1); that is, they increase the incidence of naturally occurring birth defects. The effect on the genetic locus may be associated with a direct interaction of the agent at that locus. Most frequently, the effect is mediated by the agent indirectly; for example, via physiological change such as that associated with stress. Thus, it is important to consider the incidence of spontaneous defects among animal species. Examples are as follows: among rabbits, 10% of offspring have some defect; mice, about 4%; rats, about 3%; man 2-3% and monkeys and dogs, less than 1/2%. Thus, since animal species vary greatly in their incidence of naturally occurring birth defects, it is only reasonable to expect to find species differences in susceptibility to teratogens. An example of a drug which has been erroneously used to demonstrate species differences in susceptibility is thalidomide (Slide 12). If one considers the percent response (abnormal fetuses, death fetuses or maternal toxicity) over a range of dose levels in various species, one finds that the ratio between the dose level required to cause fetal abnormalities and that required to cause maternal toxicity is fairly constant from one species to another, even though the absolute dose level required to

cause fetal abnormalities varies between species. The data are much more meaningful if one considers equivalent effects and equivalent dosages rather than identical effects at identical dosages (1,17,18,19,23). The teratogenic effect of thalidomide is dependent upon blood levels and oral dosing of small animals results in much lower blood levels of thalidomide than do the same doses in man. When the drug is given intravenously to small animals to give blood levels equal to those in man, teratogenesis results. Using my previous premise for revealing a compound constituting a teratogenic hazard, the now well known teratogenic hazard of thalidomide would have been revealed by studies conducted in any of the indicated species.

Another problem is that of false positive results, compounds which cause malformations in animals but not in man (Slide 13). For example, it has been reported that aspirin is a teratogen in rats but not in man (1). If one compares the percent response to the dose level of aspirin in mg/kg/day required to cause those responses, it is evident that the dose level required to cause fetal abnormalities and fetal deaths also cause maternal toxicity. In humans, aspirin is not usually given at dose levels which cause serious maternal toxicity. Thus, the possibility exists that aspirin given to pregnant women at toxic dose levels might be teratogenic. Cancer chemotherapeutic agents have a dose response curve in animals similar to that of aspirin, that is, embryotoxic effects occur at dose levels

where a maternally toxic effect is evident. These agents are used at toxic dose levels in humans and are teratogenic in humans.

Another important factor to consider is that decisions should not be based on single isolated tests (Slide 14). All of the available data should be considered -- pharmacological, metabolic and toxicological. Examples where the results of a single test might be misleading are as follows. Meclizine is teratogenic in rats (10,11). In the rat, meclizine is metabolized to norchlorcyclizine which has been demonstrated to be the teratogen in rats (12). In man, meclizine is excreted as the conjugate and is not converted to norchlorcyclizine (13). Imipramine is teratogenic in rabbits (7). The rabbit does not rapidly metabolize imipramine and blood levels are maintained for long periods of time after administration (8,9). In man, imipramine is rapidly demethylated to the central nervous system-active agent desmethylimipramine (6). Thus, species specificity for the metabolic alteration of a compound or for the capacity to detoxify or eliminate the agent from the body may give rise to both qualitative and quantitative differences in the response of various species.

In summary (Slide 15), there are several factors that are critical to the relevancy of teratology studies. First, the studies should be done in the appropriate species. Second, enough animals should be used to allow for appropriate

statistical evaluation. Third, appropriate dose levels should be used. Fourth, the animals should be treated during the critical period of gestation. Fifth, the material should be administered by the route that is intended or expected for man. Sixth, decisions should be based on consideration of all available data. These criteria, which are generally accepted as being very important in the design, conduct and interpretation of the results of teratology studies, should be considered in the evaluation of each of the studies reviewed in the subsequent section of this testimony.

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TERATOLOGY — the study of the toxic effects of physical, chemical and infectious agents on the developing embryo and fetus.

Experimental Teratology ⟷ Thalidomide

Thorough Testing BEFORE Approval

ULTIMATE TERATOLOGY EXPERIMENT

HUMANS

A. Prospective – obviously unlikely

B. Retrospective – case histories

Problems

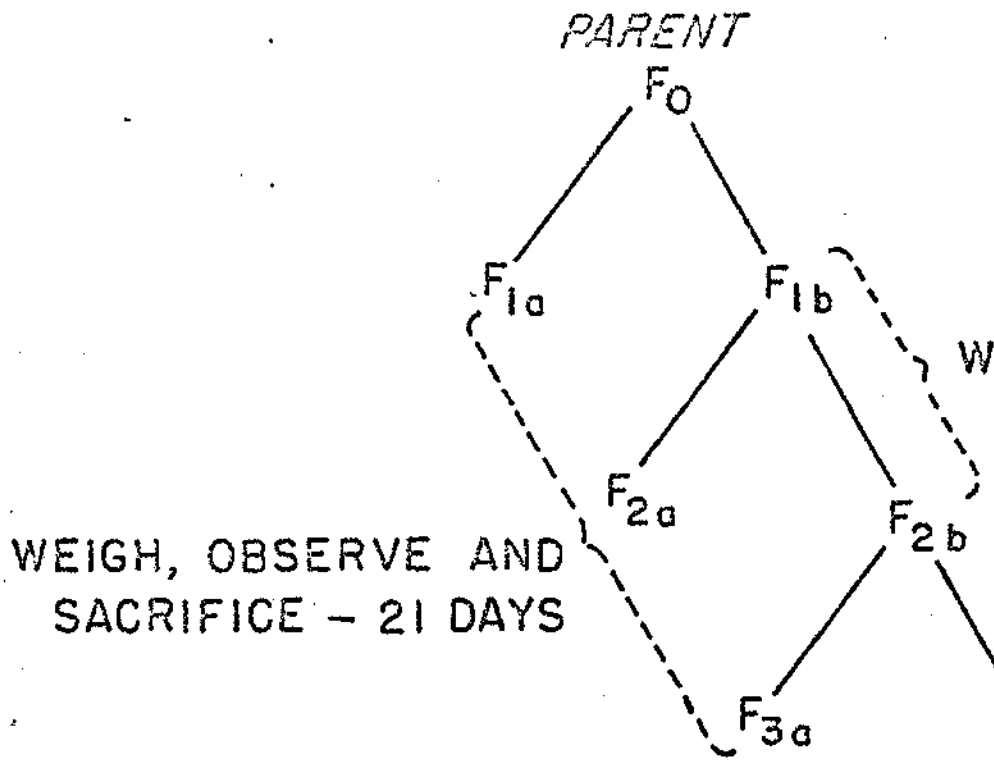
1. Use during critical period ?
2. Mothers – 3.7 potential teratogens.
3. Data source – prescription records
   women's memories.
4. Home remedies and household
   chemicals ignored or over-
   looked.
5. Number of case histories too small.

Thus, human studies not the answer.

Must accept alternate test method.

TEST PROCEDURES PRIOR TO 1966

MULTIGENERATION REPRODUCTION TEST



TEST PROCEDURES SINCE 1966

FDA GUIDELINES FOR TERATOLOGY STUDIES.

EXPERIMENTAL DESIGN

1. *SPECIES*

   at least 2

   1. mouse
   2. rat
   3. rabbit

2. *NUMBER OF ANIMALS*

   at least 20 pregnant rodents/group
   10 pregnant non rodents

3. *DOSE LEVELS*

   at least 2 levels
   1. high - maximum tolerated
   2. low - multiple of use level

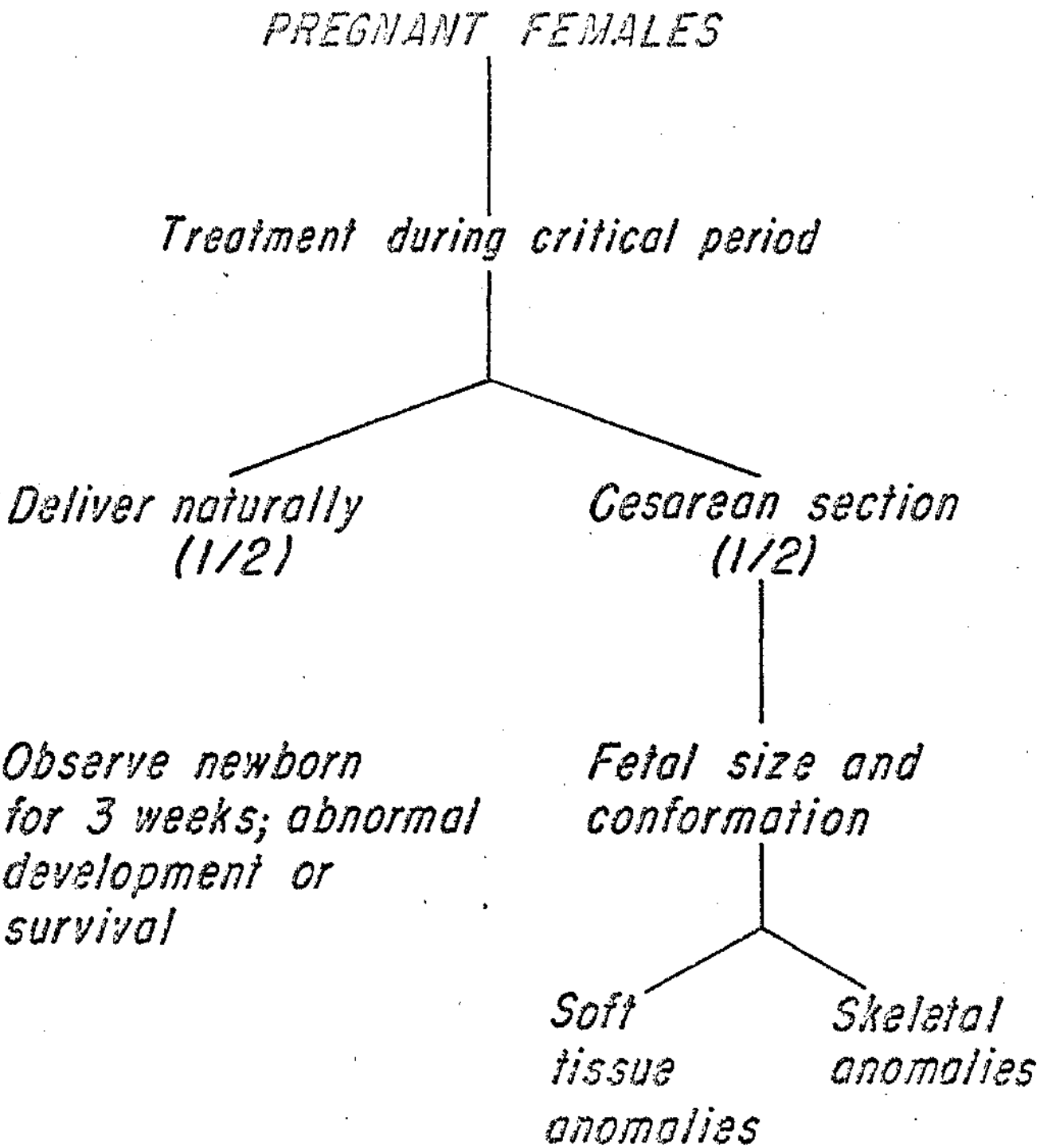
4. *PERIOD OF DOSING*

   mouse, rat - days 6-15
   rabbit — days 6-18

5. *ROUTE OF ADMINISTRATION*

   route of intended use or most likely

TERATOLOGY STUDY EXPERIMENTAL DESIGN



DEFINITIONS

EMBRYOTOXICITY

the toxic effect on an embryo caused by treating pregnant females during the period of tissue differentiation and organogenesis.

FETOTOXICITY

the toxic or degenerative effect on already formed fetal tissues & organs.

embryo- and fetotoxicity include a range of responses which extends from no functional or morphologic significance to death of the embryo or fetus.

