

3

A SUMMARY OF TOXICOLOGICAL DATA

OF THE PHENOXY HERBICIDES

William McBride, M.D.

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

C24720

2,4,5-Trichlorophenoxyacetic acid.

Registered in 1948 -

- phenoxy herbicides - are synthetic plant growth regulators.

The phenoxy compounds mimic the properties of a naturally occurring plant growth regulator, indoleacetic acid.

The military furthered investigation by studying phenoxy herbicide used as a vegetation control weapon in 1944-1945.

Phenoxy herbicides presently in use today in the U.S. include 2,4-D, 2,4,5-T, MCPA, silvex, 24-DB, MCPB, dichlorprop, mecoprop, 2,4-Dep and dichlorprop-methyl.

In 1975 the U.S.A. used 59 million pounds of 2,4-D, 6.7 million pounds of 2,4,5-T and two million pounds of silvex - Stanford Research Institute, Chemical Economics handbook Stanford Research Institute - Menlo Park, California - 1976.

In 1969, 11,626,000 pounds of 2,4,5-T acid esters and salts were produced in the United States alone. In 1970 the quantity rose to 12,335,000 pounds. Between 1971 and 1974 738,907 pounds of 2,4,5-T were imported into the U.S. averaging a total of 148,000 pounds per year - E.P.A. - Herbicide report, Document EPA-SAB-74-D01 US EPA, Washington D.C., 1974.

The phenoxy herbicide enters the plant in an imperfect and uncontrolled way. It interferes with the regulation of growth processes normally governed by the plants own naturally occurring growth hormones (Ashton, F.M., Craft, A.S. Mode of action of Herbicides, Wiley Interscience publishers. N.Y. 1973).

The phenoxy herbicides interfere with cell division (mitosis) and cell enlargement, food utilization and various other processes necessary to stabilize life.

C24721

CC FIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

Scientists do not know the precise mode of action of either synthetic or natural plant growth regulators. They know that phenoxy herbicides are far more toxic to green plants than to animals. Plant growth regulating compounds act on plant cell compounds such as cellulose and lignocellulose which are not present in animals.

2,4,5-T Chemistry:

2,4,5-Trichlorophenoxy acetic acid has a chemical formula, $C_8H_5Cl_3O_3$. The pure form occurs as white crystals and its molecular weight is 255.49 grams per mole.

2,4,5-T melts at temperatures of 156° - 158° C. It is stable at its melting point. 2,4,5-T has a solubility in water of 278 parts per million (-ppm) at 25° C. Further solubility occurs when this herbicide is mixed with acetone, ether, and ethanol.

Technical grade 2,4,5-T manufactured for agricultural application generally contains 90 to 92% of the herbicide and 8-10% impurities.

- detailed composition of the herbicide given by one producer:

91.0 ± 1.0%	2,4,5 Trichlorophenoxyacetic acid.
0.5 ± 0.1%	2,4,5-Trichloroanisole
2.0 ± 0.5%	5, methoxy 2,4-dichlorophenoxyacetic acid.
2.0 ± 0.5%	2, methoxy, 4-5 dichlorophenoxyacetic acid
0.3 ± 0.1%	2,4,5-Trichlorophenol.
3.0 ± 0.5%	bis 2,4,5-Trichlorophenoxyacetic acid
0.3 ± 0.1%	2,5-dichlorophenoxyacetic acid
0.2 ± 0.1%	SO ₄
0.2 ± 0.1%	sodium salt of 2,4,5-T
0.5 ± 0.2%	H ₂ O

000017 total = 100%

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24722

Fisher, J.R. letter, PGT12. In Final EIS, Vol 1. Vegetation management with herbicides, U.S. department of Agriculture, Forest Service 1977, - reported that the average level of dioxin in U.S. production of 2,4,5-T appears to be about 0.01 ppm - This was supported by International Agency for Research on Cancer 1977 - Chlorinated Dibenzo-dioxins, vol 15 World Health Organization and McQueen et al 2,4,5-T and Human Birth Defects, Division of Public Health, Department of Health, New Zealand, 1977.

Thermal stability - 2,4,5-T is stable at least to its melting point (156⁰-158⁰C).

Biochemical degradation - 2,4,5-T degrades slowly in soil containing organic matter under warm moist conditions. Half life for the herbicide under these optimal conditions approaches several weeks.

Metabolic degradation is caused by two concurrent processes. Binding of the herbicide molecule to other compounds, mainly glucose to form a non-toxic complex and actual degradation of the molecule.

Three months is generally accepted as the time span for the herbicide to disappear completely from the soil. Disappearance rate is independent of the amount of herbicide present. Even in the presence of sunlight 2,4,5-T remains relatively unreactive.

Residues

A residue is an accumulation of a product for a period following initial application. Residues occur when the initial product is not totally degraded. The persistence of a residue can cause problems. If use of a known toxin leaves sufficient residues in active form, the extent and duration of that toxicity must be recognised and appropriate cautions followed.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24723

of the particular chemical.

Soil residues Scientific studies demonstrate that phenoxy herbicides break down in soils - Andus, L.J. Microbiological Breakdown of Herbicides in Soils. In Herbicides and the Soil E.K. Woodford et Sugar (eds), Blackwell Sci Pub. Oxford England. 2,4,5-T and 24-D form carbon dioxide, inorganic chloride and water as final degradation products. Classified as slightly persistent 2,4,5-T takes anywhere from two to six weeks for a fifty percent disappearance rate.

The co-operative studies by Sterk et al
Soil incorporation Biodegradation of Herbicide Orange Microbial and Baseline ecological study of the U.S. airforce Logistics command test range Hill AFB Utah Fund report, Tecom project No 5Co-213-00-015 U.S. Army Dugway proving ground - Dugway Utah 1975,

Arnold et al. Three years of field studies on the soil persistence and movement of 2,4,-D, 2,4,5-T and TCDD - Weed Science Soc Am. Meet. Abst, 1976 - demonstrated the applications of 2,4,5-T and 2,4-D at massive rates not only did not sterilise the soil but indeed stimulated the growth of certain microflora, and that this stimulation may have contributed to the degradation of the herbicide.

Norris, L.A. et al, "The Persistence of 2,4,5-T in the Pacific Northwest Forest," Weed Science, 25(5) 417, 1977 - examined foliage, forest floor and soil for 2,4,5-T residue. The conclusions, summarised in the Journal of Weed Science were that within six months of application, herbicide residues declined 90 percent, within one year less than six percent of the herbicide remained. They found little leeching of 2,4,5-T from the forest floor to the soil and no residues were found deeper than 15cm.

CONFIDENTIAL

C24724

Hellin, et al. "Chlorodioxins in pesticides, soils and plant:" J. Env. Qual., 2: 171-178, 1973, found that 2,4,5-T is relatively mobile in soils. However, that movement decreases as organic content increases. 2,4,5-T therefore becomes moderately mobile in clay soils and only slightly mobile in mulch.

Water residues:

2,4,5-T acids and esters are soluble in water. In addition 2,4,5-T rapidly dissipates in streams by dilution and photodegradation in water - see Crosby & Wong "Photodecomposition of 2,4,5-T in water." J. Agr. Food Chem., 21(6) 1052-1054, 1970.

Research reports involving stream contamination from land spraying in Oregon demonstrated that peak concentration of phenoxy herbicides seldom exceed 0.1 ppm. These residues rarely last for more than a few hours in almost all the streams, see (Norris, L.A. et al. "The persistence of 2,4,5-T in a Pacific Northwest Forest," Weed Science, 25(5) 417- 1977).

The report of the Advisory Committee on 2,4,5-T to the Administration of the E.P.A. stated that all available data indicated that a small amount of 2,4,5-T is present but does not persist long because clay or the biota absorb the herbicide within a matter of days (E.P.A. Herbicide Document EPA-SAB 74-001-US EPA - Washington D.C., 1974).

C24725

Animal residues:

Animal residues would accumulate because of herbicide build-up elsewhere. Animals rapidly eliminate about 95% of ingested 2,4,5-T unchanged in the urine - see (Sauerhoff, M.W. et al. "The dose-dependent pharmacokinetic profile of 2,4,5-T following intravenous administration in rats." Tox. App. Pharm., 36: 491-501, 1974)

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

Piper et al. "Metabolism of 2,4,5-T in Rats."

