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The employment service has in the meantime been uncomplimentary about the survey which, it says, is deficient in its failure to follow up past employees and in the inadequate matching of the control populations. The survey covered 41 workers known to have been exposed to dioxin, 54 who might have been exposed and 31 intended as a control group but drawn for convenience from senior office, laboratory and works staff, most of whom were being investigated for blood lipids at the time.

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The significance of these findings is far from clear, with the consensus among physicians in the field that further investigations are needed. They argue that the persistence of the phenomena uncovered by the earlier study should be confirmed — but also that further surveys should be more carefully controlled.

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Alastair Hay

Dioxin a sarcoma risk

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The authors of the letters point out that in four industry-sponsored mortality studies of dioxin-exposed workers in the United States, 105 deaths were recorded. In four of these cases, death was caused by soft tissue sarcomas. These are rare forms of cancer and for most the aetiologies are unknown.

Soft tissue sarcomas have also been identified in another cohort of workers considered to have been exposed to dioxin. According to two Swedish epidemiologists, Dr Lennart Hardell and Anita Sandström of the Centre of Oncology at the University of Umea, exposure to the dioxin contaminated herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) or chlorinated phenols 6 to 20 years previously could be responsible for the sixfold greater incidence of soft tissue sarcomas recorded in a survey of Swedish forestry workers (*British Journal of Cancer* 39, 711; 1979).

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interferons suggest that it has a tantalizing degree of activity against a broad spectrum of viral diseases and tumours. But activity is not enough, or consistent enough, in most diseases to give a final verdict without more trials. Also, as genetically-manipulated bacteria start to yield sufficient amounts of the ten or so subtypes of alpha-interferon, the permutations of subtypes and diseases becomes huge.

Meanwhile, large-scale trials of beta-interferon (from fibroblasts) lag slightly behind. The largest has yet to start. Financed and organized by the National Institutes of Health (NIH), the trial will use 50,000 million units of beta-interferon, enough for several hundred patients. The interferon is being supplied by Flow Laboratories Inc., which won the $2 million contract a year ago. First deliveries were due a few weeks ago, but NIH appear to be in no hurry. This is lucky for Flow Laboratories, whose own production has not been going smoothly. Fortunately, they have a subcontract with the West Germany company of Dr Rentschler Arzneimittel GmbH which has continued to amass beta-interferon made by its own, less advanced, techniques.

The next big contract, also for NIH, will be for gamma-interferon (from cells of the immune system). Far less is known, at least publicly, about this type of interferon, but Biogen and Genentech are rumoured to be on the verge of cloning the relevant genes.

Other commercial interests centre on monoclonal antibodies against the interferons. Several new monoclonals against either alpha or beta-interferons were announced in Rotterdam, but only the original monoclonal (see *Nature* 285, 446; 1980) is as yet commercially available. The sellers are the British company Celltech Limited, so far its only product. It will be surprising if the Celltech catalogue does not double soon. Peter Newmark

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Chemical(s) identified: Dioxins N.S. Hardell

DR-65-0986-(100)
K-66681-(100)
DR-189-7536-(100)
[1983]

Species studied:

Dose:

Health effects:

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are two series of tricyclic aromatic compounds with similar chemical, physical and toxicological properties. In all there are 75 PCDD and 135 PCDF isomers. The chemical and environmental stability coupled with lipophility has resulted in their widespread detection throughout the global system. They have a potential for accumulation in food chains and therefore they present a threat for man and the environment. Neither PCDDs nor PCDFs occur naturally and they have never had any technical use but they are impurities in several commercial preparations. It is now evident that the phenoxy herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) are not the only sources of PCDDs and PCDFs.

Table 1. Levels of 2,3,7,8-Tetra-CDD in 2,4,5-T Acid and 2,4,5-T Ester Formulatins (Rappe et al 1978, Nerström et al 1979).

Sample	Origin	2,3,7,8-TCDD µg/g
2,4,5-T acid	1952, Sweden	1.10
2,4,5-T ester	1960, Sweden	0.40
2,4,5-T ester	1962, Finland	0.95
2,4,5-T ester	1967, Finland	0.22
2,4,5-T acid	1964, USA	4.8
2,4,5-T acid	1969, USA	6.0
Herbicide Orange	unknown, USA	0.12
Herbicide Orange	unknown, USA	5.1

Table 2. Levels of PCDDs and PCDFs in Commercial Chlorinated Phenols (µg/g). (Rappe et al 1981).

	PCDDs	PCDFs
2,4,6-Trichlorophenol, Sweden	<3	60
2,4,6-Trichlorophenol, USA	0.3	4.6
2,3,4,6-Tetrachlorophenol, Finland	12	160
Pentachlorophenol, USA	2625	190
Pentachlorophenol, USA	1500	790
Pentachlorophenol, Germany	6.8	2.1

Table 3. Levels of PCDFs in commercial PCBs (Bowes et al 1975).

Sample	Total µg/g
Aroclor 1248, 1969	2.0
Aroclor 1260, 1969	1.5
Clophen A 60	8.4
Phenoclor DP-6	13.6
Mitsubishi (used)[a]	10.0

a) Rappe and Buser, unpublished

Table 4. PCDD residues associated with food.

	Number Sampl		PCDD (ng/kg) Range detected	Comments	Ref
Cattle fat	85	2	20-60	2,4,5-T treated and untreated rangeland	a)
milk	10	9	59-7900	Seveso 1976	b)
Chicken liver	19	9	100-1420	PCP impregn.	c)
Fish many species	12	8	20-170	Locations around Midland	d)
	12	3	20-102	Lake Ontario	e)
Gelatin	15	10	80-1290	Associated with PCP residues	f)

Table 5. Human tissue concentrations of 2,3,7,8-T CDD (after Kuratsune et al 1980).

Tissue	2,3,7,8-T CDD/wet tissue (ng.kg^{-1})
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Pancreas	1040
Liver	150
Thyroid	85
Brain	60
Heart	60
Kidney	40
Blood	6

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EXPOSURE TO POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN THE ENVIRONMENT.

Lennart Hardell, M.D.

Department of Oncology, University Hospital
S-901 85 Umeå
Sweden

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are two series of tricyclic aromatic compounds with similar chemical, physical and toxicological properties. In all there are 75 PCDD and 135 PCDF isomers. The chemical and environmental stability coupled with lipophility has resulted in their widespread detection throughout the global system. They have a potential for accumulation in food chains and therefore they present a threat for man and the environment. Neither PCDDs nor PCDFs occur naturally and they have never had any technical use but they are impurities in several commercial preparations. It is now evident that the phenoxy herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) are not the only sources of PCDDs and PCDFs.

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2,4,5-T acid	1969, USA	6.0
Herbicide Orange	unknown, USA	0.12
Herbicide Orange	unknown, USA	5.1

The major identified sources are associated with the use of chlorophenols (CPs) in the wood industry.

Table 2. Levels of PCDDs and PCDFs in Commercial Chlorinated Phenols (µg/g). (Rappe et al 1981).

	PCDDs	PCDFs
2,4,6-Trichlorophenol, Sweden	<3	60
2,4,6-Trichlorophenol, USA	0.3	4.6
2,3,4,6-Tetrachlorophenol, Finland	12	160
Pentachlorophenol, USA	2625	190
Pentachlorophenol, USA	1900	790
Pentachlorophenol, Germany	6.8	2.1

Also combustion of organic materials treated with CPs and chemical dumps are sources of contamination. Pyrolysis of commercial polychlorinated biphenyls (PCBs) generates PCDFs whereas no PCDDs were identified (Buser el al 1978a).

Table 3. Levels of PCDFs in commercial PCBs (Bowes et al 1975).

Sample	Total µg/g
Aroclor 1248, 1969	2.0
Aroclor 1260, 1969	1.5
Clophen A 60	8.4
Phenoclor DP-6	13.6
Mitsubishi (used)[a]	10.0

a) Rappe and Buser, unpublished

Hexachlorophene (3,4,6-trichlorophenol) is another product contaminated with PCDDs and PCDFs. Hexachlorophene has been widely used as antiseptic and has been added to a wide variety of over-the-counter products, including soaps, shampoos, acne medications, deodorants, toothpastes, "vaginal hygiene" preparations, and shaving creams (Martin-Bouyer et al 1982).

CPs were first described in 1936 by the Dow Chemical Company under the trade name of Dowicide. Already in 1937 chloracne developed among workers manufacturing CPs (Butler 1937). PCDDs as being the responsible substances as inducers of chloracne were first described by Schulz in 1957.

Hexachlorophene was developed in 1939. The toxicity of orally ingested hexachlorophene in animals was first described in 1939 and percutaneous hexachlorophene toxicity in rabbits was described the same year (reports by Applied Research Laboratories, Inc., to Givaudan Corporation, unpublished). Human percutaneous hexachlorophene poisoning was first reported in 1959 (Herter 1959).

Sources:

In 1977 Olie et al reported on the occurrence of PCDDs and PCDFs in fly ash of municipal incernators in the Netherlands. Also Buser et al have reported the same findings (Buser et al 1978b). The variation of isomers of PCDDs found in various samples of fly ash strongly suggests commercial CPs as the precursors. Regarding PCDF isomers identified in fly ash most of them are also formed in the pyrolysis of commercial PCBs (Aroclor 1254 and 1260) strongly suggesting these commonly used products as the precursors of the PCDFs in fly ash (Rappe, Buser 1981).

It has been suggested that PCDDs, including 2,3,7,8-TCDD, can be generated by a pyrolytic de novo formation from carbon and chlorides (Bumb et al 1980). This "trace chemistries of fire" hypothesis was orginally postulated by Dow Chemical Company (Dow Chemical Co. 1978). However, the experimental data available strongly indicate that the dominating part of the PCDDs found originate from chlorinated aromatics used as industrial chemicals, the chlorinated phenols being the major source (Rappe et al 1981). Moreover, attempts to identify PCDDs in emmissions from coal-fired power-plants have failed (Kimble, Gross 1980).

The estimated yearly input of PCDDs in the Canadian environment has been estimated to about 1500 kg, the major source being CPs whereas the phenoxy herbicides and combustion sources were calculated to contribute only to a minor degree (NRCC 1981).

Persistence in the Environment:

PCDDs appear to be resistant to photolysis except in the presence of a hydrogen donor. In pure water and on soil surfaces very little photolysis occurs (Dobbs, Gr[illegible] 1979).

In fact, photoformation of PCDDs from pentachlorophenol (PCP) can occur in very low yields (Lamparski et al 1980). The PCDDs tend to be rapidly and strongly absorbed to most soils. Migration of TCDD deep into soils have been reported around Seveso in Italy (Cavallaro et al 1979). The PCDDs are, however, relatively insoluble in water (Crummett, Stehl 1973). PCDDs are considered highly persistent with a reported half-life in biologically active soils and sediments of 1-2 years (Ward, Matsumura 1978) and possibly up to 10 years (DiDomenico et al 1980).

Exposure:

PCDDs are present in various matrices including food. Primary dermal contact is difficult to assess but exposure to pesticides has shown an about 10% uptake when such chemicals are spilled on the forearm (TGOEP 1974). No estimates have been made of the exposure through secondary dermal contact, i.e. contact with treated vegetation, wood etc. PCDDs have been reported in some samples of beef fat (US), fish (Lake Ontario), chicken livers (Canada) and gelatin (US) (NRCC 1981).

Table 4. PCDD residues associated with food.

	Number Sampl	Pos	PCDD (ng/kg) Range detected	Comments	Ref
Cattle					
fat	85	2	20-60	2,4,5-T treated and untreated rangeland	a)
milk	10	9	59-7900	Seveso 1976	b)
Chicken					
liver	19	9	100-1420	PCP impregn.	c)
Fish					
many species	12	8	20-170	Locations around Midland	d)
	12	3	20-102	Lake Ontario	e)
Gelatin	15	10	80-1290	Associated with PCP residues	f)

References to table 4:
a) Harless et al (1980)
b) Fanelli et al (1980)
c) Ryan, Pilon (1981)
d) Dow Chemical Co (1978)
e) McLeod (1981)
f) Firestone (1977)

PCDDs can enter the diet by different routes (NRCC 1981):

1. Accumulation in the aquatic systems like PCBs and DDT.
2. Accidental spillage of contaminated fluids into foods or feeds during processing etc.
3. Use of CPs treated wood products or by-products in agriculture or gardens.
4. Use of treated wood waste in the pulp industry and use of potentially contaminated products in the food industry.
5. Use of herbicides contaminated with PCDDs.

Groups among the general population who may be exposed to PCDDs through inhalation are those who use wood treated with CPs and those who live within houses whose interior surfaces are treated with CP-based products. Another possibility is the contribution of incineration of CPs-treated wood. Workers in wood treatment plants, tanneries, pulp and paper mills are at occupational risk to be exposed to PCDDs and PCDFs (Klemmer et al 1980, EPA 1978). Sawmill-workers, tannery-workers, textile impregnators and seamstresses sewing treated fabric all have elevated PCP urine levels (Rappe et al 1981). In almost all cases the exposure would also include PCDDs and PCDFs. A large part results thus from extensive use of CPs, in particular PCP as a preservative. Additions are use or manufacture of phenoxy herbicides, chlorobenzenes and hexachlorophene. It is thus necessary to consider the use of products contaminated with PCDDs such as wood preservatives, as herbicides but also contaminated materials as treated lumber, mouthwashes, cleansers, etc. Also recycling of contaminated products must be considered such as contaminated wood chips for pulp or burning, inadequate disposal practices, accidents, dumping of contaminated chemicals etc. Regarding exposure to PCDFs factories manufacturing or reparing transformers or capacitors containing PCBs should be considered.

Toxicology:

Most of the data available refer to PCDDs of which 2,3,7,8-TCDD has been most investigated due to its extreme acute toxicity. However, the same toxicological pattern seems to attribute to PCDFs as to PCDDs. The highest human tissue concentration of TCDD has been found in fat whereas the highest accumulation of PCDFs is in the liver.

Table 5. Human tissue concentrations of 2,3,7,8-T CDD (after Facchetti et al 1980).

Tissue	2,3,7,8-T CDD/wet tissue ($ng \cdot kg^{-1}$)
Fat	1840
Pancreas	1040
Liver	150
Thyroid	85
Brain	60
Lung	60
Kidney	40
Blood	6

A variety of signs and symptoms have been reported in human populations following accidental exposure to PCDDs and related compounds. These have included (NRCC 1981):

- skin lesions, including chloracne, hyperpigmentation and thickening of the skin
- hair loss and hirsutism
- porphyria cutanea tarda
- central and peripheral nervous disorders
- liver, kidney and gastrointestinal disturbances
- neurasthenia, including lack of drive and vigor, sleep disturbances, emotional instability and altered basic frame of mind
- respiratory and cardiac disorders
- hypothyroidism

TCDD has been shown to be a potent inducer of the hepatic enzymes aryl hydrocarbon hydroxylase (AHH) (Poland et al 1979), 5-amino levulinic acid synthetase (ALAS) (Poland and Glover 1980) and glutathine transferase (S-GT) (Kirsch et al 1975). ALAS activity is associated with defect hepatic porphyria synthesis causing porphyria cutanea tarda. The bulk of the data suggest that PCDDs are not mutagenic. Immunosuppressive effects have been demonstrated (Vos, Moore 1974). Regarding teratogenic effects cleft palate and kidney abnormalitites predominate in mice. (Smith et al 1976).

Carcinogenicity:

During the past few years exposure to phenoxy acids or chlorophenols has attached an increasing interest as a possible cause of malignant disorders. Thus Swedish railroad workers were found to have an increased incidence of tumour mortality related to both amitrole and phenoxy acid preparations (Axelson et al 1980). Case reports on soft-tissue sarcomas (Hardell 1977) and malignant lymphomas (Hardell 1979) as possibly related to exposure to phenoxy acids, have strengthened the suspicion that these substances are carcinogenic. These clinical observations initiated two subsequent case-referent studies of soft-tissue sarcoma which demonstrated a roughly 6-fold increase in the risk after exposure to phenoxy acids or chlorophenols (Hardell, Sandström 1979, Eriksson et al 1981). A similar matched case-referent study of malignant lymphoma (both Hodgkin's disease and non-Hodgkin lymphoma) indicated an association also between that disease and exposure to phenoxy acids or chlorophenols but also to organic solvents (Hardell et al 1981).

In a cohort study of mortality among workers exposed to TCDD in a 1949 trichlorophenol (TCP) accident a suspicious increase of lymphatic and hematopoetic malignancies was observed, i.e. 3 deaths as compared to 0.88 expected (Zack, Suskind 1980). In a British plant manufacturing pentachlorophenol 2 men with NHL of the scalp have been reported versus 0.28 NHL expected (Bishop, Jones 1981). Also a study by Cantor (1982) is in agreement with the Swedish study showing an elevated risk among younger farmers for histiocytic lymphoma in counties high in summary measures of general agricultural activity (OR = 3.2), of rural grain acreage and

acres treated with insecticides (OR = 6.6), and of wheat acreage (OR = 4.4). Several studies have revealed an increased incidence of Hodgkin's disease in lumberjacks and woodworkers (Milham, Hesser 1967, Crufferman et al 1976, Greene et al 1978).

In four separate studies of cohorts of workers occupationally exposed to 2,4,5-T or [illegible]chlorophenol a total of 3 deaths (2,9%) from soft-tissue sarcoma were observed versus 0.07% expected (Honchar, [illegible] 1981). A fourth case of soft-tissue sarcoma has been reported in one of these groups of men (Cook 1981). Another employee exposed to 2,4,5-T in one of the firms concerned in these studies has recently died of neurogenic sarcoma (Moses, Selikoff 1981). Three thoracic soft-tissue sarcomas have lately been reported among Vietnam war veterans exposed to the mixture of phenoxy acids known as agent-orange, but it is not clear whether or not these cases represent an excess (Sarma, Jacobs 1982). In Vietnam an increase in the number of persons with primary liver cancer in proportion to the number of all cancers in North Vietnam during the period when herbicides were used in South Vietnam was reported (Tung 1973).

Regarding animal testing, groups of mice administered 2,7-DCDD showed increased incidence of leuchemias and lymphomas, hemangiosarcomas and hemangiomas and of hepatocellular carcinoma (NCI 1979). In a study by Van Miller et al (1977) feeding rats with TCDD various tumour types were induced among them different soft-tissue sarcomas. A NCI study (1980[a]) studying the carcinogenicity of TCDD showed in mice a significant increase in hepatocellular carcinoma but also of fibrosarcoma, histiocytic lymphoma, thyroid follicular cell adenoma and adrenal cortical adenoma. Fibrosarcoma in the integumentary system was found in female mice treated dermally with 2,3,7,8-TCDD (NCI 1980 [b]). In summary these data demonstrate that 2,3,7,8-TCDD is carcinogenic in rats and mice. Whether 2,3,7,8-TCDD acts as a promotor as suggested by Pitot et al (1980), as an initiator or as both a promotor and initiator is unclear, however.

Exposure to PCDDs and PCDFs occurs thus occupationally in different types of work. Industrial accidents may give high grade of exposure. The bioaccumulation and spreading of PCDDs and PCDFs in the ecological system result in large groups of populations being at risk of exposure. Moreover

products contaminated by PCDDs and PCDFs contribute to such exposure. Local exposure of the general population occurs where chemicals have been dumped in soil or in water as in Love Canal (USA), Tecknatorp (Sweden) or through industrial accidents as near Seveso in 1976. In the presence of the carcinogenic and toxicological data of PCDDs and PCDFs it is necessary to limit the exposure of the populations to those compounds to an absolute minimum.

References:

Axelson O, Sundell L, Andersson K, Edling C, Hogstedt C, Kling H (1980). Herbicide exposure and tumour mortality; an updated epidemiological investigation on Swedish railroad workers. Scand J Work Environ Hlth 6: 73-79.
Bishop CM, Jones AH (1981). Non-Hodgkin's lymphoma of the scalp in workers exposed to dioxins. Lancet ii: 369.
Bowes GW, Mulvihill MY, Simoneit BRT, Burlingame AL, Risebrough RW (1975). Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. Nature 256: 305-307.
Bumb RR, Crummett WB, Cutie SS, Gledhill JR, Hummel RH, Kage RO, Lamparski LL, Luoma EV, Miller DL, Nestrick TJ, Shadoff LA, Stehl RH, Woods JS (1980). Trace chemistries of fire; a source of chlorinated dioxins. Science 210: 385-390.
Butler MG (1937). Arch Derm Syph 35:251.
Buser HR, Bosshardt HP, Rappe C, Lindahl R (1978a). Identification of polychlorinated dibenzofuran isomers in fly ash and PCB pyrolysis. Chemosphere 7: 419-429.
Buser HR, Bosshardt H, Rappe C (1978b). Identification of polychlorinated dibenzo-p-dioxin isomers found in fly ash. Chemosphere 7: 165-172.
Cantor KP (1982). Farming and mortality from non-Hodgkin's lymphoma; a case-control study. Int J Cancer 29: 239-247.
Cavallaro A, Tebaldi G, DeFelice G, Colli G, Gualdi R (1979). Indagine sperimentale sulla penetrazione di TCDD nella zona A di Seveso. Boll Chim d'Unione Ital Lab Prov Sci 5: 489-541.
Crummett WB, Stehl RH (1973). Determination of chlorinated dibenzo-p-dioxins and dibenzofurans in various materials. Environ Health Persp 5: 15-25.
DiDomenico A, Silano V, Viviano G, Zapponi G (1980). Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. V. Environmental persistence of TCDD in soil. Ecotoxicol Environ Safety 4: 339-345.

Dobbs AJ, Grant C (1979). Photolysis of highly chlorinated dibenxo-p-dioxins by sunlight. Nature 278: 163-165.
Dow Chemical Co (1978). The trace chemistries of fire - a source of and routes for entry of chlorinated dioxins into the environment. The Chlorinated Dioxin Task Force. Dow Chemical Co, Midland, Michigan: p 46 plus Appendices.
EPA (1978). Pesticide programs. Rebuttable presumption against registration and continued registration of pesticide products containing pentachlorophenol. Fed Regist 43(202): 48443-48617.
Eriksson M, Hardell L, Berg NO, Möller T, Axelson O (1981). Soft tissue sarcomas and exposure to chemical substances; a case-referent study. Br J Indust Med 38: 27-33.
Facchetti S, Fornari A, Montagna M (1980). Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the tissues of a person exposed to the toxic cloud at Seveso. Adv Mass Spectrom 8B: 1405-1414.
Fanelli R, Bertoni MP, Bonfanti M, Castelli MG, Chiabrando C, Martelli GP, Noe MA, Noseda A, Garrattini S, Binaghi C, Marazza V, Pezza F, Pozzoli D, Cicognetti G (1980). 2,3,7,8-Tetrachlorodibenzo-p-dioxin levels in cow's milk from the contaminated area of Seveso, Italy. Bull Environ Contam Toxicol 24: 634-639.
Firestone D (1977). Determination of polychlorodibenzo-p-dioxins and polychlorodibenzofurans in commercial gelatins by bas-liquid chromatography. J Agric Food Chem 25: 1274-1280.
Greene MH, Brinton LA, Fraumeni JF, D'Amico R (1978). Familial and sporadic Hodgkin's disease associated with occupational wood exposure. Lancet ii: 626-627.
Grufferman S, Duong T, Cole P (1976). Occupation and Hodgkin's disease. J Nat Cancer Inst 57: 1193-1195.
Hardell L (1977). Soft tissue sarcomas and exposure to phenoxy acids; a clinical observation. Läkartidningen 74: 2753-2754.
Hardell L (1979). Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet i: 55-56.
Hardell L, Sandström A (1979). Case-control study: soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39: 711-717.
Hardell L, Eriksson M, Lenner P, Lundgren E (1981). Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids; a case-control study. Br J Cancer 43: 169-176.

Harless RL, Oswald ED, Wilkinson MK, Dupuy AE Jr, McDaniel DD, Han Tai (1980). Sample preparation and gas chromatography-mass spectrometry determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem 52: 1239-1245.
Herter WB (1959). Hexachlorophene poisoning. Kaiser Fnd Med Bull 7: 228-230.
Honchar PA, Halperin WE (1981). 2,4,5-T, trichlorophenol and soft tissue sarcomas. Lancet i: 268-269.
Kirsch R, Fleischer G, Kamisaka K, Arias JM (1975). Structural and functional studies of ligandin, a major renal organic anion binding protein. J Clin Invest 55: 1009-1019.
Klemmer HW, Wong L, Sato MM, Reichert EL, Korsak RJ, Rashad MN (1980). Clinical findings in workers exposed to pentachlorophenol. Arch Environ Contam Toxicol 9: 715-725.
Lamparski LL, Stehl RH, Johnson RL (1980). Photolysis of pentachlorophenol-treated wood. Chlorinated dibenzo-p-dioxin formation. Environ Sci Technol 14: 196-200.
Martin-Boyer G, Lebreton G, Toga M, Stolley PD, Lockhart J (1982). Outbreak of accidental hexachlorophene poisoning in France. Lancet i: 91-95.
McLeod HA (1981). Personal Communication to MJ Boddington, Health Protection Branch, Health and Welfare Canada.
Milham S, Hesser JE (1967). Hodgkin's disease in woodworkers. Lancet ii: 136-137.
Moses M, Selikoff IJ (1981). Soft tissue sarcomas, phenoxy herbicides and chlorinated phenols. Lancet i: 1370.
NCI (1979). Bioassay of 2,7-dichlorodibenzo-p-dioxin (DCDD) for possible carcinogenicity. Carcinogenesis Testing Program, National Cancer Institute. Tech Rpt Ser #123. NTIS Dept Comm PB290570.
NCI (1980a). Bioassay of 2,3,7,8-tetrachlorodibenxo-p-dioxin (gavage study). Carcinogenesis Testing Program, National Cancer Institute. DHHS Publ # (NIH) 80-1765. (original unseen).
NCI (1980b). Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity. Carcinogenesis Testing Program, National Cancer Institute. Tech Rpt 201. DHHS Publ # (NIH) 80-1757. (original unseen).
NRCC (1981). Polychlorinated dibenzo-p-dioxins; criteria for their effects on man and his environment. Ottawa, Canada: pp 23-165.
Olie K, Vermeulen PL, Hutzinger O (1977). Chlorodibenzo-p-dioxins and chlorodibenzofurans are trace components of fly ash and flue gas of some municipal incinerators in the Netherlands. Chemosphere 6: 455-459.

Pitot HC, Goldsworthy T, Poland H (1980). Promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res 40: 3616-3620.
Poland A, Glover E (1980). 2,3,7,8-Tetrachlorodibenzo-p-dioxin; studies on the mechanism in action. In The Scientific bases of toxicity assessment. Edited by H Witschi: pp 223-239.
Poland A, Greenlee WF, Kende AS (1979). Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann N.Y. Acad Sci 320: 214-230.
Rappe C, Buser HR (1981). Occupational exposure to polychlorinated dioxins and dibenzofurans. Am Chem Soc Symp Ser 149: 319-342.
Rappe C, Buser HR, Bosshardt HP (1979). Identification and quantitation of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in 2,4,5,-T-ester formulations and Herbicide Orange. Chemosphere 7: 431-438.
Rappe C, Garå A, Buser HR (1978). Identification of polychlorinated dibenzofurans (PCDFs) in commercial chlorophenol formulations. Chemosphere 7: 981-991.
Rappe C, Nygren M, Buser HR, Kauppinen T (1981). Occupational exposure to polychlorinated dioxins and dibenzofurans. In Impact of chlorinated dioxins and related compounds on the environment. Edited by O Hutzinger, RW Frei, E Merian, F Pocchiari. Pergamon Press, Oxford: pp 495-513.
Ryan JJ, Pilon JC (1981). Chlorinated-dibenzodioxins and dibenzofurans in chicken liver and litter arising from pentachlorophenol contamination of wood shavings. In Impact of chlorinated dioxins and related compounds on the environment. Edited by O Hutzinger, RW Frei, E Merian and F Pocchiari. Pergamon Press, Oxford: pp 183-189. (In press).
Sarma PR, Jacobs J (1982). Thoracic soft tissue sarcoma in Vietnam veterans exposed to agent orange. N Engl J Med 306: 1109.
Schultz KH (1957). Arch Klin Exp Derm.
Smith FA, Schwetz BA, Nitschke KD (1976). Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol Appl Pharmacol 38: 517-523.
TGOEP (1974). Occupational exposure to pesticides. Rpt to Federal Working Group or Pest Management from the Task Group on Occupational Exposure to Pesticides. NTIS, US Dept Commerce #PB-244259: p 154.
Tung TT (1973). Le cancer primaire du foie du Viet-Nam. Chirurgie 99: 427-436.

Van Miller JP, Lalich JJ, Allen JR (1977). Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 10: 625-632.
Vos JG, Moore JA, Zinkl JG (1974). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57Bl/6 mice. Toxicol Appl Pharmacol 29: 229-241.
Ward CT, Matsumura F (1978). Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch Environ Contam Toxicol 7: 349-357.
Zack JA, Suskind RR (1980). The mortality experience of workers exposed to tetrachlorodibenzo-p-dioxin in a trichlorophenol process accident. J Occup Med 22: 11-14.

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Regulation of Epidermal Growth Factor Binding in a Human Keratinocyte Cell Line by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin[1]

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Regulation of Epidermal Growth Factor Binding in a Human Keratinocyte Cell Line by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. HUDSON, L. G., TOSCANO, W. A., JR., AND GREENLEE, W. F. (1985). *Toxicol. Appl. Pharmacol.* 77, 251–259. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) decreased the binding of epidermal growth factor (EGF) by the human keratinocyte cell line SCC-12F. This response was concentration dependent (half-maximal effective concentration, EC50 = 1.8 nM) and stereospecific. Scatchard analysis of EGF binding indicated that treatment with TCDD resulted in a loss of high-affinity (K_d = 0.28 nM) binding sites. This loss was accompanied by a concomitant inhibition of EGF-stimulated DNA synthesis. The kinetics for the decrease of EGF binding by TCDD and benzo[*a*]pyrene (BP) were compared. Inhibition of EGF binding by BP was maximal by 24 hr, with 90% recovery of EGF binding apparent by 48 hr. In contrast, TCDD treatment for 72 hr was required to produce maximal inhibition, and no recovery was evident up to 10 day after removal of TCDD from the growth medium. The data indicate that modulation of EGF binding by TCDD was mediated by the *Ah* receptor. Subsequent cellular responses, for example, inhibition of EGF-stimulated DNA synthesis, may be important in the expression of altered differentiation patterns observed in human epidermal keratinocytes exposed to TCDD.

TCDD[3] is the prototype for the halogenated aromatic hydrocarbons.[4] These compounds regulate a variety of biochemical responses in target tissues, some of which are associated with changes in cell proliferation, altered patterns of differentiation, or both (Nebert and Jensen, 1979; Poland and Knutson, 1982). In certain inbred murine strains, the coordinate expression of many enzyme activities is regulated by a single genetic locus

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[3] Abbreviations used: AHH, arylhydrocarbon hydroxylase; BP, benzo[*a*]pyrene; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; Me_2SO, dimethyl sulfoxide; 2,7-DpD, 2,7-dichlorodibenzo-*p*-dioxin; EC50, half-maximal effective concentration; EGF, epidermal growth factor; Hepes, *N*-2-hydroxyethylpiperazine-*N*"-2-ethanesulfonic acid; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline; SCC, squamous cell carcinoma; TCDBF, 2,3,7,8-tetrachlorodibenzofuran; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

[4] The term "halogenated aromatic hydrocarbon" is commonly used to denote a large series of compounds isosteric with TCDD. These include halogenated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, biphenylenes, and azo- and azoxybenzenes.

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(designated the *Ah* locus) and its putative gene product, the *Ah* receptor protein (Nebert *et al.*, 1972; Poland *et al.*, 1976; Thomas *et al.*, 1982). Certain tissue specific toxic responses such as epidermal hyperplasia in hairless mice (Puhvel *et al.*, 1982) appear to be regulated by at least two gene loci, *Ah* and *hr* (Knutson and Poland, 1982; Poland *et al.*, 1982).

The proliferation of human epidermal cells in culture is regulated by several biochemical mediators including EGF (Rheinwald and Green, 1975, 1977; Green, 1978). Cellular responses to EGF, including stimulation of nucleic acid synthesis, protein synthesis, and enhanced cell proliferation, are mediated by specific cell surface receptors (Carpenter and Cohen, 1979; Adamson and Rees, 1981; Schlessinger *et al.*, 1983). In many cells responsive to EGF, EGF-receptor activity is regulated by down modulation of receptors following exposure to the ligand (Carpenter and Cohen, 1976; O'Keefe *et al.*, 1982). In addition, decreased EGF binding has been observed when cells are exposed to several other peptide growth factors (Rozengurt *et al.*, 1982; Bowen-Pope *et al.*, 1983) and xenobiotics such as the tumor promoter TPA (Lee and Weinstein, 1978; Weinstein *et al.*, 1982; Karenlampi *et al.*, 1983). Although a dose-dependent decrease in EGF binding to rat hepatic plasma membranes has been observed after prolonged *in vivo* exposure to TCDD (Madhukar *et al.*, 1984), little is known about the action of TCDD on EGF binding in human target tissues. In this study, we report on the regulation of EGF binding by TCDD in a human keratinocyte cell line (SCC-12F). This cell line was selected as a model system because it is derived from a human target tissue for TCDD, is nontumorigenic, retains many characteristics of normal human epidermal cells (Rheinwald and Beckett, 1980; 1981), and exhibits altered patterns of cell growth and differentiation after exposure to TCDD (Rice and Greenlee, 1982).

METHODS

Cell culture and xenobiotic treatment. SCC-12F cells derived from a tumor of the facial epidermis were a generous gift from Dr. J. G. Rheinwald (Dana–Farber Cancer Research Institute, Boston, Mass.). Methods of cell culture as previously described (Rheinwald and Beckett, 1980) were used with some modifications (Hudson *et al.*, 1983). Briefly, SCC-12F cells were plated at a density of 5×10^3 cells/cm^2 on a layer of lethally irradiated murine 3T3 fibroblasts (one-third confluent density) in DMEM supplemented with 5% fetal calf serum (selected lots). Cells were maintained in a humidified atmosphere of 95% air, 5% CO_2, and the growth medium was changed every third day. Cultures typically reached confluence after 10 days, at which time virtually no 3T3 cells remained on the culture dishes.

Stock solutions of TCDD, chlorinated analogs (KOR Isotopes, Inc., Cambridge, Mass.), and benzo[*a*]pyrene (Aldrich, Milwaukee, Wisc.) were prepared in Me_2SO and diluted in medium prior to addition to confluent SCC-12F cultures. The final concentration of solvent added to the culture medium did not exceed 0.1%. The purity of TCDD and chlorinated isomers was greater than 99% as assessed by gas–liquid chromatography and high-performance liquid chromatography (Neal *et al* 1981).

Epidermal growth factor. EGF purified from mouse submaxillary glands was purchased from Biomedical Technology, Cambridge, Massachusetts, and iodinated using [^{125}I]Na (ICN) and chloramine T (Sigma, St. Louis, Mo.) to a specific activity of 1.6 Ci/μmol (Carpenter and Cohen, 1976).

EGF binding assays. SCC-12F cells were grown to confluence as described above in 35-mm 6-well multidishes (Falcon, Oxnard, Calif.) and treated with xenobiotics or solvent for times indicated in the figure legends. No detectable difference in EGF binding was observed in control cultures exposed to Me_2SO for the times used. Prior to assay of EGF binding, the growth medium was removed and the cultures were washed four times with 1.0 ml per well of binding assay medium (DMEM containing 20 mM Hepes, pH 7.4, and 0.1% BSA) followed by incubation in fresh binding assay medium for 30 min at 4 or 37°C. ^{125}I-EGF (50 pM) was added to each well in the presence or absence of unlabeled EGF (100 nM), and the cultures were further incubated at the appropriate temperature for the times indicated in the figures. Following incubation the medium was removed, and the cultures were rapidly washed five times in ice-cold HBSS buffered with sodium bicarbonate (pH 7.4). The cultures were solubilized in 1.0 ml of 0.1 N NaOH and cell-associated radioactivity was measured in a Packard Model 5260 gamma counter (efficiency = 70%). Specific binding was determined by subtracting values obtained in the presence of excess unlabeled EGF (no

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specific binding) from values obtained in the absence of excess unlabeled EGF (total binding). Nonspecific binding was less than 5% of total binding in control cultures. Results are expressed as a percentage of the control cultures and represent the mean of determinations on triplicate cultures. The standard errors were generally less than 7%. Results were normalized with respect to protein in parallel cultures carried through identical procedures prior to solubilization in base.

DNA synthesis. DNA synthesis was estimated by the incorporation of [6-^{3}H]thymidine (NEN, Boston, New England Nuclear, Mass., sp act = 16.2 Ci/mmol) into trichloroacetic acid-insoluble material. Confluent cultures were exposed to TCDD (100 nM) or Me_2SO (0.1%) for 72 hr, washed in serum-free medium, and then incubated in serum-free medium for an additional 72 hr. Following serum deprivation, EGF (0.5 nM) was added directly to the culture medium, and cultures were incubated for an additional 24 hr. This concentration of EGF was determined to be optimal for the stimulation of DNA synthesis. [^{3}H]Thymidine (1 μCi/ml) was added to each well and the cultures were incubated for 1 hr at 37°C. Monolayers were washed three times with 1.0 ml ice-cold PBS per well, and incubated on ice for 30 min in 1.0 ml of 10% trichloroacetic acid. Samples were collected by scraping followed by centrifugation in a Beckman Model B microfuge for 2 min (10,000*g*). The supernatant fraction was collected and counted to determine acid-soluble [^{3}H]thymidine. The pellet was washed once with PBS and dissolved in 1 N NaOH to measure incorporation of [^{3}H]thymidine into acid-insoluble material. Radioactivity was measured in a Beckman LS 7000 scintillation counter, and the results were normalized with respect to protein. The data were analyzed by analysis of variance, with difference among means analyzed with Duncan's multiple range test. The level of significance chosen was $p < 0.05$.

Protein assays. Alkali-soluble cell protein was measured by the method of Lowry *et al.* (1951) with BSA as the standard.

RESULTS

Actions of TCDD on EGF-Specific Binding

Pretreatment of SCC-12F cultures with TCDD resulted in a concentration-dependent decrease of EGF-specific binding measured at 37°C (Fig. 1). The EC50 for inhibition of EGF-specific binding was 1.8 nM. This value agrees closely with well-established *Ah* receptor-mediated responses such as the induction of AHH activity (Nebert and Jensen, 1979; Poland and Knutson, 1982) and with the reported dissociation constant for binding of TCDD to the *Ah* receptor in murine liver (Poland *et al.*, 1976). The diminished EGF binding in cultures treated with TCDD was not due to loss of cells from the dish, as measured by total protein, or to decreased cell viability, as judged by trypan blue exclusion. Maximal inhibition of EGF-specific binding (60%) occurred after 72 hr and remained unaltered for at least 96 hr (Fig. 2). The plateau observed between 72 and 96 hr was not due to depletion of TCDD or serum components from the medium, since cultures in which the medium was replaced with fresh medium containing Me_2SO or TCDD 24 hr before collection at 96 hr did not exhibit a further decrease in EGF binding. The effect of TCDD was persistent; reduction of EGF

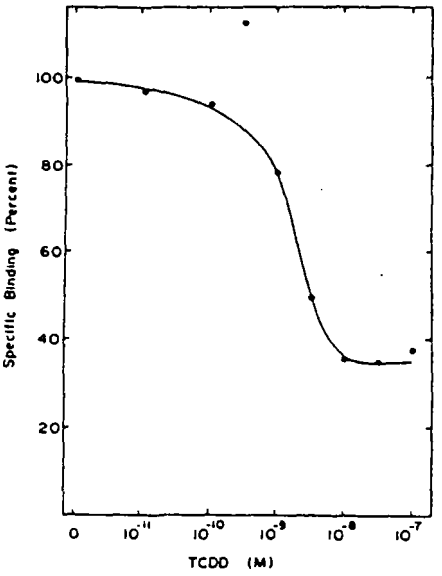

FIG. 1. Concentration-dependent inhibition of EGF-specific binding to SCC-12F cells by TCDD. Confluent cultures of SCC-12F cells were treated with Me_2SO (0.1%) or TCDD (10 pM to 100 nM) for 72 hr prior to determination of EGF-specific binding at 37°C for 50 min (see Methods). The data shown represent the mean of determinations on triplicate cultures and are expressed as a percentage of the Me_2SO control (8.9 ± .6 fmol/mg protein) as described under Methods.

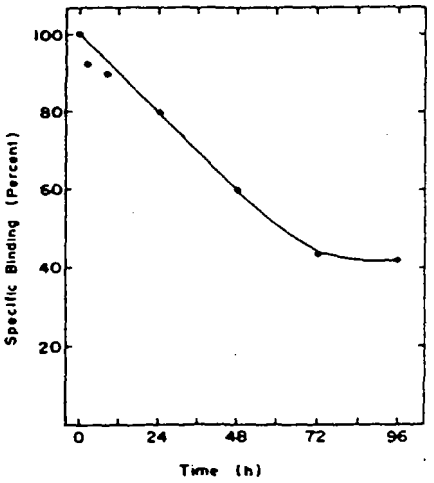

FIG. 2. Kinetics of inhibition of EGF-specific binding by TCDD. Confluent SCC-12F cultures were treated with Me_2SO (0.1%) or TCDD (100 nM) for the times indicated prior to determination of EGF specific binding at 37°C for 50 min (see Methods).

binding (60%) was observed for at least 10 days after removal of TCDD from the growth medium. In contrast, inhibition of EGF binding by BP was maximal by 24 hr with 90% recovery of EGF binding apparent by 48 hr (Fig. 3). The more rapid response observed with BP suggests that BP and TCDD modulate EGF binding by different mechanisms.

Comparable inhibition of EGF-specific binding was observed when binding was measured at 4 or 37°C. Internalization of the EGF receptor does not occur at 4°C (Carpenter and Cohen, 1976); thus, these data indicate that TCDD pretreatment alters binding of EGF rather than internalization of the ligand (Table 1). Furthermore, inhibition of EGF-specific binding was not observed when TCDD was added at the time of assay to previously untreated cultures. These results indicated that TCDD did not directly compete with EGF for the EGF receptor binding site (Table 1).

The inhibition of EGF-specific binding was stereospecific (Table 2). TCDBF, an active analog of TCDD which binds to the *Ah* receptor, elicited a response comparable to TCDD, whereas the inactive isomer 2,7-DpD did not decrease EGF binding even at a concentration 100-fold greater than the optimal concentration of TCDD. The observed concentration dependence (Fig. 1) and stereospecificity (Table 2) for the down modulation of EGF-specific binding by TCDD indicate that this response is regulated by the *Ah* receptor which has been measured in SCC-12F cells (Hudson *et al.*, 1983).

Scatchard Analysis of EGF Binding Data

The Scatchard plot of EGF equilibrium binding data obtained after exposure of SCC-12F cultures to TCDD or Me_2SO is shown in Fig. 4. Maximum binding of EGF occurred after 4 hr at 4°C. At this time, greater than 90% of the bound EGF was extractable by the acid-salt procedure of Haigler *et al.* (1980) and was trichloroacetic acid insoluble, indicating that under the conditions used, inter-

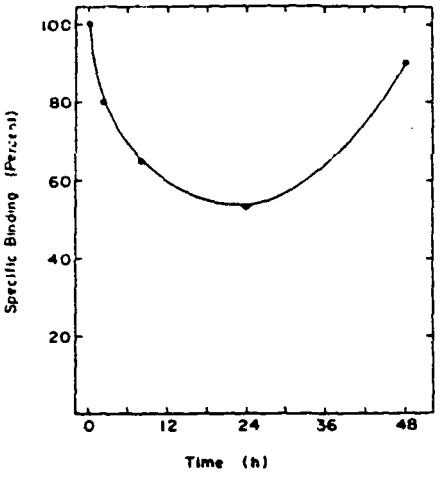

FIG. 3. Kinetics of inhibition of EGF-specific binding by BP. EGF-specific binding was measured as described for Fig. 2, except that the cultures were pretreated with BP (1 μM) for the times indicated.

Treatment	Tem atu (°C
Me_2SO	
TCDD	
Me_2SO	3
TCDD	3

[a] EGF-specific cultures of SCC- (4 hr at 4°C; Methods. Value on triplicate cul

[b] Me_2SO (0.1' added concurre untreated cultu

[c] Cultures wer (100 nM) for 72 binding as desc

nalization ar imal.

The Scatc control cultı TCDD-treat linearity was of the plot indicated tw binding cor = 0.28 nM) the other c = 46 nM) w treated with binding cor nM) with 5 the loss of l well as som

[d] Computer nonlinear least for use in Sca

TABLE 1

EFFECTS OF TEMPERATURE AND INCUBATION CONDITIONS ON INHIBITION OF EGF-SPECIFIC BINDING BY TCDD[a]

Treatment	Temperature (°C)	Specific binding [fmol/mg protein, (%)] No preincubation[b]	Specific binding [fmol/mg protein, (%)] 72 hr preincubation[c]
Me_2SO	4	6.7 ± 0.2 (100)	9.9 ± 0.7 (100)
TCDD	4	7.1 ± 0.4 (107)	3.1 ± 0.0 (31)
Me_2SO	37	7.1 ± 0.7 (100)	8.1 ± 0.2 (100)
TCDD	37	7.5 ± 0.6 (105)	3.3 ± 0.1 (41)

[a] EGF-specific binding was measured in confluent cultures of SCC-12F cells at the appropriate temperature (4 hr at 4°C; 50 min at 37°C) as described under Methods. Values shown are $\bar{x}$ ± SE of determinations on triplicate cultures normalized with respect to protein.

[b] Me_2SO (0.1%) or TCDD (100 nM) and EGF were added concurrently to the assay incubation of previously untreated cultures.

[c] Cultures were pretreated with Me_2SO (0.1%) or TCDD (100 nM) for 72 hr prior to measurement of EGF-specific binding as described.

nalization and degradation of EGF are minimal.

The Scatchard plot for EGF binding in control cultures was curvilinear, whereas in TCDD-treated cultures, no deviation from linearity was observed. Computer resolution[5] of the plot obtained from control cultures indicated two EGF binding components. One binding component was high affinity (K_d = 0.28 nM) with 2.3×10^4 sites per cell, and the other component was low affinity (K_d = 46 nM) with 2×10^6 sites per cell. Cultures treated with TCDD exhibited a single EGF binding component of low affinity (K_d = 15 nM) with 5×10^5 sites per cell, indicating the loss of high-affinity EGF binding sites as well as some low-affinity sites.

[5] Computer resolution was performed using a SAS nonlinear least squares summary statistic program adapted for use in Scatchard analysis.

Modulation of EGF-Stimulated DNA Synthesis in SCC-12F Cells by TCDD

Basal DNA synthesis and EGF-stimulated DNA synthesis were monitored in SCC-12F cells treated with TCDD for 72 hr, the time corresponding to maximum loss of high-affinity EGF binding sites (Fig. 2 and 4). EGF produced a dose-dependent increase in DNA synthesis (data not shown). At the optimal concentration (0.5 nM), EGF increased DNA synthesis nearly threefold in control cultures as judged by [^{3}H]thymidine incorporation (Fig. 5). TCDD treatment resulted in approximately a twofold increase in basal DNA synthesis, but completely inhibited EGF-stimulated DNA synthesis (Fig. 5). These results indicate that TCDD is itself potentially mitogenic, but renders cells nonresponsive to further stimulation by EGF.

DISCUSSION

The data presented in this report demonstrate that TCDD modulates EGF binding

TABLE 2

STEREOSPECIFICITY OF INHIBITION OF EGF-SPECIFIC BINDING[a]

Treatment	Specific binding [fmol/mg protein, (%)]
Me_2SO	8.9 ± 0.9 (100)
2,7-DpD	
1000 nM	8.4 ± 0.7 (94)
TCDBF	
0.3 nM	6.8 ± 0.5 (77)
3 nM	4.8 ± 0.7 (53)
30 nM	3.0 ± 0.2 (33)
300 nM	2.4 ± 0.2 (27)
TCDD	
100 nM	3.3 ± 0.5 (38)

[a] Confluent cultures of SCC-12F cells were treated with the indicated compounds for 72 hr prior to measurement of EGF-specific binding at 37°C for 50 min (see Methods). Values shown are $\bar{x}$ ± SE of determinations on triplicate cultures and normalized with respect to protein.

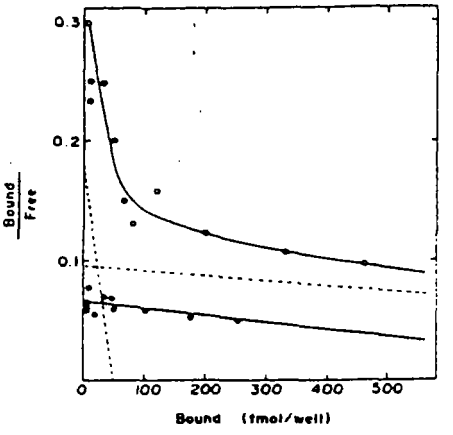

FIG. 4. Scatchard analysis of EGF binding data. Confluent cultures of SCC-12F cells were treated with 0.1% Me_2SO (O) or 100 nM TCDD (●) for 72 hr prior to incubation at 4°C for 4 hr with increasing concentrations of ^{125}I-EGF (33 pM to 5.2 nM) in the presence or absence of a 1000-fold excess of unlabeled EGF. EGF-specific binding was measured as described under Methods. Dashed lines represent the computer resolution of the curve obtained for EGF binding to Me_2SO-treated cells.

in a human keratinocyte cell line derived from a major target organ for TCDD toxicity. The Scatchard plot of EGF equilibrium binding data indicates a loss of high-affinity EGF binding sites following treatment with TCDD (Fig. 4). This loss correlates with altered biological responses to EGF, such as the inhibition of EGF-stimulated DNA synthesis (Fig. 5). Decreased EGF binding in TCDD-treated cells is not due to direct competition of TCDD with EGF for EGF receptor sites (Table 1). The concentration dependence (EC50 = 1.8 nM) and stereospecificity (Fig. 1, Table 2) strongly support that this response is mediated by the *Ah* receptor which has been detected in this cell line (Hudson *et al.*, 1983). These findings are consistent with the hypothesis of Ivanovic and Weinstein (1982) that occupancy of the *Ah* receptor results in modulation of EGF binding.

In vivo studies with hairless mice have shown that TCDD induced morphological changes consistent with terminal differentiation in skin are accompanied by increased concentrations of transglutaminase activity (Puhvel *et al.*, 1984). Similarly, keratinocytes in culture are a dynamic system composed of proliferating basal cells and additional cell layers in various stages of differentiation (Green, 1979). Reduced EGF binding capacity has been reported in response to keratinocyte differentiation (O'Keefe *et al.*, 1982), and preliminary evidence indicates that TCDD enhances differentiation in SCC-12F cells (unpublished data). However, the loss of high-affinity EGF binding sites does not solely reflect a TCDD-induced shift in the culture population to a more highly differentiated state. We have also observed a substantial reduction of EGF binding in a basal cell population isolated from SCC-12F cultures and in another squamous cell carcinoma line (SCC-4) which does not terminally differentiate (Rheinwald and Beckett, 1981). Thus it is possible that the regulation of growth factor receptors such as the EGF

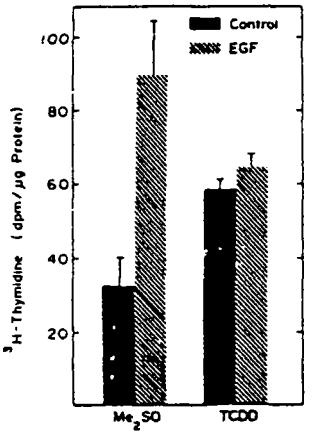

FIG. 5. Modulation of EGF-stimulated DNA synthesis in SCC-12F cells by TCDD. Confluent cultures of SCC-12F cells were pretreated with Me_2SO (0.1%) or TCDD (100 nM), then serum deprived for 72 hr prior to addition of EGF (500 pM) for 24 hr, as described under Methods. Following exposure to EGF, 1 μCi [^{3}H]thymidine/ml was added to each well for 1 hr prior to collection (see Methods). EGF-stimulated DNA synthesis is significantly different ($p < 0.05$) from control Me_2SO-treated cultures.

receptor is an important mechanism by which TCDD alters patterns of differentiation in epidermal cells.

The kinetics of inhibition of EGF binding in SCC-12F cells by TCDD and BP differ (Figs. 2 and 3). Maximum inhibition induced by BP, an agent which inhibits EGF binding in C3H10T1/2 cells (Ivanovic and Weinstein, 1982; Weinstein *et al.*, 1982) and hepatoma cell lines (Karenlampi *et al.*, 1983), occurs within 24 hr, with 90% recovery by 48 hr (Fig. 3). The observed recovery of EGF binding in these cells after 48 hr exposure to BP may be a result of further metabolism of this compound. In contrast, TCDD requires 72 hr to produce maximum inhibition (Fig. 2), and no recovery is observed 10 days after removal of TCDD from the culture medium, TCDD produces hyperkeratinization *in vivo* (Knutson and Poland, 1982; Puhvel *et al.*, 1982) and in cultured SCC-12F cells (unpublished data), and this compound is characterized by its ability to produce a sustained decrease in EGF binding. BP, which binds to the cytosolic *Ah* receptor (Poland *et al.*, 1976) but, to our knowledge, has not been reported to produce hyperkeratinization *in vivo*, produces a transient inhibition of EGF binding. These data comparing the actions of TCDD and BP in human keratinocytes suggest that a prolonged inability of keratinocytes to respond to growth factors such as EGF may be an important regulatory event in the genesis of altered differentiation responses such as hyperkeratinization.

Karenlampi *et al.* (1983) have proposed that electrophilic metabolites of polycyclic aromatic hydrocarbons are primarily responsible for the inhibition of EGF binding observed in murine hepatoma cell lines. Their proposal suggests that occupancy of the *Ah* receptor itself does not significantly affect EGF binding. In this regard, they observed a marked inhibition of EGF binding in mouse hepatoma cells after a 24-hr incubation (the longest time period examined) with BP and other polycyclic aromatic hydrocarbons, but less than a 20% inhibition following treatment with TCDD for the same length of time. Our results demonstrate that 24 hr incubation with TCDD is not sufficient to elicit maximal inhibition of EGF binding to SCC-12F cells (Fig. 2). Metabolites of polycyclic aromatic hydrocarbons may be responsible for a relatively rapid (24 hr) loss of EGF binding observed in BP-treated murine hepatoma cells (Karenlampi *et al.*, 1983) and SCC-12F cells (Fig. 3), which would not be observed upon treatment with TCDD due to its limited metabolism (Rose *et al.*, 1976; Sawahata *et al.*, 1982). Alternatively, there is evidence (Okey *et al.*, 1984; Collins and Marletta, 1984) that there may be multiple receptors for polycyclic aromatic hydrocarbons which could account for the different patterns of EGF binding inhibition by BP and TCDD.

The coordinate expression of several inducible enzymes, including cytochrome(s) P_1-450, is regulated by the *Ah* receptor (Nebert and Jensen, 1979; Poland and Knutson, 1982). Knutson and Poland (1982) have postulated that tissue-specific toxic responses (such as epidermal hyperplasia) to TCDD and isostereomers may be related to regulation of the expression of an additional battery of genes. The epidermis is a target tissue for EGF with respect to regulation of cell proliferation and differentiation and a target for TCDD toxicity as expressed in altered patterns of proliferation (acanthosis) and differentiation (hyperkeratosis) (Puhvel *et al.*, 1982, 1984). We suggest that altered regulation of the EGF receptor (and possibly other growth factor receptors) may be the consequence of the actions of TCDD on the second gene battery postulated to be controlled by the *Ah* receptor (Knutson and Poland, 1982).

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REFERENCES

ADAMSON, E. D., AND REES, A. R. (1981). Epidermal growth factor receptors. *Mol. Cell. Biochem.* **34**, 129–152.

BOWEN-POPE, D. F., DICORLETO, P. E., AND ROSS, R. (1983). Interactions between the receptors for platelet-derived growth factor and epidermal growth factor. *J. Cell Biol.* **96**, 679–683.

CARPENTER, G., AND COHEN, S. (1976). ^{125}I-Labeled human epidermal growth factor: Binding, internalization and degradation in human fibroblasts. *J. Cell Biol.* **71**, 159–171.

CARPENTER, G., AND COHEN, S. (1979). Epidermal growth factor. *Annu. Rev. Biochem.* **48**, 193–216.

COLLINS, S., AND MARLETTA, M. A. (1984). Carcinogen-binding proteins: High affinity sites for benzo(*a*)pyrene in mouse liver distinct from the Ah receptor. *Mol Pharmacol.* **26**, 353–359.

GREEN, H. (1979). The keratinocyte as a differentiated cell type. *Harvey Lect.* **74**, 101–139.

GREEN, H. (1978). Cyclic AMP in relation to proliferation of the epidermal cell: A new view. *Cell* **15**, 801–811.

HAIGLER, H. T., MAXFIELD, F. R., WILLINGHAM, M. C., AND PASTAN, I. (1980). Dansylcadaverine inhibits internalization of epidermal growth factor in Balb 3T3 cells. *J. Biol. Chem.* **255**, 1239–1241.

HUDSON, L. G., SHAIKH, R., TOSCANO, W. A., JR., AND GREENLEE, W. F. (1983). Induction of 7-ethoxycoumarin O-deethylase activity in human epithelial cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): Evidence for TCDD receptor. *Biochem. Biophys. Res. Commun.* **115**, 611–617.

IVANOVIC, V., AND WEINSTEIN, I. B. (1982). Benzo(*a*)pyrene and other inducers of cytochrome *P*450 inhibit binding of epidermal growth factor to cell surface receptors. *Carcinogenesis* **3**, 505–510.

KARENLAMPI, S. O., EISEN, H. J., HANKINSON, O., AND NEBERT, D. W. (1983). Effects of cytochrome *P*-450 inducers on the cell-surface receptors for epidermal growth factor, phorbol 12,13-dibutyrate, or insulin of cultured mouse hepatoma cells. *J. Biol. Chem.* **258**, 10378–10383.

KNUTSON, J., AND POLAND, A. (1982). Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Interaction of the Ah and hr loci. *Cell* **30**, 225–234.

LEE, L-S., AND WEINSTEIN, I. B. (1978). Tumor promoting phorbol esters inhibit binding of epidermal growth factor to cellular receptors. *Science (Washington, D.C.)* **202**, 313–315.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol Chem.* **193**, 265–275.

MADHUKAR, B. V., BREWSTER, D. W., BOMBICK, D. W., AND MATSUMURA, F. (1984). *In vivo* administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin reduces epidermal growth factor binding to rat hepatic plasma membrane. *Toxicologist* **4**, 309 (abstr).

NEAL, R. A., OLSON, J. R., GASIEWICZ, T. A., AND GUDZINOWICZ, M. (1981). The *in vivo* and *in vitro* metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the golden Syrian hamster. In *Toxicology of Halogenated Hydrocarbons: Health and Ecological Effects* (M. A. Q. Khan and R. H. Stanton, eds.), pp. 259–270. Pergamon, New York.

NEBERT, D. W., GOUJON, F. M., AND GIELEN, J. E. (1972). Arylhydrocarbon hydroxylase induction by polycyclic hydrocarbons: Simple autosomal dominant trait in the mouse. *Nature (London)* **236**, 107–110.

NEBERT, D. W., AND JENSEN, N. M. (1979). The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals by cytochrome *P*450 mediated monooxygenases. *CRC Crit. Rev. Biochem.* **6**, 401–437.

O'KEEFE, E., BATTIN, T., AND PAYNE, R., JR. (1982). Epidermal growth factor receptor in human epidermal cells: Direct demonstration in cultured cells. *J. Invest Dermatol.* **78**, 482–487.

OKEY, A. B., DUBE, A. W., AND VELLA, L. M. (1984). Binding of benzo(*a*)pyrene and dibenz(*a,h*)anthracene to the Ah receptor in mouse and hepatic cytosols. *Cancer Res.* **44**, 1426–1432.

POLAND, A., GLOVER, E., AND KENDE, A. S. (1976). Stereospecific, high affinity binding of TCDD by hepatic cytosol: Evidence that the binding species is the receptor for induction of arylhydrocarbon hydroxylase. *J. Biol Chem.* **251**, 4936–4946.

POLAND, A., AND KNUTSON, J. C. (1982). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanisms of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**, 517–554.

POLAND, A., PALEN, D., AND GLOVER, E. (1982). Tumor promotion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in skin of HRS/J hairless mice. *Nature (London)* **300**, 271–273.

PUHVEL, S. M., ERTL, D. C., AND LYNBERG, C. A. (1984). Increased epidermal transglutaminase activity following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: *In vivo* and *in vitro* studies with mouse skin. *Toxicol. Appl Pharmacol.* **73**, 32–47.

PUHVEL, S. M., SAKAMOTO, M., ERTL, D. C., AND REISNER, R. M. (1982). Hairless mice as models for chloracne: A study of cutaneous changes induced by topical application of established chloracnegens. *Toxicol Appl. Pharmacol.* **64**, 492–503.

RHEINWALD, J. G., AND BECKETT, M. A. (1980). Defective terminal differentiation in culture as a consiste

and selectable character of malignant human keratinocytes. *Cell* 22, 629–632.

RHEINWALD, J. G., AND BECKETT, M. A. (1981). Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinomas. *Cancer Res.* 41, 1657–1663.

RHEINWALD, J. G., AND GREEN, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell* 6, 331–344.

RHEINWALD, J. G., AND GREEN, H. (1977). Epidermal growth factor and multiplication of cultured human epidermal keratinocytes. *Nature (London)* 265, 421–424.

RICE, R. H., AND GREENLEE, W. F. (1982). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on cultured human epithelial target cells: Modulation by hydrocortisone. *Toxicologist* 2, 146 (abstr).

ROSE, J. Q., RAMSEY, J. C., WENTZLER, T. H., HUMMEL, R. A., AND GEHRING, P. J. (1976). The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to rat. *Toxicol. Appl. Pharmacol.* 36, 209–226.

ROZENGURT, E., COLLINS, M., BROWN, K. D., AND PETTICAN, P. (1982). Inhibition of epidermal growth factor binding to mouse cultured cells by fibroblast-derived growth factor. *J. Biol. Chem.* 257, 3680–3686.

SAWAHATA, T., OLSON, J. R., AND NEAL, R. A. (1982). Identification of metabolites of TCDD formed on incubation with isolated rat hepatocytes. *Biochem. Biophys. Res. Commun.* 105, 341–346.

SCHLESSINGER, J., SCHREIBER, A. B., LEVI, A., LAX, I., LIBERMANN, T., AND YARDEN, Y. (1983). Regulation of cell proliferation by epidermal growth factor. *CRC Crit. Rev. Biochem.* 14, 93–111.

THOMAS, G., PEREZ, J. M., SIEGMANN, M., AND OTTO, A. M. (1982). The effect of serum, EGF, PGF and insulin on S-6 phosphorylation and the initiation of protein and DNA synthesis. *Cell* 30, 235–242.

WEINSTEIN, I. B., HOROWITZ, A. D., MUFSON, R. A., FISHER, P. B., IVANOVIC, V., AND GREENBAUM, E. (1982). Results and speculations related to recent studies on mechanisms of tumor promotion. In *Carcinogenesis* (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann, eds.), Vol. 7, pp. 599–616. Raven Press, New York.

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310

Dioxins have left their mark on children from Seveso nine years ago....

"THE LINGERING (INSIDIOUS) CONTAMINATION"

Scientists (investigators) have now discovered how dioxins work in the body

The motor is an ideal dioxin-factory. This fact from Ulm's dioxin-analyst Professor Karlheinz Ballschmiter has alarmed the automobile industry. With his colleagues, Dr. Heinz Buchert has demonstrated: Autos produce highly poisonous dioxins and furans. In the exhaust of 24 German automobiles, the chemists have identified these materials.

With soot the dioxins are blown into the air, and they accumulate in the dirt of the street. One gram of dirt (dust) can contain up to 120 billion parts of a gram (nanograms) of the Seveso dioxin 2,3,7,8 TCDD, as well as other dangerous dioxins. "We would probably all be long dead," says Dr. Buchert, "if the sun did not to a great extent break down this material."

Sources of the danger are permissible mixtures, that make leaded gas antiknock and motor oil long-lived, as well as mixed PCB's, which are prohibited in recycled motor oil and were used for insulation in transformers until 1983.

The experts of health and environmental agencies in many industrial countries have recognized the general dioxin danger. As with DDT, the Seveso-poison has now also spread over the northern hemisphere. Bears at the Arctic Circle, herrings in the East Sea, crabs from Canadian waters, salmon and carp from Swedish rivers and lakes, moreover pork fat, chicken liver, or cow's milk are already tainted with this and other dioxins.

In Germany dioxins and furans concentrate in the sediments of the Rhine, Neckar and Bodensee, as the analytical Professor Hanspaul Hagenmaier from Tübingen has discovered. On the Bodensee this contamination began immediately after WWII with non-biodegradable molecules from retorts (coke ovens). At that time chemical plants first manufactured the defoliant 2,4,5-T, as well as DDT, lindane and PCP, thereby co-producing dioxin.

The dangerous materials are by no means, as industry asserts, the companions of mankind since the "origin of fire." The main producers of the worldwide contamination, in the opinion of scientists and environmental groups, is the other segment of the chemical industry which produces wood and plant preservatives containing these materials.

If dioxins arise from the smokestacks of incineration establishments, they are predominantly traced back to combustion of waste containing problem chemicals such as PCP, report Canadian authorities. "The key to the environmental problems," says Professor Ballschmiter, "are dioxins and furans. If we could successfully reduce these materials, many problems would be solved."

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While environmentalists still for the most part unsuccessfully battle single chemical products, such as lindane, DDT, or PCP, industry worldwide has long recognized that, above all, the true (substantial) danger threatens via the dioxin investigations of the health and environmental protection agencies of Canada, the U.S., Sweden, the Netherlands, and Italy.

Therefore, the American chemical giant Dow Chemical took up the pursuit itself. Its analytical team Lamparski/Tjnestrick (T.J.Nestrick) has just disclosed at the 5th International Dioxin Symposium in Bayreuth, that the company, through the production of the defoliant 2,4,5-T, has contaminated the soil of the city of Midland, Michigan, with up to 450 micrograms per kilo (ppt) of Seveso-poison. On the company property, up to 52,000 ppt have penetrated the ground. By comparison: on the property of the previously closed chemical plant Boehringer in Hamburg one finds more than one million ppt.

No German company has yet admitted, as openly (frankly) as the Dow Chemical Co., what risk its production entails for the environment.

The Seveso-TCDD can escape (out-gas) from the ground and spread through the air and also into the water. The Canadian EPA therefore warns against eating more than one fish per month out of Lake Ontario. In the body fat of Canadians, as well as Americans, up to 1000 ppt dioxins and furans have already accumulated. Included therein are up to 10 ppt Seveso-poison .

On the other hand, in Germany the federal health bureau knows virtually nothing about the burden to the population. Suitable investigations should be starting in a few months.

How do these poisons work in the body? How much can a person take up daily? In that vein, the ideas (opinions) of industrial toxicologists and scientists of the U.S. EPA were bounced around (discussed) in Bayreuth.

The EPA labels the Seveso-dioxin as an initiator and at the same time as a promotor of cancer. Certainly, for this reason, the dose should be only zero. Therefore, it seems that society accepts a certain number of cancer victims through this material.

Industry and the federal health bureau favor the theory that this poison at best potentiates the cancer-producing acitivity of other chemicals such as arsenic. Thus one could without danger daily ingest and inhale dioxin.

What a person accumulates today, he can only after decades wholly eliminate (excrete), discovered the Zurcher (?) toxicologist Dr. Hans Poiger through an experiment upon himself. Canadian and American biochemists have discovered that the

material is dangerous in small doses. Hamsters, guinea pigs, rabbits, sheep, Rhesus monkeys, which are especially sensitive to dioxin, as well as at least 10-20% of people, have in certain cells a protein, to which the Seveso-poison and similar substances bind.

Indeed this so-called A-receptor induces the production of enzymes, which should detoxify the body. However, the enzymes cannot break down the unnatural molecules. The excessive enzyme production now serves as a "surveyor's rod" (marker) for the inherent danger of dioxin-containing chemicals.

Under the influence of dioxin, the A-receptor even invades the genetic material. It alters the genes of other growth factors, of maturation as well as differentiation of replacement cells in the skin, and of the regulation of the thymus gland, which belongs to the immune system. The results : chloracne, as well as fewer active T-lymphocytes, the police of the body. That in return means weakening of the immune system. The organism becomes a victim of bacteria, viruses, as well as cancer. Moreover, dioxins cause a vitamin-A deficiency which can eventually lead to cancer.

Under the action of dioxins, the thyroid gland produces fewer hormones. Thereby nodules (growths) are formed. Metabolism is disturbed, and depression occurs. In conclusion, dioxins are the reason (cause) for this-- as detected in mice, too few hormones are also produced in the ovaries and fertility diminishes.

Nevertheless (meantime), scientists have discovered new molecules, which are similar to Seveso-dioxin, but which should be still more poisonous. Professor Ballschmiter believes therefore: "that we only stand at the beginning of a laborious search for ultra-poisons. Out of the exhaust and out of the incineration facilities come thousands of things, about which we still know nothing at all. (No end of quote.)

written by ELVIRA SPILL, STERN magazine
translated respectfully by Anita S. Knight and L.F. McCarty, 11-15-85

ASK

Dioxine haben vor neun Jahren Kinder von Seveso gezeichnet. Doch die Substanzen sind, aus vie

Die schleichende Verseuchung

177 michiga div.

Wissenschaftler haben jetzt herausgefunden, wie Dioxine im Körper wirken

Der Motor ist eine ideale Dioxin-Fabrik.« Diese Feststellung des Ulmer Dioxin-Analytikers Professor Karlheinz Ballschmiter schreckte die Autoindustrie. Mit seinem Kollegen Dr. Heinz Buchert hatte er nachgewiesen: Autos produzieren hochgiftige Dioxine und Furane. In den Auspuffanlagen von 24 deutschen Automobilen hatten die Chemiker diese Substanzen identifiziert.

Mit dem Ruß werden die Dioxine in die Luft geblasen und lagern sich im Staub der Straßen an. Ein Gramm Staub kann bis zu 120 billionstel Gramm des Seveso-Dioxins 2,3,7,8-TCDD, aber auch andere gefährliche Dioxine enthalten. »Wir wären wahrscheinlich alle schon längst tot«, sagt Dr. Buchert, »wenn nicht die Sonne einen großen Teil dieser Stoffe wieder zerstöre.«

Quelle der Gefahr sind erlaubte Zusatzstoffe, die Bleibenzin klopffest und Motoröl langlebig machen, aber auch verbotenerweise in wieder aufgearbeitetes Motoröl gemixte polychlorierte Biphenyle (PCBs), die bis 1983 zur Isolation in Transformatoren verwendet wurden.

Die Experten der Gesundheits- und Umweltbehörden vieler Industrieländer haben die generelle Dioxin-Gefahr erkannt. Denn wie einst DDT hat sich jetzt auch das Seveso-Gift vor allem über die nördliche Halbkugel verbreitet. Bären am Polarkreis, Heringe in der Ostsee, Krabben aus kanadischen Gewässern, Lachse und Karpfen aus den Flüssen und Seen Schwedens, aber auch Schweinefett, Hühnerleber oder Kuhmilch sind bereits mit diesem und anderen Dioxinen belastet.

In der Bundesrepublik reichern sich Dioxine und Furane in den Sedimenten von Rhein, Neckar und Bodensee an, wie der Analytiker Professor Hanspaul Hagenmaier aus Tübingen herausfand. Am Bodensee hat diese Verseuchung mit Molekülen aus der Retorte, die von der Natur nicht abgebaut werden können, unmittelbar nach dem Zweiten Weltkrieg begonnen. Damals hatten zum erstenmal Chemie-Fabriken das Entlaubungsmittel 2,4,5-T hergestellt, sowie DDT, Lindan und PCP – und dabei Dioxin mitproduziert.

Die gefährlichen Stoffe sind also keineswegs, wie die Industrie behauptet, von »Anbeginn des Feuers« Begleiter der Menschen. Hauptverursacher der weltweiten Verseuchung, so die Meinung von Wissenschaftlern und Umweltbehörden, ist jener Teil der chemischen Industrie, der Holz- und Pflanzenschutzmittel mit diesen Stoffen herstellt.

Wenn Dioxine aus den Schloten der Müllverbrennungsanlagen quellen, sind sie vorwiegend auf die Verbrennung von Abfall mit Problem-Chemikalien wie PCP zurückzuführen, urteilen kanadische Behörden. »Der Schlüssel für Umweltprobleme«, so Professor Ballschmiter, »sind Dioxine und Furane. Wenn es uns gelänge, diese Stoffe zurückzudrängen, würde sich vieles von selbst erledigen.«

Professor Karlheinz Ballschmiter aus Ulm spürte im Auspuff von Autos Dioxine auf

Während Umweltschützer noch immer und meist erfolglos einzelne chemische Produkte wie Lindan, DDT oder PCP bekämpfen, hat die Industrie weltweit längst erkannt, daß ihr wirkliche Gefahr vor allem durch die Dioxin-Untersuchungen der Gesundheits- und Umweltschutzbehörden Kanadas, der USA, Schwedens, der Niederlande und Italiens droht.

Der amerikanische Chemie-Riese Dow-Chemical ergriff deshalb die Flucht nach vorn. Sein Analytiker-Team Lamparski/Tjnestrick offenbarte jetzt auf dem 5. Internationalen Dioxin-Symposium in Bayreuth, daß die Firma durch die Produktion des Entlaubungsmittels 2,4,5-T die Erde der Stadt Midland im US-Bundesstaat Michigan mit bis zu 450 milliardstel Gramm pro Kilo (ppt) Seveso-Gift verunreinigt hat. Auf dem Firmengelände seien bis zu 52 000 ppt ins Erdreich gedrungen. Zum Vergleich: Auf dem Gelände der inzwischen geschlossenen Chemiefabrik Boehringer in Hamburg fanden sich mehr als eine Million ppt.

So offen wie Dow-Chemical hat noch keine deutsche Firma dokumentiert, welches Risiko ihre Produktion für die Umwelt bedeutet.

Das Seveso-TCDD kann aus der Erde ausgasen und sich durch die Luft, aber auch im Wasser ausbreiten. Die kanadische Umweltschutzbehörde warnt bereits davor, mehr als

See pro Monat zu essen. Im Körperfett von Kanadiern, aber auch von Amerikanern haben sich bereits bis zu 1000 ppt Dioxine und Furane angesammelt. Darunter sind bis zu 10 ppt Seveso-Gift.

In der Bundesrepublik weiß das Bundesgesundheitsamt dagegen so gut wie nichts über die Belastung der Bevölkerung. Erst in einigen Monaten soll mit entsprechenden Untersuchungen begonnen werden.

Wie wirken nun diese Gifte im Körper? Wieviel darf ein Mensch täglich aufnehmen? Darüber prallten die Meinungen von Toxikologen der Industrie und Wissenschaftlern der amerikanischen Umweltbehörde EPA in Bayreuth aufeinander.

Die EPA stuft das Seveso-Dioxin als Initiator und zugleich als Verstärker von Krebs ein. Sicher sei deshalb nur die Dosis null. Es sei denn, die Gesellschaft akzeptiere eine gewisse Zahl von Krebsopfern durch diese Substanz.

Die Industrie und das Bundesgesundheitsamt favorisieren die Theorie, daß dieses Gift höchstens die krebserregende Wirkung anderer Chemikalien wie etwa Arsen verstärke. Also könne man ohne Gefahr täglich weiterhin Dioxin mitessen und einatmen.

Was der Mensch heute an Dioxinen speichert, kann er erst nach Jahrzehnten vollends wieder ausscheiden, fand der Zürcher Toxikologe Dr. Hans Poiger durch einen Selbstversuch heraus. Daß die Substanz auch in geringer Dosis gefährlich ist, haben kanadische und amerikanische Biochemiker entdeckt. Hamster, Kaninchen, Hühner, Schafe, Rhesusaffen, die gegenüber Dioxin besonders empfindlich sind, aber auch mindestens zehn bis zwanzig Prozent der Menschen haben in bestimmten Zellen einen Eiweißkörper, der das Seveso-Gift und ähnliche Substanzen an sich bindet.

Dieser sogenannte Ah-Rezeptor löst zwar die Produktion von Enzymen aus, die den Körper entgiften sollen. Doch die Enzyme können die naturfremden Moleküle nicht knacken. Die übermäßige Enzymproduktion dient nun als Meßlatte für die Gefährlichkeit von dioxinhaltigen Chemikalien.

Unter dem Einfluß von Dioxin greift der Ah-Rezeptor

Er verändert die Gene jenes Wachstumsfaktors, der Reifung sowie Teilung von Nachschubzellen für die Haut und der zum Immunsystem gehörenden Thymus-Drüse steuert. Die Folge Chlorakne, aber auch weniger aktive T-Lymphozyten, der Polizei des Körpers. Das wiederum bedeutet Schwächung des Immunsystems. Der Organismus ist Bakterien, Viren, aber auch Krebs hilflos ausgeliefert. Außerdem lösen Dioxine einen Vitamin-A-Mangel aus, was ebenfalls zu Krebs führen kann.

Unter Einwirkung von Dioxinen produziert die Schilddrüse weniger Hormone. Dadurch bilden sich in ihr Knoten. Der Stoffwechsel entgleist, und es kommt zu Depressionen. Dioxine sind schließlich die Ursache dafür, daß – wie an Mäusen nachgewiesen – auch in den Eierstöcken zu wenig Hormone gebil-

Professor Hanspaul Hagenmaier aus Tübingen entdeckte Dioxine im Schlamm am Grund des Bodensees

det werden und die Fruchtbarkeit nachläßt.

Inzwischen haben die Wissenschaftler neue Moleküle entdeckt, die dem Seveso-Dioxin ähnlich sind, aber noch giftiger sein sollen. Professor Ballschmiter glaubt deshalb, »daß wir erst am Anfang einer mühseligen Fahndung nach Ultragiften stehen. Aus dem Auspuff und aus den Müllverbrennungsanlagen kommen Tausende von Stoffen, über die wir bislang überhaupt noch nichts wissen. ELVIRA SPILL

TERATOGENESIS CARCINOGENESIS and MUTAGENESIS

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Reproductive Effects of Herbicide Exposure in Vietnam: Recent Studies by the Vietnamese and Others

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INTRODUCTION

Are there adverse effects on the children of individuals exposed to such herbicides as Agent Orange and its contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin? This weighty question continues to be debated here in the United States and elsewhere, in the courts as well as in scientific meetings.

Initially, concern about possible harm to exposed human populations was prompted by observations in the laboratory: specifically, teratogenic effects among the offspring of treated females and some testicular toxicity in treated males. From the experimental data it was also apparent that the variation in response across species (small mammals, nonhuman primates) was considerable. Hence direct evidence from studies in man is clearly essential to answer questions about the reproductive risk to humans. Regrettably, such data are sparse and frequently poor in quality. [see refs. 1–4 for critical reviews of the literature]. Two relevant studies of reproductive outcomes among U.S. soldiers serving in Vietnam have recently been published [5–7]. We discuss these latest reports briefly below, but they are of course available to anyone wishing to peruse and evaluate them further.

The principal objective of this paper is to put before the interested reader as full an account as possible of related but unpublished epidemiologic investigations carried out by Vietnamese researchers. These studies, which are unlikely ever to appear in Western scientific journals, were presented in January 1983 at an International Symposium on the long-term effects of phenoxy herbicides, held in Ho Chi Minh City (formerly Saigon) and attended by the authors along with some 70 other visiting scientists from the U.S., the United Kingdom, Western and Eastern Europe, Asia, and the U.S.S.R. Because of the continuing debate, it seemed to us important to bring these studies to the attention of the wider scientific community and to provide as

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complete and unprejudiced a description of their methods and findings as possible, such that readers may make some independent assessment of the evidence contained therein.

The studies outlined below were presented formally to the Symposium's Reproductive Epidemiology Working Group,* of which one of us was rapporteur (J.D.C.) and the other a participant (M.C.H.). All conferees were assigned to one of eight such groups for in-depth discussion of the Vietnamese research. We were provided with English translations of each paper upon our arrival in Vietnam. At subsequent meetings of the working groups, the authors of the investigations presented their studies and responded to questions from the other participants. The descriptions we provide draw on information elicited in these discussions as well as on that contained in the papers circulated to us. (See subsequent footnote for fuller details.) *Tables of data are presented exactly as they appear in the original reports*. Wherever we have made additional computations, this fact is duly noted in the text.

METHODOLOGICAL PROBLEMS IN VIETNAMESE STUDIES OF THE REPRODUCTIVE EFFECTS OF HERBICIDE EXPOSURE

Before analyzing the Vietnamese investigations individually, it may be well to consider some general problems that apply to many or all of them.

Two types of studies were carried out in Vietnam to assess the reproductive effects of herbicide exposure: 1) Studies in the South compared reproductive outcomes among couples living in sprayed areas with those among couples living in unsprayed areas. 2) Studies in the unsprayed North compared reproductive outcomes among unexposed women whose husbands served in the South, and hence were potentially exposed, with those among women whose husbands remained in the North.

Specific problems which may affect either type of study include the following.

Bias in Selection and/or Reporting

Bias is a threat to the validity of any scientific research, particularly so when the investigation does not take place in the pristine environment of the laboratory but in the real world. Hence it is unfortunate that many of the Vietnamese papers prevent adequate evaluation because they are less detailed than one would wish in describing the selection of subjects and the method of data-gathering. The Vietnamese researchers do, however, appear to be aware of methodologic issues. In one investigation [9] the Northern districts chosen for study and the affected children selected as cases were determined using a truly random method. In another study carried out among Northern women in respect to exposure of their husbands [10] it was appropriate that the team determining the nature and frequency of reproductive problems was different from that inquiring about potential herbicide exposure. Blind interviews were more difficult to carry out in the South where exposure status is based on residence; knowing the respondent's domicile meant that the interviewers usually knew whether they were talking to an exposed or unexposed subject. This caveat in respect to studies

*A volume based on the proceedings of the Symposium has recently been published (Westing AH, ed: "Herbicides in War: The Long-term Ecological and Human Consequences," London and Philadelphia: Taylor and Francis, 1984, 210 pp.) The volume contains a chapter on reproductive epidemiology, which includes only a selected few of the papers presented to the Working Group, along with a brief discussion which draws on the material in our present, longer review.

carried out in the South may not apply to the investigations at a Ho Chi Minh City hospital, since the patients' residence would not be so readily apparent as in the case of home interviews.

A true double-blind study where the respondents as well as the interviewers are blind to exposure cannot even reasonably be proposed under the conditions prevailing in Vietnam.

Definition of Exposure

In virtually all of the studies to be discussed, designation of exposure is based on residence in an area sprayed with herbicides as determined by history and/or visible forest or crop destruction. Today the presumption of exposure could be confirmed by US military flight records which have become available (ie, the Defense Department's HERBS tapes). Obviously there must have been enormous variation in individual exposure; some people were outdoors at the time of spraying, others indoors, others away from the village for a day or two. Some merely passed through a sprayed area; others continued in residence there. Excepting one study where only preliminary results have been reported [11], no attempts have been made to construct an exposure index, nor has a distinction been drawn between direct exposure or secondary exposure through the diet. In no instance have the various herbicidal agents been differentiated.

The studies carried out in the North, while effective in isolating paternal exposure from exposure of the mother, consider all Northern soldiers who were posted to the South as exposed, although presumably only some of them actually were, at least to any significant degree. The inclusion of all will weigh against the demonstration of health effects of herbicide exposure in the studies in question. Exposure of the female only cannot be studied, since women living in sprayed areas generally had husbands living with them who were similarly exposed. No effort has hitherto been made to seek out exposed women with unexposed husbands, and this may not be feasible. Hence, while certain effects have been ascribed to maternal exposure only, paternal influence cannot be excluded.

Sources of Data on Reproductive Outcomes

Four pregnancy outcomes have been examined in the Vietnamese studies: miscarriage, stillbirth, congenital defects, and hydatidiform mole, or molar pregnancy.

The frequency of miscarriage has been ascertained solely by verbal report rather than through medical records. While this method is considered acceptable and is the only one used in many worldwide studies, in one of the Vietnamese papers [11] the data on miscarriage are somewhat inconsistent, suggesting that these self-reports may not be entirely reliable. Stillbirths were generally ascertained from health records. It is believed that at least 75% of all births in Vietnam take place within the Ministry of Health system, ie, at least within the knowledge of a rural midwife. Original midwives' records provide reliable data on number of live and stillbirths, sex, and weight of infant and placenta. However, the noting of congenital anomalies is very erratic, even though there is a column provided for recording them. In these studies reported birth defects were validated in so far as possible. Local health records were checked and surviving children with malformations were examined by physicians from the investigative team. In most cases, specific congenital anomalies appear to have been carefully observed and listed. However, their classification is not uniform. A few of

the studies [12,13] include among congenital anomalies aberrations of viral origin, such as infantile poliomyelitis, and obscure conditions such as enuresis.

It is impossible to be sure how complete the ascertainment of birth defects has been in these investigations, but the way the inquiries are set up makes this unimportant, providing the search was uniformly carried out in both groups: exposed and unexposed. The overall rate of congenital anomalies reported among the unexposed is at the very low end of the worldwide range (ie, 0.5%). Prior to the herbicide spraying there had been no real effort to establish the expected rates of various congenital anomalies in Vietnam, making evaluation of the figures presented in these studies difficult. To provide some context for their assessment, we include here some data from a WHO survey of several Southeast Asian nations carried out in the early 1960s (Table I). As the table shows, the overall rate of common congenital malformations in these populations is similar to that reported from Vietnam.

TABLE I. WHO Comparative Study of Congenital Malformations (Suppl. 34 Bull. WHO, pp. 1–127, Geneva, 1966)

	Rate of common congenital malformations in the Centre (per 1,000)						
	1	2a	2b	3a	3b	4	Note
Down's syndrome	0.17	0.00	0.00	0.16	0.37	0.54	(Underreported + + +)
Anencephalus	1.24	1.74	0.32	1.00	0.67	0.52	
Dto & SB	0.00	0.15	0.09	0.06	0.05	0.00	
Hydrocephalus	0.29	0.73	0.05	1.04	0.23	0.27	Standardized
Dto and SB	0.10	0.29	0.00	0.00	0.00	0.03	for mother's age
Spina bifida	0.26	0.75	0.13	0.19	0.12	0.03	
Occipital SB	0.00	0.04	0.00	0.00	0.00	0.00	
Other NTD	0.07	0.05	0.03	0.00	0.02	0.17	
(All NTD)	(1.96)	(3.75)	(0.62)	(2.29)	(2.09)	(1.02)	
Oesophageal atresia	0.00	0.08	0.00	0.06	0.00	0.03	(Including TEF)
Anal atresia	0.10	0.30	0.10	0.56	0.15	0.07	
Other gut malformations	0.20	0.00	0.00	0.19	0.00	0.13	
Exomphalos	0.20	0.20	0.05	0.12	0.07	0.07	
Cleft lip	0.41	0.35	0.31	0.31	0.20	0.34	
Cleft lip + palate	1.01	0.73	0.31	1.25	1.21	0.78	
Cleft palate	0.20	0.13	0.16	0.00	0.33	0.40	
Talipes	1.62	0.96	0.42	1.63	2.92	0.91	(All types)
Polydactyly *U*	0.00	0.10	0.10	0.19	0.00	0.00	(Isolated, ulnar)
Other polydactyly	0.61	0.25	0.26	0.13	0.38	0.24	
Radial polydactyly	0.91	0.025	0.00	0.25	0.50	0.20	
Syndactyly	0.10	0.00	0.05	0.06	0.18	0.07	
Other digital	0.20	0.03	0.00	0.12	0.00	0.24	
Reduction deformities	0.00	0.10	0.05	0.13	0.32	0.51	
Other limb deformities	0.10	0.35	0.26	0.38	0.35	0.20	
Sirenomelia	0.10	0.00	0.00	0.06	0.00	0.07	
Conjoined twins	0.00	0.00	0.00	0.00	0.00	0.03	
Total per 1,000 deliveries	7.89	7.58	2.69	7.89	8.07	5.85	

This study provides data from the following countries of SEA:
1) Hong-Kong: 10,001 total deliveries; 2) India: 59,458 total deliveries from Calcutta, and Bombay, respectively; 3) Malaysia: 56,125 total deliveries from Kuala Lumpur, and Singapore, respectively; 4) Philippines: 29,989 total deliveries from Manila.
There were 155,573 total deliveries (ie, single and multiple).
The sampling of data was organized in 1962 and 1963, respectively.