TERATOGENICITY

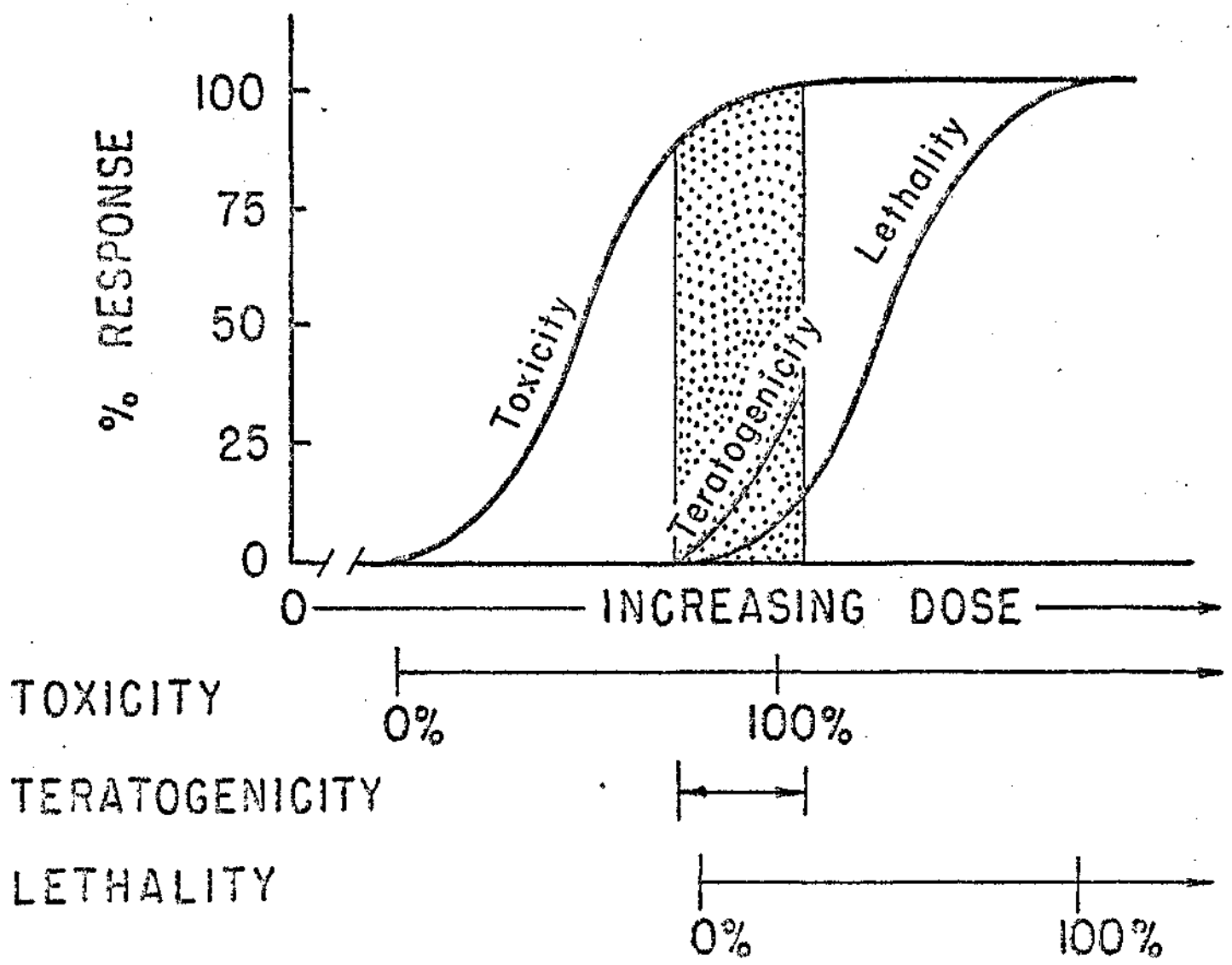
that degree of embryotoxicity which interferes with normal development or survival of the offspring.

SLIDE 7

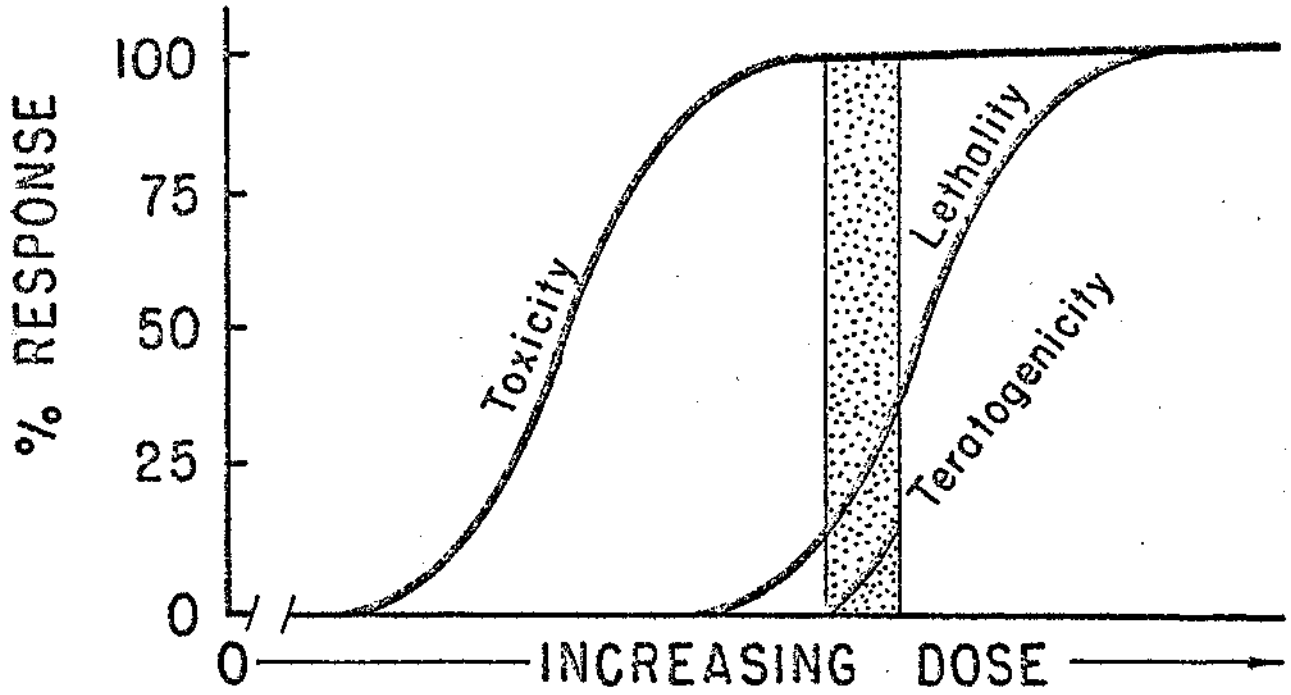
TERATOGENIC vs EMBRYOTOXIC RESPONSES

TERATOGENIC	← (Depends on Degree) →	EMBRYOTOXIC
cleft palate	microphthalmia	resorptions
aphakia	hydrocephalus	decreased body measurements
anophthalmia	hemorrhage	
renal agenesis	hydronephrosis	
malpositioned heart	hydroureter	subcutaneous edema
gastroschisis	cryptorchid	delayed ossification of bones
agnathia	hypognathia	
missing ribs, vertebrae	micrognathia	lumbar ribs
exencephaly	missing sternebrae	wavy ribs
spina bifida	extra sternebrae	
malformed limbs	fused ribs	unfused centers of ossification
missing limbs	fused sternebrae	