Health Aspects Pesticides, 6(5): 272, 1973. Piper noted a significant difference in the plasma half life of 2,4,5-T remaining in the dog as compared to the amount seen in the rat. This delayed excretion time for 2,4,5-T experienced by the dog is postulated as the cause for greater toxicity of the herbicide to dogs than to rats. Hook et al. "The renal handling of 2,4,5-T in the Dog," Fd & Cos Tox, 14: 19-23, 1976) suggest a tight binding of the herbicide to the animals plasma protein operates as the mechanism for delayed urine clearance in the dog.

A study by Gehring et al. "The fate of 2,4,5-T following oral administration to man" Tox. Appl. Pharm., 26: 352-361, 1973, suggests that human urine clearance more closely parallels the rate of excretion in the rat than the dog, see Kociba et al. "Results of 2 years chronic toxicity study and oncogenic study of rats ingesting diets containing 2,4,5-T" Fd. Cosm. Tox. 17: 205-221, 1979 who support Gehring et al.'s work.

Residues do not remain indefinitely. Their loss from the environment can be predicted through the chemical's half life in various media in the environment and a knowledge of the chemicals physical characteristics.

Polychlorinated Dioxins

Recognised in 1957 as an impurity in the manufacture of herbicide 2,4,5-T. TCDD is one of the most potent small molecule toxins known to man. Small amounts of the chemical can cause enormous changes in animal cells. Currently technical grade 2,4,5-T in the U.S. routinely contains TCDD levels at or below 0.1 ppm.

C24726

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

Chemistry 2,3,7,8-tetrachlorodibenzo-p-dioxin exists as a white crystalline solid having a molecular weight of 322 grams per mole. It melts at temperatures between 303, and 305°C. and decomposes at temperatures between 980 - 1000°C. About 99.5% of the dioxin decomposes at temperatures of 800°C. 2,3,7,8-TCDD arises during the hydrolysis of tetrachlorobenzene to form 2,4,5-Trichlorophenol, the industrial precursor of 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T).

T.C.D.D. has a low water solubility and a high fat solubility. Dioxin can accumulate in the environment under some conditions. TCDD however is readily decomposed by ultra-violet light which helps to diminish potential accumulation of the chemical.

Crosby and Wong (1977) - "Environmental Degradation of 2,3,7,8-TCDD". Science, 195: 1337-1338 - found that herbicide formulations with TCDD contaminant lose most or all their TCDD within a single day when the leaves, soil or grass of the treated foliage receive natural sunlight. Photochemical dechlorination causes this degradation of TCDD. While the stability of pure TCDD as a persistent residue in the environment is accepted, it is not stable in the presence of its herbicide vehicle. Appearing on the foliage in a thin herbicide film, the contaminant diminishes as it is exposed to sunlight. Crosby and Wong established three requirements for significant dioxin breakdown in the environment. They are (1) dissolution in a light transmitting film. (2) The presence of an organic hydrogen-donor - such as a solvent or pesticide and (3) ultraviolet light. They propose that all three conditions are normally met in applications of 2,4,5-T. Therefore they feel that these data suggest that environmental residues of TCDD often will be considerably less than previously expected.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24727

Residue experiments have monitored the TCDD levels in land, water and various species of animals. For the most part TCDD residue levels directly compare to the amount of initial TCDD application and are not detectable in many instances.

Recently the Dow chemical company of Midland Michigan, released a report demonstrating the production of TCDD as a by-product of normal combustion processes. Analyzing samples from a number of combustion products such as refuse incineration emissions, automobile exhausts, fireplace soot, charcoal grills, cigarette smoke and particulate emission from chemical plant powerhouses - researchers quantitatively measured the amount of TCDD present - (Dow chemical company - Midland, Michigan, 1979.)

If environmental presence of dioxins from combustion can be further established and supported, then it will become increasingly difficult if not impossible to link environmental residues equivocally and solely to herbicide spraying.

Toxicity:

The way of expressing a chemicals acute toxicity is through its LD⁵⁰ defined as the amount of chemical sufficient to cause death in 50% of the test animals.

An LD⁵⁰ value can differ due to such variables as age or sex of test animals, nutritional state, formulation of toxicant, time of day, route of administration and temperature. Mode of entrance and chemical form of the substance can also alter the LD⁵⁰. A non observable effect level - signifies a toxicological endpoint. Chemicals administered in this concentration cause no observable adverse effects.

C24728

Animals suffer toxic effects from 2,4,5-T in dosages ranging from 10 milligrams per kilogram of body weight per day

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

narrow effects of 2,4,5-T toxicity.

Variations are expected by differences within a species - alternate modes of chemical entrance into the body and variable lengths of time for the duration of the experiment. This is fundamental to any toxicological investigation. A large dose of chemical toxin will profoundly affect an animal in a short time.

Two studies on the toxicity of TCDD are particularly note worthy - (1) a two year study detailing the chronic exposure of TCDD in Sprague-Dawley rats by Koetba et al. (2) a nine month study describing long-term toxic effects of TCDD in Rhesus monkeys by Allen and Van Miller.

Allen and Van Miller (1977) "Morphological change in monkeys consuming a diet containing low levels of 2,4,5 tetrachlorodibenzo-p-dioxin," Food Cosm. Tox., 15: 401-410.

They housed 16 adult Rhesus monkeys each weighing 5.6kg in an environment simulating their natural habitat during the mating season. For six months, investigators monitored general health and hematologic values and established control parameters for the study. At the end of this time they divided the monkeys into two groups of eight animals each. One group was placed on a diet containing 500 ppt TCDD. The other group ate an identical diet without TCDD. Food intake was quantitated for each group over a nine month period of time. After six months on the experimental diet, the females were mated with males from the control group. After nine months the experimental animals ceased eating the TCDD contaminated diet and ingested a control diet for an additional three months. Researchers estimated total TCDD levels for these animals at $5.8 \pm 0.9\mu\text{g}$ after three months, $11.3 \pm 1.7\mu\text{g}$ after six months and $17.9 \pm 2.0\mu\text{g}$ following nine months on the diet.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

09A729

After three months on the diet, monkeys developed periorbital edema, loss of facial hair and eyelashes, acne, accentuated hair follicles and dry scaly skin. After six months these changes became prominent in six of the eight animals concerned. At the six month analysis monkeys also experienced a decreased haemoglobin and haematocrit (an decrease in red cells). These lowered haematological parameters became especially exaggerated in six of the seven monkeys that survived the nine months on experimental treatment. Animals in the experimental group lost weight despite no change in feeding habits.

After seven months, one monkey became severely pancytopenic. Prior to death the animal had virtually no platelets or immature red or white blood cells. In the following four months four additional monkeys experienced similar clinical changes and died. Of the animals that survived the regime all displayed significant hair loss and periorbital edema. During the twelfth and final month of the study, one monkey developed a reduction in both platelets and white blood cells.

Autopsy findings revealed several areas of damage from TCDD exposure. Focal areas of haemorrhage appeared in the skin, heart, lung and stomach, intestine, adrenals, skeletal musculature, brain and bone marrow. Fingernails and toe nails grew irregularly, often accompanied by gangreous necrosis (i.e. death of cells surrounded by living tissue). They also suffered from ascites (an abnormal collection of serous fluid in the peritoneal cavity, it is seen in cases of carcinoma, tuberculosis and other chronic inflammations of the peritoneum) and subcutaneous edema. Hypertrophy, dilation, edema, and hydropic degeneration of the myocardium occurred in all the animals. The biliary ducts showed marked dilation without associated jaundice

C24730

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

sebaceous glands. The Monkeys also demonstrated a marked decrease in the haematopoietic process in the bone marrow and hypocellular lymphoid tissue (i.e. the blood making process). Cellular hyperplasia appeared on many levels especially in the mucous secreting cells, epithelial lining of the biliary system and the transitional epithelium of the urinary tract and goblet cells of the palpebral conjunctivae (the conjunctiva of the eyelids).

They concluded that the primary target area for TCDD in non-human primates (monkeys) is the haematopoietic (blood producing) system. As exposure lengthens cellular degeneration involving the lymphoid system, responsible for the formation of white blood cells (leukocytes) and bone marrow responsible for red blood cells (erythrocyte) formation, increases in severity. In addition atrophy of the thymus, spleen and lymph nodes also occur. Allen and Van Miller assert that there is considerable differences in the level of TCDD necessary to produce intoxication. The LD⁵⁰ range of 50-70µg/kg body weight produces death in 50% of the test animals in an acute space of time, a considerably lower dose of 3µg TCDD per kg body weight will also yield the same results when administered continuously over a nine month period. Allen and Van Miller also demonstrate that the pathological changes caused by the two dose levels remained quite similar. Therefore they believe their data indicate that exposure to extremely low concentrations of TCDD can produce widespread deleterious effects in primates.