Hydatidiform mole is a distinct clinical entity, normally requiring hospitalization. It is therefore assumed that data currently being collected in hospital settings provide reasonably complete, accurate estimates of its occurrence.

A word is in order as to the reliability of historical hospital statistics in Vietnam as compared with those derived from study of the original health records. At least one author, Dr. Nguyen Thi Ngoc Phuong [14], states in her paper that government statistics, at least until recently, are unreliable. One of us (J.D.C.) was personally involved in studying some of these for the period from 1966 to 1972 [15]. The relation between midwives' records and figures reported by the Ministry of Health is very poor. A report by Cutting et al for the U.S. Department of Defense [16], described subsequently, is significantly flawed in this respect. For example, figures obtained from the original midwife records in Tay Ninh are at striking variance with those recorded by the Ministry of Health and subsequently reported by Cutting.

Control for Potentially Confounding Variables

Only limited efforts have been made in these studies to consider the potentially confounding effects of extraneous variables (eg, maternal age, nutrition, infection) which might distort any observed association between herbicide exposure and reproductive outcome. Variables which have been controlled, either in design or analysis, will be mentioned in summarizing the individual studies.

DESCRIPTION OF THE VIETNAMESE STUDIES

We shall now consider the nine Vietnamese investigations which were presented to us.* To help the reader follow the ensuing discussion, Table II lists each study in turn, together with a brief description of the research design.

Studies Carried Out in the South of Vietnam

Khoa. Dr. Nguyen Dinh Khoa of the University of Hanoi presented a paper entitled "Some Biologic Parameters Collected on the Groups of People in an Area Affected by Chemicals," [12]. Dr. Khoa collected retrospective histories covering the years between 1965 and 1982 from 313 families of Montagnards living in an area of heavy spraying. In this one paper the original figures as presented appeared to us so confusing that we have had to extrapolate and summarize them.

Infant mortality was reported to be 14.7% (176 death/1,196 births) and the rate of miscarriage was 10.1% (134/1,196 + 134). The incidence of birth defects was

*To properly appreciate the Working Group's evaluation of these papers, we should note some of the practical considerations that controlled our discussion. By an heroic effort on the part of the Vietnamese participants, all of the papers had been translated into English and distributed to all Congress participants at the time of registration. The members could, therefore, study the papers before their presentation in the various working groups. In some cases the authors had not been able to review fully these English translations. In other cases, either immediately after the initial presentation in English, French, or Vietnamese or on subsequent days in answer to questions posed previously, the numbers in the reports as we individually received them were corrected, emendated, or significantly augmented. In one paper, for example, all of the controls were accidentally omitted from the English version. The constraints of time, and sometimes of language, prevented these additions from being fully incorporated into our discussion and statistical evaluation of these papers. Most of our comments, therefore, are principally based, though perhaps in some cases unfairly, on the English version after the correction of some obvious errors, not upon the "final" and complete Vietnamese paper. French provided the common language for many of the questions and much of the discussion of the various reports.

TABLE II. Vietnamese Studies of the Reproductive Effects of Herbicides

Authors	Site, population studied	Research design
1. Khoa, 1983 [12]	Montagnards living in a heavily sprayed area of Southern Vietnam	Rates of obstetric events for a period during and after the spraying, 1965–1982.
2. Nguyen, 1983 [13]	Provincial hospital in a heavily sprayed area of Southern Vietnam	Rates of obstetric events, 1979–1981.
3. Trung and Chien, 1983 [17]	Families from a sprayed and an unsprayed village, Southern Vietnam	Comparison of rates of miscarriage and birth defects before and after spraying in an exposed and unexposed village
4. Huong and Phuong, 1983 [18]	Obstetric Hospital, Ho Chi Minh City (Saigon), Southern Vietnam	Time trends analysis of reproductive events, 1952–1981. Case-control study of herbicide exposure and hydatidiform mole
5. Phuong and Huong, 1983 [14]	Women from a sprayed area of Southern Vietnam and from unsprayed areas of the South and the North	Comparison of reproductive problems in women exposed and unexposed to herbicides
6. Lang, Tung, and Van, 1983a [10]	Residents of agricultural villages for veterans, Northern Vietnam	Comparison of birth defects in offspring of soldiers who did and did not serve in the South
7. Lang, Van, Dwyer, et al, 1983 [11]	Families of veterans, Northern Vietnam	Comparison of miscarriage rates according to degree of husband's herbicide exposure
8. Can, Xiem, Hong, et al, 1983 [8]	Veterans living in three areas of Northern Vietnam	Comparison of reproductive events among wives of exposed and unexposed veterans
9. Can, Xiem, Tong, et al, 1983 [9]	Malformed and normal offspring of veterans living in Northern Vietnam	Case-control study of birth defects in relation to father's service in the South

2.7% (33/1,196), but it is unclear how many cases of infantile paralysis may have been included amongst the congenital anomalies. Although unfortunately no control data were included in the paper as translated into English, these had in fact been collected in an unsprayed village; the controls demonstrated a miscarriage rate of 6.1% (38/587 + 38) and a 1.0% rate of congenital anomaly (6/587), both rates being lower than the comparable occurrence in exposed villages. No statistical evaluation of these "differences" could be attempted by the Working Group since the control figures were not available in the original paper.

Nguyen. Dr. Ho Dang Nguyen of the Tay Ninh Polyclinic Hospital presented a paper entitled "Pregnancies at the Polyclinic of Tay Ninh Province," [13] summarizing the results of a study of reproductive outcomes occurring between 1979 and 1981 in a provincial hospital serving an area heavily sprayed until about 1970. Although the province has a total population of some 700,000 living in seven districts, the Tay Ninh City Polyclinic cares primarily for a population of about 30,000 and in addition receives many difficult cases referred from the district dispensaries which handle most deliveries. Combining the figures from 1979, 1980, and 1981 and the first 6 months of 1982, there were 7,344 deliveries, resulting in 166 stillbirths (2.3%); 1,633 premature births (22.2%); 133 hydatidiform moles (1.8%); and 78 congenital defects

(1.1%), while 1,920 abortions were recorded out of a total of 9,264 pregnancies (20.7%).

To the 78 congenital defects noted from this part of the study, 57 defects referred in from the districts were added, presumably only a small proportion of those actually born. Of the 57 referred mothers, one had had malaria and two had positive tests for syphilis. Smoking and intrapregnancy use of medications were very limited. The mothers of the 57 had had 202 total pregnancies terminating in 20 abortions, 4 premature births, 52 stillbirths, and 126 fullterm babies. Among the 57 defects, two were monsters, eight showed anencephaly, four showed anopthalmia, four showed phocomelia, and four others showed severe skeletal deformities; these were anomalies referred to the polyclinic rather than representing an unselected sample of all congenital defects in the area. There is some ambiguity in the paper in that the 57 were "sent from the district" as distinguished from the 78 born at the polyclinic, but nonetheless among the six mothers (of the 57) for whom the location of the delivery is given, three were, in fact, delivered at the polyclinic. Note was also made of two mothers, each of whom delivered four children with cleft lips and another who delivered four blind children. These last are in addition to the four previously noted cases of anopthalmia (ie, not included in the 57) even though the children were apparently not born at the polyclinic and might normally have been considered as "sent from the district." This investigation is one of several presented to the working group which, while not susceptible of statistical analysis, seem to show a large number of striking and usually rare anomalies, even allowing for their being selected.

Trung and Chien. Dr. Cung Binh Trung and Nguyen Tran Chien of the Medical College of Hanoi presented a paper entitled "Spontaneous Abortions and Birth Defects in Area Exposed to Toxic Chemical Sprays in Giong Trom District, Ben Tre Province, South Vietnam" [17]. They studied the occurrence of spontaneous abortion and birth defects before and after the time of spraying. The authors surveyed 848 families who continued to live in heavily sprayed areas; families living in a nearby unsprayed village served as a comparison group. The method of selecting study participants is not described. Before the spraying, the rate of miscarriage in these 848 families was 5.6% (129 miscarriages/2,292 pregnancies); after the spraying the rate was 13.9% (217/1,562). The control families reported a miscarriage rate of 7.3% (32/436) prior to the time of spraying and 7.4% (27/365) after the time of spraying. This being a pre/post exposure comparison of the same families, the mothers were likely to be older in the postexposure period and on this basis alone might have been expected to show some increase, even in the unexposed controls.

Birth defects (apparently corrected by exclusion of cases induced by drugs or diseases such as syphilis) were also studied in the exposed villages, though not among the controls. Prior to the spraying, the rate of defects among surviving live births was 0.14% (3/2,163 births) compared with a rate of 1.78% (24/1,345) among children born after the spraying. The preexposure rate seems to reflect very limited reporting and again mothers were older in the period after exposure. Of the 24 anomalies occurring in the postexposure period, four were cases of deafness and eight involved skeletal defects.

Huong and Phuong. Drs. Le Thi Diem Huong and Nguyen Thi Ngoc Phuong of the former Tu Du Gynecological and Surgical Hospital in Ho Chi Minh City presented two papers. The first of these, entitled "The State of Abnormal Pregnancies and Congenital Malformation at the Gyneco-Obstetrical Hospital of Ho Chi Minh

City, formerly Tu Du Hospital," [18] was divided into two sections. The first section reports on time trends in the incidence of abortion, "intrauterine deaths" (? stillbirths), molar pregnancies, and congenital anomalies between 1952 and 1981, though the figures are unavailable for some years in each category (see Table III following). Figures for the years prior to 1975 are drawn from doctoral theses, since administrative data for this period are viewed by the authors as unreliable; figures for the years since 1975 come from the annual report of the hospital. The authors recognize that there is some element of error in that the denominator—total number of pregnancies—is not always consistent.

Although striking changes in rates are demonstrable, interpretation is somewhat difficult; however, the authors feel that the data, even though provided by different sources, are comparable. The abortion rate appears to remain fairly low until 1967 (the rate reported for 1952 seems contrary to general experience), then rises dramatically until 1978 and then declines slightly. These changes could be considered to be consistent with the times of heaviest spraying if a persistent effect is assumed. Stillbirths appear to rise between 1952 and 1953 (but again the figures seem very low

TABLE III. Data From Huong and Phuong, 1983 [18]

Years	Molar pregnancies and choriocarcinoma (%)		Congenital malformations (%)		Intra-uterine deaths (%)		Abortions (%)	
1952	56/6,495	0.78			38/6,495	0.58	29/6,495	0.45
1953					10/6,889	0.12	103/6,889	1.20
1959	173/14,413	1.20						
1960	160/14,076	1.13						
1962	158/12,440	1.27						
1963			93/12,538	0.73				
1964			106/16,779	0.63				
1965			96/18,463	0.52			916/19,308	4.73
1966			82/19,429	0.42			820/19,854	4.13
1967	350/24,345	1.43	128/23,509	0.57	378/24,345	1.56	3,594/24,345	14.58
1971	242/27,457	0.87			382/27,475	1.39	3,822/27,475	13.99
1976	338/16,167	2.09			216/16,167	1.33	2,732/16,167	16.89
1977	499/12,943	3.85	92/12,796	0.70	232/12,943	1.78	2,139/12,943	16.52
1978	477/13,544	3.52	101/13,430	0.75	196/13,544	1.44	2,458/13,544	18.14
1979	580/12,757	4.54	134/12,648	1.06	187/12,757	1.47	1,396/12,757	10.94
1980	460/12,766	3.60	158/12,573	1.24	185/12,766	1.45	1,624/12,765	12.73
1981	569/12,754	4.19	133/13,430	0.99	239/13,574	1.76	1,367/13,574	10.09

and only 2 years are available) and increase again in 1967, from which time they seem to remain fairly constant until 1981. Again these changes could be consistent with a herbicide exposure if one assumes a persistent effect. Moles and choriocarcinomas are among the most complete figures available and again show a sharp increase, but this is first apparent in 1976 (1971 available, but not 1972–1975), well after heavy spraying had ceased. Congenital anomalies, presumably restricted to grossly recognizable external defects, show no great changes and only a slight increase in 1979, 1980, and 1981 as compared to 1977, 1978, and the preceding years. In considering these figures, it should be remembered that Tu Du is a referral hospital and the rates of some reproductive problems may be somewhat higher than for Ho Chi Minh City as a whole.

The second section of this paper reports a case-control study comparing the frequency of herbicide exposure among 100 women with molar pregnancies (cases) and 284 women with normal deliveries (controls). Control mothers were matched to cases on maternal age and parity; however, the groups differ significantly in social conditions and diet (86% of the women with molar pregnancies were considered to have a "good" living standard against only 45% of the controls). Moreover, strikingly more of the husbands of women with molar pregnancies smoked (84 vs 70%) or drank alcohol (90 vs 35%), although it is not clear that these are relevant differences. Data on past exposure to herbicides were collected at interview. Of the 85 out of 100 cases whose exposure status was ascertained, 48 or 56.5% were considered exposed, while of the 276 controls for whom determination of exposure was made, about 27 (out of 284) or 9.8% reported exposure. (No reason is given for the lack of exposure information on 15 cases and eight controls.) The data are summarized below in a fourfold table (Table IV) to which we have added the odds ratio and 95% confidence limits.

The data suggest a strong association between herbicide exposure prior to conception and subsequent development of a hydatidiform mole. Molar pregnancies, generally more common among Asian women, are conceptions without an embryo, but with grossly swollen chorionic villi. Complete moles are androgenetic in origin, arising from fertilization of an ovum in which the nucleus is either absent or inactivated [19]. Molar pregnancy is often associated with subsequent development of choriocarcinoma. Tissue determination of dioxin levels in the fat or other tissues of the patient would be very helpful in confirming the possible association between herbicides and hydatidiform mole/choriocarcinoma. In this study, data on congenital anomalies have also been gathered, but the figures are too small as yet to draw any conclusions; fortunately, the authors plan to expand their research.

Phuong and Huong. The same authors presented another paper entitled "The Effects of Toxic Chemicals on the Pregnancy of the Women Living at Two Localities

TABLE IV. Data From Huong and Phuong, 1983 [18]*

	Exposed	Not exposed
Case	48	37
Control	27	249
Total	95	286

*OR = 11.96 (6.67, 21.46).

in the South of Vietnam" [14]. This inquiry presents a comparison of reproductive problems reported by the women from a sprayed village, in which all were considered exposed, with those recorded from a group of women in Ho Chi Minh City, amongst whom 92% were considered unexposed. The time period for these reproductive histories and the method of selecting subjects for interview are not described. The tables showing the reported results are appended here (Tables V,VI). The original paper reported a comparison by number of families interviewed: 1,249 and 1,244, respectively, the latter figure comprised of 1,126 unexposed and 98 exposed women. (These are the figures used in the original tables but they add up to 1,224 not 1,244.) A comparison of total pregnancies was subsequently added and percentages determined from these; 98 exposed families in Ho Chi Minh City were excluded from these calculations. A further comparison is then made between the figures for the exposed population and those from a number of villages in North Vietnam that show considerably lower rates (Table VI). The authors state that the number of reproductive abnormalities varies in a significant manner with respect to the degree of exposure, but this statement is not further elucidated in the paper. In contrast to their previous study, the authors give no information as to the changes in successive years nor as to the rates before spraying. Note that the rate of molar pregnancy among the exposed mothers is 1.8% (133/7,327). While considerably higher than the rate of 0.4% in the unexposed comparison group, it is only slightly higher than that of 1.20% reported in the previous paper from Tu Du for 1959 to 1962 [18]; ie, before significant spraying, and lower than the rate of 3 to 4% observed at Tu Du since spraying. Again, however, the comparison with Tu Du may not be legitimate in that complicated pregnancies are likely to have been referred there. The rate for abortion at 8% is between that for Tu

TABLE V. The State of Pathological Pregnancies at the Thang Phong Village (Ben tre) and at the 10th District of Ho Chi Minh City [14]

	Thang Phong village: exposed group, 7,327 pregnancies (%)	10th district of Ho Chi Minh city: nonexposed group, 6,690 pregnancies (%)
Congenital anomalies	81 (1.1)	29 (0.4)
Embryo death in uterus	59 (0.8)	2 (0.0)
Natural abortion	587 (8.0)	243 (3.6)
Molar pregnancy	133 (0.7)	26 (0.4)
Prenatal death of newborn	924 (12.4)	1 (4.6)

TABLE VI. Data From Phuong and Huong, 1983 [14]

	South of Vietnam Thanh Phong (%)	North of Vietnam		
		My Van	Hai Hau	Mai Chau
Birth defects	1.1	0.45 ± 0.06	0.39 ± 0.05	0.68 ± 0.12
Embryo death in uterus	0.8	1.91 ± 0.13	1.91 ± 0.12	3.85 ± 0.29
Natural abortion	8.01	5.77 ± 0.21	4.96 ± 0.018	8.74 ± 0.040
Molar pregnancy	0.73	0.09 ± 0.04	0.03 ± 0.01	0.10 ± 0.13

Du 1965–66 (4.5%) and 1967–68 (an average of 16%). That for stillbirths at 0.8% is about half the Tu Du rate for 1967–81 (average 1.5%) and that for congenital anomalies at 1.1% is about double that of Tu Du for 1963–67 and comparable to 1979 and 1980, always remembering that most of the Tu Du population was not severely exposed.

Studies Carried Out in the North of Vietnam

Lang, Tung, and Van. We come now to a consideration of those two Vietnamese investigations that at the time of their presentation seemed to be the most complete, and which concern themselves with the possible deleterious effects of male herbicide exposure. The first is that of Drs. Ton Duc Lang, Ton That Tung, and Do Duc Van from Viet Duc Hospital in Hanoi, "Mutagenic Effects on the First Generation After Exposure to Agent Orange" [10]. This work is a continuation of the seminal investigation originated by Dr. Ton That Tung, the first results of which were made available to the West, albeit in a very limited way, through his manuscript "Le Probleme des Effets Mutagenes sur la Premiere Generation apres L'Exposition aux Herbicides" early in 1980. These figures were augmented and some other changes made by Dr. Ton That Tung in a second manuscript in 1981 and are further supplemented in the report we are discussing now. The following pages will synthesize these three sources of information, though only the last was discussed and evaluated at the conference.

The investigation consists of an epidemiological survey, an analysis of the nature of the congenital anomalies encountered, and a correlation of the degree of herbicidal exposure with the rate of occurrence of congenital anomalies. The epidemiological survey was carried out in two steps: initially by studying the obstetrical statistics of veterans' wives in two villages, one containing many veterans returning from the south, and one not. This was subsequently extended to an investigation carried out at a number of agriculatural cooperatives set up principally for veterans. The husbands were divided into those who had served in the southern part of Vietnam, all of whom are assumed to be exposed (although most were Northerners returning home, the manuscript of Ton That Tung records that some may have been Southerners now going north for the first time), and those who had remained at home and were consequently unexposed. Ton That Tung states that all of the exposed fathers of the anomalous children told him of being directly exposed to spraying and that their average stay in the South was from 3 to 4 years. Marriages for exposed men normally occurred after their definitive return following at least a brief period of courtship. Therefore the delay between possible exposure and conception will have been more than the 80 days that sperm normally survive. The results of the study appear in Table VII which shows that half the congenital anomalies found at Yen Bai were born to exposed fathers, though these made up only some 16.6% of the potential breeding male population (Ton That Tung: 700 out of 4,200) and in Quy Mong all the anomalies were born to exposed fathers, even though they made up only 10% of the potential breeding male population (using the same proportions as determined for Yen Bai, since no precise figures are given). There is no explanation offered as to the cause of the somewhat higher birth rate in Yen Bai as compared to that in Quy Mong, even allowing that the figures for Yen Bai include 4 years and those of Quy Mong only 3. All 30 congenital anomalies encountered at Yen Bai are then listed and include six anencephalics of various kinds as well as two major limb deformities, all amongst

TABLE VII. Congenital Malformations in Obstetrical Services [10]

	Yen Bai	Quy Mong
Local population	35,000[a]	4,500
Local demobbed service men	700[b]	30
Time of statistics	1975–1978	1976–1978
Number of births	3,058[c]	233[e]
Number of congenital malformations	30[d]	9
Congenital malformations of civilian population	15	0
Congenital malformations of veteran's children	15	9

[a]32,000 (Ton That Tung #1).
[b]"Approximately" 700.
[c]3,058: 511 births to exposed; 2,547 births to unexposed.
[d]of the 30: 22 at term; eight premature.
[e]Division of births unknown; he guesses 90 and 143.

children of exposed fathers. At Quy Mong they reported three anencephalics, all to one pair of parents, and three major deformities, all with fathers exposed (vide supra). It should be noted again that many of these details are taken from the manuscript of Dr. Ton That Tung, not from the abbreviated text as presented to the conference, though of course this includes all of Ton That Tung's cases in the total figures reported.

In the agricultural cooperatives, which were largely composed of veterans from the South, a total of 1,142 exposed veterans produced 3,147 children with 71 anomalies (2.25%) while 613 unexposed married veterans had 2,172 children with 10 anomalies (0.46%), a difference of fivefold. Among the 71 anomalies born to exposed fathers were six anencephalics, one meningocele, two anopthalmias, three major limb deformities, and one amorphous monster. Below we have abstracted data from Ton Duc Lang's paper to compare against the data from Dr. Ton That Tung's manuscripts (Table VIII). Ton That Tung's series consists of only 47 anomalies which are presumably included in the final count of 71; hence there would appear to be some discrepancy in the definition of congenital heart anomalies.

Table IX shows Lang's data on the frequency of reproductive outcomes by gravidity. The wives of unexposed fathers demonstrate the increase in the frequency of adverse outcomes with successive pregnancies normally expected, whereas this trend is reversed among the women whose husbands were exposed. The pattern is suggestive of a toxic effect most virulent at the time of first conception and then gradually diminishing in its potency. The paper concludes with a map showing an increased percentage of anomalies among soldiers serving in particularly heavily defoliated areas, but no specific figures or controls are provided.

It is of interest that in Ton That Tung's original manuscripts he describes for the same group of cooperative veterans a striking change in reproductive endpoints other than congenital anomalies, as shown in the appended table (Table X). This was not included in the paper presented at the conference.

Dr. Tung in both his manuscripts notes apparent increases in hydatidiform moles in Hanoi. He states that at the moment of his writing there were 19 cases of hydatidiform mole in his hospital, nine of them in wives of exposed soldiers, but no other figures are given for this anecdotal report.

TABLE VIII. Comparison of Figures From Tung and From Lang

	Ton That Tung (II) out of total of 47	Lang out of total of 71
Anencephaly	6	6
Anopthalmia	1	2
Cleft lip and palate	4	9
Polydacryly	4	7
Congenital heart	18	11 (sic)
Hemangioma	0	4

TABLE IX. Frequency of Reproductive Incidents According to the Ordinal Number of Pregnancies [10]

Ordinal number of pregnancy	Affected group (%)	Nonaffected (%)
1	20.44	5.42
2	15.64	5.98
3	13.57	7.88
4	13.22	8.76
5	15.95	9.74
6	15.00	15.00
7	7.60	19.38

TABLE X. Investigation of 1,549 Vietnamese Soldiers*

	Congenital malformations		Abortions		Premature deliveries		Sterile	
Group	Number	%	Number	%	Number	%	Number	%
A	47 (out of 1,496 births)	3.14	252 (out of 1,748 pregnancies)	14.42	30 (out of 1,496 deliveries)	2.01	22 (out of 786 couples)	2.8
B	3 (out of 1,438 births)	0.21	143 (out of 1,581 pregnancies)	9.04	9 (out of 1,438 deliveries)	0.61	5 (out of 418 couples)	1.2
	$p\ 1.\ 10^{-9}$		$p\ 1.\ 10^{-5}$		$p\ 1.\ 10^{-3}$		p 0.05	

*Data from Ton That Tung, "La probleme des effets mutagenes sur la premiere generation apres l'exposition aux herbicides." Group A; 956 individuals with 786 couples in which the husband lived in the sprayed zones of South Vietnam; group B; 593 individuals with 418 couples in which the husband was not in South Vietnam (control group).

Lang, Van, Dwyer, et al [11]. An important addendum to this study was provided in a paper entitled "Self Reports of Exposure to Herbicides and Health Problems—A Preliminary Analysis of Survey Data From the Families of 432 Veterans of Northern Vietnam" [11] authored by Dr. Ton Duc Lang and others and presented by one of the authors, Dr. James Dwyer of the State University of New York at Stony Brook. This preliminary investigation describes a survey of 432 veterans from whom a medical/reproductive history was obtained by one interview team while a separate team, blind to the first, determined presumptive herbicide exposure. This included, very significantly, an estimate of the degree of exposure as indicated by whether the

individual 1) sustained direct or wet exposure, 2) lived in a defoliated area, or 3) only passed through a defoliated region. Coupled with a history of military service between 1964 and 1970, this allowed division of these respondents into high, moderate, and low exposure subgroups. The sample (N = 432) is too small as yet to provide data on rare events such as congenital anomalies and in terms of reproductive outcomes only miscarriages were analyzed. The number of miscarriages was ascertained in two ways: 1) The wife was asked to report the number of live births, miscarriages, and stillbirths that occurred prior to, during, and after her husband's military service and 2) a complete reproductive history was obtained in which pregnancies were reported by date of delivery or other termination. These two methods of obtaining what should have been the same information showed surprising disparities and their inconsistency is evidence of limited reliability. However, the general pattern of events, especially after the father's time of military service, was the same by either method of inquiry. For younger mothers, there was no apparent association between the degree of exposure of her mate and her risk of miscarriage, and for all age groups combined, the rates were within the range of freguency found in other Vietnamese study populations. However, for older women there was shown to be a strong association between the extent of paternal exposure and the rate of miscarriage. These figures are not fully convincing since the reported rates of miscarriage among the unexposed older mothers (0 out of 60 by one method of inquiry; 3 out of 66 by another) are too low to be fully credible. Also it is not immediately clear why older women would be more affected by their husband's herbicide exposure than would be those who were younger. Furhermore, as noted previously, if women of all ages are combined, the association largely disappears.

Can, Xiem, Hong, et al. We now come to the final Vietnamese report which in consideration of adherence to an appropriate protocol, completeness of figures, and the possibility of precise statistical analysis is the most convincing of those presented to us. Dr. Nguyen Can and his co-workers, from the Institute for the Protection of Mothers and Newborn in Hanoi presented "An Epidemiological Survey of Pregnancies in North Vietnam" [8] together with a very important addendum, "A Case Control Survey of Congenital Defects in My Van District, Hai Hung Province" [9]. As in the previously discussed study of Dr. Ton Duc Lang [10], a survey was made of the rate of adverse reproductive outcomes among the wives of 40,064 veterans exposed or unexposed to herbicides. Three areas in North Vietnam (ie, unsprayed) were arbitrarily selected—one in the mountains, one in the lowlands, and one on the coast. The women were 90% rice growers (the remainder medical staff, office workers, etc.) and are reported to have had no history of tuberculosis, syphilis, or malaria nor to have had drug exposure during pregnancy. It is not clear whether there were, in fact, no women found to be so afflicted or whether the investigations excluded those that were, since 40,000 women free of any such disease is hard to credit. All 40,064 interviews were carried out in 3 months (June–August 1982) using three doctors, thirty midwives, and additional ancillary personnel—a truly formidable undertaking. An effort was made to validate all reported reproductive events. Although questioning was concentrated on co–ops and production teams to increase the yield of exposed fathers (11,053 or 25%), nonetheless all married couples *present* in the locality at the time of the interview were included and it was estimated that 70–90% of those domiciled were questioned.

The results of these interviews are shown in Tables XI and XII. The data appear to show a small increase of borderline statistical significance ($\alpha = .10$, two-tailed) in the rate of miscarriages and congenital defects among women whose mates were exposed, but no increase in molar pregnancy or stillbirths. Once again, the total rate of congenital anomalies is very low, even in the exposed group (0.64%). The authors themselves point out, however, that no cardiac defects or hemangiomas are listed. Presumably most of the defects reported are grossly detectable abnormalities. It is also noted that the exposed group have fewer pregnancies on the average than did the unexposed (2.91 vs. 4.24) and that maternal age was not controlled, although it was in the case control study soon to be discussed. There is no precise breakdown as to occupation nor a distinction made between soldiers that served as officers, but there is good evidence that, at least in the countryside of North Vietnam, the general conditions of life vary little with these parameters. An analysis of the distribution of specific congenital anomalies is then given. This seems to show a slight excess among exposed fathers in the proportion of anencephalics (6.3% vs. 4.6%). A comparable proportional increase in cleft lip and palate is also shown for exposed fathers whereas in contrast to the figures in previously discussed reports, limb deformities are more frequent among children of unexposed fathers.

Can, Xiem, Tong, et al [9]. A case-control study was set up by the authors to answer some of the possible objections to the investigation previously concluded. The districts for follow-up were drawn by lot. Sixty-one case families were chosen, each with a surviving child with a congenital anomaly—cleft palate, cleft lip, limb malformations, megacolon, absence of the ears, imperforate anus*, congenital cataracts, or congenital blindness. Of these 61 anomalies, 30 were in the children of exposed fathers (49.2%). Three controls were drawn by lot for each of these 61 cases from a pool matched to the cases on maternal age (plus or minus 3 years); number of deliveries (plus or minus 2); village of residence; and age of a living child (plus or minus 2 years). Among the 183 control couples, 39 fathers (21.3%) were exposed. Almost all subjects were currently ricefield workers. None had a history of heart disease, VD, cancer, malaria, etc. None gave a history of addiction to smoking or alcohol. Most of the women were less than 36 years old (51 out of 61) and the controls and cases were well-matched as to age. The investigators calculated odds ratios and 95% confidence intervals for the association of interest. As can be seen in Table XIII, the risk of having served in the South is about 3.5 time greater for fathers of cases than for fathers of controls. Stratifying on maternal age (<36, ≥ 36) produces similiar results and no evidence of an interaction with age of the mother. Providing that this study was indeed carried out as described, then it shows a statistically significant association between paternal "exposure" to herbicides and certain congenital anomalies in the offspring.

SUMMARY OF RESULTS FROM THE VIETNAMESE STUDIES

The studies carried out in the North of Vietnam are consistent in reporting an association between presumptive paternal exposure to herbicides prior to or at con-

*Since unoperated imperforate anus is fatal, we are a little uncertain as to the true nature of the anomaly meant here.

TABLE XI. Data From Can et al, 1983 [8]

Group	Number of women investigated	Number of pregnancies	Number of deliveries	Number of abortions	Number of curetages	Number of molar pregnancies	Dead fetuses	Cases with congenital defects
A (unexposed)	29,041	121,993	114,025	7,148	750	70	2,512	521
B (exposed)	11,023	32,069	29,560	2,271	210	28	576	189
Both groups	40,064	154,062	143,585	9,419	960	98	3,088	712

TABLE XII. Statistics of Abnormal Pregnancies in Women of A and B Groups in Three North Vietnamese Districts [8]

Group	Abortions/ Pregnancies (% ± 2 SD)	Molar pregnancies/ No. of pregnancies (% ± 2 SD)	Fetuses dead before and during delivery/ No. of deliveries (% ± 2 SD)	Fetuses with congenital defects/ No. of deliveries (% ± 2 SD)
A (unexposed)	7,148/ 121,933 (5.86 ± 0.13)	70/ 121,993 (0.06 ± 0.01)	2,512/ 114,025 (2.20 ± 0.09)	521/ 114,025 (0.46 ± 0.04)
B (exposed)	2,274/ 32,069 (7.08 ± 0.28)	28/ 32,069 (0.09 ± 0.04)	576/ 29,560 (1.95 ± 0.16)	188/ 29,560 (0.64 ± 0.09)
Both groups	9,419/ 154,062 (6.11 ± 0.12)	98/ 154,062 (0.06 ± 0.01)	3,088/ 143,585 (1.15 ± 0.07)	710/ 143,585 (0.49 ± 0.03)

TABLE XIII. Data From Can et al, 1983 [9]*

	Exposed	Not exposed
Cases	30	37
Controls	39	144

*OR = 3.57 (1.91, 6.69).

ception and congenital defects in subsequent offspring, particularly certain types of anomaly (anencephaly, orofacial defects). The strength of the association demonstrated in the data varies among studies. Results in respect to miscarriage are conflicting. No association with molar pregnancy is documented.

The studies carried out in the South of Vietnam, where women as well as men were at risk of exposure, report increases in miscarriage, stillbirths, molar pregnancy, and birth defects, again with certain types predominating, among couples previously exposed to herbicides. In the case of molar pregnancy the evidence for an association with herbicide exposure is very suggestive.

OTHER STUDIES OF HERBICIDE EXPOSURE IN VIETNAM

For purposes of completeness and comparison with the results of the Vietnamese studies, we briefly describe the other investigations pertaining to herbicide exposure in Vietnam and possible reproductive damage. These include the following: two U.S. studies of South Vietnamese women [16,20]; an Australian Government study of birth defects in relation to father's Vietnam service [21]; a study of Air Force personnel associated with Ranch Hand, the U.S. Government's herbicide-spraying operation [7]; and the Centers for Disease Control study in Altanta of birth defects, Vietnam service, and potential Agent Orange exposure [5,6]. Studies of those exposed to herbicides or dioxins in other circumstances (eg, Seveso, herbicide production facilities, contaminated residential sites) will not be discussed here.

U.S. Studies of South Vietnamese Women

The study mentioned above by Cutting et al [16] for the Department of Defense utilized obstetric data from the records of selected hospitals in Vietnam to compare the rates of stillbirth, hydatidiform mole, and congenital defects in periods of light (1960–65) and heavy (1966–69) herbicide spraying. In the Coastal Plain and Delta areas of South Vietnam, rates rose in the heavy spray period but elsewhere rates were constant or declined. As discussed above, this study is flawed because of inadequacies in the data base.

A study by Kunstadter [20] for the National Academy of Science used the obstetric records of selected hospitals in Ho Chi Minh City (Saigon), supplemented by personal interviews, to gather information on birth defects and perinatal mortality. Maternal herbicide exposure was established based on information contained in the Defense Department's HERBS tapes. The data showed no consistent association between congenital malformation and maternal exposure in the first trimester. Cleft lip, however, increased relative to other defects during the heavy spraying period and remained elevated. The stillbirth rate rose, but not until after spraying ceased.

Australian Study of Birth Defects and Father's Vietnam Service

In January 1983 the Australian government published results of a large case-control study of some 8,500 children with birth defects (largely structural defects detectable at birth) and 8,500 liveborn controls, matched for maternal age and time of birth [21]. Cases with no father's name included on the birth certificate were excluded from the study. The frequency of Vietnam service among the fathers, as determined from Army lists, was virtually the same in cases as in controls. In relation to specific birth defects the report states that the odds ratios for Down's syndrome and ventricular septal defects "exceed 1.5, the minimum odds ratio that can be usefully studied in an epidemiological study," but these data are not shown.

No index of exposure of Australian troops to herbicides could be developed. Thus the results of this study apply only to service in Vietnam and not to potential effects of herbicide exposure sustained there. In fact the investigators state that "exposure to herbicides was infrequent and probably very low in Australian troops in Vietnam."

Air Force Study of Morbidity in Ranch Handers

In February 1984 the Air Force released its preliminary study of morbidity in Ranch Hand personnel, the approximately 1,200 men who conducted the herbicide spraying missions in Vietnam and who some maintain sustained the highest exposures of any American troops [7]. (Others argue that ground troops living in contaminated areas, without opportunities to shower and change clothes, may have received a dose as high or higher.) An exposure index was calculated based on the amount of herbicide used during the Ranch Hander's tour of duty. Ranch Handers were compared with cargo-mission personnel who also flew in Southeast Asia but were not exposed to Agent Orange.

The results reported are viewed as preliminary in that reproductive events were initially ascertained by self-report and are only now being verified in birth records; the results of this validation process are expected shortly. The preliminary data showed a significant excess of neonatal deaths (associated with severe defects) and of physical handicaps among children of Ranch Handers; overall, birth defects were

more frequent in the Ranch Hand group, but the increase is statistically significant only when minor anomalies (eg, skin) are included. Other reproductive parameters considered (infertility, miscarriage) are not appreciably different in the two groups.

CDC's Atlanta Study of Birth Defects and Vietnam Service

In August 1984 results of the CDC birth defects study were published [5,6]. Cases, gathered from the Metropolitan Atlanta Congenital Defects Register, include all severe defects diagnosed through the first year of life. Controls, matched for hospital, year of birth, and race, were identified from birth certificates. Case and control parents were traced with similar procedures and interviewed by telephone. For mothers the overall response rate was 70%, for fathers only 56% (with most losses reflecting a failure to locate rather than a refusal). Among whites, the losses were equivalent in cases and controls while among nonwhites significantly more cases than controls were lost to interview.

No association was found between father's reported Vietnam service and either birth defects in the aggregate or specific types of malformations. Agent Orange exposure was also assessed using (1) self-reports and (2) an exposure index based on judgments by the military about where and when the man served in Vietnam (eg, Ranch Handers were generally assigned a score of 5, representing highest exposure). These analyses are based on smaller samples than the analysis of Vietnam service. The exposure index showed no association with birth defects overall but did show statistically significant associations with spina bifida, cleft lip (± cleft palate), coloboma, and neoplasms in the first year of life.

CONCLUSIONS

Our primary intention in this paper has been to provide detailed descriptions of the unpublished research on reproductive sequelae of herbicide exposure that has been carried out by Vietnamese scientists. In order to put this in context we have also summarized the design and results of the other reproductive studies known to us that examine populations potentially exposed in Vietnam (U.S., Australian, and Vietnamese soldiers, civilian residents of Southern Vietnam). It remains for the reader to judge the quality of each study and the compatibility of the findings.

In closing, we venture three general observations and recommendations based thereon:

1) It can be argued that virtually all of the research to date has been conducted by groups that are *parti pris* to the Vietnam conflict (to a lesser extent this is also true of the studies of exposed occupational groups and Seveso residents). If further research is undertaken, it might well be under the auspices of an independent body.

2) Measurement of exposure has thus far often been lacking or quite crude. Apparent inconsistencies in research results might be resolved if exposure dose were better quantified. Route of absorption and environmental matrix (eg, soil, food) as well as amount of chemical should be considered. Biological markers (eg, adipose tissue levels) need to be developed and applied in order to determine effective or absorbed dose following exposure. Data analysis should consider carefully specified exposure-effect models.

3) Timing of exposure in relation to pregnancy has rarely been evaluated in the studies to date but needs to be taken into account. This is particularly true in the

studies of paternally mediated birth defects, where a biologic model for teratogenesis (as distinct from mutagenesis) is lacking.

REFERENCES

1. Friedman JM: Does Agent Orange cause birth defects? Teratology 29:193–221, 1984.
2. Hatch M: Reproductive effects of the dioxins. In Lowrance WW (ed) "Public Health Risks of the Dioxins." New York: The Rockefeller University, 1984, pp 255–274.
3. Pearn JH: Teratogens and the male. Med J Aust 2:16–20, 1983.
4. Young AL, Kang HK, Shepard BM: Chlorinated dioxins as herbicide contaminants. Environ Sci Tech 17:530A–540A, 1983.
5. Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams Jr. MJ: Vietnam veterans' risks for fathering babies with birth defects. JAMA 252:903–912, 1984.
6. Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams Jr. MJ: "Vietnam Veterans' Risks for Fathering Babies with Birth Defects." Atlanta: Centers for Disease Control, 1984.
7. Lathrop GD, Wolfe WH, Albanese RA, Moynahan PM: "Project Ranch Hand II. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Baseline Morbidity Study Results." San Antonio, Texas: U.S. Air Force School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, 1984.
8. Nguyen Can, Nguyen Thi Xiem, Tran Tan Hong, Nguyen Kim Tong, Do Binh Duong: "An Epidemiological Survey of Pregnancies in Viet Nam." 1983a.*
9. Nguyen Can, Nguyen Thi Xiem, Nguyen Kim Tong, Do Binh Duong: "A Case-Control Survey of Congenital Defects in My Van District, Hai Hung Province." 1983b.*
10. Ton Duc Lang, Ton That Tung, Do Duc Van: "Mutagenic Effects on the First Generation After Exposure to 'Orange Agent'." 1983a.*
11. Ton Duc Lang, Do Duc Van, Dwyer JH, Flamenbaum C, Dwyer KM, Fantini D: "Self-reports of Exposure to Herbicides and Health Problems: A Preliminary Analysis of Survey Data From the Families of 432 Veterans in Northern Viet Nam." 1983b.*
12. Nguyen Dinh Khoa: "Some Biologic Parameters Collected on the Groups of People in an Area Affected by Chemicals." 1983.*
13. Ho Dang Nguyen: "Pregnancies at the Polyclinic of Tay Ninh Province." 1983.*
14. Nguyen Thi Ngoc Phuong, Le Thi Diem Huong: "The Effects of Toxic Chemicals on the Pregnancy of the Women Living at Two Localities in the South of Viet Nam." 1983.*
15. Meselson MS, Westing AH, Constable JD: "Background Material Relevant to Presentations Concerning the Herbicide Assessment Commission for the American Association for the Advancement of Science." Washington: AAAS, 1971. Addendum to the Report.
16. Cutting RT, Phuoc TH, Ballo JM, Benenson MW, Evans CH: "Congenital Malformations, Hydatidiform Moles and Stillbirths in the Republic of Vietnam, 1960–1969." Washington, D.C.: U.S. Government Printing Office, 1970.
17. Cung Binh Trung, Nguyen Tran Chien: "Spontaneous Abortions and Birth Defects in Area Exposed to Toxic Chemical Sprays in Giong Trom District, Ben Tre Province, South Vietnam." 1983.*
18. Le Diem Huong, Nguyen Thi Ngoc Phuong: "The State of Abnormal Pregnancies and Congenital Malformations at the Gyneco-Obstetrical Hospital of Ho Chi Minh City (formerly Tu Du Hospital)." 1983.*
19. Jacobs PA, Wilson CM, Sprenkle JA, Rosenshein NB, Migeon BR: Mechanism of origin of complete hydatidiform moles. Nature 287:714–716, 1980.
20. Kunstadter P: "A Study of Herbicides and Birth Defects in the Republic of Vietnam: An Analysis of Hospital Records." National Academy of Sciences, Washington, D.C.: National Academy Press, 1982.
21. Donovan JW, Adena MA, Rose G, Batisutta D: "Case-control Study of Congenital Anomalies and Vietnam Service (Birth Defects Study)." Canberra: Australian Government Publishing Services, 1983.

*Vietnamese papers presented at the Symposium on Herbicides and Defoliants in War: The Long-term Consequences on Man and Nature Ho Chi Minh City, January 13–20, 1983.

Toxicology and Applied Pharmacology

TOXICOLOGY AND APPLIED PHARMACOLOGY 78, 147–157 (1985)

Dose-Related Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J and DBA/2J Mice[1]

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Dose-Related Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J and DBA/2J Mice. CHAPMAN, D. E., AND SCHILLER, C. M. (1985). *Toxicol. Appl. Pharmacol.* 78, 147–157. The dose-related effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were studied in B6D2F$_1$/J (B6D), C57BL/6J (C57), and DBA/2J (DBA) mice. A 14-fold difference in lethality was observed in C57 and DBA mice, based upon 30-day LD50 values of 182 and 2570 μg TCDD/kg body wt, respectively. The 30-day LD50 for B6D mice was 296 μg TCDD/kg body wt. A progressive loss of body weight in all strains of mice was observed during the 30-day LD50 studies, with maximal weight losses of 24.7, 34.0, and 33.4% prior to death of C57, B6D, and DBA mice, respectively. In separate experiments, it was found that decreased feed consumption did not contribute to weight loss in C57 mice exposed to lethal or sublethal doses of TCDD until the animals were moribund. Time-course studies in C57 mice treated with 200 μg TCDD/kg body wt indicated that decreases in serum glucose and triglyceride concentrations and increases in hepatic triglyceride content occurred within 4 to 8 days of exposure, and were maximally altered within 17 to 21 days postexposure, concomitant with a 25% body weight loss. C57 mice fasted for 24 to 96 hr lost 18% of body weight and also exhibited alterations in glucose and lipid parameters; however, these changes were substantially different than the effects of TCDD exposure. In concert, these observations demonstrate that decreased feed consumption (hypophagia) does not account for weight loss and changes in carbohydrate and lipid metabolism in TCDD-treated C57 mice. Dose–response experiments resulted in comparable changes in glucose and lipid parameters when DBA mice were exposed to 10-fold higher doses of TCDD than C57 mice. Parallel LD50 responses and parallel changes in carbohydrate and lipid metabolism, at 10- to 15-fold differences in dose range, are indicative of a common mechanism of toxicity in TCDD-treated C57 and DBA mice.

The effects of the extremely toxic dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is formed as a contaminant during trichlorophenol synthesis and is found as a component of flyash, have been reviewed (Hay, 1982). TCDD-induced lethality is characterized by a broad range of oral LD50 values, which extend from 0.6 μg TCDD/kg body wt for male guinea pigs (Schwetz *et al.*, 1973) to 5051 μg TCDD/kg body wt for male hamsters (Henck *et al.*, 1981). A marked species difference in the nature of the biological responses to TCDD has hindered identification of the specific lesion(s) responsible for the lethality of TCDD. Weight loss has been identified as a consistent response of laboratory animals to TCDD (Matsumura, 1983). Hypophagia may contribute to weight loss in rats (Seefeld *et al.*, 1984), although

[1] A preliminary report of these results was given at the annual meetings of the Society of Toxicology, March 1983 and 1984.

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pair-feeding studies suggest that hypophagia does not totally account for TCDD-induced weight loss (Gasiewicz *et al.*, 1980).

Marked changes in glucose and lipid metabolism have been reported to accompany weight loss and mortality in laboratory animals exposed to TCDD. Increased serum cholesterol or triglyceride concentrations have been reported in rats (Gasiewicz *et al.*, 1980; Schiller *et al.*, 1982, 1984), guinea pigs (Gasiewicz and Neal, 1979), and hamsters (Olson *et al.*, 1980). Similar serum results for mice have not been reported, although TCDD exposure does alter hepatic lipid metabolism in mice (Jones and Grieg, 1975). The relationships between TCDD-induced changes in lipid and glucose metabolism, weight loss, and lethality are unclear; however, the documented affects of TCDD on human lipid and carbohydrate metabolism (Oliver, 1975; Reggiani, 1978; Walker and Martin, 1979) emphasize the need to clarify these relationships.

In the present study, we have utilized C57BL/6J (C57) and DBA/2J (DBA) mice to relate TCDD-induced changes in glucose and lipid metabolism to the relative lethality of TCDD. Previous reports have suggested that various inbred strains of mice, and specifically TCDD-sensitive C57 and TCDD-insensitive DBA mice, provide a well-defined genetic model for characterizing the comparative toxicity of TCDD (Nebert, 1981). One approach to characterize the genetic component of TCDD toxicity has been to examine the association of the binding affinity of TCDD to its receptor, which is determined by the *Ah* locus, with TCDD sensitivity in mice (Poland and Glover, 1980). Our results indicate that TCDD produces dose-related hypoglycemia and hypolipidemia in both C57 and DBA mice. In C57 mice, we have examined the effects of TCDD on feed consumption as well as compared the effects of fasting and TCDD exposure to evaluate the possible contribution of altered feed consumption to the observed changes in intermediary metabolism.

METHODS

Chemicals. TCDD, obtained from the Laboratory of Chemistry, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina was >99% pure by gas chromatographic analysis. Stock TCDD solutions were prepared by dissolving TCDD in acetone at 60°C. An equal volume of corn oil was added, and the acetone was removed with a stream of N_2 while maintaining the solution at 60°C. Solution concentrations of TCDD were confirmed by neutron activation analysis of chlorine content (Crouthamel, 1975). Working solutions of TCDD were prepared by dilution of the stock solution with the appropriate volume of corn oil. Final acetone concentrations were less than 1% by volume. In corn oil solutions containing more than 250 μg TCDD/ml, a fine, pale powder precipitated within 2 to 3 days of preparation. Therefore, when TCDD concentratuons greater than 250 μg TCDD/ml were required, solutions were used within 2 to 4 hr of preparation and were maintained at 37°C in the interim. Solutions of less than 250 μg TCDD/ml did not precipitate or exhibit any loss of chlorine content, as determined by neutron activation analysis, even when stored at room temperature for more than 3 months. NIEHS safety protocols were followed during all aspects of TCDD preparation and handling. Enzymes and enzyme substrates were obtained from Boehringer-Mannheim, New York, New York. 1,5-Diphenylcarbohydrazide was obtained from Sigma Chemical Company, St. Louis, Missouri. All other chemicals were reagent grade from commercial sources.

Animals. Male C57, DBA, and B6D[3] mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and were acclimated at the NIEHS animal care facility for at least 1 week prior to use. At the time of experimentation, all animals were 10 to 12 weeks old and weighed 22 to 32 g. Mice were housed five per cage and maintained under controlled temperature (21 ± 2°C) lighting (12 hr light and dark), and humidity conditions (40 to 60%). Animals were allowed free access to water and National Institutes of Health (NIH) diet NIH-31, which was supplied in pellet form.

All animals were fasted overnight prior to receiving a single po dose of TCDD in corn oil (0.3 ml/25 g body wt) by gastric intubation. Control animals received corn oil only (0.3 ml/25 g body wt). All TCDD doses are reported as micrograms of TCDD per kilogram of body weight.

LD50 30-day experiments. The 30-day LD50 values for C57, B6D, and DBA mice were established with 10 to 15 mice per dose. C57 mice received doses of 0, 95, 145, 190, and 285 μg TCDD/kg; B6D mice received doses of 0, 170, 220, 265, 325, 370, 425, and 450 μg

[3] (C57BL/6J female × DBA/2J male)F_1.

TCDD/kg; DBA mice received doses of 0, 1370, 1870, 2610, 3500, and 4470 μg TCDD/kg. These doses were based on preliminary studies which established the minimum dose required to obtain 100% mortality in each strain. The animals were observed daily and body weights measured every 2 or 3 days. At the end of the 30-day postexposure period, surviving animals were killed and selected organs removed and weighed. The 30-day observation period has become standard for TCDD studies because the mean time to death after a single po dose of TCDD in mice is 20 to 25 days (Hay, 1982). Organ weights were obtained for each dose in which sufficient numbers of animals (4 to 5) survived. Organ weights for kidneys, spleen, heart, testes, adrenals, lungs, intestine, liver, thymus, and epididymal fat pads were obtained.

Feed intake. The effect of TCDD exposure on feed consumption by C57 mice was examined after exposure to 0, 50, 100, 200, and 400 μg TCDD/kg. Mice, 10 per dose, were housed 5 per cage. Mice were raised 2 cm above the cage floor by stainless-steel wire-mesh cage inserts. Feed was supplied in an overhead feeder. The weights of the animals, feed remaining in the feeder, and feed spilled were determined every 2 or 3 days. Feed spillage was established by separating spilled feed from fecal matter collected on absorbent paper liners placed below the wire-mesh cage inserts. The weights of feed spilled and fecal matter were recorded at each interval throughout the 27 days of the experiment.

Dose–response and time-course experiments. The dose-responses of serum glucose, total and esterified cholesterol; triglycerides, glycerol, and nonesterified fatty acid (NEFA); and hepatic triglycerides to po TCDD exposure in C57 and DBA mice were examined. C57 mice received doses of 0, 5, 20, 50, and 200 μg TCDD/kg, and DBA mice received doses of 0, 50, 250, 750, and 2500 μg TCDD/kg by gavage. One week later animals were fasted overnight (16 hr) before being killed. In a second series of experiments the time-related changes in serum triglycerides, glucose, total cholesterol, and hepatic triglycerides in C57 mice exposed to a single po dose of TCDD (200 μg TCDD/kg) were examined. C57 mice were dosed 21, 17, 12, 8, and 4 days before being killed. All animals were fasted overnight before termination between 0900 and 1100 hr of the same day.

Fasting experiments. The effects of starvation on serum glucose, triglycerides, and total cholesterol and hepatic triglycerides were examined in C57 mice. Animals were fasted for 0, 24, 48, 72, or 96 hr prior to being killed. All animals were killed between 0900 and 1100 hr of the same day.

Analytical methods. Serum was separated from clotted whole blood by centrifugation for 10 min in an Eppendorf microcentrifuge (Model No. 3200). Glucose was determined enzymatically, after perchloric acid protein precipitation, with hexokinase and glucose-6-phosphate dehydrogenase (Bergmeyer *et al.*, 1974). Cholesterol and cholesterol ester were determined with a cholesterol oxidase assay (Röschlau *et al.*, 1974); total cholesterol in the same serum samples was determined after hydrolysis of the serum in ethanolic KOH, and cholesterol ester was estimated by subtracting cholesterol determined before hydrolysis from total cholesterol. Serum triglycerides were estimated by determining glycerol concentrations before and after ethanolic KOH hydrolysis and subtracting free glycerol from total glycerol (Eggstein and Kuhlman, 1974). Nonesterified fatty acids (NEFA) were measured with the complexing agent 1,5-diphenylcarbohydrazide (Mahadevan *et al.*, 1969). Serum samples (0.1 ml) were extracted with 10 ml chloroform:methanol (2:1, v/v); the chloroform extract was evaporated to dryness, and the residue was taken up in 5.0 ml chloroform for analysis. Palmitic acid standards were used to quantify extraction and colorforming steps. Hepatic triglyceride content was estimated by measuring glycerol as described above. The entire liver was removed and minced, and 0.5 g was extracted with 20 ml chloroform:methanol (2:1, v/v). The extract was dried, and the residue was analyzed for triglyceride content as described above.

Statistical analysis. Slopes of probit responses and estimates of LD50 values were obtained by the statistical analysis system (SAS) probit procedure (Finney, 1971). Dose–response effects were established with a one-way analysis of variance. Comparisons between means were by a two-tailed *t* test, variances assumed unequal (Bailey, 1959).

RESULTS

Acute Toxicity

The sensitivity of male C57, B6D, and DBA mice to TCDD was established by determining 30-day LD50 values for a single po dose of TCDD. A 14-fold difference in the sensitivity, based on percentage mortality, of C57 and DBA mice to TCDD was observed (Fig. 1). B6D mice, the progeny of a C57 and DBA cross, resembled the C57 parent in sensitivity to TCDD. Probit analysis of the data (Fig. 1) provided LD50 values (95% confidence intervals) of 182 μg TCDD/kg (163 to 201 μg TCDD/kg), 296 μg TCDD/kg (268 to 324 μg TCDD/kg), and 2570 μg TCDD/kg (2206 to 2912 μg TCDD/kg) for C57, B6D, and DBA mice, respectively. The parallelism of the dose–response curves (Fig. 1) was confirmed by the absence of significant ($p > 0.05$) differences in the slopes of the log dose vs probit responses among the three

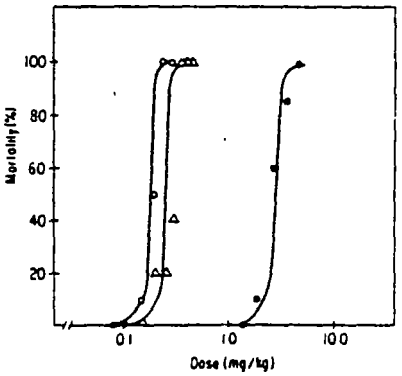

FIG. 1. Percentage mortality of C57, B6D, and DBA mice receiving a single po dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Mice, 10 to 15 animals in each group, were given a single po dose of TCDD in 0.3 ml corn oil. The animals were observed for 30 days postexposure and percentage mortality was calculated for C57 (○), B6D (△), and DBA (●) mice.

strains. The 30-day LD50 value obtained for C57 mice is comparable to values reported previously, 114 μg TCDD/kg (Vos *et al.*, 1974) and 284 μg TCDD/kg (McConnell *et al.*, 1978).

Mice exposed to TCDD exhibited a progressive dose-related loss of body weight beginning 4 to 6 days postexposure (Fig. 2). The mean body weight loss in animals that died from TCDD exposure was 24.7 ± 1.8, 34.0 ± 0.9, and 33.4 ± 1.1% ($\bar{x}$ ± SE) for C57, B6D, and DBA mice, respectively. The mean time to death was 24.4 ± 0.7, 25.4 ± 0.6, and 21.4 ± 0.7 days ($\bar{x}$ ± SE) for C57, B6D, and DBA mice, respectively. Selected organ weights were measured on Day 30 in the groups of C57 and DBA mice that survived and contained at least four animals (Table 1). Given these qualifications, several limited observations can be made about the organ weight changes. Absolute liver weights were increased significantly by TCDD exposure in both C57 and DBA mice, and because of the concomitant decrease in body weights, the relative liver/body weight ratio also increased significantly ($p < 0.05$). Thymus weight decreased markedly with dose in both strains as previously reported (Poland and Glover, 1980). The epididymal fat pads were larger in the DBA than in C57 mice, but both decreased with increasing dose. Only in the DBA mice did the epididymal fat pads/

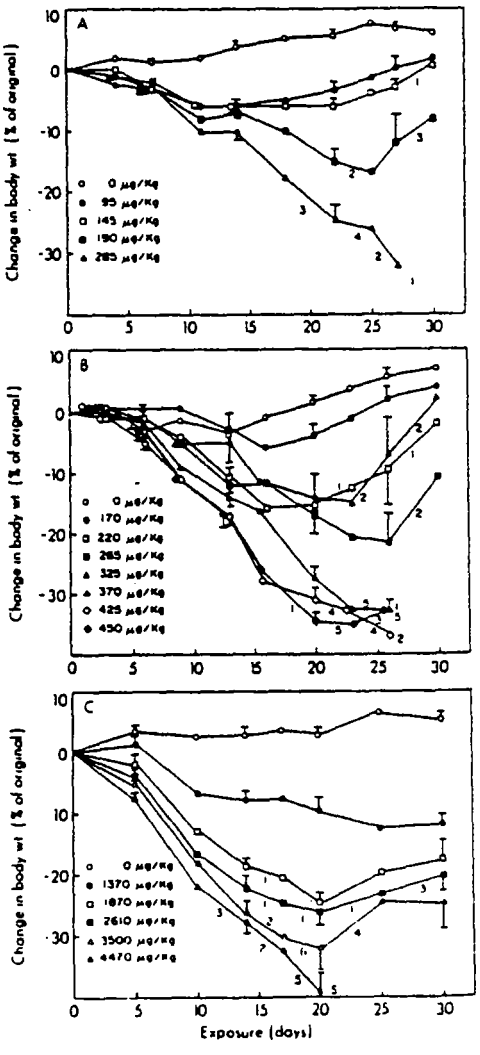

FIG. 2. Change in body weight with time in three strains of mice after exposure to a single po dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The small numbers are the number of mice that died each day. Values are $\bar{x}$ ± SE, N = 10 to 15 mice per dose. Doses are indicated in each panel: (A) C57, (B) B6D, and (C) DBA. See Methods for further details.

TABLE 1

ABSOLUTE AND RELATIVE ORGAN WEIGHTS IN C57 AND DBA MICE SURVIVING 30 DAYS IN THE LD50 STUDY[a]

Dose (μg/kg)	Initial body weight (g)	Final body weight (g)	Liver	Thymus	Epididymal fat pads
C57					
0 (5)[b]	25.8 ± 0.5	27.8 ± 0.7	1.33 ± 0.06[c] 4.96 ± 0.19[d]	0.046 ± 0.001 0.170 ± 0.009	0.36 ± 0.08 1.32 ± 0.27
95 (5)	26.3 ± 0.7	26.6 ± 0.52	1.52 ± 0.04* 5.65 ± 0.10*	0.016 ± 0.001* 0.060 ± 0.006*	0.32 ± 0.01 1.18 ± 0.36
145 (5)	25.9 ± 0.7	24.5 ± 1.5	1.70 ± 0.11* 6.56 ± 0.32*	0.011 ± 0.001* 0.042 ± 0.005*	0.30 ± 0.04 1.13 ± 0.11
190 (5)	26.1 ± 0.3	24.5 ± 1.5	1.62 ± 0.11* 7.03 ± 0.28*	0.008 ± 0.005* 0.014 ± 0.004*	0.27 ± 0.06 0.96 ± 0.17
DBA					
0 (5)	27.9 ± 0.8	28.7 ± 0.6	1.52 ± 0.10 5.27 ± 0.33	0.029 ± 0.001 0.100 ± 0.004	0.54 ± 0.06 1.86 ± 0.19
1370 (5)	27.6 ± 0.6	24.4 ± 0.8*	1.94 ± 0.09* 8.08 ± 0.28*	0.005 ± 0.001* 0.021 ± 0.002*	0.22 ± 0.14* 0.89 ± 0.15*
1870 (5)	28.2 ± 0.8	23.3 ± 1.5[e]	1.57 ± 0.13 7.21 ± 0.22*	0.007 ± 0.001* 0.033 ± 0.005*	0.19 ± 0.06* 0.82 ± 0.21*
2610 (4)	28.9 ± 0.7	23.3 ± 0.8*	1.80 ± 0.09* 7.72 ± 0.22*	0.007 ± 0.001* 0.030 ± 0.002*	0.14 ± 0.04* 0.58 ± 0.18*

[a] Data are from the 30-day LD50 study and represent those groups with at least 4 surviving animals. See Fig. 2 and Methods.
[b] Numbers in parentheses are the number of animals examined at each dose.
[c] Absolute organ weight in grams, expressed as $\bar{x} \pm$ SE.
[d] Relative organ weight (organ weight in g/body weight in g) ×100 expressed as $\bar{x} \pm$ SE.
[e] * Significantly different from control values of same strain (0 μg TCDD/kg) at $p < 0.05$.

body weight ratio decrease with dose. Other organ weights, including kidneys, spleen, heart, testes, adrenals, lungs, and intestines, were measured but showed no change in relative weights (data not shown).

Time Courses of Effects of TCDD Exposure on Glucose and Lipid Parameters in C57 Mice

The effects of TCDD (200 μg TCDD/kg) on serum glucose, total cholesterol, triglyceride concentrations, and hepatic triglyceride content were initially examined in C57 mice (Table 2). Serum glucose and triglyceride concentrations were significantly ($p < 0.05$) decreased after 4 days and remained at a reduced concentration for the remaining 21 days postexposure. Maximal reductions of 54 and 70% in serum glucose and triglyceride concentrations were observed. Serum total cholesterol concentration was significantly ($p < 0.05$) decreased within 8 days, and then increased 17 and 21 days postexposure to 60% above control values. Hepatic triglyceride content was significantly ($p < 0.05$) increased 4 days after TCDD exposure and reached maximal values (720% of control) after 21 days postexposure.

Dose–Response Effects of TCDD on Glucose and Lipid Parameters of C57 and DBA Mice

The dose–response effects of TCDD on glucose and lipid parameters of C57 and

TABLE 2

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON GLUCOSE AND LIPID PARAMETERS IN C57 MICE[a]

		Serum			
Day	Body weight (g)	Glucose (mmol/l)	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	Hepatic triglycerides (μmol/g)
0	25.7 ± 0.3 (72)	10.81 ± 0.16 (6)	3.01 ± 0.07 (5)	2.03 ± 0.19 (5)	6.38 ± 0.52 (6)
4	25.8 ± 1.3 (12)	8.80 ± 0.47 (6)*	2.53 ± 0.39 (4)	1.14 ± 0.14 (5)*	18.0 ± 1.09 (6)*
8	24.2 ± 1.4 (12)*	7.00 ± 0.22 (6)*	1.55 ± 0.13 (4)*	0.69 ± 0.08 (5)*	46.1 ± 1.89 (6)*
12	23.4 ± 1.1 (12)*	5.26 ± 0.39 (5)*	2.27 ± 0.40 (4)	0.66 ± 0.14 (5)*	48.8 ± 1.24 (6)*
17	17.7 ± 0.9 (12)*	4.93 ± 0.63 (5)*	4.80 ± 0.50 (4)*	0.70 ± 0.11 (4)*	46.3 ± 3.43 (6)*
21	18.7 ± 1.0 (12)*	4.99 ± 0.34 (5)*	4.13 ± 0.42 (4)*	0.65 ± 0.10 (5)*	54.2 ± 3.02 (6)*

[a] C57 mice were treated with a single po dose of 200 μg TCDD/kg and killed at various times postexposure. All animals were fasted overnight before being killed. Values are $\bar{x} \pm$ SE, with the number of separate samples given in parentheses. Each sample consisted of combined serum or liver from 2 animals. Number of separate samples less than six were due to insufficient serum to complete all assays.

* Significantly different from controls (Day 0), $p < 0.05$.

DBA mice were compared 1 week postexposure. Figures 3 and 4 present serum concentrations of glucose, glycerol, NEFA, total cholesterol, and esterified cholesterol, and Fig. 5 presents serum triglyceride concentrations and hepatic triglyceride content in C57 and DBA mice. The dose–response effects of TCDD were evaluated in the two strains of mice over a dose range that extended two orders of magnitude below the respective 30-day LD50 values. Serum glucose (Fig. 3), total cholesterol (Fig. 4), esterified cholesterol (Fig. 4), and triglyceride (Fig. 5) concentrations decreased in a dose-related manner ($p < 0.05$). Hepatic triglyceride content (Fig. 5) increased in a dose-related manner ($p < 0.05$). The changes in glucose and lipid parameters, at TCDD doses of equivalent lethality, were similar in both C57 and DBA mice. For example, serum glucose concentrations (Fig. 3) were reduced 29% by doses of 200 μg TCDD/kg and 2500 μg TCDD/kg in C57 and DBA mice. Serum glycerol and NEFA concentrations did not exhibit significant dose-related effects ($p > 0.05$) of TCDD exposure (Fig. 3). Serum glucose and lipid concentrations and hepatic triglyceride content in untreated C57 and DBA mice were comparable to previously reported values (Salvador *et al.*, 1970; Cuendet *et al.*, 1975; Menahan and Sobocinski, 1983).

Effects of TCDD on Feed Consumption of C57 Mice

The contribution of altered feed consumption to the observed changes in intermediary metabolism of TCDD-treated C57 mice was also evaluated. C57 mice exhibited a progressive loss of body weight beginning 4 to 6 days postexposure (Fig. 6). However, cumulative feed removed (Fig. 7A), the amount of feed removed from the feeder and not corrected for feed spillage, increased after exposure to 100, 200, and 400 μg TCDD/kg. A similar increase in feed removed, not corrected for feed spillage, by B6D mice was observed after TCDD exposure (data not shown). Cumulative feed consumed (Fig. 7B), cumulative feed removed corrected for feed spillage, was not affected by TCDD until C57 mice exposed to lethal doses of TCDD (200 and 400 μg TCDD/kg) became listless and moribund, shortly before death. The

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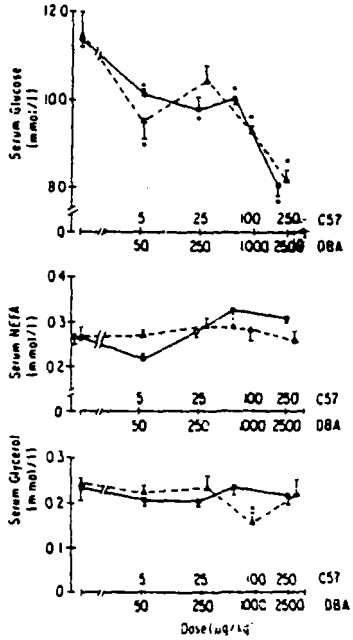

FIG. 3. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on serum glucose, NEFA, and glycerol of C57 and DBA mice. C57 (O) and DBA (Δ) mice were given a single po dose of the appropriate concentration of TCDD in 0.3 ml corn oil 1 week before termination. Control animals received corn oil only. Values are $\bar{x} \pm$ SE of three separate samples, except serum glucose which was obtained from six separate samples. Each sample consisted of pooled serum from 3 to 4 animals. Values differ significantly from controls (0 µg TCDD/kg), * = $p < 0.05$.

increased feed spillage, represented by the difference between Figs. 7A and B, was first observed 8 to 10 days postexposure. At 4 days postexposure, daily feed spillage had increased in C57 mice exposed to 200 µg TCDD/kg four- to sixfold over control values and accounted for 79% of feed removed each day. Comparable dose-related increases in feed spillage have been reported for rats exposed to TCDD (Seefeld *et al.*, 1984).

Fecal material from C57 mice was separated from the spilled feed and weighed throughout the feed consumption study. The weight, in grams, of the fecal material collected per gram of feed consumed during each 2- to 3-day interval (calculated from Fig. 7) was 0.22 ± 0.01 g/g ($\bar{x} \pm$ SE) for control C57 mice (0 µg TCDD/kg). The ratio of grams of feces per gram of feed consumed was not significantly ($p > 0.05$) altered by TCDD treatment at doses of 50, 100, 200, and 400 µg TCDD/kg (respectively 0.23 ± 0.01, 0.24 ± 0.01, 0.23 ± 0.01, and 0.24 ± 0.02 g/g).

Effects of Fasting on Glucose and Lipid Parameters in C57 Mice

To compare the effects of TCDD and fasting on glucose and lipid parameters, C57 mice were fasted for 0 to 96 hr and concentrations of serum glucose, triglyceride, and total cholesterol, and hepatic triglyceride content measured (Table 3). No deaths were observed in C57 mice until after 5 to 6 days of fasting and a 28% weight loss, which is similar to the weight loss observed in C57 mice exposed to a lethal dose of TCDD (see above). A 96-hr fast reduced serum glucose and triglyceride concentrations 30 and 70%,

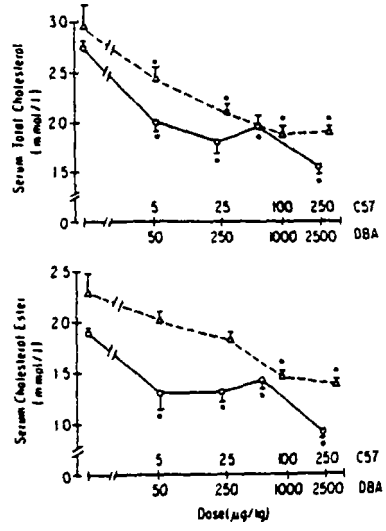

FIG. 4. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on serum total and esterified cholesterol of C57 and DBA mice. C57 (O) and DBA (Δ) mice were treated as described in Fig. 3.

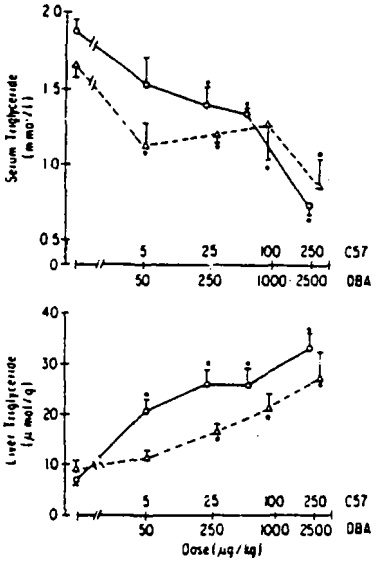

FIG. 5. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on serum and liver triglyceride of C57 and DBA mice. C57 (O) and DBA (Δ) mice were treated as described in Fig. 3.

respectively (Table 3). Serum total cholesterol concentration was increased 27% by a 24-hr fast, and then decreased to near unfasted

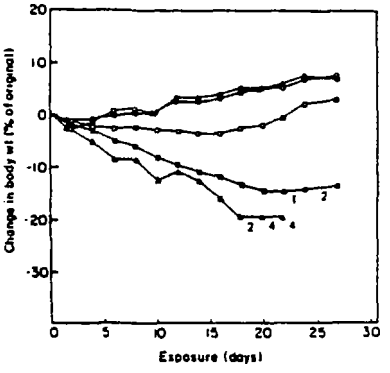

FIG. 6. Body weight change in C57 mice receiving a single po dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Mice, 10 animals in each group, were given a single po dose of 0 (O), 50 (●), 100 (□), 200 (■), or 400 (Δ) μg TCDD/kg in 0.3 ml corn oil. The animals were weighed every 2 to 3 days for 27 days postexposure. Values are $\bar{x} \pm$ SE. The small numbers are the number of animals that died during each interval.

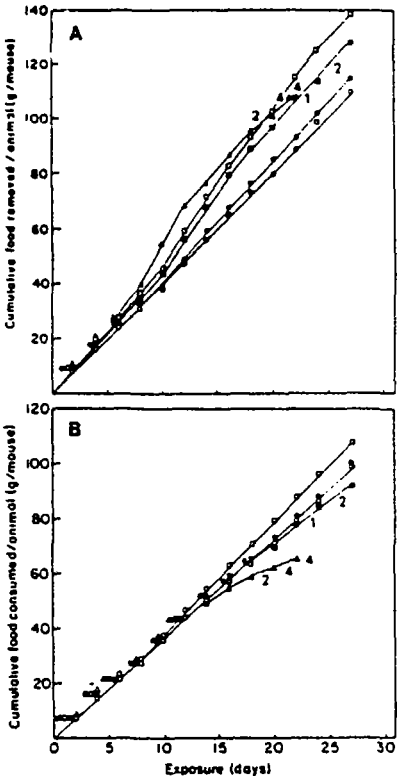

FIG. 7. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on cumulative food removed (A) and cumulative food consumed (B) by C57 mice. Mice, 10 animals in each group, were given a single po dose of 0 (O), 50 (●), 100 (□), 200 (■), or 400 (Δ) μg TCDD/kg in 0.3 ml corn oil. The animals were housed 5 per cage and each point represents the mean cumulative feed removed or consumed per animal within the two cages. Cumulative feed removed represents the total amount of feed removed from the feeder. Cumulative feed consumed represents the amount of feed removed from the feeder adjusted for the amount of feed spilled as described under Methods. The small numbers are the number of animals that died during each interval.

concentrations with prolonged fasting. Hepatic triglyceride content, which increased 115% after a 48-hr fast, also decreased when fasting was prolonged. Comparable changes in plasma glucose and triglyceride concentrations and in hepatic triglyceride content in fasted mice have been reported (Menahan and Sobocinski, 1983).

TABLE 3

EFFECT OF STARVATION ON GLUCOSE AND LIPID PARAMETERS IN C57 MICE[a]

Days	Body weight (g)	Serum			Hepatic triglycerides (μmol/g)
		Glucose (mmol/l)	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	
0	25.2 ± 0.4 (6)	12.03 ± 0.22 (6)	2.54 ± 0.13 (5)	1.82 ± 0.14 (5)	6.81 ± 0.49 (6)
1	23.0 ± 1.5 (12)*	11.36 ± 0.28 (6)	3.22 ± 0.14 (4)*	1.14 ± 0.08 (5)*	8.71 ± 0.52 (6)*
2	22.3 ± 1.8 (12)*	10.68 ± 0.05 (6)*	3.18 ± 0.08 (5)*	1.33 ± 0.05 (5)*	14.6 ± 1.54 (6)*
3	21.2 ± 1.7 (12)*	8.37 ± 0.20 (6)*	3.06 ± 0.07 (5)*	0.90 ± 0.13 (5)*	9.50 ± 0.39 (6)*
4	21.1 ± 1.1 (12)*	8.51 ± 0.30 (6)*	2.88 ± 0.08 (5)	0.63 ± 0.12 (5)*	10.3 ± 0.65 (6)*

[a] C57 mice were fasted for the appropriate period of time before being killed: controls (Day 0) were not fasted before termination. Values are $\bar{x}$ ± SE. with the numbers of separate samples given in parentheses. Each sample consisted of combined serum or liver from 2 animals. Number of separate samples less than six were due to insufficient serum to complete all assays.

* Significantly different from controls (Day 0), $p < 0.05$.

DISCUSSION

The differences in sensitivity of these strains of adult male mice toward TCDD is clearly established. The oral 30-day LD50 values for C57, B6D, and DBA mice are, respectively, 182, 296, and 2570 μg TCDD/kg (Figs. 1 and 2). This difference in sensitivity to TCDD was correlated positively to changes in glucose and lipid parameters measured in serum and liver. The changes in these parameters at doses of equivalent lethality given to C57 and DBA mice are of comparable magnitude. No qualitative differences in the dose responses of glucose and lipid parameters to TCDD were observed. However, the difference in sensitivity of C57 and DBA mice to TCDD was similar for both TCDD-induced lethality and altered intermediary metabolism. The 10-fold differences in dose–response of glucose and lipid parameters (Figs. 3–5) are comparable to the 14-fold difference in LD50 values (Fig. 1) for C57 and DBA mice. Parallel dose–percentage mortality responses and parallel dose–response effects on glucose and lipid parameters in C57 and DBA mice suggest a similar mechanism(s) of lethality and a common lesion(s) in intermediary metabolism. Further studies are necessary to determine if these alterations in intermediary metabolism contribute directly to the lethality of TCDD in C57 and DBA mice. However, these results support the hypothesis that differences in sensitivity of C57 and DBA mice to TCDD result from the influence of a genetic factor(s) which controls the magnitude of a common injury. It has been suggested previously that segregation of thymic involution and probably teratogenesis with the *Ah* locus are indicative of TCDD binding to the receptor as an essential step in the mechanism of TCDD toxicity (Poland and Glover, 1980).

Several recent studies have suggested that hypophagia (Seefeld *et al.*, 1984) or reduced feed consumption (Gasiewicz *et al.*, 1980) contribute to weight loss and toxicity in TCDD-treated rats. In the present study, hypophagia was not observed in TCDD-treated mice (Fig. 7), despite significant decreases in body weight (Fig. 6). We compared C57 mice given 200 μg TCDD/kg body wt which decreases cumulative feed consumed by 5% after 21 days (Fig. 7) to C57 mice fasted 0 to 4 days (Table 3). Our results indicated that TCDD exposure (Table 2) and fasting (Table 3) produced significantly different effects on glucose and lipid parameters

despite similar weight losses of 25 and 18% for TCDD-treated and fasted mice, respectively. In particular, hepatic triglyceride content and serum glucose concentration are affected to a much greater extent by TCDD exposure than fasting. The two treatments also produce opposite effects on serum total cholesterol concentrations. Therefore, we suggest that factors other than hypophagia or reduced feed consumption contribute significantly to the changes in intermediary metabolism and body weight loss in TCDD-treated mice.

The factor(s) responsible for hypoglycemia and altered lipid metabolism in TCDD-treated mice is (are) unknown. TCDD may produce sufficient hepatic injury to block the release of cholesterol and triglyceride-rich heptic lipoproteins. Liver injury has been reported in mice exposed to TCDD (Jones and Grieg, 1975) and a number of agents that produce hepatic injury result in the accumulation of hepatic triglyceride and decrease of serum lipids (Plaa, 1975). Alternatively, the concomitant increase in hepatic triglyceride and decrease in serum glucose in TCDD-treated mice suggests TCDD exposure may alter the normal interactions between carbohydrate and lipid metabolism. Accumulation of hepatic triglyceride represents an altered metabolic fate for gluconeogenic precursors, e.g., glycerol, and sources of metabolic energy and reducing equivalents, e.g., fatty acids. The absence of TCDD-induced hypophagia in our study suggests that loss of body weight in mice exposed to TCDD represents an increased requirement for metabolic energy. Previous studies have reported no effect of TCDD on mitochondrial respiratory control ratios or ADP/O ratios in rat hepatic mitochondria (Courtney *et al.*, 1978). Therefore, investigations into the effects of TCDD on intermediary metabolism, and specifically the interaction between carbohydrate and lipid metabolism, are crucial to understanding the metabolic basis for body weight loss and mortality in TCDD-exposed animals.

REFERENCES

BAILEY, N. J. R. (1959). *Statistical Methods in Biology*. English Universities Press, London.

BERGMEYER, H. U., BERNT, E., SCHMIDT, F., AND STORK, H. (1974). D-Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis* (H. V. Bergmeyer, ed.), Vol. 4, pp. 1196–1201. Verlag Chemie, Deerfield, Fla.

CROUTHAMEL, C. E. (1975). *Applied Gamma-Ray Spectrometry*. Pergamon, New York.

COURTNEY, K. D., PUTNAM, J. P., AND ANDREWS, J. E. (1978). Metabolic studies with TCDD (dioxin)-treated rats. *Arch. Environ. Contam. Toxicol.* 7, 385–396.

CUENDET, G. S., LOTEN, E. G., CAMERON, D. P., RENOLD, A. E., AND MARLISS, E. B. (1975). Hormone substrate responses to total fasting in lean and obese mice. *Amer. J. Physiol.* 228, 276–283.

EGGSTEIN, M., AND KUHLMANN, E. (1974). Triglycerides and glycerol. In *Methods of Enzymatic Analysis* (H. V. Bergmeyer, ed.), Vol. 4, pp. 1825–1831. Verlag Chemie, Deerfield, Fla.

FINNEY, D. J. (1971). *Statistical Methods in Biological Assay*. 2nd ed., London Griffin Press, London.

GASIEWICZ, T. A., HOLSCHER, M. A., AND NEAL, R. A. (1980). The effect of total parenteral nutrition in the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Toxicol. Appl. Pharmacol.* 54, 469–488.

GASIEWICZ, T. A., AND NEAL, R. A. (1979). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin tissue distribution, excretion, and effects on clinical parameters in guinea pigs. *Toxicol. Appl. Pharmacol.* 51, 329–339.

HAY, A. (1982). *The Chemical Scythe: Lessons of 2,4,5-T and Dioxin*. Plenum, New York.

HENCK, J. M., NEW, M. A., KOCIBA, R. J., AND RAO, K. S. (1981). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Acute oral toxicity in hamsters. *Toxicol. Appl. Pharmacol.* 59, 405–407.

JONES, G., AND GRIEG, J. B. (1975). Pathological changes in the livers of mice given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Experientia* 31, 1315–1317.

MAHADEVAN, S., DILLARD, D. J., AND TAPPEL, A. L. (1969). A modified colorimetric micromethod for long-chain fatty acids and its aplication for assay of lipolytic enzymes. *Anal. Biochem.* 27, 387–396.

MATSUMURA, F. (1983). Biochemical aspects of action mechanisms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related chemicals in animals. *Pharmacol Ther.* 19, 195–209.

MCCONNELL, E. E., MOORE, J. A., HASEMAN, J. K., AND HARRIS, M. W. (1978). The comparative toxicity of chlorinated dibenzo-*p*-dioxin in mice and guinea pigs. *Toxicol. Appl. Pharmacol.* 44, 335–356.

MENAHAN, L. A., AND SOBOCINSKI, K. A. (1983). Comparison of carbohydrate and lipid metabolism in mice

and rats during fasting. *Comp. Biochem. Physiol. B* 74, 859–864.

NEBERT, D. W. (1981). Genetic differences in susceptibility to chemically induced myelotoxicity and leukemia. *Environ. Health Perspect.* 39, 11–22.

OLIVER, R. M. (1975). Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory workers. *Brit. J. Ind. Med.* 32, 49–53.

OLSON, J. R., HOLSCHER, M. A., AND NEAL, R. A. (1980). Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Golden Syrian hamster. *Toxicol. Appl. Pharmacol.* 55, 67–78.

PLAA, G. L. (1975). Toxicology of the liver. In *Toxicology. The Basic Science of Poisons* (L. J. Cassarett and J. Doull, eds.), pp. 170. Macmillan Co., New York.

POLAND, A., AND GLOVER, E. (1980). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Segregation of toxicity with the *Ah* locus. *Mol. Pharmacol.* 17, 86–94.

REGGIANI, C. (1978). Medical problems raised by the TCDD contamination in Seveso, Italy. *Arch. Toxicol.* 40, 161–168.

RÖSCHLAU, P., BERNT, E., AND GRUBER, W. (1974). Cholesterol and esterified cholesterol. In *Methods of Enzymatic Analysis* (H. V. Bergmeyer, ed.), Vol. 4, pp. 1890–1893. Verlag Chemie, Deerfield, Fla.

SALVADOR, R. A., ATKINS, C., HABER, S., AND CONNEY, A. H. (1970). Changes in the serum concentration of cholesterol, triglycerides and phospholipids in the mouse and rat after administration of either chlorcyclizine or phenobarbital. *Biochem. Pharmacol.* 19, 1463–1470.

SCHILLER, C. M., WALDEN, R., CHAPMAN, D. E., AND SHOAF, C. R. (1984). Metabolic impairment associated with a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adult male Fischer rats. *Ranbury Report* 18, 319–331.

SCHILLER, C. M., WALDEN, R., AND SHOAF, C. R. (1982). Studies on the mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: Nutrient assimilation. *Fed. Proc.* 41(4), 1426.

SCHWETZ, B. A., NORRIS, J. M., SPARSCHU, G. L., ROWE, V. K., GEHRING, P. J., EMERSON, J. L., AND GERBIG, C. G. (1973). Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect.* 5, 87–99.

SEEFELD, M. D., CORBETT, S. W., KEESEY, R. E., AND PETERSON, R. E. (1984). Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 73, 311–322.

VOS, J. G., MOORE, J. A., AND ZIMBL, J. G. (1974). Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J mice. *Toxicol. Appl. Pharmacol.* 29, 229–241.

WALKER, A. L., AND MARTIN, J. V. (1979). Lipid profiles in dioxin-exposed workers. *Lancet* 1, 446.

K-66681-(00) PV
abstract

328

DOW215821

PROFILE ID 11467T QUERY NUMBER 001
CA96(21):179616N Journal CASCT CA117005 CCR 104

The use of epidemiology in the regulation of dioxins in the food supply. Cordle, Frank (Bur. Foods, FDA, Washington, DC 20204 USA). Regul. Toxicol. Pharmacol. [RTOPDW] 1981, 1(3-4), 379-87 (Eng).