DOSE-RESPONSE RELATIONSHIP FOR EMBRYOTOXICITY, TERATOGENICITY & LETHALITY



DOSE-RESPONSE RELATIONSHIP FOR EMBRYOTOXICITY, TERATOGENICITY & LETHALITY

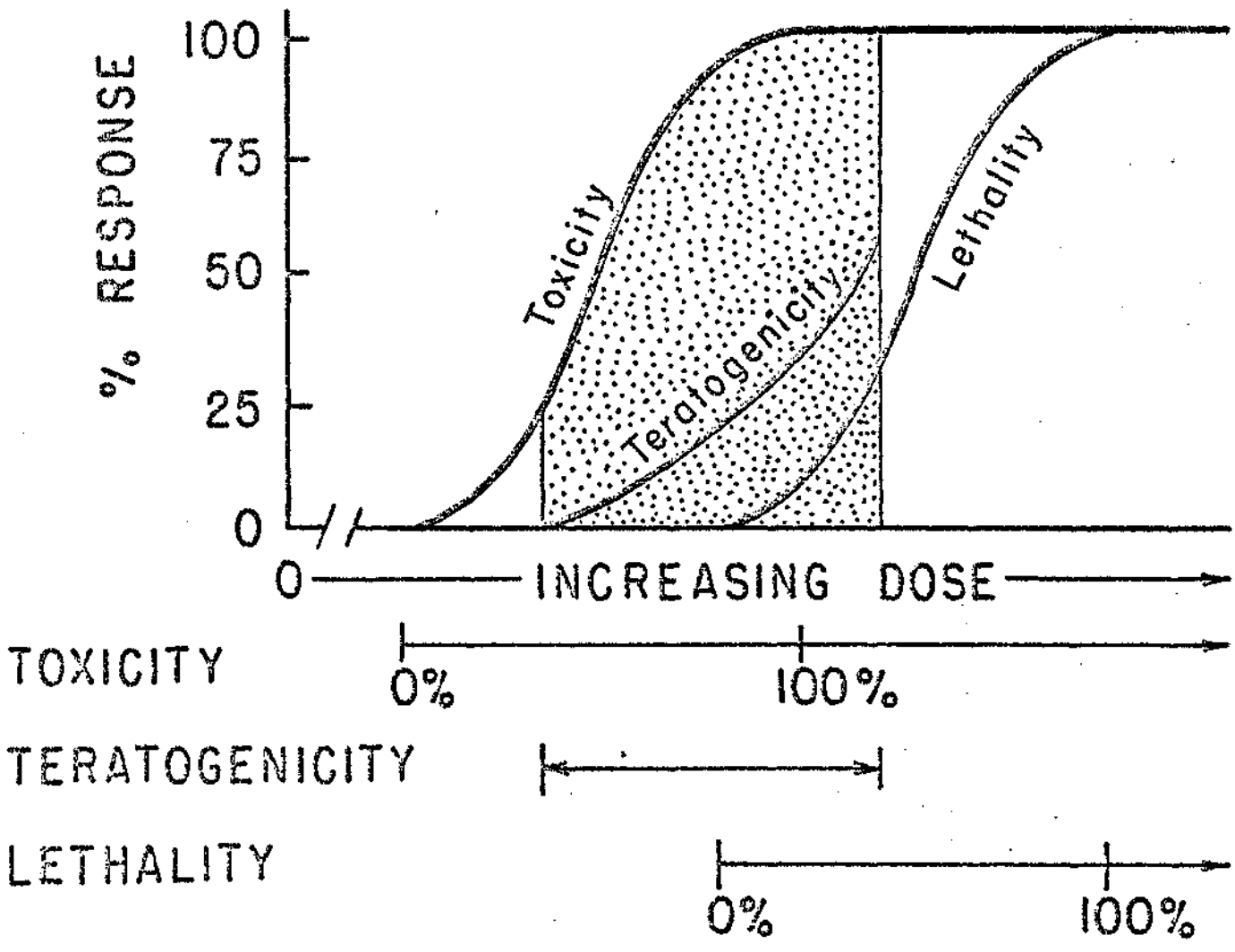


TOXICITY 0% 100%

TERATOGENICITY

LETHALITY 0% 100%

DOSE-RESPONSE RELATIONSHIP FOR EMBRYOTOXICITY, TERATOGENICITY & LETHALITY

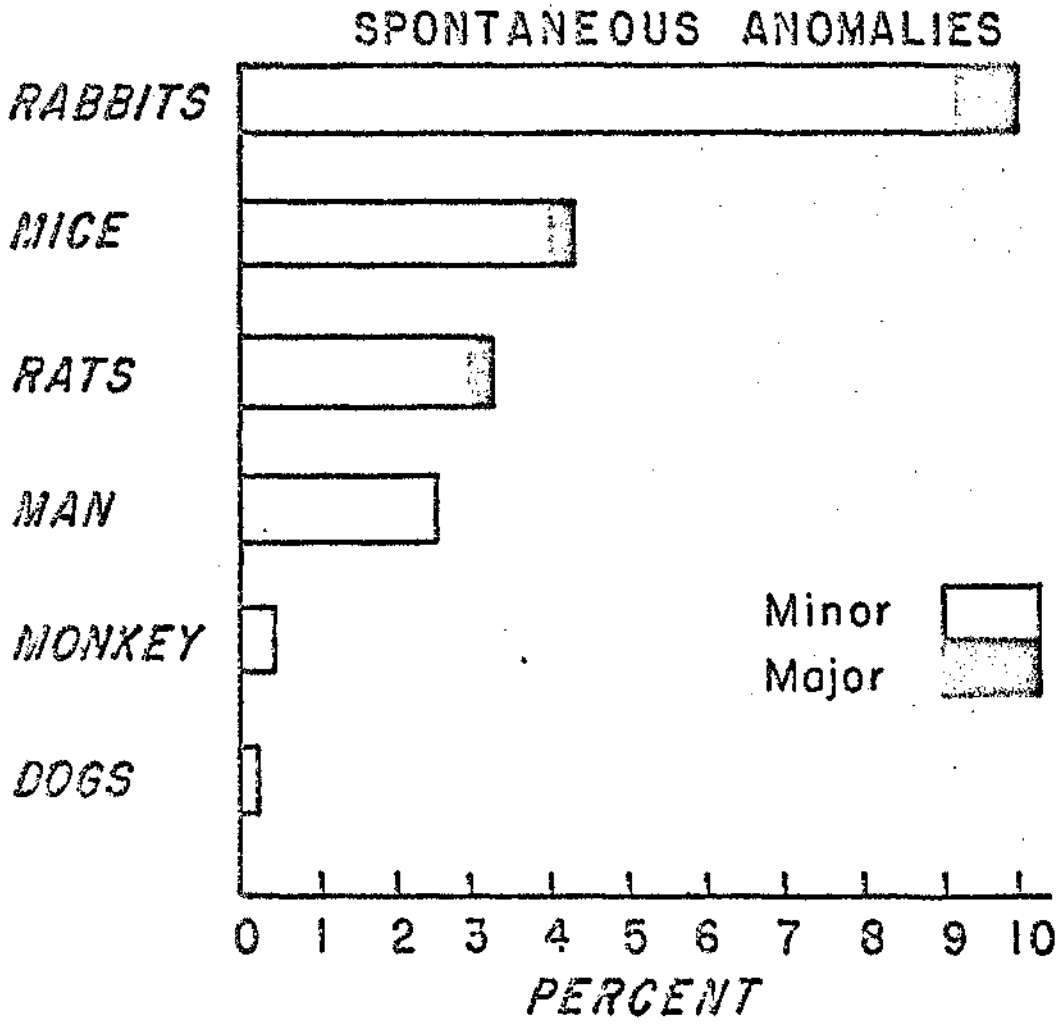


PROBLEMS INHERENT IN TERATOLOGY STUDIES

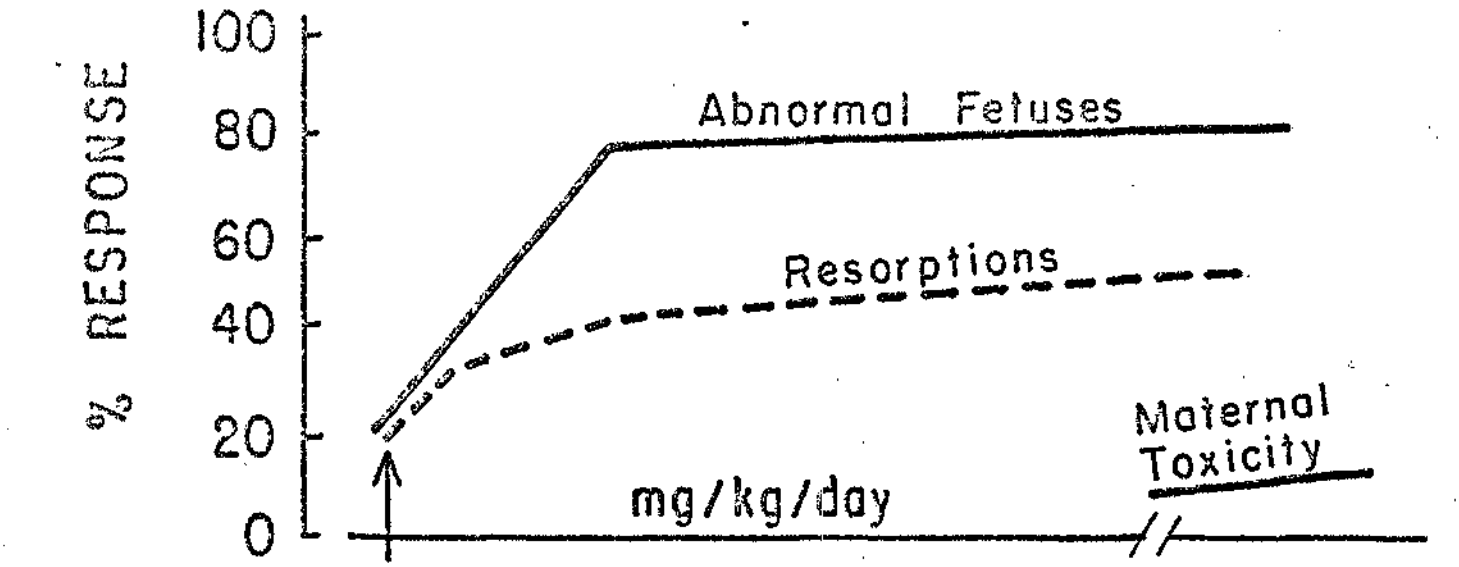
1

SPECIES DIFFERENCES IN SUSCEPTIBILITY

**Almost all Teratogens manifest their influence through a susceptible genetic locus. They increase the incidence of naturally occurring congenital malformations.*



SPECIES COMPARISON - THALIDOMIDE



RABBIT	25	150	500	5000
MOUSE	30		600	
RAT	50		1000	
MONKEY	10		200	
MAN	1		20	

Equivalent effects and equivalent dosages, NOT identical effects at identical dosages.

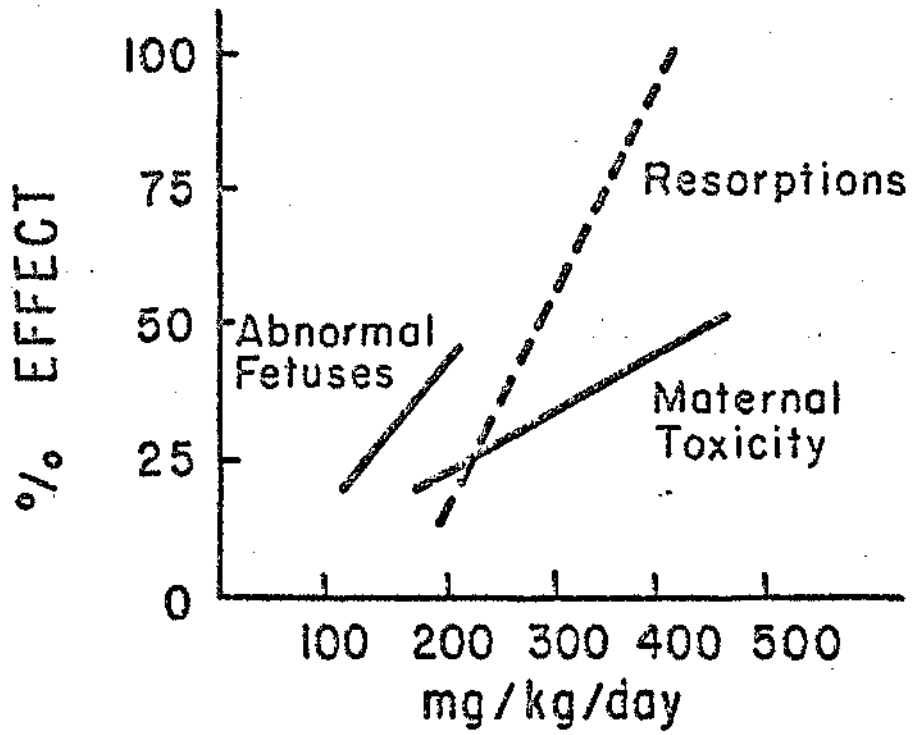
PROBLEMS

2

FALSE POSITIVES —

malformations in animals but not in man.

Example — ASPIRIN (Rats)



Anti-Tumor Agents —— similar dose response curve

1. used at toxic dose levels

2. are embryotoxic in man

Decisions should not be based on single, isolated tests —— consider all data —— pharmacological, metabolic and toxicological.

Example —— Teratogenicity of

Meclizine ——— rats
Imipramine — rabbits

Teratogenicity caused by metabolites specific to the species affected.

FACTORS CRITICAL TO THE RELEVANCY OF TERATOLOGY STUDIES

1. Species
2. Number of animals
3. Dose levels
4. Period of treatment
5. Route of administration
6. Consideration of all data

PART II, 2,4,5-T TESTIMONY: STUDIES OF THE TERATOGENIC POTENTIAL OF 2,4,5-T AND CERTAIN CHLORINATED DIBENZO-P-DIOXINS

A large number of teratology studies have been conducted using a variety of laboratory and non-laboratory animal species in order to evaluate the teratogenic potential of 2,4,5-T. Studies have been reported on mice, rats, hamsters, rabbits, sheep, rhesus monkeys and reindeer using samples of 2,4,5-T with varying degrees of purity. The content of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (hereafter referred to as TCDD) ranged from less than 0.01 ppm to approximately 30 ppm. In certain studies, 2,4,5-T and TCDD were administered simultaneously, using dose levels of 50 to 100 mg/kg/day of 2,4,5-T in combination with 0.01 to 3 µg/kg/day of TCDD. Considered overall, these studies indicate that purified 2,4,5-T is teratogenic in mice when administered at high dose levels. Dose levels of 45 mg 2,4,5-T/kg/day or higher were generally associated with cleft palate and/or kidney malformations in mice. 2,4,5-T was not found to be teratogenic in other species of mammals. Even at high dose levels, administration of 2,4,5-T to mammalian species other than the mouse was associated primarily with adverse effects on the development of the skeletal structures, changes which reflect retarded development rather than malformations and are very likely associated with the stress of being given large doses rather than the direct interaction of the agent with genetic material.

The potential for 2,4,5-T to have an adverse effect on the developing embryo was found to be dependent upon the content of TCDD in the samples as well as the dose of 2,4,5-T administered. In the studies in which animals were treated with 2,4,5-T and TCDD simultaneously, it was found that 2,4,5-T would have to contain greater than 1 ppm of TCDD before any effect could be attributed to the TCDD contaminant. The content of TCDD in currently produced 2,4,5-T rarely exceeds 0.1 ppm and usually contains considerably less than this. The dose levels of 2,4,5-T which are required to cause a teratogenic response in mice or an embryo-toxic response in other mammals are far in excess of the exposure of humans and other animals to 2,4,5-T under conditions of manufacture, application or use of 2,4,5-T.

Now that I have presented my summary of the status of the teratogenic potential of 2,4,5-T, I would like to review the existing literature which led to those conclusions. I will review in chronological sequence the reports that are available which contain information on the teratogenic potential of 2,4,5-T. Please keep in mind my previous comments regarding experimental design, the interpretation of teratology study results, terminology and dose-response relationships as I review these studies.

The following are a series of slides which will summarize the individual teratology studies using the same format for each

study as shown on the first slide (Slide 1). I will cite the complete reference for the work including the names of the authors, the title of the report and the journal in which it was published. I will then indicate the form of the 2,4,5-T that was used, the animal species and, if possible, the strain, and the period of gestation during which the animals were treated, as well as the route by which the material was administered. In some cases, 2,4,5-T was administered in the diet, in others it was injected under the skin (subcutaneously) or it was administered orally and deposited in the stomach (oral gavage). I have indicated what dose levels of 2,4,5-T were administered, expressed as mg of 2,4,5-T/kg of body weight per day on the indicated days of gestation. I've also indicated whether or not maternal toxicity, fetal abnormalities, or fetal deaths were observed at any of the indicated dose levels.

In the fall of 1969, the results of a study on a number of pesticides and industrial chemicals by the Bionetics Laboratories were released which indicated that mice treated during early pregnancy with large doses of 2,4,5-T gave birth to malformed offspring. The portion of this Bionetics report which had to do with 2,4,5-T was subsequently published in Science in 1970. That report is summarized on Slide #2. A sample of 2,4,5-T acid was used for this study which was later found to contain approximately 30 ppm of TCDD. 2,4,5-T was administered to three strains of mice at various stages of gestation. The material was given

orally or was administered under the skin in dimethylsulfoxide (DMSO). The material was injected under the skin at dose levels of 21.5 or 113 mg/kg/day, or was given orally at dose levels of 46.4 or 113 mg/kg/day. Mice of the C57 strain were treated with both dose levels by both routes of administration. The AKR strain was treated with only the high dose level by both routes. Whether or not these dose levels caused maternal toxicity was not mentioned in the report, but, based on the results of other studies in mice, the high dose must have caused serious manifestation of toxicity in the mothers. Regarding the incidence of fetal abnormalities, there were none observed upon external examination of the fetuses. The fetal abnormalities that were observed internally included cleft palate and "cystic kidney". Cleft palate was observed at the high dose level with both routes of administration in both strains of mice. Cystic kidney was found in both strains when 2,4,5-T was administered under the skin, and was found at both the low and high dose levels in C57 mice when 2,4,5-T was administered orally, but was not found in the AKR strain of mice. There were no skeletal abnormalities observed in either strain of mouse at either dose level by either route of administration. Administration of these dose levels of 2,4,5-T had no effect on the incidence of fetal deaths when administered beneath the skin, but there was a significant increase in the incidence of fetal deaths at a higher dose level of 2,4,5-T administered orally.

The authors also administered 2,4,5-T to Holtzman strain rats on days 10 through 15 of gestation (Slide #3). The material was administered orally at dose levels of 4.6, 10 or 46.4 mg/kg/day. The existence of maternal toxicity was not mentioned. There were no external abnormalities observed among the rat fetuses. Regarding internal abnormalities, cleft palate was not observed in the rat fetuses but what the authors described as "cystic kidneys" was observed at the two higher dose levels of 2,4,5-T. There were no skeletal abnormalities observed. An increased incidence of fetal deaths was observed at the two higher dose levels of 2,4,5-T.

I will describe in more detail the significance of abnormalities that were observed. Cleft palate is the absence of part of the hard palate above the tongue. The right and left halves of the palate fail to close over the roof of the mouth. This is a very serious malformation since the newborn animal cannot get any milk when it nurses its mother and usually dies of starvation. This is definitely a teratogenic response. In the case of "cystic kidneys", it has never been perfectly clear to me what malformation was observed by the authors. The term "cystic kidneys" refers to fluid filled holes or pockets in the outer portion of kidneys. These cysts would not be connected to the drainage part of the kidneys but would just take up space inside the kidneys. No other authors have reported this lesion in any teratology study using mice or rats. Others

have found what's referred to as "dilated renal pelvis." This is an enlargement of the open central part of the kidney which drains into the ureter - the tube which carries the urine from the kidney to the bladder. The kidney may be the normal size on the outside but there is less functional tissue inside the kidney. In severe cases, this is definitely a teratogenic response.

A critique of this study has been presented in a report of the Panel on Herbicides of the President's Science Advisory Committee, a report presented in March, 1971 (Slide #4). As indicated on Slide #5, the panel observed that:

> "The study involved a great number of variations in procedure which made the task of evaluation of the results difficult.
>
> "Too few animals and litters were used to allow for adequate statistical evaluation.
>
> "There appears to be an unusually high level of embryo lethality and teratogenicity among untreated and vehicle treated groups.
>
> "The strain of mouse most often used (C57BL/6) appeared to have undesirable traits as a test animal, being variably and uncertainly responsive to the substance being tested (Slide #6).
>
> "It is puzzling that virtually no skeletal malformations were encountered in either controls or test groups. Skeletal defects usually account for a substantial part of the easily detected malformations that occur spontaneously or after treatment in rodent species. Hardly a strain that has been carefully studied and properly cleared in stained specimens has failed to show vertebral and rib variations.