Kociba, R.J. et al. "Results of a 2 year chronic toxicity and oncogenic study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in rats."

Tox. and Appl. Pharm. 46: 270-303, 1978.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24731

They placed male and female Sprague-Dawley rats aged six to seven weeks into suspended wire bottom cages. Food and water were available to the animals ad libitum. Groups of 100 rats (50 males, 50 females) ingested a diet that contained 0.1, 0.01, 0.001ug TCDD/kg/day for up to two years. The diet of the control group (86 males, 86 females) 172 rats contained only the carrier, no TCDD.

The TCDD was provided by Dow chemical company and had a purity exceeding 99 percent as determined by electron capture gas chromatography.

Body weight and food consumption of 20 males and 20 females at each treatment level were routinely recorded for each week of the first three months of the study and at monthly intervals thereafter. Eight rats per sex group provided blood samples for haematological investigation at three, twelve and twenty three months of treatment. Urine samples were collected from seven to eight rats per sex per group at these same time levels to determine specific gravity, pH, sugar protein, ketones, bilirubin, and occult blood.

At terminal necropsy, investigators collected serum samples from all survivors or - maximum of 10 rats per sex group for determination.

All rats dying or killed during the course of the study underwent gross pathologic investigation and representative portions of major organs and any lesion suggestive of a significant pathologic process or tumour formation were preserved in 10% formalin.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24732

Terminal necropsy examination took place at the end of two years of treatment. The weights of the liver, kidney, brain, heart, thymus, spleen, testes and ovaries/uterus were recorded for a maximum of 10 rats per sex per group. Bone marrow smears from most rats were also prepared. They also froze portions of fat, liver and kidney from a maximum of five rats per sex per group for possible TCDD analysis.

All rats from the control and the high dose level regardless of whether they died during the course of the study or were killed upon its completion, underwent histologic investigation of an extensive list of tissues intended to include a majority of those tissues listed above and those collected at the time of terminal necropsy. All rats from the lower dose levels were subjected to histological examination of those selected tissues identified as possible target area organs in addition to gross lesions suggestive of tumor formation.

They found at the conclusion of the study (1) continuous ingestion of diets containing approximately 2200 ppt TCDD (0.1ug/kg/day) for two years caused multiple toxic effects, including increased mortality, decreased body weight gain, slight depression of certain haematological parameters, increased urinary excretion of porphyrins and λ -amino levulinic acid. Increased serum activities of Ap, GGT, and SGPT and morphological changes primarily of the hepatic lymphoid respiratory and vascular tissue of the body. This high dose level seemed to reduce the incidence and severity of chronic renal disease in aged male rats. Female rats given this high dose for two years had 24,000 ppt TCDD present in the liver. This compares with 34,600 ppt TCDD present in the liver of female rats given this dose for thirteen weeks, thus indicating attainment of steady state concentration in the early

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

C24733

Ingestion of the intermediate dose level of 0.01 μ g TCDD/kg/day (approximately 210 ppt in diet) caused a lesser degree of toxicity. Here research found:

1. increased urinary excretion of porphyrins (females)
2. liver toxicity, including an increased incidence of hepatocellular nodules
3. increased incidence of focal alveolar hyperplasia in the lungs.

Terminal liver and fat content of TCDD averaged 5100 and 1700 ppt, respectively. This compares with 3700 ppt present in the liver after thirteen weeks at this dose level.

Lifetime ingestion at a dose of 0.001 μ g/kg/day (approx. 22 ppt in the diet) caused no effects considered to be of toxicological significance. The liver and fat each contained 540 ppt of TCDD at the end of lifetime ingestion at this low level.

Kociba et al demonstrated a dose related toxic response to TCDD. At the lowest levels of TCDD tested, 0.001 μ g/kg/day, the authors could find no toxic response attributable to the inclusion of TCDD in daily diet given to rats over a two year period of time.

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

Toxicity Summary:

TCDD inevitably contaminates 2,4,5-T, no consideration of toxicity of the herbicide without proper attention to the toxicity of the contaminate TCDD can be considered complete.

Toxic response to 2,4,5-T and TCDD vary among and within species. LD⁵⁰ values for 2,4,5-T range from a single 100mg/kg dose in the dog (acid form) to 500 in the rat (acid form). In the mixed butyl ester form of 2,4,5-T, the mouse exhibits an LD⁵⁰ of 490mg/kg single dose.

C24734

For TCDD the LD⁵⁰ for the guinea pig is 0.6µg/kg. The mouse has a 120µg/kg LD⁵⁰. Dogs however show no effect at doses as high as 100µg/kg. LD⁵⁰ for dogs is 3000µg/kg.

Initial toxic response to 2,4,5-T appears at dosages of 10mg/kg in the dog and escalates to 140mg/kg in the mouse. Weight loss, muscle weakness and accompanying tissue changes represent the most common effects of 2,4,5-T toxicity.

In a chronic two year study detailing the effects of 2,4,5-T on Sprague-Dawley rats, Kociba et al. (1979) ascertained a level (3mg/kg) where the animals exhibited no toxic effects from chronic exposure to 2,4,5-T. However the herbicide used in this project was purified 2,4,5-T containing 0.3 ppb TCDD. Commercial samples of the chemical routinely contain 0.1 ppm TCDD.

The thymus and to a lesser degree, the lymphoid tissue and bone marrow (including hematological dyscrasias derived from damage there) are the primary areas which exhibit a toxic response to TCDD. Mice are least sensitive to TCDD's toxic effects. Guinea pigs and chicks suffer the greatest adverse effects. Initial toxic response appears after single doses of 3µg/kg in the guinea pig and up to a dose of 100µg/kg in the rat.

Exposure of Rhesus monkey to 500 ppt TCDD in the diet reported by Allen and Van Miller (1977). The authors detailed toxic changes leading to death in five of the eight monkeys studied, after what they determined to be daily doses of 2-3 µg/kg TCDD for a chronic period of time. Allen and Van Miller suggested that the effects noted in their experiment paralleled the changes experienced after a single administration of an acute LC dose of 50-70µg/kg.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24735

Kociba et al (1978) conducted a two year chronic toxicity study of TCDD on Sprague-Dawley rats. These experiments demonstrated a dose related toxic response when administered in daily diet at doses of 0.1, 0.01 and 0.001 µg/kg TCDD per day. The highest dose elicited multiple toxic signs, while their lowest dose (0.001 µg/kg) caused no effects of toxicological significance.

Teratogenicity: (Malformations in the Offspring).

The discipline of teratology encompasses three categories: causes, mechanisms and manifestations. A cause is an external element capable of eliciting a detrimental change (teratogenic agent). These agents act in specific ways (mechanisms) on developing cells.

Mechanisms all interact at the earliest stages of development involving many cellular processes and environmental conditions. Up to Day 17 after conception in humans there occurs a "retractory period". From day 18 to day 30 the embryo becomes very susceptible to outside interference. At this stage the early stages of tissue and organ differentiation take place (from gastrulation to organ differentiation). Thereafter the embryo shows an increasing resistance to teratogenic forces as organogenesis advances to completion in days 55 to 60 in man from day of conception.

In all species the period of organogenesis remains most fragile to outside teratogenic influences. Upon completion of organogenesis, outside influences can generate an adverse change, but these are usually in the form of growth or functional alterations.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24736

Wilson, J.G. Environmental and Birth Defects.

Academic Press, New York and London, 1973, Wilson lists 6 generalisations of teratologic susceptibility.

1. susceptibility to teratogenesis depends on the geno-type of the conceptus and the manner in which this interacts with adverse environmental factors.
2. susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence.
3. teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis).
4. the access of adverse influence to developing tissues depends on the nature of the influence (agent)
5. the four manifestations of abnormal development are death, malformations, growth retardation and functional deficit.
6. manifestations of abnormal development increase in frequency and degree as dosage increases from the no effect level to the totally lethal level.

Almost any chemical substance possesses a teratogenic potential when administered in excess amounts during a susceptible period of development. A broad spectrum of compounds are responsible for a myriad of biological and chemical effects.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24737

Some types of Drugs and other chemicals that have been shown to be teratogenic in one or more species of laboratory animal.