Residues of tetrachlorodibenzo-p-dioxin (TCDD) [41903-57-5] were detected recently in several species of freshwater fish from various areas of the Great Lakes. Concern for the potential human health risk assocd. with the consumption of these fish prompted the Food and Drug Administration to assess epidemiol. and toxicol. TCDD data in order to det. the need for regulatory action. In the assessment, pertinent animal and human data and data for the TCDD residues in the freshwater fish, the amts. of the different fish species consumed, and the no. of individuals consuming the fish were evaluated. The various methods of evaluation used to reach a regulatory decision are described.

fish TCDD
dioxin fish food regulation

18509

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Abstracts

240 QUANTITATION OF TCDD IN ANIMAL AND HUMAN TISSUES BY RADIO IMMUNOASSAY, Luster, M.I., Albro, P.W., Chae, K. and McKinney, J.D., Laboratory of Environmental Chemistry, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709. Sponsor: J.A. Goldstein

Double-antibody radioimmunoassays (RIAs) have been developed for quantitating a number of chlorinated aromatic hydrocarbons of current concern including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Antisera against the amino-derivatives of 3,7,8-TriCDD were produced in rabbits by conjugating compounds to proteins using the mixed anhydride method. Extensive characterization studies indicated the antisera were, in general, fairly specific to the non-aminated analogue and cross-reacted extensively with 2,3,7,8-TCDD. The limit of detection is approximately 25pg of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Assuming an appropriate cleanup procedure is used, chlorinated dibenzofurans are the only likely interferences and these can be distinguished through the use of the two antisera of different dibenzofuran/dibenzodioxin selectivities. A feature of the assay method is the use of nonionic detergents (Cutscum or Triton X-305). To test and validate the applicability of this assay to tissue samples, we examined liver and adipose tissues from monkeys that had been orally exposed to TCDD. TCDD levels obtained in monkey tissues by RIA analysis correlated well with gas chromatography with negative ion mass spectrometry and gas chromatography with electron capture detector. Human adipose tissue spiked with various levels of TCDD ranging from 100pg to 2.5ng per 100mg of fat were also analyzed by RIA. In a double blind study involving over 130 samples, well over 90% of the samples were accurately determined. Thus, the RIA is applicable for screening samples in order to minimize the demand for mass spectrometric screening and for routine monitoring for exposure to known chlorinated dibenzo-p-dioxins in potentially contaminated environments.

K-6c .01-(00)PV
abstract

334

DOW215020A

PROFILE ID 11467T QUERY NUMBER 001
CA96(23):194471E Journal CASCT CA104000

Epidemiological problems with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (A critical view). Tognoni, G.; Bonaccorsi, A. (Ist. Ric. Farmacol. "Mario Negri", 62-20157 Milan, Italy). Drug Metab. Rev. [DMTRAR] 1982, 13(3), 447-69 (Eng).

A discussion with 35 refs. on the epidemiol. problems assocd. with the toxicity of TCDD (I) [1746-01-6].

(This abstract has a structure in the CA issue.)

review TCDD toxicity Seveso epidemiol

18502

K-66. - (100)pV
ABSTRACTS

463 INDUCTION OF 7-ETHOXYCOUMARIN O-DEETHYLASE ACTIVITY IN HUMAN EPITHELIAL CELLS IN CULTURE: EVIDENCE FOR DIOXIN RECEPTOR. L.G. Hudson and W.P. Greenlee, Lab. of Toxicol., Harvard Sch. Pub. Hlth., Boston, MA 02115. Sponsor: John G. Dent

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of cytochrome P_1-450-mediated monooxygenase activities, including aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin O-deethylase (ECOD). In certain inbred strains of mice this response is regulated by a single genetic locus coding for a cytosolic receptor protein. In the present study the induction of ECOD activity by TCDD and several halogenated analogues was examined in 5 human cell lines derived from squamous cell carcinomas (SCC) of epidermal and oral epithelia. The cells were grown in the presence of a lethally irradiated feeder layer of mouse 3T3 cells in Dulbecco-Vogt modified Eagle's medium supplemented with 5% fetal calf serum. Addition of varying concentrations of TCDD (10^{-11} - 10^{-7} M) to confluent cultures of SCC 4, 9, 13 and 15 cells resulted in a dose-dependent increase in ECOD activity with an ED_{50} ranging from 1 to 3 nM. Near maximal induction was observed within 24 h. Under the same culture conditions, addition of TCDD to SCC 12F2 cells at concentrations up to 1 μM produced no significant increase in ECOD activity. The structure-activity relationship for the induction of ECOD activity in the four responsive lines (SCC 4, 9, 13 and 15) showed the same stereospecificity required for binding to the TCDD receptor in murine liver. Using sucrose density gradient analysis, specific binding of radiolabeled TCDD was detected in the cytosol fractions from the responsive cell lines, whereas no specific binding could be detected in the SCC 12F2 cells. These data indicate the presence of a putative receptor for TCDD in cultured human epithelial cells that appears to regulate at least one inducible enzyme activity.

The Toxicologist Vol. 2 No. 1 Jan. '82

K-66681-(100) abstracts pV

516 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON CULTURED HUMAN EPITHELIAL TARGET CELLS: MODULATION BY HYDROCORTISONE. R.H. Rice and W.F. Greenlee, Lab. of Toxicol., Harvard Sch. Pub. Hlth., Boston, MA 02115. Sponsor: J.L. Whittenberger

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an extraordinarily potent toxin, teratogen and carcinogen. In exposed humans, chloracne, a skin disorder characterized by altered patterns of growth and differentiation in the epidermis and its appendages, is one of the most common clinical findings. In initial studies of the molecular mechanisms of toxicity, several human cell lines derived from squamous cell carcinomas (SCC) of epidermal and oral epithelia have been surveyed for their response to TCDD. The cells were grown in the presence of a lethally irradiated feeder layer of mouse 3T3 cells in Dulbecco-Vogt modified Eagle's medium supplemented with 5% fetal calf serum. Under conditions of clonal growth with continuous exposure to various concentrations of TCDD (10^{-11} to 10^{-7} M) for 3 wks, colony expansion in the lines SCC 9, 12F2, 12B2 and 13 was inhibited. The ED_{50} for this response was approximately 0.1 nM, with maximal inhibition at 10 nM. Similar values were obtained for the induction of cytochrome P_1-450 mediated monooxygenase activities in these cells. SCC 4 cells, a line exhibiting the least differentiated character, were not inhibited in colony expansion by TCDD. Addition of hydrocortisone (0.4 µg/ml) antagonized the inhibitory effect of TCDD with an accompanying shift in the dose-response curves. In two lines (SCC 9 and SCC 13) a dose-related stimulatory response to TCDD was noted in the presence of hydrocortisone. We conclude that the response to TCDD depends on both the cell line and the presence of hydrocortisone in the culture medium. The dose-response parameters suggest that the observed effects are mediated by the TCDD receptor.

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K-66681-(100) abstracts 9

515 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON RNA POLYMERASE I INDUCTION IN RAT LIVER. C.L. Potter, I.G. Sipes and D.H. Russell, Toxicol. Prog. and Depts. of Pharmacol. and Anesthesiol., Univ. of Arizona, Tucson, AZ 85724.

Previously, we have reported that pretreatment of rats with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits the extent of hepatic induction of ornithine decarboxylase, the rate limiting enzyme in biosynthesis of the polyamines (putrescine, spermidine and spermine). Since increased hepatic ornithine decarboxylase activity has been positively correlated to a subsequent increase in RNA polymerase I activity, we examined the effects of TCDD on RNA polymerase I induction. Male Sprague-Dawley rats (100-150 g) received a single i.p. injection of 5 µg/kg TCDD in acetone:corn oil (1:17). Controls received vehicle only. Hepatic RNA polymerase I activity was subsequently stimulated 25-50% by partial hepatectomy or by administration of dexamethasone (0.5 mg/kg) in normal saline. At 5 hr or 16 hr after partial hepatectomy, or 4 hr after dexamethasone, times at which TCDD has been shown to strongly inhibit ornithine decarboxylase induction, animals pretreated one week earlier with TCDD exhibited RNA polymerase I activity 20-50% lower than controls; i.e. TCDD-treated rats exhibited 0-50% as much stimulation of RNA polymerase I activity as did the controls. In unstimulated liver, RNA polymerase I activity, as well as protein DNA, RNA and spermine levels, remained essentially the same in control and TCDD-treated rats one week after treatment. However, TCDD-treated rats had significantly lower putrescine and spermidine concentrations. These results indicate that TCDD pretreatment may prevent a normal growth-stimulated expression of ornithine decarboxylase and RNA polymerase I activities. (Supported by CA-14783 and 5-T32-ES07091.)

The Toxicologist Vol. 2 No. 1 Jan 1982.

K-66681-(100) Abstracts PV

461 COMPARISON OF BODY WEIGHT LOSS AND OXYGEN CONSUMPTION IN RATS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) AND PAIR-FED CONTROL RATS. M. D. Seefeld, S. W. Corbett, R. E. Keesey and R. E. Peterson, Univ. of Wisconsin, Madison, WI.

Male rats were given a single oral dose of TCDD (15, 25 or 50 ug/kg) and paired mates, matched on a body weight basis, received an equivalent volume of acetone/corn oil. Daily food intakes were corrected for spilled food since TCDD treatment caused a progressive dose-related increase in spillage. Failure to account for spillage results in overestimating the amount of food consumed by TCDD-treated rats with consequent overfeeding of pair-fed controls. Young rats (80-100 g) given 25 ug TCDD/kg displayed an immediate reduction in food intake and weight gain which persisted over the 35 day post-treatment period, and 65% of the rats in this group died. Pair-fed controls exhibited an identical weight response but only 13% of the rats died. Experiments using adult rats (275-300 g) demonstrated that pair-fed controls display identical weight loss during the first 10 days after treatment as rats given 25 and 50 ug TCDD/kg. After day 10, pair-fed rats exhibited a greater ability to maintain their body weight on the reduced food intake than TCDD-treated rats. In the 25 and 50 ug/kg dosage groups, 33 and 75% of the rats died respectively, whereas in the respective pair-fed groups 0 and 15% of the rats died. In rats housed in a thermoneutral environment, doses of 15 and 50 ug TCDD/kg depressed both total and resting oxygen consumption, however, oxygen consumption was similarly reduced in pair-fed controls. These studies demonstrate that reduced food intake is the primary cause of body weight loss and depressed oxygen consumption in TCDD-treated rats. (Supported by NIH Grant ES01332).

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The *in Vivo* Biotransformation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in the Rat[1]

The *in Vivo* Biotransformation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in the Rat. RAMSEY, J. C., HEFNER, J. G., KARBOWSKI, R. J., BRAUN, W. H., AND GEHRING, P. J. (1982). *Toxicol. Appl. Pharmacol.* 65, 180–184. This study presents evidence for the *in vivo* biotransformation of TCDD in the rat. Three male rats with indwelling bile loop cannulas were given repeated daily po doses of 15 μg [^{14}C]TCDD/kg body weight. After either two, four, or six doses, the total output of bile from one rat was collected for 24 hr following the last dose. Biliary ^{14}C activity was excreted at a rate similar to the excretion of ^{14}C activity in the feces of normal (noncannulated) rats given po doses of [^{14}C]TCDD. Therefore it is not likely that enterohepatic recycling plays a significant role in the retention of ^{14}C activity following a dose of [^{14}C]TCDD. High-pressure liquid chromatography of the bile from these rats showed the presence of at least eight radioactive peaks and very little, if any, unchanged TCDD. These metabolites were all more polar than TCDD, and the chromatographic profile was altered following incubation of the bile with β-glucuronidase. These data, in conjunction with previous studies, indicate that the metabolic transformation of TCDD in the liver may be the rate-limiting step in the elimination of TCDD from the body.

The toxicological properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have been intensively studied in recent years. A recent report of the results of a 2-year toxicity and oncogenicity study with TCDD in rats includes a concise review of the salient knowledge concerning TCDD toxicity (Kociba *et al.*, 1978). However, in spite of the deluge of literature concerning many aspects of TCDD toxicity in a variety of animal species, the mechanism of action of this highly toxic molecule has remained obscure.

Knowledge of the mechanism of toxic action of any chemical is helpful in predicting the relative sensitivity of various species and in extrapolating the results of toxicity studies to lower dose levels. An answer to the fundamental question of whether a chemical undergoes metabolic transformation within the body is necessarily one of the first steps in establishing a scientific framework within which the mechanism of action subsequently may be elucidated. The rat has been one of the species most studied concerning the effects of TCDD, and the pharmacokinetics of radiolabeled TCDD following both single and repeated oral doses have been well defined in rats (Piper *et al.*, 1973; Rose *et al.*, 1976). Most of the ^{14}C activity following an oral dose of [^{14}C]TCDD was excreted in the feces of rats in an apparent first order manner with half-life values ranging from approximately 17 to 31 days. However, these studies were not designed to identify the nature of the excreted radioactivity. Therefore, the purpose of this study was to determine whether TCDD is metabolized in the rat and, if so, to what extent.

METHODS

[^{14}C]TCDD. [^{14}C]2,3,7,8 - tetrachlorodibenzo - *p* - dioxin uniformly labeled in the ring was synthesized by Dr. A. S. Kende of the University of Rochester (Kende and Wade, 1973). The [^{14}C]TCDD had a specific activity of 148 mCi/mmol (1.02×10^6 dpm/μg). The radiochemical purity was greater than 98% as determined by high-pressure liquid chromatography with the HPLC system described in this section. This radiochemical purity was further verified by thin-layer chromatography on 0.25 mm silica gel plates[2] developed in acetone

[1] A preliminary report of this study was presented at the 18th Annual Meeting of the Society of Toxicology, New Orleans, La., March 11–15, 1979.

[2] Brinkman Instruments Inc., Westbury, N.Y.

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[a] Calculated as mi
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for the following 24 hr.
were immersed in dry ic
High-pressure liquid
system consisted of three
Model 1200 solvent prog

[3] Panax Equipment L
Kingdom.
[4] Paxton Processing Co
[5] Waters Assoc., Milfo

TABLE 1

^{14}C ACTIVITY IN BILE FROM RATS GIVEN REPEATED DAILY ORAL DOSES OF 15 μg [^{14}C]TCDD PER KILOGRAM

Rat No.	Total No. of doses	Bile collected (g)	μg eq. TCDD/g of bile[a]	Percentage of daily administered dose of ^{14}C activity excreted in bile for 24 hr	
				Observed	Predicted[b]
1	2	15.8	0.006	2.3	4.9
2	4	21.0	0.017	8.8	9.5
3	6	6.6[c]	0.024	3.8	13.9

[a] Calculated as microgram equivalents of [^{14}C]TCDD based on the specific activity of the administered TCDD.
[b] Predicted values based on the observed excretion rate of ^{14}C activity in feces of rats from previous studies (Rose *et al.*, 1976) and Eq. (1).
[c] The reason for the reduced quantity of bile collected from this rat is not known.

or in chloroform, then scanned for radioactivity with an RTLS-1A scanner.[3]

Animals. Male Sprague–Dawley rats weighing approximately 270 g were obtained from Spartan Research Animals, Inc. (Haslett, Mich.). At least 24 hr prior to dosing, the rats were surgically implanted with an indwelling cannula leading from the common bile duct through an externalized loop, back into the anterior portion of the duodenum. The rats were housed singly in plastic cages equipped with SAN-I-CELL[4] bedding, and allowed free access to Purina lab chow and water throughout the experiment. They were maintained in a room with a 12 hr light cycle, a temperature of 20 to 22°C, and a relative humidity of 40 to 60%.

Dosing and bile collection. The [^{14}C]TCDD was dissolved in a 10:1 corn oil:acetone solution at a concentration of 6.8 μg TCDD/ml. This dosing solution was orally administered to the rats with a syringe and feeding needle at a volume of 0.6 ml to achieve a dose level of approximately 15 μg TCDD/kg body weight. Three rats were dosed daily until one rat had received two doses, another four doses, and the last rat six doses. Immediately following the last dose to each rat, the external bile cannula loop was opened and the entire flow of bile from the common bile duct was collected for the following 24 hr. The bile collection containers were immersed in dry ice.

High-pressure liquid chromatography. The HPLC system consisted of three Model 6000A pumps and one Model 1200 solvent programmer.[5] The column was 3.9 mm i.d. by 30 cm μBondapak/C_{18}[5] operated at ambient temperature with a solvent flow rate of 1 ml/min. The solvent program consisted of 3% (v/v) methanol in water for 5 min, followed by a linear gradient to 100% methanol in 50 min, then a linear gradient from 100% methanol to 100% ethyl acetate in 15 min. Fractions were collected in scintillation vials at 1-min intervals, and ^{14}C activity was determined by liquid scintillation counting. The sample was applied to the column by lyophilizing a 2.5-ml aliquot of bile and reconstituting the residue in 200 μl of water. An average of approximately 100% of the ^{14}C activity applied to the HPLC column was recovered in the eluent fractions.

Enzymatic incubations. β-D-Glucuronide glucuronohydrolase (β-glucuronidase) and aryl sulfate sulfohydrolase (sulfatase) enzymes were obtained from Sigma Chemical Company.[6] An 8-ml sample of bile from rat No. 2 was lyophilized and then reconstituted in 300 μl of water. A 100-μl aliquot of this reconstituted bile was incubated with chloroform-activated β-glucuronidase at 37°C overnight, and another aliquot was incubated with sulfatase at 37°C for 7 hr. Phenolphthalein glucuronide and *p*-nitrocatechol sulfate, respectively, were used to confirm the activity of these two enzyme preparations in the presence of control rat bile. The enzymic digestion solutions were evaporated to a volume of approximately 200 μl under a stream of nitrogen before HPLC analysis as described above.

RESULTS AND DISCUSSION

The quantity of bile collected from each rat during the 24-hr period following the last

[3] Panax Equipment Ltd., Redhill, Surrey, United Kingdom.
[4] Paxton Processing Co., Paxton, Ill.
[5] Waters Assoc., Milford, Mass.
[6] St. Louis, Mo.

oral dose of [^{14}C]TCDD is shown in Table 1, as well as the concentration of ^{14}C activity expressed as microgram equivalents of [^{14}C]TCDD per gram of bile. Previous studies with [^{14}C]TCDD (Rose *et al.*, 1976) have shown that ^{14}C activity is excreted in the feces of rats following repeated po doses at an apparent first-order rate with a half-life of 23.7 days. The one-compartment open pharmacokinetic model with first-order excretion which characterizes the elimination of ^{14}C activity from rats repeatedly fed [^{14}C]TCDD predicts that the amount of the dose eliminated in the feces during one dose interval will be given by Eq. (1) where E_n is the amount eliminated following the nth dose.[7]

$$E_n = f \cdot D_0(1 - e^{-nkr}). \qquad (1)$$

Equation (1) was used to calculate the predicted percentage of the daily dose of ^{14}C excreted in the feces as shown in the last column of Table 1. The observed ^{14}C activity excreted in the bile (Table 1) was not greater than the quantity predicted in the feces. Therefore, if the fecal radioactivity is comprised mainly of biliary metabolites as suggested by the results reported here, it is unlikely that enterohepatic recycling plays a significant role in the retention of ^{14}C activity in the body following oral administration of [^{14}C]TCDD.

HPLC analysis of all three bile samples revealed numerous radioactive peaks, for which the retention volume did not correspond to the retention volume of authentic [^{14}C]TCDD. Although there were minor differences in the chromatographic patterns of the three bile samples, they exhibited a generally similar ^{14}C profile. Figure 1 shows a typical HPLC ^{14}C chromatogram of bile collected from the rat receiving four doses of [^{14}C]TCDD. At least eight radioactive

[7] D_0, administered dose; f, fraction of oral dose absorbed (0.861); n, number of doses; r, dose interval (1 day); k, first-order elimination rate constant (0.0293 day^{-1}) (from Rose *et al.*, 1976).

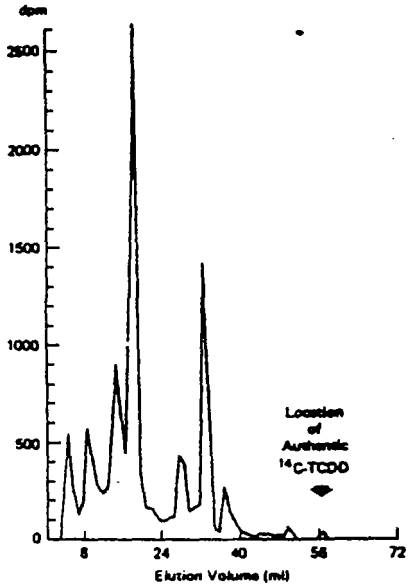
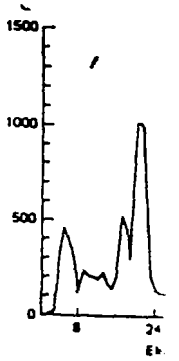


FIG. 1. HPLC chromatogram of bile obtained from a rat after four repeated daily doses of 15 μg [^{14}C]TCDD/kg.

peaks are apparent, all of which elute with a smaller retention volume than does TCDD on the reverse phase column, and are therefore more polar than TCDD itself. Little, if any, unchanged [^{14}C]TCDD was excreted in the bile. It is therefore apparent that TCDD is transformed to a variety of more polar metabolites before being excreted from the liver via the bile. Since virtually all of the liver radioactivity following administration of radiolabeled TCDD to mice, rats, and hamsters has been identified as unchanged TCDD (Vinopal and Casida, 1973; Rose *et al.*, 1976; Olson *et al.*, 1980), it seems likely that the rate-limiting step for the clearance of TCDD from the body may be its metabolic transformation in the liver; the resulting metabolites are then rapidly excreted in the bile.

The minute quantities of the various metabolites separated by the HPLC procedure precluded structural elucidation. However, the HPLC chromatographic pattern follow-



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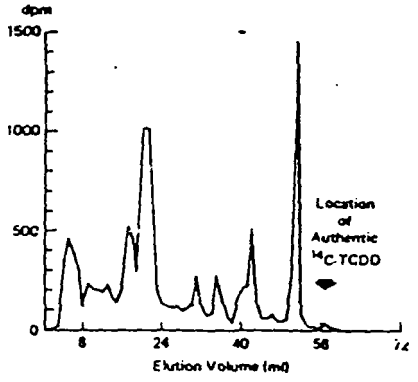

FIG. 2. HPLC chromatogram of the bile sample in Fig. 1 after incubation with β-glucuronidase.

ing incubation of the bile with β-glucuronidase (Fig. 2) implies that a portion of the biliary metabolites of [^{14}C]TCDD was excreted in the form of glucuronide conjugates. The change in the chromatographic pattern consisted of a general shift to higher retention volumes, indicating that less polar compounds were formed upon release from glucuronic acid. We infer that at least some of the biliary metabolites of TCDD may contain hydroxyl groups capable of reacting with glucuronic acid. Incubation with a sulfatase enzyme preparation did not appreciably change the chromatographic pattern.

Subsequent to the work reported here (Ramsey *et al.*, 1979), other investigators have confirmed not only that TCDD is extensively metabolized in rat liver, but also that some of the biliary metabolites are excreted in the form of glucuronide conjugates (Poiger and Schlatter, 1979; Olson *et al.*, 1981). The Syrian hamster also transforms radiolabeled TCDD to metabolites detectable in both bile and urine (Olson *et al.*, 1980).

The results reported here, coupled with the observations cited above, clearly indicate that TCDD is extensively metabolized in at least two mammalian species. In these same species, radioactivity residing in the liver following administration of radiolabeled TCDD has been shown to exist predominantly as the unchanged parent compound. Based on these observations, we tentatively conclude that the toxicity arising from TCDD may be due primarily to the parent compound rather than its metabolites, which are apparently rapidly excreted. This conclusion is in general agreement with those of Beatty *et al.* (1978) who observed an inverse relationship between hepatic MFO activity and the toxicity of TCDD in rats, and of Olson *et al.* (1981) who suggested a relationship between TCDD metabolism in isolated rat hepatocytes and hepatic MFO activity.

REFERENCES

BEATTY, P. W., VAUGHN, W. K., AND NEAL, R. A. (1978). Effect of alteration of rat hepatic mixed-function oxidase (MFO) activity on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol. Appl. Pharmacol.* 45, 513–519.

KENDE, A. S., AND WADE, J. J. (1973). Synthesis of new steric and electronic analogs of 2,3,7,8-tetrachloro-dibenzo-p-dioxin. *Environ. Health Perspect.*, Experimental Issue No. 5, 49–58.

KOCIBA, R. J., KEYES, D. G., BEYER, J. E., CARREON, R. M., WADE, C. E., DITTENBER, D. A., KALNINS, R. P., FRAUSON, L. E., PARK, C. N., BARNARD, S. D., HUMMEL, R. A., AND HUMISTON, C. G. (1978). Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. *Toxicol. Appl. Pharmacol.* 46, 279–303.

OLSON, J. R., GUDZINOWICZ, M., AND NEAL, R. A. (1981). The *in vitro* and *in vivo* metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. *Toxicologist* 1, 69–70.

OLSON, J. R., GASIEWICZ, T. A., AND NEAL, R. A. (1980). Tissue distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the golden Syrian hamster. *Toxicol. Appl. Pharmacol.* 56, 78–85.

PIPER, W. N., ROSE, J. Q., AND GEHRING, P. J. (1973). Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. *Environ. Health Perspect.*, Experimental Issue No. 5, 241–244.

POIGER, H., AND SCHLATTER, CH. (1979). Biological degradation of TCDD in rats. *Nature (London)* 281 (No. 5733), 706–707.

RAMSEY, J. C., HEFNER, J. G., KARBOWSKI, R. J., BRAUN, W. H., AND GEHRING, P. J. (1979). The *in vivo* biotransformation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. *Toxicol. Appl. Pharmacol.* 48 (No. 1, Part 2), A162.

ROSE, J. Q., RAMSEY, J. C., WENTZLER, T. H., HUMMEL, R. H., AND GEHRING, P. J. (1976). The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. *Toxicol. Appl. Pharmacol.* 36, 209-226.

VINOPAL, J. H., AND CASIDA, J. E. (1973). Metabolic stability of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian liver microsomal systems and in living mice. *Arch. Environ. Contam. Toxicol.* 1, 122-132.

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Evaluation of the Carcinogenic and Mutagenic Potential of 2,3,7,8-TCDD and Other Chlorinated Dioxins

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OVERVIEW

The carcinogenic potential of unsubstituted dibenzo-*p*-dioxin; 2,7-dichlorodibenzo-*p*-dioxin; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); and a mixture of two hexachlorodibenzo-*p*-dioxin isomers have been assessed in chronic rodent bioassays. Higher dose levels of TCDD or the hexachlorodibenzo-*p*-dioxin isomers have been associated with dose-related carcinogenic responses in the rodent bioassays. The unsubstituted dibenzo-*p*-dioxin did not elicit any carcinogenic response. The 2,7-dichlorodibenzo-*p*-dioxin did not elicit any carcinogenic response in the rat; in the mouse there was a suggestive effect in the form of increased liver tumors at the high dose level.

Various studies on tumor initiation, promotion, cocarcinogenesis, mutagenesis, and DNA interaction have been conducted with TCDD. TCDD has been shown to have a relative lack of ability to either covalently bind to DNA or to induce DNA repair synthesis. The preponderance of data indicate little mutagenic or clastogenic potential for TCDD. Recent studies have indicated TCDD to be a promoter of diethylnitrosamine (DEN)-initiated liver tumors in rats and also dimethylbenzanthracene (DMBA) or methyl-*n*-nitrosoguanidine (MNNG)-initiated skin tumors in hairless mice. These results, along with the observations that the carcinogenic response occurred at dose levels producing organ toxicity, support the concept of a nongenetic mechanism of TCDD carcinogenesis.

INTRODUCTION

Numerous experimental studies have been conducted as part of the evaluation of the carcinogenic and mutagenic potential of the chlorinated dibenzo-*p*-dioxins, especially the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These studies have included the more conventional long-term carcinogenic bioassays conducted with groups of rodents exposed to various dose levels for a lifetime, as well as studies of

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shorter duration that have addressed the issues of tumor initiation, promotion, co-carcinogenesis, DNA interaction, mutagenesis, and clastogenesis. It is the objective of this paper to review the results of these studies in a composite manner to allow evaluation of the likely mechanism of TCDD carcinogenesis.

RESULTS AND DISCUSSION

Table 1 lists the chlorinated dioxins that have been studied in more conventional long-term bioassays in rats and mice. The unsubstituted dibenzo-*p*-dioxin isomer has been evaluated in both the rat and the mouse in conventional lifetime 2-year studies. This material is relatively nontoxic in comparison to the others and thus the dosages used were relatively high—10,000 and 5000 parts per million—in the diet of both the rat and the mouse for 2 years. In both species there was no carcinogenic response (National Toxicology Program [NCI] 1979a). The 2,7-dichlorodibenzo-*p*-dioxin isomer was similarly studied at these same relatively high dose levels in both rodent species. In the rat, there was no carcinogenic response; in the mouse, there was a suggestive effect in the form of an increased incidence of hepatocellular neoplasms at the high dose level (National Toxicology Program [NCI] 1979b).

A mixture of two hexachlorodibenzo-*p*-dioxin isomers was given via oral gavage to rats and mice for 2 years (National Toxicology Program [NCI] 1980). For the rat, the NTP report stated that there was no carcinogenic response in male rats, but there was a carcinogenic response in female rats at the higher dose levels. In the mouse, there was a carcinogenic response at the high dose level. This particular study has been recently reviewed by Dr. R. Squire, who has issued a subsequent report (Squire 1983) of his histopathologic examination that supplies additional perspective on the overall interpretation of this study on this hexachlorodibenzo-*p*-dioxin mixture.

TCDD has been the most extensively studied dioxin isomer. Table 2 is a collation of the data listed in a decreasing dosage schedule from the two more definitive studies in rats (Kociba et al. 1978; National Toxicology Program [NCI] 1982a). There is good concurrence between results of these studies. The dosage range of 0.07–0.1 μg/kg·day elicited a positive carcinogenic response in both the Sprague-Dawley and Osborne-Mendel strains of rats. An intermediate dosage range of 0.007–0.01 μg/kg·day elicited a less than definitive carcinogenic response in these studies in rats. Lower dosages in the range of 0.001-0.0014 μg/kg·day for a lifetime elicited no increase in tumors in either study with rats.

Table 2 also lists the data from the two long-term studies that have been conducted on TCDD in mice (Toth et al. 1979; National Toxicology Program [NCI] 1982a). There was good concurrence wherein higher dosages were associated with an increase in hepatocellular tumors in both the B6C3F1 mouse as well as in the Swiss mouse. At dosages of 0.03 μg/kg·day and lower, there were no increases in the incidence of tumors.

Table 1
Chlorinated Dibenzo-*p*-dioxins That Have Been Studied in Long-term Carcinogenic Bioassays

Dibenzo-*p*-dioxin	Species	Dose level	Results	References
Unsubstituted	Rat	10,000 ppm in diet	No carcinogenic response	NTP (1979a)
	Rat	5,000 ppm in diet	No carcinogenic response	
Unsubstituted	Mouse	10,000 ppm in diet	No carcinogenic response	NTP (1979a)
	Mouse	5,000 ppm in diet	No carcinogenic response	
2,7-Dichloro	Rat	10,000 ppm in diet	No carcinogenic response	NTP (1979b)
	Rat	5,000 ppm in diet	No carcinogenic response	
2,7-Dichloro	Mouse	10,000 ppm in diet	Suggestive carcinogenic response	NTP (1979b)
	Mouse	5,000 ppm in diet	No carcinogenic response	
2,3,7,8-Tetrachloro	(See Table 2 for data.)			
1,2,3,6,7,8 and 1,2,3,7,8,9 Hexachloro (mixture)	Rat	5, 2.5, or 1.25 μg/kg·wk via gavage	No carcinogenic response in male rats; carcinogenic response in female rats at higher dose levels	NTP (1980)
1,2,3,6,7,8 and 1,2,3,7,8,9 Hexachloro (mixture)	Mouse	5, 2.5, or 1.25 μg/kg·day via gavage (male mice) 10, 5, or 2.5 μg/kg·day via gavage (female mice)	Carcinogenic response at high dose level	NTP (1980)
Octachloro	(Nominated for testing in NTP bioassay program.)			

Table 2
Results of Studies Assessing Carcinogenic Potential of TCDD

Species and strain	Calculated daily dose TCDD (μg/kg-day)	Response	Reference
Rats			
Sprague-Dawley	0.1	Hepatocellular carcinoma, squamous carcinoma of oropharynx and lung	Kociba et al. (1978)
Osborne-Mendel	0.07	Hepatocellular carcinoma, thyroid tumors	NTP (1982a)
Sprague-Dawley	0.01	Hepatocellular nodules	Kociba et al. (1978)
Osborne-Mendel	0.007	Questionable increase in thyroid tumors	NTP (1982a)
Osborne-Mendel	0.0014	No increase in tumors	NTP (1982a)
Sprague-Dawley	0.001	No increase in tumors	Kociba et al. (1978)
Mice			
Swiss (M)	1.0	No increase in tumors, but decreased lifespan	Toth et al. (1979)
B6C3F1 (F)	0.3	Hepatocellular tumors and thyroid tumors	NTP (1982a)
Swiss (M)	0.1	Hepatocellular tumors	Toth et al. (1979)
B6C3F1 (M)	0.07	Hepatocellular tumors	NTP (1982a)
B6C3F1 (F)	0.03	No increase in tumors	NTP (1982a)
B6C3F1 (M)	0.007	No increase in tumors	NTP (1982a)
B6C3F1 (F)	0.006	No increase in tumors	NTP (1982a)
B6C3F1 (M)	0.0014	No increase in tumors	NTP (1982a)
Swiss (M)	0.001	No increase in tumors	Toth et al. (1979)

There are a number of studies that have addressed the issue of tumor initiation, tumor promotion and cocarcinogenesis. Whereas the earlier studies gave a somewhat mixed response, the more recent studies have indicated that TCDD carcinogenesis appears to be via a promoter mechanism.

In earlier studies by DiGiovanni et al. (1977), TCDD was reported to be only a weak initiator when studied in a conventional two-stage skin bioassay in mice using t-phorbol acetate (TPA) as the promoting agent. DiGiovanni et al. (1977) also reported TCDD to be a rather inactive cocarcinogen when applied concurrently with DMBA followed by promotion with TPA in a conventional skin bioassay in CD-1 mice. Cohen et al. (1979) reported that TCDD strongly inhibited mouse skin tumors initiated by either DMBA or benzo[*a*]pyrene (B[*a*]P) followed with TPA promotion. Berry et al. (1978) also reported TCDD did not promote DMBA-initiated skin tumors in a conventional skin bioassay using CD-1 mice. Kouri et al. (1978) reported mixed response, with no clear-cut results when TCDD was given subcutaneously or intraperitoneally prior to, or in conjunction with, methylcholanthrene (MCA) given subcutaneously to mice.

Whereas most of the studies cited above used more conventional strains of mice, more recent studies by Poland et al. (1982) have utilized the HRS/J strain of hairless mouse. This strain of hairless mouse is one of the few species that does show the chloracne-like reaction of the integumentary epithelial tissue, similar to the response of the rabbit ear used as a model of the chloracnegenic response observed in humans. Using those members of this hairless strain of mouse that are genotypically heterogeneous for the hairless trait (i.e., phenotypically haired), Poland et al. (1982) reported that TCDD did not promote skin tumors initiated by DMBA. Using mice that were genotypically homozygous (phenotypically hairless), TCDD did promote skin papillomas initiated with either DMBA or MNNG. Poland et al. (1982) concluded that the skin effects of TCDD in this hairless strain of mouse are dependent on the interaction of two genetic loci: first, the *Ah* locus which is associated with the cytosol receptor in its enzyme induction, and second, the *hr* locus which dictates whether or not epidermal proliferation will occur. Poland et al. (1982) concluded that their data supported a promoter mechanism of TCDD carcinogenesis.

Another more recent study of TCDD that is pertinent here is the liver tumor promotion study reported by Pitot et al. (1980). Intact rats were subjected to partial hepatectomy in order to stimulate hepatocellular proliferation followed by subsequent systemic dosing. TCDD or phenobarbital (PB) alone did not initiate the liver foci or liver tumors in rats subjected to partial hepatectomy. The initiating agent DEN did initiate hepatocellular foci. The group of rats initiated with DEN followed by a lower dose of TCDD promoted liver foci but no liver tumors. The group of rats initiated with DEN and given a higher dosage of TCDD did lead to promotion of liver foci and liver tumors. The group of rats promoted with PB gave similar results, and Pitot et al. (1980) concluded that this demonstrated the promoter mechanism of TCDD carcinogenesis in this particular test system. The

authors stated it mimicked PB as a liver tumor promoter, but TCDD was more potent than PB in this particular test system.

Another more recent study is a 2-year skin painting study with TCDD reported by the National Toxicology Program ([NTP] 1982b). In this study, female mice were reported to have shown an increased incidence of fibrosarcoma at the topical site of application of TCDD for the 2-year treatment period.

The fibrosarcomas reported induced by treatment were limited to the local site of topical application of TCDD and were preceded or accompanied by inflammation/necrosis and ulceration of the underlying subcutaneous tissue. As mouse integument is rather delicate and at most two or three epithelial cell layers thick, it appears that repeated lifetime application of certain materials to this rather thin and delicate epithelial covering can lead to denudation and/or ulceration of the superficial epithelium. Repeated application could be anticipated to result in the material being directly applied to the underlying mesenchymally-derived tissue, which has only a limited number of ways of responding to insults. One of these limited responses is the proliferation of the mesenchymal type of tissues leading to fibrosarcoma.

The report from the National Toxicology Program ([NTP] 1982b) stated that there was no evidence to suggest that TCDD was a systemic tumorigen when applied to the backs of mice; and it suggested that caution be exercised when researchers attempt to interpret the findings of an increased incidence of localized subcutaneous fibrosarcoma in the female mice. In addition, the NTP report stated that "the tumor type is induced by implanted inert plastics." Historical research has documented the relative ease in which this fibrosarcoma type of reaction can be induced within the rodent subcutaneous tissue with a number of rather innocuous materials in both the rat and the mouse (Grasso and Golberg 1966).

Mutagenesis assays using both bacteria and plant organisms have been performed on some of the chlorinated dibenzo-*p*-dioxin isomers. Hussain et al. (1972) evaluated TCDD for mutagenicity with the *Escherichia coli* strain Sd-4, which measures reversion from streptomycin dependence to independence; the *Salmonella typhimurium* strains, which measure reversion to histidine independence; and prophage induction in *Escherichia coli* K-39. Treatment with TCDD appeared to cause some reversion to streptomycin independence in the *Escherichia coli* Sd-4; the data were not well reproduced nor did there appear to be dose-related mutagenicity. In the tests with *Salmonella typhimurium*, strain TA1530 had no mutations but TA1532 showed an increase in mutational rate, similar to that observed with acridine mustard. This result suggested reversion to histidine independence by frameshift mutation. The compound also caused some activation of prophage in the *Escherichia coli* K-39.

Seiler (1973) tested both TCDD and octachlorodibenzo-*p*-dioxin in *Salmonella* strains *his* G46, TA1530, TA1531, TA1532, and TA1534. Seiler (1973) reported that TCDD was mutagenic in strain TA1532, of doubtful mutagenicity for strains

TA1531 and TA1534, and nonmutagenic in strains G46 and TA1530. The octachlorodibenzo-*p*-dioxin was reported by Seiler (1973) to be of doubtful mutagenicity in strains TA1532 and TA1534 and nonmutagenic in strains *his* G46, TA1530, and TA1531. No dose levels, negative controls, solvents used, or indications of toxicity were included in this report.

Unpublished work by McCann, cited by Wassom et al. (1977-78), reportedly found TCDD to be negative for mutagenesis, both with and without metabolic activation in the *Salmonella typhimurium* tester strains TA1532, TA1535, TA1537, and TA1538.

Nebert et al. (1976) reported TCDD to be nonmutagenic in *Salmonella typhimurium* strains TA1535 and TA1538; and Geiger and Neal (1981) reported TCDD to be nonmutagenic in *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98. Gilbert et al. (1980) also reported TCDD to be nonmutagenic in *Salmonella typhimurium* strains G46, TA1530, TA1535, TA100, TA1950, TA1975, TA1532, TA1537, TA1538, TA98, and TA1978. However, Bronzetti et al. (1980) reported that TCDD induced a positive response in tests with *Saccharomyces cerevisiae*.

Possible cytological effects of TCDD in the African Blood Lily were investigated by Jackson (1972). Treatment caused inhibition of mitosis and chromosomal abnormalities, such as the formation of dicentric bridges and chromatin abnormalities such as the formation of multinuclei or a single larger nucleus.

Rogers et al. (1982) reportedly induced a positive response with TCDD in an in vitro mouse lymphoma cell system for evaluation of mutagenic potential. Hay et al. (1983) presented preliminary data on an in vitro cell transformation using an assay with a "preneoplastic" baby hamster kidney cell line.

Kondorosi et al. (1973) reported that any interaction of TCDD with nucleic acids would most likely be by formation of a physical complex (intercalation) rather than by direct chemical reaction. Poland and Glover (1979) found the potential for TCDD to bind to rat liver DNA, RNA, or protein to be 4–6 orders of magnitude lower than the binding potential observed for most chemical carcinogens.

Althaus et al. (1982) found TCDD did not induce unscheduled DNA repair synthesis in a procedure that used cultured hepatocytes as a screening test for carcinogenic properties. Similarly, Loprieno et al. (1983) also reported that TCDD did not stimulate unscheduled DNA synthesis in a human cell line.

Green and Moreland (1975) reported studies in which TCDD; the 2,7-dichlorodibenzo-*p*-dioxin; and the unsubstituted dibenzo-*p*-dioxin were evaluated in tests for chromosomal aberrations in bone marrow cells of male rats. Rats were given 10 μg/kg of each of these dioxins by oral intubation each day for 5 consecutive days, and then injected with colchicine prior to killing. In a subsequent study, TCDD was given orally or intraperitoneally to rats which were killed after 1 or 29 days. The authors concluded that the dioxins studied had no potential for pro-

ducing chromosomal aberrations in the bone marrow cells of male rats. In a subsequent study with TCDD, Green et al. (1977) reported a statistically significant increase in the number of chromosome breaks in the bone marrow cells of rats given a higher dose when a different experimental protocol was used. The authors cautioned that the observed statistical increase should be regarded as only weakly positive for bone marrow chromosomal effects in rats.

Khera and Ruddick (1973) performed a dominant lethal study wherein male rats were dosed orally with 4, 8, or 12 μg/kg-day of TCDD for 7 consecutive days followed by seven sequential mating trials (each of 5 days duration) in which each surviving male was mated with two virgin females. The females were killed 9 days after the end of each breeding trial, and the viable embryos, resorption sites, and corpora lutea were counted. No dominant lethal mutations were noted during the 35-day posttreatment period, even though the dose levels caused dose-related toxicity and mortality for the treated males.

Loprieno et al. (1983) treated mice intraperitoneally with TCDD and reported an enhancement of chromosomal aberrations in bone marrow cells collected after 96 hours but not in bone marrow cells collected after 24 hours. Rats treated in the same study with TCDD for 45 consecutive weeks had no increase in chromosomal aberrations.

Beatty et al. (1975) reported that TCDD caused no significant effects on growth patterns or the ultrastructural morphology of cultured normal human lymphocytes, normal mouse fibroblasts, normal human fibroblasts, HeLa human epithelial carcinoma cells, and a derivative of mouse 3T3 cells. Knutson and Poland (1980) reported TCDD to have no adverse effects on 23 cultured cell types of human or animal origin.

Cytogenetic evaluation of maternal peripheral blood cells and fetal tissues from humans exposed to TCDD at Seveso, Italy, has been performed. De Carli et al. (1977), Homberger et al. (1979), and Reggiani (1980) report no chromosomal alterations in peripheral blood cells from workers selected from the chemical plant that was the source of the TCDD or from local residents (source of fetal tissues) of the highly contaminated area in Seveso.

Overall, the proponderance of available data are more supportive of a nongenetic mechanism of TCDD carcinogenesis rather than a genetic mechanism. The data of Poland and Glover (1979) indicated a relative lack of binding of TCDD to DNA. Likewise, TCDD did not cause unscheduled DNA synthesis when tested in rat hepatocytes or a human cell line. Results of numerous tests also indicate a relatively low potential for mutagenesis or clastogenesis. Pitot et al. (1980) and Poland et al. (1982) have conducted studies leading both to conclude that TCDD carcinogenesis occurs via a promoter mechanism.

A number of the mechanisms of action proposed for TCDD biologic and toxicologic activity do not contradict the concept of a nongenetic (possible promoter) mechanism of carcinogenesis. Matsumura (1983) has proposed as a

mechanism of TCDD action the alteration of cellular membrane function and subsequent effect on cell-cell communication. Thunberg (1983) has proposed a mechanism of action involving an effect of TCDD on Vitamin A function. Stohs et al. (1983) have proposed that the toxicity of TCDD may involve membrane lipid peroxidation. Rozman et al. (1984) have published data suggesting that a thyroid hormone or hormones may play an important role in mediating the toxicity of TCDD; and Potter et al. (1983) theorize TCDD toxicity may be due to hormonal alterations. In the study by Kociba et al. (1978), TCDD elicited a carcinogenic response only at dose levels inducing organ toxicity. Thus, there appears to be no inherent contradiction between the mechanisms proposed for either TCDD toxicity or TCDD carcinogenesis in that the most likely mechanisms can all be considered to be threshold in nature.

REFERENCES

Althaus, F.R., S.D. Lawrence, G.L. Sattler, D.G. Longfellow, and H.C. Pitot. 1982. Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential chemical carcinogens. *Cancer Res.* 42: 3010.

Beatty, P.W., K.J. Lembach, M.A. Holscher, and R.A. Neal. 1975. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on mammalian cells in tissue cultures. *Toxicol. Appl. Pharmacol.* 31: 309.

Berry, D.L., J. DiGiovanni, M.R. Juchau, W.M. Bracken, G.L. Gleason, and T.J. Slaga. 1978. Lack of tumor-promoting ability of certain environmental chemicals in a two-stage mouse skin tumorigenesis assay. *Res. Commun. Chem. Pathol. Pharmacol.* 20(1): 101.

Bronzetti, G., I. Lee, E. Zeigler, H. Malling, and H. Suzuki. 1980. Genetic effects of TCDD in vitro and in vivo using D7 strain of *S. cerevisiae. Mutat. Res.* 74: 206.

Cohen, G.M., W.M. Bracken, R.P. Iyer, D.L. Berry, J.K. Selkirk, and T.J. Slaga. 1979. Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. *Cancer Res.* 39: 4027.

De Carli, L.M. Fraccaro, F. Nuzzo, B. Nicolletti, F. Cefis, P. Mocarelli, G. Zei, and G. Morganti. 1977. *Final report of cytogenetic investigations in individuals exposed to the toxic action of TCDD.* University of Milan and Pavia, Italy.

DiGiovanni, J., A. Viaje, D.L. Berry, T.J. Slaga, and M.R. Juchau. 1977. Tumor-initiating ability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and Arochlor 1254 in the two-stage system of mouse skin carcinogenesis. *Bull. Environ. Contam. Toxicol.* 19(5): 552.

Geiger, L.E. and R.A. Neal. 1981. Mutagenicity testing of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in histidine auxotrophs of salmonella typhimurium. *Toxicol. Appl. Pharmacol.* 59: 125.

Gilbert, P. G. Saint-Ruf, F. Poncelet, and M. Mercier. 1980. Genetic effects of chlorinated anilines and azobenzenes on salmonella typhimurium. *Arch. Environ. Contam. Toxicol.* 9: 533.

Grasso, P. and L. Golberg. 1966. Review section: Subcutaneous sarcoma as an index of carcinogenic potency. *Food Cosmet. Toxicol.* 4: 297.

Green, S. and F.S. Moreland. 1975. Cytogenetic evaluation of several dioxins in the rat. *Toxicol. Appl. Pharmacol.* 33: 161.

Green, S., F. Moreland, and C. Sheu. 1977. Cytogenetic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on rat bone marrow cells. *FDA By-Lines Issue No. 6.*

Hay, A., J. Ashby, J.A. Styles, and B. Elliott. 1983. The mutagenic properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Symp. on Chlorinated Dioxins and Dibenzofurans in the Total Environment. II.* Am. Chem. Soc. Meeting, Washington, D.C., August 29, 1983.

Homberger, E., G. Reggiani, J. Sambeth, and H.K. Wipf. 1979. The Seveso accident: Its nature, extent and consequences. *Ann. Occup. Hyg.* 22: 327.

Hussain, S., L. Ehrenberg, and T. Gejvall. 1972. Mutagenic effects of TCDD on bacterial systems. *Ambio* 1: 32.

Jackson, W.T. 1972. Regulation of Mitosis III. Cytological effects of 2,4,5-trichlorophenoxyacetic acid and of dioxin contaminants in 2,4,5-T formulations. *J. Cell Sci.* 10: 15.

Khera, K.S. and J.A. Ruddick. 1973. Polychlorodibenzo-*p*-dioxins: Perinatal effects and the dominant lethal test in wistar rats. In *Chlorodioxins-Origin and Fate.* Advances in Chemistry Series 120 (ed. E.H. Blair). American Chemistry Society, Washington, D.C.

Knutson, J. and A. Poland. 1980. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Failure to demonstrate toxicity in twenty-three cultured cell types. *Toxicol. Appl. Pharmacol.* 54: 377.

Kociba, R.J., D.G. Keyes, J.E. Beyer, R.M. Carreon, C.E. Wade, D.A. Dittenber, R.P. Kalnins, L.E. Frauson, C.N. Park, S.D. Barnard, R.A. Hummel, and C.G. Humiston. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol. Appl. Pharmacol.* 46: 279.

Kondorosi, A., I. Fedorcsak, F. Solymosy, L. Ehrenberg, and S. Osterman-Golkar. 1973. Inactivation of Qβ RNA by electrophiles. *Mutat. Res.* 17: 149.

Kouri, R.E., T.H. Rude, R. Jogelkar, P.M. Dansette, D.M. Jerina, S.A. Atlas, I.S. Owens, and D.W. Nebert. 1978. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin as co-carcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah locus. *Cancer Res.* 38: 2777.

Loprieno, N., I. Sbrana, D. Rusciano, Lasciafari, and T. Lari. 1983. In vivo cytogenetic studies on mice and rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In *Permagon Series on Environmental Science. Chlorinated dioxins and related compounds* (ed. O. Hutzinger et al.), vol. 5. Permagon Press, New York.

Matsumura, F. 1983. Biochemical aspects of action mechanisms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related chemicals in animals. *Pharmacol. Ther.* 19: 195.

National Toxicology Program (NCI). 1979a. Bioassay of dibenzo-*p*-dioxin for possible carcinogenicity. NCI Technical Report No. 122, National Institutes of Health, Bethesda, Maryland.

_____. 1979b. Bioassay of 2,7-dichlorodibenzo-*p*-dioxin for possible carcinogenicity. NCI Technical Report No. 123. National Institutes of Health, Bethesda, Maryland.

_____. 1980. Bioassay of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin for possible carcinogencity. NTP Report No. 80-12. NCI Report No. 198, National Institutes of Health, Bethesda, Maryland.

National Toxicology Program (NTP). 1982a. Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Osborne-Mendel rats and B6C3F1 mice (gavage study). NTP Technical Report No. 209. NIH Report No. 82-1765. National Institutes of Health, Bethesda, Maryland.

_____. 1982b. Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Swiss-Webster mice (Dermal study). NTP Technical Report No. 201, NIH Report No. 82-1757, National Institutes of Health, Bethesda, Maryland.

Nebert, D.W., S.S. Thorgeirsson, and J.S. Felton. 1976. Genetic differences in mutagenesis, carcinogenesis and drug toxicity. In *In vitro metabolic activation in mutagenesis testing* (ed. F.J. de Serres et al.). Elsevier North Holland Biomedical Press, Amsterdam.

Pitot, H.C., T. Goldsworthy, H.A. Campbell, and A. Poland. 1980. Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of hepatocarcinogenesis from diethylnitrosamine. *Cancer Res.* 40: 3616.

Poland, A. and E. Glover. 1979. An estimate of the maximum in vivo covalent binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to rat liver protein, ribosomal RNA, and DNA. *Cancer Res.* 39: 3341.

Poland, A., D. Palen, and E. Glover. 1982. Tumour promotion by TCDD in skin of HRS/J hairless mice. *Nature* 300: 271.

Potter, C.L., I.G. Sipes, and D.H. Russell. 1983. Hyperthyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administration. *Toxicol. Appl. Pharmacol.* 69: 89.

Reggiani, G. 1980. Acute human exposure to TCDD in Seveso, Italy. *J. Toxicol. Environ. Health* 6: 27.

Rogers, A.M., M.E. Andersen, and K.C. Back. 1982. Mutagenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and perfluoro-n-decanoic acid in L5178Y mouse-lymphoma cells. *Mutat. Res.* 105: 445.

Rozman, K., T. Rozman, and H. Greim. 1984. Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced toxicity. *Toxicol. Appl. Pharmacol.* 72: 372.

Seiler, J.P. 1973. A survey on the mutagenicity of various pesticides. *Experientia* 29: 622.

Squire, R. 1983. An assessment of the experimental evidence for potential carcinogenicity of hexachlorodibenzo-*p*-dioxins. Robert S. Squire Association. Ruxton, Maryland.

Stohs, S.J., M.Q. Hassan, and W.J. Murray. 1983. Lipid peroxidation as a possible cause of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity. *Biochem. Biophys. Res. Commun.* 111: 854.

Toth, K., S. Somfai-Relle, J. Sugar, and J. Bence. 1979. Carcinogenicity of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. *Nature* 278: 548.

Thunberg, T. 1983. ***Studies on the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxins on Vitamin A: A new aspect concerning the mechanism of toxicity.*** Karolinska Institute, Stockholm, Sweden.

Wassom, J.S., J.E. Huff, and N. Loprieno. 1977/1978. A review of the genetic toxicology of chlorinated dibenzo-*p*-dioxins. *Mutat. Res.* 47: 141.

COMMENTS

MOORE: Is there a model where you're got a chemical that can initiate maybe a mesenchymal tumor cell, followed by promotion?

POLAND: I am unaware of evidence that TCDD affects the morphology or growth of mesenchymal cells. Perhaps it affects epithelial surfaces derived from mesenchyme, i.e., endothelium or periosteum.

MCCONNELL: The endothelial and periosteal changes may be secondary to things like debilitation and poor nutrition. The primary lesions are epithelial in origin.

POLAND: I guess I'm saying there are some mesenchymal epithelium surfaces, the endothelium being one.

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A REVIEW OF THE TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) WITH A COMPARISON TO THE TOXICITY OF OTHER CHLORINATED DIOXIN ISOMERS[1]

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I. INTRODUCTION

Over the past 15 years a substantial amount of toxicologic information on the chlorinated Dibenzo-p-dioxins has become available. Any review of this toxicity information is facilitated by a clear understanding of the terminology commonly used in this type of discussion.

The term "chlorinated Dibenzo-p-dioxins", or more simply "Dioxins" refers to 75 isomers that differ in the number and position of attached chlorine atoms. Each of the 75 chlorinated dioxins that has been studied has its own unique identity and toxicologic properties. 2,3,7,8-Tetra-chlorodibenzo-p-dioxin (TCDD) is considered to be the most toxic of he chlorinated dioxin isomers that have been studied, and it has been comprehensively examined for its toxicological effects. There are 22 possible isomers of Tetrachlorodibenzo-p-dioxin. However, in most cases, and for the purposes of this short review, the term "TCDD" has been used to mean the 2,3,7,8-Tetrachlorodibenzo-p-dioxin isomer, thereby differentiating it from the other 21 Tetrachlorodibenzo-p-dioxin isomers.

TCDD may be formed in small quantities under certain conditions during the production of 2,4,5-Trichlorophenol, and TCDD may be one of the causes of chloracne that has been associated with industrial overexposure during the production of 2,4,5-Trichlorophenol. More recently, various poly-chlorinated dioxins and furans may be formed during various combustion processes, such as in municipal incinerators, fossil-fueled power plants, internal combustion engines, home fireplaces and cigarette smoke (1,2). The present day knowledge of the magnitude of this issue is somewhat limited.

During this presentation, my objective will be to review the toxicity of TCDD and certain other dioxins. In recognition of the very extensive toxicity data base available on TCDD, and the inherent limitations existent in a presentation of this type, I will attempt to review the most pertinent toxicity information. As certain aspects of the toxicity of TCDD, especially of the acute and short-term toxicity, have been the subject of several previous reviews (3,4,5), my review will emphasize the more recent data available on chronic toxicity, teratogenesis, mutagenesis, reproductive effects and carcino-

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genesis. The key toxicity information available on the other chlorinated Dibenzo-p-dioxin isomers will be then summarized on a comparative basis, using TCDD as the principal point for this comparison.

In general, the toxicologic pattern observed with TCDD or other chlorodioxins is not unique; it also occurs with certain halogenated dibenzofurans, chlorinated biphenyls, naphthalenes and brominated dioxins (6).

Table 1 depicts the comparative acute toxicity of TCDD following a single oral dose.

TABLE 1

SINGLE DOSE ORAL LD_{50} VALUES FOR TCDD IN VARIOUS LABORATORY ANIMAL SPECIES

Species	Single Dose LD_{50} (μg/kg)	Reference
Guinea Pig	0.6 - 2.0	[3, 7]
Rabbit	115	[3]
Monkey	~70	[4, 5, 12]
Chicken	25 - 50	[13]
Rat	22 - 45	[3]
Dog	~100 - 200	[3]
Mouse	114 - 284	[7, 11]
Bullfrog	over 1000	[10]
Hamster	1157 - 5051	[8, 9]

There are marked interspecies differences in the doses required to produce lethality. The guinea pig is the most sensitive, with an acute oral LD_{50} of 0.6 - 2.0 μg/kg (3,7). In contrast, the hamster is much less sensitive, with an oral LD_{50} of 1157-5051 μg/kg (98,9). The responses of the other species tested, as depicted in Table 1, are intermediate between the guinea pig and hamster (3, 4, 5, 10, 11, 12).

The administration of a lethal dose is typically followed by a progressive weight loss and protracted time to death of the animal. At necropsy examination, the most constant lesions noted in various species are thymic atrophy and a general loss of body condition. In primates, the earliest signs of toxicity include a swelling of the eylids followed by loss of eyelashes, facial alopecia and acne-like eruptions (4,5,12). Hepatic toxicity is a prominent observation in acute and subacute toxicity of TCDD in rats, mice and rabbits, but not in the primate, in which effects on the bone marrow and epithelial tissue may be more likely noted.

Ascites or edema have been noted in chickens (13) and mice (11) given toxic doses of TCDD. The ability of TCDD and certain other compounds to elicit edema and hydropericardium in young chicks was the basis for the development of the chick edema bioassay. In this bioassay, the amount of excess fluid was used as a biologic monitor (prior to development of analytical procedures) for the detection of these edema-producing factors in certain

materials. TCDD is also one of numerous compounds associated with a potential to induce increased excretion of porphyrins (14,15).

In acute and subacute studies with toxic doses of TCDD, the clinical observations and hematologic findings generally reflect the non-specific debilitation associated with the overall toxicity. In a subchronic study with rats (15) doses of 1.0, 0.1, 0.01, or 0.001 μg TCDD/kg were given 5 days/week for 13 weeks. At the highest dose level, there was increased mortality, decreased food consumption and body weight gains, hepatic toxicity, elevated serum levels of bilirubin and alkaline phosphatase, elevated urinary excretion rates of porphyrins and delta-amino-levulinic acid, thymic atrophy and minimal alterations of some hematopoietic components. A dose-related decrease in toxicity was noted at the intermediate dose level of 0.1 μg TCDD/kg/day, and at 0.01 and 0.001 μg TCDD/kg/day there were no adverse effects associated with the 13 weeks of exposure.

The high toxicity of TCDD in some animal species has led numerous groups to explore the possible mechanisms of its action. Comparative studies of different dioxin isomers have revealed two properties that generally correlate with a greater toxic potential of certain dioxin isomers: 1) at least 3 of the 4 lateral ring positions occupied by halogen atoms, and 2) at least one of the adjacent ring positions non-halogenated.

A protein species that binds TCDD has been identified in the cytosol by Poland and Glover (16) which they hypothesize acts as the receptor to initiate the actions of TCDD.

The numerous parameters that have been evaluated as possible mechanisms of TCDD toxicity are listed in Table 2 (13,17-21).

TABLE 2

PARAMETERS EVALUATED BUT CONSIDERED UNLIKELY MECHANISMS OF TCDD TOXICITY

Parameters Evaluated	Reference
DNA synthesis after partial hepatectomy	[17]
ATP production by liver mitochondria	[18]
Protein and lipid synthesis	[19]
Growth of mammalian cells in culture (human HeLa cells, lymphocytes or fibroblasts, mouse BALB 3T3 cells, or SV101 cells)	[20]
Thyroid function	[21]
Intracellular energy	[21]
Adrenal glucocortical function and metabolism	[21]
Fatty acid composition of liver, plasma and adipose tissue	[21]
Metabolism of ^{14}C glucose, ^{14}C alanine, ^{14}C oleate	[21]
Decreased immunocompetence	[13]

At this point in time it appears as if the toxicity of TCDD has not been explained by the following parameters: DNA synthesis, growth capabilities of mammalian cells in culture, protein and lipid synthesis, liver mitochondrial oxidative phosphorylation and intracellular energy production, thyroid function, adrenal glucocortical function, metabolism of glucose, alanine or oleate or lipid composition of liver, plasma and adipose tissue. The studies by Greig (13) have indicated that impaired immunocompetence is not a likely mechanism of TCDD toxicity.

Recent data of Poiger and Schlatter (22) indicate that the biologic uptake and toxicity of TCDD is significantly decreased if it is absorbed onto carbon or soil particles. These data, extracted from the paper by Poiger and Schlatter, are given in abbreviated form in Tables 3-5.

TABLE 3

TCDD IN LIVER 24 HOURS AFTER ORAL ADMINISTRATION OF 12.7 - 22.9 NG TCDD IN RATS

Formulations of TCDD	Percent of Dose in Liver
50% Ethanol	36.7 ± 1.2
Aqueous suspension of soil via contact with TCDD for 16 hours	24.1 ± 4.8
Aqueous suspension of soil via contact with TCDD for 8 days	
Aqueous suspension of activated carbon	≤0.07

After Poiger and Schlatter, 1980 [22]

TABLE 4

TCDD IN LIVER AFTER DERMAL APPLICATION OF 28 NG TCDD TO RATS

Formulation of TCDD	Percent of Dose in Liver
Methanol solution	14.8 ± 2.6
Vaseline	1.4 ± 0.21
Soil/water paste	0.05
Activated carbon/water paste	≤0.05

After Poiger and Schlatter, 1980 [22]

TABLE 5

CHLORACNEGENIC POTENCY OF TCDD AFTER APPLICATION TO RABBIT EAR SURFACE

Formulation of TCDD	Minimal Dose to Induce Chloracne Response, µg/ear
Acetone solution	0.6 - 1.5
Vaseline	0.8 - 1.0
Soil/water paste	2.0 - 3.0
Activated carbon/water paste	160.0

After Poiger and Schlatter, 1980 [22]

It appears that the longer the time lapse to allow for adsorption onto soil or carbon particles, the less TCDD was taken up by the liver. This is an important aspect to consider when assessing possible hazard associated with environmental exposure to TCDD.

The chlorinated Dioxins, especially TCDD, have been well-documented in regard to their potential to induce hepatic microsomal fixed functional oxidase enzymes (18,23-26). Poland and Kende (27) have noted some parallelism between the toxic potency of certain Dioxin isomers and their ability to induce certain of these enzymes. While this parallelism may exist, there is considerable difference in response from species to species with a rather potent induction of enzymes in rat and mouse, but little or no induction in guinea pig or rabbit (25). Furthermore, there are inherent contradictory factors wherein the guinea pig which has the greatest sensitivity to acute TCDD toxicity shows little or no induction of enzymes (25). Review of the research indicates that in those species in which there is induction of enzymes of TCDD, it is dose-related, with the lowest effective dose of 0.002 µg TCDD/kg for the rat (26).

HISTORICAL REVIEW OF TCDD INDUCED CHLORACNE RESPONSE IN HUMANS

Chloracne is considered the most consistent response and hallmark of TCDD toxicity in humans. This is based on the experience of the industrial production accidents which led to the inadvertent overexposure of some workers to TCDD. Although it is now recognized that chloracne can be caused by numerous materials such as polychlorinated Dibenzodioxins, polychlorinated Dibenzofurans, polychlorinated Naphthalenes, polychlorinated Biphenyls, Tetrachlorobenzene and Tetrachloroazoxybenzene (28) the best documented is TCDD overexposure associated with the industrial production accidents.

German researchers established in the late 1950's (29) that TCDD was capable of eliciting a chloracnegenic response in man as well as on the rabbit ear. The rabbit ear, which predated the analytical capabilities to detect TCDD and was published by Adams et al. in 1941 (30), has been a very useful bioassay for the detection of chloracnegenic potential for a number of

materials. With TCDD, a minimal dose in the range of 0.1 - 1.0 μg has induced the chloracnegenic response (3, 22). Pathologically, the response of the rabbit ear involves the production of hyperkeratosis and keratinaceous occlusion of the hair follicles leading to comedone formation.

A skin reaction somewhat similar to human chloracne has been noted in the rabbit, monkey and hairless mice. Greig (31) has suggested that in view of the fact that the skin areas in these species affected by TCDD all lack major hair growth, there is a possibility that the longer hair shafts on the other unaffected portions of the body may be acting as an effective wick to assist drainage of sebum and keratinaceous debris from the hair follicles.

STUDIES OF POSSIBLE EFFECTS UPON IMMUNE SYSTEM

Early toxicity studies of TCDD in animals indicated thymic atrophy to be a rather consistent observation after treatment with toxic doses. This was suggestive of a potential to alter the immunocompetence of the individual. This led to subsequent immunotoxicity studies, including both functional and morphologic assessment of humoral and cell-mediated immunity. A review of these studies indicates that with TCDD toxicity the humoral immunity is relatively unaltered, but there may be some depression of cell-mediated immunity in some animals. This potential effect is age-related, with TCDD exposure during the earlier developmental stages of the immune system (32). Various studies have been attempted to define the mechanism of TCDD upon the cell-mediated immunity. The pretreatment with thymosin (33) does not prevent the thymic atrophy induced by toxic doses of TCDD. Likewise, it is not the result of a direct cytotoxic effect on lymphocytes or altered zinc levels (33). The effect is not mediated via the pituitary or adrenal gland (34) nor is it the result of altered food intake or growth hormone (35). Data generated on the human population with documented overexposure to TCDD after the industrial explosion at Seveso, Italy does not indicate any resultant immunotoxic effects (36, 37).

TERATOLOGY AND REPRODUCTION STUDIES WITH TCDD

In the early 1970's, teratology studies with mice indicated a potential for higher doses of TCDD to induce cleft palate and dilation of the renal pelvis. In the rat, TCDD does not cause a teratogenic effect, but does cause embryo- and fetotoxicity. The key teratology studies (38-43) are tabulated in Table 6.

TABLE 6

SUMMARY OF TERATOLOGY STUDIES OF TCDD IN MICE AND RATS

Strain	Embryotoxic effects	TCDD Dose Level (μg/kg/day)	Reference
Studies in Mice:			
CD-1	Cleft Palate Kidney Abnormality	1, 3	[38]
DBA/2J	Cleft Palate Kidney Abnormality	3	[38]
C57BL/6J	Cleft Palate Kidney Abnormality	3	[38]
NMRI	Cleft Palate	3	[39]
C57BL/6	Cleft Palate Dilated Renal Pelvis	1.3	[40]
CF-1	Cleft Palate	0.001, 0.01, 0.1, 1.0, 3.0	[41]
Studies in Rats:			
Sprague-Dawley	Intestinal Hemorrhage	0.03, 0.125, 0.5, 2, 8	[42]
CD	Kidney Abnormality	0.5	[38]
Wistar	Hemorrhage	0.125, 0.25, 0.5, 1, 2, 4, 8, 16	[43]

= No-Effect-Levels

In mice, the no-adverse-effect-level for teratogenesis is 0.1 μg TCDD/kg/day. For the rat, there is no embryo- or fetotoxicity at a dose level in the range of 0.03 - 0.125 μg TCDD/kg/day.

Pregnant monkeys were used by McNulty (44) in a study wherein TCDD was given 3 times weekly for 3 weeks during gestation. A high dose level of 0.24 μg TCDD/kg/day (~5000 ppt TCDD in diet) abortions occured in 2/2 pregnant monkeys that died from severe TCDD toxicity. At an intermediate dose level of 0.048 μg/kg/day (~1000 ppt in diet) abortions occurred in 3/4 monkeys, with some slight maternal toxicity noted. At a dose level of about 0.0096 μg TCDD/kg/day (~200 ppt in diet) at which no maternal toxicity was noted, the abortion rate of 1/4 was comparable to the abortion rate of 3/11 for the control group of monkeys.

Some studies in monkeys have been reported by Barsotti et al., and Allen et al. (45, 46) who fed diets containing 500 ppt of TCDD to monkeys for approximately 9 months. Substantial toxicity, including alopecia, anemia and death were reported, along with abortion in some of the pregnant monkeys. A follow-up study (47) indicated some toxicity in monkeys given diets containing 50 ppt of TCDD for 20 months. Preliminary data presently available indicate

that breeding trials initiated after 7 months of this treatment resulted in some abortions/resorptions. At the present time, additional studies with monkeys given diets containing 25 or 5 ppt of TCDD are underway at the University of Wisconsin, with no results yet available.

A 3-generation reproduction study in rats has been reported in 1979 by Murray et al. (48). These rats were maintained on diets supplying 0.1, 0.01 or 0.001 μg TCDD/kg/day through 3 consecutive generations. There was a dose-related effect at the 2 higher dose levels, with decreases in fertility and neonatal survival. The dose level of 0.001 μg TCDD/kg/day was tolerated without impairment of reproductive capacity through the 3 consecutive generations, indicating this to be the no-adverse-effect-level.

MUTAGENESIS STUDIES WITH TCDD

TCDD has been studied for mutagenic activity in both *in vitro* plant and microbial systems as well as *in vitro* mammalian systems. The results of these studies are tabulated in Table 7.

TABLE 7
MUTAGENIC STUDIES OF TCDD IN NON-MAMMALIAN AND MAMMALIAN TEST SYSTEMS

Test System	Mutagenic Activity	Reference
Salmonella typhimurium strains to assess base pair substitutions		
G-46	Negative	[52]
TA1530	Negative, Negative	[51, 52]
TA1535	Negative, Negative	[49, 50]
Salmonella typhimurium strains to assess frameshifts		
TA1531	Questionable	[52]
TA1532	Negative, Positive, Positive	[49, 51, 52]
TA1534	Questionable	[52]
TA1537	Negative	[49]
TA1538	Negative	[49, 50]
Prophage induction in *E. coli* K39	Positive	[51]
Streptomycin Independence in *E. coli* Sd-4	Positive	[51]
Chromosomal analysis of African Blood Lily	Positive	[54]
Dominant Lethal study in rats	Negative	[43]
Cytogenetics, rat bone marrow	Negative	[57]
Cytogenetics, rat bone marrow	Questionable	[58]
Morphology of human and animal cells in culture	Negative	[26]
Cytogenetics, Human Blood Cells, Seveso, Italy	Negative	[37, 59, 60]

Overall review of these data indicate a mixed response with the *in vitro* tests, with some positive and some negative results. With the Salmonella microbial strains that test for base pair substitution, a negative response has been noted in Strain TA1538 (49, 50) and TA1537 (49). With Strain TA1531 and 1534, there has been a questionable response reported by (52) and in Strain TA1532, a negative response has been reported by (49) but a positive response has been reported by (51, 52).

Jackson (54) used a cytologic plant test system (African Blood Lily) in which he reported inhibition of mitosis and chromosomal abnormalities after treatment with TCDD. Hussain et al. (51) reported some induction of prophage in *E.coli* K-39 treated with TCDD. There was also indication of some reversion to streptomycin independence in *E. coli* strain Sd-4, but the data did not show the expected dose-response.

Kondorosi et al. (55) reported that any interaction of TCDD with DNA would more likely be by way of intercalation (formation of a physical complex) rather than by direct chemical reaction with DNA.

Poland and Glover (56) found very little potential of TCDD to bind to rat liver DNA, somewhere in the range of 4-6 orders of magnitude lower than the binding potential observed for most chemical carcinogens.

A number of *in vivo* tests of mutagenic potential have been conducted in higher animals and man, and the preponderance of data indicates little likelihood of mammalian mutagenesis with TCDD. There were no chromosomal aberrations in bone marrow cells of rats given 10 μg of TCDD for 5 consecutive days (57). When the TCDD was given orally or intraperitoneally to rats that were subsequently killed after 1 or 29 days, there were again no cytogenetic changes in the chromosomes. The authors concluded that TCDD had no potential for inducing chromosomal changes in the bone marrow cells of rats. A subsequent study by the same group (58) reported a statistical increase in the number of chromosomal breaks in bone marow cells of rats given a higher dose level of TCDD and following a different experimental protocol. Based on these findings, the authors cautioned that the observed statistical increase should be regarded as only weakly positive for bone marrow chromosomal effects in rats.

No dominant lethal mutations were noted in rats after toxic doses of TCDD were given orally to male rats for 7 consecutive days followed by 7 five-day sequential trials (43).

Beatty et al. (26) used cultures of normal human fibroblasts, HeLa human epithelial cells, SV101 cells, and mouse 3T3 cells in studies of the potential of TCDD to cause morphologic transformation of intact cells. There were no significant effects on morphology or growth patterns of mammalian cells in culture after treatment with TCDD.

Results of cytogenetic evaluation of peripheral blood cells from humans overexposed to TCDD as a result of the explosion of a trichlorophenol plant at Seveso, Italy, have indicated no mutagenic effects (37, 59, 60).

Thus, upon review of the full data base available on TCDD mutagenesis, one can conclude that there have been a few positive or questionable mutagenic responses in certain *in vitro* plant or microbial test systems, but there appear

to be no definitive correlates of mutagenic potential in higher animals or man.

CARCINOGENIC AND LONG-TERM STUDIES OF TCDD IN ANIMALS

Long-term carcinogenic studies via ingestion of TCDD have been performed in the Sprague-Dawley rat by Kociba et al. (61) and the Osborne Mendel rat by NCI (62). Carcinogenic studies of TCDD have also been conducted in the mouse by NCI (62) and Toth et al. (63). These results, which are summarized in Table 8, indicate very good correlation of these results in rats and in mice.

TABLE 8

CARCINOGENIC ASSESSMENT OF TCDD IN RATS AND MICE

Species & Strain	Calculated Daily Dose TCDD (µg/kg/day)	Response	Reference
Rats:			
Sprague-Dawley	0.1	Hepatocellular carcinoma, squamous carcinoma of oropharynx and lung	[61]
Osborne-Mendel	0.07	Hepatocellular carcinoma, thyroid tumors	[62]
Sprague-Dawley	0.01	Hepatocellular nodules	[61]
Osborne-Mendel	0.007	Questionable increase in thyroid tumors	[62]
Osborne-Mendel	0.0014	No increase in tumors	[62]
Sprague-Dawley	0.001	No increase in tumors	[61]
Mice:			
Swiss (M)	1.0	No increase in tumors, but decreased lifespan	[63]
B6C3F1 (F)	0.3	Hepatocellular tumors/ thyroid tumors	[62]
Swiss (M)	0.1	Hepatocellular tumors	[63]
B6C3F1 (M)	0.07	Hepatocellular tumors	[62]
B6C3F1 (F)	0.03	No increase in tumors	[62]
B6C3F1 (M)	0.007	No increase in tumors	[62]
B6C3F1 (F)	0.006	No increase in tumors	[62]
B6C3F1 (M)	0.0014	No increase in tumors	[62]
Swiss (M)	0.001	No increase in tumors	[63]

An oncogenic response has been reported in these studies only after long-term ingestion of higher dose levels that induce toxicity. In the rat, lifetime daily doses of 0.07 - 0.1 μg TCDD/kg/day elicited a neoplastic response, and in the mouse daily doses of 0.07 - 0.3 μg TCDD/kg/day elicited a neoplastic response. The liver was the primary target tissue for oncogenesis in both the rat and the mouse. No oncogenic response occurred in rats at dose levels of 0.001 - 0.0014 μg TCDD/kg/day or in mice at dose levels of 0.001 - 0.03 μg TCDD/kg/day.

The study by Kociba et al. (61) addressed both oncogenicity as well as other measures of chronic toxicity, and determined a daily dose level of 0.001 μg TCDD/kg/day to be tolerated by rates for 2 years without causing any toxicologic effects. This no-observed-effect-level for chronic toxicity concurs with the no-observed-effect-level reported in the multiple generation reproductive study of TCDD by Murray et al. (48).

There have been multiple studies with TCDD regarding tumor initiation, promotion and cocarcinogenesis. These have been summarized in Table 9.

TABLE 9

TUMOR INITIATION, PROMOTION AND COCARCINOGINESIS STUDIES OF TCDD

Animal Test System	Results	Reference
TCDD on mouse skin with TPA as promotor	TCCD as weak initiator	[64]
TCDD+DMBA on mouse skin with TPA as promotor	TCDD a rather inactive cocarcinogen	[64]
TCDD+BAP on mouse skin with TPA as promotor	TCDD inhibited BAP tumor initiation	[65]
TCDD+DMBA on mouse skin with TPA as promotor	TCDD inhibited DMBA tumor initiation	[65]
DMBA on mouse skin with TCDD as promotor	TCDD not a promotor of skin tumors	[66]
DMBA on mouse skin with TCDD as promotor	TCDD not a promotor of skin tumors	[67]
TCDD orally after DEN+partial hepatectomy of rats	TCDD a promotor of liver tumors	[68]
TCDD given SQ or IP prior to or with MCA given SQ to mice	Mixed response: no clear-cut results	[69]

Mouse skin painting studies by DiGiovanni et al. (64) indicate TCDD to be a rather inactive cocarcinogen when applied with Dimethylbenzanthracene (DMBA) followed by T-Phorbol Acetate (TPA). Their work also indicated TCDD to be at best a weak initiator of mouse skin tumors when followed by TPA as the promotor. TCDD inhibited mouse skin tumor initiation when applied in conjunction with either DMBA or Benzo(a)pyrene (BAP) in the studies of Cohen et al. (65). TCDD was not an active promotor of mouse skin tumors initiated by DMBA in the studies conducted by Berry et al. (66) and by NCI (67).

Using rats subjected to partial hepatectomy and given Diethylnitrosamine (DEN) to initiate liver tumors, Pitot et al. (68) gave doses of TCDD chosen to

"closely resemble" the doses found to be carcinogenic in the study with rats by Kociba et al. (61). Using this test system, they concluded that TCDD was a promotor of liver tumors in the rat.

Based on (a) the low potential for TCDD to induce mutagenesis or bind covalently to DNA, (b) the well-documented capability to induce microsomal enzymes, (c) the carcinogenic response induced in rats and mice at or above dose levels producing organ toxicity and (d) the finding of a promotional role for TCDD in the primary target tissue (liver) of rats, it appears as if the predominant mechanism for TCDD carcinogenicity is most likely non-genetic rather than genetic.

COMPARATIVE TOXICITY OF OTHER CHLORINATED DIOXIN ISOMERS

The more limited toxicity data that is available on the other chlorodioxins is best evaluated on a comparative basis with the extensive data on 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). In general, the other dioxins possess lesser degress of biologic activity when compared to TCDD.

TABLE 10

COMPARATIVE ACUTE TOXICITY OF CHLORINATED DIBENZO-P-DIOXINS

Dibenzo-p-Dioxin Isomer	Single Oral LD_{50} (30 days) µg/kg Guinea Pig	Mouse
2,8-Dichlorodibenzo-p-dioxin	> 300,000	—
2,3,7-Trichlorodibenzo-p-dioxin	29,444	>3,000
2,3,7,8-Tetrachlorodibenzo-p-dioxin	2	283.7
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	3.1	337.5
1,2,4,7,8-Pentachlorodibenzo-p-dioxin	1,125	>5,000
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	72.5	825
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	70-100	1,250
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	60-100	>1,440
1,2,3,4,6,7,8-Septachlorodibenzo-p-dioxin	> 600	—

Data from McConnell et al., 1978 [7]

Table 10 lists the comparative single oral dose LD_{50} values observed in the guinea pig and mouse for various chlorodibenzo-p-dioxin isomers (7).

TABLE 11
INDUCTION OF ALA SYNTHETASE ACTIVITY IN LIVER OF CHICK EMBRYO BY VARIOUS CHLORINATED DIBENZO-P-DIOXIN ISOMERS

Dibenzo-p-Dioxin Isomer	Dose Injected, (moles x 10^{-11}/egg)	ALA Synthetase Activity (m μ moles ALA/gm liver/hour)
Solvent Control	—	< 20
Unsubstituted Dibenzo-p-dioxin	1359	< 20
One Chlorodibenzo-p-dioxin	1144	< 20
2,3-Dichlorodibenzo-p-dioxin	989	< 20
2,7-Dichlorodibenzo-p-dioxin	989	< 20
2,8-Dichlorodibenzo-p-dioxin	989	< 20
1,2,4-Trichlorodibenzo-p-dioxin	870	< 20
1,2,3,4-Tetrachlorodibenzo-p-dioxin	777	< 20
1,3,6,8-Tetrachlorodibenzo-p-dioxin	777	< 20
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	544	< 20
1,2,4,6,7,9-Hexachlorodibenzo-p-dioxin	640	< 20
2,3,7,8-Tetrachlorodibenzo-p-dioxin	93	~270
1,2,3,4,7,8-hexachlorodibenzo-p-dioxin	256	~190
1,2,3,4,7-Pentachlorodibenzo-p-dioxin	281	~200
2,3,7-Trichlorodibenzo-p-dioxin	209	~190

Data from Poland & Glover, 1973 [70]

TABLE 12
ENZYME INDUCTION OF CHICK EMBRYO HEPATIC AHH ACTIVITY WITH VARIOUS CHLORINATED DIBENZO-P-DIOXIN ISOMERS

Dibenzo-p-Dioxin Isomer	Dose Injected, (moles x 10^{-11}/egg)	Activity expressed as AHH Units/mg/liver
Unsubstituted Dibenzo-p-dioxin	470	< 2
One Chlorodibenzo-p-dioxin	470	< 2
2,3-Dichlorodibenzo-p-dioxin	470	< 2
2,7-Dichlorodibenzo-p-dioxin	470	~2
2,8-Dichlorodibenzo-p-dioxin	470	~2
1,2,4-Trichlorodibenzo-p-dioxin	470	>2
1,2,3,4-Tetrachlorodibenzo-p-dioxin	470	~2
1,3,6,8-Tetrachlorodibenzo-p-dioxin	470	~2
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	470	~5
	47	~2
2,3,7,8-Tetrachlorodibenzo-p-dioxin	4.7	~16
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	4.7	~12
1,2,3,4,7-Pentachlorodibenzo-p-dioxin	47	~10
	4.7	~2
2,3,7-Trichlorodibenzo-p-dioxin	47	~6
	4.7	~2

Data from Poland & Glover, 1973 [70]

Tables 11 and 12 show the relative potency of other isomers compared to TCDD as reported by Poland and Glover (70) using enzyme induction in chick embryo liver. Based on their data, the 2,3,7-Tri isomer, the 1,2,3,4,7-Penta isomer, and the 1,2,3,4,7,8-Hexa isomer all possessed a portion of the biologic activity of TCDD, but the other isomers tested were relatively inactive.

TABLE 13

INDUCTION OF AHH ACTIVITY IN CULTURED RAT HEPATOMA CELLS BY VARIOUS CHLORINATED DIBENZO-P-DIOXIN ISOMERS

Dibenzo-p-Dioxin Isomer	Pmol/Plate to Induce ED_{50}
2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.5
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	22
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	31
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	128
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	189
1,3,7,8-Tetrachlorodibenzo-p-dioxin	383
1,2,3,4,6,7,8-Septachlorodibenzo-p-dioxin	551
1,2,3,8-Tetrachlorodibenzo-p-dioxin	2500
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (99.2% purity)	2500
2,3,7-Trichlorodibenzo-p-dioxin	4800
1,2,3,4,6,7,9-Septachlorodibenzo-p-dioxin	15,000 (projected value)
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (99.88% purity)	79,500 (projected value)
1,2,3,6,7,9-Hexachlorodibenzo-p-dioxin	330,000 (projected value)
1,2,4,7,8-Pentachlorodibenzo-p-dioxin	>500,000 (projected value)
Unsubstituted Dibenzo-p-dioxin 1,3-Dichlorodibenzo-p-dioxin 1,6-Dichlorodibenzo-p-dioxin 2,3-Dichlorodibenzo-p-dioxin 2,7-Dichlorodibenzo-p-dioxin 2,8-Dichlorodibenzo-p-dioxin 1,2,3,4-Tetrachlorodibenzo-p-dioxin 1,3,6,8-Tetrachlorodibenzo-p-dioxin 1,2,4,6,7,9-Hexachlorodibenzo-p-dioxin	Inactive at 50,000 Pmol/Plate

Data from Bradlaw & Casterline, 1979 [71]

Table 13 summarizes the comparative biologic activity as determined by Bradlaw and Casterline (71) in a test for AHH induction in cultured Rat Hepatoma cells. Again, the 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was the most active, with all others showing varying but lesser degrees of activity. The 1,2,3,7,8-Penta isomer, the 1,2,3,4,7,8-Hexa isomer, the 1,2,3,6,7,8-Hexa

isomer, the 1,2,3,7,8,9-Hexa isomer, and the 1,3,7,8-Tetra isomers were relatively more active than the remaining isomers tested.

A similar pattern was noted in the rabbit ear bioassay for chloracne wherein the 2,3,7,8-Tetrachlorodibenzo-p-dioxin has been the most active of the isomers tested (Table 14).

TABLE 14

RESPONSE NOTED IN RABBIT EAR BIOASSAY FOR CHLORACNE WITH VARIOUS CHLORINATED DIBENZO-P-DIOXIN ISOMERS

Dibenzo-p-Dioxin Isomer	Chloracne-type Response	Concentration of Test Material, ppm
2,7-Dichlorodibenzo-p-dioxin	-	>100,000
1,2,3,4-Tetrachlorodibenzo-p-dioxin	-	50
1,3,6,8- and 1,3,7,9- Mixed Tetrachlorodibenzo-p-dioxin	-	50
	+	5,000
2,3,7,8-Tetrachlorodibenzo-p-dioxin	-	0.004
	+	0.04
Mixed Hexachlorodibenzo-p-dioxin	+	50
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	-	>100,000

Schwetz et al. 1973 [3]

Unpublished data for mixture of 1,3,6,8 and 1,3,7,9-TCDD isomers.

Some teratology studies have been conducted in rats on the isomers listed in Table 15. Of all the isomers studied, the 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) has again been the most active, when compared with the other isomers.

Animal studies for detection of carcinogenicity have been conducted with the Unsubstituted Dibenzo-p-dioxin, the 2,7-Dichlorodibenzo-p-dioxin, the 2,3,7,8-Tetrachlorodibenzo-p-dioxin, and a mixture of 1,2,3,6,7,8 and 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxins. The results of these studies, summarized in Table 16, indicate the observed pattern predicted from a comparative review of the other toxicity data.

The Unsubstituted or the 2,7-Dichloro isomers have been negative or only suggestive for carcinogenic activity, but the Hexachloro isomers appear to be somewhat similar to TCDD wherein there was some carcinogenic response when given at higher toxic dose levels.