"There were some known teratogens used in these experiments (Trypan Blue, 6-aminonicotinamide). It is puzzling to find that these agents failed to produce significant teratogenic and embryo lethal effects consistently. This raises questions about the precision with which the teratogenicity tests were performed (Slide #7).

"2,4,5-T appeared to be clearly teratogenic in two strains of mice treated with 2,4,5-T at 100 mg/kg via either of the two routes of administration. In rats, 2,4,5-T appeared only equivocally teratogenic at any dosage, but clearly embryo lethal from 10-46 mg/kg.

"The lack of an unequivocally-defined dose-response relationship renders these results less than completely satisfying."

Following the release of the 2,4,5-T data in the original report from the Bionetics Laboratories, an effort was made to trace the identity of the sample of 2,4,5-T that had been used in these studies which were sponsored by the National Cancer Institute. A sample of the 2,4,5-T used in the National Cancer Institute's study was analyzed and was found to contain about 27 ppm of TCDD. Subsequently, teratology studies were begun with 2,4,5-T as well as the TCDD impurity in Dow Toxicology Laboratories. The first experiments were conducted while representatives of the Department of Health, Education and Welfare were present in the laboratories. The first of these studies is summarized on the next slide (Slide 8). In a study reported by Emerson and coworkers, commercial grade 2,4,5-T containing less than 1 ppm of TCDD was administered to Sprague-Dawley rats on days 6-15 of gestation. 2,4,5-T in this study was administered orally at dose levels of 1, 3, 6, 12 or 24 mg/kg/day. No clinical or gross pathological signs of adverse

effects were observed in treated dams during the period of treatment or gestation. Visual and microscopic examination of the fetuses revealed no teratogenic or embryotoxic effects. These dose levels of 2,4,5-T did not increase the incidence of fetal deaths. A copy of the published report of this study is included at the end of this testimony.

In a subsequent study conducted in our Dow Toxicology lab at Midland, Michigan, the same sample of 2,4,5-T was administered to Sprague-Dawley rats at higher dose levels, 50 and 100 mg/kg/day (Slide #10). Administration of 50 mg/kg/day caused no maternal toxicity, but at the 100 mg/kg/day dose level, a high incidence of maternal deaths occurred and most of the fetuses of surviving mothers died. No abnormalities were observed at either dose level upon external examination of the fetuses or examination of the soft tissues internally. Skeletal abnormalities were observed at both dose levels which were indicative of retarded fetal development. To summarize these three studies, no teratogenic effects were observed among rats treated with dose levels as high as 100 mg/kg/day, a dose level which caused some treated mothers to die. Administration of the same sample of 2,4,5-T to pregnant rabbits caused no signs of embryo toxicity or teratogenicity at dose levels up to 40 mg/kg/day. A copy of the published report of this study is also attached.

In a later study in our laboratory, purified 2,4,5-T acid was administered to Sprague-Dawley rats on days 6-15 of gestation

at one dose level, 50 mg/kg/day. In addition, these rats were also treated with varying dose levels of TCDD (Slide #11). Rats were treated with 50 mg 2,4,5-T/kg/day plus 0.01, 0.03, 0.06, 0.125, 0.5 or 1 µg of TCDD/kg/day. Both materials were administered orally. Maternal toxicity in the form of decreased body weight was observed at 1 µg/kg/day of TCDD but not at lower dose levels. Regarding fetal abnormalities, none were observed upon external examination of the fetuses. Soft tissue abnormalities including subcutaneous edema and hemorrhage in the walls of the intestines were observed at dose levels of 0.06 µg/kg/day or higher. Skeletal abnormalities were observed at dose levels of 0.125 and higher; these consisted primarily of retarded development of bones of the skull and the sternum. Cleft palate occurred at 0.5 and 1 µg/kg/day. An increased incidence of fetal death was observed at dose levels of 0.5 and 1 µg/kg/day. In the previous study in this lab, it was demonstrated that 50 mg 2,4,5-T/kg/day was a threshold dose level for causing observable embryotoxic effects in rats. In this study, this same threshold dose level of 50 mg 2,4,5-T/kg/day was used and it was found that at this dose level, 2,4,5-T would have to contain more than 10 ppm TCDD to cause intestinal hemorrhage and would require more than 100 ppm to cause the teratogenic effect, cleft palate. These levels of 10 and 100 ppm of TCDD are 100 and 1000 times the highest concentration of TCDD that would be expected in currently produced 2,4,5-T. The control group for this study was a group of rats treated with 50 mg 2,4,5-T/kg/day with no TCDD administered. The effects of this treatment were comparable

to those observed in the previous study in which rats were treated similarly. In another study in our laboratory which has been published, groups of rats were treated with varying dose levels of TCDD alone. In that study as in this study, the no-effect dose level of TCDD for intestinal hemorrhage was 0.03 µg/kg/day. This suggests that a large dose of 2,4,5-T does not contribute to the fetal.toxicity of TCDD.

In the previous teratology studies using purified 2,4,5-T, no cleft palates were observed when either 50 or 100 mg/kg/day was administered. Also, when TCDD was administered alone, cleft palates were not observed. When given TCDD and 2,4,5-T concurrently, cleft palates occurred, suggesting the quality of the response seen when the two are administered simultaneously may be altered. However, the quantity of the response to administration of the combination may be predicted by the quantity of the response to either when given alone. To reiterate, it appears that the effects of the TCDD contaminant of 2,4,5-T will be discernible only when the concentration exceeds 10 ppm.

In studies conducted subsequent to the Bionetics studies, Courtney administered technical or analytical grade 2,4,5-T to mice on days 6-15 of gestation (Slide 12). Again, the 2,4,5-T was administered under the skin in dimethyl sulfoxide at dose levels of 50, 100, 125 and 150 mg/kg/day. CD-1 strain mice were treated with each dose level and two other strains of mice were treated with 100 mg/kg/day. Among the CD-1 mice, no

maternal toxicity was observed at any dose level of 2,4,5-T. No maternal toxicity was observed among the DBA mice at 100 mg/kg but toxicity was observed among the C57 mice at this dose level. Cleft palate was observed among all strains of mice at dose levels of 100 mg/kg/day and higher. The technical grade of 2,4,5-T did not cause kidney anomalies in any strain at any dose level of 2,4,5-T. The analytical grade of 2,4,5-T did cause kidney anomalies at 100 and 125 but not at 150 mg/kg/day in the CD-1 strain mice. Fetal deaths were observed at a higher incidence than control at 150 mg/kg/day but not at lower levels. An additional group of mice was treated with 100 mg/kg/day of analytical 2,4,5-T plus 1 µg/kg/day of TCDD. The treatment with TCDD did not potentiate the effect of 2,4,5-T given alone. This would be the equivalent of using 2,4,5-T with 10 ppm TCDD.

In the same study, 2,4,5-T was administered to CD strain rats on days 6-15 of gestation (Slide #13). It was given orally at dose levels of 10, 21.5, 46.4 and 80 mg/kg/day. Maternal toxicity in the form of reduced weight gain was observed at the two higher dose levels. No fetal abnormalities were observed in the offspring of these rats at any dose level of 2,4,5-T. Fetal deaths were observed only at the high dose levels. These authors did a postnatal study in which groups of rats were treated with 50 mg 2,4,5-T/kg/day on days 6-15 of gestation and were then allowed to deliver their offspring normally. Among the rats treated with this dose level of 2,4,5-T, the survival and

growth of these naturally born litters were normal. Thus, 2,4,5-T was not teratogenic in this strain of rat and administration before birth did not impair growth and development after birth.

On the next slide (Slide #14) is the summary of a study done at the Food and Drug Administration.by Collins and Williams. In this study, 2,4,5-T was administered to Golden Syrian Hamsters on days 6-10 of gestation. It was administered orally at dose levels of 20, 40, 80 and 100 mg/kg/day. Whether or not maternal toxicity was evident was not mentioned in the report. No fetal abnormalities were observed at dose levels less than 100 mg/kg/day. At 100 mg/kg/day, the abnormalities consisted of missing eyelids, missing cover over the brain and delayed development of the bones of the skull. The incidence of fetal deaths was also increased at 100 mg/kg. Additional groups of hamsters were treated with 2,4,5-T containing 0.1, 0.5, 2.9 and 45 ppm of TCDD. A TCDD content of 2.9 ppm or less contributed very little to the embryotoxic effects of 2,4,5-T. A TCDD content of 45 ppm, however, clearly increased the toxicity of 2,4,5-T to hamster embryos and fetuses.

On the next slide (Slide #15) is summarized a study done by Roll of the Federal Health Office, Berlin, Germany. Dr. Roll administered 2,4,5-T acid to NMRI strain mice on days 6-15 of gestation. It was given orally at dose levels of 20, 35, 60, 90 and 130 mg/kg/day. Maternal toxicity in the form of decreased body

weight was observed at 90 and 130 mg/kg/day and maternal deaths were observed at the higher dose levels. No external abnormalities or skeletal abnormalities were observed at any dose level of 2,4,5-T. The only abnormality observed was cleft palate which was found at dose levels of 35 mg/kg/day or higher. The incidence of fetal death was increased at 90 and 130 mg/kg/day of 2,4,5-T. The author concluded that a dose of 20 mg/kg/day was established as the "teratogenic no-effect level".

On the next slide (Slide #16) is summarized a study conducted by Dr. Wilson of the Childrens Hospital Research Foundation and the Departments of Pediatrics and Anatomy of the University of Cincinnati, College of Medicine, Cincinnati, Ohio. In this study, 2,4,5-T acid was administered to Rhesus monkeys 3 times per week from days 20-48 of gestation. It was given orally at dose levels of 5, 10, 20 or 40 mg/kg/day. Maternal toxicity was not observed among the monkeys treated 3 times weekly with these dose levels of 2,4,5-T. However, among monkeys treated with the high dose level 7 days per week, maternal deaths were observed. Regarding fetal abnormalities of mothers receiving 10 mg/kg or higher, offspring had a decreased body weight at dose levels of 10 mg/kg or higher. The offspring were normal based on external examination and internal examination of soft tissues. The deviations from normal skeletal development were the same as those observed in control offspring. One abortion occurred among 5 monkeys treated with 40 mg/kg. No abortions were observed at lower dose levels or among the control monkeys. Among 220

pregnancies in the authors' colony, the spontaneous incidence of abortion was 26%.

The next slide (Slide #17) summarizes a teratology study that was done in sheep by Binns of the Poisonous Plant Research Laboratory, USDA, Logan, Utah. The author administered 100 mg 2,4,5-T acid/kg/day or 113 mg of the propylene glycol butyl ether ester of 2,4,5-T/kg/day to pregnant sheep on days 14-36 of gestation. The material was given orally. The occurrence of maternal toxicity was not mentioned by the author. No fetal abnormalities or deaths were observed following treatment with either form of 2,4,5-T. When given during this period of gestation, Veratrum alkoloids, natural occurring substances in some range land plants, cause serious malformations in lambs which attests to the temporal susceptibility of sheep to the administration of teratogenic agents.

The next slide (Slide #18) summarizes a study done by Dr. Khera of the Food and Drug Directorate of Canada. 2,4,5-T acid and the butyl ester of 2,4,5-T were given to Wistar strain rats on days 6-15 of gestation. The material was given orally at dose levels ranging from 25 to 150 mg/kg/day. Maternal toxicity in the form of decreased weight gain during pregnancy was observed at the high dose level of 2,4,5-T acid but not among any other treatment group. No external or internal soft tissue abnormalities were observed in the fetuses at any dose level of either

form of 2,4,5-T. Skeletal abnormalities were observed at 100 and 150 mg/kg/day of 2,4,5-T acid. The skeletal abnormalities involved primarily the ribs, sternum and bones of the skull. There was an increase in the incidence of normally occurring minor skeletal abnormalities as well as a low incidence of abnormalities not observed in control litters (fused ribs, distorted scapula and long bones of the forelimbs). The incidence of fetal deaths was increased at 100 and 150 mg/kg/day of 2,4,5-T acid.

In a postnatal study by the same author, groups of rats were treated with 2,4,5-T acid (12.5-100 mg/kg) or the butyl ester of 2,4,5-T (50 or 150 mg/kg) on days 6-15 of gestation and were allowed to deliver their offspring normally. The survival and growth of offspring that were delivered normally were not different from control offspring. Thus, the skeletal abnormalities noticed in the teratology study were not incompatible with life. No changes in behavior or subsequent reproductive performance were observed.

The next slide (Slide #19) summarizes a study done at the Frei University in Berlin, Germany. Dr. Neubert administered 2,4,5-T acid, the butyl ester of 2,4,5-T or 2,4,5-trichlorophenol to NMRI strain of mice on days 6-15 of gestation. 2,4,5-T was given orally at dose levels which ranged from 8 to 120 mg/kg/day. Maternal toxicity in the form of decreased body weight was

observed at 90 and 120 mg/kg/day but not at lower dose levels. Fetal body weight was decreased at all dose levels of 2,4,5-T. Cleft palate was observed at dose levels of 45 mg/kg and higher. The results of the skeletal examination were not reported. The incidence of fetal deaths was increased at dose levels of 60 mg/kg and higher. The authors also did some studies in which pregnant mice were treated with TCDD as well as 2,4,5-T. It was concluded that a TCDD content of greater than 1 ppm would be required to contribute to the embryotoxic effects of 2,4,5-T administered to NMRI mice.