<u>Drug type</u>	<u>Examples</u>
salicylates	asprin, oil of wintergreen
certain alkaloids	caffeine, nicotine, colchicine
tranquilizers	meprobamate, chlorpromazine, reserpine
antihistamines	bucizine, meclizine, cyclizine
antibiotics	chloramphenicol, streptomycin, penicillin, tetracycline
hypoglycemics	carbutamide, tolbutamide, hypoglycins
steroid hormones	triamcinolone, cortisone, testosterone
alkylating agents	busulfan, chlorambucil, cyclophosphamide
antimalerials	chloroquine, quinacrine, pyrimethamine
anesthetics	halothane, urethane, nitrous oxide, pentobarbital
antimetabolites	folic acid, purine, and pyrimidine analogs
solvents	benzene, dimethylsulfoxide, propylene glycol.
pesticides	2,4,5-T, carbaryl, captan, folpet
industrial effluent	some compounds of Hg, Pb, As, Li, Cd.
miscellaneous	trypan blue, trypanol, diazox

Teratogenic effects were usually seen only at doses well above therapeutic levels for the drugs, above or likely exposure levels for the environmental chemicals. Source Wilson, J.G. Handbook of Teratology Vol. I. General principles and etiology, James Wilson, Clarke Fraser (eds), Plenum Press, New York and London, 1977.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24738

Wilson in his book Environment and Birth Defects (1973) opcit, lists the necessary criteria for recognising a new teratogenic agent in man.

1. an abrupt increase in the incidence of a particular deficit or association of defects
2. coincidence of this increase with a known environmental change, i.e. widespread use of a new drug
3. known exposure to the environmental change early in pregnancies yielding characteristically defective infants
4. absence of other factors common to all pregnancies yielding infants with the characteristic defect.

Since chemical response varies with dosage route of administration and particular agent, Wilson (1977) opcit. has established criteria and categories for classifying chemicals according to their embryotoxic (causing damage in early pregnancy) potential (assuming exposure to therapeutic or higher dose during 1st trimester) in man.

1. established as being embryotoxic
 - (a) unquestionably produces a higher percentage of developmental defects or intrauterine death in infants of exposed women than otherwise similar nonexposed women.
2. suspected of being embryotoxic
 - (a) apparent increase above background level of developmental defects or intrauterine death among infants of exposed women, based on large numbers of accumulated case reports.
 - (b) retrospective surveys indicate significant increase of defects or death among infants of exposed women, but "memory bias" of mothers introduces uncertainty
 - (c) prospective surveys indicate significant increase among

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24739

Numerous studies have been conducted detailing the teratogenic embryotoxic and fetotoxic effects generated by 2,4,5-T and its contaminant dioxin. Researchers experimented with a variety of species and have demonstrated both no effect and lethal levels in many cases. The prime target areas for teratogenic response from 2,4,5-T and dioxin are cleft palate and kidney anomalies.

Mutagenicity

The ability of a substance to induce genetic alterations. Because of the similarities of basic molecular mechanisms by which chemical mutagens and most chemical carcinogens appear to induce genetic effects (i.e. molecular lateralization of DNA) researchers postulate that mutagenic effects can be predictive of carcinogenicity -

- Scientists rely on short term tests to indicate the mutagenic potential, a chemical possesses. These tests include:
- 1. tests for mutation in bacteria and eukaryotic microorganisms
- 2. tests for mutations in somatic mammalian cells
- 3. tests on chromosomal effects in higher eukaryotes including mammals.
- 4. evaluation of DNA repair synthesis.

All of the 2,4,5-T mutagenicity studies have drawn either negative or inconclusive results in tests covering primary DNA damage, gene mutations and chromosome effects. The scientific dispute resolution conference on 2,4,5-T, 1979 August, stated that reasonably adequate experimental data has been generated regarding 2,4,5-T and that these data are not suggestive of 2,4,5-T induced mutagenic activity.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24740

The scientific dispute resolution conference on 2,4,5-T 1979 also felt that results from several microbial studies have determined that TCDD increases the frequency of gene mutations. Microbial data suggests that DNA repair processes are involved in the mechanism of action since no mutagenic effects can be elicited in studies using repair defective bacterial strains. The bacteria data suggests that TCDD acts as a direct initiator of malignant transformation since most mutagenic carcinogens are of the initiator class. Few promoters or cocarcinogens show mutagenic activity.

Carcinogenicity

Animal model studies have long been used for predicting carcinogenic potential in man. The species of animals used in these studies must be as carefully considered as the data resulting from the experiment. Rodents meet certain specifications that make them highly desirable for carcinogenicity experiments. Their lifespan is short - which allows the fullest expression of a positive carcinogenic response. Rodents are small, relatively inexpensive, easily bred, all of which encourage large study samples and confirmation of test results by other investigators.

Yet results obtained using rats or mice cannot be unequivocally adapted to man because of certain biological differences. Second the Sprague-Dawley rat and several mouse strains noted for their high rate of spontaneous tumor incidence are often used in these experiments so care must be taken to discriminate between spontaneous tumors and those caused by carcinogenic mechanisms. Also a carcinogenic response can become apparent long after the original cancer causing stimulus has been removed. Manifestations of cellular alterations can take years to develop - By the time a carcinogenic

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

094711

because that would not fit with the classic carcinogenic mechanism.

All these factors must be considered when analyzing a chemical for its possible carcinogenic properties.

Research conducted on animal models for potential carcinogenic effects of 2,4,5-T have failed to yield positive results. The E.P.A. Carcinogen Assessment group, report in February 1979 acknowledged these conclusions. "On the basis of the completed mice and rat studies there is no significant evidence of carcinogenicity for 2,4,5-T". They added however, that testing had not been done sufficiently in mice.

TCDD is an extremely potent inducer of hepatic aryl hydrocarbon -(AHH) activity in the rat -(see Poland & Glover "Comparison of 2,3,7,8-tetrachlorobenzo-p-dioxin - a potent inducer of aryl hydrocarbon hydroxylase with 3-methylcholanthrene," Molec. Pharmacol., 10: 349-359, 1974). This enzyme system is proposed as the promoter of carcinogenic activity. Further, in the presence of known carcinogen DMSO, TCDD is weak tumor initiator in the two stage system of mouse skin tumorigenesis.

Investigations of the carcinogenic potential of TCDD have produced dose-dependent results. At relatively high, long term doses, a diet containing TCDD has been found to cause increased incidence of liver and lung tumor as well as nasal turbinate palate tumors in certain rat species.

Two animal studies are important in the analysis of carcinogenesis potential of TCDD.

1. a two year chronic study by Kociba et al, 1978.

Tox. and Appl. Pharm., 46, 279-303,

2. A 78 week investigation conducted by Van Miller et al.

"Increased incidence of neoplasms in rats exposed to low level

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24742

In both studies Sprague-Dawley rats were used. At the highest dosage tested their experiments yielded similar results. At highly toxic levels of 2200 ppt and 500 ppt respectively these reports documented an increase in the incidence of liver and lung tumors in Sprague-Dawley rats.

At the lower dose levels their results differed. Kocibas group considered the lower dose ranges negative for carcinogenic effect citing the appearance of hepatic hyperplastic modules from a medium dose as the only cellular change observed.

Van Miller reported significant carcinogenic potential at doses well below the 500 ppt dose. Tumors, he claimed, appeared with TCDD levels of 1000 ppt, 500 ppt, 50 ppt and 5 ppt. Only at 1 ppt did Van Miller acknowledge no chemical related effects.

There have been several criticisms of Van Miller's investigation. The carcinogen assessment group audited the study and found that it had major shortcomings in design and conduct that limited the reliability of data developed at dose levels lower than 100 ppt (EPA 1977). Therefore its carcinogenic effects could only be verified at the two highest dose levels. In addition the "investigators in the study reported no tumors in the control animals a highly unlikely phenomenon when using Sprague-Dawley rats" (EPA, 1979). This strain of rats is particularly susceptible to spontaneous tumor induction and lack of tumors in controls is highly suspect. Also the areas of tumor induction at lower dose ranges of TCDD did not correlate with the expected target organs determined from the higher doses - i.e. lung and liver. Also the experiment only involved 10 animals per test group. This small number is below the minimum number recommended by the National Cancer Institute.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24743

The Kociba study has also been criticised. Kociba et al found hyperplastic alterations in the liver at the 210 ppt dose and considered this a negative finding. In an audit of the Kociba study by the Carcinogen assessment group, a conflict of classification of hyperplastic nodules became evident. The CAG group re-evaluated the study and concluded that the combined incidence of hepatocellular hyperplastic nodules and the hepatocellular carcinomas is statistically significant for both the 2200 ppt and 210 ppt level.