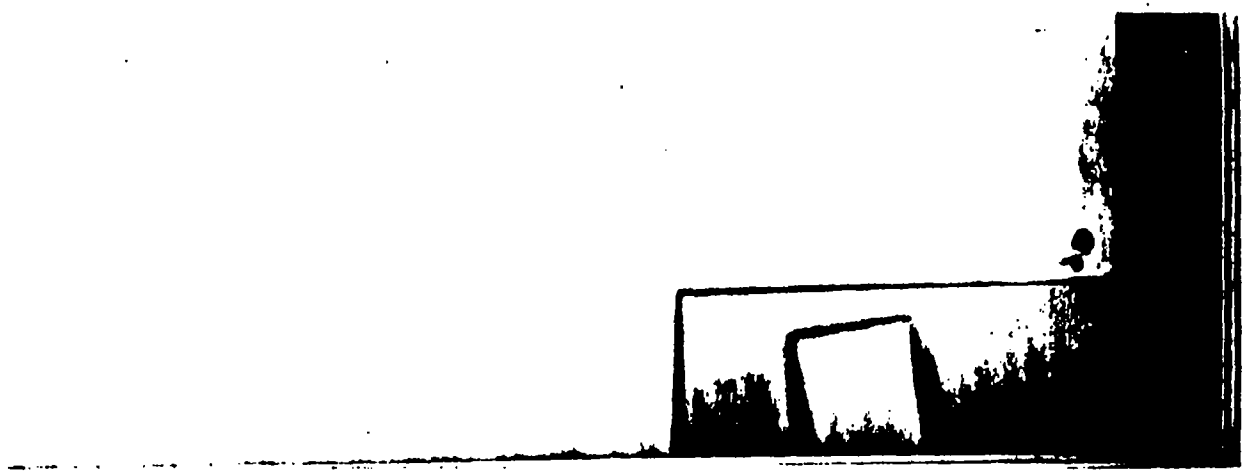

TABLE 15

COMPARISON OF RESULTS OF TERATOLOGY STUDIES OF CHLORODIBENZO-P-DIOXIN ISOMERS IN RATS

Chlorodioxin Isomer	µg/Kg/Day	Effect	Reference
2-Chlorodibenzo-p-dioxin	2,000-1,000	No fetal anomalies	[43]
2,7-Dichlorodibenzo-p-dioxin	2,000-1,000	Slight myocardial edema	[43]
	500-250	No fetal anomalies	[43]
2,3-Dichlorodibenzo-p-dioxin	2,000-1,000	No fetal anomalies	[43]
1,2,3,4-Tetrachlorodibenzo-p-dioxin	800-50	No fetal anomalies	[43]
2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.125	No fetal anomalies	[43]
	0.25-2	Fetal edema, hemorrhage	[43]
	>4	Embryolethal, toxic to dams	[43]
2,7-Dichlorodibenzo-p-dioxin	100,000	No fetal anomalies	[3]
2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.03	No fetal anomalies	[42]
	>0.125	Loss in weight, edema, hemorrhage, embryolethal	[42]
Mixed Hexachlorodibenzo-p-dioxin	100	Fetotoxic, fetal anomalies	[3]
	10-1	Subcutaneous edema	[3]
	0.1	No fetal anomalies	[3]
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	100,000	No fetal anomalies	[3]
	500,000	No fetal anomalies	[3]

TABLE 16

SUMMARY OF AVAILABLE ANIMAL DATA ON CARCINOGENICITY OF CHLORINATED DIBENZO-P-DIOXINS

Dioxin	Species	Dose Level	Results	Reference
Unsubstituted Dibenzo-p-dioxin	Rat	10,000 ppm in diet	No carcinogenic response	[72]
	Rat	5,000 ppm in diet	No carcinogenic response	
Unsubstituted Dibenzo-p-dioxin	Mouse	10,000 ppm in diet	No carcinogenic response	[72]
	Mouse	5,000 ppm in diet	No carcinogenic response	
2,7-Dichlorodibenzo-p-dioxin	Rat	10,000 ppm in diet	No carcinogenic response	[73]
	Rat	5,000 ppm in diet	No carcinogenic response	
2,7-Dichlorodibenzo-p-dioxin	Mouse	10,000 ppm in diet	Suggestive carcinogenic response	[73]
	Mouse	5,000 ppm in diet	No carcinogenic response	
2,3,7,8-Tetrachlorodibenzo-p-dioxin (See Table 8 for data.)				
1,2,3,6,7,8 & 1,2,3,7,8,9 Hexachlorodibenzo-p-dioxins (mixture)	Rat	5, 2.5 or 1.25 μg/kg/week via gavage	No carcinogenic response in male rats; carcinogenic response in female rats at higher dose levels	[74]
1,2,3,6,7,8 & 1,2,3,7,8,9 Hexachlorodibenzo-p-dioxins (mixture)	Mouse	5, 2.5 or 1.25 μg/kg/week via gavage (male mice)	Carcinogenic response at high dose level	[74]
		10, 5 or 2.5 μg/kg/week via via gavage (female mice)		

SUMMARY

The 2,3,7,8-Tetrachloro isomer of the Dibenzo-p-dioxins has been the most extensively studied of the series, and has been used as a reference point for comparison of the biologic activity of the other chlorinated Dibenzo-p-dioxins. The toxicity of the 2,3,7,8 TCDD isomer has been comprehensively examined in multiple acute, subchronic and chronic animal studies. Acute toxicity studies have shown marked species differences, with up to a 10,000-fold difference between the single oral LD_{50} dose for the guinea pig (as low as 0.6 μg/kg) and hamster (over 5000 μg/kg). With overexposure, TCDD is capable of causing an acnegenic response in man, and a somewhat similar skin response in certain animals. It is also reported to be a potent inducer of microsomal enzymes in some, but not all species. A dose-related suppression of cell-mediated immunity has been observed at higher dose levels in laboratory animals, but not in humans overexposed to TCDD and manifesting TCDD-induced acnegenic response.

TCDD causes a dose-related teratogenic response (primarily cleft palate) in mice, with the no-adverse-effect-level of 0.1 μg TCDD/kg/day. In rats, TCDD does not cause teratogenic effects, but it does cause embryo- and feto-toxicity at higher dose levels. Dose-related adverse effects on reproductive performance have also been noted in monkeys at doses that elicit maternal toxicity, and additional studies are presently underway. Multigeneration reproduction and lifetime chronic toxicity studies have been completed with TCDD in rats with the no-adverse-effect-level found to be 0.001μg TCDD/kg/day. Various mutagenic studies have been performed using *in vitro* plant and microbial test systems as well as *in vivo* tests in mammals and man. A mutagenic response was noted in a few of the *in vitro* plant and microbial test systems, but there are no definitive *in vivo* correlates of TCDD mutagenicity in higher mammals or man.

TCDD has been studied for its carcinogenic potential in both rats and mice. The results show good correlation, with a carcinogenic response noted in both species only after long-term ingestion of higher dose levels that induce organ toxicity. No carcinogenic response occurred at continuous dose levels of 0.001 - 0.0014 μg TCDD/kg/day in rats and 0.001 - 0.03 μg TCDD/kg/day in mice.

Numerous studies of tumor initiation, promotion and cocarcinogenesis have also been conducted, and data presently available are more supportive of a nongenetic (possibly promotional) rather than a genetic mechanism of TCDD carcinogenesis.

Recently reported research indicates that the biologic uptake and toxicity of TCDD may be significantly decreased if the TCDD is adsorbed onto carbon or soil particles. This newly available information is helpful in hazard assessment of exposure to TCDD.

In general, the other Chlorobenzene-p-dioxins possess lesser degrees of toxicity when compared to the 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Those isomers with (a) halogen atoms occupying at least 3 of the 4 lateral ring positions (2,3,7,8 positions), and (b) with at least one of the adjacent ring positions non-halogenated, are more likely to possess a higher degree of biologic activity when compared to the remaining isomers.

REFERENCES

(1) J. W. A. Lustenhouwer, L. Olie, and O. Hutzinger, *Chemosphere*, *5*, 501 (1980).

(2) R. R. Bumb, W. B. Crummett, S. S. Cutie, J. R. Gledhill, R. H. Hummel, R. O. Kagel, L. L. Lamparski, E. V. Luoma, D. L. Miller, T. H. Nestrick, L. A. Shadoff, R. H. Stehl, and J. S. Woods, *Science*, *210*, 385 (1980).

(3) B. A. Schwetz, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson, and C. G. Gerbig, *Environ. Hlth. Perspect.* Exp. Issue No. *5*, 87 (1973).

(4) J. A. Moore, in *Chlorinated Phenoxy Acids and Their Dioxins* (C. Ramel, ed.), *Ecol. Bull.* (Stockholm) *27*, 134 (1978).

(5) E. E. McConnell and J. A. Moore, in *Dioxin: Toxicological and Chemical Aspects*, Monograph of the Giovanni Lorenzini Foundation, *1*, 137 (1978). (Flaminio Cattabeni, Aldo Cavallaro and Giovanni Galli, eds.), Spectrum Publications, Inc., New York.

(6) E. E. McConnell and J. A. Moore, *Annals N. Y. Acad. Sci.*, *320*, 138 (1979).

(7) E. E. McConnell, J. A. Moore, J. K. Haseman, and M. W. Harris, *Toxicol. Appl. Pharmacol.*, *44*, 335 (1978).

(8) J. R. Olson, M. A. Holscher, and R. A. Neal, *Toxicol. Appl. Pharmacol.*, *55*, 67 (1980).

(9) J. W. Henck, M. A. New, R. J. Kociba, and K. S. Rao, in press, *Toxicol. Appl. Pharmacol.* (1981).

(10) P. W. Beatty, M. A. Holscher, and R. A. Neal, *Bull. Environ. Contam. & Toxicol.*, *5*, 578 (1976).

(11) J. G. Vos, J. A. Moore, and J. G. Zinkl, *Toxicol. Appl. Pharmacol.*, *29*, 229 (1974).

(12) E. E. McConnell, J. A. Moore, and D. W. Dalgard, *Toxicol. Appl. Pharmacol.*, *42*, 175 (1978).

(13) J. B. Greig, Glenys Jones, W. H. Butler, and J. M. Barnes, *Fd. Cosmet. Toxicol.*, *11*, 585 (1973).

(14) J. A. Goldstein, P. Hickman, H. Bergman, and J. G. Vos, *Rev. Comm. in Chem. Pathol. & Pharmacol.*, *6*, 919 (1973).

(15) R. J. Kociba, P. A. Keeler, C. N. Park, and P. J. Gehring, *Toxicol. Appl. Pharmacol.*, 35, (1976).

(16) A. Poland and E. Glover, *J. Biol. Chem.*, *251*, 4936 (1976).

(17) J. B. Greig, O. H. Taylor, and J. D. Jones, *Chem. Biol. Interact.*, *8*, 31 (1974).

(18) G. W. Lucier, O. S. McDaniel, G. E. R. Hook, B. A. Fowler, B. R. Sonawane, and E. Faeder, *Environ. Hlth. Perspect.*, Exp. Issue No. *5*, 199 (1973).

(19) H. M. Cunningham and D. T. Williams, *Bull. Environ. Contam. & Toxicol.*, *7*, 45 (1972).

(20) P. W. Beatty, K. J. Lambach, M. A. Holscher, and R. A. Neal, *Toxicol. Appl. Pharmacol.*, *51*, 309 (1979).

(21) R. A. Neal, P. W. Beatty, and T. A. Gasiewicz, *Annals N. Y. Acad. Sci.*, *320*, 204 (1979).

(22) H. Poiger and Ch. Schlatter, *Fd. Cosmet. Toxicol.*, *18*, 477 (1980).

(23) A. Poland and E. Glover, *Mol. Pharmacol.*, *10*, 349 (1974).

(24) A. Aitio and M. G. Parkki, *Toxicol. Appl. Pharmacol.*, *44*, 107 (1978).

(25) G. E. R. Hook, J. K. Haseman, and G. W. Lucier, *Chem.-Biol. Interact.* *10*, 199 (1975).

(26) K. T. Kitchen and J. S. Woods. *Toricol. Appl. Pharmacol.*, *47*, 537 (1979).

(27) A. Poland and A. Kende. *Fed. Proc. 35*, 2404 (1976).

(28) K. Crow. *New Scientist (England)*, pg. 78 (1978).

(29) H. Bauer, K. H. Schulz, and U. Spiegelberg. *Archiv fur Gewerbepath. und Gewerbehyg. 18*, 538 (1961).

(30) E. M. Adams, D. D. Irish, H. C. Spencer, and V. K. Rowe. *Indust. Med. & Ind. Hyg.*, *2*, 1 (1941).

(31) J. B. Greig. *Ann. Occup. Hyg.*, *22*, 411 (1979).

(32) J. G. Vos, J. G. Kreeftenberg, and L. Kstar, in Dioxin: *Toricological and Chemical Aspects*, Monograph of the Giovanni Lorenzini Foundation, *1*, 163 (1978). (Flaminio Cattabeni, Aldo Cavallara and Giovanni Galli, eds.), Spectrum Publications, Inc., N.Y.

(33) J. G. Vos, J. G. Kreeftenberg, H. W. B. Engel, A. Minderhoud, and L. M. Van Noorlejansen, *Toricol. Appl. Pharmacol.*, *9*, 75 (1978).

(34) J. G. Vos and J. A. Moore, *Int. Arch. Allergy*, *47*, 777 (1974).

(35) M. J. van Logten, B. N. Gupta, E. E. McConnell, and J. A. Moore, *Toricol. 15*, 135 (1980).

(36) E. Homberger, G. Reggiani, J. Sambeth, and H. Wipf, *The Seveso Accident: Its Nature, Extent and Consequences*, Report of the Givaudan Research Company, Ltd. (1979).

(37) G. Reggiani, *J. Toxicol. & Environ. Hlth.*, *6*, 27 (1980).

(38) K. D. Courtney and J. A. Moore, *Toricol. Appl. Pharmacol.*, *20*, 396 (1971).

(39) D. Neubert and I. Dillman, *Naunyn Schmeid. Arch. Pharmacol. 272*, 243, (1972).

(40) J. A. Moore, B. N. Gupta, J. G. Zinki, and J. G. Vos, *Environ. Hlth. Perspect.*, Exp. Issue No. *5*, 81 (1973).

(41) F. A. Smith, B. A. Schwetz, and K. D. Nitschke, *Toxicol. Appl. Pharmacol.*, *38*, 517 (1976).

(42) G. L. Sparschu, F. L. Dunn, and V. K. Rowe, *Fd. Cosmet. Toxicol.*, *9*, 405 (1971).

(43) K. S. Khera and J. A. Ruddick, "Polychlorodibenzo-p-dioxins: Perinatal Effects and the Dominant Lethal Test in Wistar Rats", in *Chlorodioxins - Origin and Fate*, Advances in Chemistry Series 120, (Eteyl H. Blair, ed.), American Chemistry Society (1973).

(44) W. P. McNulty, Unpublished data submitted to U. S. EPA (1980).

(45) Barsotti, D. A., L. J. Abrahamson and J. R. Allen, *Bull. Environ. Contam. Toxicol. 20*, 463 (1979).

(46) J. R. Allen, D. A. Barsotti, J. P. van Miller, L. J. Abrahamson and J. J. Lalich, *Fd. Cosmet. Toxicol.*, *15*, 401 (1977).

(47) S. L. Schantz, D. A. Barsotti, and J. R. Allen, *Toricol. Appl. Pharmacol.*, *48* A180 (1979).

(48) F. J. Murray, F. A. Smith, K. D. Nitschke, C. G. Humiston, R. J. Kociba, and B. A. Schwetz, *Toxicol. Appl. Pharmacol.*, *50*, 241 (1979).

(49) J. McCann (unpublished research cited by Wassom et al., in Ref. 53).

(50) D. W. Nebert, S. S. Thorgeirsson, and J. S. Felton, "Genetic Differences in Mutagenesis, Carcinogenesis, and Drug Toxicity", in *In Vitro Metabolic Activation in Mutagenesis Testing*, p. 105, (F. J. de Serres, J. R. Fouts, J. R. Bend, R. M. Philpot, eds.), Elsevier/North-Holland Biomedical Press, Amsterdam (1976).

(51) S. Hussain, L. Ehrenberg, G. Loiroth, and T. Gejvall, *Ambio. 1*, 32 (1972).

(52) J. P. Seiler, *Experientia, 29*, 622 (1973).

(53) J. S. Wasnom, J. E. Huff, and N. Loprieno, *Mut. Res., 47*, 141 (1977-1978).

(54) W. T. Jackson, *J. Cell. Sci. 10*, 15 (1972).

(55) A. Kondorosi, I. Fedorcsak, F. Solymosy, L. Ehrenberg, and S. Osterman-Golkar, *Mut. Res., 17*, 149 (1973).

(56) A. Poland and E. Glover, *Can. Res., 39*, 3341 (1979).

(57) S. Green and F. S. Moreland, *Toricol. Appl. Pharmacol., 33*, 161 (1975).

(58) S. Green, F. Moreland, and C. Shou, *FDA By-Lines, 6*, 292 (1977).

(59) M. L. Tenchini, G. Giorgi, C. Crimando, G. Simoni, F. Nuzzo, and L. DeCarli, "Approaches to Examination of Genetic Damage After a Major Hazard in Chemical Industry: Preliminary Cytogenetic Findings on TCDD-Exposed Subjects After Severe Accident". Special Project of Investigations on TCDD-Exposed Pregnancies - Prof. G. B. Candiani and Prof. L. DeCarli, University of Milan, (1977).

(60) H. Rehder, L. Sanchioni, F. Cefis, and A. Gropp, *Schweiz. Med. Wschr. 108*, 1617 (1978).

(61) R. J. Kociba, D. G. Keyes, J. E. Beyer, R. M. Carreon, C. E. Wade, D. A. Dittenber, R. P. Kalnins, L. E. Frauson, C. N. Park, S. D. Barnard, R. A. Hummel, and C. B. Humiston, *Toricol. Appl. Pharmacol., 46*, 279 (1978).

(62) National Cancer Institute, DHHS Public No. NIH80-1765 (1980).

(63) K. Toth, S. Somfai-Relle, J. Sugar, and J. Bence, *Nature, 278*, 548 (1979).

(64) J. DiGiovanni, A. Viaje, D. L. Berry, T. J. Slaga, and M. R. Juchau, *Bull. Environ. Contam. & Toricol., 18*, 552 (1977).

(65) G. M. Cohen, W. M. Bracken, R. P. Iyer, D. L. Berry, J. K. Selkirk, and T. J. Slaga, *Can Res., 39*, 4027 (1979).

(66) D. L. Berry, J. DiGiovanni, M. R. Juchau, W. M. Bracken, G. L. Gleason, and T. J. Slaga, *Res. Comm. in Chem. Path. and Pharmacol., 20*, 101 (1978).

(67) National Cancer Institute, DHHS Public No. NIH80-1757 (1980).

(68) H. C. Pitot, T. Goldsworthy, H. A. Campbell, and A. Poland, *Can. Res., 40*, 3616 (1980).

(69) R. E. Kouri, T. H. Rude, R. Jogiekar, P. M. Dansette, D. M. Jerina, S. A. Atlas, I. S. Owens, and D. W. Nebert, *Can. Res., 38*, 277 (1978).

(70) A. Poland and E. Glover, *Mol. Pharmacol., 9*, 736 (1973).

(71) J. Bradlaw and J. Casterline, *J. Assoc. Off. Analyt. Chem., 62*, 904 (1979).

(72) National Cancer Institute, Tech. Report No. 122 (1979).

(73) National Cancer Institute, Tech. Report No. 123 (1979).

(74) National Cancer Institute, Tech. Report No. 198 (1980).

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COMPARATIVE TOXICITY AND BIOLOGIC ACTIVITY OF CHLORINATED DIBENZO-P-DIOXINS AND FURANS RELATIVE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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ABSTRACT

An assessment of the comparative toxicity and biologic activity of the various chlorinated dibenzo-p-dioxins and furans indicates a range of potency extending from $\sim 10^{-1}$ to $<10^{-6}$ relative to TCDD.

INTRODUCTION

The purpose of this paper will be to perform a comparative assessment of the toxicity and biologic activity of certain of the chlorinated dibenzo-p-dioxins and furans relative to TCDD. As TCDD has been most comprehensively studied, it has been used as a reference for the other dioxins and furans.

For the sake of brevity, only limited reference will be made to specific data available in previous publications (1,2,3). The emphasis herein will be on the comparative assessment of the various isomers as measured by certain differential responses in studies of toxicity and/or biologic activity.

COMPARATIVE TOXICITY AS MEASURED BY ACUTE LETHALITY IN LABORATORY ANIMALS

Tables 1 and 2 list the comparative single oral dose LD_{50} values for sixteen different dioxins and five different furans relative to TCDD. Evaluation of the data available from as many as seven different laboratory animals in which these studies have been performed indicates some rather substantial differences in single dose oral LD_{50} values. This holds true when evaluated on the basis of interspecies response (for the same isomer) or intraspecies response (for the same species of animal tested). For example, the interspecies differential response for TCDD as measured by single oral dose LD_{50} data indicates an LD_{50} value for the guinea pig of 0.6-2 ug/kg, whereas in the hamster it is 1157-5051 ug/kg. The intraspecies differential response to different isomers is typified by the greater than six orders of magnitude difference between the LD_{50} values of 0.6-2 ug/kg for TCDD and the LD_{50} value of $>15 \times 10^6$ ug/kg for the 1,3,6,8-tetrachlorodibenzo-p-dioxin when tested in the same species (guinea pig).

The limited data presently available on the furans indicates some parallelism to the dioxins in regard to those structural characteristics associated with toxicity or the lack of toxicity as measured by acute oral lethality.

RELATIVE BIOLOGIC ACTIVITY AS MEASURED BY IN VITRO TESTS SUCH AS ENZYME INDUCTION OR EPITHELIAL KERATINIZATION

Several groups of researchers (4,5,6,7,8,9,10,11,12) have utilized certain in vitro tests whereby the biologic activities of various dioxin and furan isomers have been ranked relative to TCDD. Tables 3 and 4 are tabulations of the comparative biologic activities as measured by in vitro enzyme induction or epithelial keratinization for various dioxins and furans relative to TCDD.

Due to the overexpanding data base now being generated with various in vitro test systems, the tabulation in Tables 3 and 4 is understandably not intended to be all inclusive. More recent data such as that from the laboratory of Safe (11, 12) or Gierthy (10) will likely be presented in companion papers at this symposium. The comparative assessment in Tables 3 and 4 indicates the considerable range of biologic activity for the dioxin isomers and furan isomers that have been evaluated for biologic activity as measured by in vitro enzyme induction or epithelial keratinization.

For the chlorinated dioxins, the 1,2,3,7,8,9-Hexa-, the 1,2,3,4,7,8-Hexa-, the 1,2,3,4,7-Penta-, the 1,2,3,7,8-Penta-, the 1,3,7,8-Tetra- and the 2,3,7-Trichloro-dioxins exhibited some biologic activity relative to TCDD. Of the lesser number of chlorinated furans tested, the 2,3,7,8-Tetra-, the 1,2,3,7,8-Penta- and the 2,3,4,7,8-Pentachloro-furans exhibited biologic activities in the range of one to two orders of magnitude less than TCDD.

COMPARATIVE ASSESSMENT FOR CHLORACNEGENIC ACTIVITY IN THE RABBIT EAR BIOASSAY

The rabbit ear bioassay has been used to evaluate the acnegenic potential of a limited number of chlorinated dioxins and furan isomers listed in Table 5.

As expected, TCDD exhibited the greatest activity, with a positive rabbit ear bioassay noted when a 0.04 ppm concentration was tested. No acnegenic response occurred with TCDD when tested at a lower concentration of 0.004 ppm. A mixture of two unspecified hexachlorodioxins gave a positive response, but at a higher concentration of 10-50 ppm. A mixture of 1,3,6,8- and 1,3,7,9-tetrachlorodioxin gave a positive acnegenic response at a concentration of 5000 ppm and a negative response at a concentration of 50 ppm. The remaining dioxin and furan isomers listed in Table 5 are reportedly negative for acnegenesis in the rabbit ear bioassay.

COMPARATIVE EVALUATION OF TERATOGENICITY AND RELATED END POINTS

Studies to evaluate the potential for teratogenicity and related end points have been performed on the chlorodioxins and furans listed in Table 6. A comparative evaluation of the quantitative Lowest-Observed-Effect-Levels (LOEL) and No-Observed-Effect-Levels (NOEL) as defined in studies using the mouse or the rat is included. Relative to the data on TCDD, most of those tested thus far have been considerably less active. The exceptions are a mixture of two unspecified hexachlorodioxins and also the 2,3,7,8-tetrachlorofuran which exhibited some fractional activities relative to TCDD.

COMPARATIVE ASSESSMENT OF MUTAGENIC, CLASTOGENIC AND RELATED END POINTS

In regard to mutagenic and clastogenic potential, numerous studies of various types have been conducted with TCDD that indicate little or no potential for mutagenesis, clastogenesis or interaction with DNA. These various studies on TCDD and other chlorodioxins have been recently reviewed (2,3) and, for the sake of brevity, will only be briefly reviewed herein.

Table 7 is a compilation of data from various chlorodioxins (other than TCDD) and furans that have been evaluated. For both the chlorodioxins and furans listed in Table 7 there is an overall concurrence indicative of a relative lack of potential for mutagenesis, clastogenesis or related end points evaluated in these various types of tests.

COMPARATIVE ASSESSMENT OF CARCINOGENICITY AND LONG-TERM TOXICITY

In regard to carcinogenicity and long-term toxicity, the data base presently available is essentially that reviewed in previous papers (2,3). Thus, for the sake of brevity, only a brief review of these data will be given here.

At this time, these chlorodioxins have animal bioassay data that can be summarized as follows:

1) The unsubstituted dibenzo-p-dioxin given to rats and mice at 10,000 or 5,000 ppm in the diet elicited no carcinogenic response (13).

2) The 2,7-dichlorodibenzo-p-dioxin was also given to rats and mice at 10,000 or 5,000 ppm in the diet. No carcinogenic response was noted in either species, except for a suggestive response in mice given the 10,000 ppm level (14).

3) TCDD has elicited a tumorigenic response in rats during lifetime ingestion of 0.07 - 0.1 ug/kg/day and in mice during lifetime ingestion of 0.07 - 0.3 ug/kg/day (15,16,17). Daily dose levels of 0.001 - 0.014 ug/kg/day (rats) or 0.001 - 0.003 ug/kg/day (mice) of TCDD were tolerated for a lifetime without eliciting any increase in tumors in these studies. In the lifetime study with TCDD wherein both carcinogenicity and other chronic toxicity were evaluated (15), a lifetime No-Observed-Effect-Level of 0.001 ug/kg/day was defined for the rat species.

4) A mixture of the 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins has been given by gavage to rats and male mice (5, 2.5 or 1.25 ug/kg/week) and female mice (10, 5 and 2.5 ug/kg/week).

   The initial report of this bioassay (18) reported a carcinogenic response at the higher dose level for the female rat, the male mouse and the female mouse. However, several re-examinations of the histologic slides from this study have been stimulated by the results of a re-examination (19) rendering differing diagnoses for certain of the tumors in the study.

The various studies with 2,3,7,8-TCDD on tumor initiation, promotion and cocarcinogenesis have been reviewed previously (2,3). One of the most pertinent of these studies (20) found TCDD to be a promoter of rat tumors initiated by diethylnitrosamine. Another study (21) using the hairless HRS/J strain of mouse (those capable of chloracnegenic-like reaction of the skin), found that TCDD was a promoter of skin tumors initiated by either Dimethylbenz-anthracene or Methyl-N-Nitrosoguanidine.

Other mechanistic studies (22) indicated a relative lack of binding of TCDD to DNA (4-6 orders of magnitude less than for most chemical carcinogens). Likewise, TCDD did not stimulate unscheduled DNA synthesis when tested in rat hepatocytes (23) or in a human cell line (24).

Overall, there is a substantial amount of data available on TCDD, including the results from the lifetime bioassays, the mechanistic studies describing it as a promoter, as well as the studies finding little or no potential for either mutagenesis or DNA interaction. Evaluation of all these pertinent data supports the concept of a nongenetic (possible promoter) mechanism of carcinogenesis for TCDD.

SUMMARY

Of all the chlorinated dibenzo-p-dioxins and furans, the 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been evaluated most extensively in regard to its biologic activity and toxicologic properties. Thus, TCDD has been used as the reference for comparative evaluation of the other dioxins and furans.

A compilation of the results of various studies wherein single dose oral LD_{50} data have been generated for sixteen different dioxins and five furans relative to TCDD in as many as seven different animal species indicates marked differences in acute toxicity when evaluated on the basis of interspecies differential response (same isomer, different animal species) or on the basis of intraspecies differential response (same animal species, different isomers).

Marked differences in response have also been noted for those chlorinated dibenzo-p-dioxins and furans that have been comparatively evaluated in studies of the potential for chloracne-genesis, teratogenesis or carcinogenesis.

When evaluated for comparative biologic activity as measured by various in vitro tests for enzyme induction or epithelial keratinization, a similar wide range of differential response has been noted for the various chlorinated dibenzo-p-dioxins and furans.

Overall, this assessment of the comparative toxicity and/or biologic activity of the various chlorinated dibenzo-p-dioxins and furans indicates a range of potency extending from $\sim 10^{-1}$ to $<10^{-6}$ relative to TCDD.

REFERENCES

1. R.J. Kociba and B. A. Schwetz, Assoc. Fd. Drug Offic. Qrtrly. Bull. 46, 168 (1982).
2. R.J. Kociba, in Public Health Risks of the Dioxins, W. Lowrance, ed., William Kaufmann Public., Los Altos, Calif. (1984).
3. R.J. Kociba, in Banbury Report 18, J. Blum, Ed. Cold Spring Harbor Press, N.Y. (1984).
4. J. Bradlaw and J. Casterline, J. Assoc. Off. Analyt. Chem. 62, 904 (1979).
5. J. Bradlaw, L. Garthoff, H. Hurley and D. Firestone, Fd. Cosmet. Toxicol. 18, 627 (1980).
6. A. Poland and E. Glover, Mol. Pharmacol. 9, 736 (1973).
7. A. Poland and E. Glover, J. Biol. Chem. 251, 4936 (1976).
8. A. Poland and A. Kende, Fed. Proc. 35, 2404 (1976).
9. J. Knutson and A. Poland. Cell. 22, 27 (1980).
10. J. Gierthy, D. Crane and G. Frenkel, Fund. Appl. Toxicol. In Press (1984).
11. S. Safe, L. Safe, J. Agric. Fd. Chem. 32, 68 (1984).
12. S. Bandiera, T. Sawyer, M. Romkes, B. Zmudzka, L. Safe, G. Mason, B. Keyes and S. Safe, Toxicology 32, 131 (1984).
13. National Toxicology Program, NTP/NCI Report No. 122, NIH, Bethesda, MD (1979).
14. National Toxicology Program, NTP/NCI Report No. 123, NIH, Bethesda, MD (1979).
15. R.J. Kociba, D.G. Keyes, J.E. Beyer, R.M. Carreon, C.E. Wade, D.A. Dittenber, R.P. Kalnins, L.E. Frauson, C.N. Park, S.D. Barnard, R.A. Hummel and C.G. Humiston, Toxicol. Appl. Pharmacol. 46, 279 (1978).
16. National Toxicology Program, NTP/NCI Report No. 209, NIH, Bethesda, MD (1982).
17. K.S. Toth, S. Somfai-Relle, J. Sugar and J. Bence, Nature 278, 548 (1979).
18. National Toxicology Program, NTP/NCI Report No. 198, NIH, Bethesda, MD (1980).
19. R. Squire, R. Scuire Associates, Ruxton, MD (1983).
20. H.C. Pitot, T. Goldsworthy, H.A. Campbell and A. Poland, Cancer Res. 40, 3616 (1980).
21. A. Poland, D. Palen and E. Glover, Nature 300, 271 (1982).
22. A. Poland and E. Glover. Cancer Res. 39, 3341 (1979).
23. F.R. Althaus, S.D. Lawrence, G.L. Sattler, D.G. Longfellow and H.C. Pitot, Cancer Res. 42, 3010 (1982).
24. L. Loprieno, I. Sbrana, D. Rusciano, Lasciafari, and T. Lari, in Permagon Series on Environmental Science. Chlorinated Dioxins and Related Compounds (ed. R. Hutzinger), Vol. 5, Permagon Press, New York (1983).
25. B.A. Schwetz, J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson and C.G. Gerbig, Environ. Health. Perspect. Exp. Issue No. 5, 87 (1973).
26. E.E. McConnell, J.A. Moore, J.K. Haseman and M.W. Harris, Toxicol. Appl. Pharmacol. 44, 335 (1978).
27. E.E. McConnell, J.A. Moore and O.W. Dalgard, Toxicol. Appl. Pharmacol. 43, 175 (1978).
28. J.W. Henck, M.A. New, R.J. Kociba and K.S. Rao, Toxicol. Appl. Pharmacol. 59, 405 (1981).
29. J.R. Olson, M.A. Holscher and R.A. Neal, Toxicol. Appl. Pharmacol. 55, 67 (1980).
30. K.D. Courtney, Bull. Environ. Contam. Toxicol. 16, 674 (1976).
31. M. Bernstein, personal communication, Ciba-Geigy Corp. (1981).

32. S. Saint-Ruff, in Dioxin: Toxicological and Chemical Aspects, F. Cattabeni, A. Cavallaro and G. Galli, eds., Spectrum Public. (1978).
33. K. Kawamura, R. Sato and M. Kashima, Oyo Yakuri 25, 703 (1983).
34. J.A. Moore, B.M. Gupta and J.G. Vos, EPA Report 56016-75-004 (1976).
35. J.A. Moore, E.E. McConnell, O.W. Dalgard and M.W. Harris, in Annals N.Y. Acad. Sci. 320, 151 (1979).
36. J. McKinney and E.E. McConnell, in Chlorinated Dioxin and Related Compounds, O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiara, eds., Permagon Press, N.Y. (1981).
37. F.A. Smith, B.A. Schwetz and K.D. Nitschke, Toxicol. Appl. Pharmacol. 38, 517 (1976).
38. K.S. Khera and J.A. Ruddick, in Chlorodioxins - Origin and Fate: Advances in Chemistry Series 120, Etcyl H. Blair, ed., American Chemistry Society (1973).
39. G.J. Sparschu, F.L. Dunn and V.K. Rowe, Fd. Cosmet Toxicol. 9, 405 (1971).
40. K. Kamata, Oyo Yakuri 25, 713 (1983).
41. S. Green and F.S. Moreland, Toxicol. Appl. Pharmacol. 33, 161 (1975).
42. J.P. Seiler, Experientia 29, 622 (1973).
43. R. Schoeny, Mutat. Res 101, 45 (1982).
44. H. Weber, J.C. Lamb, M.W. Harris and J.A. Moore, Toxicol. Letters, In Press (1984).

TABLE I

COMPARATIVE SINGLE ORAL DOSE LD_{50} VALUES FOR CHLORODIBENZO-P-DIOXIN ISOMERS

Chlorodibenzodioxin	Oral LD_{50} Values (µg/kg)							
	Guinea Pig	Mouse	Rat	Monkey	Hamster	Rabbit	Dog	References
2,3,7,8-Tetra	0.6-2	114-284	22-45	~70	1157-5051	115	>300,<3,000	(25.26.27.28.29)
Unsub		>50,000	>1,000,000					(30) (32)
2,3-Di			>1,000,000					(32)
2,7-Di		>2,000,000	>1,000,000					(25)
2,8-Di	>300,000	847,000,000	>5,000,000					(26) (31)
1,3,7-Tri		>15,000,000	>5,000,000					(31)
2,3,7-Tri	29,444	>3,000	>1,000,000					(26) (32)
1,2,3,4-Tetra			>1,000,000					(32)
1,3,6,8-Tetra	>15,000,000	>2,987,000	>10,000,000					(33)
1,2,3,7,8-Penta	3.1	337.5						(26)
1,2,4,7,8-Penta	1,125	>5,000						(26)
1,2,3,4,7,8-Hexa	72.5	825						(26)
1,2,3,6,7,8-Hexa	70-100	1250						(26)
1,2,3,7,8,9-Hexa	60-100	>1440						(26)
1,2,3,4,6,7,8-Hepta	>600							(26)
Octa		>4,000,000	>1,000,000					(25)

TABLE 2

COMPARATIVE SINGLE ORAL DOSE LD_{50} VALUES FOR CHLORODIBENZOFURANS COMPARED TO TCDD

	Oral LD_{50} Values (µg/kg)							
Chlorodioxin/furan	Guinea Pig	Mouse	Rat	Monkey	Hamster	Rabbit	Dog	References
2,3,7,8-Tetradioxin	0.6-2	114-284	22-45	~70	1157-5051	115	>300,<3000	(25,26,27,28,29)
Chlorodibenzofuran								
2,8-DI		>15,000,000	>15,000,000					(31)
2,4,8-Tri		>15,000,000	>5,000,000					(31)
2,3,7,8-Tetra	5-10	>6000	>1000	1000				(34,35)
2,3,4,7,8-Penta	<10							(36)
2,3,4,6,7,8-Hexa	120							(36)

TABLE 3

COMPARATIVE BIOLOGIC ACTIVITY (IN VITRO) OF CHLORODIBENZO-P-DIOXINS RELATIVE TO TCDD

Chlorodibenzo-p-dioxin	AHH Activity in Rat Hepatoma Cells	AHH Activity in Chick Embryo Liver	ALA Synthetase in Chick Embryo Liver	Keratinization of X8/3T3 Cells
2,3,7,8-Tetra	1/1	1/1	1/1	1/1
Unsub.	Inactive	Inactive	Inactive	Inactive
1-Chloro		Inactive	Inactive	
1,3-Di	Inactive			
1,6-Di	Inactive	Inactive		Inactive
2,3-Di	Inactive	Inactive	Inactive	Inactive
2,7-Di	Inactive	Inactive	Inactive	Inactive
2,8-Di	Inactive	Inactive	Inactive	
1,2,4-Tri		Inactive	Inactive	
2,3,7-Tri	1/920-1/3060	1/1666	Active	1/100
1,3,7,8-Tetra	1/57-1/242	1/12		1/100
1,2,3,8-Tetra	1/1666-1/5900			
1,2,3,4-Tetra	Inactive	Equiv.	Inactive	
1,3,6,8-Tetra	Inactive	Inactive	Inactive	Inactive
1,2,3,7,8-Penta	1/5-1/53			1/2
1,2,3,4,7-Penta	1/21-1/132	Active	Active	
1,2,4,7,8-Penta	Inactive			
1,2,3,6,7,9-Hexa	Inactive			
1,2,4,6,7,9-Hexa	Inactive	Inact./Equiv.	Inact./Equiv.	
1,2,3,4,7,8-Hexa	1/10-1/20	Active	Active	
1,2,3,7,8,9-Hexa	1/114-1/523	1/5		1/200
1,2,3,6,7,8-Hexa	1/71-1/947			
1,2,3,4,6,7,9-Hepta	1/10,200			
1,2,3,4,6,7,8-Hepta	1/282-1/367			
Octa (99.2%)	1/1666-1/4594			
Octa (>99%)	1/53,000	Inactive	Inactive	
	(Refs. 4,5)	(Refs. 6,7,8)		(Ref. 9)

Biologic Activity expressed as fractions relative to TCDD (1/1).

TABLE 4

COMPARATIVE BIOLOGIC ACTIVITY (IN VITRO) OF CHLORODIBENZOFURANS RELATIVE TO TCDD

Chlorodioxin/furan	AHH Activity in Rat Hepatoma Cells	AHH Activity in Chick Embryo Liver	Keratinization of XB/3T3 Cells
2,3,7,8-Tetra Dioxin	1/1	1/1	1/1
Chlorodibenzofuran			
Unsub.	Inactive	Inactive	Inactive
2,8-Di	Inactive	Inactive	
2,4-Di		Inactive	
2,4,8-Tri		Inactive	
2,3,8-Tri	1/20,714		
2,4,6-Tri	Inactive		
1,4,6,8-Tetra	Inactive		
1,3,6,7-Tetra		Inactive	
2,3,6,8-Tetra	Inactive		
2,4,6,8-Tetra	Inactive		
2,3,7,8-Tetra	1/92	2/3	1/20
1,2,3,7,8-Penta		1/7	
1,3,4,7,8-Penta	1/1,928		
2,3,4,7,8-Penta		7/10	
1,2,4,7,8-Penta	1/31,428		
1,2,3,4,6,8,9-Hepta	1/24,286		
	(Ref. 4)	(Ref. 7)	(Ref. 9)

Biologic Activity expressed as fractions relative to TCDD (1/1).

TABLE 5

RABBIT EAR BIOASSAY FOR CHLORACNEGENIC ACTIVITY

Chlorodioxin or Furan		Response to Conc. (ppm) Positive	Negative	Reference
2,3,7,8-Tetra	Dioxin	0.04	0.004	(25)
Unsub.	"		Unspecif.	(32)
2,7-Di	"		100,000	(25)
2,8-Di	"		Unspecif.	(31)
2,3-Di	"		Unspecif.	(32)
1,3,7-Tri	"		Unspecif.	(31)
1,2,3,4-Tetra	"		50	(25)
1,3,6,8+ 1,3,7,9-Tetra (mixture)	"	5000	50	(1)
Unspecified Hexas (mixture of 2)	"	10-50		(25)
Octa	"		100,000	(31)
2,8-Di	Furan		Unspecif.	(31)
2,4,8-Tri	"		Unspecif.	(31)

TABLE 6

COMPARATIVE EVALUATION OF QUANTITATIVE DATA ON
FETOTOXICITY/TERATOGENICITY OF CHLORODIBENZO-P-DIOXINS AND FURANS

Chlorodibenzo Dioxin or Furan		Fetotoxicity/Teratogenicity Dosage (µg/kg/day) LOEL	NOEL	Reference
Mouse Studies:				
2.3.7.8-Tetra	Dioxin	1.0	0.1	(37)
1,2,3,4-Tetra	"		1000	(30)
Octa	"		20,000	(30)
2,3,7,8-Tetra	Furan	10-30		(44)
Rat Studies:				
2,3,7,8-Tetra	Dioxin	0.125-0.25	0.03-0.125	(38,39)
2-Mono	"		2000	(38)
2,3-Di	"		2000	(38)
2,7-Di	"	1000	500	(38)
2,7-Di	"		100,000	(25)
2,8-Di	"		not specif.	(31)
1,3,7-Tri	"		not specif.	(31)
1,3,6,8-Tetra	"		3,000,000	(40)
1,2,3,4-Tetra	"		800	(38)
Unspec. Hexas (mixture of 2)	"	1-10	0.1	(25)
Octa				
2,8-Di	Furan		not specif.	(31)
2,4,8-Tri	"		not specif.	(31)

TABLE 7

COMPARATIVE DATA ON EVALUATION OF POTENTIAL FOR MUTAGENESIS, CLASTOGENESIS AND RELATED END POINTS

Chlorodioxin or Furan		Tests and Results
2,3,7,8-Tetra	Dioxin	See (2,3) for Reviews
Unsub.	Dioxin	No chromosomal aberrations in rats (41)
2,7-Di	Dioxin	No chromosomal aberrations in rats (41)
2,8-Di	Dioxin	No cytogenetic or dominant lethal effects in Chinese hamster or mouse; No mutagenic response in Salmonella sp. (31)
1,3,7-Tri	Dioxin	Same tests and results as given above for 2,8-Di Isomer (31)
Octa	Dioxin	No mutagenic response in strains TA1530, TA1531, G46; "doubtful mutagenicity" in strains TA1532 and TA1534 (42)
Unsub.	Furan	No mutagenic response with 8/8 strains of Salmonella sp. (43)
2,8-Di	Furan	No mutagenic response with 11/11 strains of Salmonella sp. (43)
		No cytogenetic or dominant lethal effects in Chinese hamster or mouse; No mutagenic response in Salmonella sp. (31)
3,6-Di	Furan	No mutagenic response with 10/10 strains of Salmonella sp. (43)
2,4,8-Tri	Furan	No cytogenetic or dominant lethal effects in Chinese hamster or mouse; No mutagenic response in Salmonella sp. (31)
2,3,7,8-Tetra	Furan	No mutagenic response with 5/5 strains of Salmonella sp. (43)
Octa	Furan	No mutagenic response with 9/9 strains of Salmonella sp. (43)

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DEATHS AMONG TCDD-EXPOSED WORKERS FOUND NOT TO EXCEED NATIONAL AVERAGE

Deaths among a group of 121 workers exposed to TCDD (dioxins) in 1949 were not in excess of national mortality figures, according to a study noted by Monsanto this week.

All of the employees had developed chloracne following an accident at a Monsanto plant, but through long-range follow-up of plant records, workmen's compensation files and death certificates, it was found that total deaths (32) were less than expected compared to national averages and that there was no apparent excess in deaths from cancer or cardiovascular disease.

The study of the 121 workers is part of a larger study of 400 workers by Dr. Raymond Suskind, Director of the Institute of Environmental Health at the University of Cincinnati Medical Center, and Monsanto epidemiologist Judith Zack.

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Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, Kepone, and Polybrominated Biphenyls on Transport Systems in Isolated Rat Hepatocytes[1]

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Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, Kepone, and Polybrominated Biphenyls on Transport Systems in Isolated Rat Hepatocytes. EATON, D. L., AND KLAASSEN, C. D. (1979). *Toxicol. Appl. Pharmacol.* 51, 137–144. Adult male rats recived intraperitoneal injections of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 10 μg/kg), polybrominated biphenyls (PBBs, 200 mg/kg), Kepone (5 mg/kg/day for 5 days), or vehicle, and isolated hepatocytes were prepared 10, 3, or 1 day after injection of TCDD, PBBs, or Kepone, respectively. All treatments significantly increased the hepatic microsomal content of cytochrome *P*-450. The initial velocity of uptake (V_0; measured within the first 2 min), steady-state concentration (SS, measured over 1 hr), and an indirect estimate of an efflux rate constant, k_{ef}, were determined for ouabain and procaine amide ethobromide (PAEB). TCDD decreased V_0 and SS of ouabain but had no effect on k_{ef} for ouabain or any parameter of PAEB transport. PBBs tended to increase k_{ef} and significantly decreased SS of ouabain. The k_{ef} for PAEB was significantly increased, but V_0 and SS were not changed in PBB-treated rats. Kepone had no effect on V_0 of either substrate but significantly decreased SS and increased k_{ef} for ouabain. These data demonstrate that the ouabain transport system is selectively inhibited by TCDD and that PBBs may nonspecifically increase the rate of efflux of actively transported substrates from cell to medium.

Considerable attention has recently been devoted to the hepatotoxic and enzyme inducing effects of several environmental contaminants. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a very toxic contaminant of various technical chlorinated compounds such as the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which are widely used in agriculture (Schwetz *et al.*, 1973). TCDD has been shown to both induce and suppress a variety of microsomal enzyme systems (Poland and Glover, 1974; Hook *et al.*, 1975; Beatty and Neal, 1976). Pretreatment with a single dose (5 to 25 μg/kg) causes an increase in liver microsomal enzyme activity that generally reaches a peak 3 to 10 days after administration. Histologic damage of the liver is not evident at these doses, in contrast to higher doses of TCDD which induce jaundice and degenerative necrotic and regenerative changes in the liver of rats (Gupta *et al.*, 1973; Greig *et al.*, 1973).

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[2] Supported by U.S.P.H.S. Training Grant GM-07044.

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In contrast to the stimulatory effects of phenobarbital on biliary drug clearance via increased excretion into the bile (Klaassen, 1970), Hwang (1973) demonstrated that TCDD pretreatment produced a decrease in biliary excretion and an increase in hepatic content and plasma concentration of an administered dose of indocyanine green (ICG) and thus suggested that TCDD caused a reduction in both hepatic uptake and biliary excretion of ICG. Yang *et al.* (1977) examined the effects of pretreatment with a low (10 μg/kg) and high (25 μg/kg) dose of TCDD on the plasma disappearance of sulfobromophthalein (BSP), phenol-3,6-dibromphthalein disulfonate (DBSP), and ouabain in rats at several periods after TCDD administration. Both doses of TCDD decreased hepatic uptake and biliary excretion of ouabain 10 days after administration, but the biliary excretion of BSP and DBSP was enhanced by the low dose and depressed by the high dose 10 days after treatment.

Kepone[4] and mirex are organochlorine insecticides which are quite persistent in the environment. Fabacher and Hodgson (1976) found a dose-related increase in hepatic microsomal protein, cytochrome *P*-450, and *N*-demethylase activities in the mouse after dietary exposure to Kepone. Similar results were reported by Mehendale *et al.* (1978) in the rat. Mehendale (1977a,b) examined the effects of pretreatment with mirex and Kepone on biliary excretion of imipramine and BSP in the isolated perfused rat liver. Both Kepone and mirex produced a marked suppression in the biliary excretion of imipramine metabolites without altering the rate of its hepatic biotransformation. BSP excretion was reduced by preexposure to mirex (Mehendale, 1977a), but Kepone exposure had no effect on the biliary excretion of BSP (Mehendale, 1977b).

Polybrominated biphenyls (PBBs), manufactured as a flame retardant in plastics and electronic parts, have been identified as environmental contaminants. When compared to phenobarbital and 3-methylcholanthrene, the hepatic microsomal enzyme induction produced by a commerical mixture of PBBs was found to have some properties of both agents, but also exhibited some differences in stimulating activity (Dent *et al.*, 1978). The broad range of enzyme induction is apparently due to different inducing capabilities of several isomers within the commerical mixture, since the primary isomer, 2,2',4,4',5,5'-hexabromobiphenyl, has enzyme-inducing effects which are different from those of the mixture of isomers (Moore *et al.*, 1978). Studies by Cagen *et al.* (1977) in the mouse demonstrated that dietary exposure to PBBs resulted in enhanced plasma disappearance of ICG. In another study, Cagen and Gibson (1977) found that the plasma disappearance of ouabain was increased in developing rats (up to 21 days of age), but not in adults, after exposure to a mixture of PBBs, suggesting that these compounds may stimulate the development of the ouabain excretory system which is underdeveloped in newborn rats (Klaassen, 1972).

The purpose of the present study is to utilize isolated hepatocytes to study the effects of these toxicants on the carrier-mediated transport systems responsible for ouabain and PAEB accumulation in the liver.

METHODS

Animals. Male Sprague–Dawley rats (Bio-Lab, White Bear, Minn.) were used as liver donors in all studies. Rats were housed in a temperature-controlled environment with a 12-hr light cycle. Food was withheld for 12 hr prior to the isolation of hepatocytes and water was provided *ad libitum*.

Chemicals and treatments. [^{3}H]Ouabain (12 Ci/mmol) and [^{14}C]PAEB (4.13 mCi/mmol) were obtained from New England Nuclear Corp (Boston, Mass.). TCDD (99%+ purity) was a gift from Dow Chemical Company (Midland, Mich.). A commercial mixture of PBBs (Firemaster BP-6, PBBs) was kindly supplied by Pesticide Research Center, Michigan State University (Lansing, Mich.), and Kepone (chlordecone) was a gift from Dr. H. M. Mehendale, (University of Mississippi, Jackson, Miss.). TCDD,

[4] Kepone is the trade name for a commercial pesticide, decachloroactahydro-1,3,4-metheno-2 cyclobuta[cd]-pentalen-2-one (chlordecone).

PBBs, and Kepone were dissolved or suspended in acetone/corn oil (0.5/9.5 v/v) and were administered intraperitoneally in a volume of 1.0 ml/kg. Rats receiving TCDD were administered a dose of 10 μg/kg 10 days before the experiment was to be performed. PBB-Treated rats received 200 mg/kg PBBs 3 days prior to isolation of hepatocytes. Kepone-treated rats were given 5 mg/kg/day for 5 consecutive days, and hepatocytes were isolated about 24 hr after the last dose. Control animals received the vehicle either 3 days before cell preparation or once daily for 5 days, and the cells were then isolated 24 hr after the last administration.

Isolation of hepatocytes. Suspensions of purified hepatic parenchymal cells were prepared by the method of Baur *et al.* (1975) as described in detail previously (Eaton and Klaassen, 1978a). Cell viability was determined by trypan blue exclusion, and in every preparation viability was greater than 90% with most preparations between 94 and 97%. Cells were suspended in a medium containing: 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1.2 mM $CaCl_2$, 3.0 mM Na_2HPO_4, and 10 mM Tris–HCl at pH 7.40.

Experimental design. Cell suspensions (1.5–2.5 mg protein/ml) were preincubated at 37°C for 10 min under 95% O_2/5% CO_2 prior to addition of substrate. After preincubation, 200 μl of a substrate solution was added to 7.8 ml of cell suspension to bring the final substrate concentration to the desired level. The substrates and final concentrations used were: ouabain (150 μM, 250 nCi/ml) and PAEB (50 μM, 125 nCi/ml).

After addition of the substrate at zero time, duplicate 200-μl samples of each cell suspension were removed at 0.5, 1.0, 1.5, 2.0, 5, 10, 20, 30, 45, and 60 min and were placed in 400-μl polyethylene microcentrifuge tubes layered with 50 μl of 3 M KOH and 50 μl of silicone oil ($d = 1.05$). The samples were centrifuged at the designated times and the amount of substrate within the cell fraction was quantitated by cutting the polyethylene microcentrifuge tubes at the oil–KOH interface and counting the radioactivity in the pellet fraction. Little or no radioactivity was found in the oil layer. Fifty-microliter samples obtained from the remainder of each cell suspension were also counted, and the amount of substrate within the pellet fraction was calculated based on the known concentration and radioactivity in the total cell suspension. The pellet fractions containing KOH were neutralized with HCl before counting.

Calculations. Initial velocities of uptake (V_0) were calculated as the slope of the least squares regression line of the plot of substrate vs time obtained with the 0.5-, 1.0-, 1.5-, and 2.0-min points. Steady-state concentrations were determined by averaging the 45- and 60-min time points for each experiment. Efflux rate constants were obtained indirectly by computing the ratio of initial velocity of uptake to steady state, according to the following assumptions:

(1) all uptake and efflux processes are first order at the substrate concentrations used, and the external substrate concentration, $(S)_1$, is not appreciably changed by the uptake;

(2) the V_0 obtained experimentally is an estimate of the total uptake process, such that $V_0 \approx k_{in} \cdot (S)_1$;

(3) at steady state, uptake equals efflux, such that

$$k_{in} \cdot (S)_1 = k_{ef} \cdot (S)_2;$$

(4) at steady state, the concentration of substrate in the cellular fraction approximate $(S)_2$, thus,

$$\frac{k_{in}(S)_1}{(S)_2} \approx k_{ef} \text{ or } \frac{V_0}{SS} \approx k_{ef},$$

where SS is the steady-state concentration.

Assays. Cellular protein was determined by the method of Lowry *et al.* (1951). Cell counts were determined by counting at least three different cell suspensions, and each cell suspension was counted in duplicate using four 1 × 1 × 0.1-mm fields on an improved Neubauer counting chamber. Cytochrome *P*-450 was determined by homogenizing 5.0 ml of a stock cell suspension (35–50 mg protein/ml) with a Polytron homogenizer/sonicator (Brinkman, Lucerne, Switzerland). The cell homogenate was centrifuged at 7000*g* for 20 min, and the supernatant from this was then centrifuged at 100,000*g* for 60 min. The microsomal pellet was resuspended in 10 ml of incubation buffer, and the amount of cytochrome *P*-450 was quantitated according to the method of Omura and Sato (1964) as described by Mazel (1971).

Statistical analysis. All data were compared using a one-way analysis of variance. Individual differences between treatment groups and the control were determined with the least significant difference (LSD) test (Steel and Torrie, 1960). The level of statistical significance was set at the 95% level using a two-tailed comparison.

RESULTS

The effects of treatment with TCDD, Kepone, and PBBs on several parameters indicative of alterations in hepatic architecture are shown in Table 1. TCDD and PBBs tended to increase both liver mass and the protein content of the cells, but these were not statistically significant. All three treatments significantly increased hepatic cytochrome *P*-450. PBBs were the most effective inducers at the dose employed, increasing the *P*-450 value to 2.5 times the control. All treatments also tended to increase microsomal protein content (Table 1).

TABLE 1

EFFECT OF TOXICANTS ON VARIOUS PARAMETERS OF CELL SIZE[a]

Treatment	Liver weight (g/kg)	mg Protein / 10^6 cells	Cytochrome P-450 nmol / mg microsomal protein	mg microsomal protein / mg total protein
Control	37.3 ± 1.5	2.14 ± 0.10	0.71 ± 0.08	0.110 ± 0.015
TCDD	46.4 ± 5.4	2.50 ± 0.35	1.54 ± 0.14[b]	0.161 ± 0.017
Kepone	38.4 ± 1.9	2.17 ± 0.21	1.36 ± 0.08[b]	0.159 ± 0.022
PBBs	41.7 ± 0.5	2.62 ± 0.09	1.97 ± 0.08[b]	0.180 ± 0.021

[a] Each value represents the mean and SE of three or four experiments.
[b] Statistically significant ($p < 0.05$).

TABLE 2

EFFECTS OF TOXICANTS ON THE INITIAL VELOCITY OF UPTAKE (V_0) OF OUABAIN AND PAEB[a]

Treatment	Ouabain		PAEB	
	nmol/min / mg protein	nmol/min / 10^6 cells	nmol/min / mg protein	nmol/min / 10^6 cells
Control	0.811 ± 0.059	1.744 ± 0.172	0.077 ± 0.006	0.163 ± 0.014
TCDD	0.453 ± 0.060[b]	1.060 ± 0.028[b]	0.081 ± 0.009	0.197 ± 0.022
Kepone	0.726 ± 0.055	1.540 ± 0.047	0.066 ± 0.002	0.143 ± 0.016
PBBs	0.621 ± 0.043	1.632 ± 0.150	0.082 ± 0.008	0.215 ± 0.024

[a] Concentrations of ouabain and PAEB were 150 and 50 μM, respectively. Each value represents the mean and SE for three or four experiments.
[b] Statistically significant ($p < 0.05$).

Because of the tendency for TCDD and PBBs to increase cellular protein content, it was necessary to express uptake data on the basis of cell number in addition to the commonly used index of protein content. Table 2 shows the effects of toxicant treatment on the initial uptake velocities (V_0) for 150 μM ouabain and 50 μM PAEB. TCDD decreased the V_0 for ouabain by 44% but had little effect on PAEB uptake velocity. Neither Kepone nor PBB treatment significantly affected uptake velocity of either substrate (Table 2).

Previous studies have demonstrated that the accumulation of both ouabain and PAEB approach a steady state after 45–60 min of incubation (Eaton and Klaassen, 1978a,b), and similar results were obtained in this study (data not shown). Steady-state concentrations of both substrates in hepatocytes from control and treated rats were obtained by averaging data obtained at 45 and 60 min of incubation. Expressed on the basis of cell number, all treatments significantly decreased the steady-state concentration of ouabain, but none affected PAEB steady state (Table 3). Identical statistical differences were obtained when the data were expressed on the basis of cellular protein (data not shown).

Indirect estimates of the efflux rate constants for ouabain and PAEB were obtained by computing the ratio of V_0 to steady state for each treatment. Kepone treatment significantly increased the calculated efflux rate constant (k_{ef}) for ouabain but had no effect on that for PAEB. PBBs significantly increased the efflux rate for PAEB and also tended to increase ouabain k_{ef}, though this was not statistically significant (Table 3).

TABLE 3

EFFECTS OF TOXICANTS ON STEADY-STATE CONCENTRATIONS AND EFFLUX RATE CONSTANTS FOR OUABAIN AND PAEB[a]

	Ouabain		PAEB	
Treatment	Steady state (mmol/10⁶ cells)	$k_{ef} \times 10^{2}$[b] (min^{-1})	Steady state (mmol/10⁶ cells)	k_{ef} (min^{-1})
Control	32.1 ± 1.5	5.4 ± 0.5	2.31 ± 0.17	7.2 ± 0.5
TCDD	24.2 ± 0.2[c]	4.4 ± 0.1	2.70 ± 0.13	7.3 ± 0.6
Kepone	22.7 ± 1.1[c]	6.8 ± 0.3[c]	2.11 ± 0.13	6.7 ± 0.4
PBBs	24.7 ± 2.0[c]	6.6 ± 0.5	2.17 ± 0.11	9.9 ± 1.1[c]

[a] Each value represents the mean ± SE of three or four experiments.
[b] Calculated at the ratio of V_0/steady state.
[c] Statistically significant ($p < 0.05$).

DISCUSSION

A previous study from this laboratory demonstrated that there is no correlation of induction of microsomal proteins with alterations in hepatic transport (uptake) systems in isolated rat hepatocytes (Eaton and Klaassen, 1979). The present study extends those observations to include the effects of three environmental contaminants on microsomal protein induction and hepatic transport processes.

By experimentally determining both the initial velocity of uptake and the steady-state concentration of a substrate in hepatocytes, an indirect estimate of the effects of treatments on the efflux of substrate from cell to medium can be obtained. This efflux rate will be the composite of at least three possible processes: (1) backflux of the uptake carrier system, (2) "forward" transport of substrate from cell to medium via canalicular excretory processes, and (3) simple diffusion of substrate from cell to medium. Thus alterations in efflux rate constants do not necessarily reflect alterations in biliary excretory processes, since changes in membrane permeability associated with the treatment may also alter diffusion processes.

Consideration of the composite effects of treatments on V_0, steady-state concentrations and k_{ef} values for ouabain and PAEB lead to the following conclusions.

TCDD. TCDD pretreatment decreased the V_0 of ouabain by 44% and the steady content by 32% but had no effect on PAEB transport. Because the decrease in steady state was of a magnitude similar to that for V_0, no effect on the k_{ef} was apparent. The selective toxicity of TCDD on the ouabain transport system is in agreement with the studies of Yang *et al.* (1977), who found that TCDD inhibited the plasma disappearance and biliary excretion of ouabain *in vivo*. Later studies by Hamada and Peterson (1978) further demonstrated that, *in vivo*, TCDD inhibits uptake and excretion of ouabain, but that this effect is reversed by pretreatment with pregnenolone-16α-carbonitrile (PCN) and spironolactone, but not phenobarbital or 3-methylcholanthrene. Because of the number of variables involved in *in vivo* studies of this nature, these authors were not able to experimentally define the exact site of action of TCDD on ouabain uptake and excretion or the mechanism by which PCN reversed this effect. However, their data did demonstrate that: (1) the beneficial effect of PCN and spironolactone on hepatic clearance of ouabain does not involve a reduction in the hepatic content of TCDD, (2) these agents do not reverse or protect against the decrease in bile flow or loss of body weight produced by TCDD, and (3) the positive effect of PCN and spironolactone on ouabain transport is not solely dependent

on the presence of these compounds in the liver, but rather chronic treatment is necessary for this effect to occur. From these data these authors suggested that TCDD may damage or inhibit the carrier system for ouabain in some way and that pretreatment with PCN might increase the *de novo* synthesis of ouabain carrier proteins, thus reversing the inhibitory effect of TCDD.

The present study with isolated hepatocytes demonstrates that TCDD does inhibit, apparently specifically, the uptake carrier system for ouabain. Recent studies from our laboratory (Eaton and Klaassen, 1979) have also demonstrated that pretreatment of rats with PCN does indeed selectively stimulate the synthesis of carrier proteins for the ouabain transport system. Thus the data obtained in this and a previous study are in excellent agreement with the hypothesis of Hamada and Peterson (1978) that TCDD selectively inhibits the ouabain transport system and that this effect is reversed by PCN via an increase in synthesis of ouabain carrier units. The quantitative relationship of the effects of TCDD and PCN on ouabain transport units is also in agreement with the *in vivo* studies of Peterson and co-workers (Yang *et al.*, 1977; Hamada and Peterson, 1978). We found about a 50% decrease in the transport of ouabain after TCDD and a two fold increase in the V_{max} of the ouabain carrier system after PCN treatment. Although we have not experimentally determined the effects of these combined treatments on ouabain transport in isolated hepatocytes, from the quantitative relationship of the two separate effects, one might expect that combined treatments would result in essentially "normal" transport characteristics for ouabain in isolated hepatocytes. This is, in fact, what Hamada and Peterson (1978) demonstrated *in vivo* after the combined treatments.

Kepone. Although Kepone significantly increased microsomal cytochrome *P*-450, no effect of this treatment on the V_0 of ouabain or PAEB was evident at the dose employed in this study. However, steady-state concentration of ouabain was significantly decreased, apparently because of an increase in the rate of efflux of ouabain from cell to medium. This did not appear to be the result of a nonspecific effect on membrane permeability because the efflux of PAEB was not increased by Kepone treatment.

No published studies have examined the effects of Kepone on ouabain or PAEB clearance in rats, but Mehendale (1977a,b) did show that both Kepone and mirex induce hepatobiliary dysfunction as determined by clearance of imipramine and its metabolites in the isolated perfused rat liver, but have no effect on the clearance of BSP. The data presented in this study also suggest that the effect on imipramine noted by Mehendale (1977a,b) was not a result of general impairment of hepatic membrane function.

PBB. Treatment with PBBs had no significant effect on the V_0 values for ouabain or PAEB, but the steady state of ouabain was significantly decreased and the k_{ef} for PAEB was increased. The significant decrease in ouabain steady state was apparently the result of the tendency for PBBs to both decrease the V_0 and increase k_{ef}, though neither change was statistically significant. PBBs tended to increase the V_0 of PAEB and significantly increased the efflux rate constant, thus resulting in no significant effect on steady-state concentration of PAEB.

Previous *in vivo* studies suggest that PBB exposure will enhance the plasma elimination of ouabain in mice (Cagen and Gibson, 1977), but other studies showed no effect of PBBs on the ouabain transport system of adult rats (Cagen *et al.*, 1977). However the study of Cagen *et al.* (1977) did demonstrate that PBBs enhanced the rate of maturation of the ouabain transport system in neonatal rats, which is not fully developed until about 28 days of age (Klaassen, 1972). The present study also suggests that PBBs have little or no effect on ouabain transport in mature rats. However, the significant increase in k_{ef} for PAEB and the tendency for an increase in the

k_{ef} for ouabain suggest that PBBs may nonspecifically increase the permeability of the isolated hepatocyte membrane, resulting in an increase in diffusion of substrates from cell to medium at later time points when the concentration of substrates in the cell becomes high (Eaton and Klaassen, 1978a,b).

In conclusion, the data in this study demonstrate that the hepatic membrane transport process for ouabain may be selectively damaged by TCDD and thus support the suggestion by Peterson and co-workers (Yang *et al.*, 1977; Hamada and Peterson, 1978) that such information may eventually prove useful in the development of a simple clinical assay for the detection of hepatocellular dysfunction produced by TCDD. The selectivity of such an assay for TCDD is also suggested since Kepone and PBBs were not as effective as TCDD in altering the ouabain transport system.

REFERENCES

BAUR, H., KASPEREK, S., AND PFAFF, E. (1975). Criteria of viability of isolated liver cells. *Hoppe-Seyler's Z. Physiol. Chem.* 356, 827–838.

BEATTY, P., AND NEAL, R. A. (1976). Induction of DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Biochem. Biophys. Res. Commun.* 68, 197–204.

CAGEN, S. Z., PREACHE, M. M., AND GIBSON, J. E. (1977). Enhanced disappearance of drugs from plasma following polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* 40, 317–325.

CAGEN, S. Z., AND GIBSON, J. E. (1977). Ouabain lethality as a measure of biliary function in developing mice and rats and effects of polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* 40, 327–334.

DENT, J. G., ELCOMBE, C. R., NETTER, C. J., AND GIBSON, J. E. (1978). Rat hepatic microsomal cytochrome(s) *P*-450 induced by polybominated biphenyls. *Drug Metab. Dispos.* 6, 96–101.

EATON, D. L., AND KLAASSEN, C. D. (1978a). Carrier-mediated transport of ouabain in isolated hepatocytes. *J. Pharmacol. Exp. Ther.* 205, 480–488.

EATON, D. L., AND KLAASSEN, C. D. (1978b). Carrier-mediated transport of the organic cation procaine amide ethobromide into isolated rat liver parenchymal cells. *J. Pharmacol. Exp. Ther.* 20, 595–606.

EATON, D. L., AND KLAASSEN, C. D. (1979). Effects of microsomal enzyme inducers on hepatic transport systems in isolated rat hepatocytes. *J. Pharmacol. Exp. Ther.* 208, 381–385.

FABACHER, D. L., AND HODGSON, E. (1976). Induction of hepatic mixed-function oxidase enzymes in adult and neonatal mice by Kepone and mirex. *Toxicol. Appl. Pharmacol.* 38, 71–77.

GREIG, S. B., JONES, G., BUTLER, W. H., AND BARNES, J. M. (1973). Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Food Cosmet. Toxicol.* 11, 589–595.

GUPTA, B. N., VOS, J. G., MOORE, J. A., ZINKL, S. G., AND BULLOCK, B. C. (1973). Pathologic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. *Environ. Health Perspect.* Exp. Issue No. 5, 125–140.

HAMADA, N., AND PETERSON, R. E. (1978). Effect of microsomal enzyme inducers on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced depression in the biliary excretion of ouabain in rats. *Drug Metab. Dispos.* 6, 456–464.

HOOK, G. E. R., ORTON, T. C., MOORE, J. A., AND LUCIER, G. W. (1975). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced changes in the hydroxylation of biphenyl by rat liver microsomes. *Biochem. Pharmacol.* 24, 335–340.

HWANG, S. W. (1973). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the biliary excretion of indocyanine green in rat. *Environ. Health Perspect.* Exp. Issue No. 5, 227–231.

KLAASSEN, C. D. (1970). Effects of phenobarbital on the plasma disappearance and biliary excretion of drugs in rats. *J. Pharmacol. Exp. Ther.* 175, 289–300.

KLAASSEN, C. D. (1972). Immaturity of the newborn rat's hepatic excretory function for ouabain. *J. Pharmacol Exp. Ther.* 183, 520–526.

KLAASSEN, C. D. (1974). Effect of microsomal enzyme inducers on the biliary excretion of cardiac glycosides. *J. Pharmacol. Exp. Ther.* 191, 201–211.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.

MAZEL, P. (1971). General principles and procedures for drug metabolism *in vitro*. In *Fundamentals of Drug Metabolism and Drug Disposition* (B. N. LaDu, H. G. Mandel, and E. L. Way, eds.), pp. 546–582. Williams and Wilkins, Baltimore.

MEHENDALE, H. M. (1977a). Mirex-induced impairment of hepatobiliary function: Suppressed biliary excretion of imipramine and sulfobromophthalein. *Drug. Metab. Dispos.* 5, 56–62.

MEHENDALE, H. M. (1977b). Effects of preexposure to Kepone on the biliary excretion of imipramine and sulfobromophthalein. *Toxicol. Appl. Pharmacol.* 40, 247–259.

MEHENDALE, H. M., TAKANAKA, A., DESAIAH, D., AND HO, T. K. (1978). Effect of preexposure to Kepone on hepatic mixed-function oxidases in the female rat. *Toxicol. Appl. Pharmacol.* 44, 171–180.

MOORE, R. W., SLEIGHT, S. D., AND AUST, S. D. (1978). Induction of liver microsomal drug-metabolizing enzymes by 2,2',4,4',5,5'-hexabromobiphenyl. *Toxicol. Appl. Pharmacol.* 44, 309–321.

OMURA, T., AND SATO, R. (1964). The carbon monoxide binding pigment of liver microsomes. *J. Biol. Chem.* 239, 2370–2378.

POLAND, A., AND GLOVER, E. (1974). Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* 10, 349–359.

SCHWETZ, B. A., NORRIS, J. M., SPARSCHU, G. L., ROWE, V. K., GEHRING, P. J., EMERSON, J. L., AND GERBIG, C. G. (1973). Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect.* Exp. Issue No. 5, 87–100.

STEEL, R. G., and TORRIE, J. H. (1960) *Principles and Procedures of Statistics*, McGraw–Hill, New York.

YANG, K. H., CROFT, W. A., AND PETERSON, R. E. (1977). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on plasma disappearance and biliary excretion of foreign compounds in rats. *Toxicol. Appl. Pharmacol.* 40, 485–496.

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Environmental Contamination and Toxicology

Determination and Measurement of Human Exposure to the Dibenzo-P-dioxins

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One of the major problems in the conduct of epidemiologic studies involving dioxins is in the identification of an exposed population. This is true whether one is talking about Vietnam veterans, Times Beach residents, children of Seveso, Italy, or individuals who consume dioxin-contaminated fish in Michigan or New York. Because dioxin, especially 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), occurs as an environmental contaminant only in trace quantities, its measurement in environmental substrates and human tissue requires complicated extraction procedures and highly sophisticated instrumentation, namely high resolution gas chromatography and high resolution mass spectrometry. To properly address the issue of documenting dioxin exposure, a review of literature is necessary.

Accidental exposure to TCDD. Confirmation that TCDD accumulates in human tissues was documented by Facchetti et al. (1981). This was the first case of human exposure to TCDD in which an analysis was made of cadaveric tissue to detect and study the distribution of dioxin. The subject of the study was a 55-year-old woman who had died from a pancreatic adenocarcinoma seven months after the ICMESA accident in Seveso, Italy in July, 1976. Although the cancer was not a result of the exposure to TCDD, the woman was significantly exposed to the toxic cloud. During the passage of the toxic cloud, the woman was eating a meal in her home with doors and windows open. In the four days after the event, the woman consumed vegetables from the garden attached to her home. Animals reared by the woman's family in an area adjacent to the home began to die over a period of 15 days after the event. The woman was evacuated from her home and associated area after 16 days. Subsequent tests for TCDD indicated that the subject had lived in a sector of zone A which had a mean soil concentration of 185 ug/m^2. On the basis of the circumstances recorded, it was presumed that the woman absorbed toxic substances contained in the cloud by inhalation, ingestion and contact. Although she did not develop toxic symptoms from the exposure, two young nephews living with her at the time of the accident devloped serious chloracne. The results (means of three independent determinations) for the GC-MS analysis for 2,3,7,8-TCDD in selected human tissues are shown in Table 1.

Table 1. Tissue concentrations, parts per trillion, of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the organs of a woman who died of cancer seven months after exposure, Seveso, Italy[a]

Sample	2,3,7,8-TCDD/wet tissue (ppt)
Fat	1840[b]
Pancreas	1040[bc]
Liver	150[bc]
Thyroid	85[c]
Brain	60[b]
Lung	60[b]
Kidney	40[b]
Blood	6[c]

[a] Data from Fachetti et al., 1981

[b] Values obtained at a resolution of 2,500

[c] Values obtained at a resolution of 10,000

As noted, 2,3,7,8-TCDD was present in all of the tissues analyred. On the basis of concentrations observed, it was possible to distinguish four groups of tissues: adipose tissue and pancreas tissue with levels between 1,000 and 2,000 parts per trillion (ppt); liver tissue with levels between 100 and 200 ppt; other tissues (thyroid, brain, lungs, kidneys) with levels between 10 and 100 ppt; and blood with levels less than 10 ppt. The levels in the pancreas may have been abnormally high due to the presence of cancerous cells.

The data suggest that blood levels are one-tenth the level of TCDD in liver tissue and one one-hundredth the level in adipose tissue. Reggiani (1981), estimated that the TCDD body burden in the above subject at the time of death was 40 ug. Because human milk has a high fat content where TCDD could reside, Reggiani examined TCDD levels in human milk from those exposed to TCDD while living in Zones A and B of Seveso at the time of and immediately after the release of three deaths of lung cancer by 1967. In any event, not one of the 87 died of lung cancer. When they died, their lungs were examined and were found to be full of asbestos. They did not die of lung cancer. On the other hand, instead of three deaths among the cigarette smokers, there were 24. So the problem came up of multiple factor interaction.

When we did the study of the 17,800 men in the asbestos union, we again took smoking histories. For those who did not smoke and did not work with asbestos, the rate was 11 per 100,000 per year. For those who worked with asbestos but did not smoke, it was five times as much, 58. Five times a very low figure is still very little. On the other hand, for those who smoked but did not work with asbestos it was 122, and in those who smoked and worked with asbestos it was

601 per 100,000, an this extraordinary increase. These rates, by the way, are different for every tissue. It was true for lung cancer, for the esophagus and for the larynx. It was not true for mesothelioma and it was not true for the remainder of the gastrointestinal with levels between 1,000 and 2,000 parts per trillion (ppt); liver tissue with levels between 100 and 200 ppt; other tissues (thyroid, brain, lungs, kidneys) with levels between 10 and 100 ppt; and blood with levels less than 10 ppt. The levels in the pancreas may have been abnormally high due to the presence of cancerous cells.

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Table 2. TCDD levels, parts per trillion, in human milk from breastfeeding mothers exposed to TCDD in Seveso, Italy and South Vietnam

Sample Location	2,3,7,8-TCDD Level (Whole Milk Basio) (ppt)	Reference
Seveso (Zones A & B)	2.3 - 28.0	Reggiani, 1981
South Vietnam (Area sprayed with Agent Orange)	40.0 - 50.0	Baughman, 1976

Environmental Exposure to TCDD. The Environmental Protection Agency (EPA) has analyzed human adipose tissue for TCDD. Kutz (1981) reported on six specimens of human adipose tissue collected from residents of an urban Ohio county to serve as control specimens for some analytical studies done bythe EPA Dioxin Monitoring Program. These specimens were excised during post-mortem examinations from individuals with no recorded or known exposure to 2,4,5-T or Silvex. Subsequently, they were analyzed in duplicate following the EPA Dioxin Monitoring Program protocol. Instrumental determinations were conducted at two independent laboratories.

The results, shown in Table 3, demonstrated that all specimens contained residues of 2,3,7,8-TCDD. Levels ranged between five and 12 ppt, with a detection limit below 5 ppt. Kutz emphasized that all studies conducted to date, including this one, have been accomplished utilizing small sample sizes and deliberate specimen selection criteria. Consequently, these few data cannot be construed as being representative of the general population.

Table 3. Levels of 2,3,7,8-TCDD ppt, in human adipose tissue

Source	Total Number of Samples[a]	Number Positive	Range (ppt)	Mean + SD[b]	Reference
EPA Ohio Monitoring Program	6	6	5-12		Kutz
Great Lakes Area, Canada	23	22	4.1-21.8,130	10.7 + 5.4 (n = 21)	Ryan and Williams
			Kingston	12.4 ± 5.8 (12)	
			Ottawa	8.6 ± 4.4 (9)	
U.S. Veterans Administration	33	25	3-29,99	7.7 ± 5.5 (24)	Hobson et al.
			Vietnam Experience	8.3 ± 6.9 (13)	
			No Vietnam Experience	5.7 ± 3.1 (11)	
Total	62	53	3-30	10 ppt	

[a] Detection limits defined in text

[b] Excluding outlying high samples

Recently, the Canadian scientists Ryan and Williams (1983) released data on the analysis of human fat tissue from the Great Lakes Area for 2,3,7,8-TCDD residues. The fat samples (10 to 20 g) were obtained from deceased elderly hospital patients from the communities of Kingston and Ottawa, Ontario. The results obtained by Ryan and Williams are also shown in Table 3. Levels of 2,3,7,8-TCDD were found in 22 of 23 samples analyzed. Values ranged from 4.1 to 130 ppt. Excluding the one outlying high sample, average values found were 10.7 + 5.4 (n = 21) with the highest value being 21.8 ppt. Grouping of the 22 samples which had been analyzed in a blind fashion with regard to origin showed that the 12 Kingston samlpes had an average of 12.4 + 5.8 ppt (n = 12) and the Ottawa samples 8.6 + 4.4 ppt (n = 9) but the difference was only significant at about $P = 0.1$ level. Ryan and Williams concluded from these data it would appear that most human fat tissues from older patients in the Great Lakes Area have low but measurable amounts of TCDD.

The issue of Agent Orange and the Vietnam veteran prompted a study of TCDD in human adipose tissue collected from U.S. Vietnam-Era veterans. This study, reported by Hobson et al. (1983), was initiated in 1979 with with the selection of two groups of adult males: (1) 21 Vietnam veterans, all but two of whom claimed health problems related to Agent Orange exposure, and who volunteered for the fat biopsy; and (2) 12 veterans with no service in Vietnam. Ten of the latter group had no exposure to any herbicides, were undergoing elective abdominal surgery and volunteerred to serve as controls. The other two individuals were active duty U.S. Air Force officers with known heavy and relatively recent exposure in connection with herbcide disposal operations. Each of the volunteers had a medical history, physical examination and routine clinical chemistry. The details of military service in Vietnam from the volunteer's report and his service record were examined to evaluate his potential exposure to herbicides using the dates, location and nature of his service. From these a rough estimate of the likelihood of exposure to TCDD was made without knowledge of the assay results.

The results of this study are also shown in Table 3. Fourteen of the 21 Vietnam veterans had levels of TCDD in their adipose tissue at or above the detection limit. Three of these men had detectible material that could not be validated as TCDD or the measured value was only questionably above the detection limit. Six Vietnam veterans had TCDD in amounts from 5 to 7 ppt. Three Vietnam veterans had TCDD in amounts from 9 to 13 ppt. One veteran had 63 and 99 ppt, and another had 23 and 35 ppt.

Of the 13 individuals who had never served in Vietnam, five had TCDD identified in their fat (4, 6, 7, 7 and 14 ppt). Six had values low enough to be considered equivocal or the detected material was not validated as TCDD. The remaining veteran had no detectible TCDD. In the two Air Force officers with known heaviest exposure, TCDD measured was never more than 3 ppt above the limit of detection.

Among the 21 Vietnam veterans there was no uniformity of symptoms, either immediately after exposure, at the time of biopsy, or during the intervening period. No one symptom or group of symptoms was common to veterans with detectible TCDD in their fat. The presence of TCDD did not mean ill health, nor did its absence indicate good health. No detailed statistical analysis of this small pilot series was attempted.

Hobson et al. (1983) concluded that the results of the very complex and technically difficult analysis indicated that very low levels of TCDD, believed to be 2,3,7,8-TCDD, could be detected in human adipose tissue in the range 3-99 ppt. The levels, however, did not correlate well with known exposure and nonexposure, and there was no correlation with health status. The study results did indicate that the assay method was feasible, but would serve no clinically or administratively useful purpose until additional data are available on background levels of TCDD in the general United States population.

The available data on TCDD residues in human tissue suggest that TCDD can be distributed throughout body tissues following accidental exposures. In such situations, a major site for storage of TCDD in the human body is in the adipose tissue. However, significant levels of residue (albeit, much lower than in adipose tissue) may be found in the liver and human milk. The monitoring of persons not involved in episodic events involving TCDD suggests that adult human adipose tissue may have a "background" level of TCDD that may reflect a generalized contamination of our environment. This "background" level may be very low (approximately 10 ppt) and detectible only through the use of sophisticated analytical methods.

Intentional Exposure to TCDD. In a review of 23 industrial episodes involving human exposure to TCDD, the one consistent medical finding was "chloracne" (Young, 1978). Chloracne is a skin reaction characterized by an acniform dermatitis with comedones (blackheads) and inclusion cysts or papules, and frequently pustules so severe that they cause permanent scarring. Morphologically it is similar to adolescent acne, but it is usually more severe particularly on the upper face, ears and neck. Active chloracne lesions have been reported many years after exposure to TCDD, but the condition usually clears up spontaneously in a few months. The confirmation that the chloracnigen encountered in the industrial production of trichlorophenol was 2,3,7,8-TCDD involved the dermal testing of TCDD on humans.

Bauer et al. (1961) reported on an experiment in which an investigator applied a 0.01 percent solution containing TCDD to his forearm and developed lesions characteristic of chloracne. Mild dermatitis developed in two days, followed by comedones several days later.

In the mid-1960s, prisoners (volunteers) at Holmesburg Prison in Philadelphia were dermally exposed to 2,3,7,8-TCDD in an experiment designed to determine the dose required to induce chloracne. The dermatologist that administered the material, Dr. Albert Kligman,

never published the results, but the Dow Chemical Company, sponsor of the studies, released them in testimony in the EPA 2,4,5-T/Silvex Cancellation Hearing (Towe 1980). A one percent suspension of TCDD in alcohol/chloroform was applied to the backs of 10 subjects on alternate days for one month. This protocol resulted in the application of a cumulative dose of 7,500 ug of TCDD. Eight of the 10 subjects developed chloracne, which lasted four to seven months. No other adverse health effects were noted from urinalysis or chemical tests monitoring blood, liver and kidney function. A cumulative dose of 16 ug applied in the same manner was ineffective; no intermediate doses were tested. Assuming that the entire quantity of TCDD was absorbed by the skin and if the average volunteer weighed 70 kg, then the dose of TCDD dermally-applied required to produce chloracne would be approximately 100 ug TCDD/kg body weight.

Earlier it was noted that the amount of TCDD in the women at Seveso (whose organs were analyzed at autopsy) was approximately 40 ug. She did not have chloracne but the blood and adipose tissues contained detectable levels of TCDD.

Discussion and Conclusion. There can be no doubt that exposure to TCDD occurred to individuals involved in the trichlorophenol industrial episodes where chloracne has been reported. Data by Rowe (Kligman Report) and Reggiani suggest that there is a threshold level at which chloracne occurs. Although there are no TCDD data on human tissue levels from individuals currently having chloracne, data by Rappe et al. (1983) suggest that the chloracnigens 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran persist for many years in body tissues. For the epidemiologist, it is important to know how long these chemnicals persist after acute and chronic exposures and what tissues should be monitored. The removal of adipose tissue during autopsy permits retrospective study, but similar surgical procedures for prospective studies may require the resolution of a series of medico-legal issues.

Blood analysis for TCDD has been effectively employed by Rappe et al. and Facchetti (1981), but Bickel et al. (1983) has pointed out that "fat storage is the chief contributor to the body burden of the polychlorinated xenobiotics." Characteristically, the concentrations of polychlorinated compounds in adipose tissue may be 1000 fold greater than in plasma. This suggests that the plasma levels of TCDD in individuals reported earlier by Hobson et al. and Ryan and Williams would be in the femtogram (1 X 10-15 gram) concentration, a level below the current state of the art. Although the analytical chemist may soon perfect techniques to measure such low concentrations, the biologist, physician and epidemiologist will likely conclude that it has no biologic significance in view of the overwhelming effects of life style and other chemicals in our environment.

REFERENCES

Bauer H, Schulz KH, Spiegelburg V (1961) Occupational intoxication in the manufacture of chlorophenol compounds. Arch Gewerbepath 18:538-555.

Baughman RW (1976) Tetrachlorodibenzo-p-dioxins in the environment high resolution mass spectometry at the picogram levels. Diss Abstr Inc 36:3380B.

Bickel MH, Jondorf WR, Muhlebach S, Wyss PA (1983) Adipose tissue storage of polychlorinated compounds. Environ Sci Res 26:477-482.

Facchetti S, Fornari A, Montagna M (1981) Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the tissues of a person exposed to the toxic cloud at Seveso. Forensic Environ App 1:1405-1414.

Hobson LB, Lee LE, Gross ML, Young AL (1983) Dioxin in body fat and health status: A feasibility study. Presented to the Division of Environmental Chemistry, American Chemical Society.

Kutz FW (1981) Chemical exposure monitoring in the EPA Office of Pesticides and Toxic Substances. Prepared statement to the VA Advisory Committee on Health Effects of Herbicides, November 19, Washington, DC.

Rappe C, Nygren M, Gustafsson G (1983) Human exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans. In: Choudhary G, Keith LH, Rappe C (eds), Chlorinated dioxins and dibenzofurans in the total environment. Butterworth, Boston, pp 355-365.

Reggiani G (1981) Medical survey techniques in the Seveso TCDD exposure. J Appl Toxicol 1:323-331.

Ryan JJ, Williams DT: Analysis of human fat tissue from the Great Lakes Area for 2,3,7,8-tetrachlorodibenzo-p-dioxin and -furan residues. Extended Abstracts, Division of Environmental Chemistry, American Chemical Society 23:157-158.

Towe VK (1980) Direct testimony before US Environmental Protection Agency, FIFRA Docket No. 415 et al., November 13.

Young AL, Calcagni JA, Thalken CE, Tremblay JW (1978) The toxicology, environmental fate and human risk of Herbicide Orange and its associated dioxin. Air Force technical report OEHL-TR-78-92, USAF Occupational and Environmental Health Laboratory, Brooks AFB, TX. (Available from the National Technical Information Service, Springfield, VA.)