On the next slide (Slide #20) are the results of the studies in which the butyl ester of 2,4,5-T or 2,4,5-trichlorophenol itself were given to pregnant mice. No maternal toxicity was observed among mice treated with either dose level of the butyl ester. No externally visible malformations were found but the body weight of the fetuses was decreased at both levels of the ester. The incidence of cleft palate was increased at the high dose level of the butyl ester but not at the low dose level. Again the results of the skeletal examination were not reported. Neither dose level of the ester caused an increase in the incidence of fetal deaths. Regarding 2,4,5-trichlorophenol, there was no evidence of maternal toxicity among groups of mice receiving 0.9 or 9 mg/kg/day. Fetal body weight was decreased at the low dose level but not at the high dose level. No external or internal abnormalities were observed. The incidence of fetal deaths

was increased at the high dose level of trichlorophenol but not at the low dose level.

On the next slide (Slide #21) is a summary of a study done at the Neurophysiological Laboratory at Ulleraker Hospital, Uppsala, Sweden. Female rats were treated orally with a single dose of 2,4,5-T acid during early pregnancy. All of the rats were allowed to deliver their offspring which were subsequently subjected to open-field behavior tests for two consecutive days at 90 days of age. Dose levels of 2,4,5-T ranged from 4 to 100 mg/kg. Maternal toxicity was not observed at any dose level. There were no fetal abnormalities observed among any of the offspring at birth. The incidence of fetal death was higher at dose levels of 40 mg/kg and higher. Regarding the behavioral observations, it was found that male offspring of treated mothers were significantly more explorative than male offspring of control mothers. No effect of treatment on emotional reactivity was observed in male offspring and no change in the open-field behavior of female offspring was noted. Although the exploratory behavior of male offspring of female rats treated with 2,4,5-T increased, no clue as to the possible significance of this effect was given.

On the next slide (Slide #22) is a summary of a study with phenoxy herbicides in reindeer. Dr. Erne conducted a study in which a commercial preparation consisting of 2,4-D and 2,4,5-T in a ratio of 2:1 was sprayed on birch leaves which were then fed to

reindeer during the last 1 to 1-1/2 months of gestation (too late to induce embryotoxicity). The sprayed leaves contained about 45 ppm total phenoxy acid and the reindeer consumed about 1 mg/kg/day of phenoxy acid. No maternal toxicity was observed and all fetuses were alive and normally developed at delivery. The authors also determined that both 2,4-D and 2,4,5-T went across the placenta and neither one accumulated in the fetus.

The next slide (Slide #23) summarizes a study in which 2,4,5-T was mixed with the diet and given to rats throughout pregnancy. The diets contained 250 and 1000 ppm 2,4,5-T. When given in this manner, the rats were ingesting approximately 20 and 80 mg/kg/day of 2,4,5-T. Rats were maintained on a diet containing a lower than normal amount of protein as well as a normal level of protein. With a normal level of protein in the diet, rats that were fed the high level of 2,4,5-T showed a decrease in food consumption and weight gain. In these rats, the incidence of fetal deaths was unaltered but the offspring of rats treated with the high level of 2,4,5-T had a lower body weight than control offspring. In animals receiving 2,4,5-T in a diet that was low in protein, the adverse effects associated with 2,4,5-T were enhanced (reduced body weight and tissue weights as well as DNA and protein content of certain tissues).

The next slide (Slide #24) is a summary of a study conducted at the Karolinska Institute, Stockholm, Sweden. Commercial preparations of 2,4,5-T alone or a combination of 2,4-D and 2,4,5-T in

the ratio 2:1 were administered to NMRI strain mice on days 6-14 of gestation. The compounds were given under the skin in dimethylsulfoxide at dose levels of 50 or 110 mg/kg/day of chlorophenoxyacetic acids. No maternal toxicity was observed at either dose level of either commercial preparation. No external abnormalities were observed. Soft tissue abnormalities, primarily cleft palate, were observed at both dose levels of both preparations. Skeletal abnormalities were observed at the high dose level of both preparations. The skeletal abnormalities involved the ribs and vertebrae. No kidney malformations were observed in this study. The authors concluded that "the results of the present and other studies do not substantiate any special risk to the human embryo from the regular use of phenoxy herbicides," because of the extremely high dose levels required to cause a teratogenic effect.

On the next slide (Slide #25) is a summary of a study in which technical grade 2,4,5-T was administered to Rhesus monkeys on days 22-38 of gestation. The material was administered orally at dose levels of 0.05, 1 and 10 mg/kg/day. No maternal toxicity, fetal abnormalities or fetal deaths were observed at any dose level of 2,4,5-T. The authors concluded that "under the conditions of this experiment, there is no evidence that 2,4,5-T is teratogenic in the rhesus monkey, nor that it interferes in any way with normal development of the young."

On the next slide (Slide #26) is a summary of a study conducted by Hart and Valerio in which commercial grade 2,4,5-T was given to pregnant CD-1 mice. The material was given on days 6-15 of gestation and was given as a solution in DMSO or propylene glycol under the skin. Mice received either 10 or 100 mg/kg/day. The complete information on this study is not available and the information that I have is gathered from an abstract. The presence or absence of maternal toxicity or the incidence of fetal deaths was not mentioned. Cleft palate was observed at 100 mg/kg/day but not at 10. No other anomalies were observed at either dose level.

On the next 2 slides are summarized studies that were conducted in the laboratory of C. H. Boehringer in Germany. The results of these studies have not been published. In the first study (Slide #27), a sample of purified 2,4,5-T containing less than 0.02 ppm TCDD was given to rats on days 6-15 of gestation by oral gavage. Rats received dose levels of 25, 50, 100 or 150 mg/kg/day. Maternal toxicity was observed at the two higher dose levels. No fetal abnormalities were observed at any dose level. An increased incidence of fetal deaths was observed at the three higher dose levels but not among rats treated with 25 mg/kg/day. The results of a study using technical grade 2,4,5-T containing 0.08 ppm TCDD are indicated on the next slide (Slide #28). The same strain of rats was used and the same experimental design was used except that in this study, the dose levels

were 25, 50, 75 and 100 mg/kg/day. Maternal toxicity was observed at 75 and 100 mg/kg/day. Fetal abnormalities were observed at the high dose level but were not considered a teratogenic response. Instead, they consisted of minor skeletal deviations. Again, an increased incidence of fetal deaths was observed at dose levels higher than 25 mg/kg/day but not at 25 mg/kg/day.

The next series of slides will summarize the teratology studies that have been conducted on certain of the chlorinated dibenzo-p-dioxins. In the first study summarized on Slide #29, TCDD was administered to pregnant Sprague-Dawley rats on days 6-15 of gestation by oral gavage in a study conducted by Sparschu et al., here at The Dow Chemical Company. Rats were administered dose levels of 0.03 to 8 µg/kg/day. Maternal toxicity was observed at dose levels of 0.5 µg/kg/day and higher. One of the dams died among the group receiving 8 µg/kg/day. Among the surviving dams, there were no live fetuses at the time of cesearean section, day 20 of gestation. At dose levels of 0.5 or 2 µg/kg/day, the incidence of fetal deaths was significantly higher than among control rats. No effects were observed at 0.03 µg/kg/day. No anomalies were revealed by external examination of the fetuses at any dose level of TCDD. Internally, intestinal hemorrhage and edema beneath the skin were observed at dose levels of 0.125 and higher. Skeletal anomalies were observed at the same dose levels, consisting primarily of

a retarded development of the bones rather than a teratogenic response. The primary toxic effect observed in the fetuses in this study, intestinal hemorrhage, is most likely a manifestation of toxicity to the fetus rather than to the embryo. No teratogenic effects were observed. In this study, TCDD was lethal to embryos and fetuses and caused toxicity among fetuses but did not cause any serious malformations.

A similar response to TCDD was observed by Dr. Khera using Wistar rats, Slide #30. TCDD was administered on days 6-15 of gestation by oral gavage. Dose levels ranged from 0.125 to 16 µg/kg/day. External malformations were not observed at any dose level of TCDD. Internally, soft tissue anomalies consisting primarily of intestinal hemorrhage and edema were observed at dose levels of 0.25 µg/kg/day and higher. Hemorrhage was also observed in other tissues. Skeletal anomalies were not observed at any dose level of TCDD. Fetal deaths were observed at dose levels of 1 µg/kg/day and higher, being 100% of all implantations at 4 µg and higher. The observations in this study were quite similar to those of Sparschu in our own laboratory in that no teratogenic effects were observed in the rats. Evidence of toxic effects to the fetus were noticed. In a postnatal study conducted by Khera in which rats that were treated on days 6-15 of gestation were allowed to deliver their young normally, growth and survival of the newborn rats to 21 days of life were decreased at dose levels of 0.5 µg/kg/day or more.

In contrast to the lack of teratogenic effects of TCDD in rats, a significant incidence of cleft palate was observed among mice treated with TCDD as reported by Neubert and Dillman, shown in Slide #31. NMRI strain mice were given TCDD by oral gavage on days 6-15, 9-13 or on single days from day 6 to day 15 of gestation. The presence or absence of maternal toxicity among mice in any of these treatment categories was not mentioned. Among mice treated with 0.3 µg/kg/day on days 6-15 of gestation, no evidence of toxic effects was observed. A significant incidence of cleft palate was observed at 3 µg/kg/day or higher among mice treated on days 6-15 or 9-13 of gestation. The incidence of fetal deaths was significantly higher among mice receiving 9 µg/kg/day on days 6-15 of gestation but not among any other treatment group. Among mice treated with 23 µg/kg/day on day 9, 10, 11 or 12 of gestation, a significant incidence of cleft palate was observed if TCDD was given on days 11 or 12. Among mice receiving 45 µg/kg/day on 1 single day from days 6-15 of gestation, a significant incidence of cleft palate was observed when TCDD was administered on any one of the days between 7 and 13 of gestation.

The results of a teratology study using hexachlorodibenzo-p-dioxin in rats is reported on the next slide, #32. This is a study that I conducted in our Toxicology Lab at Dow. Sprague-Dawley rats were given hexachlorodibenzo-p-dioxin on days 6-15 of gestation by oral gavage. Dose levels of 0.10, 1, 10 or 100 µg/kg/day were used. Maternal toxicity was observed

at dose levels of 10 and 100 µg/kg/day. A significant incidence of cleft palate was observed at the highest dose level, but not at lower dose levels. Soft tissue anomalies, mainly edema under the skin, were observed at dose levels of 1 µg/kg and higher. Minor variations in skeletal development were observed at the highest dose level. A significant incidence of fetal deaths was observed at the 2 higher dose levels. No effects were seen at 0.1 µg/kg. A copy of the published report of this study is attached.

The results of this study suggest that hexachlorodibenzo-p-dioxin causes a more serious embryotoxic response in rats than does TCDD. A significant anomaly was observed, cleft palate, but at dose levels considerably higher than a dose of TCDD that would be lethal to rats. No other studies of the teratogenic potential of hexachlorodibenzo-p-dioxin in any species have been reported.

On the next slide (Slide #33) is a summary of the teratology studies using TCDD in rats and mice. Studies have been reported in 3 different strains of rats. In the Sprague-Dawley rat, the highest dose level which caused no toxic effect to the embryo or fetus was 0.03 µg/kg/day. No teratogenic effects were observed at any dose level of TCDD, but higher dose levels were highly lethal to the embryos and fetuses. In a study conducted in CD rats, only 1 dose level was tested, 0.5 µg/kg/day. The

TCDD was administered under the skin. Abnormal kidney development was observed at this dose level. Lower dose levels were not tested. In the Wistar rat, hemorrhage and edema were observed as in Sprague-Dawley rats. The highest dose level which caused no toxic effect to the developing embryo and fetus in this study was 0.125 µg/kg/day.

In mice, cleft palate has been observed in 4 different strains. Dose response studies have not been conducted in mice. The lowest dose level used, 1 µg/kg/day, was associated with embryo toxicity. Lower dose levels have not been tested. In these studies conducted by Courtney and Moore, TCDD was administered under the skin.

A summaury of studies using dioxins other than TCDD is presented on Slide #34. In studies conducted by Dr. Khera using Wistar rats, no embryotoxic effects were observed with 1,2,3,4-tetrachlorodibenzo-p-dioxin at dose levels as high as 800 µg/kg/day. Higher dose levels were not tested. With the 2,7-dichlorodibenzo-p-dioxin, dose levels of 250 or 500 µg/kg/day had no effect on developing embryos and fetuses. At dose levels of 1,000 or 2,000 µg/kg/day, microscopic examination of the heart muscle revealed separation of the muscle fibers of the heart by edema fluid. The significance of this observation is not known. The 2,3-dichlorodibenzo-p-dioxin had no effect at dose levels as high as 2,000 µg/kg/day. Likewise, the 2-chlorodibenzo-p-dioxin had no effect at 2,000 µg/kg/day. In studies conducted

in our laboratory, dose levels of 2,7-dichlorodibenzo-p-dioxin as high as 2,000 µg/kg/day had no effect on the developing embryo or fetus of Sprague-Dawley rats. Microscopic examination of the heart muscle of fetuses revealed no changes similar to those described by Khera. Among rats treated with octachlorodibenzo-p-dioxin, edema under the skin was observed among fetuses of rats treated with 500 mg/kg/day but no effect was observed at 100 mg/kg/day. The complete report on these studies is in the attached paper by Schwetz et al., 1973.