Studies by Hirota and Williams 1979 - "Persistence and Growth of Rat Liver Neoplastic Nodules Following Cessation of Carcinogen Exposure." J. NCI, 63(5), 1257-1265, 1979 - They investigated the persistence and growth of rat neoplasms and concluded that these nodules represent a different type of neoplasm than carcinoma possibly a benign neoplasm. They assert that these neoplastic nodules are not obligatory precursors of carcinoma and may in fact be at a stage in neoplastic development that is less autonomous than carcinoma. Their interpretation of nodule therefore is an end stage, benign neoplasm that is capable of continued growth but is not invasive or metastatic and may only infrequently acquire these properties. In their study, the authors detected no transition of nodules to carcinomas. At intervals after cessation of carcinogen exposure, nodules displayed changes suggestive of phenotype reversion or degeneration that were not present in carcinomas.

Those who support the Kociba conclusion favour this interpretation of hyperplastic nodules as non-proof of carcinogenicity. Further support for such rationale lies in the lack of carcinogenic character of nodules to tumors within the two year test period of the Kociba et al study.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24744

The EPA, FIFRA Scientific Advisory Panel, Review of FIFRA Section 6(6) 2 Action on 2,4,5-T and Silvex, September 27, 1979. Also has reservations as to whether hyperplastic nodules are precursors, per se of hepatocellular carcinoma.

HUMAN EXPERIENCE WITH 2,4,5-T and TCDD

1. Dow chemical plant workers.

The study examines the mortality of 204 persons exposed to 2,4,5-T during its manufacture from 1950 to 1971 at the Dow chemical plant in Midland Michigan. The health status of 2,4,5-T workers exposed for a period of over one year. Investigators concentrated on the crew directly involved in the 2,4,5-T manufacture process. In general, at exposure concentration of 2,4,5-T (containing less than 1 ppm TCDD) experienced by the workmen in the study, no adverse mortality effects have been observed attributable to the work environment. Mortality compared well with that of all U.S. white males, and also compares well with the background mortality experience in the geographical area see Gitt et al. "A Mortality analysis of employees engaged in the manufacture of 2,4,5-Trichlorophenoxyacetic acid." J. Occ. Med. 22(1) 47-50, 1980. The single observed cancer death occurred as a result of respiratory malignancy discovered in a 63 year old retiree who had been exposed to 2,4,5-T for eight years. The employee also had a past history of smoking up to two packs of cigarettes per day.

2. Monsanto plant accident, Nitro, West Virginia

A study of the chronic health effects of exposure to TCDD as reflected in the mortality experience of a group of Monsanto workers who developed symptoms of chloracne following a trichlorophenol process accident in Nitro, West Virginia, 1949.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24745

Chloracne is a skin disease characterised by comedones, cysts, pustules and abscesses. Accompanying this may be hepatic dysfunction (impairment of function of the liver) peripheral neuritis (paralysis of the facial nerve) disorders of fat metabolism and porphyria cutanea tarda, a chronic skin lesion condition caused by the disturbances in porphyrin metabolism. Chloracne is primarily associated with exposure to TCDD.

In March 1949, a reaction accident occurred at the plant discharging TCDD into the atmosphere and interior of the building.

Employees involved in the preliminary cleanup developed immediate symptoms - including eye and respiratory tract irritation, headache, dizziness and nausea and severe irritant reaction of exposed skin. After these symptoms subsided chloracne symptoms appeared.

A four year follow up revealed

1. acne from lesions, 2. severe pains in muscles of upper and lower extremities, shoulders and thorax on exertion 3. Fatigue, nervousness and irritability 4. decrease of libido 5. dyspnoea, 6. vertigo and intolerance to cold. One year after the accident, four of the six workers examined had liver enlargement. Also found were liver impairment indicated by hepatomegaly (liver enlargement), tenderness in the right upper quadrant and epigastrium and a delayed prothrombin time.

1953, four of the six original workers were again examined. Investigators observed a general regression of both cutaneous and non cutaneous symptoms. All workers exhibited a marked improvement in their skin lesions.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24746

The Journal of Occupational Medicine report in January, 1980, carried out a 29 year follow-up of 121 members of the original study cohort - 89 members were verified as living and 32 were verified deceased by death certificate. The results of standardised mortality ratio for all deaths was 0.69 with 32 deaths observed, and 46.41 deaths expected. The report by the American Council on Science and Health 1980 - felt this was the only statistically significant value determined. There were nine malignant neoplasm with 9.04 expected. No deaths resulted from stomach or liver cancer. Five lung cancer deaths occurred versus 3.02 expected. One skin cancer (a fibrous histiocytoma) proved fatal. Exceedingly rare the expected incidence of its appearance is 0.15. Three deaths from lymphatic and hematopoietic neoplasms resulted with 0.88 expected. There were 17 deaths from diseases of the circulatory system with 25.01 expected.

Because the study cohort was small and only 32 deaths were observed the results from this study cannot be considered conclusive. Nevertheless, this is the best long term study of TCDD effects to date. The analysis of mortality experiences of personnel at this point indicate no apparent excesses of total mortality or of deaths due to malignant neoplasms, or circulatory diseases.

3. Arkansas cleft palate study.

C24747

A study to determine what, if any, human effects were experienced after exposure to 2,4,5-T. A retrospective study on the incidence of cleft palate in areas sprayed with 2,4,5-T. Researchers analyzed facial cleft rates and concurrent use of 2,4,5-T in Arkansas during 1948-1974. Estimated levels of exposure to the herbicide was categorised as high, medium or low exposure on the basis of rice acreage during 6 - 7 year intervals beginning

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

than two fold. A two fold increase in any anomaly would be extremely large. We believe more powerful epidemiological studies of 2,4,5-T are needed to more conclusively resolve questions concerning its developmental toxicity under conditions of regulated use".

4. Humboldt county

The California department of Health carried out an epidemiological investigation to assess the claims of abnormal outcome of pregnancies in the Orleans California area, which includes a phenoxy herbicide spray area.

The methods of investigation Orleans included:

- 1) Review of Humboldt county vital record for information concerning all live births, fetal and infant deaths, and congenital malformations occurring among infants born in Orleans and nearby areas during the years 1974-1978
- 2) Determination of the dates of spraying phenoxy herbicides within - five mile radius of Orleans
- 3) Review of Hoopa Medical Clinic records for live births and

C24743

Nelson et al. "Retrospective study of the relationship between agricultural uses of 2,4,5-T and cleft palate occurrence in Arkansas," Teratology, 19(3): 377-303, 1979. Nelson found that facial cleft rates presented by sex, race, time period and exposure group generally rose over time. No significant differences were detected in any race or sex grouping.

The authors concluded that the increase in incidence of this anomaly was due to better case assessment rather than actual increase in incidence. They cautioned that "The present study does not implicate any overt causal relationship between 2,4,5-T use and facial clefts". Yet they added "It is important to recognise that such an effect, if it were to exist, would not have been detected, if the increase in facial clefts was less

- 4) Interviews with affected mothers in Orlenas for dates and details of pregnancy problems.

Within the study period, 87 pregnancies occurred. Of these, eight, 9% ended in spontaneous abortion, one (1 percent) in fetal death (placenta previa) and the remaining 78 in live births. - Reed, D. "California department of Health Services memorandum, Further investigations into reports of abnormal outcome of pregnancies in Humboldt and trinity counties, April 27, 1979." Of the live births, six (7.7%) children were born with birth defects (two hypospadias, two with incomplete hip socket formation, one cleft lip and palate, one mild talipes equinovarus) one sudden infant death syndrome at age seven months.

Phenoxy herbicide spraying has been carried out in the fall of the year since 1961 with the exception of 1962, 1972, and 1973. Spraying ceased in November 1976. Therefore spraying consistency cannot be established in this case.

When the 16 abnormal pregnancies were compared to the total pregnancy outcome for that region, the picture came into perspective:

- 1) The spontaneous abortion rate of 9% is less than the expected rate of 15%.
- 2) Major congenital malformations occur at the rate of 7% of live births, while inclusion of minor defects brings the percentage up to 15%. The Orlenas rate of occurrence is 7.7% - so the figures were within the expected boundaries.
- 3) One fetal death and one sudden infant death also reflect the patterns established for the state.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24749

Reed concluded that "it appears unlikely that there has been an unusually high rate of pregnancy wastage in Orleans".

5. St. Louis Missouri, Horse arena.

Beale et al. 1977. Long-term effects of dioxin exposure.

Letter to editor, Lancet, 1(8014): 748.