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Immunologic Effects of TCDD Exposure in Humans

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD),[1] a contaminant found in the manufacture of a number of useful halogenated hydrocarbons, has been shown to have marked toxicity in experimentally exposed animals (McConnell and McKinney 1978). In all animal species studied, significant thymic atrophy with cortical thymic depletion has been observed (McConnell 1980). In addition, depletion of lymphocytes in T-dependent regions of peripheral lymphoid tissue, such as the paracotical area in lymph nodes and periarterial sheath in the spleen, is uniformly observed (Vos et al. 1980; Vos and Moore 1974). These changes have been observed in both acute and chronic TCDD exposed animals and are most pronounced in neonatal animals (McConnell 1980). Subsequent studies of cellular immunity showing that T cell functions are suppressed have corroborated these histological findings. TCDD exposed mice have depressed delayed hypersensitivity skin responses, prolonged allograft rejection and increased susceptibility to certain infectious agents (Vos and Moore 1974; Vos et al. 1974). In vitro studies of isolated lymphocytes have shown depressed lymphoproliferative responses to T-dependent mitogens (Vos and Moore 1974; Vos et al. 1974; Luster et al. 1980; Faith et al. 1978) and depressed generation of cytotoxic T lymphocyte responses (Clark et al. 1981).

An intriguing observation in TCDD exposed mice has been the demonstration of different strain susceptibility to TCDD induced immunotoxicity. Poland and Glover (1980) and Vecchi et al. (1983) have shown that certain strains of mice exposed to TCDD were extremely sensitive to TCDD whereas others were less sensitive, and F_1 hybrids of the two strains had intermediate susceptibility. Further studies have demonstrated that the immunotoxicity to TCDD segregated with its ability to induce the arene hydrocarbon hydroxylase (AHH) system (Poland and Glover 1980; Vecchi et al. 1983). TCDD is first bound by a "receptor" in the cytosol of cells in many organs, including the liver and thymus, which then results in induction of AHH. Sensiti-

[1] A report of work done in collaboration with Raymond G. Slavin (Department of Internal Medicine) and Stanford T. Roodman, (Department of Pathology), St. Louis University School of Medicine, 1325 South Grand Boulevard, St. Louis, MO 63104.

vity to TCDD induced AHH activity correlated well with immunotoxic effects. In humans, Kouri et al. (1975) have shown that TCDD stimulated AHH activity in in vitro lymphocyte cultures, and others have demonstrated polymorphism of the AHH system (Kellerman et al. 1973).

B lymphocytes are also suppressed in TCDD exposed animals, though generally at higher doses than those required to suppress T cell responses (Luster et al. 1979; Vecchi et al. 1980). Lymphoproliferative responses to the B cell mitogen, lipopolysaccharide, in exposed mice are reduced in a dose dependent fashion. In vivo both primary and secondary antibody responses are decreased in TCDD exposed mice immunized to SRBC (Vecchi et al. 1980), and in vitro their plaque forming cells are also depressed (Kellerman et al. 1973). Strain susceptibility and TCDD inducibility of AHH is also seen in humoral responses similar to those seen in T cell-mediated immune responses (Poland and Glover 1980).

Vos et al. (1978) demonstrated that part of the increased mortality to infectious agents in TCDD exposed mice was related to a marked increase in sensitivity to endotoxin from gram negative bacteria. Studies to explain this phenomenon examined macrophages, which are principal cells in endotoxin detoxification. In summary, macrophage dependent functions, including phagocytosis, killing and nitroblue tetrazolium reduction of peritoneal macropahges have been found to be normal in TCDD exposed adult mice. Further studies are needed to understand this phenomenon.

Though TCDD does have toxic effects in humans (Reggiani 1978; Pazderova-Vejlupkova et al. 1981), little is known regarding effects on human immunity. Reggiani (1980) reported on the immune status of 44 children (20 of whom had chloracne) in the Seveso, Italy accident and found normal quantitative serum immunoglobulins and complement concentrations, normal percentages of T and B lymphocytes and normal lymphoproliferation to mitogens. No increased incidence of infections was reported. However, Bekesi et al. (1978) found that in Michigan dairy farm residents exposed to a related halogenated hydrocarbon, a polybrominated biphenyl (PBB), in the form of contaminated meat and dairy products there was significant suppression of cellular immunity. These subjects had decreased percentages and absolute numbers of T cells, decreased lymphocyte transformation to alloantigens and an increased percentage of lymphocytes with no detectable membrane markers. The study was repeated approximately one year later by Silva et al. (1979), who detected no abnormalities. Immune function may recover in sublethal TCDD exposed mice after several months once exposure is discontinued (Faith et al. 1978), and perhaps this explains the disparity of immune findings in the Michigan farmers.

In Feburary, 1983 the Centers for Disease Control, the Missouri Division of Health and St. Louis University Medical Center conducted a pilot medical health survey of Missouri residents exposed to TCDD

contaminated soil in the Times Beach area. Based on previous animal studies, cell mediated immunity was examined by measuring delayed hypersensitivity skin tests, T cell subsets and lymphocyte proliferative responses.

Delayed hypersensitivity skin tests (DTH) were performed by the intracutaneous application of seven recall antigens (tetanus, diphtheria, old tuberculin, proteus, streptokinase, Candida and Tricophyton) using a Multi-Test device (Merieux Institute USA, Miami, FL) (Kniker et al. 1979). Skin test sites were read at 48 hours. Both the number of positive skin tests ($\geq$ 2 mm induration) and the sum of the diameters of induration were recorded.

T lymphocyte subset analysis was performed using FITC labeled monoclonal antibodies (Ortho Pharmaceuticals, Raritan, NJ) to detect T cell surface antigens and analyzed in a Spectrum III flow cytometer (Ortho Diagnostics, Westwood, MA) (Hoffman et al. 1981; Lanier and Warner 1981). OKT3 (pan T lymphocytes), OKT4 (T inducer) and OKT8 (T suppressor cytotoxic) monoclonal antibodies were used to detect total T lymphocytes and subsets.

Lymphocyte proliferation studies were performed in vitro on mononuclear cells isolated from heparinized venous blood by Ficoll-Hypaque density centrifugation (Schiff et al. 1974). Mononuclear cells were cultured either with medium alone or with the mitogens phytohemagglutinin (PHA, Difco Laboratories, Detroit, MI), conconavalin A (Con A, Sigma Chemical Company, St. Louis, MO) and pokeweed mitogen (PWM, Grand Island Biologicals, Grand Island, NY) or with the antigen tetanus toxoid (Massachusetts State Health Department).

The results of the DTH skin tests are illustrated in Table 1. The patients were placed in either high or low risk TCDD exposed groups as discussed by Dr. Webb. Since earlier studies had determined less skin reactivity for adult females, we further divided the exposure groups into adults ($\geq$17 years old), male and female, and children. No statistical differences were found comparing the various high versus low exposure groups, although several trends were noted for less DTH reactivity in high TCDD exposed groups. There were fewer positive skin tests found in high risk children (2.7 vs 3.5) and in high risk adults (3.3 vs 3.9) and decreased induration in the two groups (8.7 vs 10.6 and 12.6 vs 17.1 respectively). No differences were observed in the adult female groups.

Analysis of T lymphocyte subsets revealed no significant differences of either percentages or absolute numbers of T cells (OKT3), T helper (OKT4), T suppressor cells (OKT8) or T helper/suppressor ratios (T4/T8), comparing the high versus low exposure groups (Table 2). However, two trends were noted in the high exposure group that indicated the possibility of altered T cell surface markers. There was a greater percentage of individuals with a T4/T8 ratio $\leq$ 1.0 in the high risk group compared to the low risk group (15% vs 6% respectively). In addition, using the calculation (T4 + T8)/T3 to examine the

Table 1. Delayed hypersensitivity skin test results with multi-test in subjects exposed to TCDD

	Low Risk			High Risk		
	Male	Female	Children[1]	Male	Female	Children[1]
Total Subjects	12	10	8	27	22	15
No. Anergic	0	1 (10.0%)	0	0	1 (4.6%)	0
Geom. Mean Score, mm	17.1	8.2	10.6	12.6	9.1	8.7
Mean No. Antigens Positive[2]	3.9[3] ± 2.0	2.9 ± 1.9	3.5 ± 1.8	3.3 ± 1.5	2.8 ± 1.7	2.7 ± 1.3

[1] Children less than 17 years old.

[2] Induration greater than 2 mm at 48 hours is considered a positive reaction

[3] Data expressed in the mean number of antigens positive ± 1 S.D.

Table 2. T-lymphocyte profile in subjects exposed to TCDD

	Low Risk (32)	High Risk (68)
Lymphocyte/µl	1462 ± 227	1502 ± 67
OKT3/µl	945 ± 64	1086 ± 56
percent	70.3 ± 1.3	71.6 ± 0.8
OKT4/µl	588 ± 45	652 ± 35
percent	43.5 ± 1.2	43.4 ± 1.1
OKT8/µl	338 ± 26	425 ± 30
percent	25.6 ± 1.3	27.7 ± 1.1
T4/T8 ratio	1.85 ± 0.11	1.80 ± 0.10

Data are presented as means ± S.E.M.

percentage of OKT3 cells with both T4 and T8 antigens on their surface, 7/68 (10%) of individuals in the high risk group had a ratio > 1.17 compared to none in the low risk groups, indicating the possibility of dual surface markers on OKT3+ cells. During normal maturation of thymocytes, cells express both OKT4 and OKT8 antigens, but prior to release into the peripheral blood express only T4 or T8 (Reinherz et al. 1983; Janossy et al. 1981). This unusual phenotype (OKT 3+ 4+ 8+ cells) is seen following recovery of the T lymphocyte populations in renal (Cosimi et al. 1981) or cardiac transplantation patients following cessation of anti-thymocyte antibody therapy (unpublished observation). This finding and the increased incidence of low T4/T8 ratios in highly exposed individuals suggested altered T cell surface markers. Beseki, using sheep red blood cells and complement rosetting techniques, had found an increased incidence of lymphocytes devoid of surface markers in PBB exposed Michigan residents (Beseki et al. 1978). Since TCDD is preferentially toxic to cortical thymocytes, it is feasible that altered T cell surface markers may result from release of immature T cells into the peripheral blood. Future studies, which include examination of thymocyte surface markers, need to be done to determine the significance, if any, of these observations.

Lymphoproliferative responses of cultured mononuclear cells to mitogens and antigens were performed to examine T cell function. Previous studies have shown that PHA and antigens stimulate T helper cells, Con A stimulates both T helper and suppressor cells and PWM stimulates T and B cells (Reinherz and Schlossman 1980). No statistical differences were observed comparing high and low risk groups for lymphoproliferative responses (Table 3). A trend was noted that high risk children had lower tetanus toxoid stimulated blastogenesis compared to their low risk counterparts (7,256 cpm vs 42,485 cpm).

In summary, we observed no statistically significant alterations of cellular immunity in Missouri residents exposed to TCDD over a long

Table 3. Lymphocyte proliferative responses in subjects exposed to TCDD

Exposure		^{3}H-thymidine incorporation, cpm			
		PHA	Con A	PWM	Tetanus
High	Adult	63,834[1] 48.81 +/- .18[2] 29[3]	48,124 4.68 + .20 28	37,540 4.57 + .21 29	12,979 4.11 + .69 29
	Children	83,878 4.92 + .18 8	68,188 4.83 + .20 8	41,053 4.61 + .17 8	7,256 * 3.86 + 104 8
	Group	67,717 4.83 + .18 37	51,999 4.72 + .20 36	38,278 4.58 + .20 37	11,446 4.06 + .77 37
High	Adult	56,261 4.77 + .19 15	44,370 4.65 + .17 13	34,014 4.53 + .25 14	7,077 3.5 + .71 15
	Children	81,340 4.91 + .14 5	58,482 4.77 + .19 5	52,643 4.72 + .12 5	42,485 * 4.63 + .20 5
	Group	63,330 4.80 + .19 20	47,908 4.68 + .18 18	38,157 4.58 + .23 19	11,084 4.04 + .70 20

[1] Mean cpm of optimum response

[2] Log geometric mean cpm +/- 1 S.D.

[3] Number in each group

period of time, which was comparable to the studies of Reggiani. However, several trends were noted which suggested that perhaps TCDD does affect human CMI. There were decreased DTH responses in children and adult males and decreased lymphoproliferative responses in tetanus toxoid in children. There was also evidence for altered T cell surface markers as indicated by a greater percentage of high risk subjects with low T4/T8 ratios and by an increased percentage of individuals with T3+ T4+ T8+ cells. Is TCDD responsible for these trends? Immunotoxicity in humans exposed to TCDD might be expected to occur extrapolating from the animal data and from Bekesi's study. Likewise, we might also expect varying sensitivity to TCDD immunotoxicity, which seems to be linked to TCDD inducibility of AHH in animal models. Certainly, there are also differences of inducibility of the AHH system in humans (Kellerman et al. 1973). The route of exposure might be significant. In animal experiments and in the Michigan farmers, exposure was by ingestion of the halogenated hydrocarbons. Perhaps cutaneous contact does not cause as significant internal organ dysfunction as ingestion because of decreased absorption. The question of bioavailability of TCDD contaminated soil was recently studied by McConnell et al. (1984) and found to induce symptoms in animals fed the soil.

Future studies need to address actual TCDD exposure, perhaps by measuring TCDD in fat tissue. In addition, subjects should be examined during the exposure, as recovery of the immunotoxicity is possible. Finally, inclusion of other immune studies such as CTL responses, which may be a more sensitive assay of TCDD immunotoxicity, should be performed. Finally, are there long-term health effects of TCDD exposure, such as the development of soft tissue sarcomas? Though a number of papers have suggested an increased risk of soft tissue sarcomas in occupationally TCDD exposed individuals (Hardell and Eriksson 1981; Moses and Selikoff 1981; Cook 1981; Honchar and Halperin 1981; Hardell and Sandstrom 1979; Sarma and Jacobs 1982), this remains unproven. Further long-term epidemiological studies should address these questions.

REFERENCES

Beseki JG, Holland JF, Anderson, HA, et al. (1978) Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science 199:1207-1209.

Clark DA, Gauldie J, Szewczuk MR, Sweeney G (1981) Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (41275). Proc Soc Exp Biol Med 168: 290-299.

Cook RR (1981) Dioxin, chloracne and soft tissue sarcoma. Lancet 1:618-619.

Cosimi AB, Colvin RB, Burton RC, et al. (1981) Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. New Engl J Med 305:308-314.

Faith RE, Luster MI, Moore JA (1978) Chemical separation of helper cell function and delayed hypersensitivity responses. Cellular Immunol 40:275-284.

Hardell L, Eriksson M (1981) Soft-tissue sarcomas, phenoxy herbicides and chlorinated phenols. Lancet 1:250.

Hardell L, Sandstrom A (1979) Case-control study: Soft-tissue sarcomas and exposure to phenoxyacetic acids and chlorophenols. Br J Cancer 39:711-717.

Hoffman RA, Kung PC, Hansen WP, Goldstein G (1981) Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA 77:4914-4917.

Honchar PA, Halperin WE (1981) 2,4,5-T, trichlorophenol, and soft tissue sarcoma. Lancet 1:268-269.

Janossy G, Tidman N, Papageroriou ES, Kung PC, Goldstein G (1981) Distribution of T lymphocyte subsets in human bone marrow and thymus: An analysis with monoclonal antibodies. J Immunol 125:1608-1613.

Kellerman G, Luyten-Kerreman M, Shaw CR (1973) Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. Am J Human Gent 25:327-331.

Kniker WT, Anderson CT, Roumiantzeff M (1979) The Multi-Test system: A standardized approach to evaluation of delayed hypersensitivity and cell-mediated immunity. Ann Allergy 43:73-79.

Kouri E, Ratrie H III, Atlas SA, Nebert DW (1975) Aryl hydrocarbon hydroxylase induction in human lymphocytes cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sci 15:1585-1595.

Lanier LL, Warner NL (1981) Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometer (FACS) analysis. J Immunol Methods 47:25-30.

Luster MI, Boorman GA, Dean JH, et al. (1980) Examination of bone marrow, immunologic parameters and host susceptibility following pre-and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Int J Immunopharmacol 2:301-309.

Luster MI, Faith RE, Clark G (1979) Laboratory studies on the immune effects of halogenated aromatic. Ann NY Acad Sci 320:473-486.

McConnell EE (1980 Acute and chronic toxicity, carcinogenesis, reproduction, teratogenesis and mutagenesis in animals. In: Kimbrough RD, ed. Halogenated biphenyls, terphenyls, napthalenes, dibenzodioxins and related products. Elsevier/North-Holland, Amsterdam, pp 109-150.

McConnell EE, Lucier GW, Rumbaugh RC, et al. (1984) Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science 223:1077-1079.

McConnell EE, McKinney JD (1978) Exquisite toxicity in the guinea pig to structurally similar halogenated dioxins, furans, biphenyls, and naphthalenes. Toxicol Appl Pharmacol 45:299.

Moses M, Selikoff IJ (1981) Soft-tissue sarcomas, phenoxy herbicides and chlorinated phenols. Lancet 1:1370.

Pazderova-Vejlupkova J, Nemcova M, Pickova J, Jirasek L (1981) The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in men. Arch Environ Health 36:5-11.

Poland A, Glover E (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Molecular Pharmacol 17: 86-94.

Reggiani G (1978) Medical problems raised by the TCDD contamination in Seveso, Italy. Arch Toxicol 40:161-188.

Reinherz EL, Kung PC, Goldstein G, Levy RH, Schlossman SF (1980) Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 77:1588-1592.

Reinherz E, Schlossman SF (1980) Regulation of the immune response-inducer and suppressor T-lymphocyte subsets in human beings. New Engl J Med 303:370-373.

Sarma PR, Jacobs J (1982) Thoracic soft-tissue sarcoma in Vietnam veterans exposed to Agent Orange. New Engl J Med 306:1109.

Schiff RI, Buckley AH, Gilbertson RB, Metzger RS (1974) Membrane receptors and in vitro responsiveness of lymphocytes in human immunodeficiency. J Immunol 112:376-386.

Silva JA, Kaufman CA, Simon DG, et al. (1979) Lymphocyte function in humans exposed to polybrominated biphenyls. J Retic Soc 26:341-347.

Vecchi A, Mantovani A, Sironi M, et al. (1980) Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on humoral antibody production in mice. Chem Biol Interactions 30:337-342.

Vecchi A, Mantovani A, Sironi M, et al. (1980) The effect of acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral antibody production and cell-mediated activities in mice. Arch Toxicol Suppl 4:163-165.

Vecchi A, Sironi M, Canegrati MA, Recchia M, Garattini S (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol Appl Pharmacol 68:434-441.

Vos JG, Faith RE, Luster MI (1980) Immune alterations. In: Kimbrough RD, ed. Halogenated biphenyls, terphenyls, napthalenes, dibenzodioxins and related products. Elsevier/North-Holland, Amsterdam, pp 241-266.

Vos JG, Kreeftenberg JG, Engel HWB, Minderhoud A, Van Noorle Jansen LM (1978) Studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced immune suppression and decreased resistance to infection: endotoxin hypersensitivity, serum zinc concentrations and efects of thymosin treatment. Toxicology 9:75-86.

Vos JG, Moore JA (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int Arch Allergy Appl Immunol 47:777-794.

Vos JG, Moore JA, Zinkl JA (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 mice. Toxicol Appl Pharmacol 29:229-241.

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Environmental Contamination and Toxicology

Dioxin in Missouri: 1971–1983

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From April, 1970 through January, 1972 NEPACCO, Northeastern Pharmaceutical and Chemical Company, in Verona, Missouri leased a chemical plant to produce hexachlorophene from 2,4,5-trichlorophenol (Figure 1). During this production, 2,3,7,8-tetrachloro-dibenzo-p-dioxin was formed and concentrated in still bottoms and in filter cakes. Approximately 12,000 gallons of the still bottoms were incinerated in, presumably, an environmentally safe way. Forty three hundred gallons remains stored on-site, and 18,000 gallons was removed by a waste oil hauler and subsequently sprayed on roads, truck lots and riding arenas primarily located in eastern Missouri. Also, approximately 25,000 gallons of waste water was sent to the Waste Management School in Neosho, Missouri and filter cake was sent to local farms for burial. In the latter case, farmers were paid to dispose of filter cake in barrels and drums on their land.

While the filter cake and the wash water does pose a major problem in southwest Missouri, the site receiving the most attention in that area is the Spring River. In 1982 a health advisory was issued by the FDA alerting the public as to possible health effects which might result from eating fish from the Spring River. The data upon which this advisory was based indicated that in seven samples of whole fish, the concentration of TCDD was 26 parts per trillion (ppt) and in eight samples of filets, 18 ppt of TCDD was found.

These two major points concerning southwest Missouri aside, the story of dioxin contamination in Missouri is really the story of the widespread contamination of dioxin in soil in the eastern part of the state: dirt roads, unpaved truck lots, horse arenas. From February 16, 1971 to October 25, 1971, six truck loads of still bottoms of approximately 3,000 gallons each were hauled to eastern Missouri. Almost all of this material was either sprayed directly or in a mixture with waste oil as a dust suppressant. In all, the EPA estimates that approximately 100 times the total amount of TCDD was sprayed on Missouri that was released during the Seveso incident (American Institute of Chemical Engineers 1983). The total amount of TCDD released in Missouri was about 20–25% of the amount that was

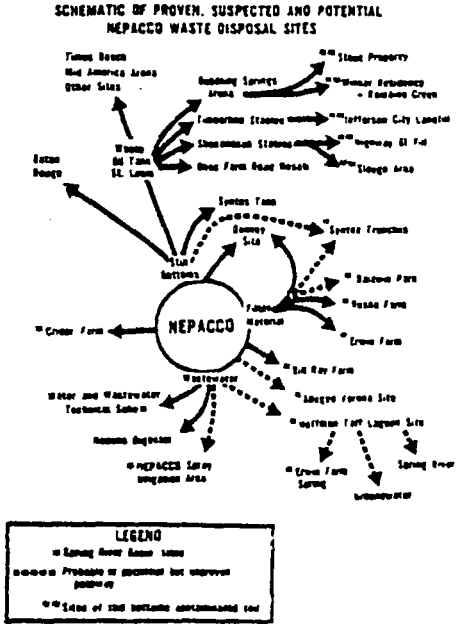

Figure 1. Schematic showing the fate of dioxin from the NEPACCO plant.

sprayed on Vietnam during the period that Agent Orange was being used as a defoliant.

There are three eastern Missouri sites which are important in understanding the history of dioxin contamination in Missouri. First is the Shenandoah stable site (see Dr. Kimbrough's article) where on May 25, 1971 undiluted still bottoms containing TCDD at concentrations up to 350 parts per million (ppm) were sprayed on Shennandoah stables. The total dose of TCDD has been estimated to be in excess of six pounds. The following day, horses became sick and eventually nearly 75 died or had to be destroyed. Within a week sparrows were literally falling out of the rafters and many other domestic and other wild animals were sick or dying. In August of 1971, a six year old girl was admitted to St. Louis Children's Hospital. Two days later the Centers for Disease Control (CDC) was consulted. In August of 1971 the samples were taken. Before any conclusions were drawn from the tests, the stables were excavated and, in November of 1971, animals were returned. According to some reports they continued to get sick and die. Fortunately, one of the co-owners of the stables

had become suspicous and trailed the waste oil hauler. Her documentation of his visits provided crucial evidence revealing other dioxin sites. Results of CDC's tests on the soil samples were not available for three years.

Shenandoah stables represents the first place where there was a documented illness in humans. It was also where TCDD was conclusively identified. Today, it still has the highest levels of contamination, about 1200 parts per billion (ppb) in the slough area (Missouri Dioxin Task Force, 1983).

Another site which is very important in understanding our dioxin history is the Bubbling Springs site. This is another arena sprayed in 1971 at which several horses died. This site was excavated and the excavated soil was hauled away by Mr. Stout, who used it for fill at the Minker residence and at the Stout residence. Hence, we have the Minker/Stout site, which was one of the first sites announced for the EPA "buy out" under "Superfund." It has been sampled at between 85 and 740 ppb and has been put on the national priority list. A recent study (McConnell et al. 1984) has shown that the soil is toxic to certain animal species.

One of the biggest problems at the Minker site is the severe erosion which has caused considerable contamination in Romaine creek. TCDD levels in the creek have been reported up to 270 ppb and dioxin has been detected two feet underneath the surface of the creek. Furthermore, bottom feeding whole fish taken from the Meramec River near the mouth of Romaine Creek have shown levels of TCDD above 78 ppt.

Along with the Minker site goes the Stout site. Soil was hauled from Bubbling Springs to the Stout site and used for deep fill. Testing down to 20 feet indicates TCDD contamination. When combined with the Minker site, the Stout site represents a total of between 5,000 and 8,000 cubic yards of soil which must be removed in order to decontaminate the area.

The other major site which receives the most press and seems to be running a good race to unseat Love Canal as being known as the environmental disaster of the 20th century is Times Beach, where roads were sprayed in 1972 and 1973. Times Beach was first sampled in November and December of 1982 and the advisory to evacuate Times Beach was issued December 23, 1982. On February 22, 1982, the EPA announced that they would buy the entire city of Times Beach, making it unique in the history of environmental disasters. Once purchase is completed, the city will no longer exist.

Times Beach is in the flood plain of the Meramec River. This river had two 100 year floods in 1982 and 1983, each of which flooded Times Beach. Fortunately, this flood plain is not a scouring area. Sampling done before and after the two major floods indicated that there were no appreciable changes in the TCDD contamination. Times Beach houses over 60% of the known TCDD contaminated soil in Missouri.

In summary, there are over 25 miles of roads, most at Times Beach. The recent discovery of contaminated fish in the Meramec River has focused attention on Romaine Creek. Potentially, several miles of the Spring River will require remedial action. There are depths of contamination of twenty feet. Besides the Minker residence where severe erosion caused the contamination at Romaine Creek, erosion at one of the other horse arenas has possibly caused the contamination of another stream which feeds the Meramec River. At the Jones truck site in the City of St. Louis, samples of rafter dust have indicated the presence of TCDD. This opens the possibility of an inhalation route as a source of exposure. This has further been stressed by the discovery of a soft tissue sarcoma in a former truckyard worker.

Usual methods call for for dealing with hazardous waste in a waste stream; however, most of the dioxin in Missouri is not a waste stream, it is contaminated soil. It is distributed over 38 known sites and over 100 more sites are currently under investigation. It adheres to soil, implying that any method that one would use to destroy it would first require disassociating it from the soil. Incineration would probably require an initial volatilization step and then destruction in its volatilized form. In solvent extraction, there is a unit operation explicitly involving the removal of the TCDD from the soil with a subsequent destruction operation. Any of these methods must be capable of handling large quantities, greater than a half million cubic yards and a large range of contamination levels.

Besides how it got there and what is there, the other major question I wish to address is "Why have we not done anything about it until now, especially when TCDD was positively identified in 1974?" Probably the main reason for this is the misapplication of the concept of "half life." This issue was specifically addressed by the Missouri Governor's Dioxin Task Force, which concluded that the concept as applied to dioxin contaminated soil was invalid. A typical sample of surface soil is shown in Figure 2. Considering this as a control volume, we ask the question "What are the fundamental mechanisms by which TCDD enters and leaves the control volume?" A number of mechanisms are noted, including: surface movement of soil particles, erosion, vaporization (which is believed to be a mechanism at the very early stages of spraying), transport by living organisms, transport by solubilization, motion of small particles containing TCDD, molecular diffusion and, finally, the rate of decay. Here, the rate of change of TCDD in the region of interest is governed by many factors including the rate of decay, which is the basis for the concept of half life. In soil analysis, one measures the combined effects of all of these factors, not only the half life.

The confusion of the scientific basis of the use of the term "half life" was partly the basis on inaction. On March 31, 1975, a CDC report (CDC, 1975) indicated that the "half life," i.e. the rate of disappearance, was one year. A Missouri Department of Natural

Resources report in July of the same year also used this figure. Another report (Missouri Department of Natural Resources, 1975) indicated that the "half life" of TCDD was between 0.24 and one year. Hence, the conventional wisdom through the mid-1970s was that this material would decay in the short term.

The realization that TCDD does not "decay" brings us to the modern history of TCDD contamination in the state of Missouri, which begins during the fall of 1981 through the spring of 1982. At that time EPA consolidated its internal records. New sites were discovered in

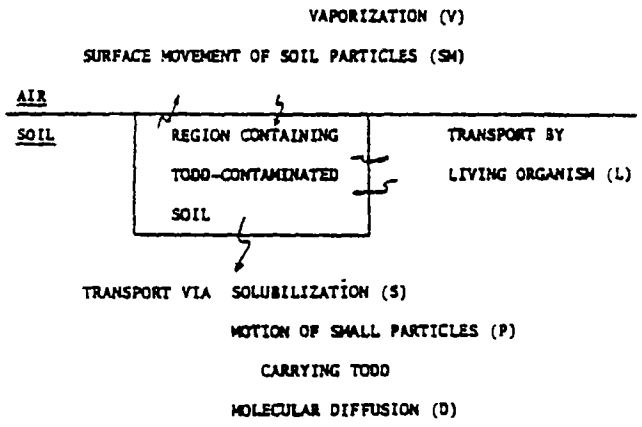

Figure 2. Control volume of soil along with many probably transport mechanisms.

southwest Missouri. In March, 1982 the health advisory relating to Spring River fish was issued by the Food and Drug Administration. There was a new round of sampling in June, 1982, and exchanges of information among the Division of Health, Department of Natural Resources, EPA and CDC. All of this activity came to a head on October 27 when the Environmental Defense Fund released EPA internal documents listing possible sites. At that time, there were 14 confirmed sites and 41 more under investigation. Today we have the 38 confirmed sites and approximately 200 under investigation.

As a result of these events there have been several responses. The media response has ranged from an excellent supplement that appeared in the St. Louis Post Dispatch (1983) to a confused and rhetorical editorial in the Wall Street Journal (1983).

More important has been the response of the Missouri State Legislature in passing two major pieces of legislation: a "cancer registry bill" requiring that hospital administrators file with the State information concerning reported cancers; and the "State Superfund" which has instituted a head tax on all employees of hazardous waste generators and has put a $25 per ton on all hazardous wastes that are land filled.

Regarding the citizen response, there are two major trends. One is the formation of residents/victims groups and the other is the interaction of these groups with environmentalists. The residents/victims groups are demanding contracts with government agencies concerning health studies and clean-up criteria. In eastern Missouri, there is the Contaminated Sites Residents Committee representing about ten sites. In southwest Missouri there is the Spring River Dioxin Committee. For the truck sites in St. Louis city, the Teamsters Local 600 has instituted a dioxin task force. The Missouri Coalition for the Environment, a state-wide environmental group, has been providing legal and office support and has been coordinating among all of these groups. In the future it appears that there may be a coalescing of groups and there will be new issues raised.

On the administrative front, at the state level, the Governor's Dioxin Task Force recommended the development of an overall strategy for dealing with all of the sites at one time. It was recommended that all the soil be consolidated at a single location and the TCDD destroyed when the technology catches up. Times Beach has been suggested as the site of the central storage facility.

As a member of the Task Force, I believe there were a couple of faults with its final report. First, specific statements concerning economic compensation cannot be found. Second, the long-term physiological effects on affected individuals were not addressed.

Finally, as this conference shows, there is the educational aspect or the educational response to the dioxin crisis. Most attending this conference are from St. Louis. Some of the out-of-state speakers who have come for this conference have also spent much time here in the last year helping us overcome this problem. It is good for us, as Missourians who have been affected, to educate those who are here today concerning the uniqueness and magnitude of the Missouri dioxin problem and to try to progress beyond our current knowledge.

REFERENCES

2,3,7,8-TCDD contamination of salvage motor oil. Centers for Disease Control (1975) Centers for Disease Control Report EPI-75-31-3, Atlanta.

Exner JH, Johnson JD, Ivins OD, Wass MN, Miller RA (1982) In: Exner JH, ed. Detoxification of hazardous waste. Ann Arbor: Ann Arbor Science, p 269.

Final report of the Missouri Dioxin Task Force. October 31, 1983.
McConnell EE, Lucier GW, Rumbaugh RC, et al. (1984) Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science 223:1077-1079.
Missouri Department of Natural Resources Memo, July 24, 1975. Jefferson City, MO: Department of Natural Resources.
St. Louis Post Dispatch (1983) Dioxin: Quandry for the '80s. November 14, 1983.
St. Louis Section of the American Institute of Chemical Engineers (1983) Dioxin risk assessment: A panel discussion, June 1, 1983.
Wall Street Jounal (1983) Dioxin hysteria (editorial). New York, May 31, 1983.

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DIOXIN, AGENT ORANGE AND HUMAN HEALTH

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April 1984

DIOXIN, AGENT ORANGE, AND HUMAN HEALTH

Summary

Agent Orange, the United States military code name for a 50/50 mixture of the herbicides 2,4,5-T and 2,4-D, was used during the Vietnam War from 1965-1970 to defoliate jungle vegetation to protect U.S. servicemen from enemy ambush. Both of these herbicides had been used domestically without noteworthy adverse effect for about 20 years at the time they were selected by the military as the components of Agent Orange.

Present at trace levels in the 2,4,5-T component of Agent Orange is an unwanted contaminant, the dioxin compound 2,3,7,8-tetrachlorodibenzo-para-dioxin, commonly known as TCDD or dioxin. TCDD is extremely toxic and has been shown to cause a number of serious health conditions in laboratory animals, including birth defects, cancer, and death. It is not a product, and no one makes it on purpose. TCDD has also been shown to cause a serious skin disorder known as chloracne and reversible signs of toxicity in workers accidentally exposed to extremely high levels on the job.

In spite of the acknowledged toxicity of TCDD, the consensus of scientific opinion is that it has not been shown to cause harm in people at the trace levels at which it has been present in herbicides. Among the organizations which share this view are the American Medical Association; the United Kingdom's Ministry of Agriculture, Fisheries, and Foods; the World Health Organization of the United Nations and the Council for Agricultural Science and Technology.

During the Vietnam War, The Dow Chemical Company supplied about 32 percent of the Agent Orange applied. As a quality control measure, Dow analyzed the 2,4,5-T in all shipments of Agent Orange to the government to ensure the absence -- no detectable level -- of TCDD. Today, with better analytical techniques, we know that the level of TCDD in Dow-supplied Agent Orange was less than 0.5 parts per million.

In early 1979, the first of multiple lawsuits was filed against seven manufacturers of Agent Orange: Dow, the Monsanto Company, Diamond Shamrock Corporation, Uniroyal Inc., T.H. Agricultural and Nutrition Company, Thompson Chemical, and Hercules Inc. These claims have been consolidated into a class action suit in which thousands of Vietnam veterans and their family members will be represented by selected cases. These cases will be tried individually to determine whether Agent Orange has caused health problems to these veterans and their families. The trial is scheduled to begin in early May 1984 in the courtroom of Chief Judge Jack B. Weinstein of the Federal District Court for the Eastern District of New York (Brooklyn, NY).

We all appreciate that Vietnam veterans have been through tough, demanding duty, a difficult experience. Although preliminary findings, such as the Ranch Hand studies and the Veterans' Administration's Agent Orange Registry, have been reassuring, scientific research at this time is insufficient to determine definitively whether the health of the Vietnam veteran and his family is any different from the general U.S. population. We do know that overwhelming scientific evidence demonstrates that Agent Orange is neither a likely nor plausible cause of the health effects some Vietnam veterans and their families have been suffering. Studies which have examined this issue and found no link between Agent Orange and ill health include the Ranch Hand mortality and morbidity studies, the Australian birth defect study released in 1983, and the Veterans' Administration Agent Orange Registry.

There are presently about 95 studies ongoing to evaluate the potential health impacts of TCDD. Essentially, only one of these studies, presently being conducted by the U.S. Centers for Disease Control, considers health effects in the Vietnam veteran from any potential cause other than Agent Orange.

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DIOXIN, AGENT ORANGE AND HUMAN HEALTH

Approximately 2.8 million servicemen served in Southeast Asia during the Vietnam War, according to the Veterans' Administration. Today, some of these veterans are experiencing a wide variety of health problems. The Centers for Disease Control (CDC) is attempting to determine whether the Vietnam experience is related to these health concerns. One aspect of the Vietnam experience being explored by the CDC, and the one which has received the greatest public and scientific attention, is the claim that the ill health of these veterans is related to Agent Orange exposure.

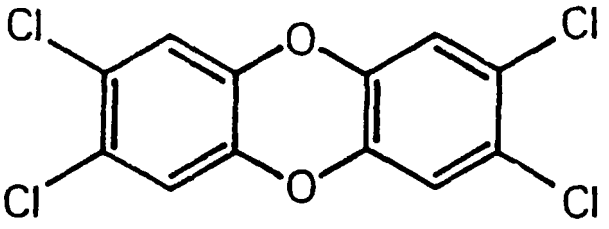
Agent Orange was a defoliant used by the U.S. military from 1965 to 1970 to eliminate dense jungle vegetation and protect allied troops from enemy ambush. This defoliant was a 50/50 mix of 2,4,5-T* and 2,4-D, two herbicides developed as a result of military research during World War II. Both of these herbicides had been used domestically, and without noteworthy adverse effect, for about 20 years at the time they were selected by the military as the components of Agent Orange. Code named by the orange identification band painted on the storage drum, Agent Orange was usually sprayed from fixed wing C-123 military aircraft. A small amount of the herbicide was also applied from ground sources, such as boats, trucks and backpack sprayers.

The source of today's public controversy is an unwanted trace contaminant, the dioxin compound 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD),

*Note: Underlined terms are defined in the glossary at the back of this paper.

present at trace levels in the 2,4,5-T component of Agent Orange. It should be noted that TCDD is not a commercial product, but rather an unavoidable manufacturing process contaminant. It is not shipped in drums or railcars across the United States.

Considerable controversy surrounds TCDD even today, primarily because of its high acute toxicity. TCDD has been shown to cause a number of serious conditions in laboratory animals, including birth defects, cancer and death. However, according to numerous independent scientific reviews, with the exception of the skin disorder chloracne and reversible signs of toxicity, none of these health conditions have been documented in people, even in cases of severe overexposure.



2,3,7,8-TCDD

We must be concerned with establishing safe levels for human exposure to TCDD in our environment. However, there is extensive scientific knowledge about TCDD which does not suggest that the trace levels of this

contaminant found in herbicides or in the general environment pose a health threat to people.

We must also be concerned about the health problems of individual veterans and their families. These veterans have endured difficult, demanding duty in the service of their country. They deserve answers to their very real concerns. Although preliminary findings should be encouraging to veterans, scientific research is insufficient at this time to determine definitively whether the health of these veterans and that of their families is any different from the general population. However, a great deal of public anxiety and misunderstanding has developed about alleged links between health problems and Agent Orange. According to overwhelming scientific evidence, Agent Orange is not a plausible cause of the various illnesses experienced by some veterans.

The bulk of the scientific studies conducted in the United States, Europe, Australia, and New Zealand among industrial workers, herbicide sprayers, and military personnel demonstrates that the presence of TCDD at trace levels has not shown any long-term adverse health effects in people.

INDEPENDENT SCIENTIFIC REVIEWS

The American Medical Association Review

In 1981, the American Medical Association (AMA) published a technical report reviewing the medical evidence regarding the toxicity and long-term health effects of the TCDD contaminant in Agent Orange. As the report's preface states:

> "In spite of the voluminous data . . . there is still very little substantive evidence for many of the alleged claims that have been made against these compounds (Agent Orange, 2,4,5-T, *and* TCDD). *The most serious of these allegations* assert that Agent Orange, or compounds of a like nature, have caused malignant tumors, spontaneous abortions, and birth defects. Although data from studies on experimental animals tend to support some of these claims, it is not certain that the animal data are extrapolatable to man. No laboratory animal can fully substitute for man; we must, therefore, depend on the results of ongoing epidemiological studies on persons who have been exposed."[1]

Some of what we know of the potential health and environmental effects of TCDD exposure has been due to scientific studies conducted on laboratory animals. Yet as the AMA Advisory Panel on Toxic Substances which produced the report concluded, "there are significant differences between some of the toxic effects of TCDD in experimental animals and the human experience; thus the animal data cannot be translated directly to man."[2]

In terms of acute toxicity, a wealth of epidemiological data support the position that people seem less susceptible to TCDD than several of the animal species which have been studied.

According to the AMA Panel, one of the more pronounced biological effects of heavy exposure to TCDD, as well as a number of other chlorinated compounds, is chloracne which occurs in humans and some animals. This particular skin condition is regarded as the clinical indicator of TCDD overexposure in people. The AMA Panel noted that human systemic disorders from exposures to TCDD are unlikely to occur in the absence of chloracne. Such findings as impaired liver and kidney function, gastrointestinal irritation, muscle and nerve disorders, depression and fatigue, and central nervous system effects have been reported after exposure to relatively large amounts of TCDD. However, these disorders "have not been progressive" and have diminished with time after exposure ceased, the AMA report stated. In short, if there is no chloracne, other persistent toxic effects from exposure to TCDD are "unlikely".[2]

The AMA report also stated that TCDD may act as a promoter of carcinogenesis in some strains of rats and mice. For cancer to start, it must go through several distinct steps. The first stage, called initiation, is produced by a carcinogen (an initiator) and probably involves irreversible mutational (genetic) changes. Later growth and development of an actual cancer may require promotion, which could be

the result of additional applications of a carcinogen, or of other noncarcinogenic materials. Promotion may involve additional unknown processes which could aid the growth of the cancer or reduce resistance to the developing cancer.

By way of an analogy, the process of carcinogenesis could be compared to the events leading up to the rupture of a dam. Initiation could be compared to an event which produces a slight crack (genetic damage) in the dam, which in and of itself does not significantly jeopardize the dam's structure. Promotion could be compared to an unusually violent storm which raises the water level in the river, increasing the water pressure on the crack causing increased flow, and widening of the crack to the point at which the structure becomes undermined. Implicit in this analogy is the concept of a threshhold in terms of promotion: that is, there is a water level below which the well-being of the structure is maintained, even though a small leak (initiation) was present.

TCDD is among the group of chemicals that may secondarily influence the formation of cancer in animals by promotion. The carcinogenicity associated with TCDD in laboratory animals is typically accompanied by other signs of systemic toxicity, in contrast to some other carcinogens for which cancer is the only observable toxic effect.

In terms of mutagenicity, TCDD has reportedly been studied in various tests using certain bacterial, viral, yeast and tissue cell cultures.

While these in vitro tests have given mixed results, tests using intact animals or human cells in culture indicate little potential for mutagenic effects from TCDD to occur in living higher animals.

The AMA Panel made clear in its summary that the extensive information collected for more than 30 years provides "no conclusive evidence" that either TCDD or 2,4,5-T are "mutagenic, carcinogenic, or teratogenic in man, nor that they have caused reproductive difficulties in the human."[3]

An updated report from AMA is expected in late 1984.

Other Independent Scientific Reviews

Other independent scientific groups have reviewed the studies on TCDD.

United Kingdom Pesticide Advisory Committee Report: According to this 1980 report, updated in 1983, and commissioned by the Ministry of Agriculture, Fisheries, and Foods, "human health risks posed by dioxin contamination in 2,4,5-T formulations may hitherto have been overestimated." The report states that its authors "cast far and wide" in search of evidence that 2,4,5-T herbicide formulations, used properly, posed a threat to either humans or the environment; however, no such evidence was found. This report considered the presence of the TCDD contamination in those

herbicide formulations, recommended strict control over the contaminant, but found "no valid medical or scientific evidence that 2,4,5-T herbicides harm humans, animals, or the environment if they are used in the recommended way and for the recommended purposes."[4]

Queensland Cabinet Committee Report: "No evidence exists that the continuation of present and approved use of 2,4-D and 2,4,5-T will in any way harm the health and well being of any members of the general public," according to this 1981 Australian report. Regarding Agent Orange, the report also states that no evidence exists that use of the defoliant "caused any physical disability of the local population" and further adds that the military use of these two herbicides provided no indication "that their approved use in peacetime would cause any hazard to life."[5]

Council for Agricultural Science and Technology Report: On the presence of TCDD in Agent Orange, this report states that, "One is at first likely to be so overwhelmed by the enormity of the toxicity of the compound that he fails to comprehend the infinitesmal levels of exposure." This early, 1975 report did not examine the issue of carcinogenicity but found "no conclusive evidence of association between exposure to herbicides and birth defects in humans."[6]

INDUSTRIAL EXPOSURES TO TCDD

From the late 1940s to recent years, a number of chemical process upsets and industrial incidents have occurred around the globe unintentionally exposing people to high levels of TCDD. While these incidents are unfortunate, the knowledge gained by studying the exposed populations is important in evaluating the potential for TCDD to cause human harm. In addition, such knowledge should provide understanding needed to prevent future harm.

The Seveso Incident

The best known and most widely studied incident of community exposure to TCDD followed the July 10, 1976, process accident at the ICMESA trichlorophenol plant at Seveso, Italy. As a result of the accidental release, approximately seven square miles of the countryside were contaminated by chemicals, including TCDD, exposing as many as 30,000 people.[7,8] The heaviest contamination involved an area of 180 acres (Zone A), occupied by 736 people. TCDD was reportedly detected on soil at levels in excess of 5.5 parts per million (ppm).[9,7] This concentration is 5,000 times the level of concern later set for TCDD by the Centers for Disease Control (CDC) for evaluating the hazard of residential soil at Times Beach, Missouri. (See Appendix D for chart comparing various reported concentrations of TCDD.) It is also potentially tens of thousands of times greater than any TCDD exposure ground troops could have had to TCDD from the spraying of Agent Orange in Vietnam.

At Seveso minimal effect was noted on plant life as a result of this high exposure; but some wild animals especially herbivorous species did die, particularly in Zone A (nearest the chemical plant). About four percent of the domestic animals in contaminated zones died. (Virtually all were small animals.)[7] Some of these deaths may have been due to other chemicals released at the same time.

Chloracne, which has generally been regarded as the hallmark, or sentinel sign, of TCDD exposure,[10] was observed in the Seveso population although at generally low rates and primarily in children.[11,12,7,8] The highest incidence of reported chloracne was about 13 to 14 percent among elementary school children in Seveso, and this was generally mild and rapidly resolved in most affected people.[7,8,13] Other than chloracne (of which there were ultimately 100 to 200 cases), transient nausea, vomiting, itching, and headache were the most commonly observed symptoms[8] readily attributable to the exposure, which again may have involved chemicals other than TCDD. Headache, stomach and intestinal upset were more commonly noted in individuals with chloracne.[11,8] In addition, very slight peripheral nerve impairment was reported, and there were also tentative signs of liver toxicity, both of which reversed over time. Immunoresponse was not diminished, nor was susceptibility to infectious diseases increased.[14]

Two years after this incident, 12 researchers involved in the study of reproductive effects at Seveso concluded that no "major event" had

occurred with respect to birth defects, miscarriages (spontaneous abortions), births and deaths.[15] However, changes in birth defect reporting procedures and in the reporting of spontaneous abortions may have obscured any small changes which might have occurred.[16,7,8,17,11] Data on reproductive outcomes are limited, but one researcher reported that examination of fetuses in 34 cases of medically-induced abortion revealed no evidence of fetal injury attributable to TCDD.[13] Pregnancy outcome in the years following 1976 appears to be comparable with the Western experience.[8]

It is of particular interest that no birth defects were observed during 1977 among the 70 births in Zones A and B. Only those pregnancies coming to term after January 1977 would have been relevant, i.e., those in which July 1976 maternal exposure would have occurred during the critical period for the developing fetus. It should also be noted that many factors are known to cause human birth defects, which occur in three to six percent of live births: for example viral infections, genetic predisposition, smoking, alcohol, and even medications, including excess quantities of certain vitamins.

A long-term epidemiological survey of the 220,000 residents of the Seveso area is being conducted by the National Institute of Environmental Health Sciences (NIEHS) and the International Agency for Research on Cancer (IARC) working group.

Other Exposures From Industrial Incidents

While the Seveso incident provides considerable insight as to the reproductive effects and acute toxicity of TCDD, the latency period is insufficient to draw reliable conclusions about carcinogenicity. Studies of other industrial incidents are more revealing in terms of the potential of TCDD to cause cancer in people.

Nitro, West Virginia: In 1949, an industrial accident occurred at a Monsanto trichlorophenol plant resulting in 122 cases of chloracne among the workers. Of those exposed 121 were monitored for 30 years after their high peak exposure to TCDD. To date, there is no apparent excess in terms of total deaths, deatns from cancer or deaths from diseases of the circulatory system.[18] Of the total cancers, there was one case of soft tissue sarcoma. A recently reported 1979 cross-sectional study of herbicide production workers at this plant concluded that it is "unlikely" that "permanent, severe, and debilitating" toxic effects are "inevitable" after exposure to TCDD sufficient to produce chloracne. The study also noted that individual susceptibility may make certain heavily exposed workers more vulnerable but found that "even severe acute toxicological effects of TCDD were reversible or markedly improved over time."[19]

Midland, Michigan: In 1964, a process change in a Dow trichlorophenol plant resulted in the unintentional exposure of 61 workers

to TCDD levels potentially as high as 6,000 to 10,000 parts per million. Of those exposed, 49 workers had some evidence of chloracne. All 61 of these workers have been monitored over the 20 years since their exposure. When last reported in 1980, no excess had occurred in terms of total deaths or deaths from diseases of the circulatory system. (There were, in fact, fewer deaths in both categories than statistically expected.) Cancer deaths have been slightly elevated (3 deaths v. 1.6 expected), but no single type of tumor was predominant.[20] Of the three cancer deaths, there was one case of soft tissue sarcoma.

An additional study including both Dow and Monsanto occupationally exposed groups is being conducted by the National Institute of Occupational Health and Safety (NIOSH).

Other exposures to TCDD from industrial incidents have occurred outside the United States.

Bolsover, United Kingdom: As a result of a 1968 explosion in a Coalite and Chemical Products, Ltd. plant manufacturing 2,4,5-T from trichlorophenol, 79 workers developed chloracne, which was the only consistent finding.[21] Initial findings on these men suggested some change in white blood cell counts, and in three

cases, an elevation of glucose in the urine, suggesting possible liver and kidney damage. But a repeat screening 10 days later did not confirm this pattern: all of the tests gave values within normal limits.[21,22] It should be noted that the design of this study has received strong criticism, since less than half of the workers who developed chloracne were included.[23] No findings on mortality are reported. While most cases of chloracne were resolved within a matter of months following the accident, new cases were noted in a report nine years later which found that one-half the workers studied who had once had chloracne were still suffering from "minor" cases of the skin disorder. Other than the chloracne, the study noted "no evidence" that the workers had been "adversely affected in any way."[24]

Ludwigshafen, Germany: Due to a process accident in 1953 at a BASF Aktiengesellschaft trichlorophenol plant, 55 workers developed chloracne.[25] Peripheral nerve damage and liver toxicity were reported.[26] Due to insufficient decontamination, an additional case of chloracne developed five years later.[27] Follow-up studies 27 years later reported no excess in total deaths, but did find an excess of total cancer deaths (7 v. 4.1 expected). Of these, three deaths were due to stomach cancer (v. 0.6 expected).[27] However, the study's authors note that, "Many of the workers in the factory for some time had probably been exposed to other chemicals and the exposure to dioxin was an additional experience of temporary duration."[27]

Amsterdam, The Netherlands: As a result of an explosion at an NV Philips herbicide factory in 1963, 145 people were exposed to TCDD, 69 of whom had definite signs of chloracne.[28] Liver function tests did not indicate damage.[26] Twenty years later, "no significant differences" were found between those workers and a matched control group.[28] Data on cancers did not show any organ-related pattern and are not considered to represent any excess mortality.

Times Beach and Imperial, Missouri: Mismanagement of waste in 1971 by a now defunct company which produced hexachlorophene led to the spraying of TCDD-contaminated waste oils for dust control on southern Missouri roads and two horse arenas. Analysis 20 years later found that the waste oils may have contained as much as 350 parts per million of TCDD.[29] In both of the arenas, a number of animals died; and in one of them, in which later analysis revealed about 33 parts per million of TCDD in the soil, reports indicate that two children developed lesions resembling chloracne.[29] (See Appendix D for chart comparing various reported concentrations of TCDD.) Testing in Times Beach and Imperial in 1983 revealed TCDD in soil ranging from non-detectable levels to 300 parts per billion, a hundred times less than that found in the horse arena.[30] Recent testing of 68 residents with presumed high exposure to TCDD found no cases of chloracne and no difference, in comparison with a control group, in birth defects, infertility, impotence, headaches, loss of memory or cancer rates.[31,32] Slightly more urinary

tract problems and enlarged livers were found in the test group, but the differences were not statistically significant. Continued health surveillance of the Times Beach residents is planned.

Other Industrial Exposures: In addition to these accidental industrial exposures, a number of occupational exposures have occurred at levels high enough to produce adverse effects. Limited knowledge is available on occupational exposures at 2,4,5-T plants in Spolana, Czechoslovakia, from 1965 to 1968; and in New Jersey from 1963 to 1969. In both of these cases, porphyria cutanea tarda, a liver dysfunction resulting in lesions, was reported in addition to chloracne.[33,34] These findings are obviously in conflict with those at Seveso and also with the six other industrial incidents previously reported in this review in which porphyria cutanea tarda symptoms were not identified. As pointed out by the authors of the New Jersey study, the exposures there probably involved chemicals other than TCDD.

A limitation of the preceding studies is that they were conducted after TCDD exposures sufficiently high to cause obvious signs of illness. A Dow Chemical report on herbicide workers within one of its chemical complexes provides information on the results of lesser exposures. This report examined 204 employees with potential TCDD exposure from 1950 to 1971, and found no chloracne and no other observable adverse effects.[35] Efforts were made to minimize TCDD contamination of the

product at this facility resulting in an internal standard developed in 1965 of less than one part per million, which was undetectable by analytical standards at the time.

It is important to note that most of these industrial incidents represent essentially worst-case scenarios involving exposures considerably higher than any that could be derived by ground troops, even if directly sprayed with Agent Orange: in some cases, the exposures were potentially from 350,000 to ten million times greater. The only lasting, consistent finding documented in these industrial incidents is chloracne.

HYPOTHESES REGARDING THE HEALTH EFFECTS OF TCDD

Soft Tissue Sarcoma

Perhaps the most publicized hypothesis about TCDD and human health involves the possible causal relationship between the compound and soft tissue sarcomas. In some studies based on detailed technical knowledge of the chemical process and clinical evidence of excessive exposure (chloracne) there is evidence that TCDD is at least one of a number of common exposures. In other reports the evidence is more presumptive, and in some cases it is totally lacking. To date, the bulk of the evidence does not demonstrate a causal link between TCDD and soft tissue sarcomas.

Soft tissue sarcoma is a generic term for a group of lesions that include more than 100 different types of rare cancers.[36] Most experts believe that if TCDD were acting as a carcinogen it would cause an increase in one type of soft tissue sarcoma rather than causing an increased risk across the board. For example, one of the reasons vinyl chloride is recognized as a human carcinogen is the specific way it acts. At high doses it has been associated with a single type of soft tissue sarcoma: angiosarcoma of the liver.

The soft tissue sarcoma hypothesis derives from the epidemiological work reported in 1979 by Dr. Lennart Hardell and his associates in Sweden.[37] No one has been able to replicate Dr. Hardell's findings, and his studies have been strongly criticized because of the potential for observer and recall bias in addition to other confounding elements.[38,39]

Dr. Hardell first administered mail questionnaires in his studies then followed up with telephone interviews on a selected subset of subjects. While the questionnaire was designed with the idea of masking the intent of the investigation, the telephone inquiry specifically focused on the exposures of pertinent interest, which included 2,3,5-T and 2,4-D. Interviewers were given extensive information on the purpose of the study and could easily have known which subjects were soft tissue sarcoma cases. Interviewers probed respondents carefully only about occupations in which exposures to TCDD were likely.[38]

Recall bias is also involved in that workers are unlikely to remember with accuracy the chemicals they encountered some years in the past, and it is also extremely difficult to estimate the extent or duration of the exposures. It should be noted that out of 15 chemicals evaluated by Dr. Hardell, only one, sodium chlorate, did not show an elevated risk. Nor is it known what elevations would have been shown for these other agents if they had been probed for in the targeted approach as for 2,4,5-T and 2,4-D.

Additional problems are posed by the rareness and diversity of these tumors. Few pathologists have been able to achieve a high degree of consistency in their diagnoses.[40] Further, the identification and classification of soft tissue sarcomas have not been consistent over time.

The significance of this last point is illustrated by findings announced by Dr. Marilyn Fingerhut, an epidemiologist for the National Institute for Occupational Safety and Health (NIOSH), at an October 1983 dioxin conference at Rockefeller University. Prior to the conference, seven cases of soft tissue sarcoma had been reported in the literature among United States chemical plant employees with presumptive exposures to herbicides. These reports seemed to support the findings of Dr. Hardell. At the conference, however, a different picture emerged. NIOSH announced, after detailed evaluation of the employees' work histories and medical

records and microscopic examination of the tumors, that two of the seven had been misdiagnosed: the two deaths were due to carcinomas, not soft tissue sarcomas. In addition, of the remaining five, three were judged by NIOSH not to have had documented TCDD exposure. Further, neither of the remaining two cases had been exposed to 2,4,5-T but rather to the reedstock trichlorophenol. Furthermore, both of these cases had been exposed during upset operating conditions involving relatively high TCDD exposures sufficient to cause chloracne. Both of remaining cases were malignant fibrous histiocytomas.

At present, there is a growing body of literature which does not support Dr. Hardell's hypothesis.

New Zealand: No association between the use of these phenoxy herbicides and soft tissue sarcoma has been found in an ongoing case control study in New Zealand, where herbicide application is a registered profession. According to preliminary results published in 1982, not one of the soft tissue sarcoma patients had ever worked as a licensed applicator of these herbicides.[41]

Washington State: No consistent patterns of death due to soft tissue sarcomas were found among occupations related to the use of these herbicides. The two occupations found to be at highest risk in this 1982 review were marine engineers and bankers.[42]

Finland: No soft tissue sarcomas were found among 1,900 Finnish herbicide applicators in this 1982 review. Nor was the death rate for these applicators different from any natural cause in comparison with the Finnish total male population.[43]

Midland, Michigan: A statistically significant excess of connective soft tissue cancer has been found by the Environmental Protection Agency among white females in Midland County (where Dow has production facilities). The excess amounted to 13 deaths over a 20-year period. In some cases onset of the disease had begun before the woman moved to Midland. In its 1983 preliminary report, the Michigan Department of Public Health (MDPH) was unable to link the excess with any environmental factor, including TCDD. As part of this study the MDPH reviewed data for 28 other counties in which TCDD or other dioxins were likely to be produced as a chemical manufacturing contaminant. No increase in connective and soft tissue cancers was found in these counties versus those that did not have industrial manufacturing sources suspected of generating such dioxins.[44] Research continues on the problem by the MDPH, funded by a $250,000 grant by Dow to the state of Michigan.

Contrary to expectations based on Dr. Hardell's findings, even in Sweden recent reports from Stockholm's Karolinska Institute indicate that Swedish farmers, about 15 percent of whom use the herbicides Hardell

probed for, have experienced slightly fewer cases of soft tissue sarcoma than expected, in comparison with the overall Swedish data.[45] The only way that Dr. Hardell's findings and those of the Karolinska Institute could both be correct would be if those Swedish farmers not exposed to these herbicides developed soft tissue sarcomas at somewhere between one quarter and one half of the rest of the Swedish population. This seems unlikely.

In addition to the above, it should also be noted that no deaths from soft tissue sarcomas were noted in the U.S. Air Force Ranch Hand mortality study of servicemen who sprayed Agent Orange in Vietnam;[46,47] nor were any cases of soft tissue sarcoma noted in the subsequent morbidity study. Further, examinations by the U.S. Veterans' Administration of 85,000 self-selected Vietnam veterans have found fewer cases of soft tissue sarcomas than would be expected from the national average.[48] In the latter case, however, documented exposure to Agent Orange is uncertain; and the registry was not intended as an epidemiological study. Finally, the hypothesis that an association exists between TCDD exposure and soft tissue sarcomas is not supported by research on laboratory animals: animals fed TCDD during laboratory experiments did not develop soft tissue sarcomas.

Additional research is underway in the United States and elsewhere which should provide additional perspective on the soft tissue sarcoma hypothesis.

Reproductive Effects

Laboratory tests have shown that certain levels of TCDD can cause a variety of reproductive disorders in pregnant animals. In pregnant mice certain levels of TCDD can cause birth defects. In pregnant rats exposure to certain levels can be toxic to the fetus causing conditions such as low birth weight. In pregnant women, however, studies to date have found none of these effects from TCDD. In addition, there is no medical or scientific support for claims that toxic effects in a male from exposure to TCDD could impact a developing fetus in the female.

The most widely publicized claims that the spraying of herbicides contaminated with TCDD could cause reproductive disorders stems from the 1978 Alsea II study by the Environmental Protection Agency (EPA) which reportedly found a link between herbicide spraying and spontaneous abortions in Alsea, Oregon. But this study has been severely and almost universally criticized in the scientific community. At least 18 separate critiques of the study have found that its conclusions are not supported by its data.[49] While some of these are brief assessments, the longest is a 100-page report by an interdisciplinary task force at Oregon State University, which concluded that, "If there is a relationship between herbicide use and miscarriages in the 'Alsea Basin'..., it is not apparent and cannot be tested using the data from the Alsea II study."[50] More recently, EPA's handling of the incident also came under criticism by the Agency's inspector general.[51] Among the limitations

of the Alsea II study was the lack of evidence that the affected women ever came in contact with the herbicide. In addition, there was no dose/response effect: miscarriages did not increase substantially when herbicide application was doubled.

The EPA's 1979 Alsea II study findings resulted in the suspension of the major uses of the herbicide 2,4,5-T. Dow no longer manufactures 2,4,5-T in the United States. (A partially owned Dow subsidiary manufactures 2,4,5-T in New Zealand.) The following studies were conducted to determine whether TCDD causes reproductive disorders in humans. None of the studies support the conclusions of Alsea II.

Yarraw District, Australia: This 1978 investigation by the Australian Commission of Public Health failed to find any link between the use of 2,4,5-T and 2,4-D and birth defects in people or domestic animals.[52]

Arkansas, 1948 to 1974: This 1979 investigation found no association between 2,4,5-T and facial cleft defects in children.[53]

Long Island Railroad: This study by the National Institute for Occupational Health and Safety (NIOSH), also conducted in 1979, found no definite excess of birth defects related to 2,4,5-T exposure in maintenance workers exposed to the herbicide, which was used for weed control along the railroad tracks.[54]

Hungarian Agriculture and Forestry Workers: This 1980 study found that despite a large increase in the use of 2,4,5-T in that nation between 1969 to 1975, levels of various birth defects either remained stable or declined.[55]

New Zealand 2,4,5-T Applicators: This 1982 study conducted by researchers at the Wellington Clinical School of Medicine found no statistically significant increase in relative risk among male professional 2,4,5-T sprayers in terms of birth defects or miscarriages. Wives involved with spraying activities or who washed contaminated clothes also had no detectable reproductive effects.[56]

Midland, Michigan: This 1983 investigation by the Michigan Department of Public Health (MDPH), updating a previous report on an increase of birth defects in Midland County from 1971 to 1974, found that the increases "may not have been unusual" and speculated that the fluctuations may have been due to changes in classification and reporting of birth defects.[57]

Further light is shed on the issue of reproductive disorders and the potential for toxic effects of TCDD to be transmitted from the male to the fetus by a study conducted by Dow Chemical on the wives of employees involved in herbicide production. In this 1982 study, the test group consisted of wives of employees with potential TCDD exposure, while the

control group consisted of wives of employees without such potential exposure.

One phase of analysis dealt with potential effects on fertility. The average number of pregnancies among wives of 370 employees with potential exposure to TCDD were compared to those among wives of 345 employees without such presumed potential exposure. Results showed no decrease in pregnancies among the test group: in fact, the test group had more pregnancies than the control.[58]

Another phase of analysis examined miscarriages, stillbirths, birth defects and infant deaths in terms of potential TCDD exposure. (In this phase, it was necessary to redefine the births in the test and control groups, because some pregnancies in the test group took place before the father's presumed dioxin exposure. This required the transfer of 766 births from the test group to the control.) Analysis found no statistically significant associations between TCDD exposure and pregnancy outcomes.[59]

Synergism and TCDD

Unsubstantiated claims have been made that TCDD exposure could account for any number of ill effects in humans, because of its ability to enhance the toxic effects of other compounds. A Dow Chemical mortality

report on 8,181 employees working in a large production complex involving as many as 500 distinct chemical processes found no observable adverse effects. This 24-year follow-up from 1954 to 1978 found overall mortality to be 19 percent less than that expected for the corresponding U.S. population.[60] While the healthy-worker factor could have played a part here, if low-level TCDD exposure could cause a variety of increased illnesses, an increase in mortality should have been found, not a decrease.

AGENT ORANGE AND THE VETERAN

On the basis of the considerable knowledge of the human health impact of TCDD gained from instances of high-level industrial exposure and long-term occupational exposure, it might seem surprising that eight years after the herbicide program in Southeast Asia ceased health claims were filed by veterans alleging a link between their current health status and what they believe to have been exposure to Agent Orange. An excess in total cancers has not been seen in workers exposed to high levels of TCDD at Nitro, West Virginia; Amsterdam, the Netherlands; or Times Beach and Imperial, Missouri. Nor have studies found reproductive effects from low-level TCDD exposures as a result of herbicide spraying in Australia; Hungary; New Zealand; or Times Beach and Imperial, Missouri; nor from male railroad worker exposures on Long Island, or from the exposures of male herbicide production workers in Midland, Michigan.

Further, male mice fed simulated Agent Orange containing two parts per million TCDD (the average TCDD concentration in the Agent Orange used in Vietnam) did not show any reproductive disorders when mated with female mice after eight weeks of exposure.[61] (See Appendix C.) In addition, in contrast to other documented cases of TCDD poisoning, in which chloracne serves as an indicator of overexposure, no cases of chloracne have been documented by government physicians in any of the Vietnam veterans examined.[48]

On the basis of the available information, Agent Orange does not seem either a very likely or plausible explanation for the health problems experienced by some veterans and their families. Nor is there any scientific documentation to date that the health of Vietnam veterans as a group is any different from that of the general population. Nor until now has any concerted scientific effort been made to determine if the veterans of the Vietnam conflict or any other war have suffered any long-term health problems from any wartime experiences.

The plaintiffs in the present litigation represent less than one-tenth of one percent of the 2.8 million veterans who served in Vietnam. Many of these plaintiffs are very ill, from undetermined causes, and need help. While their problems are real and legitimate, their link to Agent Orange has yet to be established.

There are about 95 ongoing state and federal studies relating to TCDD and its potential health impact; however, few of these studies have relevance to the Vietnam veteran exposed to Agent Orange. Essentially, only one of these studies, which is being conducted by the Centers for Disease Control, examines in detail the potential for adverse health effects in Vietnam veterans from a variety of potential causes other than Agent Orange. The following studies have already been conducted to examine the potential for Agent Orange to cause harm, among Vietnam veterans allegedly exposed.

Ranch Hand Studies

In a 1983 study of more than 1,200 servicemen who applied Agent Orange in Vietnam and who were deemed by the U.S. military to have had the highest exposures to TCDD, there was no indication that herbicide exposure had any effect to date on their mortality.[47] These servicemen are estimated by the military to have had exposures to Agent Orange lasting from ten to 12 hours a day, five to six days a week, for periods of at least a year.[62] Each of these servicemen was matched in this study with five other comparisons based on similar job, race, age and month of birth, wherever possible. In addition, the Ranch Handers were compared with the 1978 Department of Defense Nondisability Retired Life Table and the mortality experience of the West Point class of 1956.

Results showed no adverse mortality trends among the Ranch Hand veterans. The Ranch Hands were found to have 40 percent fewer deaths than expected, compared to the statistics for U.S. white males.

While chloracne has been the most consistent finding among workers exposed to high levels of TCDD, a morbidity study recently completed on the Ranch Hands has found no chloracne in the group. While soft tissue sarcomas and porphyria cutanea tarda have been hypothesized as possible adverse outcomes of TCDD exposure, the recent study noted neither of these disorders among the Ranch Hands nor was there any excess in total cancers.

Some differences did exist between the Ranch Hands and the control, however. Among these findings were apparent increases in self-reported neonatal deaths and minor birth defects and an excess of nonmelonoma skin cancers. While these findings merit further investigation of the potential health impacts of the Vietnam experience, it should be noted that no exposure/response relationship with Agent Orange could be established. That is, those Ranch Hands with the highest presumed exposures to Agent Orange did not have a greater frequency of disease, neonatal deaths or birth defects when compared to less-exposed Ranch Hands.

Further, it should be noted that because the neonatal deaths and birth defects were self-reported, medical confirmation is needed before they

can be adequately interpreted. In addition, the skin cancers were of a type that is highly curable and which appears to be related to sunlight. That is, geographical areas with greater sunlight exposure have more cases of these skin cancers. The Air Force study notes that these apparent excesses of skin cancers among Ranch Hands have not been adjusted for sunlight in terms of geographical location of residence.[47].

Australian Birth Defect Study

According to this 1983 study, commissioned by the Australian Ministry for Veterans' Affairs, no evidence was found that army service in Vietnam has increased the risk of birth defects. The study was conducted by searching the records of 34 Australian hospitals and cytogenetics laboratories for birth defective children born to any of that country's veterans who served in Vietnam between 1962 and 1972. The study "gives persuasive evidence that Vietnam service has not been associated with any important increase in the risk of birth defects in children of veterans."[63]

A similar study of U.S. Vietnam veterans living near Atlanta, Georgia, is scheduled to be released in spring of 1984.

Australian Senate Standing Committee Report

"No convincing evidence" was found in this 240-page report of the Australian Standing Committee on Science and the Environment in its investigation of the potential for Agent Orange to cause harm that the rates of birth defects or mortality were excessive among Vietnam veterans. Further, the Committee concluded that Agent Orange is an improbable cause of birth defects in children of Vietnam veterans. "The Committee believes," the report states, "that there is no biologically plausible mechanism whereby the father's exposure in Vietnam can lead, years later, to exposure of the fetus in the uterus, as would be required to produce teratogenic birth abnormalities."[64] The report also noted the "striking similarities of the veterans' disorders in comparison with those found among veterans of World War I, World War II, the Korean War, and the Arab/Israeli Wars."[64] No conclusion on carcinogenicity was reached by the report; the Committee believed there was insufficient evidence to make an assessment either way.[64]

U.S. Agent Orange Registry Findings

No "unusual long-term morbidity or mortality associated with Vietnam service or Agent Orange exposure" was found in this recent Veterans'

Administration evaluation of 85,000 Vietnam servicemen who presented themselves to the agency for examination. While this assessment cannot be considered a full-fledged epidemiological study, it should be noted that "of the several thousand veterans complaining of dermatologic problems, only one may possibly turn out to be chloracne."[48] While the report notes "a wide variety of health problems," they were "of the sort one sees in a population of males growing older."[65]

National Health Statistics and the Veteran

The studies just cited attempt to determine if the health effects of Vietnam veterans can be related to their potential exposure to Agent Orange. Results of these studies should be encouraging to the veteran, and as noted previously, further study continues. Statistics indicate, however, that serious medical conditions do occur in the normal course of events. Given a large enough population, these conditions occur with statistical regularity.

Presently there is no certainty that the 2.8 million Vietnam veterans as a group are, or are not sick, or dying from unusual causes or at unusual rates. There is some certainty, however, what the vital statistics of a group the same size, age, and sex as the veterans would be: that is, national health statistics suggest how many people in a similar population would die within a given time frame, and from what causes.

On the basis of previous research, over the ten year period 1970 to 1979, 48,592 veterans would have been expected to die. Accidents, homicides and suicides would be expected to account for the largest portion of these deaths: 30,972. An additional 17,620 deaths would occur from various physical disorders:

Heart disease and stroke	**4973**
Cancer	**3819**
Disease of the digestive system	**2109**
Diseases of the respiratory system	**1193**
Mental disorders	**1010**
Diseases of the nervous system	**817**
Diseases of the endocrine system	**633**
Other physical disorders	**3066**

It should be noted that these are statistics based on the mortality of U.S. white males.[66] These are deaths that could be expected to occur in the "normal" course of events. In effect, what the study suggests is that many of the claims being made by the plaintiffs in the present litigation are no different from the types of ailments that would be expected in a similar group from the general population.

Recent Developments

In July 1983, TCDD was described as a probable human carcinogen by a panel called by the Environmental Protection Agency (EPA) to review the literature on the compound. A primary reason for this determination was the presence of seven cases of soft tissue sarcoma in a registry maintained by the National Institute for Occupational Safety and Health (NIOSH) of about 6,000 workers potentially exposed to TCDD. However, in October of the same year, NIOSH announced that three of this seven did not have significant dioxin exposure, and two of the remaining four had been misdiagnosed: they had died of carcinomas, not soft tissue sarcomas. Neither EPA's nor NIOSH's findings in this regard have been published yet in the scientific literature.

In September 1983, the Supreme Court of Nova Scotia ruled in favor of the continued use of 2,4,5-T in that province, denying plaintiffs an injunction to prevent the spraying. According to Justice D. Merlin Nunn, "This court is of the opinion that these spraying operations can be carried out in safety and without risk to the citizens of this province." In his decision Justice Nunn criticized witnesses for the plaintiffs for partisanship and refusing to accept criticism of studies supporting their points of view. By contrast, Justice Nunn stated that he did not detect partisanship on the part of the defendant, Nova Scotia Forest Industries, and accepted the evidence of the defendant's witnesses as representing the generally accepted view of responsible scientists.

In December 1983, a group of scientists attending an international conference on TCDD at Michigan State University concluded that humans appear to be less sensitive to TCDD than some laboratory animals, and that insufficient evidence exists to indicate TCDD is a human carcinogen. While the report noted the potential for TCDD to cause reproductive effects in pregnant women exposed to high levels, such as the most contaminated areas of Seveso, Italy, it is also noted that there is no evidence that TCDD can cause birth defects through paternal exposure.[67]

According to press accounts, researchers attending this conference stated that TCDD does not warrant "an exceptionally high public policy priority which diverts resources and public attention from other more widespread and dangerous compounds." However, the announcement noted that "hot spots" where major concentrations of TCDD exist should continue to be studied.[68]

Conclusion

Considerable research is being directed towards determining whether TCDD at trace levels poses a risk to human health. A considerable amount of research has already been conducted. This research suggests that:

1. TCDD causes chloracne, the most consistent indicator of a toxic exposure, at relatively high levels;

2. At higher levels of exposure, TCDD causes transient liver and nerve dysfunction;

3. TCDD does not adversely affect human reproduction from toxic exposures sustained by the father;

4. No deaths from TCDD exposure have been documented, nor has exposure to the compound been shown to increase the risk of dying at some later period, whether from all diseases in aggregate or total cancers; and

5. More research is needed to clarify the relationship, if any, between TCDD exposure and soft tissue sarcoma.

Based on the conclusions of this research, Agent Orange exposure as a result of military spraying to protect American servicemen in Vietnam does not seem a likely cause of the health effects presently suffered by some veterans and their families. Although preliminary findings to date should be encouraging to veterans, scientific research is insufficient to determine definitively whether the health of Vietnam veterans as a whole is any different from that of the general population. Further research is needed, and is now underway, on the potential health impact of the entire Vietnam experience on our veterans, who endured tough, demanding duty in the service of their country.

APPENDIX A

A BRIEF HISTORY OF THE AGENT ORANGE CONTROVERSY

Herbicide Use In Vietnam

Agent Orange, the United States military code name for a 50/50 mixture of herbicides 2,4,5-T and 2,4-D was used during the Vietnam War from 1965-1970 to defoliate jungle vegetation and to protect U.S. servicemen from enemy ambush.

During that time it is estimated that 10 percent of Vietnam was defoliated with herbicides and 60 percent of that area was sprayed with Agent Orange. (In terms of perspective, it might be noted that in 1982 the gypsy moth defoliated eight million acres in the Northeastern states according to the U.S. Forest Service -- twice as many acres as were defoliated during the entire Vietnam War.) Agent Orange was usually sprayed from C-123 (fixed-wing) military aircraft. A small amount of Agent Orange was applied from ground sources such as boats, trucks and backpack sprayers. The total amount of Agent Orange used during the herbicide program is estimated at between 10 and 12 million gallons.[69]

Studies indicate that only about six percent of the Agent Orange sprayed over Vietnam ever penetrated the dense foliar canopy to reach the jungle floor; and of the 94 percent caught in the dense foliage, direct exposure

to sunlight would have degraded most of the TCDD within 24 hours.[69] The remaining TCDD which might have reached the jungle floor would have been tightly bound to the soil and therefore been less available for human contact. (Under these conditions, TCDD levels in Vietnamese soil from Dow-supplied Agent Orange would have been more than 100 times less than the level of concern recently set at Times Beach by the Centers for Disease Control for residential areas. See Appendix D.)

Development of Agent Orange

During World War II, the United States military began studying the use of 2,4,5-T and 2,4-D, during field trials to determine their capability in defoliating large areas of vegetation in tropical battlegrounds. Military experiments with these herbicides continued through the 1940s and 1950s. In 1961, a defoliant similar to Agent Orange was selected by the U.S. Secretary of Defense to be tested as the herbicide of choice for use in Vietnam.

In the early 1960s the Office of the Surgeon General, Department of the Army, conducted research on the trace contaminant TCDD. The published results acknowledged the work of German scientists Drs. Kimmig and Schulz who observed cases of chloracne among workers engaged in the production of 2,4,5-T from trichlorophenol in the late 1950s. Kimmig and Schulz had reported TCDD to be a contaminant of this manufacturing process.[70]

In 1962, the U.S. Government published research sponsored by the Research and Development Division of the Office of the Surgeon General, Department of the Army and by the U.S. Public Health Service which reported on the process to determine the existence of TCDD in certain chemical products. This method, known as the rabbit ear test, was designed by Dow to measure skin response (chloracne) from TCDD exposure, as well as from other chemicals.

In 1965, the U.S. military decided upon a 50/50 mixture of 2,4,5-T and 2,4-D which later came to be known as Agent Orange. The military subsequently determined the rates and frequencies of Agent Orange application throughout the Vietnam herbicide program as well as the areas to be sprayed. As used in Vietnam Agent Orange has not been prescribed for any domestic weed control applications.

Dow supplied about 32 percent of the Agent Orange to the U.S. Government for the war effort. As a quality control measure Dow analyzed the 2,4,5-T in all shipments of the Agent Orange it supplied the government to insure the absence -- no detectable level -- of TCDD, which given the technology of the day meant less than 0.5 part per million in the Agent Orange.

Agent Orange Lawsuits

In early 1979, the first of multiple lawsuits was filed against seven manufacturers: Dow, the Monsanto Company, Diamond Shamrock

Corporation, Uniroyal Inc., T.H. Agriculture and Nutrition Company, Thompson Chemical, and Hercules Inc. alleging a connection between the spraying of Agent Orange in Vietnam and various maladies reported by some Vietnam veterans and their families.

These claims have been consolidated into a class action suit in which thousands of Vietnam veterans and their family members will be represented by selected cases. These model cases will be tried individually to determine whether Agent Orange has caused health problems to these veterans and their families. The trial is scheduled to begin in early May 1984 in the courtroom of Chief Judge Jack B. Weinstein of the Federal District Court for the Eastern District of New York.

In the meantime, more than 95 studies are underway to determine the validity of health claims involving dioxin and Agent Orange.

The results of these and other ongoing studies should help to address public anxiety over the alleged link between Agent Orange and human health problems.

APPENDIX B

CONSPIRACY OF SILENCE CONTENTION IN THE AGENT ORANGE CONTROVERSY

Much of the present plaintiffs' case has centered around the so-called "conspiracy of silence" claim. In brief, this theory claims that Dow knew that Agent Orange was harmful to users and met with other chemical producers deliberately to conceal this information from the government. The theory does not hold up under scrutiny.

An examination of the chronology of events surrounding the selection of Agent Orange by the military for use in Vietnam shows 1) that the United States government had extensive knowledge of both the components of Agent Orange as well as the potential occupational health effects of the contaminant TCDD; 2) this knowledge on the part of the government was concurrent with that of Dow and the other co-producers; and 3) that Dow's concerns about TCDD centered around the potential of relatively high levels of the compound to cause harm in the workplace not on the end uses of the finished product which contained vastly lower levels of the contaminant.

Chronology

1949 The U.S. Public Health Service (USPH) investigates the chloracne incident at a trichlorophenol production plant at Nitro, West Virginia.

1957 Having determined TCDD to be the cause of chloracne in its trichlorophenol workers, the German firm **Boehringer Sohn** sends a letter to **Dow Chemical** regarding the availability of technology to address the problem. Dow has no chloracne problem at the time of receipt of the information, and the letter is filed for future reference.

1959-60 United States military personnel at **Edgewood Arsenal, Fort Detrick,** and the **Army Corps Research and Development Laboratory** become aware of the chloracne potential in trichlorophenol manufacture, through the research of two German scientists, Kimmig and Schulz. TCDD is considered briefly by the military as a weapon of war.

An article published by the **USPHS** indicates knowledge that chloracne was associated with 2,4,5-T production.

1962 Military personnel at **Fort Detrick** recommend a defoliant formulation, later to become known as Agent Orange, for use in Vietnam.

Research supported by the **Research and Development Division of the Office of the Army Surgeon General** indicates both knowledge of TCDD's acnegenic potential and that the contaminant could be produced at high temperatures from trichlorophenol.

1963 The **USPHS** completes investigation of a chloracne incident at a New Jersey 2,4,5-T production plant.

1964 A process change in **Dow Chemical** trichlorophenol production at Midland, Michigan, exposes 61 workers to high levels of TCDD. Of those exposed, 49 workers have some evidence of chloracne. The trichlorophenol plant is shut down for a period to resolve the problem. TCDD is first suspected, then confirmed, as the cause of the problem.

1965 Dow establishes an internal standard for detectable TCDD at an analytical limit of one part per million.

Dow calls a meeting with its competitors to discuss the presence of the acnegen in trichlorophenol.

Dow also informs the **USPHS**, the **Michigan State Department of Public Health**, the **University of Michigan Institute for Industrial Health** and other Michigan universities, the **United States Army Biological Laboratories at Fort Detrick**, the **Ontario Department of Labor**, and other government agencies of the Midland trichlorophenol chloracne incident. Also notified were a number of physicians and health officials from private industry, and from foreign countries including the Netherlands, England, South America and the Soviet Union.

Military personnel at **Fort Detrick** request toxicity data on TCDD from **Edgewood Arsenal.**

Late in the year, Dow supplies the military with its first shipment of Agent Orange.

1966 A new Dow trichlorophenol plant comes on line at Midland, Michigan, built with technology licensed from **Boehringer Sohn** in Europe.

The **Army Surgeon General's Office** requests toxicity information on 2,4,5-T from the **National Academy of Sciences (NAS);** the NAS response references porphyria and chloracne as effects of exposure.

Personnel at **Eglin Air Force Base** learn of the potential for TCDD to cause occupational harm as the military considers construction of its own 2,4,5-T plant, due to short supply of Agent Orange.

1967 With regard to the government's plan to build its own Agent Orange production facilities, **Dow Chemical** informs **Edgewood Arsenal** and the **Department of Defense** of the "serious potential health hazard" (chloracne) to 2,4,5-T industrial plant workers.

The **Rand Corporation** reports that infants could potentially receive lethal doses from the wartime crop-destruction program. The report is passed by **Secretary of Defense Robert McNamara** to the **Joint Chiefs of Staff**, who recommend that the herbicide program continue.

1968 **Thompson Chemical Company**, in the course of meetings relating to the military's plan to build its own Agent Orange facilities, informs military personnel of the availability of technology to reduce TCDD in Agent Orange. Also discussed are the hazards of TCDD.

1969 Military personnel at **Fort Detrick** receive data, prior to its official release, on the teratogenicity of 2,4,5-T in animals based on animal studies conducted by **Bionetics Laboratories.** After the information has been made available to the public, the **Department of Defense** orders the restriction of Agent spraying to areas of Vietnam remote from population.

1970 Dow advises **Edgewood Arsenal** personnel that the TCDD in the 2,4,5-T used in the Bionetics study probably accounted for the teratogenic findings. Spraying of Agent Orange in Vietnam is suspended by the government.

1971 **Edgewood Arsenal** personnel indicate that stockpiled Agent Orange containing less than one part per million of TCDD is

still safe for use by the military. Stockpiles are ultimately incinerated at sea.

Note: plaintiffs have argued that the Department of Defense should have been notified directly by Dow. Dow has no proof that such notification did or did not take place prior to 1967. However, Dow's concerns centered around occupational exposure to TCDD not exposure to Agent Orange or any other finished product. It should also be noted that Dow was not a supplier of Agent Orange to the government at the time of the Midland chloracne incident, and measures to correct the problem had been taken before Dow's first shipment to the government. In addition, the record shows that Dow notified government agencies of its occupational problem with TCDD. Such a wide sharing of knowledge can hardly be equated with the so-called "conspiracy of silence." Finally, the chronology just given reveals extensive knowledge on the part of the government, and by military personnel within the government, regardless of when notification to the Department of Defense by Dow did or did not take place.

APPENDIX C

ANIMAL STUDIES

The Toxicity of TCDD in Animal Species

There are wide variations in how different animal species are affected by TCDD. For example, the single oral dose LD_{50} (the amount that it would take to kill 50 percent of the test animals) in guinea pigs is 0.6-2.0 ug/kg* (micrograms per kilogram or every 2.2 pounds of body weight [a microgram is one millionth of a gram; a kilogram is 1,000 grams, or 2.2 pounds]).[71,72] In hamsters, however, TCDD is much less toxic with an oral LD_{50} of 1157-5051 ug/kg.[73,74]

Therefore, the guinea pig is approximately 5,000 times more sensitive than the hamster, although both species are commonly used for laboratory experiments. The direct relevance of any single animal experiment to people must be evaluated with respect to all other available data.

In acute and subchronic studies, liver toxicity is a prominent component of TCDD toxicity in rats, mice and rabbits, but not in monkeys where effects on the bone marrow and epithelial tissue are more prominent.

*See conversion chart, Appendix E.

Thymic atrophy in early toxicity studies suggested that TCDD might decrease the immune response. In laboratory animals, scientists can induce immunotoxic effects with high doses of TCDD. However, as the Seveso incident shows, these effects have not been demonstrated in exposed humans.[7,14]

Teratogenicity/Animal Species

Of the various toxic responses of animals to TCDD, the potential for teratogenicity (birth defects) has perhaps received the most attention. Teratogenic effects resulting from TCDD exposure have been realized only in mice which show an increased frequency of cleft palate and a kidney abnormality. It is well known that many factors can cause birth defects in mice including the stress of being transported by air during pregnancy or being deprived of drinking water overnight. In rats TCDD does not cause a true teratogenic effect, but sufficiently high doses can cause embryo- and fetotoxicity. In both rats and mice, dose levels of TCDD have been identified at which these effects do not occur.

Many of the studies with TCDD in monkeys have been conducted at the University of Wisconsin. In experiments where the high dose level of about 0.011 ug/kg/day* TCDD in the diet was given to the monkeys for up

*See conversion chart, Appendix E.

to 9.3 months, there were substantial toxic effects.[75,76] A preliminary abstract of a follow-up study[77] showed that monkeys given diets containing approximately 0.0017 ug/kg/day* of TCDD had only slight toxicity. There are studies currently in progress at the University of Wisconsin at lower doses.