On the next slide I have summarized the teratology studies that have been done with 2,4,5-T and have indicated the dose level of 2,4,5-T in mg/kg which was the highest dose level causing no embryotoxic effect. The dose level which was associated with no embryotoxic effect among the mouse studies was generally in the range of 20 to 30 mg/kg and in some studies was as high as 50. At higher dose levels in mice, definite teratogenic effects were observed, specifically, cleft palate and dilated renal pelvis in nearly all strains of mice studied. Among the rat studies, the no-effect dose level is in the range of 25-50 mg/kg/day. The first study indicated by Courtney et al. had a no-effect dose level of about 5 mg/kg/day but that sample of 2,4,5-T contained a high level of TCDD. In the other rat studies, at dose levels which did cause an embryotoxic effect, the responses were generally limited to the skeletal system and were an indication of reversible

retardation of fetal development rather than a specific irreversible and persistent effect. The effects were like those seen in fetuses of mothers subjected to various forms of stress including physical, nutritional and behavioral factors - high or low temperature, air travel, starvation or fasting, physical restraint; Golberg (1971), Fd. Cosmet. Toxicol. 9, 65-80.

Teratogenic effects were found in neither of the monkey studies that have been reported. In the study by Wilson, the only observation was decreased body weight at dose levels as high as 40 mg/kg. In the one rabbit study that has been done, no effects were observed at the highest dose level employed, 40 mg/kg/day. In the one hamster study that has been reported, there was no effect at 80 mg/kg/day and malformations were observed at 100. In the one study that has been reported in sheep, no effects were found at 100 mg/kg/day, the highest dose level used.

My overall conclusions upon consideration of all of these teratology studies are shown in the last slide. (1) 2,4,5-T is teratogenic in some strains of mice at high dose levels but has not been shown to cause malformations in rats, rabbits and monkeys. (2) The dose levels of 2,4,5-T required to cause any kind of embryotoxic effect are far in excess of the exposure level among individuals manufacturing or using 2,4,5-T. Thus, it is extremely unlikely that 2,4,5-T is a teratogenic hazard in humans. (3) The embryotoxic

potential of 2,4,5-T in susceptible animals varies with the content of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin. Levels of TCDD greater than 1 ppm are required to enhance the embryotoxic potential of 2,4,5-T. Thus, the TCDD content of currently produced 2,4,5-T (a maximum of 0.1 ppm) does not represent a teratogenic hazard.

AUTHORS:

TITLE:

JOURNAL:

CHEMICAL:

ANIMAL SPECIES:

TREATMENT PERIOD: ROUTE:

1. MATERNAL TOXICITY:

2. FETAL ABNORMALITIES:

A. EXTERNAL:

B. INTERNAL: SOFT TISSUE

SKELETAL

3. FETAL DEATHS:

4. COMMENTS:

AUTHORS: COURTNEY, K. D., GAYLOR, D. W., HOGAN, M. D., FALK, H. L.

TITLE: TERATOGENIC EVALUATION OF 2,4,5-T

JOURNAL: SCIENCE 168: 864-866, 1970

CHEMICAL: 2,4,5-T ACID (CONTAINED 30 PPM 2,3,7,8-TCDD)

ANIMAL SPECIES: MICE, C57BL/6 AND AKR STRAINS

TREATMENT PERIOD: DAYS 6-14 or 9-17 6-15 OF GEST. ROUTE: ORAL OR UNDER THE SKIN IN DMSO

		DOSE LEVELS, MG/KG/DAY			
		UNDER THE SKIN		ORAL	
		21.5	113.0	46.4	113.0
1. MATERNAL TOXICITY:		NOT MENTIONED			
2. FETAL ABNORMALITIES:					
A. EXTERNAL:		NO	NO	NO	NO
B. INTERNAL:	CLEFT PALATE	NO	YES	NO	YES
	"CYSTIC KIDNEY"	NO	YES	YES	NO - AKR YES - C57BL/6
	SKELETAL	NO	NO	NO	NO
3. FETAL DEATHS:		NO	NO	NO	YES

4. COMMENTS: THE AKR STRAIN WAS TESTED ONLY AT 113 MG/KG

AUTHORS: COURTNEY, K.D., GAYLOR, D. W., HOGAN, M. D., FALK, H. L.

TITLE: TERATOGENIC EVALUATION OF 2,4,5-T

JOURNAL: SCIENCE 168: 864-866, 1970

CHEMICAL: 2,4,5-T ACID (CONTAINED 30 PPM 2,3,7,8-TCDD)

ANIMAL SPECIES: RATS, HOLTZMAN STRAIN

TREATMENT PERIOD: DAYS 10-15 OF GEST. ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY		
	4.6	10.0	46.4
1. MATERNAL TOXICITY:	NOT MENTIONED		
2. FETAL ABNORMALITIES:			
A. EXTERNAL:	NO	NO	NO
B. INTERNAL: "CYSTIC KIDNEY"	NO	YES	YES
SKELETAL	NO	NO	NO
3. FETAL DEATHS:	NO	YES	YES

4. COMMENTS:

SLIDE 4

REPORT ON 2,4,5-T

A Report of the Panel on Herbicides of the President's Science Advisory Committee

EXECUTIVE OFFICE OF THE PRESIDENT
OFFICE OF SCIENCE AND TECHNOLOGY

MARCH 1971

SLIDE 5

Comments of the screening study contracted for by the National Cancer Institute have already been published (*18*). The following observations by the Panel are concerned with the portion of this study in which the teratogenic potential of 2,4,5-T was tested.

1. The study involved a great number of variations in procedure (strain of mice used, dates when tests were performed, and routes of administration). These variations make the task of evaluation difficult.

2. Too few animals and litters of animals were used. Since biological variability is considerable, the sample size must be adequately large to demonstrate specific effects of the chemical agents in question. At least 10 pregnant females to assure at least 100 conceptions is suggested. The Food and Drug Administation suggests 20 females per test group.

3. There appears to be an unusually high level of embryo lethality and teratogenicity among untreated and vehicle-treated groups. Either the experimental conditions were less than optimal or the strain of animals was developmentally unstable.

4. The strain of mouse most often used (C57BL/6) appeared to have had undesirable traits as a test animal, being variably and uncertainly responsive to the substance being tested. A reasonably homogeneous, colony-bred stock which has been maintained in the laboratory long enough for the investigator to have accumulated substantial background data on fecundity, spontaneous malformation and intrauterine death rates is generally regarded as preferable to inbred stocks for teratological testing.

SLIDE 6

5. It is puzzling that virtually no skeletal malformations were encountered in either controls or test group. Skeletal defects usually account for a substantial part of the easily detectable malformations that occur spontaneously or after treatment in rodent species. Hardly a strain that has been carefully studied in properly cleared and stained specimens has failed to show vertebral and rib variations.

6. There were some known teratogens used in these experiments (trypan blue, 6-aminonicotinamide). It is puzzling to find that these agents failed to produce significant teratogenic and embryo-lethal effects consistantly. This raises questions about the precision with which these teratogenicity tests were performed.

SLIDE 7

7. 2,4,5-T appeared to be clearly teratogenic in two strains of mice treated with 2,4,5-T at 113 mg/kg via either of the two routes of administration. In rats, 2,4,5-T appeared only equivocally teratogenic at any dosage but clearly embryo-lethal from 10.0–46.4 mg/kg.

8. The lack of an unequivocally defined dose-response relationship renders these results less than completely satisfying.

AUTHORS: EMERSON, J. L., THOMPSON, D. J., STREBING, R. J., GERBIG, C. G., ROBINSON, V. B.

TITLE: TERATOGENIC STUDIES ON 2,4,5-TRICHLOROPHENOXYACETIC ACID IN THE RAT AND RABBIT

JOURNAL: FD. COSMET. TOXICOL. 9, 395-404, 1971

CHEMICAL: 2,4,5-T ACID

ANIMAL SPECIES: RATS, SPRAGUE-DAWLEY STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

DOSE LEVELS, MG/KG/DAY

1 3 6 12 24

1. MATERNAL TOXICITY: ← NONE →

2. FETAL ABNORMALITIES:

A. EXTERNAL:

B. INTERNAL: SOFT TISSUE NONE

SKELETAL

3. FETAL DEATHS:

4. COMMENTS:

AUTHORS: EMERSON, J. L., THOMPSON, D. J., STREBING, R. J., GERBIG, C. G., ROBINSON, V. B.

TITLE: TERATOGENIC STUDIES ON 2,4,5-TRICHLOROPHENOXYACETIC ACID IN THE RAT AND RABBIT

JOURNAL: FD. COSMET. TOXICOL. 9, 395-404, 1971

CHEMICAL: 2,4,5-T ACID

ANIMAL SPECIES: RABBITS, NEW ZEALAND WHITE STRAIN

TREATMENT PERIOD: DAYS 6-18 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY		
	10	20	40
1. MATERNAL TOXICITY:	←	NONE	→
2. FETAL ABNORMALITIES:		↗	
A. EXTERNAL:			
B. INTERNAL: SOFT TISSUE		NONE	
SKELETAL			
3. FETAL DEATHS:		↘	
4. COMMENTS:			

AUTHORS: SPARSCHU, G. L., DUNN, F. L., LISOWE, R. W., ROWE, V. K.

TITLE: STUDY OF THE EFFECTS OF HIGH LEVELS OF 2,4,5-T ON RAT FETAL DEVELOPMENT

JOURNAL: FD. COSMET. TOXICOL 9, 527-530, 1971

CHEMICAL: 2,4,5-T ACID

ANIMAL SPECIES: RATS, SPRAGUE DAWLEY STRAIN

TREATMENT PERIOD: DAYS 6-15 (50 MG/KG) OR 6-10 (100 MG/KG) ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY	
	50	100
1. MATERNAL TOXICITY:	NO	YES
2. FETAL ABNORMALITIES:		
A. EXTERNAL:	NO	NO
B. INTERNAL: SOFT TISSUE	NO	NO
SKELETAL	YES	YES
3. FETAL DEATHS:	NO	YES

4. COMMENTS:

AUTHORS: SPARSCHU, G. L., DUNN, F. L., LISOWE, R. W. AND ROWE, V. K.

TITLE: THE EFFECTS OF VARIOUS DOSES OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ADMINISTERED WITH 2,4,5-TRICHLOROPHENOXYACETIC ACID ON RAT FETAL DEVELOPMENT

JOURNAL: UNPUBLISHED, THE DOW CHEMICAL CO., MIDLAND, MICH.

CHEMICAL: 2,4,5-T ACID, PURIFIED, PLUS VARIOUS DOSE LEVELS OF TCDD

ANIMAL SPECIES: RATS, SPRAGUE DAWLEY STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS OF TCDD, UG/KG/DAY					
50 MG 2,4,5-T/KG/DAY PLUS	0.01	0.03	0.06	0.125	0.5	1.0
1. MATERNAL TOXICITY:	NO	NO	NO	NO	NO	YES
2. FETAL ABNORMALITIES:						
A. EXTERNAL:	←		NONE			>
B. INTERNAL: SOFT TISSUE*	NO	NO	YES	YES	YES	YES
SKELETAL	NO	NO	NO	YES	YES	YES
3. FETAL DEATHS:	NO	NO	NO	NO	YES	YES

*INTESTINAL HEMORRHAGE AND CLEFT PALATE

4. COMMENTS: AT 50 MG/KG, 2,4,5-T WOULD HAVE TO CONTAIN >10 PPM TCDD TO CAUSE INTESTINAL HEMORRHAGE OR >100 PPM TO CAUSE CLEFT PALATE.

AUTHORS: COURTNEY, K. D., MOORE, J. A.

TITLE: TERATOLOGY STUDIES WITH 2,4,5-TRICHLOROPHENOXYACETIC ACID AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

JOURNAL: TOXICOL. APPL. PHARMACOL. 20, 396-403, 1971

CHEMICAL: 2,4,5-T (TECHNICAL AND ANALYTICAL)

ANIMAL SPECIES: MICE - 3 STRAINS

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: UNDER THE SKIN IN DMSO

	DOSE LEVELS, MG/KG/DAY					
	50	100			125	150
	CD-1 STRAIN	CD-1 STRAIN	DBA/2J STRAIN	C57B1/6J STRAIN	CD-1 STRAIN	CD- STRAIN
1. MATERNAL TOXICITY:	NO	NO	NO	YES	NO	NO
2. FETAL ABNORMALITIES:						
A. CLEFT PALATE	NO	YES	YES	YES	YES	YES
B. KIDNEY ANOMALIES	NO	NO-TECH. YES-ANAL.	NO-TECH.	NO-TECH.	YES-ANAL.	NO-TECH.
3. FETAL DEATHS:	NO	NO	NO	NO	NO	YES

4. COMMENTS: 1 µG/KG TCDD + 100 MG/KG ANALYTICAL 2,4,5-T DID NOT RESULT IN POTENTIATION OF THE EFFECT OF 2,4,5-T ALONE.

AUTHORS: COURTNEY, K. D., MOORE, J. A.