August 1972, the Missouri Division of Health and the Center for Disease Control reported an investigation of a horse arena in Eastern Missouri where 54 of 57 horses exposed to the arena had died of an illness characterised by skin lesions, severe weight loss, and hepatotoxicity. Birds, dogs, cats, insects and rodents were also found dead in and around the arena. A six year old girl who frequented the arena as a playground, developed epistaxis, a gastrointestinal complaint and severe hemorrhagic cystitis characterised by hematuria. Bacterial and viral urine cultures yielded negative results. Three other persons suffered from milder illnesses with symptoms of headaches and nausea. They also visited the arena though for shorter periods than the young girl.

Subsequent soil analysis showed that both TCP (trichlorophenol) and TCDD were the probable toxic substances causing the adverse health effects. It was found that the arena soil contained a TCDD level of 31.8 to 33µg/g(ppm) of soil. The contamination was traced back to waste oil used in the area to control dust.

C24750

On reexamination of the young girl after a five year interval since exposure, the patient had grown normally. Both height and weight exceeded the 75th percentile. Detailed physical, neurological and chemical examinations revealed no abnormalities. The same studies were conducted on the patients' sister and mother exposed simultaneously though less extensively to TCDD and the results were

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

Beale et al concluded "Our experience demonstrates that people exposed to dioxin can recover completely with no apparent sequela from the toxin. It remains to be seen whether the exposure to dioxin in these children will result in abnormal pregnancies or affect their offspring.

Seveso, Italy:

The Icmesa (Industrie Chimiche Meda Societa Anonima) chemical plant did not produce herbicide 2,4,5-T but made hexachlorophene, an anti bacterial agent. The herbicide and hexachlorophene share a common denominator, 2,4,5-trichlorophenol in their manufacturing process.

The Icmesa factory explosion occurred in a reactor that produced 2,4,5-trichlorophenol by combining tetrachlorobenzene with sodium hydroxide in an ethyleneglycol solvent. The explosion propelled forth a toxic white cloud which when cooled, descended slowly over the surrounding area. Among the chemicals that contaminated the area during cooldown was the chemical dioxin (TCDD). Based on determinations made by production officials, an estimated 4,000 to 5,000 kg of reaction product was lost into the atmosphere (di Domenico, 1977). Estimates of the TCDD dispersion ranged from 650 grams to 1,700 grams - Kearney, P.C. et al. (TCDD in the environment, sources, fate and decontamination. Environmental Health Perspective, 5, 273-277, 1973). A sample of the escaped product taken from the reactor head for analysis revealed the presence of 3.5% 35,000 ppm TCDD.

C24751

Reggiani - "The estimation of the TCDD toxic potential in the light of the seveso accident." Paper presented at the 20th Congress of the European Society of Toxicology, Berlin (West) June 25-28, 1978. - Reggiani reported that birds, rabbits and

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

of TCDD poisoning or were selectively killed to ward off further contamination. By the following Monday, leaves from plants and trees turned brown and fell off trees in an area measuring 1,000 to 3,000 meters in a triangle with a base of 400 meters and a vertex of around 100 meters centered around the factory. Measurements of TCDD residue on vegetation in this area ranged from 1 to 65 ppm, with one reading as high as 50 ppm.

Children and adults exposed to the descending dust complained of nausea and developed skin lesions accompanied by redness and swelling. Some experienced the burns and blisters common in exposure to caustic material.

Garattini- 1977. "TCDD poisoning at Seveso," Biomedicine, 26: 28-29. reported the highest TCDD content was determined in the herbivorous animals. People complained of nervous symptoms, mood changes and above all a serious rash-like skin lesion - later diagnosed as chloracne.

The Seveso area being divided into two zones - Giovanardi, A. (Decontamination progress for the dioxin contaminated areas of Seveso and Media, Cuihta Resionale Della Lombardia, Ministero Della Sanita, Milan, Italy, August 18, 1976 min. p.17). described the first zone "A" as a triangular area covering approximately 100 hectares (ha). Zone A included land located south, southeast of the Icmesa factory and downwind at the time of the accident. Reggiani (1978 opcit) determined that soil levels registered TCDD levels approximately 10 pphs. The inhabitants of this area were evacuated in three stages - on July 26, July 28 and August 2, 1976.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24752

Zone B had detectable soil levels of TCDD. Reggiani (1978 opcit) later described this area as having residue levels between 0.1 and 10 ppbs for TCDD. This area covered approximately 250 ha and was divided between a large urban center and an extensive rural area with some small residential aggregates. The population of 4,900 was not evacuated. Recommendations were made for the women and children in order to reduce the possibility of exposure.

A third Zone, Zone R, covered a total of about 1,430 ha. Occasional residue concentrations, all less than 0.1 ppbs TCDD in soil, characterised this zone. This population of Zone R approximated 40,000 people.

An extensive health surveillance system was set up to determine the mid-term and long-term range effects of TCDD exposure. Medical surveillance included laboratory tests, medical examinations, monitoring of pregnancies and abortions for any abnormal outcomes, rate of stillbirths, population morbidity and mortality, and cancer registry. The medical surveillance program was extended to 11 Districts with a population of 216,000.

Reggiani, G. (The estimation of the TCDD toxic potential in the light of the Seveso accident, Paper presented at the 20th Congress of the European Society of Toxicology, Berlin (West) June 25-28, 1978). He concluded "The Seveso accident has not revealed up to now, toxic effects in humans which have not been observed in other episodes. Chloracne, the typical skin lesion, has occurred in children with the tendency to spontaneous and rapid healing. The peripheral nervous system has perhaps been attacked and reacted with subclinical signs of impairment. Signs of involvement of the liver without apparent functional disorders

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

024753

and loss, no gross malformations, no growth retardation at term and no cytogenetic abnormalities. The immunocapability of the population not even of the children with cancer, has not been attained.

At the dispute resolution conference on 2,4,5 T in 1979, Doctor L. Sanchioni and H. Rehder presented data on abortion experiences following the explosion:

- 1) Twenty-eight of the 35 induced abortions were represented by products of conception that were partially fragmented during curettage and incomplete for morphologic evaluation. No morphologic malformations were noted in any of these fetuses.
- 2) A slight increase in the incidence of spontaneous abortions was determined in the B Zone. These increases might have been due to improved registration (some of the abortions also may have been self induced). Fourteen of the 57 cases of spontaneous abortions and perinatal mortality examined were malformed structurally (24.6%). This incidence was within the range that might be expected generally, but could not be compared to the rate in this particular area prior to the accident as no data are available. The types of malformations seen were consistent with those which would be expected spontaneously. All the reported malformations were in the R and RT Zones; where the soil concentrations of dioxin were below detectable limits.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24754

and loss, no gross malformations, no growth retardation at term and no cytogenetic abnormalities. The immunocapability of the population not even of the children with chloracne, has not been attained.

At the dispute resolution conference on 2,4,5 T in 1979, Doctor L. Sanchioni and H. Rehder presented data on abortion experiences following the explosion:

- 1) Twenty-eight of the 35 induced abortions were represented by products of conception that were partially fragmented during curettage and incomplete for morphologic evaluation. No morphologic malformations were noted in any of these fetuses.
- 2) A slight increase in the incidence of spontaneous abortions was determined in the B Zone. These increases might have been due to improved registration (some of the abortions also may have been self induced). Fourteen of the 57 cases of spontaneous abortions and perinatal mortality examined were malformed structurally (24.6%). This incidence was within the range that might be expected generally, but could not be compared to the rate in this particular area prior to the accident as no data are available. The types of malformations seen were consistent with those which would be expected spontaneously. All the reported malformations were in the R and RT Zones; where the soil concentrations of dioxin were below detectable limits.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24755

Alsea I.

The study deals with a limited number of women residing in the Alsea Basin Area of Oregon. A number of women living in this 2,4,5-T treated forest area experienced miscarriages, which they said were correlated with the spraying season. Initially 10 miscarriages were reported by eight women. Subsequently 13 miscarriages were reported from nine women. Twelve of the 13 miscarriages occurred within the first 20 weeks of gestation.

When this data was compared to the live birth rate in Alsea (86 in the six year period studied) a miscarriage rate of 12-13 lies well within the limits of an expected one miscarriage for every seven live births.

The report noted a seasonal variation in miscarriage incidence when the Alsea values were compared with those from nearby Corvallis, Oregon. However, insufficient evidence precluded any establishment of proof or non-proof for a relation between miscarriages and 2,4,5-T. Mantel, N. (An evaluation of the statistical methods used in EPAS report of assessment of a field investigation of six years spontaneous abortion rates in three Oregon areas in relation to forest 2,4,5-T spray practices. (Alsea 11 report) George Washington University, March 26, 1979) - commented that "the possibility thus arises that at least some of these miscarriages were experienced by miscarriage-prone women. He also felt the "publicity about 2,4,5-T spraying and spontaneous abortion could have caused more previous spontaneous abortion to be recalled and reported".