In a three-generation reproduction study[78] of rats exposed to incremental dose levels of TCDD in the diet high doses caused decreased fertility and decreased neonatal survival. The intermediate dose level caused decreased fertility and other effects in the first two generations but not in the third. At the low-dose level there was no impairment of reproductive capacity through the three consecutive generations, indicating that 0.001 ug/kg/day* was a no-adverse-effect level over multiple generations.

In addition, animal studies done to date do not suggest that birth defects can be passed from the male to the female as a result of a toxic TCDD exposure in the male. In a study conducted by the National Toxicology Program, laboratory mice were fed simulated Agent Orange containing two parts per million of TCDD (the average concentration of TCDD in the Agent Orange used in Vietnam) for eight weeks as part of their diet. Definite signs of toxicity, in terms of reduced weight gain and liver toxicity, were noted in the exposed males. Yet, when these males were

*See conversion chart, Appendix E.

mated to unexposed virgin females, "no significant decrease in fertility or reproduction" was noted. Survival of offspring was "apparently unaffected by paternal exposure to the simulated mixtures of Agent Orange."[55]

Mutagenicity/Animal Species

A number of mutagenic studies of TCDD have been conducted with various *in vitro* test systems using bacterial yeast, plant and tissue culture tests. Additional tests have been conducted with intact animals as well as human cells.[14, 79, 80, 81, 82, 83, 84, 85]

While a few positive or questionable mutagenic responses have been observed in certain plant or microbial test systems, there appear to be no definitive correlates of mutagenicity in higher animals or people.

Carcinogenicity/Animal Species

Carcinogenic studies following ingestion of TCDD have been conducted in rats and mice.[86,87,88] Review of these data indicates that the results in rats and mice correlate well with a carcinogenic response associated only with lifetime ingestion of higher dose levels that also induce other toxicity. The liver was the primary target for cancer in both rats and mice. No cancer response occurred at dose levels of 0.001-0.0014 ug/kg/day* in the rats and 0.001-0.03 ug/kg/day* in mice.

TCDD does cause cancer in animals. However, this happens only at high dose levels -- i.e., dose levels that are higher than those that elicit other kinds of toxicity. Cancer induced by exposure to TCDD would be preceded by substantial signs of toxicity. This toxicity (which is reversible when exposure is discontinued) would act as a sentinel, or warning, at dose levels below those that might cause cancer.

*See conversion table, Appendix E.

APPENDIX D

ENVIRONMENTAL HAZARD EVALUATION

Dioxin in Soil

Potential human exposure from TCDD environmental contamination concerns many people. The U. S. Centers for Disease Control (CDC) has set a level of concern for TCDD in residential soil at one part per billion. This recommendation is based in part on the hypothetical cancer risk mathematically derived from laboratory animal studies. Implicit in this recommendation is the recognition that the amount of chemical absorbed into the body determines whether a harmful response will occur. That is, dose makes the poison. Even for very toxic chemicals such as TCDD there can be concentrations within the body which will cause no harm.

The amount of TCDD absorbed from the soil depends upon 1) duration of contact with the skin and 2) ingestion of the soil. The known soil binding tendencies of TCDD reduces the amount that can be absorbed through the skin or internally via ingestion. Animal studies show that only a fraction of the TCDD is absorbed from contaminated soil. In animals studies when such soil contained about 0.5 part per million of TCDD and the soil was held in contact with skin for 24 hours (covered with aluminum foil), less than 0.2 percent of the TCDD in the soil was absorbed.[89]

In 1983, CDC announced a one part per billion level of concern at Times Beach for TCDD in residential soil.[90] According to the CDC a prime consideration in establishing this guideline was the possibility of children at play in residential areas who might eat dirt.[91]

Based on the CDC's assumptions and calculations and those of Dr. Kingsley Stevens,[92] it appears that general environmental soil contamination in the range of low parts per billion would not pose a health hazard to the general population, including the unborn, children, pregnant women or the old and infirm.

Dioxin in Fish

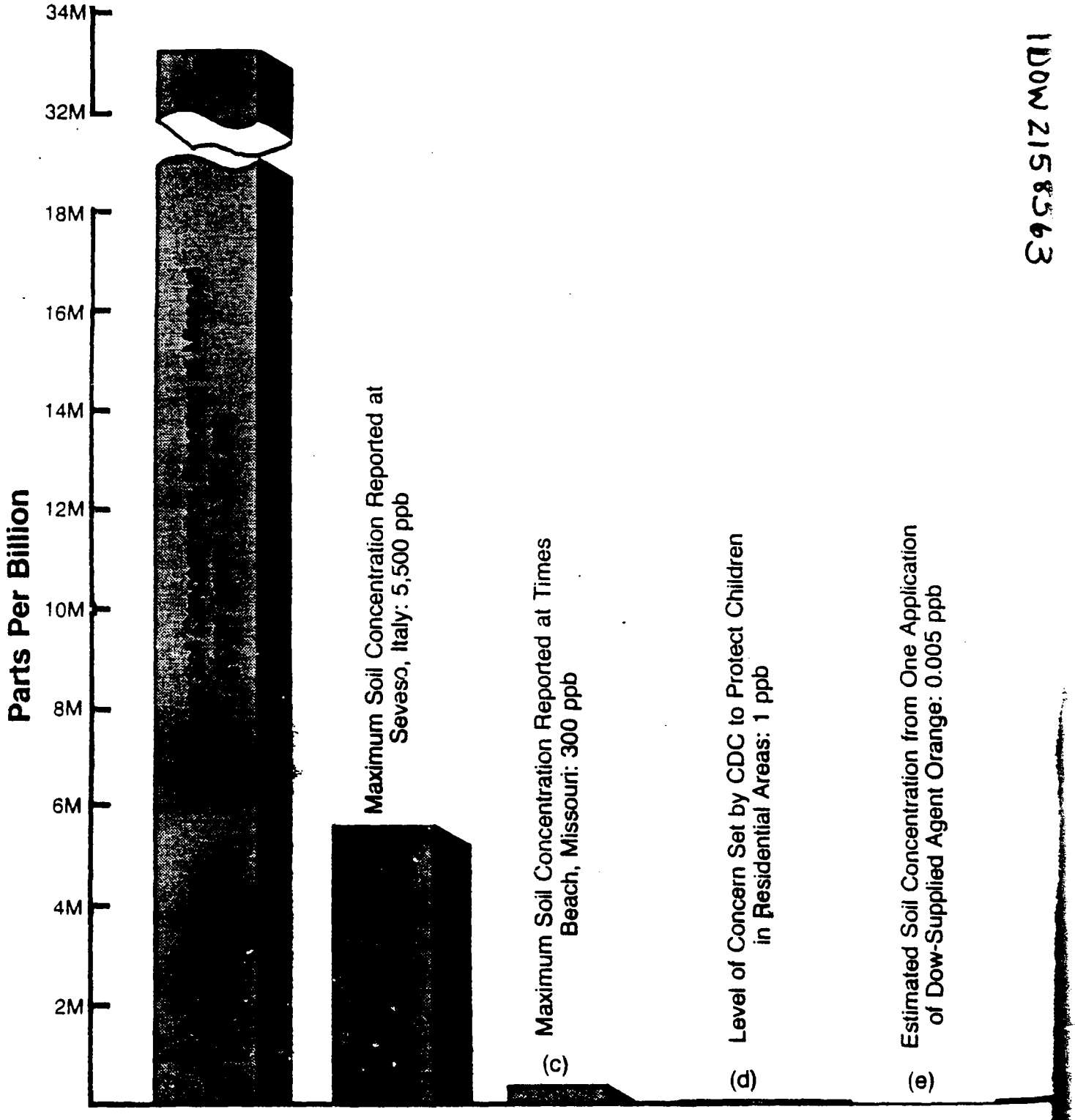
Rats consuming a diet containing 22 parts per trillion (ppt) TCDD (1 nanogram per TCDD to kilogram of body weight per day) for a lifetime showed no adverse health effects. Rats consume about 10-20 percent of their body weight in food each day, while people consume about two to three percent of their body weight. Thus, for a given TCDD concentration in dietary components, rats will ingest relatively more of the compound than people. A person eating about two to three pounds of food per day uniformly contaminated with 25 parts per trillion of TCDD would ingest about 0.5 nanogram per kilogram of body weight per day* or about one-half the dose (weight of TCDD per unit of body weight) shown

*See conversion chart, Appendix E.

to produce no adverse health effects when fed to rats for a lifetime. Again, this is true because people eat less food in proportion to their body weight compared to laboratory rats.

Most people in the United States (99 percent according to 1981 U.S. Food and Drug Administration figures) eat less than 0.6 pounds of fish per week. Nine out of ten people in the United States eat less than 0.3 pounds of fish per week, while the "average" person eats still less. Since fish is a relatively small part of the American diet, 99 percent of the American public would receive less than 1/70 of the amount of TCDD shown to produce no effect in laboratory rats, fed TCDD daily for their lifetimes, if those people ate fish containing 25 parts per trillion of TCDD. Or said another way, a person could eat nearly one ton of fish per year containing 25 parts per trillion of TCDD and not exceed the no-effect level established by laboratory animal experiments. The fish consumption guideline of 25 parts per trillion established by the Food and Drug Administration assures an adequate margin of safety to protect people from overexposure to TCDD.[93]

TCDD Concentrations in Soil



(a) Carter, **et. al.**, (1975): Science, 138: 738-740.
(b) Fanelli, **et. al.**, (1982): Drug Metabolism Review 13(3): 407-422.
(c) Interim Report of the Missouri Dioxin Task Force, June 1, 1983
(d) Kimbrough, **et. al.**, (1983): Centers for Disease Control.

(e) Estimate based on 0.5 parts per million TCDD in the defoliant and assuming 94% photodegredation in the jungle canopy, with remaining TCDD distributed throughout the top centimeter of soil (**cf.**, Young, **et.al.**, 1978 : U.S. Air Force, p. I-20, for documentation on photodegredation estimate.

APPENDIX E

CERTAIN UNITS OF MEASURE

1 milligram (1mg) = 1 gram x 10^{-3} = 0.001 g. (1000 milligrams - 1 gram)

1 microgram or 1 ug = 1 gram x 10^{-6} = 0.000001 g.

1 nanogram = 1 gram x 10^{-9} = 0.000000001 g.

1 picogram = 1 gram x 10^{-12} = 0.000000000001 g.

1 milligram = 1000 micrograms = 1 million nanograms (1,000,000) = 1 billion picograms (1,000,000,000)

1 milligram per kilogram or per liter is 1 part per million

1 microgram per kilogram is 1 part per billion

1 nanogram per kilogram is 1 part per trillion

1 microgram per gram is 1 part per million

1 microgram per milliliter is 1 part per million

1 nanogram per gram is 1 part per billion

1 picogram per gram is 1 part per trillion

1 picogram per liter is 1 part per quadrillion

mg/kilogram	ppm	ppb	ppt
1.0	1.0	1000.0	1,000,000
0.1	0.1	100.0	100,000
0.01	0.01	10.0	10,000
0.001	0.001	1.0	1,000
0.0001	0.0001	0.1	100

APPENDIX F

GLOSSARY

Agent Orange	a 50/50 herbicide mixture of 2,4,5-T and 2,4-D produced according to U.S. government specifications for use as a defoliant in Vietnam. It contained trace elements of TCDD as a result of the 2,4,5-T manufacturing process.
Carcinogen	any agent or substance which produces cancer, accelerates the development of cancer or acts upon a population to change its total frequency of cancer in terms of numbers or distribution by site and age.
Chloracne	an acneform eruption caused by exposure to chlorinated hydrocarbons. Chloracne is considered to be the hallmark or sentinel symptom of human overexposure to the chemical contaminant TCDD.
Immunoresponse	the capacity of an organism to respond to any substance which is capable, under appropriate conditions, of inducing a specific immune response and of reacting with the products of that response, such as specific antibodies.
Initiator	a carcinogen responsible for producing the first stage of a cancer. This step usually involves irreversible mutational changes, which do not necessarily cause cancer.
Kilogram	a unit of weight of the metric system, being 1,000 (10^3) grams, or the equivalent of 2.20 pounds.
Malignant fibrous histiocytoma	A soft tissue sarcoma composed of cells of fibrous connective tissue.
Microgram	a unit of weight of the metric system, being one-millionth of a gram (10^{-6} gm.), or one one-thousandth of a milligram (10^{-3} mg.).
Morbidity	the condition of being diseased.
Nanogram	a unit of weight of the metric system, being one one-billionth (10^{-9}) gram.

No-effect level	the amount of chemical that has been determined by testing to cause no harm to the subject.
Peripheral nerve	the part of the nervous system comprising the cranial nerves, the spinal nerves and the sympathetic nervous system, excluding the brain (central nervous system) and spinal cord.
Porphyria cutanea tarda (PCT)	a member of a group of uncommon diseases, the porphyrias, that comprise disturbances in the body's formation of hemoglobin, the red pigment in the blood. A specific pattern of chemicals called porphyrins, excreted in the urine and stool, characterizes PCT and reflects a deficiency of one of the liver's enzymes involved in hemoglobin formation. The disease manifests itself in the skin where small and large blisters form on the exposed parts of the body, probably as a slow response to sunlight. The skin becomes very fragile and easily rubs off to produce sores that scab over and sometimes leave scars.
Promotion	the second stage of the carcinogenic process which may be the result of additional applications of a carcinogen, or of other, noncarcinogenic materials; or it may involve additional unknown processes which could aid the growth of the cancer or reduce resistance to the developing cancer. TCDD is among the group of chemicals that may secondarily influence the formation of cancer by promotion in some strains of rats and mice.
Soft tissue sarcoma	a relatively rare group of about 100 tumors in the body's muscles, tendons and other connective tissues, in fat tissues, blood vessels and nerves.

TCDD	acronym for 2,3,7,8-tetrachlorodibenzo-p-dioxin, an unwanted trace contaminant formed during the synthesis of 2,4,5-trichlorophenol.
Teratogenic	embryonic maldevelopment leading to serious congenital defects. I.e., causing birth defects.
Thymus	a ductless gland-like structure, situated just behind the top of the breastbone, that plays some part in building resistance to disease.
Toxicity	the inherent property of being poisonous, especially the degree to which any substance can cause acute or chronic injury to living things, or which is suspected of being able to cause disease or injury under some conditions. All substances, man-made or natural, are toxic to some degree; the likelihood of harm depends on toxicity and the amount taken into the body.
2,4-D	also known as 2,4-dichlorophenoxyacetic acid, this chemical compound is a widely used herbicide and a chemical relative of 2,4,5-trichlorophenoxyacetic acid. A 50/50 mixture of these two chemicals, known as Agent Orange, was used as a defoliant during the Vietnam conflict. 2,4-D has been widely used as a herbicide for broadleaf weed control in cereal crops, sugar cane and citrus fruits, and on turf, pastures and noncrop land. At present levels of analytical detection, 2,3,7,8-TCDD has not been identified in any 2,4-D formulations.
2,4,5-T	2,4,5-trichlorophenoxyacetic acid is a selective weedkiller which has root inducing, growth inhibiting and herbicidal properties comparable to those of the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid). 2,4,5-T is known to be particularly active against woody shrubs and trees. It has been used in forestry, on grassland, for industrial and rice and sugar cane weed control.
Trichlorophenol	also known as 2,4,5-TCP. Used as a starting material in the manufacture of a series of industrial and agricultural chemicals, the most notable of which is the herbicide 2,4,5-T and its related products including silvex. Most of the TCDD present in 2,4,5-T is formed during the synthesis of trichlorophenol.

2,3,7,8-tetrachloro-dibenzo-p-dioxin	also known as TCDD or dioxin. It is formed as an unwanted contaminant during the synthesis of 2,4,5-trichlorophenol. Considered to be the most toxic of the 75 chemical compounds that make up the family known as dioxins. Also occurs, with other members of the family, as a result of the process of combustion.

INDEX

REFERENCES

1. The Health Effects of Agent Orange and Polychlorinated Dioxin Contaminates, American Medical Association, October 1981.

2. Ibid.

3. Ibid.

4. Further Review of the Safety for Use in the U.K. of the Herbicide 2,4,5-T, Advisory Committee on Pesticides of the Ministry of Agriculture, Fisheries and Foods (United Kingdom), February 7, 1983.

5. A Report on 2,4-D and 2,4,5-T and Human Health, Interdepartmental Committee Appointed by Queensland Cabinet (Australia), 1981.

6. The Phenoxy Herbicides: Second Edition, Council for Agricultural Science and Technology, Report No. 77, August 1978.

7. Homberger, E., Reggiani, G., Sambeth, J., and Wipf, H.K., (1979): The Seveso Accident: Its Nature, Extent, and Consequences. **Ann. Occup. Hyg. 22:** 327-370.

8. Pocchiari, F. et. al., (1979): Human Health Effects From Accidental Release of Tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. **Ann. N.Y. Acad. Sci.,** 311-320.

9. Fanelli, F., et. al., (1982): TCDD Contamination in the Seveso Incident. **Drug Metab. Rev. 13:** 407-422.

10. Crow, K., (1978): The Chemical Disease. **New Scientist:** April 13, 1978.

11. Caramaschi, F. et. al., (1981): Chloracne Following Environmental Contamination by TCDD in Seveso, Italy. **Int. J. Epidemiol., 10:** 135-143.

12. Fara, G. M., et al., (1980); Chloracne After Release of TCDD at Seveso, Italy. In: **Chlorinated Dioxins and Related Compounds: Impact on the Environment,** Oxford: Pergamon Press, pp. 545-559.

13. Reggiani, G., (1979): Estimation of the TCDD Toxic Potential in the Light of the Seveso Incident. **Arch. Toxicol. (Suppl.),** 291-302.

14. Reggiani, G., (1980): Acute Human Exposure to TCDD in Seveso, Italy. **J. Toxicol. Environ. Health, 6:** 27-43.

15. Bisanti, L., Bonnetti, F., Caramaschi, F., Del Corno, G., Favaretti, C., Giambelluca, S., Marni, E., Montesarchio, E., Puccinelli, V., Remotti, G., Volpato, C., and Zambrelli, E., (1978): Experience of the Accident of Seveso, proceedings from the Sixth EST Conference: 1-32.

16. Abate, L., et. al., (1980): Mortality and Birth Defects From 1976 to 1979 in the Population Living in the TCDD Polluted Area of Seveso. In: **Chlorinated Dioxins and Related Compounds: Impact on the Environment**, Oxford: Pergamon Press, pp. 571-587.

17. Reggiani, G., (1977): Toxic Effects of TCDD in Man. NATO Workshop in Ecology, Guilford, England, July-August, 1977.

18. Zack, J. A., and Suskind, R. R., (1980): The Mortality Experience of Workers Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident. **J. Occup. Med., 22:** 11-14.

19. Moses, M., Lills, R., Crow, K. D., Thornton, J., Fischbein, A., Anderson, H. A., and Selikoff, I. J., (1984): Health Status of Workers With Past Exposure to 2,3,7,8-Tetrachlorodibeno-p-dioxin in the Manufacture of 2,4,5-Trichlorophenoxyacetic Acid: Comparison of Findings With and Without Chloracne. **Am. J. Ind. Med., 5:** 161-182.

20. Cook, R. R., Townsend, J. C., Ott, M. G., and Silverstein, L. G., (1980): Mortality Experience of Employees Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). **J. Occup. Med., 22:** 530-532.

21. May, G., (1973): Chloracne From the Accidental Production of Tetrachlorodibenzodioxin. **Br. J. Ind. Med., 30:** 276-283.

22. Hay, A., (1982): Industrial Accidents in Trichlorophenol Manufacture. In: **The Chemical Scythe: Lessons of 2,4,5-T and Dioxin,** Plenum Press, pp. 109-121.

23. Office of Technology Assessment Review of Two Papers Reporting on Long-Term Effects of Exposure to Dioxin. In: Congressional Record/Senate, September 29, 1983, S13220-S13221.

24. May, G., (1982): Tetrachlorodibenzodioxin: A Survey of Subjects Ten Years After Exposure. **Br. J. Ind. Med., 39:** 128-135.

25. Goldman, P. J., (1973): Severe Acute Chloracne: A Mass Intoxication by 2,3,7,8-Tetrachlorodibenzodioxin. **Hautarzt, 24:** 149-152.

26. Huff, J. E., Moore, J. A., Saracci, R., Tomatis, L., (1980): Long-Term Hazards of Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans. **Environ. Health Perspect. 36:** 221-240.

27. Thiess, A. M., Frentzel-Beyme, R., and Link, R., (1982): Mortality Study of Persons Exposed to Dioxin in a Trichlorophenol-Process Accident that Occurred in the BASF AG on November 17, 1953. **Am. J. Ind. Med., 3:** 179-189.

28. Dalderup, L. M. and Zellenrath, D., (1983): Dioxin Exposure: 20 Year Follow-up. **Lancet, ii:** 1134-1135.

29. Carter, C. D., Kimbrough, R. D., Liddle, J. A., Cline, R. E., Zack, M. M., and Barthel, W. F., (1975): Tetrachlorodibenzodioxin: An Accidental Poisoning Episode in Horse Arenas. **Science, 188:** 738-740.

30. Interim Report of the Missouri Dioxin Task Force, June 1, 1983.

31. Donnell, H. D., and Hoffman, R. E., (1983): Findings of a Pilot Epidemiological Study of Missouri Residents. In a letter from the Missouri governor's office to state physicians.

32. Webb, K., Ayres, S., Slavin, R., Knutsen, A., Roodman, R., Gedney, W. B., Schramm, W., Hotchkiss, R. L., Miller, R., and Donnell, H. D., (1984): Results of a Pilot Study of Health Effects Due to 2,3,7,8-Tetrachlorodibenzodioxin Contamination -- Missouri. J. **Am. Med. As., 251:** 1139-1140.

33. Pazderova-Vejlupkova, J., Nemcova, M., Pickova, J., Jirasek, L., Lukas, E., (1981): The Development and Prognosis of Chronic Intoxication by Tetrachlorodibenzo-p-dioxin in Men. **Arch. Environ. Health, 36:** 5-11.

34. Bleiberg, J., Wallen, H., Brodkin, R., Applebaum, I., (1964): Industrially Acquired Prophyria. **Arch. Dermatol., 89:** 793-797.

35. Ott, M. G., Holder, B. B., and Olson, B. D., (1980): A Mortality Analysis of Employees Engaged in the Manufacture of 2,4,5-Trichlorophenoxyacetic Acid. **J. Occup. Med., 22:** 47-50.

36. Hajdu, S. I., (1981): Soft Tissue Sarcomas: Classification and Natural History, **Ca Cancer J. Clin., 31:** 271-280.

37. Hardell, L., Sandstrom, A., (1979): Case-Control Study: Soft Tissue Sarcomas and Exposure to Phenoxyacetic Acids or Chlorophenol. **Br. J. Cancer, 39:** 711-717.

38. Hobson, L. B., (1983): Epidemiology of Soft-Tissue Sarcoma and Related Human Research, Veterans Administration, March.

39. Cole, P., (1980): Testimony before the Environmental Protection Agency (U.S.) in re: The Dow Chemical Company, et. al.

40. Rubin, P., ed., and Bakemeir, R. F., Assoc. ed., (1978): Soft Tissue Sarcoma, W. B. Patterson, In: **Clinical Oncology for Medical Students and Physicians.** Chapter XIX, American Cancer Society, pp. 210-217.

41. Smith, A. H., Fisher, D. O., Giles, H. J., and Pierce, N., (1982): The New Zealand Soft Tissue Sarcoma Case-Control Study: Interview Findings Concerning Phenoxyacetic Acid Exposure, presentation before the Third International Symposium on Chlorinated Dioxins.

42. Milham, S., (1982): Herbicides, Occupation, and Cancer. **Lancet,**: 1464-1465.

43. Riihimaki, V., Asp, S., and Hernberg, S., (1982): Mortality of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid Herbicide Applicators in Finland: First Report of an Ongoing Prospective Cohort Study. **Scand. J. Work Environ. Health, 8:** 37-42.

44. Evaluation of Soft and Connective Tissue Cancer Mortality Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide, Michigan Department of Public Health, May 4, 1983.

45. Eklund, G., (1983): Does Occupational Exposure to Chemical Pesticides Increase the Cancer Risk?, proceedings of Weed and Plant Protection Conferences, Swedish University of Agricultural Sciences: 6-12.

46. Project Ranch Hand II: An Epidemiological Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. U.S. Air Force, June 30, 1983.

47. Project Ranch Hand II: An Epidemiological Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. U.S. Air Force, February 24, 1984.

48. Flicker, M. R., and Young, A. L., (1983): Evaluation of Veterans for Agent Orange Exposure, in proceedings of the American Chemical Society, 186th national meeting, 2: 152-154.

49. Witt, J. M., (1980): A Discussion of the Suspension of 2,4,5-T and the EPA Alsea II Study. In: Supplement to the 34th annual meeting of the Northeastern Weed Science Society, January 8, 34: 9-25.

50. Sheldon, L. W., Witt, J. M., Logan, A. N., Higgins, J. E., Agresti, A., and Ortiz, M., (1979): A Scientific Critique of the EPA Alsea II Study and Report, Oregon State University.

51. Martin, J. C. (1983): Report on OIG Review and Inquiry into the Five Rivers Incident. Environmental Protection Agency, November 22.

52. Report of the Consultative Council on Congenital Abnormalities in the Yarram District, Commission of Public Health (Australia).

53. Nelson, C. J., Holson, J. F., Green, H. C., and Gaylor, D. W., (1979): Retrospective Study of the Relationship Between Agricultural Use of 2,4,5-T and Cleft Palate Occurrence in Arkansas. **Teratology, 19:** 377-384.

54. Honchar, P., (1982): Long Island Railroad Investigation, National Institute for Occupational Safety and Health.

55. Thomas, H. F., (1980): 2,4,5-T Use and Congenital Malformation Rates in Hungary. **Lancet,** i: 214-215.

56. Smith, A. H., Fisher, D. O., Pierce, N., and Chapman, C. J., (1982): Congenital Defects and Miscarriages Among New Zealand 2,4,5-T Sprayers. **Arch. Environ. Health,** 37: 197-200.

57. Evaluation of Congenital Malformation Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide, the Michigan Department of Public Health, May 4, 1983.

58. Townsend, J. C., Bodner, K. M., Van Peenen, P.D.F., Olson, R. D., and Cook, R. R., (1982): Survey of Reproductive Events of Wives of Employees Exposed to Chlorinated Dioxins. **Am. J. Epidemiol., 115:** 695-713.

59. Cook, R. R. and Bodner, K. M., (1983): Dioxin and Reproductive Effects. In: **Human and Environmental Risks of Chlorinated Dioxins and Related Compounds,** ed., Tucker, R. E. et. al. Plenum Publishing Corporation.

60. Ott, M. G., (1983): Mortality Among Men in a Chemical Manufacturing Company Dissertation, University of Michigan, Lansing, Michigan.

61. Lamb, J. C., Moore, J. A., Marks, T. A., (1980): Evaluation of 2,4-Dichlorophenoxyacetic Acid (2,4-D), 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T), and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Toxicity in C57BL Mice, National Toxicology Program.

62. Chesney, M. A., (1983): Presentation to the Science and Technology Committee Subcommittee on Natural Resources, Agriculture Research and Environment, U.S. House of Representatives, July 28.

63. Case Control Study of Congenital Abnormalities and Vietnam Service (Birth Defects Study), Ministry of Veterans' Affairs (Australia), January 1983.

64. Jessop, D. S. et. al., (1982): Pesticides and the Health of Australian Vietnam Veterans: First Report of the Senate Standing Committee on Science and the Environment (Australia), November.

65. "Study of 85,000 Veterans Calls Pesticide Effect Vague," **The New York Times**, August 30, 1983.

66. Moore, F., (1980): Expected Mortality for a Population the Size and Age Distribution of Vietnam Theater Veterans 1970 Through 1979. The Dow Chemical Company.

67. Human Health and Toxicity Workshop on Dioxins in the Environment: Summary, University of Michigan, December 9, 1983.

68. "Scientists Say Dioxin 'Not a High Priority'," UPI Domestic Newswire, December 10, 1983, Update: 10.

69. Young, A. L., Calcagni, J. A., Thalken, C. E., Tremblay, J. W., (1978): The Toxicity, Environmental Fate, and Human Risk of Herbicide Orange and Its Associated Dioxin. United States Air Force, report OEHL TR-78-92.

70. Kimmig, J., Schultz, K. H., (1957): Chlorinated Aromatic Cyclic Ether as Cause of So-Called Chloracne. **Naturwissenschaften, 44**: 337-338.

71. Schwetz, B. A., et. al., (1973): Toxicology of Chlorinated Dibenzo-p-dioxins. **Environ. Health Perspect., 5**: 87.

72. McConnel, E. E., et. al., (1978): The Comparative Toxicity of Chlorinated Dibenzo-p-dioxins in Mice and Guinea Pigs. **Toxicol. Appl. Pharmacol., 44**: 335.

73. Olson, J. R., et. al., (1980): Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin to the Golden Syrian Hamster. **Toxicol. Appl. Pharmacol., 55**: 67.

74. Henck, J. W., et. al., (1981): 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Acute Oral Toxicity in Hamsters. **Toxicol. Appl. Pharmacol., 59**: 405.

75. Allen, J. R., et. al., (1977): Reproductive Dysfunction in Non-human Primates Exposed to Dioxins. **Toxicol. Appl. Pharmacol., 41**: 177.

76. Allen, J. R., et. al., (1977): Morphological Changes in Monkeys Consuming a Diet Containing Low Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin. **Food Cosmet. Toxicol., 15**: 401.

77. Schantz, S. L., (1979): Toxicological Effects Produced in Non-human Primates Chronically Exposed to 50 Parts per Trillion of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). **Toxicol. Appl. Pharmacol., 48**: A180.

78. Murray, F. J., et. al., (1979): Three Generation Reproduction Study of Rats Given 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the Diet. **Toxicol. Appl. Pharmacol., 50**: 241.

79. Green, S., Moreland, F. S., (1975): Cytogenic Evaluation of Several Dioxins in the Rat. **Toxicol. Appl. Pharmacol.**, 33: 161.

80. Green, S., et. al., (1977): Cytogenic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on Rat Bone Marrow Cells. **FDA By-Lines, 6:** 292.

81. Wassom, J. S., et. al., (1977-78): A Review of the Genetic Toxicology of Chlorinated Dibenzo-p-dioxins. **Mutat. Res., 47:** 141.

82. Khera, K. S., Ruddick, J. A., (1973): Polychlorodibenzo-p-dioxins: Perinatal Effects and the Dominant Lethal Test in Wistar Rats. In: Chlorodioxins -- Origin and Fate, **Advances in Chemistry Series, No. 120** (ed. Blair), American Chemical Society, Washington, D.C.

83. Beatty, P. W., et. al., (1975): Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on Mammalian Cells in Tissue Culture. **Toxicol. Appl. Pharmacol.**, 31: 309.

84. Tenchini, M. L., et. al., (1977): Approaches to Examination of Genetic Damage After a Major Hazard in Chemical Industry: Preliminary Cytogenetic Findings on TCDD-Exposed Subjects After Seveso Accident, Special Project of Investigations on TDCC-Exposed Pregnancies (Prof. G. B. Candini and Prof. L. DeCarli), University of Milan, Italy.

85. Rehder, H. et. al., (1978): **Schweiz. Med. Wochenschr., 108:**1617.

86. Kociba, R. J., et. al., (1978): Results of a Two Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rats. **Toxicol. Appl. Pharmacol., 46:** 279.

87. National Cancer Institute, (1980): Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-siozin (TCDD) in Rats. DHHS Public No. NIH80-1765.

88. Toth, K., et. al., (1979): Carcinogenicity Testing of the Herbicide 2,4,5-Trichlorophenoxy Ethanol Containing Dioxin or of Pure Dioxin in Swiss Mice. **Nature, 278:** 548.

89. Poiger, H., and Sclatter, C., (1980): Influence of Solvents and Adsorbents on Dermal and Intestinal Absorption of TCDD. **Food Cosmet. Toxicol., 18:** 477-481.

90. Kimbrough, R. D., Falk, H., Stehr, P., and Fries, G., (1983): Health Implications of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) Contamination of Residential Soil, U.S. Centers for Disease Control in Atlanta.

91. Health-Risk Estimates for 2,3,7,8-Tetrachlorodibenzodioxin in Soil. **Morbidity and Mortality Weekly Report**, January 27, 1984.

92. Stevens, K., (1981): Agent Orange: A Quantitative Perspective. **Hum. Toxicol., 1:** 31-39.

93. Cordle, F., (1981): The Use of Epidemiology in the Regulation of Dioxins in the Food Supply. **Regul. Toxicol. Pharmaco., 1:** 379-387.

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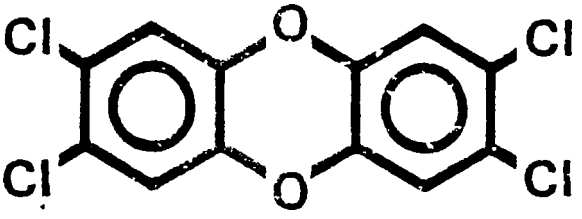
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, "dioxin")



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

FOREWORD

Current Intelligence Bulletins are reports issued by the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control, Atlanta, Georgia, for the purpose of disseminating new scientific information about occupational hazards. A Current Intelligence Bulletin may draw attention to a hazard previously unrecognized or may report new data suggesting that a known hazard is either more or less dangerous than was previously thought.

Current Intelligence Bulletins are prepared by the staff of the Division of Standards Development and Technology Transfer, NIOSH, (Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio, 45226) and are distributed to representatives of organized labor, industry, public health agencies, academic institutions, and public interest groups as well as to those federal agencies, such as the Department of Labor, which have responsibilities for protecting the health of workers. It is our intention that anyone with the need to know should have ready access to the information contained in these documents; we welcome suggestions concerning their content, style, and distribution.

Because of the recent attention given to human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, "dioxin") contaminated materials and published reports on the toxicity of TCDD, NIOSH staff consider it necessary to present a review of the pertinent data and a summary of findings related to the human hazard potential of TCDD. Because of the compression in this bulletin of the voluminous literature on TCDD, it is suggested that readers wanting to know more of the details of the reported studies consult the appended references.

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CURRENT INTELLIGENCE BULLETIN #40

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, "DIOXIN")

January 23, 1984

ABSTRACT

In animals, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, "dioxin") causes various systemic effects at a wide range of exposure concentrations, including tumorigenesis, immunological dysfunction, and teratogenesis. Studies of humans exposed to TCDD-contaminated materials suggest that TCDD is the cause of observed chloracne, metabolic disorders (porphyria), and other systemic problems and are suggestive of TCDD's ability to cause cancer.

TCDD occurs as a contaminant of materials such as 2,4,5-trichlorophenol (TCP), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-(2,4,5-trichlorophenoxy)propionic acid (silvex). Occupational exposure may occur through contact with these materials during use or from the past contamination of worksites.

The National Institute for Occupational Safety and Health (NIOSH) recommends that TCDD be regarded as a potential occupational carcinogen, that occupational exposure to TCDD be controlled to the fullest extent feasible, and that decontamination measures be used for TCDD-contaminated work environments. This recommendation is based on a number of reliable studies demonstrating TCDD carcinogenicity in rats and mice.

BACKGROUND

Physical and Chemical Properties of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

TCDD is one of a family of isomers known chemically as dibenzo-p-dioxins. The chemical and physical properties are summarized in Table I. TCDD is a colorless crystalline solid at room temperature. It is sparingly soluble in most organic solvents and essentially insoluble in water. TCDD is stable to heat, acids, and alkali and will decompose when exposed to ultraviolet light, including sunlight [1].

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF TCDD [2,3]

CAS Registry No.: 1746-01-6		
Empirical formula		$C_{12}H_4Cl_4O_2$
Percent by weight	C	44.7
	O	9.95
	H	1.25
	Cl	44.1
Molecular weight		322
Vapor Pressure mm Hg at 25°C		1.7×10^{-6}
Melting point, °C		305
Decomposition temperature, °C		>700
Solubilities, g/liter		
o-Dichlorobenzene		1.4
Chlorobenzene		0.72
Benzene		0.57
Chloroform		0.37
n-Octanol		0.05
Methanol		0.01
Acetone		0.11
Water		2×10^{-7}

Formation and Use of TCDD

TCDD forms as a stable by-product or contaminant during the production of TCP. Run-away reactions at high temperature, in which excess TCDD was produced, have occurred at TCP production sites in the United States and elsewhere [4]. Normally, TCDD persists as a contaminant in TCP in relatively small, variable amounts (0.07-6.2 mg/kg) [5]. TCP has been utilized primarily as a feedstock for production of the phenoxy herbicides 2,4,5-T and silvex, resulting in the contamination of these products with TCDD. Production of 2,4,5-T and silvex ceased in the United States in 1979. However, stockpiles of both products are still being distributed and

used. TCP also is used in the production of hexachlorophene, a bactericide and fungicide.

The combustion of 2,4,5-T can result in its conversion to small amounts (0.6 ppt TCDD/1 ppm 2,4,5-T burned) of TCDD. Also, the burning or heating of commercial and purified chlorophanates and pyrolysis of polychlorinated biphenyls (PCBs) contaminated with trichlorobenzenes have resulted in the production of TCDD [6,7]. The formation of TCDD from trace chemical reactions in fires has been postulated but has not been verified [8,9].

Existing Regulations and Guides

No occupational exposure standard exists for TCDD. The United States Environmental Protection Agency (U.S. EPA) temporarily suspended or banned most uses of 2,4,5-T and silvex in 1979, although their use was allowed on sugarcane, orchards and for miscellaneous non-crop uses [10]. On October 18, 1983 EPA published its intent to cancel registration of pesticide products containing 2,4,5-T and silvex and to prohibit the transfer, distribution, sale or importation of any unregistered pesticide product containing 2,4,5-T or silvex or their derivatives [11].

Nature of Occupational Exposure to TCDD

It is not possible to estimate accurately the number of U.S. workers currently at risk of exposure to TCDD. Occupational exposure to TCDD may occur during production of TCP; in decontamination of worksites from prior production or use of TCP, 2,4,5-T, or silvex; from waste materials (such as reclaimed oil) contaminated with TCDD; or from cleanup after fires in transformers containing polychlorinated aromatics.

Dust or soil particles contaminated with TCDD can remain airborne or accumulate on indoor or outdoor work surfaces and may present a potential exposure hazard. Exposure to TCDD as a vapor will normally be negligible because of its low vapor pressure. Contact with TCDD-contaminated liquids is possible through the handling of drums or tanks containing the liquid or through dispersion of the liquid.

TOXICITY

Results of Studies of TCDD in Animals

Acute and Chronic Toxicity

There is wide variation in the dosage of TCDD required to cause death among animal species (oral LD_{50} 0.6-5,000 µg TCDD/kg body weight (bw)) [12,13]. Progressive weight loss with death several weeks later is reported to characterize the response in experimental animals after administration of a lethal dosage of TCDD [12,14,15]. Animals given single or repeated oral dosages of TCDD of 0.1 to 25 µg/kg bw demonstrated increased liver weights and lipid accumulation, thymic atrophy, and histopathological changes in liver and thymus [12,16-18].

TCDD is reported to be at least three times more potent than any other known compound in stimulating production of aminolevulinic acid synthetase (ALA), the rate-limiting enzyme in porphyrin and heme synthesis [19,20]. Varied effects on hematological functions have been reported in rats and mice dosed with TCDD: increased numbers of erythrocytes and leucocytes, increased hemoglobin concentration, decreased blood platelets in rats [21,22], and decreased hemoglobin concentration in mice [23].

Effects on Reproductive Function

TCDD administered at dosages of 0.125-3.0 µg TCDD/g bw to mice and rats induced fetotoxicity that included cleft palates and kidney anomalies [24-26], intestinal hemorrhages and excessive tissue/organ fluid (edema), and prenatal mortality [27,28].

Impairment of reproduction has been reported for rats ingesting 0.01 µg TCDD/kg bw/day. Significant decreased fertility, litter size, number of pups alive at birth, postnatal survival, and postnatal body weight of pups were evident in two successive generations delivered from male and female rats that ingested TCDD 90 days prior to first mating, during pregnancies, and for the durations of time between pregnancies [29]. No significant dose-related reproductive effects were observed in male mice treated with up to 2.4 µg TCDD/kg bw/day and mated with untreated female mice [30,31].

Immunological Effects

TCDD induced immunological function alterations, expressed by decreased thymus-to-body weight ratios, in nursing newborn rats exposed through dosing of the lactating mother [32]. Other reports have shown that pre- and post-natal maternal dosing of rats and mice with TCDD caused thymic atrophy

and suppression of cellular immunity in the offspring [33]. TCDD administered intraperitoneally or orally to mice induced a strong immunosuppressive effect on antibody production and cell-acquired immune responses [34].

Mutagenic Effects

Results of mutagenicity tests are inconclusive. In two studies TCDD was mutagenic in Salmonella typhimurium TA 1532 without activation [35,36]. In another study, which used a more sensitive mutant strain, Salmonella typhimurium TA 1537, TCDD was not a mutagen [37]. There is weak evidence of chromosomal aberrations in bone marrow of rats given dosages of 0.25 to 4 µg TCDD/kg bw [38,39].

Carcinogenic Effects

Male rats fed dosages of 0.001 µg TCDD/kg bw/week for 78 weeks and sacrificed at week 95 of the study showed a variety of neoplastic tumors (ear duct carcinoma; lymphocytic leukemia; kidney adenocarcinoma; malignant peritoneal histiocytoma; skin angiosarcoma; hard palate, tongue and nasal turbinate carcinoma) [40]. Female rats that had ingested TCDD for two years at a dosage of 0.1 µg/kg bw/day developed carcinomas of the liver and squamous cell carcinomas of the lung, hard palate, nasal turbinates, or tongue [41]. Male and female rats orally dosed with 0.5 µg TCDD/kg bw/week for two years demonstrated neoplastic nodules of the liver and thyroid adenomas [42].

Male mice fed dosages of TCDD of 0.05 or 0.5 µg/kg/week for two years developed liver cancer; female mice fed 0.2 or 2.0 µg/kg/week for the same duration developed liver cancer and thyroid follicular cell adenomas [42]. TCDD applied to the skin of female mice for two years (0.005 µg/kg bw/application; 3 days/week) resulted in a significantly higher incidence ($p=0.007$) of skin cancers (fibrosarcomas) when compared to untreated controls. An increase in the same tumor type, although not statistically significant ($p=0.084$), was also observed in the male mice that received a maximum dosage of 0.001 µg TCDD per application [43].

Human Health Effects

The only information on the health effects in humans from exposure to TCDD is from clinical or epidemiological studies of populations who were occupationally and non-occupationally exposed to 2,4,5-T and TCP contaminated with TCDD. Because of the coincidental exposure to 2,4,5-T and TCP and to other herbicides as well as to TCDD, it is not possible to

attribute the observed health effects solely to TCDD exposure. To date, no studies of humans include a quantitation of exposure to TCDD.

Chloracne and Other Systemic Effects

Chloracne is a chronic and sometimes disfiguring skin eruption caused by exposure to halogenated aromatic compounds including TCDD. Chloracne is possibly a result of systemic effects of these compounds, although it also may occur as a contact dermatitis [44,45].

There are numerous cases of chloracne reported following accidental exposure to chlorinated aromatic chemicals which were probably contaminated with TCDD [46-48]. The most notable recent exposure occurred in Seveso, Italy in 1976 [49]. In most incidences of chloracne, there are a variety of signs and symptoms (ranging from gastrointestinal disturbances to metabolic disorders) which accompany the appearance of the skin eruptions and persist for varying lengths of time [50-54].

Reproductive Effects In Humans

Reproductive effects resulting from possible human exposure to TCDD are inconclusive. Data on male workers who applied agricultural sprays of 2,4,5-T or who produced TCDD-contaminated materials are consistent with the animal data which suggest no reproductive effects in males from TCDD exposure [55-57]. To date, no study of reproductive effects in women or in offspring of males or females with defined exposure to TCDD has been reported.

Studies of birth defects in populations that may have been exposed non-occupationally to TCDD have been conducted in Australia where a correlation was observed between 2,4,5-T use and seasonal variation in the rate of spinal cord and spine formation defects; no causal association could be drawn [58]. In a similar study in Hungary, an increased incidence of congenital malformations including spine formation defects could not be correlated with increased use of 2,4,5-T [59]. A study based on incomplete fetal tissue samples from the Seveso, Italy population found no mutagenic, teratogenic, or fetotoxic effects in 30 interrupted pregnancies and four spontaneous abortions in women believed to have been exposed to TCDD [60]. A U.S. EPA study found a positive relationship between spontaneous abortions and 2,4,5-T use in the Alsea, Oregon area [61]. The study, however, has been severely criticized because of its numerous limitations: inaccurate comparisons of the study and control areas; inaccuracies in the collection of data on spontaneous abortions; incomplete and inaccurate data on 2,4,5-T usage; and failure to recognize that the rate of spontaneous abortions was not greater than would be expected [62].

Studies of Mortality and Carcinogenesis in Humans

Findings have been inconclusive in many mortality studies of workers with occupational exposure to TCDD-contaminated materials because of the small size of the study population and concomitant exposures to other substances.

No excess mortality or tumor incidence was observed among Swedish railroad workers exposed to unknown amounts of 2,4-D, 2,4,5-T, and other herbicides but believed to have been exposed primarily to phenoxy acid herbicides for at least 45 days [63]. In a subsequent analysis of mortality in this group of workers, 45 deaths (49 expected) were observed in the total population. A significant excess of tumors also was observed among those believed to be exposed primarily to Amitrol® (3-amino-1,2,4-triazole), a suspect carcinogen, as well as to phenoxy herbicides. Two cases of stomach cancer (0.33 expected) were observed among those exposed primarily to phenoxy herbicides [64].

Among Swedish forestry workers exposed to phenoxy herbicide preparations, supervisors, who had more extensive exposure to herbicides than the other forest workers, had a nonsignificant excess of deaths from all cancers. Mortality associated with the presence of tumors was, however, lower than expected for the total group of exposed workers [65].

In a group of 74 workers involved in an accident during TCP production in Germany, 21 deaths occurred during the following 27 years. Seven (7) malignant neoplasms vs. 4.2 expected and a significant excess of stomach cancer (3 observed vs. 0.61 expected) were observed [66].

Several case control studies of cancer patients have yielded data on the carcinogenicity of phenoxyacetic herbicides. Two studies were conducted in Sweden following a clinical observation of patients with soft tissue sarcoma who had previous occupational exposure to the herbicides [67]. The first study of 52 cases of soft tissue sarcoma concluded that the sarcoma cases were 5.3 times more likely than the 206 controls to have had occupational exposure to phenoxyacetic acids (primarily 2,4,5-T and 2,4-D) [68]. The second study of 110 cases of soft tissue sarcomas indicated that this population was 6.8 times more likely to have had exposure to phenoxyacetic acids than the 219 controls [69]. In neither study was it possible to demonstrate the relative risk related to exposure to TCDD-contaminated 2,4,5-T because of the presence of impurities such as chlorinated dibenzodioxins and dibenzofurans which were part of the phenoxyacetic herbicides.

In other reports from Sweden, 11 of 17 patients with malignant lymphoma reported occupational exposures to phenoxyacetic acids or chlorophenols

[70]; a case control study with 169 malignant lymphoma cases found a significantly higher occupational exposure to phenoxyacetic acids (primarily 2,4,5-T, and 2,4-D) associated with the sarcoma cases than did the 338 controls. Analysis by individual herbicide exposure was not possible [71].

Two additional studies conducted in Sweden for colon cancer and nasal and nasopharyngeal cancer did not demonstrate an elevated risk for occupational exposure to phenoxyacetic acids [72,73].

Among four small groups of U.S. production workers exposed to TCP and 2,4,5-T a total of 105 deaths were observed [74-76]. In these, three deaths were attributed to soft tissue sarcoma (43 times the number expected for this age group of U.S. white males) [77]. Later, four additional cases were reported to have soft tissue sarcomas [78-81]. However, a detailed review of work records and expert review of pathological tissue specimens have shown only two of the seven cases with both confirmed exposure to TCP or 2,4,5-T and diagnosis of soft tissue sarcoma [82].

Summary of Toxicity in Animals and Humans

TCDD causes a variety of systemic and immunological effects in animals with wide variation among species in the dosage required to cause death. Studies using rats and mice have demonstrated that TCDD is an animal teratogen and carcinogen. Results of tests for mutagenicity are inconclusive.

Humans exposed to materials reported to be contaminated with TCDD have developed chloracne and other signs of systemic poisoning. Soft tissue sarcoma has been observed in excess among workers exposed to phenoxy herbicides. These data are inconclusive regarding TCDD toxicity in humans because the populations studied had mixed exposures making causal relationships between exposure and effect unclear. The data are, however, suggestive of an association between exposure to phenoxyacatic herbicides contaminated with TCDD and excess lymphoma and stomach cancer. Attempts to associate reproductive effects with TCDD exposure are inconclusive because of the inadequately defined populations studied and the difficulties of defining exposure.

RECOMMENDATIONS

There are several classifications for identifying a substance as a carcinogen. Such classifications have been developed by the U.S. National Institute of Environmental Health Sciences, National Toxicology Program [83], the International Agency for Research on Cancer [84], and OSHA [85]. NIOSH considers the OSHA classification the most appropriate for use in identifying carcinogens in the workplace. This classification is outlined

in 29 CFR 1990.103.* Since TCDD has been shown to carcinogenic in experimental studies with rats and mice, and studies are suggestive of an association between human exposure to TCDD-contaminated materials and carcinogenicity, NIOSH recommends that TCDD be considered as a potential occupational carcinogen and exposure to TCDD in all occupational settings should be controlled to the fullest extent feasible. While observations to date do not confirm a causal relationship between TCDD exposure and soft tissue sarcoma, they suggest a need for continued investigations.

Because of the variety of situations likely to be encountered in TCDD-contaminated worksites, it is not possible to offer in this bulletin detailed procedures for assessing exposures or decontamination. Based on NIOSH hazard evaluations of TCDD-contaminated sites, the following general guidelines are recommended until more specific procedures can be developed [86,87].

Assessment of Exposure

Workers may be exposed to TCDD derived from a variety of sources: the production of TCP, residues from prior production or use of 2,4,5-T or silvex, waste materials contaminated by TCDD, or contamination resulting from transformer fires. The first step in assessing workplace contamination should be environmental sampling to determine the presence of TCDD contamination, keeping in mind the possible routes of exposure, with later sampling conducted to define the quantity of TCDD in the environment. The assessment may include sampling of soil and settled dust for TCDD, air sampling for TCDD-contaminated particles, and wipe sampling of surfaces [86,87].

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*"'Potential occupational carcinogen' means any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory or dermal exposure, or any other exposure which results in the induction of tumors at a site other than the site of administration. This definition also includes any substance which is metabolized into one or more potential occupational carcinogens by mammals."

Decontamination and Worker Protection Programs

In general, decontamination procedures must provide an organized process in which levels of contamination are reduced. This requires containment, collection, and disposal of contaminated solutions and residues generated during the cleanup. Separate facilities should be provided for decontamination of large equipment.

Each stage of decontamination, such as gross decontamination and repetitive wash/rinse cycles, should be conducted separately, either by using different locations or by spacing in time. Personnel decontamination locations used should be physically separated to prevent cross-contact and should be arranged in order of decreasing level of contamination. Separate entry/exit routes and locations should be provided for workers when it is necessary to isolate them from different contamination areas containing incompatible waste. Entry and exit points to these areas should be well marked and controlled. Access to the decontamination area should be separate from the path between the contaminated and clean areas. Dressing stations for entry should be separate from re-dressing areas for exit.

Protective Clothing and Equipment

All workers who may be exposed to TCDD should be equipped with adequate chemical protective clothing and equipment to ensure their protection. In the selection of protective clothing, consideration should be given to the utilization of disposable apparel due to the uncertainty of decontamination of clothing.

The protective apparel should consist of both outer and inner garments. The outer garments should consist of a zippered coverall with attached hood and draw string or elastic sleeves, gloves and closure boots. If exposure is to particulate or dust, the coveralls should be made of a non-woven fabric such as spunbonded polyethylene, Tyvek®. In cases of exposure to liquids, the coveralls, gloves and boots should be made of chemically resistant materials such as disposable laminates, e.g., Saranax® coated Tyvek®, or synthetic elastomers such as butyl, nitrile or neoprene rubber. The inner garments should consist of cotton coveralls, undershirts, undershorts, gloves, and socks and should be disposed of after use. The effectiveness of the protective clothing should be evaluated under simulated use conditions, regardless of the type of clothing used. All disposable clothing should be placed in marked and approved containers and disposed of appropriately. All reusable clothing and equipment should be thoroughly cleaned and checked for residual contamination before reuse or storage.

Respiratory Protection

The use of respiratory protection requires that a respiratory protection program be instituted according to the requirements of 29 CFR 1910.134 [88] and that the respirators have been approved by the Mine Safety and Health Administration (MSHA) and by NIOSH. This program should include training on proper fit testing and use and procedures for respirator maintenance, inspection, cleaning and evaluation.

For situations where TCDD contamination is low (e.g., exposure to dust contaminated with low levels of TCDD), air purifying respirators should provide sufficient protection until the extent and characterization of the exposure can be determined. Where quantities of materials highly contaminated with TCDD have been released and have contaminated an area (e.g., production accidents), all workers who may be exposed to TCDD should wear respirators that consist of a self-contained breathing apparatus with a full facepiece operated in pressure-demand or other positive pressure mode. An alternate method utilizes a combination Type C supplied air respirator, with full facepiece, operated in pressure-demand mode and equipped with auxiliary positive pressure self-contained air supply.

Post-Decontamination Testing

The adequacy of the decontamination effort should be determined by conducting follow-up sampling and analysis of the contaminated areas and protective equipment. This testing should be conducted as each area is decontaminated and after the entire facility has been cleaned.

REFERENCES

1. Crosby DG, Moilanen KW, Wong AS: Environmental generation and degradation of dibenzodioxins and dibenzofurans. Environ Health Perspect 5:259-266 (1973).

2. Crummett WB, Stehl RH: Determination of chlorinated dibenzo-p-dioxins and dibenzofurans in various materials. Environ Health Perspect 5:17-25 (1973).

3. National Research Council of Canada: Polychlorinated Dibenzo-p-dioxins: Criteria for Their Effects on Man and His Environment. Pub. No. NRCC 18574: NRCC/CNRC Assoc. Comm. on Scientific Criteria for Environmental Quality, Ottawa, Canada (1981).

4. Hay A: Accidents in trichlorophenol plants: A need for realistic surveys to ascertain risks to health. Ann NY Acad Sci 320:321-324 (1979).

5. Firestone D, Ress J, Brown NL, et al.: Determination of polychlorodibenzo-p-dioxins and related compounds in commercial chlorophenols. J Assn Off Anal Chem 55(1):85-92 (1972).

6. Stehl RH, Lamparski LL: Combustion of several 2,5,5-trichlorophenoxy compounds: Formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Science 197:1008-1009 (1977).

7. Rappe C, Marklund S: Formation of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) by burning or heating chlorophenates. Chemosphere 3:269-281 (1978).

8. Bumb RR, Crummett WB, Cutie SS, et al.: Trace chemistries of fire: A source of chlorinated dioxins. Science 210(4468):385-389 (1980).

9. Kimble BJ, Gross ML: Tetrachlorodibenzo-p-dioxin quantitation in stack-collected coal fly ash. Science 207:59-61 (1980).

10. Federal Register, Environmental Protection Agency, Part III, 44(52):15874-15920 (March 15, 1979).

11. Federal Register, Environmental Protection Agency, Part X, 48(202):48434-48437 (October 18, 1983).

12. McConnell EE, Moore JA, Haseman JK, et al.: The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol Appl Pharmacol 44:335-356 (1978).

13. Henck JM, New MA, Kociba RJ, et al.: 2,3,7,8-tetrachlorodibenzo-p-dioxin: Acute oral toxicity in hamsters. Toxicol Appl Pharmacol 59:405-407 (1981).

14. McConnell EE, Moore JA, Dalgard DW: Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Rhesus monkeys (Macaca mulatta) following a single oral dose. Toxicol Appl Pharmacol 43:175-187 (1978).

15. Kociba RJ, Keiler PA, Park CN, et al.: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-week oral toxicity study in rats. Toxicol Appl Pharmacol 35:553-574 (1976).

16. Gupta GN, Vos JG, Moore JA, et al.: Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 5:125-140 (1973).

17. Kimbrough RD: Morphology of lesions produced by the dioxins and related compounds. In: Tucker RE, ed.: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds. Proceedings of an international symposium on chlorinated dioxins and related compounds, held October 25-29, 1981, in Arlington, Virginia. pp. 527-538 (1983).

18. Matthiaschk G: Survey about toxicological data of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In: Paoletti R, ed.: Monographs of the Giovanni Lorenzini Foundation, Volume 1, Dioxin: Toxicological and Chemical Aspects. pp. 123-136 (1978).

19. Poland A, Glover E: 2,3,7,8-tetrachlorodibenzo-p-dioxin: A potent inducer of delta-aminolevulinic acid synthetase. Science 179:476-477 (1973).

20. Poland A, Glover E: Studies on the mechanism of toxicity of the chlorinated dibenzo-para-dioxins. Environ Health Perspect 5:245-251 (1973).

21. Weissberg JB, Zinkl JG: Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon hemostasis and hematologic function in the rat. Environ Health Perspect 5:119-123 (1973).

22. Zinkl JG, Vos JG, Moore JA, et al.: Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ Health Perspect 5:111-118 (1973).

23. Vos JG, Moore JA, Zinkl JG: Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 mice. Toxicol Appl Pharmacol 29:229-241 (1974).

24. Courtney KD, Moore JA: Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 20:396-403 (1971).

25. Smith FA, Schwetz BA, Nitschke KD: Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol Appl Pharmacol 38:517-523 (1976).

26. Moore JA, Gupta BN, Zinkl JN, et al.: Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ Health Perspect 5:81-85 (1973).

27. Sparschu GL, Dunn FL, Rowe VK: Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Fd Cosmet Toxicol 9:405-412 (1971).

28. Khera KS, Ruddick JA: Polychlorodibenzo-p-dioxins: Perinatal effects and the dominant lethal test in Wistar rats. Toxicology 120:70-84 (1973).

29. Murray FJ, Smith FA, Nitschke KO, et al.: Three generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol Appl Pharmacol 50(2):241-252 (1979).

30. Lamb JC IV, Moore JA, Marks TA, et al.: Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 8:835-844 (1981).

31. Lamb JC IV, Marks TA, Gladen BC, et al.: Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 8:825-834 (1981).

32. Luster MI, Faith RE, Clark G: Laboratory studies on the immune effects of halogenated aromatics. Ann NY Acad Sci 320:473-486 (1979).

33. Vos JG, Moore JA: Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int Arch Allergy Appl Immunol 47:777-794 (1974).

34. Garattini S, Vecchi A, Sironi M, et al.: Immunosuppressant activity of TCDD in mice. In: Hutzinger O, ed.: Chlorinated Dioxins and Related Compounds: Impact on the Environment. Proceedings of a workshop, Institute Superiore di Sanita, Rome, Italy, held 22-24 October 1980. pp. 403-409 (1982).

35. Hussain S, Ehrenberg L, Lofroth G, et al.: Mutagenic effects of TCDD on bacterial systems. Ambio 1:32-33 (1972).

36. Seiler JP: A survey on the mutagenicity on various pesticides. Experimenta 15(5):622-623 (1973).

37. Geiger LE, Neal RA: Mutagenicity testing of 2,3,7,8-tetrachlorodibenzo-p-dioxin in histidine auxotrophs of Salmonella typhimurium. Toxicol Appl Pharmacol 59:125-129 (1981).

38. Green S, Moreland F, Sheu C: Cytogenic effects of 2,3,7,8-tatrachlorodibenzo-p-dioxin on rat bone marrow cells. FDA By-Lines 6:242-294 (1977).

39. Loprieno N, Sbrana I, Rusciano D, et al.: In vivo cytogenic studies on mice and rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In: Hutzinger O, ed.: Chlorinated Dioxins and Related Compounds: Impact on the Environment. Proceedings of a workshop, Institute Superiore di Sanita, Rome, Italy, held 22-24 October 1980. pp. 419-428 (1982).

40. Van Miller JP, Lalich JJ, Allen JR: Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tatrachlorodibenzo-p-dioxin. Chemosphere 9:537-544 (1977).

41. Kociba RJ, et al.: Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tatrachlorodibenzo-p-dioxin in rats. Toxicol Appl Pharmacol 46:279-303 (1978).

42. National Toxicology Program: Technical Report Series No. 209. Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Osborn-Mendel Rats and B6C3F$_1$ Mice (Gavage Study). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health Publication No. 82-1765, (February 1982).

43. National Toxicology Program: Technical Report Series No. 201. Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Swiss-Webster Mice (Dermal Study). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health Publication No. 82-1757, (February 1982).

44. Crow KD: Chloracne. Semin Dermatol 1(4):305-313 (1982).

45. Jones EL, Krizek H: A technic for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo -p-dioxin. J Invest Dermatol 39:511-517 (1962).

46. May G: Chloracne from the accidental production of tetrachlorodibenzodioxin. Br J Ind Med 30:276-283 (1973).

47. Goldmann PJ: [Severe acute chloracne. A mass intoxication by 2,3,6,7-tetrachlorobenzodioxin]. Hautarzt 24:149-152 (1973) (Ger.).

48. Reggiani G: Acute human exposure to TCDD in Seveso, Italy. Toxicol Environ Health 6:27-43 (1980).

49. Pocchiari F, Silano V, Zampieri A: Human health effects from accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. Ann NY Acad Sci 320:311-320 (1979).

50. Pazderova-Vejlupkova J, Nemcova M, Pickova J, et al.: The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in men. Arch Environ Health 36(1):5-11 (1981).

51. Singer R, Moses M, Valciukas J, et al.: Nerve conduction velocity studies of workers employed in the manufacture of phenoxy herbicides. Environ Res 29:297-311 (1982).

52. Oliver RM: Toxic effects of 2,3,7,8 tetrachlorodibenzo 1,4 dioxin in laboratory workers. Br J Ind Med 32:49-53 (1975).

53. Caramashi F, Del Corono G, Favaretti C, et al.: Chloracne following environmental contamination by TCDD in Seveso, Italy. Int J Epidemiol 10(2):135-143 (1981).

54. Kimbrough RD, Carter CD, Liddle JA, et al.: Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. Arch Environ Health 32(2):77-85 (1977).

55. Smith AH, Matheson DP, Fisher DO: Preliminary report of reproductive outcomes among pesticide applicators using 2,4,5-T. NZ Med J 93(680):177-179 (1981).

56. Smith AH, Fisher DO, Pearce N, et al.: Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch Environ Health 37(4):197-200 (1982).

57. Townsend JC, Bodner KM, Van Peenen PFD, et al.: Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Amer J Epidemiol 115(5):695-713 (1982).

58. Field B, Kerr C: Herbicide use and incidence of neural tube defects. Lancet I (June 23):1341-1342 (1979).

59. Thomas HF: 2,4,5-T use and congenital malformation rates in Hungary. Lancet I (July 26):214-215 (1980).

60. Rehder H, Sanchoni L, Cefis F, et al.: [Pathologic-Embryologic studies in cases of abortion connected with the accident at Seveso]. Schweiz Med Wochenschr 108(42):1617-1625 (1978) (Ger.).

61. Report of Assessment of a Field Investigation of Six-Year Spontaneous Abortion Rates in Three Oregon Areas in Relation to Forest 2,4,5-T Spray Practices. Environmental Protection Agency. Prepared by the Epidemiologic Studies Program, Human Effects Monitoring Branch, Benefits and Field Studies Division, OPP, OTS, EPA, (1979).

62. Wagner S, Witt JM, Norris LA, et al.: A Scientific Critique of the EPA ALSEA II Study and Report. Environmental Health Sciences Center, Oregon State University, Corvallis, (October 25, 1979).

63. Axelson O, Sundell L: Herbicide exposure, mortality, and tumor incidence: An epidemiological investigation on Swedish railroad workers. Work Environ Health 11:21-28 (1974).

64. Axelson O, Sundell L, Andersson K, et al.: Herbicide exposure and tumor mortality: An updated epidemiologic investigation on Swedish railroad workers. Scand J Work Environ Health 6:73-79 (1980).

65. Hogstadt C, Westerlund B: [Mortality of forest workers exposed and not exposed to phenoxy acid preparations.] Lakartidningen 77(19):1828-1831 (1980) (Swe.).

66. Thiess AM, Frentzel-Beyme R, Link R: Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on November 17, 1953. Am J Ind Med 3:179-189 (1982).

67. Hardell L: [Malignant mesenchymal tumors and exposure to phenoxy acids: A clinical observation.] Lakartidningen 74:2753-2754 (1977) (Swe.).

68. Hardell L, Sandstrom A: Case control study: Soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711-717 (1979).

69. Eriksson M, Hardell L, Berg NO, et al.: [Case-control study on malignant mesenchymal tumors of the soft parts and exposure to chemical substances.] Lakartidningen 76:3872-3875 (1979) (Swe.).

70. Hardell L: Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet I (January 6):55-56 (1979).

71. Hardell L, Eriksson M, Lenner P, et al.: Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. Br J Cancer 43:169-176 (1981).

72. Hardell L: Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand J Work Environ Health 7:119-130 (1981).

73. Hardell L, Johansson B, Axelson O: Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid of chlorophenol exposure. Am J Ind Med 3:247-257 (1982).

74. Zack JA, Suskind RR: The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J Occup Med 22(1):11-14 (1980).

75. Ott MG, Holder BB, Olson RD: A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J Occup Med 22(1):47-50 (1980).

76. Cook RR, Townsend JC, Ott MG, et al.: Mortality experience of employees exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J Occup Med 22(8):530-532 (1980).

77. Honchar PA, Halperin WE: 2,4,5-trichlorophenol and soft tissue sarcoma. Lancet I (January 31):268-269 (1981).

78. Cook RR: Dioxin, chloracne, and soft tissue sarcoma. Lancet I (March 1):618-619 (1981).

79. Moses M, Selikoff LJ: Soft tissue sarcomas, phenoxy herbicides, and chlorinated phenols. Lancet I (June 20):1370 (1981).

80. Johnson FE, Kugler MA, Brown SM: Soft tissue sarcomas and chlorinated phenols. Lancet II (July 4):40 (1981).

81. Fingerhut MA, Halperin WE: Dioxin exposure and sarcomas. JAMA 249(23):3176 (1983).

82. Statement by J. Donald Millar, M.D., Assistant Surgeon General, Director, National Institute for Occupational Safety and Health, Centers for Disease Control, Public Health Service, Department of Health and Human Services, Before the United States House of Representatives Committee on Public Works and Transportation Subcommittee on Investigations and Oversight. (November 9, 1983).

83. Matthews HB: NTP Technical Report on the Toxicity and Carcinogenicity of Tris(2-ethylhexyl)phosphate (Cas. No. 78-42-2) in F344/N rats and B6C3F$_1$ mice (gavage study), National Toxicology Program, Research Triangle Park, North Carolina, p. 4, (unpublished report, September 8, 1983).

84. World Health Organization: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. IARC Monographs, Supplement 1 (1979).

85. Code of Federal Regulations, U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR 1990.103 (1982).

86. Health Hazard Evaluation - Determination Report No. 83-395, Overnight Transportation Company, St. Louis, Missouri. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Cincinnati, Ohio (1983).

87. Health Hazard Evaluation - Determination Report No. 83-394, P.J. Hamil Transfer Company, St. Louis, Missouri. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Cincinnati, Ohio (1983).

88. Code of Federal Regulations, U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR 1910.134 (1982).

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RESULTS FROM 86 TWO-YEAR CARCINOGENICITY STUDIES CONDUCTED BY THE NATIONAL TOXICOLOGY PROGRAM

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Five categories of evidence of carcinogenicity in rats and mice were used to group interpretative results on 86 chemicals studied in recent carcinogenicity tests carried out by the National Toxicology Program (NTP). Of these studies, 50% (44/86) were regarded as showing carcinogenic effects, 42% (36/86) gave no evidence of carcinogenicity, 6% (5/86) showed equivocal evidence of carcinogenicity, and 2% (2/86) were regarded as inadequate experiments. The liver was the most frequent site of cancer in male and female Fischer-344 rats and in male and female B6C3F$_1$ mice. Male rats appeared more sensitive than female rats to the induction of neoplasia, while for mice the females seemed more responsive. The routes of administration yielding the highest percentage (30–83%) of positive studies were gavage and inhalation, approximately one-third of the feed, drinking water, and dermal studies showed carcinogenic effects. In feeding studies, overall survival in dosed and control groups were similar, while the majority of gavage studies showed significantly reduced survival in one or more dosed groups relative to the corresponding controls. The overall percentage of studies showing carcinogenic effects (50%) agrees closely with the rate reported by other investigators for nearly 200 earlier carcinogenicity experiments conducted by the National Cancer Institute.

INTRODUCTION

Since the National Toxicology Program (NTP) was given responsibility for the National Cancer Institute (NCI) Carcinogenesis Bioassay Program in July 1981, the results of approximately 86 chronic studies in rodents have been published as technical reports or prepared in draft form (Huff et al., 1985; Huff and Moore, 1984). The majority of these studies are 2-yr feeding or gavage experiments involving groups of 50 male and

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female Fischer-344/N (F344) rats and B6C3F$_1$ mice and used experimental protocols similar to those employed by the NCI in earlier carcinogenicity studies (Sontag et al., 1976; Huff, 1982). A summary of the test results from approximately 200 of these earlier experiments has been given by Griesemer and Cueto (1980) and by Chu et al. (1981). The purpose of this paper is to provide similar summary information for the more recent NTP studies and to examine other factors such as species and sex sensitivity, route of administration differences, survival effects, and relative frequency of site-specific carcinogenicity.

Chemicals have been nominated, selected, and tested for carcinogenicity by both the NCI and the NTP using criteria such as human exposure, use and level of production, chemical structure, and available mutagenicity data (Huff, 1982). Priority for testing has often been given to those chemicals for which there is some a priori suspicion of a toxic or carcinogenic response. However, selection per se is not and should not be taken as an indicator of a chemical's ultimate carcinogenic potential in experimental animals or in humans.

MATERIALS AND METHODS

The Griesemer and Cueto (1980) summary of test results includes all chemicals with technical report (TR) numbers 1–196; the summary provided by Chu et al. (1981) extends from TR 1 to 190. The evaluation in this paper begins with TR 197 and includes all chemicals whose TRs have been peer reviewed by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee and Associated Panel of Experts (Hart et al., 1983; National Toxicology Program, 1984) as of July 1984. Approximately two-thirds of these reports have been printed; the remaining one-third have had their conclusions approved by the peer review process. Certain of these chemicals are presently undergoing a data audit by the NTP, but it is unlikely that these ongoing audits will alter the basic conclusions of the reports. In only one instance (methylene chloride; not included in this evaluation) have the audits to date revealed problems that would render the studies inadequate or otherwise affect the final interpretation.

The 86 NTP carcinogenicity studies were divided into four groups: studies regarded as showing carcinogenic effects (Table 1), studies with equivocal evidence of carcinogenicity, i.e., studies showing some suggestion of carcinogenicity, but the strength of the evidence was judged insufficient for a definitive conclusion of carcinogenic (Table 2), studies interpreted as showing no carcinogenic effects (Table 3), and inadequate studies (Table 4). These groupings are similar to the five categories of evidence of carcinogenicity recently adopted by the NTP (Huff and Moore, 1984), with the following exception: rather than attempt to classify retrospectively the NTP studies considered positive regarding whether they showed "clear" or "some" evidence of carcinogenicity, these classifications were pooled (Table 1).

TABLE 1. NTP Studies Interpreted as Showing Carcinogenic Effects

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat		Mouse		TR no.
				M	F	M	F	
Allyl isothiocyanate (57-06-7)	F344 B6C3F$_1$	Gavage: 12 or 25 mg/kg	Urinary bladder: transitional-cell papillomas	+				234
			Subcutaneous tissue: fibrosarcomas				E[b]	
Allyl isovalerate (2835-39-4)	F344	Gavage: 31 or 62 mg/kg	Mononuclear cell leukemia					253

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TABLE 1. NTP Studies Interpreted as Showing Carcinogenic Effects

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat M	Rat F	Mouse M	Mouse F	TR no.
Allylisothiocyanate (57-06-7)	F344 B6C3F$_1$	Gavage: 12 or 25 mg/kg	Urinary bladder: transitional-cell papillomas	+				234
			Subcutaneous tissue: fibrosarcomas		E[b]			
Amyl acetate (2835-39-4)	F344 B6C3F$_1$	Gavage: 31 or 62 mg/kg	Mononuclear cell leukemia	+				253
			Malignant lymphomas				+	
11-Aminoundecanoic acid (2432-99-7)	F344 B6C3F$_1$	Feed: 7500 or 15,000 ppm	Liver: neoplastic nodules	+				216
			Urinary bladder: transitional-cell carcinomas	+				
Asbestos, chrysotile; Intermediate grade (12001-29-5)	F344	Feed: 1% (lifetime)	Large intestine: adenomatous polyps	+				295
Benzene (71-43-2)	F344 B6C3F$_1$	Gavage: 50, 100 or 200 mg/kg (male rats); 25, 50 or 100 mg/kg (female rats, mice)	Zymbal gland: carcinomas	+	+	+	+	289
			Oral cavity: squamous-cell papillomas	+	+			
			Oral cavity: squamous-cell carcinomas	+	+			
			Skin: squamous-cell papillomas and carcinomas	+				
			Malignant lymphomas			+	+	
			Lung: alveolar/bronchiolar adenomas			+	+	
			Lung: alveolar/bronchiolar carcinomas			+	+	
			Harderian gland: adenomas			+		
			Preputial gland: carcinomas			+		
			Ovary: granulosa-cell tumors				+	
			Ovary: benign mixed tumors				+	
			Mammary gland: carcinomas				+	
			Mammary gland: carcinosarcomas				+	
Benzyl acetate (140-11-4)	F344 B6C3F$_1$	Gavage: 250 or 500 mg/kg (rats); 500 or 1000 mg/kg (mice)	Pancreas: acinar-cell adenomas	E				250
			Liver: adenomas			+	+	
			Forestomach: squamous-cell papillomas			+	+	

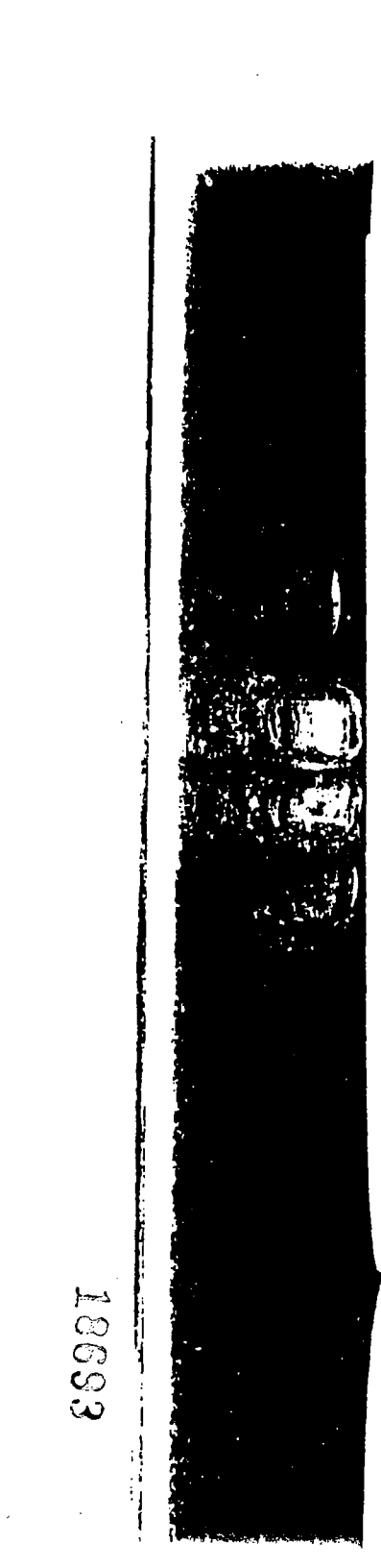

TABLE 1. NTP Studies Interpreted as Showing Carcinogenic Effects (*Continued*)

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat M	Rat F	Mouse M	Mouse F	TR no.
2-Biphenylamine HCl (2185-92-4)	F344 B6C3F$_1$	Feed: 1000 or 3000 ppm	Circulatory system: hemangiosarcomas			E	+	233
Bis(2-chloro-1-methyl ethyl) ether (108-60-1)	B6C3F$_1$	Gavage: 100 or 200 mg/kg	Lung: alveolar/bronchiolar adenomas Liver: carcinomas Forestomach: squamous-cell papillomas or carcinomas (combined)			+ +	+ E	239
1,3-Butadiene (106-99-0)	B6C3F$_1$	Inhalation: 625 or 1,250 ppm	Heart: hemangiosarcomas Malignant lymphomas Lung: alveolar/bronchiolar adenomas Lung: alveolar/bronchiolar carcinomas Stomach: papillomas Mammary gland: acinar-cell carcinomas Ovary: granulosa-cell tumors Liver:carcinomas or adenomas (combined)			+ + + + +	+ + + + + + + +	288
Chlorobenzene (108-90-7)	F344 B6C3F$_1$	Gavage: 60 or 120 mg/kg (rats and female mice); 30 or 60 mg/kg (male mice)	Liver: neoplastic nodules	+				261
Chlorodibromomethane (124-48-1)	F344 B6C3F$_1$	Gavage: 40 or 80 mg/kg (rats); 50 or 100 mg/kg (mice)	Liver: carcinomas Liver: adenomas			E	 +	282
C.I. disperse yellow 3 (2832-40-8)	F344 B6C3F$_1$	Feed: 5000 or 10,000 ppm (rats); 2500 or 5000 ppm (mice)	Liver: neoplastic nodules Liver: adenomas Stomach: all tumors Malignant lymphomas	+ E			 + E	222
C.I. solvent yellow 14 (842-07-9)	F344 B6C3F$_1$	Feed: 250 or 500 ppm (rats); 500 or 1000 ppm (mice)	Liver: neoplastic nodules	+	+			226
Cytembena (21739-91-3)	F344 B6C3F$_1$	Intraperitoneal injection, 3 times/wk: 7 or 14 mg/kg (rats); 12 or 24 mg/kg (mice)	Tunica vaginalis: mesotheliomas Multiple organs: mesotheliomas Mammary gland: fibroadenomas	+ +	 +			207
D and C red no. 9 (5160-02-1)	F344 B6C3F$_1$	Feed: 1000 or 3000 ppm (rats); 1000 or 2000 ppm (mice)	Spleen: sarcomas Liver: neoplastic nodules	+ +				225
1,2-Dibromo-3-chloropropane (DBCP)	F344	Inhalation, 6 h/d, 5 d/wk;	Nasal cavity tumors	+	+	+	+	20

Chlorodibromomethane (124-48-1)	F344 B6C3F$_1$	Gavage: 40 or 80 mg/kg (rats); 50 or 100 mg/kg (mice)
C.I. disperse yellow 3 (2832-40-8)	F344 B6C3F$_1$	Feed: 5000 or 10,000 ppm (rats); 2500 or 5000 ppm (mice)
C.I. solvent yellow 14 (842-07-9)	F344 B6C3F$_1$	Feed: 250 or 500 ppm (rats); 500 or 1000 ppm (mice)
Cytembena (21739-91-3)	F344 B6C3F$_1$	Intraperitoneal injection, 3 times/wk: 7 or 14 mg/kg (rats); 12 or 24 mg/kg (mice)
D[illegible]C red no. 9 (5160-02-1)	F344 B6C3F$_1$	Feed: 1000 or 3000 ppm (rats); 1000 or 2000 ppm (mice)
1,2-Dibromo-3-chloropropane (DBCP) (96-12-8)	F344 B6C3F$_1$	Inhalation, 6 h/d, 5 d/wk: 0.6 or 3.0 ppm
1,2-Dibromoethane (ethylene dibromide) (106-93-4)	F344 B6C3F$_1$	Inhalation, 6 h/d, 5 d/wk: 10 or 40 ppm
2,6-Dichloro-*p*-phenylenediamine ([illegible]20-1)	F344 B6C3F$_1$	Feed: 1000 or 2000 ppm (male rats); 2000 or 6000 ppm (female rats); 1000 or 3000 ppm (mice)
1,2-Dichloropropane (78-87-5)	F344 B6C3F$_1$	Gavage: 62 or 125 mg/kg (male rats); 125 or 150 mg/kg (mice and female rats)

Liver: carcinomas			E		282
Liver: adenomas				+	
Liver: neoplastic nodules	+				222
Liver: adenomas				+	
Stomach: all tumors	E				
Malignant lymphomas				E	
Liver: neoplastic nodules	+	+			226

Tunica vaginalis: mesotheliomas	+				207
Multiple organs: mesotheliomas	+				
Mammary gland: fibroadenomas		+			
Spleen: sarcomas	+				225
Liver: neoplastic nodules	+				
Nasal cavity tumors	+	+	+	+	206
Tongue: squamous-cell papillomas	+	+			
Tongue: squamous-cell carcinomas	+	+			
Adrenal glands: cortical adenomas		+			
Lung: alveolar/bronchiolar adenomas			+	+	
Nasal-cavity tumors	+	+		+	210
Circulatory system: hemangiosarcomas	+	+		+	
Tunica vaginalis: mesotheliomas	+				
Lung: alveolar/bronchiolar adenomas			+	+	
Lung: alveolar/bronchiolar carcinomas			+	+	
Lung: alveolar/bronchiolar adenomas or carcinomas (combined)		+			
Subcutaneous fibrosarcomas				+	
Mammary gland: fibroadenomas		+			
Mammary gland: adenocarcinomas				+	
Liver: adenomas			+		219
Liver: carcinomas or adenomas (combined)				+	
Liver: adenomas			+	+	263
Mammary gland: adenocarcinomas		E			

TABLE 1. NTP Studies Interpreted as Showing Carcinogenic Effects (*Continued*)

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat M	Rat F	Mouse M	Mouse F	TR no.
Di(2-ethylhexyl) adipate (DEHA) (103-23-1)	F344 B6C3F$_1$	Feed: 12,000 or 25,000 ppm	Liver: adenomas Liver: carcinomas			+	+	212
Di(2-ethylhexyl) phthalate (DEHP) (117-81-7)	F344 B6C3F$_1$	Feed: 6000 or 12,000 ppm (rats); 3,000 or 6,000 ppm (mice)	Liver: carcinomas or neoplastic nodules (combined) Liver: carcinomas	+	+	+	+	217
Diglycidyl resorcinol ether (DGRE) (101-90-6)	F344 B6C3F$_1$	Gavage: 12, 25, or 50 mg/kg (rats); 50 or 100 mg/kg (mice)	Stomach/forestomach: squamous-cell papillomas Stomach/forestomach: squamous-cell carcinomas	+	+	+	+	257
Dimethyl hydrogen phosphite (868-85-9)	F344 B6C3F$_1$	Gavage: 100 or 200 mg/kg (male rats, mice); 50 or 100 mg/kg (female rats)	Lung: alveolar/bronchiolar adenomas Lung: alveolar/bronchiolar carcinomas Lung: squamous-cell carcinomas Forestomach: squamous-cell papillomas or carcinomas (combined)	+ + + +	E E			287
Ethyl acrylate (140-88-5)	F344 B6C3F$_1$	Gavage: 100 or 200 mg/kg	Forestomach: squamous-cell papillomas Forestomach: squamous-cell carcinomas Forestomach: squamous-cell papillomas or carcinomas (combined)	+	+	+ +	 +	259
HC blue no. 1 (2784-94-3)	F344 B6C3F$_1$	Feed: 1500 or 3000 ppm (rats, male mice); 3000 or 6000 ppm (female mice)	Liver: carcinomas Liver: carcinomas or neoplastic nodules (combined) Lung: alveolar/bronchiolar adenomas or carcinomas (combined) Thyroid: follicular cell adenomas	E	+	+	+	271
Hexachlorodibenzo-*p*-dioxins, 1,2,3,6,7,8- and 1,2,3,7,8,9- (57653-85-7X and 19408-74-3)	Osborne-Mendel B6C3F$_1$	Gavage: 1.25, 2.5, or 5 μg/kg·wk (rats, male mice); 2.5, 5, or 10 μg/kg·wk (female mice)	Liver: neoplastic nodules Liver: adenomas Liver: carcinomas Liver: carcinomas or neoplastic nodules (combined)	E	+	+	+	198
Melamine (108-78-1)	F344 B6C3F$_1$	Feed: 2250 or 4500 ppm (male rats, mice); 4500 or 9000 ...	Urinary bladder: transitional-cell carcinomas (associated with	+				245

HC blue no. 1 (2784-94-3)	F344 B6C3F$_1$	Feed: 1500 or 3000 ppm (rats, male mice); 3000 or 6000 ppm (female mice)
Hexachlorodibenzo-*p*-dioxins, 1,2,3,6,7,8- and 1,2,3,7,8,9- (57653-85-7X and 19408-74-3)	Osborne-Mendel B6C3F$_1$	Gavage: 1.25, 2.5, or 5 μg/kg•wk (rats, male mice); 2.5, 5, or 10 μg/kg•wk (female mice)
[illegible]mine (108-78-1)	F344 B6C3F$_1$	Feed: 2250 or 4500 ppm (male rats, mice); 4500 or 9000 ppm (female rats)
4,4'-Methylenedianiline dihydrochloride (13552-44-8)	F344 B6C3F$_1$	Drinking water: 150 or 300 ppm
[illegible]uron (150-68-5)	F344 B6C3F$_1$	Feed: 750 or 1500 ppm (rats); 5000 or 10,000 ppm (mice)
4,4'-Oxydianiline (101-80-4)	F344 B6C3F$_1$	Feed: 200, 400, or 500 ppm (rats); 150, 300, or 800 ppm (mice)
Pentachloroethane (76-01-7)	F344 B6C3F$_1$	Gavage: 75 or 150 mg/kg (rats); 250 or 500 mg/kg (mice)

cinomas					
Forestomach: squamous-cell papillomas or carcinomas (combined)				+	
Liver: carcinomas			+	+	271
Liver: carcinomas or neoplastic nodules (combined)	E				
Lung: alveolar/bronchiolar adenomas or carcinomas (combined)		+			
Thyroid: follicular cell adenomas			+		
Liver: neoplastic nodules		+			198
Liver: adenomas			+	+	
Liver: carcinomas		+			
Liver: carcinomas or neoplastic nodules (combined)	E				
Urinary bladder: transitional-cell carcinomas (associated with bladder stones)	+				245
Thyroid: follicular-cell adenomas		+	+	+	248
Thyroid: follicular-cell carcinomas	+				
Thyroid: c-cell adenomas		+			
Liver: neoplastic nodules	+				
Liver: carcinomas			+	+	
Adrenal: pheochromocytomas			+		
Malignant lymphomas				+	
Kidney: tubular-cell adenomas	+				266
Kidney: tubular-cell adenocarcinomas	+				
Liver: neoplastic nodules	+				
Liver: neoplastic nodules	+	+			205
Liver: adenomas				+	
Liver: carcinomas	+	+		+	
Liver: adenomas or carcinomas (combined)			+		
Thyroid: follicular-cell adenomas	+	+		+	
Thyroid: follicular-cell carcinomas	+	+			
Harderian gland: adenomas			+	+	
Liver: adenomas				+	232
Liver: carcinomas			+	+	
Kidney: tubular-cell adenomas	E				

TABLE 1. NTP Studies Interpreted as Showing Carcinogenic Effects (*Continued*)

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat M	Rat F	Mouse M	Mouse F	TR no.
Polybrominated biphenyl mixture (Firemaster FF-1) (67774-32-7)	F344 B6C3F$_1$	Gavage: 0, 0.1, 0.3, 1.0, 3.0, or 10 mg/kg	Liver: neoplastic nodules	+	+			244
			Liver: carcinomas	+	+	+	+	
			Liver: cholangiocarcinomas	+	+			
Propylene oxide (75-56-9)	F344 B6C3F$_1$	Inhalation: 200 or 400 ppm	Nasal turbinates: papillary adenomas	+	+			267
			Nasal turbinates: hemangiomas or hemangiosarcomas			+	+	
Telone II (542-75-6)	F344 B6C3F$_1$	Gavage: 25 or 50 mg/kg (rats); 50 or 100 mg/kg (mice)	Forestomach: squamous-cell papillomas	+	+	I		269
			Forestomach: squamous-cell carcinomas	+				
			Forestomach: squamous-cell papillomas or carcinomas (combined)				+	
			Liver: neoplastic nodules	+				
			Urinary bladder: transitional-cell carcinomas				+	
			Lung: alveolar/bronchiolar adenomas			+	+	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (1746-01-6)	Osborne-Mendel B6C3F$_1$	Gavage: 0.01, 0.05, or 0.5 μg/kg·wk (rats and male mice); 0.04, 0.2 or 2.0 μg/kg·wk (female mice)	Thyroid: follicular-cell adenomas	+			+	209
			Liver: neoplastic nodules		+			
			Liver: carcinomas			+	+	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (1746-01-6)	Swiss-Webster	Dermal: 0.001 μg (male mice); 0.005 μg (female mice) 3 d/wk	Integumentary system: fibrosarcomas			E	+	201
1,1,1,2-Tetrachloroethane (630-20-6)	F344 B6C3F$_1$	Gavage: 125 or 250 mg/kg (rats); 250 or 500 mg/kg (mice)	Liver: adenomas			+	+	237
			Liver: carcinomas				+	
Toluene diisocyanate (2,4- and 2,6-isomers) (26471-62-5)	F344 B6C3F$_1$	Gavage: 30 or 60 mg/kg (male rats); 60 or 120 mg/kg (female rats); 120 or 240 mg/kg (male mice); 60 or 120 mg/kg (female mice)	Subcutaneous fibromas or fibrosarcomas (combined)	+	+			251
			Pancreas: acinar-cell adenomas	+				
			Pancreas: islet-cell adenomas		+			
			Liver: neoplastic nodules		+			
			Liver: adenomas				+	
			Mammary gland: fibroadenomas		+			
			Circulatory system: hemangiomas or hemangiosarcomas (combined)				+	

	$B6C3F_1$	and male mice); 0.04, 0.2 or 2.0 μg/kg•wk (female mice)	Liver: carcinomas			+	+	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (1746-01-6)	Swiss-Webster	Dermal: 0.001 μg (male mice); 0.005 μg (female mice) 3 d/wk	Integumentary system: fibrosarcomas			E	+	201
1,1,1,2-Tetrachloroethane (630-20-6)	F344 $B6C3F_1$	Gavage: 125 or 250 mg/kg (rats); 250 or 500 mg/kg (mice)	Liver: adenomas Liver: carcinomas			+	+ +	237
Toluene diisocyanate (2,4- and 2,6-isomers) (26471-62-5)	F344 $B6C3F_1$	Gavage: 30 or 60 mg/kg (male rats); 60 or 120 mg/kg (female rats); 120 or 240 mg/kg (male mice); 60 or 120 mg/kg (female mice)	Subcutaneous fibromas or fibrosarcomas (combined) Pancreas: acinar-cell adenomas Pancreas: islet-cell adenomas Liver: neoplastic nodules Liver: adenomas Mammary gland: fibroadenomas Circulatory system: hemangiomas or hemangiosarcomas (combined)	+ +	+ + + + +		+ + +	251
Trichloroethylene (without epichlorohydrin (79-01-6)	F344 $B6C3F_1$	Gavage: 500 or 1000 mg/kg (rats); 1000 mg/kg (mice)	Liver: adenomas Liver: carcinomas	I		+	+ +	243
Tris(2-Ethylhexyl) phosphate (78-42-2)	F344 $B6C3F_1$	Gavage: 2000 or 4000 mg/kg (male rats); 1000 or 2000 mg/kg (female rats); 500 or 1000 mg/kg (mice)	Adrenal: pheochromocytomas Liver: carcinomas	E			+ +	274
2,4-Xylidine (87-62-7)	CD-1	Feed: 0, 300, 1000 or 3000 ppm	Nasal cavity: papillary adenomas Nasal cavity: carcinomas	+ +	+ +			278
Zearalenone (17924-92-4)	F344 $B6C3F_1$	Feed: 25 or 50 ppm (rats); 50 or 100 ppm (mice)	Pituitary: adenomas Liver: adenomas			+	+ +	235
Ziram (137-30-4)	F344 $B6C3F_1$	Feed: 300 or 600 ppm (rats); 600 or 1200 ppm (mice)	Thyroid: c-cell carcinomas Lung: alveolar/bronchiolar adenomas	+			 E	238

Rat strains: F344 (Fischer-344); Osborne-Mendel; CD-1 (Charles River CD). Mouse strains: $B6C3F_1$; Swiss-Webster.
E: Observed effect considered equivocal; I: study judged inadequate.