TITLE: TERATOLOGY STUDIES WITH 2,4,5-TRICHLOROPHENOXYACETIC ACID AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

JOURNAL: TOXICOL. APPL. PHARMACOL. 20, 396-403, 1971

CHEMICAL: 2,4,5-T (TECHNICAL AND ANALYTICAL)

ANIMAL SPECIES: RATS, CD STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY			
	10.0	21.5	46.4	80.0
1. MATERNAL TOXICITY:	NO	NO	YES	YES
2. FETAL ABNORMALITIES:				
A. CLEFT PALATE	← NO →			
B. KIDNEY ANOMALIES	← NO →			
3. FETAL DEATHS:	NO	NO	NO	YES

4. COMMENTS: POSTNATAL STUDY: 50 MG/KG/DAY ON DAYS 6-15 OF GEST; SURVIVAL AND GROWTH OF NATURALLY BORN LITTERS WERE NORMAL

AUTHORS: COLLINS, T.F.X., WILLIAMS, C.H.

TITLE: TERATOGENIC STUDIES WITH 2,4,5-T AND 2,4-D IN THE HAMSTER

JOURNAL: BULL. ENVIRONM. CONTAM. TOXICOL. 6(6), 559-567, 1971

CHEMICAL: 2,4,5-T (NO DETECTABLE 2,3,7,8-TCDD)

ANIMAL SPECIES: HAMSTERS, GOLDEN SYRIAN

TREATMENT PERIOD: DAYS 6-10 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY			
	20	40	80	100
1. MATERNAL TOXICITY:		NOT MENTIONED		
2. FETAL ABNORMALITIES:				
A. EXTERNAL:				YES
B. INTERNAL: SOFT TISSUE		NONE		NO
SKELETAL				YES
3. FETAL DEATHS:				YES

4. COMMENTS: 2,4,5-T WITH 0.1,0.5,2.9, AND 45 PPM 2,3,7,8-TCDD WAS ALSO STUDIED. ABNORMALITIES WERE CLEARLY RELATED TO THE LEVEL OF DIOXIN IN 2,4,5-T.

AUTHORS: ROLL, R.

TITLE: INVESTIGATIONS CONCERNING THE TERATOGENIC EFFECT OF 2,4,5-T IN MICE

JOURNAL: FD. COSMET. TOXICOL. 9, 671-676, 1971

CHEMICAL: 2,4,5-T ACID, <0.1 PPM TCDD

ANIMAL SPECIES: MICE, NMRI STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY				
	20	35	60	90	130
1. MATERNAL TOXICITY:	NO	NO	NO	YES	YES
2. FETAL ABNORMALITIES:					
A. EXTERNAL:	←		NONE		→
B. INTERNAL: CLEFT PALATE	NO	YES	YES	YES	YES
SKELETAL	←		NONE		→
3. FETAL DEATHS:	NO	NO	NO	YES	YES

4. COMMENTS:

AUTHORS: WILSON, J. G.

TITLE: ABNORMALITIES OF INTRAUTERINE DEVELOPMENT IN NON-HUMAN PRIMATES

SOURCE: SYMPOSIUM ON THE USE OF NON-HUMAN PRIMATES FOR RESEARCH ON PROBLEMS OF HUMAN REPRODUCTION SUKHUME, USSR, 13-17 DEC 1971

CHEMICAL: 2,4,5-T

ANIMAL SPECIES: RHESUS MONKEY

TREATMENT PERIOD: 3 TIMES/WK FROM DAY 20-48 ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY			
	5	10	20	40
1. MATERNAL TOXICITY:				
2. FETAL ABNORMALITIES:	← DEC. WT. →			
A. EXTERNAL:	← NONE →			
B. INTERNAL: SOFT TISSUE	← NONE →			
SKELETAL	← SAME AS CONTROL →			
3. FETAL DEATHS:	NO	NO	NO	YES

4. COMMENTS:

AUTHORS: BINNS, W. AND BALLS, L.

TITLE: NONTERATOGENIC EFFECTS OF 2,4,5-TRICHLOROPHENOXYACETIC ACID AND 2,4,5-T PROPYLENE GLYCOL BUTAL ESTERS HERBICIDES IN SHEEP

JOURNAL: TERATOLOGY , , 1971;(BIOSCIENCE 21, 899-905, 1971)

CHEMICAL: 2,4,5-T ACID (100 MG/KG/DAY); PGBE ESTER OF 2,4,5-T (113 MG/KG/DAY)

ANIMAL SPECIES: SHEEP

TREATMENT PERIOD: DAYS 14-36 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY	
	2,4,5-T ACID, 100 MG/KG	PGBE ESTER, 113 MG/KG
1. MATERNAL TOXICITY:	?	?
2. FETAL ABNORMALITIES:		
A. EXTERNAL:		
B. INTERNAL: SOFT TISSUE	NONE	NONE
SKELETAL		
3. FETAL DEATHS:		

4. COMMENTS:

AUTHORS: KHERA, K. S. AND MCKINLEY

TITLE: PRE- AND POSTNATAL STUDIES ON 2,4,5-TRICHLOROPHENOXYACETIC ACID, 2,4-DICHLOROPHENOXYACETIC ACID AND THEIR DERIVATIVES IN RATS

JOURNAL: TOXICOL. APPL. PHARMACOL. 22, 14-28, 1972

CHEMICAL: 2,4,5-T ACID AND BUTYL ESTER (<0.5 PPM TCDD)

ANIMAL SPECIES: RATS, WISTAR STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY					
	2,4,5-T ACID				BUTYL ESTER OF 2,4,5-T	
	25	50	100	150	50	150
1. MATERNAL TOXICITY:	NO	NO	NO	YES	NO	NO
2. FETAL ABNORMALITIES:						
A. EXTERNAL:	NO	NO	NO	NO	NO	NO
B. INTERNAL: SOFT TISSUE	NO	NO	NO	NO	NO	NO
SKELETAL	NO	NO	YES	YES	NO	NO
3. FETAL DEATHS:	NO	NO	YES	YES	NO	NO

4. COMMENTS: POSTNATAL STUDY: 12.5-100 MG/KG OF THE ACID OR 50 OR 150 MG/KG OF THE ESTER ON DAYS 6-15 OF GEST.; SURVIVAL AND GROWTH OF NATURALLY BORN LITTERS WERE NORMAL.

AUTHORS: NEUBERT, D. AND DILLMANN, I.

TITLE: EMBRYOTOXIC EFFECTS IN MICE TREATED WITH 2,4,5-TRICHLOROPHENOXYACETIC ACID AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

JOURNAL: NAUYN - SCHMEIDEBERG'S ARCH. PHARMACOL. 272, 243-264, 1972

CHEMICAL: 2,4,5-T ACID AND BUTYL ESTER; 2,4,5-TRICHLOROPHENOL

ANIMAL SPECIES: MICE, NMRI STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY 2,4,5-T ACID						
	8	15	30	45	60	90	120
1. MATERNAL TOXICITY:	NO	NO	NO	NO	NO	YES	YES
2. FETAL ABNORMALITIES:							
A. EXTERNAL:	← DEC BODY WT →						
B. INTERNAL: SOFT TISSUE*	NO	NO	NO	YES	YES	YES	YES
SKELETAL	NOT REPORTED						
3. FETAL DEATHS:	NO	NO	NO	NO	YES	YES	YES

4. COMMENTS: *CLEFT PALATE; COMBINATION STUDIES, 2,4,5-T + TCDD: >1 PPM OF TCDD REQUIRED TO CONTRIBUTE TO THE EMBRYOTOXIC EFFECTS OF 2,4,5-T IN NMRI MICE.

AUTHORS: NEUBERT, D. AND DILLMANN, I.

TITLE: EMBRYOTOXIC EFFECTS IN MICE TREATED WITH 2,4,5-TRICHLOROPHENOXYACETIC ACID AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

JOURNAL: NAUYN - SCHMEIDEBERG'S ARCH. PHARMACOL. 272,243-264,1972

CHEMICAL: 2,4,5-G ACID AND BUTYL ESTER; 2,4,5-TRICHLOROPHENOL

ANIMAL SPECIES: MICE, NMRI STRAIN

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY			
	BUTYL ESTER OF 2,4,5-T		2,4,5-TRICHLOROPHENOL	
	12	74	0.9	9.0
1. MATERNAL TOXICITY:	NO	NO	NO	NO
2. FETAL ABNORMALITIES:				
A. EXTERNAL:	——— DEC BODY WT ———			
B. INTERNAL: SOFT TISSUE*	NO	YES	NO	NO
SKELETAL	NOT REPORTED			
3. FETAL DEATHS:	NO	NO	NO	YES

4. COMMENTS: *CLEFT PALATE

AUTHORS: SJODEN, P., SODERBERG, U.

TITLE: SEX-DEPENDENT EFFECTS OF PRENATAL 2,4,5-TRICHLOROPHENOXYACETIC ACID ON RATS OPEN-FIELD BEHAVIOR

JOURNAL: PHYSIOL. BEHAVIOR 9, 357-360, 1972

CHEMICAL: 2,4,5-T (<1 PPM 2,3,7,8-TCDD)

ANIMAL SPECIES: RATS - BEHAVIORAL STUDY - OPEN FIELD

TREATMENT PERIOD: DAY 7,8 OR 9 OF GESTATION ROUTE: ORAL - SINGLE DOSE

	DOSE LEVELS, MG/KG/DAY				
	4	8	16	40 ——>	100
1. MATERNAL TOXICITY:	NONE	<——		>	NONE
2. FETAL ABNORMALITIES:					
A. EXTERNAL:	NONE AMONG OFFSPRING AT BIRTH				
B. INTERNAL: SOFT TISSUE					
SKELETAL					
3. FETAL DEATHS:	NONE	NONE	NONE	YES	YES

4. COMMENTS:

BEHAVIOR:	EXPLORATIVE	EMOTIONAL
MALE OFFSPRING:	INCREASED	NO EFFECT
FEMALE OFFSPRING:	NO EFFECT	NO EFFECT

AUTHORS: ERNE, K. AND NORDKVIST, M.

TITLE: TOXICITY STUDIES WITH PHENOXY HERBICIDES ON REINDEER

JOURNAL: SVENSK VETERINARTIDNING 7, 1-3, 1972

CHEMICAL: HORMOSLYR 64 (2,4-D AND 2,4,5-T 2:1)

ANIMAL SPECIES: REINDEER

TREATMENT PERIOD: 1-1.5 MONTHS BEFORE DELIVERY. ROUTE: SPRAYED BIRCH LEAVES WITH ABOUT 45 PPM 2,4,5-T

	DOSE LEVELS, MG/KG/DAY
	ABOUT 1 MG/KG
1. MATERNAL TOXICITY:	NO
2. FETAL ABNORMALITIES:	"ALL EMBRYOS WERE
A. EXTERNAL:	
B. INTERNAL: SOFT TISSUE	ALIVE AND NORMALLY
SKELETAL	
	DEVELOPED."
3. FETAL DEATHS:	

4. COMMENTS: BOTH 2,4-D AND 2,4,5-T WENT ACROSS THE PLACENTA AND NEITHER ACCUMULATED IN THE FETUS.

AUTHORS: HALL, S.M.

TITLE: EFFECTS ON PREGNANT RATS AND THEIR PROGENY OF ADEQUATE OR LOW PROTEIN DIETS CONTAINING 2,4,5-T OR P,P-DDT

JOURNAL: FED. PROC. 31(2), 1972

CHEMICAL: 2,4,5-T

ANIMAL SPECIES: RATS

TREATMENT PERIOD: THROUGHOUT GESTATION ROUTE: DIET

	DOSE LEVELS, PPM IN DIET	
	250*	1000
1. MATERNAL TOXICITY:	NO	YES
2. FETAL ABNORMALITIES:	NO	DEC WT
A. EXTERNAL:		
B. INTERNAL: SOFT TISSUE		
SKELETAL		
3. FETAL DEATHS:	NO	NO

*250PPM ≙ 20 MG/KG/DAY, 1000PPM ≙ 80 MG/KG/DAY

4. COMMENTS: GROWTH AND FOOD INTAKE OF THE MOTHER ARE REFLECTED IN THE DEVELOPMENT OF PROGENY

AUTHORS: BAGE, G., CEKANOVA, E. AND LARSSON, K. S.

TITLE: TERATOGENIC AND EMBRYOTOXIC EFFECTS OF THE HERBICIDES DI- AND TRICHLOROPHENOXYACETIC ACIDS (2,4-D AND 2,4,5-T)

JOURNAL: ACTA PHARMACOL. ET. TOXICOL 32, 408-416, 1973

CHEMICAL: HORMOSYLR 500-T (BUTOXYETHYLESTER OF 2,4,5-T ACID)
HORMOSYLR 64 (2,4-D AND 2,4,5-T 2:1) <1 PPM TCDD

ANIMAL SPECIES: MICE, NMRI STRAIN

TREATMENT PERIOD: DAYS 6-14 OF GESTATION ROUTE: UNDER THE SKIN IN DMSO

	DOSE LEVELS, MG/KG/DAY			
	2,4,5-T		2,4-D and 2,4,5-T 2:1	
	50	110	50	110
1. MATERNAL TOXICITY:	NO	NO	NO	NO
2. FETAL ABNORMALITIES:				
A. EXTERNAL:	NO	NO	NO	NO
B. INTERNAL: SOFT TISSUE*	YES	YES	YES	YES
SKELETAL	NO	YES	NO	YES
3. FETAL DEATHS:	YES	YES	NO	YES

4. COMMENTS: *CLEFT PALATE

AUTHORS: DOUGHERTY, W. H., COULSTON, F. AND GOLBERG, L.