Alsea II

A continuation of the Alsea study, this epidemiologic investigation further analyzed the possible relation between spraying of herbicide 2,4,5-T and reproduction events. The results of this study provided the basis for the EPA suspension order in February 28,

C24756

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER

The Alsea II investigators collected data from three areas of Oregon. The study area, which included an area of approximately 1600 square miles of Oregon coast and coastal mountain range. The "urban" area which is contiguous with the study area and included the inland towns of Corvallis and Albany, and the rural "control" area which is in sparsely populated eastern Oregon on the Idaho border.

The information gathered in the study came from hospital records and included coded diagnoses, age of patient, spontaneous abortion data, gestation period and zip code of patients residence. The investigators limited themselves to spontaneous abortions of less than 20 weeks gestation. Treated in hospitals in a six year period from 1972-1977. These data were then compared to live births for the respective areas. Conclusions revealed by the EPA study are summarised as follows.

- 1) the spontaneous abortions index (hospitalised miscarriages per 1000 births) for the Alsea study area where 2,4,5-T was used significantly greater than the index for the urban and control areas, where there was no known use of 2,4,5-T.
- 2) There was a dramatic increase in the spontaneous abortion index for the study area relative to the urban and control areas in the month of June and July. This increase followed by approximately two months a period in March and April when 2,4,5-T was used to control vegetation in the study area. Relative to the urban and control areas in the month of June and July. This increase followed by approximately two months a period in March and April when 2,4,5-T was used to control vegetation in the study area.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24757

- 3) Statistical analysis of these data indicate that there was a significant correlation between the amounts of 2,4,5-T used in the study area during spraying season and a subsequent increase in the spontaneous abortion index in the study area.

"The Ageway's systematic survey of the occurrence of spontaneous abortions in an area of 2,4,5-T use, indicates that there was an unusually high number of spontaneous abortions in the area, and that the incidence of spontaneous abortions may reasonably be related to the use of 2,4,5-T in that area. The data further indicates that the miscarriage experiences which the eight Alsea women reported to the Agency were representative of the experiences of the large population of women living in the study area".

Numerous critics of the Alsea II study

- 1) Mantal, N. An evaluation of the statistical methods used in EPA report of Assessment of a field investigation of six year spontaneous abortion rates in the three Oregon areas in relation to forest 2,4,5-T spray pesticides (the Alsea II report) George Washington University, March 26, 1979.
- 2) Lamm, 1979. An epidemiologic assessment of the Alsea II report, Tabershaw Associates, August, 1979.
- 3) Witt, 1979. A discussion of the suspension of 2,4,5-T and the EPA Alsea II study, Oregon State University, 1979.
- 4) Wagner, S.L. et al. A scientific critique of the EPA Alsea II study and report, Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon, October 25, 1979.

C24753

These criticisms have revealed fragment statistical and epidemiological flaws that the majority of the scientific community has accorded little if any weight to the EPA conclusions

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

1) Spontaneous abortions, or miscarriages frequently occur in the first three months of pregnancy. Statistics cite a spontaneous miscarriage incidence of 12 to 25%. With a 15% rate most commonly referred to in literature. In the U.S.A. hospitalization rate for spontaneous abortion approaches 8% or 82 per 1000 maternity admissions.

Whether a spontaneous abortion will be hospitalised depends on:

1. whether the women recognized the event as a miscarriage.
2. how anxious the woman is about abortion and the social support system her life style provides her.
3. whether the woman is financially able to secure treatment.
4. whether the treating physician is a specialist who deals regularly with spontaneous abortion cases.
5. the availability of treatment in an out-patient clinic.
6. the standard of health care in the community
7. the gestational age of the conceptus and the completeness of the abortion (Lamm, 1979).

Alsea II, considered only hospitalised spontaneous abortions. Attended abortions in clinics, unattended abortions, and those events that took place at area hospitals not included in the study area were not considered. The study numbers, thus don't accurately reflect the miscarriage incidence in either the control or study group.

The Alsea II study stated that obstetricians in the area hospitalised only 10 percent of persons experiencing spontaneous abortion, while general practitioners faced with the same problem hospitalised approximately 70% of these women.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24759

The study versus control areas is a further complaint. The medical effect being measured is common to the general population. All variables must be considered. Alsea II did not take into account:

1. level of medical practice
2. age of mother
3. nutritional state, diet, a variety of infectious agents, anatomic or genetic abnormalities, medicine and other drugs, consumption of alcohol or tobacco, socio economic status etc.

Thus the effects of the stress agent, 2,4,5-T could not be adequately assessed.

Also the towns involved in the study Alsea (study) and Corvallis (control) do not maintain constant populations.

Alsea a coastal resort town has an influx of people during the summer months. Corvallis a college town, experiences a summer exodus at the end of the school year.

The EPA cited "a dramatic increase in miscarriages which have consistently occurred at an alarming rate. This they attribute to the spraying of herbicide 2,4,5-T.

Yet critics say on closer examination of the data. The dramatic June peak was apparent in only one of the six years studied. There was consistency to the abortion pattern and there was no solid establishment of background miscarriage events to clarify a stress related occurrence. In addition there was no increase in the number of spontaneous abortions in the latter years of the study, even though there was a significant increase in the amount of 2,4,5-T sprayed in the test area. Other peaks and troughs appearing on the Alsea graphs randomly occur throughout the year and could not be attributed to spraying - a strictly seasonal event.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24760

Wagner stated when the data was corrected to a common hospitalisation rate (100%) and the total number of hospitalised spontaneous abortions are expressed as a percentage of live births the values are:

study area - 11.4%

urban control- 14.0%

control area - 9.3%

At best the Alsea II study could only represent the in hospital spontaneous abortion index for its test area. The study has been discredited to such an extent that it is no longer considered a useful study for analysis of the alleged problem, that is the effects of herbicide 2,4,5-T on human reproduction.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24761

Scientific Studies

The first reports linking possible adverse health effects to Agent Orange appeared in Vietnamese newspapers between June 26, 1969 and July 5, 1969 (Advisory Committee on 2,4,5-T). The public response to these reports prompted the retrospective studies of Vietnamese hospital records conducted independently by Cutting (1970) and Meselson (1971).

The President's Advisory Committee on 2,4,5-T (1971) evaluated these studies and concluded the following:

"Summarizing the Vietnam data on human embryotoxicity, it can be said that (1) the sample of births surveyed was from year to year a variable but usually very small fraction of the total number, (2) it was quite unrepresentative of the geographic and ethnic distributions, (3) the heavily sprayed and otherwise exposed areas were greatly under-represented, and (4) the birth records were not trustworthy and, therefore, the rates of stillbirth, and especially of congenital malformation, derived from them were equally unrealizable. For example, the overall congenital malformation rate found in South Vietnam, 4.91 per 1000 livebirths, is about half of what was reported in other studies in various parts of Asia, and possibly a quarter of what might actually exist at term. A further indication that the newborn children were not carefully examined is the absence of Down's syndrome in the list of specific malformations compiled by the Army survey (Cutting, et al.) (1970) despite the fact that some Oriental populations have been reported to have an incidence of this condition not unlike that in Western populations.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24762

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

Finally, there is, and can be, no precise knowledge or reasonable approximation of the exposure to 2,4,5-T (and hence, TCDD) experienced by pregnant Vietnamese women, including what amounts they ingested or absorbed and when this may have occurred during pregnancy. Thus, any attempt to relate birth defects or stillbirths to herbicide exposure is predestined to failure. It can only be concluded that the birth records that have been surveyed, and probably any that will be surveyed in the future, for South Vietnam for the period 1960-1970 cannot answer positively the questions about possible adverse prenatal effects following human exposure to 2,4,5-T. It must be emphasized, however, that the searches that have been made almost certainly would have revealed any marked increase in the incidence of birth defects or the introduction of a striking defect such as that produced by thalidomide. In spite of considerable effort, no such occurrences were found".