TABLE 2. NTP Studies Interpreted as Showing Equivocal Evidence of Carcinogenicity

Chemical (CAS no.)	Animal strain[a]	Route/dose	Site or type of tumor	Rat M	Rat F	Mouse M	Mouse F	TR no.
Butyl benzyl phthalate (85-68-7)	F344 B6C3F$_1$	Feed: 6000 or 12,000 ppm	Mononuclear-cell leukemia	I	E[b]			213
C.I. acid yellow 73 (fluorescein sodium) (518-47-8)	F344 B6C3F$_1$	Drinking water: 2500 or 5000 ppm (rats); 5000 or 10,000 ppm (mice)	Pancreas: islet-cell adenomas or carcinomas (combined)	E				265
Diallyl phthalate (131-17-9)	F344	Gavage: 50 or 100 mg/kg	Mononuclear-cell leukemia		E			284
	B6C3F$_1$	Gavage: 150 or 300 mg/kg	Malignant lymphoma			E		242
Eugenol (97-53-0)	F344 B6C3F$_1$	Feed: 6000 or 12,500 ppm (female rats); 3000 or 6000 ppm (male rats and mice)	Liver: adenomas or carcinomas (combined)			E	E	223
Sodium (2-ethylhexyl) sulfate (126-92-1)	F344 B6C3F$_1$	Feed: 10,000 or 20,000 ppm (rats and female mice); 5000 or 10,000 ppm (male mice)	Liver: adenomas or carcinomas (combined)				E	256

[a]Rat strain: F344 (Fischer-344). Mouse strain: B6C3F$_1$.
[b]Observed effect considered equivocal; I: study judged inadequate. For butyl benzyl phthalate (only), the actual language employed in technical report for female rats was "probably carcinogenic."

TABLE 3. NTP Stud

Chemical (CAS no.
Agar (9002-18-0)
Arabic gum (9000-01-
Amosite asbestos (12172-73-5)
Amosite asbestos (12172-73-5)
Asbestos, chrysotile; short and intermedia range (12001-29-5)
Asbestos, chrysotile; short range (12001-2
Asbestos, crocidolite (12001-28-4)
L-Ascorbic acid (50-81
Benzoin (119-53-9)
Bisphenol A (80-05-7)
Caprolactam (105-60-2
2-Chloroethanol (107-07-3)
C.I. acid orange 10 (1936-15-8)
C.I. acid red 14 (3567-69-9)
1,2-Dichlorobenzene (95-50-1)
Ethoxylated dodecyl alcohol (9002-92-0)
FD and C yellow no. 6 (2783-94-0)
Geranyl acetate (105-87-3)
Gitonin (12002-43-6)

TABLE 3. NTP Studies Interpreted as Showing No Carcinogenic Effects

Chemical (CAS no.)	Animal strain[a]	Route	Doses	TR no.
Agar (9002-18-0)	F344 $B6C3F_1$	Feed	25,000 or 50,000 ppm	230
Arabic gum (9000-01-5)	F344 $B6C3F_1$	Feed	25,000 or 50,000 ppm	227
Amosite asbestos (12172-73-5)	Syrian golden	Feed	1% (Lifetime)	249
Amosite asbestos (12172-73-5)	F344	Feed	1% (Lifetime)	279
Asbestos, chrysotile; short and intermediate range (12001-29-5)	Syrian golden	Feed	1% (Lifetime)	246
Asbestos, chrysotile; short range (12001-29-5)	F344	Feed	1% (Lifetime)	295
Asbestos, crocidolite (12001-28-4)	F344	Feed	1% (Lifetime)	280
L-Ascorbic acid (50-81-7)	F344 $B6C3F_1$	Feed	25,000 or 50,000 ppm	247
Benzoin (119-53-9)	F344 $B6C3F_1$	Feed	125 or 250 ppm (male rats) 250 or 500 ppm (female rats) 2500 or 5000 ppm (mice)	204
Bisphenol A (80-05-7)	F344 $B6C3F_1$	Feed	1000 or 2000 ppm (rats) 1000 or 5000 ppm (male mice) 5000 or 10,000 ppm (female mice)	215
Caprolactam (105-60-2)	F344 $B6C3F_1$	Feed	3750 or 7500 ppm (rats) 7500 or 15,000 ppm (mice)	214
2-Chloroethanol (107-07-3)	F344 Swiss CD-1	Dermal	50 or 100 mg/kg (rats) 7.5 or 15 mg/animal (mice)	275
C.I. acid orange 10 (1936-15-8)	F344 $B6C3F_1$	Feed	1000 or 3000 ppm (rats) 3000 or 6000 ppm (mice)	211
C.I. acid red 14 (3567-69-9)	F344 $B6C3F_1$	Feed	6000 or 12,500 ppm (male rats) 12,500 or 25,000 ppm (female rats) 3000 or 6000 ppm (mice)	220
1,2-Dichlorobenzene (95-50-1)	F344 $B6C3F_1$	Gavage	60 or 120 mg/kg	255
Ethoxylated dodecyl alcohol (9002-92-0)	F344 $B6C3F_1$	Feed	3000 or 6000 ppm (rats) 6000 or 12,000 ppm (mice)	264
FD and C yellow no. 6 (2783-94-0)	F344 $B6C3F_1$	Feed	12,500 or 25,000 ppm	208
Geranyl acetate (105-87-3)	F344 $B6C3F_1$	Gavage	1000 or 2000 mg/kg (rats) 500 or 1000 mg/kg (mice)	252
Gilsonite (12002-43-6)	F344 $B6C3F_1$	Feed	20,000 or 40,000 ppm	270

[a] Rat strain: F344 (Fischer-344). Mouse strain: $B6C3F_1$.
[b] Observed effect considered equivocal; I: study judged inadequate. For butyl benzyl phthalate (only), the actual language employed in technical report for ...le rats was "probably carcinogenic."

...ppm (rats and female mice); 5000 or 10,000 ppm (male mice) (continued)

TABLE 3. NTP Studies Interpreted as Showing No Carcinogenic Effects (*Continued*)

Chemical (CAS no.)	Animal strain[a]	Route	Doses	TR no.
Guar gum (9000-30-0)	F344 B6C3F$_1$	Feed	25,000 or 50,000 ppm	229
Hamamelis water (68916-39-2)	F344 B6C3F$_1$	Dermal	50% or 100% in deionized water 5 d/wk	286
HC blue no. 2 (33229-34-4)	F344 B6C3F$_1$	Feed	5000 or 10,000 ppm (male rats, male mice) 10,000 or 20,000 ppm (female rats, female mice)	293
Hexachlorodibenzo-*p*-Dioxins, 1,2,3,7,8,9- and 1,2,3,6,7,8- (57653-85-7 and 19408-74-3)	Swiss-Webster	Dermal	0.01 μg, 3 times/wk	202
8-Hydroxyquinoline (148-24-3)	F344 B6C3F$_1$	Feed	1500 or 3000 ppm	276
Locust bean gum (9000-40-2)	F344 B6C3F$_1$	Feed	25,000 or 50,000 ppm	221
D-Mannitol (69-65-8)	F344 B6C3F$_1$	Feed	25,000 or 50,000 ppm	236
Phenol (108-95-2)	F344 B6C3F$_1$	Drinking water	2500 or 5000 ppm	203
Propylene (115-07-1)	F344 B6C3F$_1$	Inhalation	5000 or 10,000 ppm	272
Propyl gallate (121-79-9)	F344 B6C3F$_1$	Feed	6000 or 12,000 ppm	240
Selenium sulfide (7446-34-6)	ICR Swiss	Dermal	0.5 or 1.0 mg/application, 3 times/wk	197
Selsun (trade name, no CAS no.)	ICR Swiss	Dermal	0.05 ml of 25% or 50% 3 times/wk	199
Stannous chloride (7772-99-8)	F344 B6C3F$_1$	Feed	1000 or 2000 ppm	231
Tara gum (39300-88-4)	F344 B6C3F$_1$	Feed	25,000 or 50,000 ppm	224
2,6-Toulenediamine dihydrochloride (15481-70-6)	F344 B6C3F$_1$	Feed	250 or 500 ppm (rats) 50 or 100 ppm (mice)	200
Tremolite (4567-73-8)	F344	Feed	1% (Lifetime)	277
Vinylidene chloride (1,1-dichloroethylene) (75-35-4)	F344 B6C3F$_1$	Gavage	1 or 5 mg/kg (rats) 2 or 10 mg/kg (mice)	228

[a]Rat strain: F344 (Fischer-344). Mouse strains: B6C3F$_1$; Swiss-Webster; ICR Swiss; Swiss CD-1. Hamster strain: Syrian golden.

(Continued)

	TR no.
Doses	
,000 ppm	229
in deionized water	286
30 ppm (male rats,	293
,000 ppm (female mice)	
es/wk	202
) ppm	276
,000 ppm	221
,000 ppm	236
) ppm	203
00 ppm	272
00 ppm	240
:/application, 3	197
:% or 50% 3 times/wk	199
) ppm	231
,000 ppm	224
pm (rats) m (mice)	200
:)	277
(rats)	228
e (mice)	

obster; ICR Swiss; Swiss CD-1.

TABLE 4. NTP Studies Regarded as Inadequate

Chemical (CAS no.)	Animal strain[a]	Route/dose	Reason for inadequacy	TR no.
Asbestos (chrysotile) and 1,2-dimethylhydrazine (DMH) (12001-29-5)	Syrian golden	1% in diet (asbestos) 4 mg/kg By gavage every other week for 5 doses (DMH)	Combination study in male and female Syrian golden hamsters considered inadequate because no increase in DMH-induced intestinal neoplasia was observed (DMH is known to induce gastrointestinal tumors)	246
2,3,7,8-TCDD and dimethylbenzanthracene (DMBA) (1746-01-6)	Swiss-Webster	Dermal: DMBA, 50 μg 1 wk prior to TCDD; 0.001 μg TCDD (males), 0.005 μg TCDD (females), per application; 3 applications/wk	Initiation-promotion study using Swiss-Webster mice was considered inadequate because DMBA was not tested alone and DMBA–TCDD-induced fibrosarcomas incidence not greater than that observed with TCDD alone	201

[a]Hamster strain: Syrian golden. Mouse strain: Swiss-Webster.

DISCUSSION

Of the 86 NTP studies, 50% were found to produce carcinogenic responses. Using the results of the NCI studies reported in TR 1–196 as summarized by Griesemer and Cueto (1980), Hottendorf and Pachter (1982) reported a similar percentage (51%, 98/192). They also noted that 28 (15%) of these studies were found to be equivocal and 66 (34%) demonstrated no evidence of carcinogenicity.

When interpreting the results of these latter studies, Hottendorf and Pachter (1982) note that only three of the negative experiments were categorized by Griesemer and Cueto (1980) as showing no evidence in two animal species. Thus, Hottendorf and Pachter (1982) propose that "the positive to negative ratio in the evaluable bioassays was 33:1." We disagree and feel that this misrepresentation of the NCI carcinogenesis testing results should be corrected.

Griesemer and Cueto (1980) actually listed 66 studies showing no evidence of carcinogenicity under the conditions used for testing and as reported. They regarded 53 studies as showing no evidence in limited animal experiments, 10 as showing no evidence in 1 animal species, and 3 as showing no evidence in 2 animal species. When reporting these results, Griesemer and Cueto (1980) stated that "unless the data indicated that the compound had been fully tested in both sexes of one species at or near the maximum tolerated dose, the evidence was considered to be too limited to draw conclusions about noncarcinogenicity without further tests."

Hottendorf and Pachter (1982) apparently misinterpreted this statement to imply that 63 of the 66 negative studies were considered by the NCI to be inadequate, or "unevaluable." While further testing for certain selected chemicals may have clarified the issue of whether or not the lack of a carcinogenic response was due to inadequate dose selection, these studies all employed doses that were estimated to be maximum tolerated doses, often far in excess of anticipated human exposure levels. Thus, while these negative studies did not "prove" with absolute certainty that these chemicals have no carcinogenic potential, all 66 studies should be considered as being in the category of no evidence of carcinogenicity. This was the position of the NCI, who did not regard these studies as "limited." Furthermore, regardless of the exact "limitations" that may have been present, the studies were *not* "unevaluable" as stated by Hottendorf and Pachter (1982). Each of these 66 NCI technical reports concluded that "under the conditions of this bioassay (chemical name) was not carcinogenic." Studies regarded as inadequate or unevaluable by the NCI were clearly identified as inadequate and often were not published as technical reports. Thus, for comparative purposes, the actual ratio of positive to negative or equivocal results is approximately 1:1, not 33:1, for both the NCI and NTP carcinogenicity studies.

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creased incidences of mouse liver neoplasms agrees with the 19% figure from the early NCI studies as reported by Hottendorf and Pachter (1982). The liver was also the site of carcinogenicity for the Fischer-344 rat more often than any other organ (Table 5). The finding that 63% (27/43) of the positive NTP studies include liver tumor effects agrees closely with the corresponding figure (64%; 54/85) reported by Hamm (1983) for the earlier NCI studies.

The finding that the liver is the primary tumor site in rodents is not surprising, because chemicals administered by feed or gavage are generally metabolized in the liver prior to transport to other organs. One should also recognize that more than two-thirds of the chemicals tested did not cause a carcinogenic response in the rodent liver (Table 5), even though experimental conditions utilizing high doses were employed. Although the spontaneous incidence of liver tumors in male B6C3F$_1$ mice is high (31%), the corresponding control rates in female B6C3F$_1$ mice (8%), male Fischer-344 rats (4%), and female Fischer-344 rats (3%) are relatively low (Haseman et al., 1984). For a more detailed discussion of rodent liver tumors, see Maronpot and Boorman (1982) and the Nutrition Foundation (1983).

Some authors (Von Wittenau and Estes, 1983) have suggested that carcinogenicity testing in the mouse is "redundant" and that male and female rats are sufficient for the detection of most carcinogens. The present data suggest that if one wishes to halve the number of sex–species groups tested, a better choice would be male rats in conjunction with female mice. This choice would have detected all 43 carcinogens in the NTP data, while the use of male and female rats would have missed 10 and possibly 13 carcinogens (3 chemicals were positive in mice, but not tested in rats).

A review of the earlier NCI studies produced similar results. Use of male rats in conjunction with female mice would have detected all but 16 of the 98 carcinogens; use of male and female rats only would have missed 30 carcinogens. Despite these results, we feel that it is premature to propose any reduction from the current two-sex, two-species experimental protocol for routine carcinogenicity testing.

The implications of the finding that inhalation and gavage routes of administration tend to produce more carcinogenic responses than feed, drinking water or dermal exposures are unclear. One factor that may have contributed to the relatively high proportion of gavage studies showing carcinogenic responses is the disproportionately large number of halogenated hydrocarbons tested by this route of administration. Among the 86 NTP chemicals were 20 halogenated hydrocarbons; 80% (16/20) produced carcinogenic responses, primarily in the liver. The majority of these chemicals were tested utilizing the gavage route of administration, and the proportion of studies showing carcinogenic effects was similar, regardless of how the chemical was administered: 85% (11/13) positive studies for the gavage route of administration, compared with 71% (5/7) for all other routes. However, even if halogenated hydrocarbons are excluded, the proportion of gavage

studies showing carcinogenic responses (9/11, 82%) remains elevated relative to the corresponding rate in feeding studies (14/43, 33%).

In the early NCI studies, 46% (70/152) of the feeding studies resulted in carcinogenic responses compared with 59% (17/29) positive gavage studies. The only other route of administration with more than one study was intraperitoneal (ip), in which all 9 studies produced carcinogenic responses (Griesemer and Cueto, 1980).

Haseman (1984) noted that overall dosed-group survival in the NTP feeding studies has been essentially the same (or slightly exceeded) the survival in corresponding control groups. In contrast, in NTP gavage studies there is often increased mortality in dosed groups relative to controls. In only 38% (9/24) of the NTP gavage studies were no significant reductions in survival observed in some dosed group relative to controls.

One factor that may have contributed to the poor survival in gavage studies is the inherent toxicity of the class of chemicals that requires utilization of this particular route of administration. Generally, NTP does not use the gavage route of administration unless the test chemical is volatile, unpalatable, or unstable, or the route mimics a potential human exposure (e.g., drugs taken in single, daily doses). As already noted, more than half of the NTP gavage studies involved halogenated hydrocarbons, which tend to be hepatotoxic.

For a variety of reasons (including poor survival of dosed groups), the NTP is limiting the number of new studies in which gavage is used as a route of administration. Other disadvantages of gavage testing include the possibility of accidental overdoses, the likelihood of gavage-related trauma (e.g., puncturing the animal's lung, esophagus, or stomach with the gavage needle, thereby debilitating or killing the animal), possible vehicle (e.g., corn oil) effects, and the fact that a single, daily gavage administration (bolus dose) may not mimic human exposure (Haseman, 1984).

A number of chemicals produced reduced survival in dosed groups showing increased tumor incidence. These included benzene, 1,3-butadiene, chlorobenzene, cytembena (male rats), DBCP, 1,2,-dibromoethane, dimethyl hydrogen phosphite (male rats), DGRE, HC blue no. 1 (female mice) melamine, 4,4'-oxydianiline (female rats), pentachloroethane, propylene oxide (mice), 1,1,1,2-tetrachloroethane, toluene diisocyanate, trichloroethylene (male mice), and 2,6-xylidine (male rats). However, reduced survival per se does not necessarily mean that the doses employed were too high, since in many cases carcinogenic responses were the direct cause of reduced survival. Moreover, the majority of carcinogenic effects were *not* associated with a corresponding decrease in survival.

Although approximately 50% (141/278) of NCI/NTP studies have shown carcinogenic effects in laboratory animals, caution must be exercised in the interpretation of this figure. Many chemicals selected for testing have a high a priori suspicion of carcinogenicity. Moreover, since chemicals are not selected randomly, but rather on the basis of the criteria outlined

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REFERENC

Chu, K. C., Cueto,
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Griesemer, R. A., a
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of Biological
York: Plenum.
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above, certain chemical classes may be over- (or under-) represented in these figures. Furthermore, these tabulations include certain chemicals tested twice by different routes of administration, in different species, or in different strains of rats. With recognition of these limitations, these data nevertheless suggest that the proportion of chemicals found to be carcinogenic has been relatively constant over the past 8–10 yr.

REFERENCES

Chu, K. C., Cueto, C., and Ward, J. M. 1981. Factors in the evaluation of 200 National Cancer Institute carcinogen bioassays. *J. Toxicol. Environ. Health* 8:251–280.

Griesemer, R. A., and Cueto, C. 1980. Toward a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals for animals. In *Molecular and Cellular Aspects of Carcinogen Screening Tests*, eds. R. Montesano, H. Bartsch, and L. Tomatis, pp. 259–281. Lyon: IARC Scientific Publications.

Hamm, T. E., Jr. 1983. The occurrence of neoplasms in long-term *in vivo* studies. In *Application of Biological Markers to Carcinogen Testing*, eds. H. A. Milman and S. Sell, pp. 9–23. New York: Plenum.

Hart, L. G., Huff, J. E., Moore, J. A., and Rall, D. P. 1983. The National Toxicology Program's research and testing activities. In *Hazard Assessment of Chemicals, Current Developments*, vol. 2, ed. J. Saxena, pp. 191–244. New York: Academic.

Haseman, J. K. 1984. Dose selection issues in carcinogenicity testing. *Fundam. Appl. Toxicol.*, in press.

Haseman, J. K., Huff, J., and Boorman, G. A. 1984. Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* 12:126–135.

Hottendorf, G. H., and Pachter, I. J. 1982. An analysis of the carcinogenesis testing experience of the National Cancer Institute. *Toxicol. Pathol.* 10:22–26.

Huff, J. E. 1982. Carcinogenesis bioassay results from the National Toxicology Program. *Environ. Health Perspect.* 45:185–198.

Huff, J. E., and Moore, J. A. 1984. Carcinogenesis studies design and experimental data interpretation/evaluation at the National Toxicology Program. In *Industrial Hazards of Plastics and Synthetic Elastomers*, eds. J. Jarvisalo, P. Pfaffli, and H. Vainio, pp. 43–64. New York: Alan R. Liss.

Huff, J. E., Haseman, J. K., McConnell, E. E., and Moore, J. A. 1985. The National Toxicology Program, toxicology data evaluation techniques, and long-term carcinogenesis studies. In *Safety Evaluation of Drugs and Chemicals*, ed. W. E. Lloyd, Washington, D.C., Hemisphere.

Maronpot, R. R., and Boorman, G. A. 1982. Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* 10:71–80.

National Toxicology Program. 1984. Fiscal year 1984 annual plan. U.S. Department of Health and Human Services, Public Health Service, Washington, D.C.

Nutrition Foundation. 1983. The relevance of mouse liver hepatoma to human carcinogenic risk. A report of the International Expert Advisory Committee to the Nutrition Foundation, September 1983. Washington, D.C.: The Nutrition Foundation.

Sontag, J. M., Page, N. P., and Saffiotti, U. 1976. Guidelines for carcinogen bioassay in small rodents. DHHS Publication (NIH) 76-801, National Cancer Institute, Bethesda, Md.

Von Wittenau, M. S., and Estes, P. C. 1983. The redundancy of mouse carcinogenicity bioassays. *Fundam. Appl. Toxicol.* 3:631–639.

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NTC. NO.
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COMPTES RENDUS HEBDOMADAIRES
DES SEANCES. SERIE D. SCIENCES
NATURELLES.
V.280( )2709-11(1975)

Similarities of the biochemical effects of 2,3,7,8-tetrachloro dibenzo-p-dioxine and 2,3,7,8-tetrabromo dibenzo-p-dioxine in rats.

AUTHOR: Saint-Hui, Germain

TRANSLATOR: Sunlone, Inc.
DATE: November, 1975

SIMILARITIES OF THE BIOCHEMICAL EFFECTS OF 2,3,7,8-TETRACHLORO DIBENZO-P-DIOXINE AND 2,3,7,8-TETRABROMO DIBENZO-P-DIOXINE IN RATS.

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Comptes Rendus hebdomadaires de l'Académie des Sciences (Paris)Series D, Sciences Naturelles 280 p. 2709-2711 (1975)

Translated from French by:

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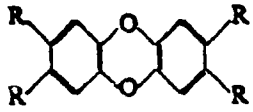
Biochemical experiments in the study of chemical carcinogens showed that 2,3,7,8-tetrabromo-p-dioxine is also active, if not more so, than its chlorinated analogue, "dioxine", in the induction of the biosynthesis of zoxazolamine hydroxylase in the rat or in the reduction of the amount of liver arginase in this animal. The biological properties of these molecules are connected with their perfectly symmetrical molecular structure.

With an LD-50 value of the order of 0.6 µg/kg in the guinea-pig, 2,3,7,9-tetrachloro-dibenzo-p-dioxine or "dioxine" (I) is undoubtedly one of the most toxic synthetic substances known.(1) This compound may contaminate certain herbicide preparations based on polychlorophenols, or trichlorophenoxy-alkanoic acids, as an industrial impurity.(2) This compound causes profound changes in the enzyme systems of animals(3), results in the appearance of pseudo-acinous structures in the liver, and in the disappearance of pericalicular (? péricaliculaire) ATP-ase(4), which indicates that these substances have a certain carcinogenic activity. More precisely, we showed that the effect of "dioxine" on the induction of the biosynthesis of zoxazolamine hydroxylase, which reaches a maximum above a dose of 0.1 mg/kg, is 60 times larger than that of benzopyrene, and that the lowering of the amount of liver arginase, provoked by this molecule is comparable to that produced by the most powerful carcinogens.(3)



1, R = Cl
2, R = Br

The other chloro-dioxines are less active from this point of view, and therefore we thought that the pronounced biological effects are linked to the particular geometrical structure of the molecule (with a high degree of symmetry), which makes the molecule more apt to be fixed on the cell receptors. If this is true, then the brominated analogue should possess similar biological properties to those of "dioxine", and this was found to be the case in the study of the activity of 2,3,7,8-tetrabromo-dibenzo-p-dioxine(2), in the biosynthesis

of zoxazolamine hydroxylase in the rat and on the amount of arginase in the hepatic tissue of this animal. The brominated substance is prepared by the direct action of bromine on dibenzo-p-dioxine.(5)

1. EFFECT ON THE BIOSYNTHESIS OF ZOXAZOLAMINE HYDROXYLASE

Our studies were carried out on Wistar ♂ rats, which were 3 months old and weighed about 100 g, using the technique described in our previous note(3), but replacing corn oil with dimethylsulfoxide as solvent (DMSO). The results, which are given in Table I, show that 2,3,7,8-tetrabromo-dibenzo-p-dioxine possesses a very high inductive power for zoxazolamine hydroxylase, similary to "dioxine". The effect, which was determined by the percentage reduction of the paralysis produced by zoxazolamine, remained very large even at a dose of the order of micrograms per kilogram.

Table I. Inductive effect of 2 on zoxazolamine hydroxylase

dose mg/kg	Duration of paralysis (min) treated rats*	control	reduction of duration %	P
20	11 ± 4 (6)	211 ± 30 (6)	~ 95	< 0,01
10	12 ± 2 (6)	194 ± 21 (6)	~ 94	< 0,01
5	14 ± 1 (6)	194 ± 21 (6)	~ 93	< 0,01
2,5	14 ± 1 (6)	194 ± 21 (6)	~ 93	< 0,01
1,25	17 ± 2 (6)	194 ± 21 (6)	~ 91	< 0,01
0,50	23 ± 6 (5)	202 ± 54 (7)	~ 89	< 0,01
0,25	25 ± 8 (6)	202 ± 54 (7)	~ 88	< 0,01
0,125	26 ± 10 (6)	202 ± 54 (7)	~ 87,5	< 0,01
0,06	28 ± 4 (7)	202 ± 54 (7)	~ 86,5	< 0,01
0,025	33 ± 7 (5)	180 ± 22 (6)	~ 82	< 0,01
0,0125	58 ± 3 (5)	180 ± 22 (6)	~ 68	< 0,01
0,003	74 ± 17 (5)	180 ± 22 (6)	~ 59	< 0,01
0,0015	79 ± 14 (5)	180 ± 22 (6)	~ 56	< 0,01

* The numbers in the parentheses correspond to the number of rats. The first number represents the average, the second is the standard deviation.

2. EFFECT ON THE AMOUNT OF ARGINASE IN THE LIVER

The 48 Sprague Dawley ♂ rats used, weighing between 300 and 350 g at the beginning of the experiment, received a normal diet regime. They were divided

into 4 lots, each containing 5 controls and 7 treated animals. The latter received compound 2, dissolved in DMSO at a concentration of 2 mg/kg, by intraperitoneal injection. The injections were given every 5 hours. The first lot received one injection, the 2nd lot received two injections, the 3rd 3 injections, and the fourth 4 injections. The controls only received the solvent at a rate of 0.25 ml/kg. The determination of the liver arginase was carried out after sacrificing each lot, using the method of Ng-Huy and coworkers[6]. The results are expressed in arginase units per milligram of the weight of fresh liver (under the conditions of the experiment, one unit of arginase hydrolyzes one nanomole of arginine per minute). The results are reported in Table II.

Table II

Day of sacrifice after the 1st injection	No. of injections	mean weight of animals* controls	treated	average amount of arginase controls	treated
7th	1	320 (5)	300 (7)	406	288
12th	2	340 (5)	230 (7)	530	210
17th	3	370 (5)	195 (7)	411	121
22nd	4	385 (5)	170 (7)	473	102

* The numbers in parentheses indicate the number of animals sacrificed.

These results show the following:

a) There is a distinct decrease in the weight of the animals treated, while at the same time, the weight of the controls increases normally. In this way, at the end of the 22nd day, the animals of the 4th lot lost on the average 150 g from their initial weight, which represents a weight deficiency of 55% with respect to the controls.

b) There is a clear decrease in the arginase activity starting after the first injection. As in the case of "dioxine" and of other chemical carcinogens which affect the liver[7], the rate of the decrease of the arginase in the liver

increases very rapidly as the duration of the treatment increases. Finally, it should be noted that among the animals treated in the 4th lot, two died before the planned date of sacrifice. The autopsy revealed lesions of necrotic appearance on the middle lobe of the liver, with generalized dot-shaped (punctiform) nodules.

In conclusion, 2,3,7,8-tetrabromo-benzo-p-dioxine provokes, even at very low doses, modificiations in the hepatocyte enzyme systems, which are very similar to those produced by "dioxine", both in quality and intensity. These results should be compared with those obtained by Kende and coworkers [8], who showed that compounds 1 and 2 have the same activity in the induction of arylhydroxylase in chick embryos. This represents an additional argument for the idea that a direct relationship exists between the biological properties of "dioxine" and the perfectly symmetrical model of its molecular geometry.

REFERENCES

see among others

(1) Cf. entre autres : J. Verrett, *in* : Effects of 2,4,5-T on Man and the Environment, Hearings before the Subcommittee on Energy, Natural Resources, and The Environment of the Committee on Commerce (U. S. Senate), April 7 and 15, 1970, U. S. Government Printing Office, Washington D. C., 1970, p. 190 ; N. P. Buu-Hoi, Pham-Huu-Chanh, G. Siesoue, M. C. Azum-Gelade et G. Saint-Ruf, *Die Naturwissenschaften*, 59, 1973, p. 173 ; B. A. Schwetz, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson et C. G. Gerbig, *Adv. Chem. Ser.*, 120, 1973, p. 55.

(2) *Chlorodioxins Origin and Fate*, Advances in Chemistry Series 120, E. H. Blair, American Chemical Society, Washington DC, 1973.

(3) N. P. Buu-Hoi, D. P. Hien, G. Saint-Ruf et J. Servoin-Sidoine, *Comptes rendus*, 272, Série D, 1971, p. 1447.

(4) R. Truhaut, Pham-Huu-Chanh, G. van Haverbeck, M. C. Azum-Gelade, G. Saint-Ruf et L. Lareng, *Comptes rendus*, 279, Série D, 1974, p. 1565.

(5) H. Gilman et J. J. Dietrich, *J. Amer. Chem. Soc.*, 79, 1957, p. 1439.

(6) H. Nguyen-Huy, R. Bockel, G. Prunner et A. Stahl, *Ann. Biol. Clin.*, 30, 1972, p. 309.

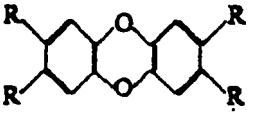
(7) S. Sato, *Tohoku Igaku Zasshi*, 67, 1963, p. 597 ; C. Wu et H. R. Eurel, *Cancer Research*, 27, 1967, p. 956 ; E. E. Elliason, *Exp. Cell. Res.*, 48, 1967, p. 1.

(8) A. S. Kende, J. J. Bergman et G. H. Haas, *Can. J. Chem.*, 49, 1971, p. 583.

TOXICOLOGIE BIOCHIMIQUE. — *Similitudes des effets biochimiques, chez le Rat, de la tétrachloro-2,3,7,8 dibenzo-p-dioxine et de la tétrabromo-2,3,7,8 dibenzo-p-dioxine.* Note (*) de MM. **Germain Saint-Ruf** et **Do-Phuoc Hien**, présentée par M. René Truhaut.

Des épreuves biochimiques pour l'étude des cancérogènes chimiques ont montré que la tétrabromo-2,3,7,8 dibenzo-*p*-dioxine est aussi active, sinon plus, que son analogue chloré, la « dioxine », dans l'induction de la biosynthèse de la zoxazolamine hydroxylase chez le Rat ou dans la réduction du taux d'arginase hépatique chez cet animal. Les propriétés biologiques de ces molécules sont liées à leur structure moléculaire parfaitement symétrique.

Avec une DL 50 de l'ordre de 0,6 µg/kg chez le Cobaye, la tétrachloro-2,3,7,8 dibenzo-*p*-dioxine ou « dioxine » (*1*) constitue sans doute l'une des substances de synthèse les plus toxiques qui soient connues (1). Ce composé, qui peut souiller, comme impureté industrielle, certaines préparations herbicides à base de polychlorophénols ou d'acides trichlorophénoxyalcanoïques (2), entraîne, d'autre part, chez l'animal, des perturbations profondes de l'équipement enzymatique (3), l'apparition de structures pseudo-acineuses dans le foie et la disparition de l'ATPase péricaliculaire (4) incitant à suspecter, chez ce corps, une certaine potentialité cancérogène. Plus précisément, nous avions montré que l'effet de la « dioxine »



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sur l'induction de la biosynthèse de la zoxazolamine hydroxylase, qui atteint son maximum dès la dose de 0,1 mg/kg, est 60 fois supérieur à celui du benzopyrène et que l'abaissement du taux d'arginase dans le foie, provoqué par cette molécule, est comparable à celui des plus puissants cancérogènes (3).

Les autres chlorodioxines étant, de ce point de vue, beaucoup moins actives, nous avons pensé que des effets biochimiques aussi importants chez la « dioxine » pourraient être liés à la structure géométrique particulière de la molécule (avec un haut degré de symétrie) qui la rend plus apte à se fixer sur les récepteurs cellulaires. S'il en est ainsi, l'analogue bromé de la « dioxine » devrait présenter des propriétés biologiques assez voisines de celles de cette dernière ; c'est ce que nous avons constaté en étudiant l'action de la tétrabromo-2,3,7,8 dibenzo-*p*-dioxine (*2*), préparée par action directe du brome sur la dibenzo-*p*-dioxine (5), à la fois sur la biosynthèse de la zoxazolamine hydroxylase chez le Rat et sur le taux de l'arginase dans le tissu hépatique de ce dernier.

1. Effet sur la biosynthèse de la zoxazolamine hydroxylase. — Nos essais ont porté sur des rats Wistar ♂ de 3 mois pesant 100 g environ selon la technique décrite dans notre précédente Note (3) en remplaçant toutefois l'huile de maïs par du diméthylsulfoxyde (DMSO) comme solvant. Les résultats, consignés dans le tableau I, montrent que la tétrabromo-2,3,7,8 dibenzo-*p*-dioxine possède, tout

TABLEAU I. — *Effet inducteur de 2 sur la zoxazolamine hydroxylase*

Dose (mg/kg)	Durée de paralysie (mn) rats traités (*)	Durée de paralysie (mn) témoins	Réduction de la durée (%)	P
20	11 ± 4 (6)	211 ± 30 (6)	~ 95	< 0,01
10	12 ± 2 (6)	194 ± 21 (6)	~ 94	< 0,01
5	14 ± 1 (6)	194 ± 21 (6)	~ 93	< 0,01
2,5	14 ± 1 (6)	194 ± 21 (6)	~ 93	< 0,01
1,25	17 ± 2 (6)	194 ± 21 (6)	~ 91	< 0,01
0,50	23 ± 6 (5)	202 ± 54 (7)	~ 89	< 0,01
0,25	25 ± 8 (6)	202 ± 54 (7)	~ 88	< 0,01
0,125	26 ± 10 (6)	202 ± 54 (7)	~ 87,5	< 0,01
0,06	28 ± 4 (7)	202 ± 54 (7)	~ 86,5	< 0,01
0,025	33 ± 7 (5)	180 ± 22 (6)	~ 82	< 0,01
0,0125	58 ± 3 (5)	180 ± 22 (6)	~ 68	< 0,01
0,003	74 ± 17 (5)	180 ± 22 (6)	~ 59	< 0,01
0,0015	79 ± 14 (5)	180 ± 22 (6)	~ 56	< 0,01

(*) Les chiffres entre parenthèses correspondent au nombre de rats ; le premier nombre représente la moyenne, le second est l'écart standard.

comme la « dioxine », un très haut pouvoir d'induction de la zoxazolamine hydroxylase, l'effet (déterminé en fonction du pourcentage de réduction de la paralysie provoquée par la zoxazolamine) demeurant très important même à la dose du microgramme par kilogramme.

2. EFFET SUR LE TAUX D'ARGINASE DANS LE FOIE. — 48 rats Sprague Dawley ♂, pesant de 300 à 350 g au début de l'expérience et recevant un régime alimentaire normal, sont répartis en 4 lots, chaque lot comprenant 5 témoins et 7 animaux traités. Ces derniers reçoivent, par voie intrapéritonéale, le composé *2* dissous dans du DMSO à raison de 2 mg/kg par injection, les injections étant pratiquées à 5 jours d'intervalle : le 1er lot reçoit une injection, le 2^{e} deux injections, le 3^{e} trois injections et le 4^{e} quatre injections. Les témoins ne reçoivent que le solvant à raison de 0,25 ml/kg. Le dosage de l'arginase du foie réalisé après sacrifice de chaque lot a été effectué selon la technique de Ng-Huy et coll. (6). Les résultats sont exprimés en

TABLEAU II

Jour du sacrifice après la 1re injection	Nombre d'injections	Poids moyens des animaux (*) témoins	Poids moyens des animaux (*) traités	Taux moyen en arginase (unités/mg de foie) témoins	Taux moyen en arginase (unités/mg de foie) traités
7^{e}	1	320 (5)	300 (7)	406	288
12^{e}	2	340 (5)	230 (7)	530	210
17^{e}	3	370 (5)	195 (7)	411	121
22^{e}	4	385 (5)	170 (5)	473	102

(*) Les chiffres entre parenthèses indiquent le nombre d'animaux sacrifiés.

unités arginases par milligramme de poids de foie frais (dans les conditions de l'expérience, une unité d'arginase hydrolyse une nanomole d'arginine par minute).

Les résultats, consignés dans le tableau II, montrent :

a. une nette diminution du poids des animaux traités, alors que, dans le même temps, celui des témoins s'accroît normalement ; c'est ainsi qu'au bout du 22e jour, les animaux du 4e lot ont perdu en moyenne 150 g par rapport au poids initial, ce qui représente une déficience pondérale de 55 % environ par rapport aux témoins ;

b. une très nette diminution de l'activité de l'arginase dès la première injection. Comme dans le cas de la « dioxine » et dans celui des cancérogènes chimiques à tropisme hépatique (7), le taux d'arginase du foie décroît très rapidement quand la durée du traitement s'accroît. Il faut noter enfin, que parmi les animaux traités du 4e lot, deux sont morts avant la date prévue pour le sacrifice ; l'autopsie a révélé des lésions d'apparence nécrotique sur le lobe médian du foie avec des nodules punctiformes généralisés.

En conclusion, la tétrabromo-2,3,7,8 dibenzo-*p*-dioxine provoque, même à des doses très faibles, des modifications de l'équipement enzymatique de l'hépatocyte tout à fait semblables, en qualité et en intensité, à celles provoquées par la « dioxine ». Ces résultats sont à rapprocher de ceux obtenus par Kende et coll. (8) qui ont montré que les composés *1* et *2* avaient une activité équivalente dans l'induction de l'arylhydroxylase chez l'embryon de poussin. Ils apportent un argument supplémentaire à l'idée de l'existence de relations très étroites entre les propriétés biologiques de la « dioxine » et le modèle parfaitement symétrique de sa géométrie moléculaire.

(*) Séance du 5 mai 1975.

(1) Cf. entre autres : J. Verrett, *in* : Effects of 2,4,5-T on Man and the Environment, Hearings before the Subcommittee on Energy, Natural Resources, and The Environment of the Committee on Commerce (U. S. Senate), April 7 and 15, 1970, U. S. Government Printing Office, Washington D. C., 1970, p. 190 ; N. P. Buu-Hoï, Pham-Huu-Chanh, G. Siesque, M. C. Azum-Gelade et G. Saint-Ruf, *Die Naturwissenschaften*, 59, 1973, p. 173 ; B. A. Schwetz, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson et C. G. Gerbig, *Adv. Chem. Ser.*, 120, 1973, p. 55.

(2) *Chlorodioxins Origin and Fate*, Advances in Chemistry Series 120, E. H. Blair, American Chemical Society, Washington DC, 1973.

(3) N. P. Buu-Hoï, D. P. Hien, G. Saint-Ruf et J. Sevoin-Sidonie, *Comptes rendus*, 272, Série D, 1971, p. 1447.

(4) R. Truhaut, Pham-Huu-Chanh, G. van Haverbeck, M. C. Azum-Gelade, G. Saint-Ruf et L. Lareng, *Comptes rendus*, 279, Série D, 1974, p. 1565.

(5) H. Gilman et J. J. Dietrich, *J. Amer. Chem. Soc.*, 79, 1957, p. 1439.

(6) H. Nguyen-Huy, R. Bockel, G. Peruhner et A. Stahl, *Ann. Biol. Clin.*, 30, 1972, p. 309.

(7) S. Sato, *Tohoku Igaku Zasshi*, 67, 1963, p. 597 ; C. Wu et H. R. Euriel, *Cancer Research*, 27, 1967, p. 956 ; E. E. Elliason, *Exp. Cell. Res.*, 48, 1967, p. 1.

(8) A. S. Kende, J. J. Bergman et G. H. Haas, *Can. J. Chem.*, 49, 1971, p. 583.

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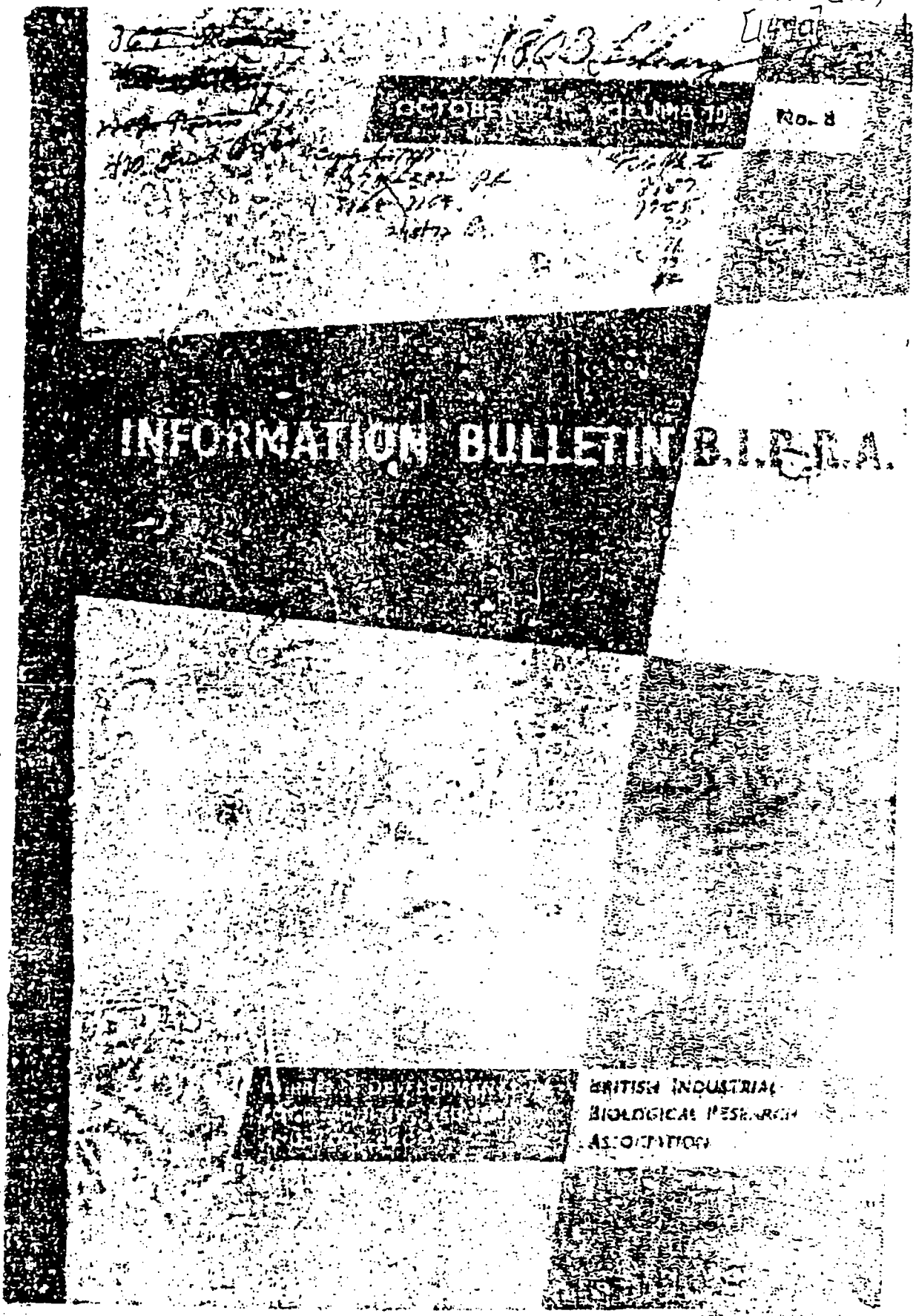
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2265. CONTRASTING STUDIES ON 2,4-D

Berwick, P. (1970). 2,4-Dichlorophenoxyacetic acid poisoning in man. Some interesting clinical and laboratory findings. J. Am. med. Ass. 214, 1114.

Kolberg, J., Helgeland, Kristen, Jonsen, J. & Tjeltveit, O. (1971). The herbicide 2,4-dichlorophenoxyacetic acid. I: Effects on L cells. Acta pharmac. tox. 29, 81.

Accidental human poisoning by 2,4-dichlorophenoxyacetic acid (2,4-D) is not common, despite the compound's widespread use as a selective weed killer. There have, however, been reports of peripheral neuropathy following dermal exposure. The first paper cited above describes the clinical and laboratory findings in a farmer who swallowed a mouthful of concentrated weed killer thinking it was iced tea! It was estimated that the preparation actually swallowed contained 7.2 g 2,4-D, 14.7 g S-ethyl dipropylthiocarbamate and 0.15 g epichlorohydrin. Any involvement by the carbamate was ruled out by a lack of cholinesterase inhibition and the intake of epichlorohydrin was considered insufficient to be of toxicological significance. Hence the effects seen were attributed to 2,4-D. These consisted of signs of acute gastritis with frequent vomiting, fibrillary twitching, muscle spasm and weakness, paralysis of the intercostal muscles causing laboured breathing, considerably elevated serum levels of lactic dehydrogenase, glutamic-oxalacetic and glutamic-pyruvic transaminases, aldolase and creatine phosphokinase indicative of generalized skeletal muscle damage and the presence of oxymyoglobin in the urine. The latter finding has apparently not been reported in previous cases of 2,4-D poisoning, although it is not surprising in view of the other indications of muscle damage. There was no evidence of cardiac involvement in this case.

The second paper cited above describes the effect of 2,4-D (50-500 µg/ml) on the growth and morphology of mouse fibroblasts (strain L 929) in monolayer cell cultures. Growth inhibition occurred at all levels in a concentration-dependent manner, starting 1 day after incubation. Complete inhibition occurred with the 350 and 500 µg/ml levels of 2,4-D. Withdrawal of 2,4-D even after 12 days' exposure to 500 µg/ml led to normal resumption of cell multiplication. Lipid-containing cytoplasmic vacuoles were observed with concentrations of 250-500 µg/ml. These appeared earlier and in greater numbers with the highest concentrations, but disappeared when incubation was continued for 2-3 days. The presence of these vacuoles was considered not to be necessarily indicative of degeneration. Study of the mode of action of 2,4-D on mammalian cells assumed a special significance in view of the suggested teratogenicity of this compound (Science, New York 1969, 166, 977). However, a study published recently provided no evidence of teratogenic activity in rats, although signs of embryotoxicity and foetotoxicity, including a decrease in foetal weight, subcutaneous oedema, delayed ossification and lumbar and wavy ribs, were observed at high (50-87.5 mg/kg) doses (BIBRA Bull. 1971, 10, xlviii).

Selective Herbicides and Growth Substances. Pathologic Effects on Man During the Manufacture of the Ester of 2,4-D. M. Assouly. Arch. Mal. Profess. 12, 26-30, (1951). (French).

Workers employed in the manufacture of an ester of 2,4-dichloro phenoxyacetic acid complain of somnolescence with heaviness of the legs, irritation of the upper respiratory passages, gastralgia with loss of appetite, of a sweet taste in the mouth with increased salivation, a sensation of drunkenness, and hypersensitivity of hearing, the least sound causing them to jump. In animal experimentation it was shown that when 2,4-D was ingested or injected intravenously, high doses were required to produce intoxication.

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SUSPECTED POISONING OF DOGS FROM EATING GRASSES TREATED WITH 2,4-D

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Frequently dogs are admitted to the veterinary clinic with the history that the owner feels that the animal has been poisoned from the effects of having consumed grass treated with 2,4-D. It has been commonly observed that dogs, on occasion, will eat various quantities of grass. We were unable to find any information in the literature concerning field cases of the toxicity of 2,4-D-treated grasses in dogs. Hill and Carlisle[1] have noted that, by intravenous daily administration of from 25 to 200 mg. of 2,4-D per Kg. of body weight, effects ranging from subacute intoxications to death may be produced. They further noted that dogs injected with 2,4-D showed a considerable susceptibility to the development of liver damage. Other work concerning 2,4-D poisoning in various animals has been done by other workers.[2-6] Since there is so little information available on 2,4-D poisoning in dogs, in which the 2,4-D was consumed on treated grasses or administered per os, the following trials were conducted.

Individual, heavy stands of lush growing bluegrass, orchard grass, timothy, and quack grass were sprayed with the water emulsion ester (butyl ester 4 lb./gal.) form of 2,4-D, at the rate of 4 pounds per acre. These are the most common grasses that dogs are most likely to eat. The spraying was at least twice as heavy as ordinarily recommended and sufficient to cause foliar damage to the grasses. The spraying was done on June 18, 1952, and the grasses were harvested in four separate lots (112 Gm. each) 48 hours later, on June 20, 1952. These grasses were chopped in small pieces and mixed in proportionately equal amounts with fresh horsemeat and dog meal. This mixture was fed to two healthy one-year-old mongrel dogs, weighing 20 to 25 pounds each, in equal amounts, in three consecutive feedings, two on June 20 at morning and evening meals, and the last one at the morning meal on June 21. Apparently the palatability of the food was not altered appreciably, as the dogs immediately consumed all of the food. The dogs were observed but no change in attitude or signs of any ill effects could be detected at any time within 96 hours following the first meal.

On June 24, both animals appeared to be in excellent health, and a subsequent trial was instigated. Each animal was given 5 cc. of the same ester concentrate of 2,4-D orally in a gelatin capsule. This dosage represents approximately 500 mg. of 2,4-D per Kg. It is noteworthy that this was a single dose given per os. No clinical deleterious effects were observed at any time within 96 hours following this oral administration.

On June 28, one dog was sacrificed. Necropsy examination of this animal failed to reveal any macroscopic lesions. The other animal, under daily observation, remained in apparently excellent health for the following 82 days, at which time these experiments were considered concluded.

We have not arrived at any definite conclusions with this meager information. However, in most instances of suspected 2,4-D poisoning entered at this clinic, some other cause has been definitely established. We are not cognizant of a proved field case of 2,4-D poisoning in dogs having occurred in this area.

Dr. Baker is associate professor of veterinary medicine, Dr. Ramsey is associate professor of veterinary pathology in the Division of Veterinary Medicine and Dr. Sylwester is extension professor of botany and plant pathology.

References

1. Hill, E. V., and Carlisle, H.: Ind. Hyg. & Toxicol. 29 (1947) 85-95.

2. Ennis, W. B.; Thompson, H. E., and Smith, H. H.: Tributyl phosphate as a solvent for preparing concentrated and oil miscible solutions of 2,4-D acid and similar substances. Science 103 (1946) 476.

3. Grigsby, B. H., and Curlson, R. F.: Some effects of herbicides on pasture and grazing livestock. Mich. Agr. Exper. Sta. Quar. Bull. 32 (1950) 378.

4. Hildebrand, E. M.: War on weeds. Science 103 (1946) 465.

5. Hildebrand, E. M.: Herbicidal action of 2,4-D on the water hyacinth (*Eichornia crassipes*) Science 103 (1946) 477.

6. Smith, F. G.; Hammer, C. L., and Carlson, R. F.: Control of ragweed pollen with 2,4-D. Science 103 (1946) 473.

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brought up to date the recommendation of the WHO Techn. Rep. Ser. No. 114 [this *Bulletin*, 1957, v. 32, 262]. Little can be said yet whether any special precautions are necessary concerning the inhabitants of treated dwellings. It is emphasized that the Authority carrying out public health insecticide programmes must take care by legislation and education to safeguard against accidental poisoning, to ensure the proper labelling of the containers and their safe disposal after use.

It was concluded that DDT should remain the insecticide of choice on the grounds of safety and that other insecticides should be used only when absolutely necessary. BHC is acceptable but dieldrin appeared too toxic to be used safely in programmes of this type. Malathion was considered a safe substitute for DDT.

H. B. Stoner

WASSERMANN, M., MIHAIL, G., VANCEA, G., MANDRIC, G., ILIESCU, S., RAILEANU, I., SAVA, V., IOSUBAS, S. & NESTOR, L. Recherches sur les conditions de milieu et sur la pathologie professionnelle des desanophélisateurs. L'intoxication chronique par l'hexachlorocyclohexane (H.C.H.). IIIe Note. Corrélation entre les troubles cliniques, chronaximétriques et biochimiques. [**Studies on the Occupational Hazards of Anti-Mosquito Sprayers. Chronic Intoxication by BHC. III. Correlation between Clinical Manifestations, Chronaxie Measurements and Biochemical Changes**] *Arch. Mal. Professionnelles.* Paris. 1962, Jan.–Feb., v. 23, Nos. 1/2, 18–31. [22 refs.]

The report follows up previous observations [this *Bulletin*, 1962, v. 37, 25] on the health of about 150 operators applying BHC in mosquito control programmes in Rumania. While in some operators new symptoms had appeared, in none of them had previous complaints disappeared or regressed.

Details of chronaxie measurements are recorded. In 38·5% chronaxie was abnormal and clinical signs were present but in 9·5% chronaxie was abnormal in the absence of symptoms.

Among the operators blood catalase activity was depressed on an average by 28% and plasma cholinesterase by 14%. There is a long discussion of these findings.

[No clear picture of the condition of these workers emerges. Polyneuritis is described as a common condition, yet the victims appear to have gone on working year after year. Unfortunately no search appears to have been made for BHC metabolites in the urine as an index of absorption.] *J. M. Barnes*

MUTALIK, G. S., WADIA, R. S. & PAI, V. R. **Poisoning by Diazinon, an Organophosphorous Insecticide.** *J. Indian Med. Ass.* 1962, Jan. 16, v. 38, No. 2, 67–71. [15 refs.]

The clinical features of 25 cases of poisoning by diazinon are summarized. All the patients had swallowed a commercially available liquid preparation [illegible] were aged 15–24 years and 4 were infants [illegible] suggestion that any of the victims were poisoned as result of an occupational exposure to this insecticide. Vomiting, abdominal cramps, stupor and restlessness were the commonest symptoms. Hypertension was noted in 12 patients and of the 12 showing pupillary changes 5 showed constriction and 7 dilatation. There were 2 fatalities.

The treatment given was atropine, gastric lavage with 2% potassium permanganate and oxygen bubbled through alcohol.

[The safety of diazinon compared with parathion when swallowed is underlined by the low mortality in this series.] *J. M. Barnes*

T-CN

VAN RAALTE, H. L'intoxication par les insecticides organophosphorés, notamment par le Phosdrin. [**Poisoning by Organophosphorus Insecticides especially Phosdrin**] *Arch. Mal. Professionnelles.* Paris. 1962, Mar., v. 23, No. 3, 132–7.

A comparison is made between phosdrin and parathion which are both organophosphorus compounds used as insecticides. Phosdrin is soluble in both water and lipids, making it rapidly transported in the body after absorption. It is a direct inhibitor of cholinesterase and reacts to form the labile di-methoxy phosphorylated cholinesterase. For these reasons phosdrin poisoning in contrast to parathion poisoning comes on rapidly after exposure, soon reaches its crisis and recovery is correspondingly fast. Atropine must be given immediately poisoning is suspected and the need for oxime therapy in human poisoning has not yet arisen.

J. M. Barnes

T-CN-2Y-D

DÉSI, I., SÓS, J., OLASZ, Júlia, SÜLE, F. & MÁRKUS, Vera. Vegyi gyomirtószer által okozott mezogazdasági munkaártalom lehetösége. [**Harmful Effects on Agricultural Workers caused by Chemical Weed-Killers**] *Egészségtudomány.* Budapest. 1962, v. 6, No. 1, 43–51, 8 figs. [16 refs.]

The English summary appended to the paper is as follows:—

"Authors have observed that the parenteral administration of 2,4-dichlorophenoxyacetic acid (2,4-D) weed-killer has caused a reversible inhibition of the cerebral electric functions in acute animal experiments while this inhibition has been of increasing tendency in chronic experiments. Toxic EEG symptoms are present. According to the conditioned reflex experiments the higher nervous functions are seriously damaged. The changes may be most likely brought about by the 2,4-D molecule itself and not by any decomposition product. In our opinion the attack might be localised in the formatio reticularis. The changes observed in the higher nerve functions as well as in the central nervous system are to be seen within a very short time, yet within 24 hours. Other pathogenic symptoms in the experimental animals

are to be seen on the *3rd* or *4th* day following the experiment only.

"No pathogenic histological changes were observed in the brain, demyelinisation was found in the spinal marrow, pyramidal track and in the Goll and Burdach area.

"All the changes observed in animal experiments lead to the conclusion that increased protection and specific neurological examination of workers is needed and increased care should be taken in using 2,4-D."

GAROFANO, M. & MOLINA, V. Indagine sulla probabile dose alle gonadi ricevuta dalla popolazione di Pavia per effetto degli esami roentgendiagnostici. [**The Dose of Radiation to the Gonads received by the Population of Pavia in the Course of Diagnostic X-Ray Examinations**] *Riv. Italiana d'Igiene.* 1961, Sept.-Oct., v. 21, No. 5, 420–26. [13 refs.]

The English summary appended to the paper is as follows:—

"A research has been made about the quantity of radiations to the gonads received by the population of Pavia during 1959 in consequence of roentgen-diagnostic examinations.

"The research has shown a quantity of mrem 59,39 per head to the gonads, without regard to age and fertility."

SCHULZ, K. H. Untersuchungen über die sensibilisierende Wirkung von Inhaltsstoffen exotischer Hölzer. [**Investigations concerning the Sensitizing Effect of the Components of Tropical Woods**] *Berufsdermatosen.* Aulendorf. 1962, Feb., v. 10, No. 1, 17–27.

This paper is extracted from Volume IV of the Monograph Series of the journal *Berufsdermatosen.* This volume is concerned with the relations between chemical structure and the allergenic action of compounds of low molecular weight.

In the last few decades timber from the tropics has come to be imported into Europe in no inconsiderable amount. Tropical woods have certain technical advantages over European woods but it has been found that workers, particularly those exposed to fine wood dust, tend to suffer from acute irritation of the mucous membranes of the upper respiratory tract, mouth and eyes. Disturbances of the digestive system have also been described in these workers as well as allergic contact eczemas. The skin effects have been found to be localized mainly on the face, forearms and elbows, neck and genital region.

There is mention of a considerable number of tropical woods which appear to have been the cause of illness in between 30 and 40 workers. Of these woods 3 have been investigated in considerable detail, particularly to discover the chemical substances contained in them and to determine by skin tests on the workers whether these substances are sensitizing. The chemical findings in examination of the 3 timbers are described in detail and are summarized as follows.

(1) Kambala (*Chlorophora excelsa*) [this *Bulletin*, 1958, v. 33, 965] contained the substance chlorophorine which gave positive cutaneous reactions in 7 joiners who had worked with this wood and suffered from contact eczema.

(2) *Mansonia* (*Mansonia altissima*) yielded 4 different chemical compounds of which benzoquinone was one and was found to give a positive allergic contact reaction.

(3) Teak (*Tectona grandis*) yielded a sensitizing substance lapachole, a derivative of naphthoquinone, but it is probable that other sensitizing components are also present. *M. E. Delaficld*

SAMITZ, M. H., GROSS, S. & KATZ, S. **Inactivation of Chromium Ion in Allergic Eczematous Dermatitis.** *J. Investigative Dermat.* 1962, Jan., v. 38, No. 1, 5–12, 6 figs.

Illustrations of a number of patch tests demonstrate that allergic reactions were prevented in 2 subjects sensitive to chromate. This was achieved by means of a reagent designed to reduce hexavalent Cr to trivalent with formation of a chelate complex. It had been confirmed in 12 patients with dichromate sensitivity that patch tests with trivalent Cr compounds, the nitrate and basic sulphate, were negative, whereas with the hexavalent compound they were positive.

The reagent contained sodium pyrosulphite as reducing agent and tartaric acid as chelating agent, with glucose and ammonium chloride, parts by weight, 2, 1, 1 and 1. It was used in 10% aqueous solution and ointments. Water, carbowax and hydrophilic ointment were used as controls.

Prevention is illustrated by 9 patch tests on the back. In each of the 2 subjects, after 48 hours, 4 tests were positive and 5 negative, with results unchanged after 5 days: positive, from 0·25% potassium dichromate alone and applied over each control; negative, from the dichromate applied over both 10% ointments and over linen soaked in the 10% solution; negative from 0·25% chromium nitrate and 0·2% basic chromium sulphate alone.

The effect of applications after dichromate contact is illustrated by 5 columns of tests, 34 in each subject. Contact for 15 minutes or less with 0·25% will elicit the eczematous reaction in such subjects. The ointment blocked the reaction after 15 and also 30 minutes' contact; the control did so slightly after 15, and the 10% solution, water and also 1% sodium lauryl sulphate solution did so if applied within 15 minutes of contact. Otherwise all were ineffective after longer contact. The earliest clinical manifestation of eczematous reaction was after 6 hours.

The findings suggest a practical approach to the prophylactic management of chromate dermatitis, and a study is being made under working conditions.

[See also this *Bulletin*, 1959, v. 34, 463.]

M. Patricia Fitzsimons

RODIER, J. Contributions à l'étude de la pathologie du tétryl. [**Study of the Pathological Effects of Tetryl**] *Arch. Malad. Professionnelles.* Paris. 1962, Mar., v. 23, No. 3, 151–60.

Tetryl is generally produced by nitration and partial

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

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ABSTRACT

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

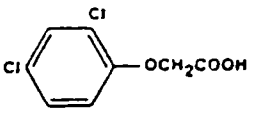
2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

INTRODUCTION

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

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

0048-9697/83/$03.00



2, 4-D

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

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

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

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

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

FDA 2,4-D RAT STUDY

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

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

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

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

[a] Some rats had both carcinoma and sarcoma and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

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

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

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

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

Malignant neoplasms at all sites in male rats

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

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

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

[a] Test for positive trend.

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

Sarcomas in male rats

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

Carcinomas in male rats

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

Malignant neoplasms at all sites in female rats

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

Lesions of the lymphoreticular system in female rats

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

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

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

[a] Some rats had both carcinomas and sarcomas and are counted only once.
[b] Test for positive trend.
[c] Departure from linear trend.

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

Carcinomas at all sites in female rats

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

Neoplasms of the mammary gland in female rats

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

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

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

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

[a] Test for positive trend.
[b] Departure from linear trend.

TABLE 5

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

Dose (ppm)	Gross[b]	p	Histologic[c]	p	Histologic[d]	p
0	10/22 (45%)		10/22 (45%)		8/22 (36%)	
5	8/20 (40%)		7/20 (35%)		7/20 (35%)	
25	13/21 (86%)	0.0064	11/21 (52%)		11/21 (50%)	
125	8/23 (35%)		10/23 (43%)		9/23 (39%)	
625	20/24 (83%)	0.0080	14/24 (58%)		15/24 (63%)	0.070
1250	16/23 (70%)	0.091	15/23 (65%)		11/23 (45%)	
5—1250	70/111 (63%)		57/111 (52%)		53/111 (47%)	
		0.0233[e]		0.0334[e]		
		0.0008[f]				

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

Comments

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

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

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

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

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

Summary

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

Conclusions

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

FDA 2,4-D DOG STUDY

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

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

Comments

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

Summary

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

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

1. 2,4-D

The maximum tolerated dose of 2,4-D was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" mice [4, 7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. 46.4 mg/kg was given in 0.5% gelatin daily by stomach tube beginning at 7 days of age. After the mice were weaned at 28 days of age, 149 ppm of 2,4-D was mixed directly in the diet and provided ad libitum. "Strain B" male and female mice were also given 100 mg/kg, followed by 323 ppm. Treatment was continued approximately 18 months.

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

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

2. 2,4-D isopropyl ester

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

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

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

TABLE 6
NUMBER OF MALE AND FEMALE MICE INGESTING 2,4-D ISOPROPYL ESTER WITH NEOPLASMS OF THE LUNG (INNES et al. [4, 7], MOUSE STUDY)

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

3. 2,4-D butyl ester

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

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

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

4. 2,4-D isooctyl ester

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

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

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

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

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

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

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

A single subcutaneous injection of 2,4-D, or its esters, was given in the nape of the neck to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1 designated as "strain A" and (C57BL/6 x AKR)F_1 designated as "strain B" at approximately the 28th day of age [7]. There were 18 treated mice and 18 untreated controls of each strain and each sex. They were killed after approximately 18 months.

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

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

Reticulum cell sarcomas in "strain B" female mice

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

Summary

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

ARCHIPOV AND KOZLOVA MOUSE AND RAT STUDIES

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

TABLE 8

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

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

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

Neoplasms of the skin

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

Comments

Groups of rats also ingested 1/10th the LD_{50} of the amine salt of 2,4-D [5]. The dose was not given.

Summary

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

BOUTWELL AND BOSCH 2,4-DICHLOROPHENOL MOUSE SKIN STUDY

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

Neoplasms of the skin

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

Conclusion

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

ACKNOWLEDGEMENT

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REFERENCES

1 W. H. Hansen, R. T. Haberman and O. G. Fitzhugh, Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs, Toxicol. Appl. Pharm., 20 (1971) 122—192.
2 T. Haberman, FDA Memorandum entitled: Pathological Changes in Dogs Fed 2,4-Dichlorophenoxy Acid for Two Years, A. J. Lehman dated November 5, 1963.
3 T. Haberman, FDA Memorandum entitled: Pathological Changes in Rats Fed 2,4-Dichlorophenoxy Acid for Two Years, A. J. Lehman dated March 20, 1964.
4 J. R. M. Innes, B. M. Ulland, M. G. Valerio, L. Petrucelli, L. Fishbein, E. R. Hart, A. J. Pallota, R. R. Bates, H. L. Falk, J. J. Gart, G. M. Klein, I. Mitchell and J. Peters, Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note, J. Natl. Cancer Inst., 42 (1969) 1101—1114.
5 G. N. Archipov and I. N. Koziova, Study of the carcinogenicity properties of the herbicide amine salt of 2,4-D, Voprosy Pitaniya, 5 (1974) 83—84 (English translation from Russian).

6 R. K. Boutwell and D. K. Bosch, The tumor-promoting action of phenol and related compounds for mouse skin, Cancer Res., 19 (1959) 413—424.

7 Bionetics Research Labs, Inc.: Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals, Vol. 1 Carcinogenicity, National Technical Information Service, U.S. Department of Commerce, 1968.

8 M. D. Reuber, Preliminary review of some oncogenicity studies for 2,4-dichlorophenoxyacetic acid, -Appendix of the Environmental Protection Agency and the Regulation of Pesticides, U.S. Government printing office, Washington, DC, 1977, pp. 471—476.

9 M. D. Reuber, Review of toxicity test results submitted in support of pesticide tolerance petitions, Sci. Total Environ., 9 (1977) 135—148.

10 M. D. Reuber, Review of additional toxicity test results submitted in support of pesticide tolerance petitions. Appendix of the Environmental Protection Agency and the Regulation of Pesticides, U.S. Government printing office, Washington, DC, 1977, pp. 460—470.

11 M. D. Reuber, Carcinogenicity testing of chemicals with particular reference to organochlorine pesticides, Sci. Total Environ., 10 (1978) 105—115.

12 R. W. Shearer, Personal communication (1979).

13 B. A. Schwetz, G. L. Sparschu and P. J. Gehring, The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development, Food. Cosmet. Toxicol., 9 (1971) 801—817.

14 T. K. Konstantinova, L. P. Ephimenko and T. A. Antonenko, The embryotropic effect of the dissociation products of herbicides based on 2,4-D, Gigiena I. Sanitariya, 11 (1976) 102—105 (English translation from Polish).

15 K. S. Khera and W. P. McKinley, Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats, Toxicol. Appl. Pharmacol., 22 (1972) 14—26.

16 T. F. X. Collins and C. H. Williams, Teratogenic studies with 2,4,5-T and 2,4-D in the hamster, Environ. Contam. Toxicol., 6 (1971) 559—567.

17 D. L. Courtney, Prenatal effects of herbicides: Evaluation by the prenatal development index, Arch. Environ. Contam. Toxicol., 6 (1977) 33—46.

18 Z. A. Alekaashina, S. Y. Buslovich and V. M. Koloeovskaya, Embryotoxic action of the diethylamine salt of 2,4-D, Gigiena I. Sanitariya, 2 (1973) 100—101 (English translation from Polish).

19 L. M. Fedorova and R. S. Belova, 2,4-D detected in reproductive organs and fetuses of treated pregnant rats, ways and dynamics of its removal, (English translation from Russian).

20 S. V. Basrur, R. A. Fletcher and P. K. Basrur, In vitro effects of 2,4-dichlorophenoxy acetic acid (2,4-D) on bovine cells, Can. J. Comp. Med., 40 (1976) 410—415.

21 R. W. Hart, S. Hays, D. Brash, F. B. Daniel, M. T. Davis and N. J. Lewis, In vitro assessment and mechanism of action of environmental pollutants, Ann. N. Y. Acad. Sci., 298 (1977) 141—158.

22 F. E. Ahmed, R. W. Hart and N. J. Lewis, Pesticide-induced DNA damage and its repair in cultured human cells, Mutat. Res., 42 (1977) 161—174.

23 J. C. Hadder, Endothal induced mutations in droeophila melanogaster, Trans. Ill. State Acad. Sci., 63 (1970) 157—159.

24 D. F. Paris and D. L. Lewis, Chemical and microbial degradation of ten selected pesticides in aquatic systems, Residue Rev., 46 (1973) 107.

25 J. D. Kohli, R. N. Khanna, B. N. Gupta, M. M. Dhar, J. S. Tandon and K. P. Sircar, Absorption and excretion of 2,4-dichlorophenoxyacetic acid in man, Xenobiotica, 4 (1974) 97—100.

26 S. Khana and S. C. Fang, Metabolism of C^{14}-labeled 2,4-dichlorophenoxyacetic acid in rats, J. Agr. Food Chem., 14 (1966) 500—503.

27 K. Erne, Distribution and elimination of chlorinated phenoxyacetic acid in animals, Acta Vet. Scand., 7 (1966) 240—256.

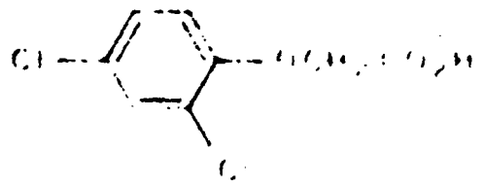
28 M. W. Sauerhoff, W. H. Braun, G. E. Blau and P. J. Gehring, The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man, Toxicology, 8 (1977) 3—11.
29 J. A. Burton, T. H. Gardiner and L. S. Schanker, Absorption of herbicides from the rat lung, Arch. Environ. Health, 29 (1974) 31—33.
30 A. D. Radionov, A. N. Chumachemko and I. I. Kirilenko, The toxic properties of the herbicide 2,4-D, Hyg. Sanit., 32 (1967) 116—118 (English translation from Russian).
31 N. P. Goldstein, P. H. Jones and J. R. Brown, Peripheral neuropathy after exposure to an ester of dichlorophenoxyacetic acid, J. Amer. Med. Assoc., 171 (1959) 1306—1309.
32 M. C. Berkley and K. R. Magee, Neuropathy following exposure to a dimethylamine salt of 2,4-D, Arch. Int. Med., 111 (1963) 351—352.
33 A. W. Dudley, Jr. and N. T. Thapar, Fatal human ingestion of 2,4-D, a common herbicide, Arch. Pathol., 94 (1972) 270—275.
34 L. Hardell and A. Sandstrom, Case-control study: Soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols, Br. J. Cancer, 39 (1979) 711—717.
35 M. Eriksson, L. Hardell, N. O. Berg, T. Moller and O. Axelson, Case control study on malignant mesenchymal tumors of the soft-tissue and exposure to chemical substances, Lakartidningen, 76 (1979) 3872—3875.
36 L. Hardell, M. Eriksson and P. Lenner, Malignant lymphoma and exposure to chemical substances especially organic solvents, chlorophenols and phenoxy acids, Lakartidningen, 77 (1980) 208—210.

AU - Kolny H
AU - Kita K
AA - Z II Oddzia:u Chor:ob Wewn:etrznych Szpitala Miejskiego w Pszczynie.
TI - [2,4-D poisoning]
SI - TOXBIB/78/155742
SO - Med Pr; VOL 29, ISS 1, 1978, P61-3
LA - Pol
AB - The paper presents 2 cases of intoxication with herbicides--chlorophenoxyacetic acid derivatives. In the cases observed, the following symptoms were shown in the clinical picture; general weakness, dizziness, headache, abdominal pains, nausea. Clinical observation revealed changes in blood circulation with pathological changes in EKG and transitory reduction of RR. We also found some changes in laboratory examination, indicating noxious effects of chlorophenoxyacetic acid derivatives upon parenchymatous organs.

K-2372-(109) 1976

[illegible] mg/kg) administered [illegible] (133-06-2) was rapidly metabolized and excreted in [illegible] of oral and ip I being excreted within 9 hr and [illegible] The urinary distribution of oral I was urine (51.5%), expired air (29.4%), feces (15.5%), and tissues (0.6%). Three urinary metabolites of oral I were identified including *thiazolidine-2-thione-4-carboxylic acid* (II) [2933-67-9] (18.6%); II was the only metabolite of ip I detected. Metab. of I via thiophosgene and thiophosgene detoxication are discussed.

73107x Absorption and excretion of 2,4-dichl[redacted]henoxyacetic acid in man. Kohli, J. D.; Khanna, R. N.; [redacted] U. N.; Dhar, M. M.; Tandon, J. S.; Sircar, K. P. (Ind[redacted]al Res. Cent., Lucknow, India). *Xenobiotica* 1974, [illegible](2): [illegible] 100 (Eng).



2,4-D (I) [94-75-7] (5 mg/kg, orally) was quickly absorbed in

r man, being detectable in the plasma 1 hr after ingestion. Within
96 hr of administration, 75% of the I dose was excreted
unchanged in urine, suggesting that cumulative toxicity of I is
unlikely.

[redacted]73108y Interaction between toxicant and [redacted]

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R & D REPORT

J.W. Henck 12/31/79

DOW CHEMICAL U.S.A.

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DATE ISSUED: 12/19/79

DEPARTMENT: TOXICOLOGY RESEARCH LABORATORY

LAB. NO.:

PROBLEM NO.:

TITLE: ACNEGENIC POTENTIAL OF 2,4-DICHLOROPHENOXY ACETIC ACID SAMPLES

15 PAGES IN FULL REPORT

AUTHOR(S): J. W. Henck, K. S. Rao and G. C. Jersey

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REVIEWER'S SIGNATURE: K. J. Olson 12/24/79

This report is: ☐ INTERIM ☐ FINAL and mainly: ☐ NEW ☐ REVIEW

DATA REFERENCES (book and page):

(Refer also to earlier related reports and publications.)

PATENT STATUS: ☐ disclosure submitted ☐ case filed ☐ no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

Eight samples related to 2,4-dichlorophenoxy acetic acid (2,4-D) were submitted to the Toxicology Research Laboratory for testing of chloracnegenic potential. These samples were identified as follows: Sample A-recrystallized 2,4-D acid; Sample B - 2,4-D acid, 948 Building (made by the "new" process); Sample C - 2,4-D acid, 489 Building (made by the "old" process); Sample D - E6E (the isooctyl alcohol ester of 2,4-D made by the acid-ester process in December, 1978); Sample E - E99C (the DOWANOL* PiB glycol ether product ester made primarily by the direct ester process from 8/4/78 to 8/30/78); Sample F-DMA-4 (dimethylamine salt of 2,4-D from 948 Building, 7/13/78 to 7/30/78); Sample G - DMA-4 (dimethylamine salt of 2,4-D from 489 Building, 10/75); and Sample H - a perchloroethylen recycle stream.

For the rabbit ear bioassay, the test material was applied to the internal aspect of the left external ear of each New Zealand albino rabbit (Langshaw Farms, Augusta, Michigan) 5 days/week for a total of 19 or 20 applications. The right ear of each rabbit remained untreated to serve as a control. Samples A, B, C and H were applied as 10% solutions in 95% ethanol, while Samples D, E, F, and G were applied undiluted. One additional rabbit received 19 applications of 95% ethanol to serve as a control. The appearance of the test ear of each rabbit was observed once weekly, and the degree of hyperemia, edema, exfoliation, hyperplasia, folliculitis, necrosis, and scab formation was recorded. At the end of the test each rabbit was subjected to a gross pathological examination, with histopathology being performed on the ears of all rabbits on test.

Severe topical responses were not observed in any of the rabbits on test. None of the test materials evaluated in this study elicited an acnegenic or comedogenic response. Therefore, none of the materials tested should be considered potential human chloracnegens.

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18741

ACNEGENIC POTENTIAL OF 2,4-DICHLOROPHENOXY ACETIC ACID SAMPLES

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SUMMARY

Eight samples related to 2,4-dichlorophenoxy acetic acid (2,4-D) were submitted to the Toxicology Research Laboratory for testing of chloroacnegenic potential. These samples were identified as follows: Sample A - recrystallized 2,4-D acid; Sample B - 2,4-D acid, 948 Building (made by the "new" process); Sample C - 2,4-D acid, 489 Building (made by the "old" process); Sample D - E6E (the isooctyl alcohol ester of 2,4-D made by the acid-ester process in December, 1978); Sample E - E99C (the DOWANOL* PiB glycol ether product ester made primarily by the direct ester process from 8/4/78 to 8/30/78); Sample F - DMA-4 (dimethylamine salt of 2,4-D from 948 Building, 7/13/78 to 7/30/78); Sample G - DMA-4 (dimethylamine salt of 2,4-D from 489 Building, 10/75); and Sample H- a perchloroethylene recycle stream.

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to a gross pathological examination, with histopathology being performed on the ears of all rabbits on test.

Severe topical responses were not observed in any of the rabbits on test. None of the test materials evaluated in this study elicited an acnegenic or comedogenic response. Therefore, none of the materials tested should be considered potential human chloracnegens.

INTRODUCTION

Eight samples related to 2,4-dichlorophenoxy acetic acid (2,4-D) were submitted to the Toxicology Research Laboratory for testing of chloroacnegenic potential. The Dow Chemical Company has used 2 different processes to produce 2,4-D acid. The "new" process is conducted in 948 Building, and may lead to the generation of impurities in 2,4-D acid such as 2,4,5,7-tetrachloroxanthone (a known chloracnegen). The "old" process, which was conducted in 489 Building but is no longer in operation, generated no impurities. Similarly, the esters of 2,4-D acid are also made by 2 processes: the direct-ester process, which may generate some impurities, and the acid-ester process, which does not generate impurities. The following study was initiated to determine if 2,4-D or samples related to it were chloracnegenic, and also if the different processes by which these chemicals are made resulted in a difference in chloracnegenic potential.

TEST ANIMALS

Rabbit ear bioassay tests were conducted on male and female New Zealand albino rabbits (Langshaw Farms, Augusta, Michigan). All rabbits were maintained on a 12-hour light and dark cycle in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care and were supplied with commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri) and water ad libitum. All rabbits were acclimated to the laboratory environment at least 2 weeks prior to testing, and were housed singly.

TEST MATERIALS

To determine if the known chloracnegen 2,4,5,7-tetrachloroxanthone was present in any of the samples tested, each was analyzed for this impurity. The results of this analysis, as well as sample name and identification, follow:

Sample	Identification	2,4,5,7-Tetrachloroxanthone Content
A	Recrystallized 2,4-D acid	Not detectable[a,e]
B	2,4-D acid, 948 Building (made by the "new" process)	20-25 ppm[e]
C	2,4-D acid, 489 Building (made by the "old" process)	Not detectable[c,d]
D	E6E, the isooctyl alcohol ester of 2,4-D (made by the acid-ester process, December, 1978)	16±2 ppm[d]
E	E99C, the DOWANOL* PiB glycol ether product ester (made primarily by the direct ester process, from 8/14/78 to 8/30/78)	Not detectable[b,d]
F	DMA-4, dimethylamine salt of 2,4-D (948 Building, 7/13/78 to 7/30/78)	20±5 ppm[d]
G	DMA-4, dimethylamine salt of 2,4-D (489 Building, 10/75)	Not detectable[c,d]
H	Perchloroethylene recycle stream	20±1 ppm[d]

a) Limit of detection: 5 ppm
b) Limit of detection: 2 ppm
c) Limit of detection: 1 ppm
d) Wisniewski, D. F., Midland Analytical Laboratories report 79-00676, March 15, 1979.
e) Humbert, D. K. and K. L. Krumel, Midland Analytical Laboratories report 79-00176, February 15, 1979.

METHOD

One rabbit per compound was treated with the samples listed previously. Samples A, B, C, and H were applied as 10% solutions in 95% ethanol, while Samples D, E, F, and G were applied undiluted. One additional rabbit received 19 applications of 95% ethanol only to serve as a control. The test materials were applied once daily, 5 days/week for a total of 19 or 20 applications as 0.1 ml/application to the inner aspect of the left external ear. The site of application was near the tip of the ear. The right external ear of each rabbit remained untreated throughout the study. The appearance of the test ear of each rabbit was observed once weekly, and the degree of hyperemia, edema, exfoliation, hyperplasia, folliculitis, necrosis, and scab formation was recorded. At the end of the 4-week test period, all rabbits on test were rendered unconscious by exposure to an atmosphere of CO_2 and then killed by decapitation. Each was examined externally and internally for gross pathologic alterations, and the observations were recorded. Special attention was given to the appearance of the integument on the inner aspect of the external ears. Approximately the distal one-half of both external ears from each rabbit were preserved in phosphate-buffered 10% formalin. Hematoxylin and eosin stained paraffin sections were prepared from each ear and examined microscopically.

RESULTS

Gross and histopathological observations on individual rabbits are summarized in Table 1.

95% Ethanol

Repeated applications of 95% ethanol resulted in no discernible irritation on the ear of the test rabbit. Upon histopathological examination, both the test and control ears were found to have very slight focal subacute perifollicular inflammation.