TITLE: NON-TERATOGENICITY OF 2,4,5-TRICHLOROPHENOXYACETIC ACID IN MONKEYS (MACACA MULATTA)

JOURNAL: TOXICOL. APPL. PHARMACOL. 25, 442, 1973 (ABSTRACT)

CHEMICAL: 2,4,5-T, TECHNICAL GRADE <0.05 PPM TCDD

ANIMAL SPECIES: RHESUS MONKEY

TREATMENT PERIOD: DAYS 22-38 OF GESTATION ROUTE: ORAL

	DOSE LEVELS, MG/KG/DAY		
	0.05	1.0	10.0
1. MATERNAL TOXICITY:	NO	NO	NO
2. FETAL ABNORMALITIES:			
A. EXTERNAL:	NO	NO	NO
B. INTERNAL: SOFT TISSUE	NO	NO	NO
SKELETAL	NO	NO	NO
3. FETAL DEATHS:	NO	NO	NO

4. COMMENTS:

AUTHORS: Hart, E. R. and Valerio, M. G. SLIDE 26

TITLE: Teratogenic effects of 2,4,5-T in Mice

JOURNAL: Toxicol. Appl. Pharmacol. 22, 317, 1972 (abstract)

CHEMICAL: 2,4,5-T (commercial, >99% pure)

ANIMAL SPECIES: Mice, CD-1

TREATMENT PERIOD: Days 6-15 ROUTE: Subcutaneous (DMSO, propylene glycol)

	MG/KG/DAY	
	10	100
1. MATERNAL TOXICITY:	NOT MENTIONED	
2. FETAL ABNORMALITIES:		
A. EXTERNAL:	NONE	CLEFT PALATE
B. INTERNAL:	NONE	NONE
3. FETAL DEATHS:	NOT MENTIONED	

4. COMMENTS:

AUTHORS: STÖTZER, H. AND NIGGERSCHULZE, A. (C. H. BOEHRINGER SOHN INGELHEIM AM RHEIN)

TITLE:

JOURNAL: PRIVATE COMMUNICATION - DECEMBER 10, 1970

CHEMICAL: 2,4,5-T - PURIFIED, < 0.02 PPM TCDD

ANIMAL SPECIES: RATS (STRAIN FW 49)

TREATMENT PERIOD: DAYS 6-15 ROUTE: ORAL GAVAGE

MG/KG/DAY

	25	50	100	150
1. MATERNAL TOXICITY:	NO	NO	YES	YES
2. FETAL ABNORMALITIES:	NO	NO	NO	NO
3. FETAL DEATHS:	NO	YES	YES	YES

4. COMMENTS:

AUTHORS: STÖTZER, A. AND NIGGESCHULZE, A. (C. H. BOEHRINGER SOHN INGELHEIM AM RHEIN)

TITLE:

JOURNAL: PRIVATE COMMUNICATION - JUNE 18, 1971

CHEMICAL: 2,4,5-T - TECHNICAL (0.08 PPM TCDD)

ANIMAL SPECIES: RATS (STRAIN FW 49)

TREATMENT PERIOD: DAYS 6-15 ROUTE: ORAL GAVAGE

MG/KG/DAY

	25	50	75	100
1. MATERNAL TOXICITY:	NO	NO	YES	YES
2. FETAL ABNORMALITIES:	NO	NO	NO	YES (SKELETAL, MINOR)
3. FETAL DEATHS:	NO	YES	YES	YES

4. COMMENTS:

AUTHORS: SPARSCHU, G. L., DUNN, F. L. AND ROWE, V. K. SLIDE 29

TITLE: STUDY OF THE TERATOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN THE RAT

JOURNAL: FD. COSMET. TOXICOL, 9, 405-412, 1971

CHEMICAL: 2,3,7,8-TCDD

ANIMAL SPECIES: RAT (SPRAGUE-DAWLEY)

TREATMENT PERIOD: DAYS 6-15 ROUTE: ORAL GAVAGE

		UG/KG/DAY			
	0.03	0.125	0.5	2	8
1. MATERNAL TOXICITY:	NO	NO	YES	YES	DEATH
2. FETAL ABNORMALITIES:					
A. EXTERNAL:					
B. INTERNAL: SOFT TISSUE*	NO	YES	YES	YES	
SKELETAL**		YES	YES	YES	
3. FETAL DEATHS:	NO	NO	YES	YES	YES (100%)

4. COMMENTS: *INTESTINAL HEMORRHAGE AND EDEMA

**RETARDED DEVELOPMENT

AUTHORS: KHERA, K. S. AND RUDDICK, J. A. SLIDE 30

TITLE: POLYCHLORODIBENZO-P-DIOXINS: PERINATAL EFFECTS AND THE DOMINANT LETHAL TEST IN WISTAR RATS

JOURNAL: ADVANCES IN CHEMISTRY, VOL 120, 70-84, 1973

CHEMICAL: 2,3,7,8-TCDD

ANIMAL SPECIES: RATS (WISTAR)

TREATMENT PERIOD: DAYS 6-15 OF GESTATION ROUTE: ORAL GAVAGE

	μG/KG/DAY							
	0.125	0.25	0.5	1	2	4	8	16
1. MATERNAL TOXICITY:	NO	NO	YES	YES	YES	YES	YES	YES
2. FETAL ABNORMALITIES:								
A. EXTERNAL:	NO	NO	NO	NO	NO			
B. INTERNAL: SOFT TISSUE*	NO	YES	YES	YES	YES			
SKELETAL	NO	NO	NO	NO	NO			
3. FETAL DEATHS:	NO	NO	NO	YES	YES	100%	100%	100%

4. COMMENTS: *INTESTINAL HEMORRHAGE AND EDEMA

POSTNATAL STUDY: GROWTH AND SURVIVAL TO 21 DAYS DECREASED AT 0.5 μG/KG OR MORE.

AUTHORS: NEUBERT, D. AND DILLMAN, I. SLIDE 31

TITLE: EMBRYOTOXIC EFFECTS IN MICE TREATED WITH 2,4,5-T AND 2,3,7,8-TCDD

JOURNAL: NAUNYN-SCHMEIDEBERG'S ARCH. PHARMACOL. 272, 243-264, 1972

CHEMICAL: 2,3,7,8-TCDD

ANIMAL SPECIES: MICE (NMRI) ROUTE: (ORAL GAVAGE)

DAYS	6-15				9-13	9,10,11 OR 12	6,7,...OR 15
µG/KG/DAY	0.3	3	4.5	9	9	23	45
1. MATERNAL TOXICITY:	NOT MENTIONED						
2. FETAL ABNORMALITIES:*							
CLEFT PALATE:	NO	YES	YES	YES	YES	DAYS 11&12	DAYS 7-13
3. FETAL DEATHS:	NO	±	±	YES	±	NO	NO

4. COMMENTS: *ONLY CLEFT PALATE WAS REPORTED

AUTHORS: SCHWETZ, ET AL. SLIDE 32

TITLE: TOXICOLOGY OF CHLORINATED DIBENZO-P-DIOXINS

JOURNAL: ADVANCES IN CHEMISTRY 120, 55-69, 1973

CHEMICAL: HEXACHLORODIBENZO-P-DIOXIN

ANIMAL SPECIES: RAT - SPRAGUE-DAWLEY

TREATMENT PERIOD: DAYS 6-15 ROUTE: ORAL GAVAGE

		UG/KG/DAY			
		0.1	1	10	100
1. MATERNAL TOXICITY:		NO	NO	YES	YES
2. FETAL ABNORMALITIES:					
A. EXTERNAL:	CLEFT PALATE	NO	NO	NO	YES
B. INTERNAL:	SOFT TISSUE (EDEMA)	NO	YES	YES	YES
	SKELETAL	NO	NO	NO	YES
3. FETAL DEATHS:		NO	NO	YES	YES

4. COMMENTS:

TERATOLOGY SUMMARY - TCDD IN RATS AND MICE

SLIDE 33

SPECIES	STRAIN	EMBRYOTOXIC EFFECT	DOSE LEVELS TESTED UG/KG/DAY	ROUTE	AUTHORS
RAT (DAYS 6-15)	SPRAGUE-DAWLEY	INTESTINAL HEMORRHAGE, (EDEMA)	[0.03], 0.125, 0.5, 2, 8	ORAL	SPARSCHU ET AL., 1971
	CD	KIDNEY ABNORMALITY	[<0.5] 0.5	SUBCUTANEOUS	COURTNEY & MOORE, 1971
	WISTAR	HEMORRHAGE	[0.125], 0.25, 0.5, 1, 2, 4, 8, 16	ORAL	KHERA & RUDDICK, 1973
MOUSE (DAYS 6-15)	CD-1	CLEFT PALATE KIDNEY ABNORMALITY	[<1] 1,3	SUBCUTANEOUS	COURTNEY & MOORE, 1971
	DBA/2J	CLEFT PALATE KIDNEY ABNORMALITY	[<3] 3	SUBCUTANEOUS	COURTNEY & MOORE, 1971
	C57BL/6J	CLEFT PALATE KIDNEY ABNORMALITY	[<3] 3	SUBCUTANEOUS	COURTNEY & MOORE, 1971
	NMRI	CLEFT PALATE	[<3] 3	ORAL	NEUBERT & DILLMAN, 1972

[ ] = NO EFFECT

TERATOLOGY SUMMARY - DIOXINS OTHER THAN TCDD

-DIOXIN	STRAIN OF RAT	EMBRYOTOXIC EFFECT	DOSE LEVELS TESTED µG/KG/DAY	ROUTE	AUTHORS
1,2,3,4-TETRA ~	WISTAR	NONE	50,100,200,400, [800]	ORAL	KHERA & RUDDICK, 1973
2,7-DI ~	WISTAR	MYOCARDIAL EDEMA	250, [500], 1000,2000	ORAL	KHERA & RUDDICK, 1973
2,3-DI ~	WISTAR	NONE	1000, [2000]	ORAL	KHERA & RUDDICK, 1973
2-CHLORO ~	WISTAR	NONE	1000, [2000]	ORAL	KHERA & RUDDICK, 1973
2,7-DI ~	SPRAGUE-DAWLEY	NONE	250,500,1000, [2000]	ORAL	SCHWETZ ET AL., 1973
OCTA ~	SPRAGUE-DAWLEY	EDEMA	[100,000] AND 500,000	ORAL	SCHWETZ ET AL., 1973

[ ] = NO EFFECT

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SUMMARY - NO-EFFECT DOSE LEVELS OF 2,4,5-T[A]

AUTHOR	SPECIES	NO EMBRYOTOXIC EFFECT
COURTNEY ET AL, 1970[B]	MICE	21.5 MG/KG
COURTNEY & MOORE, 1971	MICE	50
ROLL, 1971	MICE	20
NEUBERT & DILLMAN, 1972	MICE	30
BAGE ET AL, 1972	MICE	<50
HART & VALERIO,1972	MICE	10 (NEXT HIGHER DOSE,
COURTNEY ET AL, 1970[B]	RATS	4.6 100)
EMERSON ET AL, 1971	RATS	>24
SPARSCHU ET AL, 1971	RATS	<50
COURTNEY & MOORE, 1971	RATS	46.4
KHERA & MCKINLEY, 1972	RATS	50
BOEHRINGER, 1970	RATS	25
WILSON, 1971	MONKEY	5 (DEC WT AT 10-40)
DOUGHERTY, 1972	MONKEY	>10
EMERSON ET AL, 1971	RABBIT	>40
COLLINS & WILLIAMS, 1971	HAMSTER	80
BINNS & BALLS, 1971	SHEEP	>100

[A]STUDIES IN WHICH 2,4,5-T WAS ADMINISTERED THROUGHOUT THE PERIOD OF ORGAN DEVELOPMENT.

[B]2,4,5-T SAMPLE CONTAINED 30 PPM TCDD

SLIDE 36

CONCLUSIONS

1. 2,4,5-T IS TERATOGENIC IN SOME STRAINS OF MICE IN HIGH DOSE LEVELS BUT HAS NOT BEEN SHOWN TO CAUSE MALFORMATIONS IN RATS, RABBITS AND MONKEYS.

2. THE DOSE LEVELS OF 2,4,5-T REQUIRED TO CAUSE ANY KIND OF EMBRYOTOXIC EFFECT ARE FAR IN EXCESS OF THE EXPECTED EXPOSURE LEVELS AMONG INDIVIDUALS MANUFACTURING OR USING 2,4,5-T. THUS, IT IS EXTREMELY UNLIKELY THAT 2,4,5-T IS A TERATOGENIC HAZARD IN HUMANS.

3. THE EMBRYOTOXIC POTENTIAL OF 2,4,5-T IN SUSCEPTIBLE ANIMALS VARIES WITH THE CONTENT OF THE CONTAMINANT 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN. LEVELS OF TCDD GREATER THAN 1 PPM ARE REQUIRED TO ENHANCE THE EMBRYOTOXIC POTENTIAL OF 2,4,5-T. THUS, THE TCDD CONTENT OF CURRENTLY PRODUCED 2,4,5-T (A MAXIMUM OF 0.1 PPM) DOES NOT REPRESENT A TERATOGENIC HAZARD.