A further study on birth defects in South Vietnam was performed by Dr. Ton That Tung, et al. (1971). They reported that out of 903 South Vietnamese seeking shelter in the North, in hospitals and lodgings in Hanoi, 19 adult women, including four mothers and 70 children between the ages of 6 and 14 had experienced direct exposure to herbicide sprays while living in the South. Of the four mothers, two had given birth to children with Downs syndrome. Among the 70 children, numerous cases of deformations (ocular lesions, lumps on forehead, valgus feet (feet that extended outward) and chromosomal aberrations became evident. Tung concluded: (translation in Young, et al., 1978)

"Though still limited in number, our clinical observations confirm the results obtained on animals by American researchers. The massive and prolonged utilization of deformatants besides

C24766

Scientific Studies

The first reports linking possible adverse health effects to Agent Orange appeared in Vietnamese newspapers between June 26, 1969 and July 5, 1969 (Advisory Committee on 2,4,5-T). The public response to these reports prompted the retrospective studies of Vietnamese hospital records conducted independently by Cutting (1970) and Meselson (1971).

The President's Advisory Committee on 2,4,5-T (1971) evaluated these studies and concluded the following:

"Summarizing the Vietnam data on human embryotoxicity, it can be said that (1) the sample of births surveyed was from year to year a variable but usually very small fraction of the total number, (2) it was quite unrepresentative of the geographic and ethnic distributions, (3) the heavily sprayed and otherwise exposed areas were greatly under-represented, and (4) the birth records were not trustworthy and, therefore, the rates of stillbirth, and especially of congenital malformation, derived from them were equally unrealizable. For example, the overall congenital malformation rate found in South Vietnam, 4.91 per 1000 livebirths, is about half of what was reported in other studies in various parts of Asia, and possibly a quarter of what might actually exist at term. A further indication that the newborn children were not carefully examined is the absence of Down's syndrome in the list of specific malformations compiled by the Army survey (Cutting, et al.) (1970) despite the fact that some Oriental populations have been reported to have an incidence of this condition not unlike that in Western populations.

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24764

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

Finally, there is, and can be, no precise knowledge of reasonable approximation of the exposure to 2,4,5-T (and hence, TCDD) experienced by pregnant Vietnamese women, including what amounts they ingested or absorbed and when this may have occurred during pregnancy. Thus, any attempt to relate birth defects or stillbirths to herbicide exposure is predestined to failure. It can only be concluded that the birth records that have been surveyed, and probably any that will be surveyed in the future, for South Vietnam for the period 1960-1970 cannot answer positively the questions about possible adverse prenatal effects following human exposure to 2,4,5-T. It must be emphasized, however, that the searches that have been made almost certainly would have revealed any marked increase in the incidence of birth defects or the introduction of a striking defect such as that produced by thalidomide. In spite of considerable effort, no such occurrences were found".

A further study on birth defects in South Vietnam was performed by Dr. Ton That Tung, et al. (1971). They reported that out of 903 South Vietnamese seeking shelter in the North, in hospitals and lodgings in Hanoi, 19 adult women, including four mothers and 70 children between the ages of 6 and 14 had experienced direct exposure to herbicide sprays while living in the South. Of the four mothers, two had given birth to children with Downs syndrome. Among the 70 children, numerous cases of deformations (ocular lesions, lumps on forehead, valgus feet (feet that extended outward) and chromosomal aberrations became evident. Tung concluded: (translation in Young, et al., 1978)

C24765

"Though still limited in number, our clinical observations confirm the results obtained on animals by American researchers. The massive and prolonged utilization of deformants besides

to ancestral soil and these alterations can provoke among their progeny congenital malformations the importance of which remains to be determined. In the abominable history of wars, have we ever seen such an inhuman fate reserved for the survivors except in the case of atomic war?"

A study similar to that of Dr. Tung was performed by Rose and Rose (1972). The authors reported monstrous births, spontaneous abortions and death among man and animals following exposure to the defoliants.

The linkage of Down's Syndrome and exposure to 2,4,5-T suggested by Tung has been supported by Grummer (-- in a report by Honoroff (1973)). Grummer's comments after inspection of both North and South Vietnam were:

"Provided that it be understood that this estimate cannot be anything but a cautious one, it may be assumed that there are at least 25,000 children with hereditary defects in South Vietnam. This does not include all the unborn babies whose mothers were sprayed during the missions that were flown in recent months. It does not include those who were stillborn, or died soon after birth, on account of their serious chromosomal defects. Even after the war, it will probably be possible to arrive at only an approximation of the entire scale of this crime since one will only be able to examine the survivors when the time comes".

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER.

C24766

Tung, et al., also reported on another adverse health effect he attributed to defoliant exposure. In 1973, Tung reported an increase in the number of persons diagnosed with primary liver cancers in proportion to all cancer patients admitted to Hanoi hospitals during the period 1962-1968. He compared the 790 liver cases out of 7,911 total cancer cases (10%), with those documented in earlier pre-spraying years, 1955-1961. --- 159 liver cases out of total cancer incidence of 5,492 (2.9%).

In a 1977 International Agency for Research on Cancer (IARC) monograph, (1977), the Tung study was criticized for its limitations in reporting which they considered resulted in an inadequate assessment between herbicide spraying and liver cancer frequency.

Ford, et al., (1978), offered a further factor worthy of consideration, the consumption of aflatoxin-contaminated food in parts of the East. Aflatoxin occurs naturally as a contaminant in cereal grains and has been suggested as an explanation for the increase in liver cancer incidence. War conditions would be expected to increase aflatoxin risks in available food grains.

In 1974, the National Academy of Science (NAS) announced results of its study of Vietnam during 1972-1973. The NAS could find no conclusive evidence of association between exposure to herbicides and birth defects in humans. An analysis of hospital records of three Vietnamese hospitals showed no consistent pattern of association between malformations and annual amounts of herbicides disseminated.

To date there is still a great debate about the harmful effects resulting from human exposure to Agent Orange in Vietnam. Studies performed by Tung have generally been met

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER

C24767

Tung, et al., also reported on another adverse health effect he attributed to defoliant exposure. In 1973, Tung reported an increase in the number of persons diagnosed with primary liver cancers in proportion to all cancer patients admitted to Hanoi hospitals during the period 1962-1968. He compared the 790 liver cases out of 7,911 total cancer cases (10%), with those documented in earlier pre-spraying years, 1955-1961. --- 159 liver cases out of total cancer incidence of 5,492 (2.9%).

In a 1977 International Agency for Research on Cancer (IARC) monograph, (1977), the Tung study was criticized for its limitations in reporting which they considered resulted in an inadequate assessment between herbicide spraying and liver cancer frequency.

Ford, et al., (1978), offered a further factor worthy of consideration, the consumption of aflatoxin-contaminated food in parts of the East. Aflatoxin occurs naturally as a contaminant in cereal grains and has been suggested as an explanation for the increase in liver cancer incidence. War conditions would be expected to increase aflatoxin risks in available food grains.

In 1974, the National Academy of Science (NAS) announced results of its study of Vietnam during 1972-1973. The NAS could find no conclusive evidence of association between exposure to herbicides and birth defects in humans. An analysis of hospital records of Vietnamese hospitals showed no consistent pattern of association between malformations and annual amounts of herbicides disseminated.

To date there is still a great debate about the harmful effects resulting from human exposure to Agent Orange in Vietnam. Studies performed by Tung have generally been met

CONFIDENTIAL

SUBJECT TO PROTECTIVE ORDER.

IN-10M (REV)
C24767

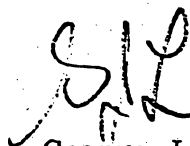
DATE March 8, 1982
SUBJECT Agent Orange Litigation
REFERENCE Memo 3/2/82, TMB/GJL
TO T.M. Bistline, E2NJ

"R.T. Berendt, E2ND
H.S. Scott, E2ND

The document "A Summary of Toxicological Data of the Phenoxy Herbicides" by Dr. William McBride contained 45 pages. Since the last page ended in the middle of a sentence, I am not sure how much of that document I did not see. Nevertheless, the part that I read was well written. It is a good, objective review of the subject. It is a balanced presentation with which I have no quarrel.

On page 27, I noted a question mark that you had put alongside the phrase "four of the six original workers were examined" in 1953. I do not know if that is correct or incorrect. While the statement can be checked, it is unlikely that we can get an answer back to you before the scheduled March 19 submission to the Australian Parliament. One difficulty in checking out that statement is the absence of a specific reference to it. As I noted earlier, the document appears to be incomplete. Certainly, no bibliography was attached.

I suggest that Dr. McBride's review be given to Charlie Love who is defending Monsanto in the Nitro litigation.



George J. Levinskas

alw

CONFIDENTIAL
SUBJECT TO PROTECTIVE ORDER