Sample A (Recrystallized 2,4-D Acid)

The test ear exhibited slight exfoliation only upon repeated application of recrystallized 2,4-D acid. Upon histopathology, the test ear exhibited a very slight increase in the thickness of the epidermis, with a slightly increased amount of keratinized debris adherent to the surface. These changes were probably due to a mild local irritant effect of Sample A.

Sample B (2,4-D Acid, 948 Building)

Repeated application of 2,4-D acid from 948 Building resulted in slight exfoliation only on the test ear. The test ear exhibited a very slight increase in the thickness of the epidermis, with very slightly increased amounts of keratinized debris adherent to the surface observed upon histopathological examination. This rabbit also had lesions of the liver and kidneys characteristic of encephalitozoonosis- a common, spontaneously occuring disease in the laboratory rabbit.

Sample C (2,4-D Acid, 489 Building)

When 2,4-D acid from 489 Building was initially applied to the ear of a rabbit, after several applications the ear was found to be markedly swollen, thickened, and hyperemic, with several scratch-like abrasions near the site of application of the test material. These abrasions

appeared to be self-inflicted. Upon incision of the ear there were copious accumulations of purulent exudate consistent with an infectious process unrelated to application of the test material. Histopathological examination of the infected ear revealed severe chronic suppurative inflammation with acanthosis or hyperplasia of the epidermis secondary to the inflammatory reaction. The character of the lesions was typical of that caused by an infectious organism. The scratch-like abrasions described previously were almost certainly the portal of entry for the infectious organism. Subsequent to necropsy of this rabbit, a second rabbit was treated with Sample C. The treated ear of this second rabbit exhibited very slight redness and slight exfoliation during the dosing period. Gross and histopathological examination of this ear revealed no visible lesions. It is concluded that the lesions in the left ear of the first rabbit were not related to treatment with Sample C.

Sample D (E6E, Isooctyl Alcohol Ester of 2,4-D)
Treatment with E6E resulted in slight redness and slight exfoliation on the test ear of a rabbit. Upon histopathological examination, the epidermis of this ear was very slightly thickened and had a very slight excess accumulation of keratinized debris at the surface.

Sample E (E99C, DOWANOL PiB Glycol Ether Product Ester)
Application of E99C to the ear of a rabbit resulted in slight redness and very slight exfoliation. The epidermis of this ear was slightly thickened with very slight excess accumulation of keratinized debris at the surface. The dermis of this ear had a slight scattered infiltration of mononuclear inflammatory cells.

Sample F (DMA-4, Dimethylamine Salt of 2,4-D, 948 Building)
Slight redness and slight exfoliation were observed on the test ear of a rabbit treated with DMA-4 (7/13-7/30/78). Upon histopathological examination, the untreated, right ear of this rabbit was observed to have very slight focal infiltration of inflammatory cells in the dermis. The treated ear of this rabbit exhibited slight acanthosis of the epidermis involving the superficial aspect of some hair follicles; in addition, slightly increased amounts of keratinized debris were adherent to the surface and within an occasional hair follicle. The dermis of this ear had slight, diffuse infiltration of inflammatory cells.

Sample G (DMA-4, Dimethylamine Salt of 2,4-D, 489 Building)
Application of DMA-4 (10/75) to the ear of a rabbit resulted in slight redness and slight exfoliation. Histopathology revealed a very slight increase in the thickness of the epidermis of this ear and a very slight accumulation of keratinized debris at the surface.

Sample H (Perchloroethylene Recycle Stream)
The perchloroethylene recycle stream was originally applied to the ear of a rabbit undiluted. Because an irreversible burn was produced, this rabbit was sacrificed and a second rabbit was treated with a 10% solution in 95% ethanol. No visible lesions resulted from applications of this dilution.

CONCLUSIONS

None of the test materials evaluated in this study elicited an acnegenic or comedogenic response, which in the rabbit ear bioassay is characterized

principally by the development of follicular hyperkeratosis. Occasionally in the rabbit ear bioassay a material, while not inducing a full blown acnegenic response may induce alterations such as distinct epidermal acanthosis and hyperkeratosis suggestive of acnegenic potential. In this study, none of the materials caused a response suggestive of even a weak acnegenic potential. All responses observed were attributed to the mild local irritant effects of the test materials. These data indicate that none of the materials tested should be considered potential chloracnegens.

REFERENCES

Humbert, D. K. and K. L. Krumel (February 15, 1979) Analysis of 2,4-D from 948 building submitted for 90 day animal feeding study. Midland Analytical Laboratories, Dow Chemical, U.S.A., report ML-AL 79-00176.

Wisniewski, D. F. (March 15, 1979) Tetrachloroxanthone analysis for 2,4-D formulations. Midland Analytical Laboratories, Dow Chemical, U.S.A., report AL 79-00676.

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TABLE 1

GROSS PATHOLOGIC AND HISTOPATHOLOGIC OBSERVATIONS ON RABBITS USED TO EVALUATE THE ACNEGENIC POTENTIAL (RABBIT EAR BIOASSAY) OF SEVERAL 2,4-D COMPOUNDS AND RELATED MATERIALS

Pathology Number Ear Number Treatment	Pathologic Observations
79-450 824 95% Ethanol (Vehicle Control)	Gross: No visible lesions. Histopathologic: Right external ear - very slight focal subacute perifollicular inflammation. Left external ear - very slight focal subacute perifollicular inflammation.
79-451 701 Recrystallized 2,4-D Acid, 10% in Ethanol	Gross: Left external ear - very slight scaliness and exfoliation at site of application on distal 1/3 of pinna. Hair follicles not enlarged or more prominent than normal. Histopathologic: Left external ear - very slight increase in thickness of epidermis with slightly increased amount of keratinized debris adherent to surface.
79-452 723 2,4-D Acid-948 Building, 10% in Ethanol	Gross: Left external ear - very slight exfoliation and scaliness at site of application. Hair follicles not enlarged or more prominent than normal; kidneys - markedly roughened and pitted over capsular surface, consistent with the lesions associated with encephalitozoonosis; liver - occasional pinpoint to 1 mm subcapsular pale focus, probably due to encephalitozoonosis. Histopathologic: Left external ear - very slight increase in thickness of epidermis with very slight increased amounts of keratinized debris adherent to surface; focal infiltration of mononuclear inflammatory cells in dermis.

TABLE 1 (Continued)

GROSS PATHOLOGIC AND HISTOPATHOLOGIC OBSERVATIONS ON RABBITS USED TO EVALUATE THE ACNEGENIC POTENTIAL (RABBIT EAR BIOASSAY) OF SEVERAL 2,4-D COMPOUNDS AND RELATED MATERIALS

Pathology Number Ear Number Treatment	Pathologic Observations
79-453 725 2,4-Acid-489 Building, 10% in Ethanol	Gross: Left external ear - entire ear swollen, thickened and hyperemic with scratch-like abrasions of epidermis; copious subcutaneous accumulations of purulent exudate which appeared to extend from the abraded areas; slight exfoliation and scaliness at site of application of test material without discernible enlargement of hair follicles. Kidneys - moderately roughened and pitted over surface consistent with the lesions of encephalitozoonosis. Liver - occasional pinpoint pale subcapsular foci probably due to encephalitozoonosis. Histopathologic: Left external ear - severe chronic suppurative inflammation resulting from abrasions described at necropsy; acanthosis of epidermis on both surfaces of ear occurring secondary to the severe inflammation; the lesions in the left ear of this rabbit were considered to be due to an infectious agent which entered via self-inflicted abrasions on the ear. A re-test of this formulation using another rabbit was requested.
79-1361 707 2,4-D Acid-489 Building, 10% in Ethanol (Re-test using second rabbit because of non-treatment-related complications in first rabbit.)	Gross: No visible lesions. Histopathologic: No visible lesions.

TABLE 1 (Continued)

GROSS PATHOLOGIC AND HISTOPATHOLOGIC OBSERVATIONS ON RABBITS USED TO EVALUATE THE ACNEGENIC POTENTIAL (RABBIT EAR BIOASSAY) OF SEVERAL 2,4-D COMPOUNDS AND RELATED MATERIALS

Pathology Number Ear Number Treatment	Pathologic Observations
79-454 722 E6E (Isooctyl Alcohol Ester of 2,4-D), 100%	Gross: Left external ear - very slight exfoliation and scaliness at site of application of test material; hair follicles not enlarged or more prominent. Histopathologic: Left external ear - epidermis very slightly thickened with very slight excess accumulation of keratinized debris at surface.
79-455 719 E99C (Dowanol PiB Ester of 2,4-D), 100%	Gross: Left external ear - very slight exfoliation at site of application of test material. Histopathologic: Left external ear - epidermis slightly thickened with very slight excess accumulation of keratinized debris at surface; slight scattered infiltration of mononuclear inflammatory cells in dermis.
79-456 1233 DMA-4 (7/13-7/30-78) Dimethylamine Salt of 2,4-D (948 Building), 100%	Gross: Left external ear - very slight exfoliation and scaliness at site of application of test material; hair follicles not enlarged or more prominent. Histopathologic: Right external ear - very slight focal infiltration of inflammatory cells in dermis. Left external ear - slight acanthosis of epidermis involving the superficial aspect of some hair follicles; slightly increased amounts of keratinized debris adherent to surface and within an occasional hair follicle; slight diffuse infiltration of inflammatory cells in dermis.

TABLE 1 (Continued)

GROSS PATHOLOGIC AND HISTOPATHOLOGIC OBSERVATIONS ON RABBITS USED TO EVALUATE THE ACNEGENIC POTENTIAL (RABBIT EAR BIOASSAY) OF SEVERAL 2,4-D COMPOUNDS AND RELATED MATERIALS

Pathology Number Ear Number Treatment	Pathologic Observations
79-457 1332 DMA-4 (10-75) Dimethylamine Salt of 2,4-D (489 Building), 100%	Gross: Left external ear - very slight exfoliation and scaliness of epidermis at site of application of test material; hair follicles not enlarged or more prominent. Histopathologic: Left external ear - very slight increase in thickness of epidermis, very slight accumulation of keratinized debris at surface.
79-936 608 Perchloroethylene Recycle Stream, 10% in 95% Ethanol	Gross: No visible lesions. Histopathologic: No visible lesions.

DOW CHEMICAL U.S.A.

TOXICOLOGY RESEARCH LABORATORY
MIDLAND, MICHIGAN

REQUEST FOR PRELIMINARY TOXICOLOGICAL STUDIES — 1803 BLDG.

NET

TOX. OR K NO. K-2372-(25)
FILE K-2372-(25)

MOLECULAR FORMULA	NAME OF CHEMICAL OR FORMULATION	# OF SAMPLES
$C_8H_6Cl_2O_3$	2,4-Dichlorophenoxy Acetic Acid Samples	8
MOLECULAR WEIGHT	SYNONYMS	
	2,4-D	

STRUCTURAL FORMULA · OR COMPOSITION
See back for descriptions.

PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT °C / mmHg	EXPLOSIVE LIMIT (% BY VOL. IN AIR)	FLASH POINT °F	IGNITION TEMP. °C	MELTING POINT °C	VAPOR PRESSURE mmHg 25°C

CORROSIVENESS (To Common Metals)	pH	PHYSICAL STATE	COLOR

CHEMICAL REACTIVITY	ODOR (Include Concentration in air)

STABILITY (To pH Change, Heat, Light)	SOLUBILITY

ACCOUNT OR PROBLEM FOR TOXICOLOGY

ACCOUNT NUMBER LEDGER	LOCATION GRP / FUNCTION	SECTION / SUB FUNC	SUFFIX	PROBLEM INDEX NUMBER LAB	PROBLEM
6	46 / 2561			17431	04330

☐ CLASS I SCREEN FOR TOXICITY
☐ CLASS IA FOR REGISTRATION
☐ OTHER

SAMPLE REFERENCE AND SOURCE

ESTIMATED COST FOR TESTS

REPORT TO (ADDRESS)
D. Buzzelli, 934
L. Smith, 9005
~~P. Enos, 1210~~
J. Wickman 9005
Stan McGregor - 1710
E.C. Jersey, Freeport

SUBMITTED BY (Signature) Peter W. Chester
DATE 1/10/79
BLDG. NO. 1910
PHONE NO. 636-4529

DO NOT WRITE BELOW THIS LINE - COMPLETE INFORMATION ON REVERSE SIDE See record of work request 4515 for

TEST	ANIMAL	SEX	SOLVENT	DOSE (g/kg.)	ANIMALS ON TEST	TESTS DONE	ADDITIONAL WORK	DONE	TOX. RESULTS	PHONED-TO DATE
ACUTE ORAL ☐ RANGE ☐ LD_{50}	RAT, GUINEA PIG, RABBIT, MOUSE		CORN OIL, WATER, UNDILUTED							
EYE ABSORPTION	RABBIT									
EYE CONTACT	RABBIT									
SKIN CONTACT REPEATED	RABBIT									
SKIN CONTACT SINGLE EXPOSURE ☐ D.O.T. ☐ F.H.S.A.										
SKIN CONTACT · CHLORACNE										
SENSITIZATION ☐ GUINEA PIG ☐ HUMAN										
SKIN ABSORPTION SINGLE EXPOSURE ☐ RANGE ☐ LD_{50}	RABBIT									
ACUTE INHALATION	RAT		SAT VAPOR ☐ PPM ____ PRESSURIZED CAN ____ AEROSOL ____							

DOW 2156517

20756

REPORT DRAFTED ____
REPORT ISSUED ____
SAMPLE RETURNED ____
SAMPLE TO K STORAGE ____
SAMPLE DESTROYED ____

(FRONT)

REASON OR NEED FOR TOXICOLOGICAL TESTS:

- ☐ DATA SHEET FOR SAFE HANDLING
- ☐ MATERIAL SAFETY DATA SHEET
- ☐ IS TOXICITY COMPATIBLE WITH INTENDED USE
- ☐ IN-PANEL TESTING
- ☐ SAMPLES TO PROSPECTIVE CUSTOMERS
- ☐ LABEL
- ☐ REGISTRATION
- ☐ OTHER - PLEASE EXPLAIN

STAGE OF DEVELOPMENT:

☐ RESEARCH ☐ BENCH ☐ PILOT PLANT ☐ SEMI PLANT ☐ PLANT ☐ REGISTRATION

PROPOSED AND POTENTIAL USE:

METHOD OF HANDLING:

AMOUNTS TO BE HANDLED:

HUMAN EXPERIENCE IN HANDLING:

DOW215618

NOTES:

Sample No	Description	Remarks
(1)	Recrystallized 2,4-D Acid	Obtained from Stan Gorzinski
(2)	948 Bldg. 2,4-D Acid	" from Stan Gorzinski
(3)	489 Bldg. 2,4-D Acid	Made By The CKL Process
(4)	E & E	Iso-octyl alcohol ester of 2,4-D made by "acid-ester" process in Dec. 1978. (6 lbs/gal acid equivalent.)
(5)	E99C	Butoxyethyl ester of 2,4-D made mostly by the "direct-ester" process from 8/1/78 to 8/30/78. (4 lbs./gal)
(6)	DMA-4 (2/3-7/30/78)	Dimethylamine salt of 2,4-D made from 948 Bldg. 2,4-D from July, 1978. (~ 4 lbs/gal
(7)	DMA-4 (10/[illegible])	Dimethylamine salt of 2,4-D made from 489 Bldg. 2,4-D during Oct, 1978
(8)	Perc. Recycle Stream from V402 Tank	Perchloroethylene recycle stream from 948 Bldg. taken on 1-11-79 (~20 ppm TCX)
(9)	[illegible]	

18757

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-454

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
722	☒M ☐F	RABBIT	☒ YES ☐ NO	49	305	4

MATERIAL AND ROUTE OF ADMINISTRATION	DOSE OR CONCENTRATION
E b E (ISOOCTYL ALCOHOL ESTER OF 2,4-D) - CHLORACNE TEST	100%

COMMENTS: T 3803

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - this ear contains the ear tag. It is unremarkable. Left external ear - shows very slight scaliness and exfoliation at the site of application near the tip of the pinna. The hair follicles are not enlarged or more prominent in appearance.

internally NVL

2/22/79
George C. Jersey DVM,PhD

DOW215519

18758

2/28/79

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE: Diane M Williams HT

PATH FILE NUMBER: 26 N L

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-454 3-15-79 M584Aja

*ATHOLOGY NUMBER

Right External Ear — Two sections examined - NVL

Left External Ear — Two sections examined - the epidermis on one section of the ear appears to be very slightly thickened and there appears to be very slight excessive accumulations of keratinized material at the surface. Overall, these barely discernible alterations of the epidermis are graded as very slight.

3/15/79
George C. Jersey DVM,PhD

DOW215520

18759

PATHOLOGIST | DATE

FORM C-32030 PRINTED 8-12-76 *See footnote on (back)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-455

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
719	☒M ☐F	RABBIT	☒YES ☐NO	49	305	5

MATERIAL AND ROUTE OF ADMINISTRATION: E99C (DOWANOL PiB ESTER OF 2,4-D) - CHLORACNE TEST

DOSE OR CONCENTRATION: 100%

COMMENTS: T3804

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - contains the ear tag. It is unremarkable.
Left external ear - shows very slight exfoliation at the site of application on the distal aspect of the pinna.

internally NVL

George C. Jersey DVM,PhD 2/22/79

18760

2/24/79

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE David M. Williams HT

PATH FILE NUMBER 26-44

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-455 3-15-79 M584Aja

PATHOLOGY NUMBER

ht External Ear	Two sections examined - NVL
eft External Ear	Two sections examined - there is slight thickening of the epidermis with very slight excessive accumulations of keratinized material at the surface. There are also slight scattered infiltrations of mononuclear inflammatory cells in the dermis. Overall, the alterations in this ear are graded as slight.

3/15/79
George C. Jersey DVM,PhD

DOW215656522

18761

PATHOLOGIST | DATE

FORM C-32030 PRINTED 8-12-76

*See footnote on front

(BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-456

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J. W. HENCK	194-8007376	K 2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
1233	☒M ☐F	RABBIT	☒ YES ☐ NO	49	305	6

MATERIAL AND ROUTE OF ADMINISTRATION	DOSE OR CONCENTRATION
DMA-4 (7/13-7/30/78) DIMETHYLAMINE SALT OF 2,4-D (948 BLDG.) CHLORACNE TEST	100%

COMMENTS: T 3805

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES - DECAP	2-14-79 G435A1a

GROSS PATHOLOGY REPORT*

externally Right external ear - this ear contains the ear tag. It is unremarkable. Left external ear - shows very slight scaliness and exfoliation at the site of application. The hair follicles are not enlarged or more prominent than normal appearance.

internally NVL 2/27/79
George C. Jersey DVM,PhD

18762

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE 2/27/79
David M. Williams HT

PATH FILE NUMBER

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-456 3-15-79 M584Aja

PATHOLOGY NUMBER

ght External Ear	Two sections examined - there are very slight focal infiltrations of a mixed population of inflammatory cells in the dermis.
Left External Ear	Two sections examined - there is slight thickening or acanthosis of the epidermis which extends to involve the more superficial aspect of the follicles. There is slight diffuse infiltration of a mixed population of inflammatory cells in the dermis. There are slightly increased amounts of keratinized material adherent at the surface. Occasionally, within a hair follicle there are slightly increased amounts of keratinized debris. Overall, these alterations are slight.

3/15/79
George C. Jersey DVM,PhD

DOW215524

18763

PATHOLOGIST | DATE

FORM C-32030 PRINTED 8-12-76

*See footnote on front

(BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-457

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K 2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
1332	☒M ☐F	RABBIT	☒ YES ☐ NO	49	305	7

MATERIAL AND ROUTE OF ADMINISTRATION: DMA-4 (10/75) DIMETHYLAMINE SALT OF 2,4-D (489 BLDG.) CHLORACNE TEST

DOSE OR CONCENTRATION: 100%

COMMENTS: T3804

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - this ear contains the ear tag. It is unremarkable. Left external ear - shows very slight scaliness and exfoliation at the site of application. The hair follicles are not enlarged or more prominent than in normal appearance.

internally NVL

George C. Jersey DVM,PhD 2/22/79

DOW215656525

18784

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE: David M. Williams HT 2/23/79

PATH FILE NUMBER: 26-414

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-457 3-15-79 M584Aja

PATHOLOGY NUMBER

ght External Ear	Two sections examined - NVL
Left External Ear	Two sections examined - there appears to be a barely discernible increase in the thickness of the surface epithelium at the sight of application of the test material with perhaps a very slight accumulation of keratinized material at the surface. Overall, these alterations are barely discernible and are graded as very slight.

GC 3/15/79
George C. Jersey DVM, PhD

DOW215526

18765

PATHOLOGIST | DATE

FORM C-32030 PRINTED 8-12-76

*See footnote on front

(BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79 - 936

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
~~SEE~~ 608	☒M ☐F	RABBIT	☒ YES ☐ NO	49	300	8

MATERIAL AND ROUTE OF ADMINISTRATION	DOSE OR CONCENTRATION
PERCHLORETHYLENE RECYCLE STREAM T-3809	—

COMMENTS: 10% in 95% ETHANOL

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/16/79	CO_2 ANES - DECAP	2-27-79 G438Aja

GROSS PATHOLOGY REPORT*

A GROSS PATHOLOGIC EXAMINATION WILL BE CONDUCTED WITH SPECIAL ATTENTION TO THE APPEARANCE OF THE EXTERNAL EARS. PORTIONS OF BOTH EXTERNAL EARS WILL BE PRESERVED IN FORMALIN. NO OTHER TISSUES WILL BE PRESERVED IN FORMALIN.

externally — Ears - the right ear - contains the ear tag. It is unremarkable. The left external ear - NVL.

The hair has been clipped from the midabdominal region. The integument in this region is unremarkable.

internally — NVL

GCJ 2/28/79
George C. Jersey DVM, PhD

DOW 2156527

18786

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

2/25/79
HISTOLOGIST/DATE: Daniel M. McKeown HT

PATH FILE NUMBER: 25NL

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-936 3-15-79 M584Aja

PATHOLOGY NUMBER

,ht Ear Two sections examined - NVL
eft Ear Two sections examined - NVL

VCJ 3/15/79
George C. Jersey DVM,PhD

DOW2156528

18767

ATHOLOGIST DATE

ORM C-32030 PRINTED 8-12-76 *See footnote on front (BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-450

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K 2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
824	☐M ☒F	RABBIT	☒ YES ☐ NO	49	300	1

MATERIAL AND ROUTE OF ADMINISTRATION	DOSE OR CONCENTRATION
95% ETHANOL - CHLORACNE TEST	100%

COMMENTS: T 3808

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES. - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

THE RABBITS WILL BE EXAMINED FOR EXTERNAL AND INTERNAL GROSS PATHOLOGIC OBSERVATIONS, PARTICULAR EMPHASIS BEING PLACED UPON THE EXTERNAL PORTION OF THE EARS, THE LEFT EXTERNAL EAR CONSTITUTING THE SITE OF APPLICATION OF TEST MATERIAL. ONLY THE EXTERNAL EARS WILL BE PRESERVED IN FORMALIN.

externally Right external ear - this ear contains the ear tag - NVL
Left external ear - NVL

internally NVL

GJC 2/22/79
George C. Jersey DVM,PhD

DOW215656529

18768

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE 2/25/79 Vincent M. McKenna HT

PATH FILE NUMBER 25NL

FORM C-32030 PRINTED 8-12-76

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-450 3-15-79 M584Aja

PATHOLOGY NUMBER

ght External Ear	Very slight subacute ~~interstitial~~ inflammation limited to a single focus located in the perifollicular region.
Left External Ear	Two sections examined - very slight isolated focal subacute inflammation observed in the region of one or two hair follicles. There was no discernible effect of the treatment regimen.

GCJ 3/15/79
George C. Jersey DVM,PhD

DOM2156530

18769

PATHOLOGIST | DATE

FORM C-32030 PRINTED 8-12-76

*See footnote on front

BACK

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-451

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K-2372-(R5)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
701	☒M ☐F	RABBIT	☒YES ☐NO	49	305	1

MATERIAL AND ROUTE OF ADMINISTRATION: RECRYSTALLIZED 2,4-D ACID - CHLORACNE TEST

DOSE OR CONCENTRATION: 10% IN ETHANOL

COMMENTS: T3800

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES. - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - contains the ear tag - NVL
Left external ear - shows very slight scaliness and exfoliation at the site of application in the distal 1/3 of the pinna. The hair follicles are not enlarged or more prominent than normal in appearance.

internally NVL

George C. Jersey DVM,PhD 2/22/79

DOW215653 1

18770

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE: Daniel M. Hewitt 2/24/79

PATH FILE NUMBER: 2541

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-451 3-15-79 M584Aja

PATHOLOGY NUMBER

ight External Ear	Two sections examined - NVL
Left External Ear	Two sections examined - there appears to be a very slight increase in the thickness of the epidermis with slightly more keratinized material adhering to the surface than was observed in the right untreated ear. Overall, this is a barely discernible alteration.

GCJ 3/15/79
George C. Jersey DVM,PhD

DOW215965532

18771

PATHOLOGIST | DATE

FORM C-32036 PRINTED 8-12-76

*See footnote on front

(BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-452

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-80073376	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
723	☒M ☐F	RABBIT	☒ YES ☐ NO	49	305	2

MATERIAL AND ROUTE OF ADMINISTRATION: 2,4-D ACID, 948 BLDG. - CHLORACNE TEST

DOSE OR CONCENTRATION: 10% IN ETHANOL

COMMENTS: T 3801

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES. - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - contains ear tag - NVL
Left external ear - shows a very slight exfoliation and scaliness at the site of application in the distal 1/3 to 1/2 of the pinna. The hair follicles are not enlarged or more prominent than normal in appearance.

internally Kidneys - both are markedly roughened and pitted over the surface consistent with the lesions associated with encephalitozoonosis.

Liver - contains an occasional pinpoint to 1 mm subcapsular pale focus in all probability also associated with the encephalitozoonosis.

George C. Jersey DVM,PhD GCJ 2/22/79

DOW215665333

18772

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE: 2/23/79

PATH FILE NUMBER: 25412

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-452 3-15-79 M584Aja

PATHOLOGY NUMBER

Right External Ear — Two sections examined - NVL

Left External EAr — Two sections examined - there appears to be a very slight, barely discernible increase in the thickness of the epidermis with very slightly increased amounts of keratinized material adherent to the surface There is also focal infiltration of mononuclear inflammatory cells in the dermis. Overall, the alterations are barely discernible and graded as very slight.

GCJ 3/15/79
George C. Jersey DVM,PhD

DOW215653

18773

PATHOLOGIST | DATE

FORM C-32936 PRINTED 8-12-76

*See footnote on front

(BACK)

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-453

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-8007376	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
725	☒M ☐F	RABBIT	☒YES ☐NO	49	305	3

MATERIAL AND ROUTE OF ADMINISTRATION: 2,4-D ACID, 489 BLDG. - CHLORACNE TEST

DOSE OR CONCENTRATION: 10% IN ETHANOL

COMMENTS: T3802

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
2/9/79	CO_2 ANES. - DECAP	2-14-79 G435Aja

GROSS PATHOLOGY REPORT*

externally Right external ear - contains the ear tag - NVL
Left external ear - the entire ear is swollen and thickened. It is hyperemic. It contains some abrasions on the outer aspect near the tip in the region opposite the site of application. There is also slight abrasion and scab formation within the region of the site of application near the margin of the ear on the inner face. The lesions on the outer surface show a pattern and are suggestive of possible self-mutilation by scratching of the ear by the rabbit.

~~Integument~~ Examination of the integument at the site of application reveals slight scaliness and exfoliation of the epidermis. The hair follicles do not appear to be enlarged or more prominent than normal. Upon incision, there is observed an accumulation of purulent exudate which is located between the integument on the outer surface of the ear and the central cartilage within the ear. This appears to be a direct extension from the abraded area on the outer surface of the ear. In all probability, the marked inflammatory process in this rabbit is a secondary infectious process resulting from the trauma to the ear, in all probability self-inflicted trama, due to scratching.

internally Kidneys - both are moderately roughened and pitted over the surface consistent with the lesions caused by infection with the cause of organism of encephalitozoonosis.

Liver - contains an occasional subcapsular pinpoint pale focus, in all probability associated with the encephalitozoonosis.

2/12/79
George C. Jersey DVM,PhD

DOW 1565535

18774

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

2/20/79
HISTOLOGIST/DATE: David M. Williams III

PATH FILE NUMBER: 26N2

LIGHT MICROSCOPIC EXAMINATION REPORT* Animal Number 79-453 3-15-79 M584Aja

PATHOLOGY NUMBER

Right External Ear — Two sections examined - NVL

Left External Ear — Two sections examined - there is severe chronic suppurative inflammation which affects both sections. This is characterized by copious accumulations of purulent exudate, within the dermal layers of the ear with proliferation of fibroblastic elements near the margins of the suppurative inflammatory process. The ear shows a considerable amount of inflammatory edema in other areas. There are thrombi within some of the vessels. The surface epithelium shows diffuse thickening and acanthosis, possibly associated with the inflammatory changes in the ear. In all probability, the cause of these lesions was that described at necropsy. It is noted that the acanthosis of the epidermis is present on both surfaces of the ear, indicating that this was, in all probability, not at all related to the treatment regimen but rather, is a secondary reaction associated with the extensive inflammatory changes in the ear of this rabbit.

3/15/79
George C. Jersey DVM,PhD

DOM2156536

18775

PATHOLOGIST | DATE

FORM C-32930 PRINTED R-12-76

*See footnote on first

PATHOLOGY SHEET

DOW - TOXICOLOGY

PATH NUMBER: 79-1361

REQUESTED BY	CHARGE NUMBER	FILE NUMBER
ACUTE TOXICITY - J.W. HENCK	194-80073?6	K-2372-(25)

AN. IDENT. OR NUMBER	SEX	SPECIES	SAVE TISSUES	ROOM	RACK	CAGE
707	☒M ☐F	RABBIT	☒ YES ☐ NO	49	341	1

MATERIAL AND ROUTE OF ADMINISTRATION	DOSE OR CONCENTRATION
2,4-D ACID (489 GLDG.) - 10% in 95% ethanol - CHLORACNE TEST	—

COMMENTS: 20 APPLICATIONS IN 25 DAYS

DATE OF NECROPSY	MODE OF DEATH	DATE TYPED AND CASSETTE NUMBER
3/9/79	OVERDOSE CO_2, DECAP	3-26-79 G442Aj

GROSS PATHOLOGY REPORT*

externally NVL

internally NVL

Both ears were saved in formalin.

C. D. BLOGG DVM

27 Mar 79

DOW215656537

*Unless noted, no visible Lesions (NVL) were observed. For specific procedures followed during pathologic examinations refer to protocol and/or "Pathology Procedures Manual."

PATHOLOGIST/DATE

HISTOLOGIST/DATE: 5/1/79

PATH FILE NUMBER: 4 NV * 4 NV

LIGHT MICROSCOPIC EXAMINATION REPORT*

ATHOLOGY NUMBER

Right Ear – No visible lesions.
Left Ear – No visible lesions.
JCL Sept. 4, 1979

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18778

HOLOGIST | DATE

M C-32030 PRINTED R-12-76

*See footnote on front

(BACK)

Witold Senczuk, Halina Pogorzelska,
Monika Debska

ABSORPTION OF [redacted] THROUGH THE SKIN

Texicology Department of the Institute
of Bioanalysis and medium analysis of
the Medical Academy of Poznan
Director: Prof. Dr. W. Senczuk

NOTE: The numbers on the graphs in this article are not clear.

Percutaneous absorption of 2.4-dichlorphenoxyacetic acid by indirect method was investigated, determining the amount of 2.4-D in the urine of rats.

The production of pesticides and their application give rise to wide possibilities of poisoning with these compounds (1,2,3,5), and this prompted the determination of the degree of percutaneous absorption of one of most frequently used protective agents, namely 2.4-dichlorphenoxyacetic acid (2.4-D).

Experimental Part

Material and Method

The tests were carried out on 28 rats of the Wistar species, weighing each 200 g ± 10g. The animals were divided into seven groups of four rats each group.

Commercial preparation "Pielik"(2.4-dichlorphenoxyacetic acid: translator) containing about 85% of sodium salt of 2.4-dichlorphenoxyacetic acid was used for the tests. The preparation was used in the form of aqueous solutions of different 2.4-D concentrations.

Results of Determinations

1. Determination of 2.4-D acid in the urine depending on the concentration of the tested compound in the solution

The tests were carried out with the use of aqueous solutions of "Pielik" containing 0.5, 1, 2, 4 and 5% of 2.4-D acid. After six hours there was determined the amount of 2.4-D acid excreted with urine. The results are shown in table 1 and in Fig. 1.

2. Determination of 2.4-D acid in urine depending on the period of exposure

The influence of the exposure period (2, 4 and 6 hours) on the excretion of 2.4-D with urine was determined by using aqueous solution of "Pielik" containing 4% of the tested acid. The results of the determination of 2.4-D acid in the collected portions of urine are shown in table II. Fig. 2 graphically

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Witold Senczuk, Halina Pogorzelska,
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illustrates the results

Table 1. Results of the determination of 2.4-D acid in urine depending on the concentration of the compound in the solution

Solution %	Animal No	Amount of 2.4-D acid in urine (mg) 1st day (24 hours)	2nd day	3rd day	jointing after three days
0.5	1	0,9	1,4	1,2	3,5
	2	1,0	1,5	1,0	3,5
	3	1,0	2,8	1,0	4,6
	4	1,0	1,8	1,1	3,6
average	Średnia	0,97	1,77	1,07	3,81
1.0	1	3,4	2,4	1,0	6,8
	2	1,0	1,6	1,2	3,8
	3	7,6	2,2	0,0	9,8
	4	2,5	1,9	0,0	3,5
average	Średnia	3,62	1,80	0,55	5,97
2.0	1	1,2	5,1	1,8	8,1
	2	2,0	8,7	1,7	12,4
	3	1,6	4,8	2,6	9,0
	4	2,5	1,8	1,1	5,4
average	Średnia	1,82	5,10	1,80	8,72
4.0	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
average	Średnia	4,45	4,00	3,80	12,25
5.0	1	5,5	4,7	4,9	15,1
	2	2,7	2,6	2,1	7,4
	3	3,6	5,8	6,8	16,2
	4	4,6	6,8	7,2	18,6
average	Średnia	4,10	4,97	5,25	14,32

Table II. Results of the determination of 2.4-D acid in urine depending on the exposure period

Period of Exposure	Animal No	Amount of 2.4-D acid in urine (mg) 1st day (24 hours)	2nd day	3rd day	jointing after three days
2	1	1,6	0,8	0,9	3,3
	2	4,7	3,8	1,7	10,2
	3	2,5	1,7	0,8	5,0
	4	3,0	1,6	0,8	5,4
	Średnia	2,95	1,97	1,05	5,97
average					
4	1	1,8	1,3	1,4	7,5
	2	1,1	3,3	1,5	8,2
	3	1,9	2,5	2,2	6,6
	4	3,2	10,5	4,0	17,7
	Średnia	2,07	5,15	2,27	9,10
average					
6	1	6,0	1,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
average	Średnia	4,45	4,00	3,80	12,25

Discussion of the Results

It has been determined that the amount of the substance excreted with urine is proportional to the 2.4-D concentration in the tested solutions(Fig.1). As the 2.4-D concentration increased, the amount of this compound excreted with urine, as compared with the preceding group, i.e. of lower 2.4-D concentration of 2.4-D increased by about 50%. This related to 0.5 to 4% concentrations, but after exposure to 5% concentration, in comparison with the group of animals exposed to 4% solution, 17% less 2.4-D acid were excreted with urine.

Also the period of exposure influenced the amount of 2.4-D acid absorbed by skin. This relation was proportional for 2, 4 and 6 hour exposure (Fig. 2). This can provide a basis for further research in determining the biochemical absorption index.

Figure1. Excretion with urine of 2.4-D acid depending on the concentration of the compound in the solution

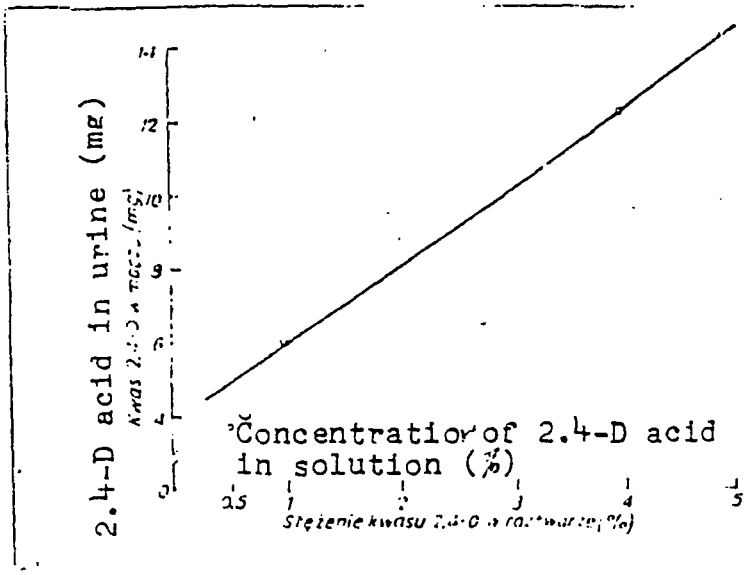
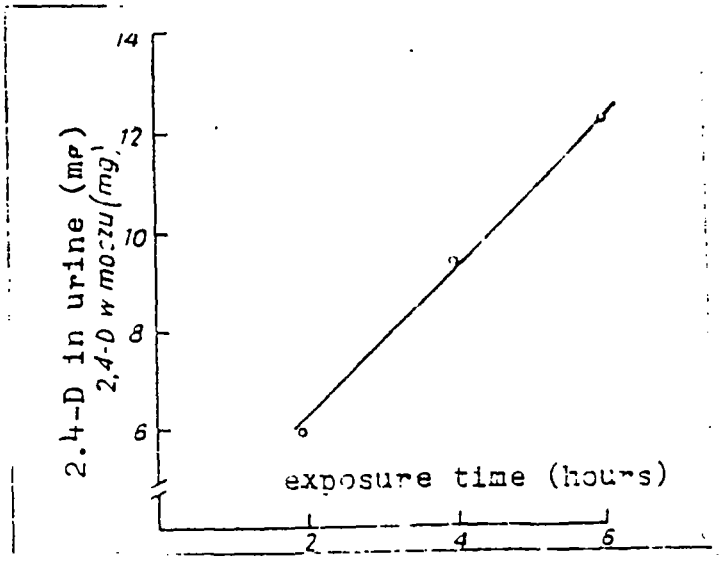


Figure 2. Excretion with urine of 2.4-D acid depending on the period of exposure.



A Russian summary corresponds to the following English summary.

W. Seńczuk, H. Pogorzelska, M. Dębska

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The degree of percutaneous absorption of one of the most frequently used herbicides — 2,4-dichlorphenoxyacetic acid — was investigated. The investigations were carried out with different concentrations of this acid in aqueous solutions of „Pielika" and in relation to the duration of exposure. *Wistar* rats were used in these experiments.

The dermal exposure was done by the tail method. The urinary excretion of the acid was determined by gas chromatography after previous extraction of the acid from urine and its estrification.

PIŚMIENNICTWO

1. *Bobiagin D. A., Syrkin A. B., Łapinosow J. W., Zajcewa L. A.:* Diejstwije na organizm czielowieka i żiwotnych gierbicidow-proizwodnych chlorfiemoksiuksusnoj kisłoty. Farmakol. i Toksikol., 1969, 32, 747. — 2. *Jirasek L., Kalensky J., Kubec K.,* Acne chlorina a porphyria cutanea tarda pri vyrobe herbicid. Cs. dermatol., 1973, 48, 306. — 3. *Radionow A. D., Chromachenko A. N., Kurilenko I. L.:* Sanitary — toxicologic features of a herbicide of sodium, ammonium and dimethylammonium salts of 2,4-dichlorophenoxyacetic acid. Gig. Sanit., 1967, 32, 100. — 4. *Seńczuk W., Pogorzelska H.:* Budowa chemiczna a toksykodynamiczne właściwości pochodnych kwasów fenoksykarboksylowych. Część II. Metody oznaczania pochodnych kwasu fenoksyoctowego i fenoksypropionowego w moczu i krwi. Roczniki PZH. Praca oddana do druku. — 5. *Zankiewicz W., Rutkowska N.:* Zatrucie Pielikiem. Medycyna Pracy, 1967, 18, 446.

Literature

1. Influence on the organism of humans and animals of herbicides on the basis of chlorphenoxyacetic acid, Pharmacology and Toxicol 1969, 32, 747.
2. Percutaneous absorption in the production of herbicides
3. see above in English
4. Chemical structure and toxidynamic properties of derivatives o phenoxycaboxylic acids. Part II . Method for determination of derivatives of phenoxyacetic and phenoxypropionic acid in urine and blood. PZH Annuals. Paper remitted to printing.
5. Poisoning with 'Pielik', Occupational Medicine

July 17, 1980

60-780 Poznan, Grunwaldzka street 6

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WITOLD SENCZUK, HALINA POGORZELSKA, MONIKA DĘBSKA

WCHŁANIANIE KWASU 2,4-DWUCHLOROFENOKSYOCTOWEGO PRZEZ SKÓRĘ

Zakład Toksykologii Instytutu Bioanalizy i Badania Środowiska
Akademii Medycznej w Poznaniu
Kierownik: prof. dr hab. W. Seńczuk

Zbadano wchłanianie przez skórę kwasu 2,4-dwuchlorofenoksyoctowego metodą pośrednią oznaczając ilość 2,4-D w moczu szczurów.

Produkcja pestycydów i ich stosowanie stwarza duże możliwości zatrucia tymi związkami [1, 2, 3, 5], stąd celowym było określenie stopnia wchłaniania przez skórę, jednego z częściej używanych środków ochrony roślin, a mianowicie kwasu 2,4-dwuchlorofenoksyoctowego (2,4-D).

CZĘŚĆ DOŚWIADCZALNA

Materiał i metodyka

Badania wykonano na 28 szczurach, szczepu *Wistar*, o masie ciała 200 g ± 10 g. Zwierzęta podzielono na 7 grup po 4 szczury.

Do badań użyto preparatu handlowego „Pielik" zawierającego około 85% soli sodowej kwasu 2,4-dwuchlorofenoksyoctowego. Preparat używano w postaci roztworów wodnych o różnych stężeniach 2,4-D.

Ogony zwierząt po dokładnym umyciu i obliczeniu powierzchni, zanurzano w probówkach zawierających około 20 cm³ badanego roztworu o temp. 37 - 40 C. Po określonym czasie ekspozycji (p. niżej) zwierzęta przenoszono do klatek metabolicznych typu „Simax", po czym przez 72 godziny zbierano dobowe porcje moczu. Dwa razy dziennie podawano zwierzętom sondą do żołądka po 5 cm³ wody. Zawartość kwasu 2,4-D w moczu określano metodą chromatografii gazowej, po uprzednim wyekstrahowaniu badanego związku z moczu i jego estryfikacji [4].

WYNIKI OZNACZEŃ

1. Oznaczanie kwasu 2,4-D w moczu w zależności od stężenia badanego związku w roztworze

Badania wykonano przy użyciu wodnych roztworów „Pielika" zawierających 0,5, 1, 2, 4 i 5% kwasu 2,4-D. Po 6 godzinach określano ilość wydalonego z moczem kwasu 2,4-D. Wyniki przedstawiono w tabeli I oraz na rycinie 1.

2. Oznaczanie kwasu 2,4-D w moczu w zależności od czasu ekspozycji

Wpływ czasu ekspozycji (2, 4 i 6 godzin) na wydalanie z moczem 2,4-D określono stosując wodny roztwór „Pielika" zawierający 4% badanego

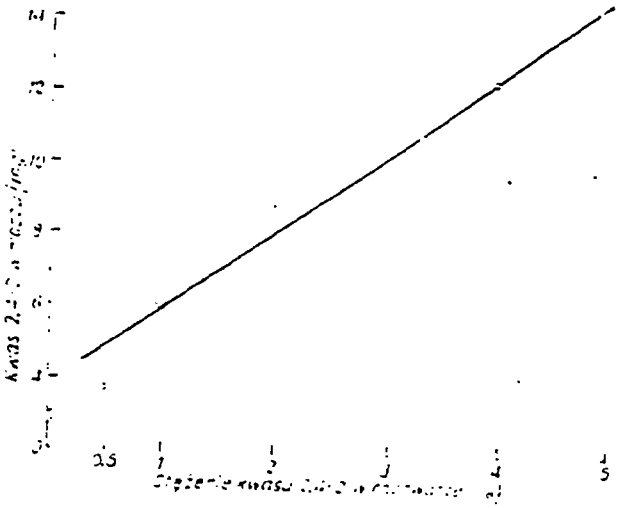
Tabela I. Wyniki oznaczania kwasu 2,4-D w moczu w zależności od od stężenia związku w roztworze

Roztwór %	Zwierzę Nr	Ilość kwasu 2,4-D w moczu (mg) I doba	II doba	III doba	Łącznie po trzech dobach
0,5	1	0,9	1,4	1,2	3,5
	2	1,0	1,5	1,0	3,5
	3	1,0	2,8	1,0	4,8
	4	1,0	1,6	1,1	3,6
	Średnia	0,97	1,77	1,07	3,81
1,0	1	3,4	2,4	1,0	6,8
	2	1,0	1,6	1,2	3,8
	3	7,6	2,2	0,0	9,8
	4	2,5	1,0	0,0	3,5
	Średnia	3,62	1,80	0,55	5,97
2,0	1	1,2	5,1	1,8	8,1
	2	2,0	8,7	1,7	12,4
	3	1,6	4,8	2,6	9,0
	4	2,5	1,8	1,1	5,4
	Średnia	1,82	5,10	1,80	8,72
4,0	1	6,0	4,8	7,2	18,0
	2	5,0	5,8	2,6	13,1
	3	4,8	2,1	2,7	9,6
	4	2,0	3,3	2,7	8,0
	Średnia	4,45	4,00	3,80	12,25
5,0	1	5,5	4,7	4,9	15,1
	2	2,7	2,6	2,1	7,1
	3	3,6	5,8	6,8	16,2
	4	4,6	6,8	7,2	18,6
	Średnia	4,10	4,97	5,25	14,32

kwasu. Wyniki oznaczania kwasu 2,4-D w zebranych porcjach moczu przedstawiono w tabeli II. Ilustracją graficzną wyników jest rycina 2.

OMÓWIENIE WYNIKÓW

Stwierdzono, że ilość substancji wydalonej z moczem jest proporcjonalna do stężenia 2,4-D w badanych roztworach (ryc. 1). W miarę wzrostu stężenia 2,4-D ilość tego związku wydalonego z moczem, w porównaniu z grupą poprzednią, tzn. o niższym stężeniu 2,4-D wzrastała o ok. 50%. Dotyczyło to stężeń od 0,5 do 4%; natomiast po ekspozycji na roztwór 5%, w porównaniu z grupą zwierząt eksponowanych na roztwór 4%, z moczem wydaliło się o ok. 17% mniej kwasu 2,4-D.



Ryc. 1. Wydalanie z moczem kwasu 2,4-D w zależności od stężenia związku w roztworze.

Czas ekspozycji wpływał również na ilość kwasu 2,4-D wchłoniętą przez skorę. Zależność ta była proporcjonalna dla 2, 4 i 6 godzinnej ekspozycji (ryc. 2). Może to stanowić podstawę do dalszych badań nad opracowaniem biochemicznego wskaźnika wchłaniania.

Tabela II. Wyniki oznaczania kwasu 2,4-D w moczu w zależności od czasu ekspozycji

Czas ekspozycji (h)	Zwierzę Nr	Ilość kwasu 2,4-D w moczu (mg) I doba	II doba	III doba	Łącznie po trzech dobach
2	1	1,6	0,8	0,9	3,3
	2	4,7	3,8	1,7	10,2
	3	2,5	1,7	0,8	5,0
	4	3,0	1,6	0,8	5,4
	średnia	2,95	1,97	1,05	5,97
4	1	1,8	1,3	1,1	7,5
	2	1,1	3,3	1,5	8,2
	3	1,9	2,5	2,2	6,6
	4	3,2	10,5	4,0	17,7
	średnia	2,07	5,15	2,27	9,10
6	1	6,0	1,8	7,2	18,0
	2	5,0	5,8	2,6	13,4
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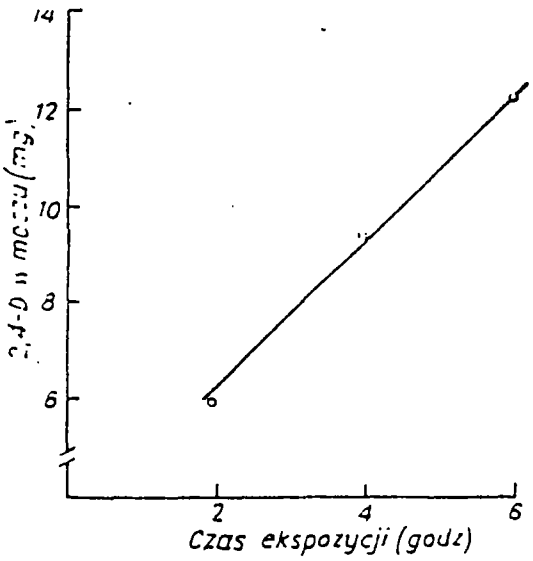

Ryc. 2. Wydalanie z moczem kwasu 2,4-D w zależności od czasu ekspozycji.

B. Seńczuk, H. Pogorzelska, M. Dębska

РЕЗОРБЦИЯ ЧЕРЕЗ КОЖУ 2,4-ДИХЛОРФЕНОКСИУКСУСНОЙ КИСЛОТЫ

Резюме

Определялась степень резорбции через кожу одного из наиболее часто применяемых гербицидов, 2,4-дихлорфеноксиуксусной кислоты (2,4-D).

Исследования проводились в зависимости от концентрации этой кислоты в водном растворе препарата „Пелик" а также от времени экспозиции хвоста применяемых животных (крыс линии Вистар) на действие 2,4-D.

Количество выделяемого с мочой 2,4-D определяли методом газовой хроматографии после его экстракции из мочи и этерификации.

W. Seńczuk, H. Pogorzelska, M. Dębska

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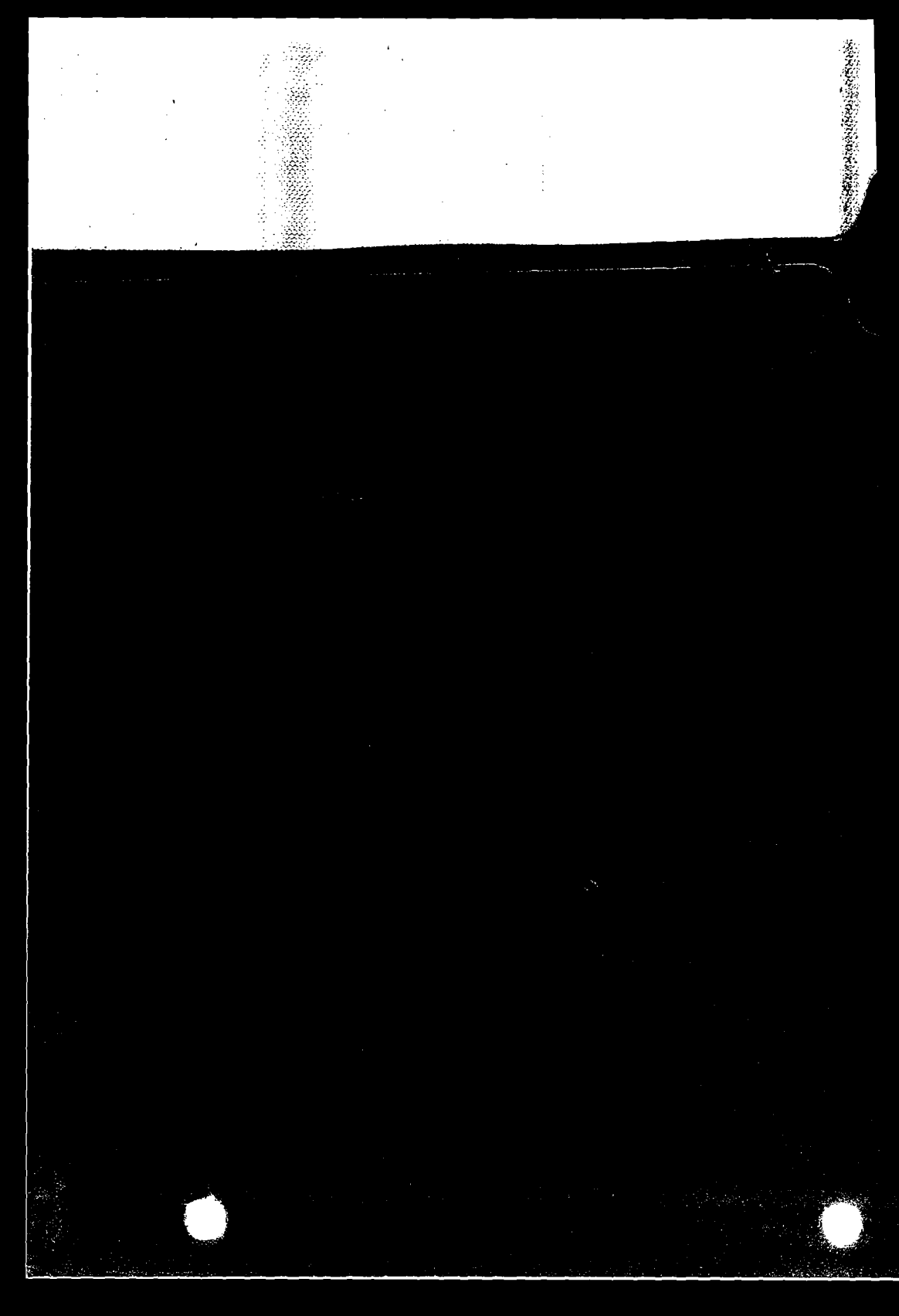
60-780 Poznań, ul. Grunwaldzka 6



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Dioxin not cause of poor health, 3 doctors say

Testimony in Vertac trial points to diabetes as the likely cause of worker's physical problems

By Gregg Jones
Gazette Staff

Three area doctors testified Monday in a federal dioxin exposure trial that diabetes, rather than dioxin, probably had caused the deteriorating health of a former Jacksonville chemical plant worker.

A fourth doctor, Dr. Jim Kizziar, a Little Rock cardiologist, testified that the worker, Billy Honey, asked him in 1984 if chemicals were responsible for his physical problems.

"I told him I didn't think this was the problem at all," Kizziar told the jury.

Lawyers for Hercules Inc. and the Vertac Chemical Corp., the defendants in the dioxin exposure lawsuit, spent the trial's 21st day trying to buttress their argument that dioxin is not responsible for the plaintiffs' health problems.

The plaintiffs, all of whom are former Jacksonville plant workers or family members, are accusing the chemical companies of fraud and negligence for failing to warn them of the dangers of dioxin.

> *"My conclusions were that I felt his fatigue ... was probably associated with his poor control of diabetes."*
>
> —Dr. Raymond Marecek
> Diabetes specialist

They are claiming that dioxin has caused, or will cause, serious medical disorders.

Since Honey's health problems and his exposure to dioxin are the most extreme of the nine plaintiffs, the defense focused its efforts Monday on attempting to explain his deteriorating health.

Dr. Raymond Marecek, a diabetes specialist who examined Honey in 1987, testified that Honey had a family history of diabetes. He also testified that Honey's diabetes appeared to be poorly controlled.

A neurological exam showed that Honey suffered from impaired sensation in his feet and toes, possibly as a result of diabetes, Marecek testified. He also testified that Honey's use of chewing tobacco could have exacerbated his diabetes.

"My conclusions were that I felt his fatigue ... was probably associated with his poor control of diabetes," Marecek testified.

During cross-examination, Marecek testified that he was "not aware of any information" that dioxin could cause, or contribute to, nerve damage.

Previously, expert medical witnesses for the plaintiffs testified that dioxin has probably caused Honey's nerve impairment, which includes tingling and numbness in his arms and legs.

Dr. Jan Sullivan, a Little Rock neurologist, testified that Honey was referred to her in 1983 after complaining of pain in his left arm. She testified that Honey suffered from neuritis, an inflammation of the nerves, which she believed was caused by diabetes.

Asked during cross-examination if she knew whether chemicals could cause nerve disorders, Sullivan responded, "They can."

Dr. Stephen D. Holt, a rheumatologist who examined Honey several times in 1983 and 1984, said some of the aches and pains Honey suffered could have resulted from diabetes. He also testified that diabetes causes the type of nerve damage from which Honey suffers.

THE DOWNFALL OF A CEO

THE INSIDE STORY OF BILL BRICKER'S REIGN AT DIAMOND SHAMROCK

KNOWLES/PICTURE GROUP

When William H. Bricker stepped to the podium to answer questions from the press on Feb. 2, it sure seemed that he had some explaining to do. Diamond Shamrock Corp. had just announced that Bricker was stepping down as chief executive officer after launching the floundering company's third major restructuring in less than three years. Now it was time to sum up Bricker's decade at the top.

It was time to explain how a once-profitable chemical company with a modest oil operation and a solid future became a debt-ridden energy conglomerate with large, persistent losses. Time to explain why Diamond's shareholders missed out on Wall Street's most stunning rally in a generation (charts). And time to explain why, three times in two years, Bricker and his board of directors had rejected offers to buy the company. What's more, each successive offer was worth less than the one before. Even news of Bricker's resignation and the latest reorganization plan had failed to push the stock above the $15 a share that raider T. Boone Pickens Jr. was offering for 20% of Diamond's stock.

Bricker didn't flinch. "I am proud of the value we have delivered to shareholders," he said calmly. "I have completed my challenge. I have completed my assignment. If this doesn't make you

DIAMOND'S BUMPY DECADE

1976 After a quick, seven-year ascent through the corporate ranks at Diamond Shamrock, Bricker, 44, becomes the company's chief executive. Diamond earns $140 million on sales of $1.4 billion; its stock ends the year at $34 a share.

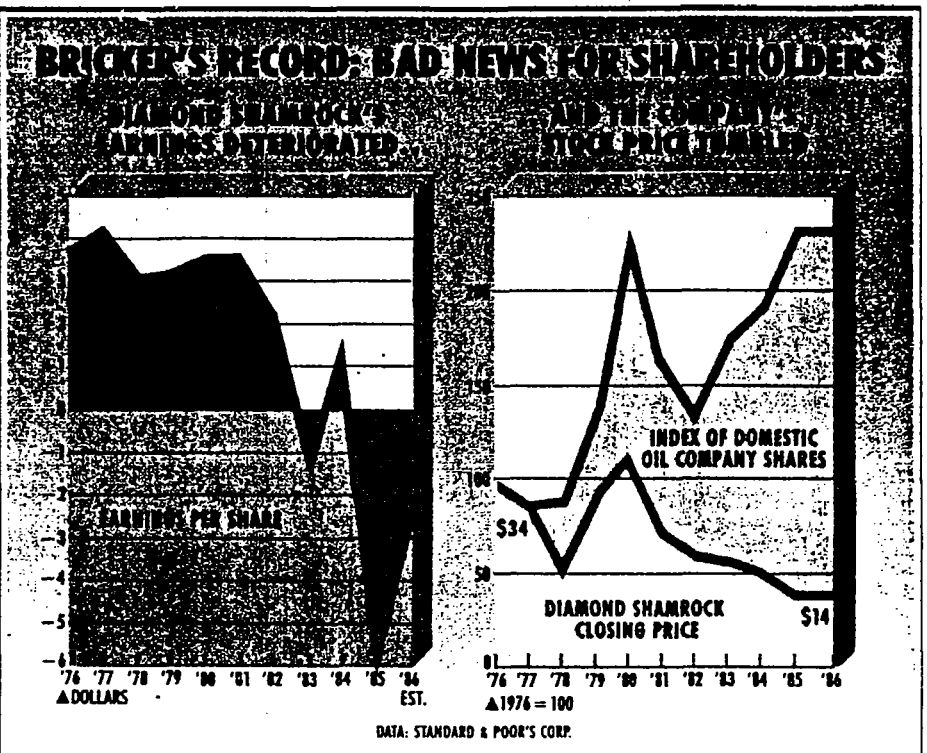
1979 Bricker sets out to make Diamond a major energy company. He buys Falcon Seaboard, a coal producer, for $250 million and moves the corporate headquarters to Dallas from Cleveland.

proud as a professional manager, I don't know what would. If I stayed on for 20 y[illegible] I'm not sure I could top this."

[illegible]was a sadly familiar performance to people who watched Bill Bricker shoot up the ranks at Diamond, first by deftly managing its chemical business, later by serving an apprenticeship as president. Raymond F. Evans, Bricker's mentor and a predecessor as CEO, liked Bricker's canny instincts. Evans especially admired his affinity for the team spirit and participatory management that Diamond prized. But moving up that one rung on the corporate ladder in 1976 made all the difference. "He changed 180 degrees when he became CEO," Evans complains. "He just became a different guy. I guess his ego got him."

COMPLIANT BOARD. What Bricker wanted was a big-league energy company. And by the time he was done, Diamond had the trappings of the status he aspired to: a lavish 12,000-acre ranch on the Texas prairie, a $1 million box at the Dallas Cowboys' home stadium, and a fleet of airplanes to whisk him and his directors around the world. Bricker's compensation was lavish, too. In 1985, he earned $891,700 in cash compensation before taking pay cuts two years running. But Diamond's profits failed to measure up to Bricker's aspirations.

[illegible]ree-month investigation by this ma[illegible]zine, including interviews with more than 40 former and current Diamond executives, reveals deep management problems. Under Bricker and a remarkably forgiving board, Diamond took unusual risks for its size. Write-offs associated with the purchase of Natomas Co. and with a dry hole in Alaska's Beaufort Sea cost some $800 million. By comparison, sales never topped $4.5 billion annually and now are only $2.5 billion. Bricker didn't sway from his goal, but his tactics for getting there became erratic. In 1978, for instance, he sold Diamond's gasoline service stations to Sigmor Corp. In 1983, he bought Sigmor back for $162 million. In the most recent restructuring, Sigmor's refineries and stations are being spun off from the exploration and production unit that will be the core of the new company.



In some cases, Bricker and other directors faced potential conflicts of interest involving Diamond and their personal investments. In one instance, Diamond purchased a stake in a prized bull partially owned by Bricker. In another, a 50%-owned Diamond unit invested in a biotechnology company partially owned by a Diamond director. In some of his dealings, Bricker's business judgment may have been influenced by friendship (page 79).

In the end, Bricker, 55, offered to step down in an effort to allay any concerns that he had become a big part of the problem at Diamond, one insider says. But whether his resignation or Diamond's new plan will lead to better results remains to be seen. After the breakup, each holder of 100 shares of the current Diamond will own 100 shares in a company with oil, natural gas, and coal production activities and 25 shares in a second company with refining and marketing operations. The latest moves are a more drastic version of a 1985 restructuring, in which Diamond spun off its oil-drilling and production operations in the Gulf of Mexico. The price of shares in both companies has dropped since then.

The centerpiece of the breakup is a $300 million investment in a new issue of Diamond convertible preferred stock by Prudential Insurance Co. The money will be used to buy back 20 million common shares, or about 18% of the common outstanding, at $17 a share. In return, Prudential gets an annual dividend amounting to 9.75%, three seats on Diamond's board, and veto power over any takeover bid.

Pickens couldn't seem to resist one last effort to foul up the deal, however. On Feb. 4 he made Diamond an offer it seemed certain to refuse: $15 a share in cash for the company.

For Bricker, the world of raiders,

[illegible]81 In a second expansion move, Bricker buys Amherst Coal Co. for $220 million. Diamond posts record earnings of $230 million on sales of $3.4 billion. Investors are impressed, and Diamond's stock nearly matches its all-time high of $40.

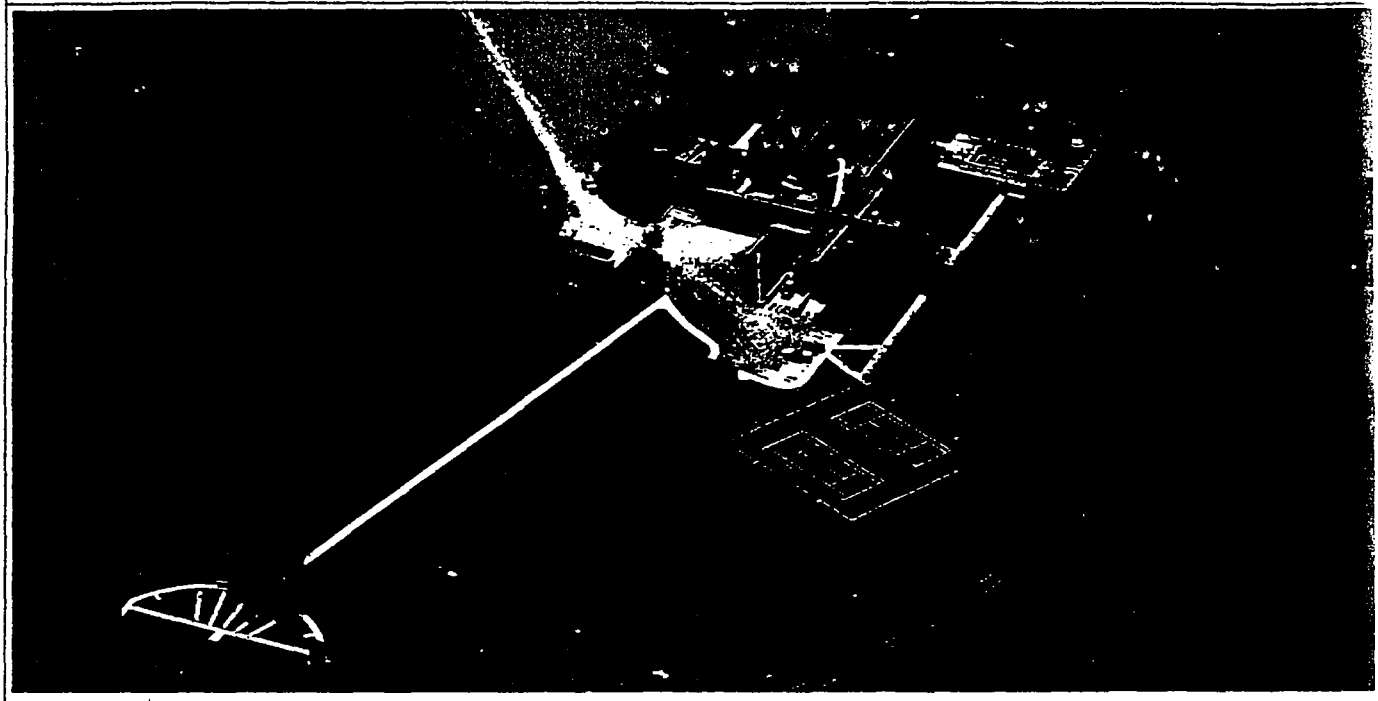
[illegible]82 The combination of a recession and [illegible]wer prices clobbers energy [illegible]mpanies. Even though Diamond's [illegible]arnings fall 35%, to $150 million, the company spends $161 million to buy drilling rights in Alaska's Beaufort Sea. Shareholders suffer as Diamond's stock drops as low as $17.

1983 Bricker buys Natomas, a struggling oil company, for $1.5 billion. Diamond's drilling venture in the Beaufort Sea produces a dry hole and a big writedown. Diamond loses $60 million for the year.

1985 Bricker tentatively agrees to sell Diamond to Occidental Petroleum for Oxy stock worth $28 a share, then backs away. Diamond restructures. Bricker buys back stock and takes $891 million in write-offs, most because of energy prices and Natomas. Losses for the year total $605 million. Stock price at yearend: $14.

1986 Boone Pickens offers to acquire Diamond for limited partnership units valued at roughly $16 per share. For a second time, however, Bricker refuses to sell. Despite another round of buybacks, Diamond shares hover at $14.

1987 Pickens offers to buy 20% of Diamond's stock for $15 a share. Bricker announces a plan to split the company in two, launches another stock buyback, and resigns as CEO. Diamond's stock is unchanged at just under $15 on the news. Mesa offers $15 a share for all Diamond shares.



THE LAVISH ENTERTAINMENT AT THE COMPANY'S $9 MILLION RIVERSIDE FARMS INCLUDED REGULAR PHEASANT HUNTS

takeover defenses, and risky oil ventures was a long way from the western Michigan farm where he spent his boyhood. He studied agriculture at Michigan State University, then sought broader horizons after getting a master's degree in horticulture. He landed a series of international assignments with chemical giant Bayer and still describes his days in Hong Kong and Egypt as the most rewarding of his career. When Bayer brought him back to headquarters in Germany, he recalls, he bridled under the regimentation.

AUTOCRATIC STYLE. In the mid-1960s, he joined Velsicol Chemical Corp. to head international sales. His work there caught the eye of Evans, whose grandfather had founded Diamond Alkali Co., which merged with Shamrock Oil & Gas Corp. in 1967. Bricker went to work for Diamond Shamrock at its Cleveland headquarters in 1969 and quickly set about bolstering its agricultural-chemical businesses.

Seven years later, Bricker, then 44, was a popular choice for chief executive. He got off to a strong start by buying new technology that made Diamond's plants more efficient. He also dumped several plastics businesses that were under pressure because of rising oil prices.

By late 1978, though, Bricker's autocratic style and bold ideas were alienating some of his former supporters. More important, he was getting Diamond into deals that would come back to haunt him. Bricker figured the outlook for energy prices meant that Diamond should plunge into production of oil, gas, and coal. So he proposed buying Falcon Seaboard Inc., a coal producer. But Evans and other directors opposed the deal—so strenuously that the board sent Bricker back to renegotiate. He got the price down to $250 million in stock, which the board accepted. Even then, however, Evans and at least one other director opposed the deal.

Evans eventually resigned when Bricker persuaded the board to move the company's headquarters from Cleveland to Dallas, to be closer to the oil patch. "I had the feeling from personal contact that he wasn't going to listen to anyone," Evans says. As old-timers on the board either resigned or retired, dissent became scarcer. Questions stopped bubbling up from management, as well. Many longtime executives concluded it was useless to fight Bricker and the flamboyant new culture he was creating at Diamond. Says one executive, now retired: "I look at group pictures from those days, and I can see in my face and my posture how little I fit in."

A BULL OWNED JOINTLY BY BRICKER'S PRIVATE RANCH AND DIAMOND SOLD FOR $1.5 MILLION

In 1982 oil prices sagged as inflation slowed sharply. Energy stocks took a pasting, and Diamond, whose earnings fell 35%, was especially hard hit. But Bricker was still stalking a big oil acquisition. That search led him to San Francisco-based Natomas, a $1.6 billion oil and gas producer. Its shares were selling at 15½—one-third of their price during the oil boom. Earnings were off, too, and Natomas had cut its dividend to conserve cash. Bricker's hostile tender offer caught Natomas' chairman, Dorman L. Commons, in a bad spot. Investors loved Diamond's offer of $23 in cash, and Commons couldn't find a more acceptable suitor that would be willing to pay more.

Six days later, Bricker and Commons met alone to resolve an impasse over Diamond's offer. Commons recounts in his 1985 book, *Tender Offer*, that Bricker asked for a counteroffer, and Commons responded with a proposal that seemed to him out of the question. Commons asked for 1.05 shares of Diamond—then trading at about 24—for each share of Natomas. Commons also proposed letting Natomas first spin off its American President Lines Ltd. and

CHARLES HATCHER

other shipping and real estate interests. Commons was incredulous when Bricker ...eed. For his part, Bricker defends his ...otiating skills, arguing that the final price was closer to his initial offer than to Commons' opener.

Analysts figured Bricker paid the equivalent of $12.50 a bbl. for Natomas' oil—or twice what reserves were fetching on the market those days. Diamond's stock dropped sharply in response to the announcement. It is now clear that Bricker paid too much—by a wide margin. Diamond has already logged a loss of some $600 million because it had to write down the value of Natomas properties in 1985. President Arthur Smith, of oil analysts John S. Herold Inc., recently called the Natomas purchase "one of the worst oil deals of the decade."

Diamond tried to fight back when Wall Street balked at the Natomas deal. The company ran a series of advertisements that pictured its executives as mavericks bucking "the conventional wisdom" of energy retrenchment. Featured prominently in the ads was Diamond's $161 million purchase in 1982 of leases in the Beaufort Sea off the North Slope of Alaska. It wasn't long before the first well, Mukluk I, turned out to be a dry hole—one of the most expensive the industry has ever seen. Bricker had ...eport his first annual loss, a $60 million deficit for 1983.

'PERFECT VISION.' Plenty of oil companies, of course, were taking heavy losses in those days from investments initiated under much more favorable economic assumptions. Still, "the investment Bricker was making was much larger in relation to the size of the corporation," says analyst Andrew Gray of Pershing Securities. "He was betting the ranch, and he lost it." Counters Bricker: "With hindsight, you can get perfect vision."

In fact, Mukluk was Bricker's last high-stakes roll. He began selling assets and cutting expenses in Diamond's first round of retrenchment during 1984.

Despite the financial pressures on the company, its layoffs and asset sales, Diamond's lavish executive perks seemed immune from the cost-cutting. One example: Riverside Farms, the company ranch outside of Hamilton, Tex. Bricker defends it as typical of the hunting camps favored by oil companies. But Riverside Farms is far more than that. The local tax assessor values the sprawling ranch and its spacious lodge at $9 million.

Besides raising Black Angus cattle to defray expenses and making pecan brit... ...r guests, Diamond used the ranch t... corporate meetings and entertainment. Regular visitors included Sir Richard Musgrave, a professional shoot manager whom Diamond flew in regularly from Ireland to organize English-style

DID BRICKER GIVE A LITTLE TOO MUCH HELP TO A FRIEND?

Bill Bricker's management style was unusual in many ways, but nothing is more curious than his apparent eagerness to do deals with Vittorio de Nora, a 75-year-old Italian businessman. De Nora, an innovator of chemical production processes, first came into contact with Diamond Shamrock Corp. in the 1950s. By 1973, he owned 4% of the company's stock and sat on the board. But fellow directors asked him not to stand for reelection the next year because he was trying to do business with competitors.

Bricker and de Nora, however, are longtime friends, and the company's relationship with de Nora didn't end when he left the board. Two transactions are especially unusual: In one, Diamond sold a subsidiary to de Nora at what appears to be an attractive price. In another, Bricker involved Diamond in de Nora's efforts to sell a business.

In 1982, Diamond and de Nora set up a joint venture called Eltech Systems Corp. to make chlorine production equipment. Two years later, however, Bricker staged an about-face: Diamond sold its interest in Eltech to de Nora. A Diamond attorney explains that the decision to sell came because "the chemical industry was flat on its back."

Even so, de Nora got what looks like a bargain. He paid $108 million, or $13 million less than the value carried on Diamond's books. Eltech, what's more, had considerable potential. It earned $29 million on sales of $64 million in 1981. A former Diamond executive argues that the company, where Bricker is now an unpaid director, is a guaranteed moneymaker. "It is a near-monopoly," he says.

PRESSURE. The relationship between Bricker and de Nora also crops up in transactions involving Vertac Chemical Corp., a Memphis producer of agricultural chemicals. De Nora bought Vertac in 1980, after Bricker introduced him to the men who were selling it. But later Bricker seemed unusually interested in helping his friend sell out.

Vertac, in fact, may have sparked a conflict at SDS Biotech Corp., a joint venture between Diamond and Showa Denko, a Japanese company. Bricker wanted SDS to acquire Vertac, even though SDS managers say the price was too high. But one of the joint venture's officials says Bricker pushed the issue to the top, and that at a 1984 meeting Showa Denko's chairman rejected the purchase. The official says he was told that Bricker answered with an ultimatum: If SDS did not buy Vertac, then the joint venture was over.

Bricker remembers the meeting differently. He says that Vertac wasn't discussed. The company, by his account, was just one of a slate of SDS acquisitions he recommended. "Vertac was turned down," Bricker says, "and that was the end of it."

Diamond, however, soon bought out its Japanese partner—creating new problems. With cutbacks elsewhere, executives say they didn't want to confuse analysts and make them think Diamond was expanding in biotech. So Diamond used a shell corporation called Vanderbilt Development Corp. to hold the additional SDS interest.

Diamond executives immediately began trying to sell the business. Late in 1986, they found a buyer—Fermenta, a Swedish pharmaceutical company. But

> He involved Diamond in deals that may have benefited Vittorio de Nora

Fermenta, as it turns out, bought more than SDS. It acquired Vertac from de Nora at almost the same time. Diamond's 1985 annual report says only that Diamond and "its joint-venture partner" sold SDS.

These events raise troubling questions. Did Bricker jeopardize a relationship with Japanese partners and pull out of a joint venture just to help a friend? Was Diamond's sale of SDS somehow linked to Fermenta's purchase of Vertac from de Nora?

As for de Nora, his deal quickly soured. He took stock instead of cash and wound up owning 14% of Fermenta. But a scandal soon erupted. Fermenta's founder, Refaat El-Sayed, had falsified academic credentials, and there were bookkeeping improprieties at his company. Fermenta shares lost about 90% of their value, and the company was delisted by the Stockholm Stock Exchange in January.

When BUSINESS WEEK caught up with de Nora in Geneva, he declined an interview. "I have been retired for 10 years," he explained.

By Todd Mason in Dallas, with bureau reports

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pheasant hunts for customers and Diamond executives. Says Bricker: "If you're going to take time to entertain a customer, you try and do it in such a way that it's not an ordinary event."

In 1984, with earnings on a short-lived rebound, Diamond continued to entertain in style. During the Republican National Convention, company-chartered helicopters ferried guests to Riverside for a media reception honoring Nevada Senator Paul Laxalt. The company says that the reception was its contribution, as a corporation in the host city, to the convention.

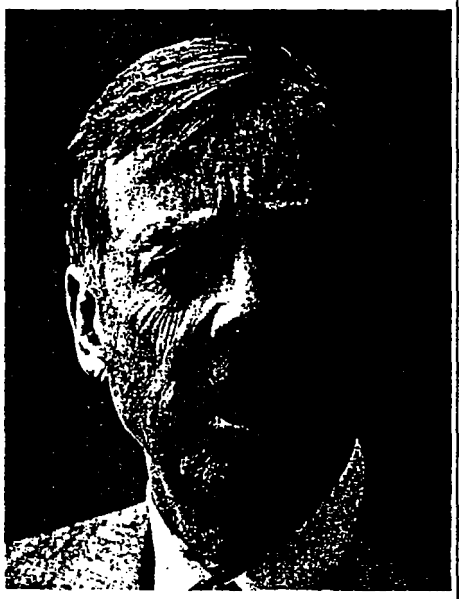
As earnings fell, Diamond cut back its original fleet of five planes to three, including a 727 that is now up for sale. But the company at times augmented its own fleet with chartered planes. Diamond chartered a Boeing 707 to help fly directors and their wives to Indonesia with a side trip to Hong Kong in 1984. In other years, there were similar flights to Europe, Alaska, and Brazil. Diamond says that many multinational companies hold meetings overseas. Not all bring officers and spouses, however. "We go when we have something major in the offing," explains Bricker.

NO COMMENT. Bricker kept company planes on call for personal trips, too. Over a two-year period, Diamond's former manager of advertising, Donald Yeskoo, says he flew several times with Bricker from Dallas to Bricker's family ranch in Montana, often in the company of Mrs. Bricker and one of the couple's three sons. Diamond says that the board of directors requires Bricker for security reasons to use the corporate jet when traveling. It adds that the company routinely bills employees for services or reports them to the Internal Revenue Service as added compensation.

Bricker's Bear Creek Ranch in Montana brought him into a potential conflict of interest in 1982. Like Riverside Farms, Bear Creek raises Black Angus cattle, and Bricker bought a one-third interest in a bull called High Voltage. Later, Riverside Farms bought a third of the bull, too. Eventually, the animal was sold—at a profit for all parties—for $1.5 million. The transactions were all disclosed in Diamond's Securities & Exchange Commission filings. Diamond policy forbids employees to invest in oil wells, because of the potential conflict of interest. But Bricker says he had no conflict in the High Voltage dealings because he is not directly in charge of Riverside.

Another potential conflict at Diamond involved consultant John T. Kimbell, who is one of the company's outside directors. In 1983, Kimbell extended a $300,000 loan to a new biotechnology company called Amtron Corp., received a stake in the company, and agreed to find venture capital, according to Amtron executives. Kimbell also became Amtron's chairman and president. Former research staffers at a Diamond Shamrock joint venture called SDS Biotech Corp. say the venture invested $300,000 in Amtron in mid-1984, even though staffers recommended against it. Amtron staffers say the money was used, among other things, to repay Kimbell's loan. Former SDS President Allan J. Tomlinson says he knows of no recommendation against the investment. The links between Kimbell and Amtron were disclosed in Diamond's SEC filings. Kimbell did not respond to written and telephone requests for comment.

OCCIDENTAL'S HAMMER would have bought Diamond, but Diamond's board of directors backed out. Now, Oxy's not complaining



BOONE PICKENS' second bid was the blow that forced Bricker's resignation. The CEO called himself an 'easy target' for the raider

For other Diamond directors, the question of where loyalties lay was more subtle. As in many companies, the links among the board members constituted what Pickens scorns as a "good ol' boys club," instinctively protective of their station. LTV Corp. Chairman Raymond A. Hay is a Diamond board member, while Bricker is an LTV director. Diamond, which sold coal to LTV, is one of the creditors now seeking repayment under LTV's Chapter 11 reorganization in bankruptcy court. Bricker was on the board of AMF Inc. until Irwin L. Jacobs took the company over, and former AMF Chairman W. Thomas York is still a Diamond board member.

What particularly irks at least one shareholder is how small a stake the officers and directors hold in the company. According to the latest SEC filings, they owned fewer than 75,000 shares outright, or less than one-tenth of one percent of total shares outstanding. At last report Bricker himself owned 35,700. Comments former director Fred S. Strauss, who says he still holds "in excess of" 100,000 shares: "I would rather have a company where the people on the firing line have their money at stake."

If the officers and directors held larger stakes, Strauss figures the shareholders would have come out better in the series of chances to sell the company. The first was in early 1985, when Bricker negotiated a tentative deal with Chairman Armand Hammer of Occidental Petroleum Corp. to have Oxy acquire Diamond. The deal involved a simple swap of one share of Occidental, then worth $28, for each share of Diamond, then worth $17. The companies announced that a deal was in the works.

NO REGRETS. Oxy's stock fell by 4½ points in response to the deal: Investors feared that Diamond would sap Oxy's earnings. Bricker had failed to negotiate a "collar," or range, for an acceptable value of Oxy stock, so the Diamond directors scotched the deal the very afternoon it was announced. Recently, Oxy traded at about 32, some 17 points higher than Diamond stock. The deal still rankles Oxy executives, but they do not regret its demise. "Diamond Shamrock would have been a terrible drag on our stock," says one.

Diamond followed up the aborted deal with sizable stock buybacks, and Bricker jettisoned whole divisions to help finance them while furthering his strategy of becoming purely an energy company. Curiously, Bricker was also in the market to buy. In late 1986, only months

(TOP) GEORGE ROSE; (BOTTOM) BRUCE HOERTEL

after selling its chemical business, Diamond sent a team to Sweden to look into the purchase of Fermenta, a chemical company that had been shaken by disclosures of improper bookkeeping. Nothing came of the trip.

Meanwhile, Bricker had his hands full back home. In November, Pickens offered to buy Diamond for units in his Mesa Ltd. partnership. The offer was valued at $16 a share, compared with Diamond's $13.50 at the time. Diamond's board rejected the offer as inadequate. Pickens refused to go away, however. In January he came with another bid, this time for 20% of Diamond's shares at $15 a share. Diamond officers had no doubts that Pickens would wind up with an even larger stake and make life miserable for the company, even though antitakeover provisions would kick in if he moved above 25%.

The board assembled in Dallas on Thursday, Jan. 29. Pickens' offer was due to expire in six days, and specialists from Diamond's investment banker, First Boston Corp., made a presentation on the breakup and the Prudential investment. According to one insider, Bricker asked the bankers to estimate how much his staying on would affect the value of the deal. The bankers told him the company would be worth more if he resigned.

FINAL DETAILS. Bricker offered his resignation to the board. "My staying makes it easy for Boone to turn a proxy fight into a personal fight, and on that basis he could possibly win," one source quoted Bricker as saying. "I'm an easy target for him." The board assembled again over the weekend to iron out final details and accepted Bricker's resignation. Bricker declined to be interviewed by BUSINESS WEEK after he resigned.

Roger R. Hemminghaus, 50, who will head the refining and marketing spinoff, has long run those operations, which are profitable. Charles L. Blackburn, 59, a Shell Oil Co. executive who joined Diamond recently, is regarded as a no-nonsense manager who could shake up the unprofitable exploration operations.

With a heavy investment and three seats on the board of Blackburn's company, Prudential is unlikely to be as protective of the status quo as current Diamond board members are. But Pickens' latest offer increased the pressure on Diamond to sell its breakup to shareholders. Diamond's stock did not move after the deal was announced. If the estimates by Diamond's investment bankers are accurate, Bricker's departure may have at last created the shareholder value that eluded him for so long.

By Todd Mason in Dallas, with G. David Wallace in New York and bureau reports

Permit No. [illegible]

[illegible] MISC.

February [illegible]

Mr. Kenneth J. Howard
Manager of Manufacturing
Vertac, Inc.
Post Office Box [illegible]
Jacksonville, [illegible]

Dear Mr. [illegible]:

This will acknowledge receipt of your letter dated January [illegible] 1979 advising that Transvaal's operations will now be known as Vertac Inc. Jacksonville Plant.

I note that all pertinent staff members within our Department received copies of your communication however I will remind them to follow through to assure that proper changes are effected.

Sincerely yours,

Jarrell E. Southall
Director

bec

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VERTAC CHEMICAL CORPORATION
24th Floor • 5100 Poplar • Memphis, TN 38137 • 901-767-6851 TELEX 53527

January 30, 1985

To: All Vertac Employees

Over the years we have worked together on many projects which have enhanced Vertac's position as a major supplier to the chemical industry, and I am pleased to announce that we are evaluating another such venture.

We have entered into discussions with SDS Biotech Corporation regarding their possible purchase of Vertac's stock. SDS is a worldwide agricultural chemical company with excellent technical and financial resources; and, if we can get together, there is much that SDS and Vertac could accomplish. I feel very good about the prospects that this transaction could hold for all employees and will advise you when we know something more definitive.

It will take some time to complete all of the legal reviews and negotiations surrounding such a venture. Meanwhile, it will be business as usual for both companies.

C. P. Bomar, Jr.
President and
Chief Executive Officer

CPB:ap

18798

Nerve conduction velocity studies of workers exposed to phenoxy herbicides

Raymond Singer, Ph.D., Marion Moses, M.D.
José Valciukas, Ph.D., Ruth Lilis, M.D.
and Irving J. Selikoff, M.D.

Report to the
National Institute of
Environmental Health Sciences
May 15, 1981

ENVIRONMENTAL SCIENCES LABORATORY
MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK

NERVE CONDUCTION VELOCITY STUDIES OF WORKERS EXPOSED TO PHENOXY HERBICIDES

by

Raymond Singer, Marion Moses, José Valciukas, Ruth Lilis and Irving J. Selikoff

Environmental Sciences Laboratory
Mount Sinai School of Medicine
New York, New York

ABSTRACT

Conduction velocities (NCV) of the median motor, median sensory, and sural nerves were measured in 56 workers employed in the manufacture of 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 2,4-dichlorophenoxy acetic acid (2,4-D). Mean age was 35 years and mean duration of employment was 7 years. The control group consisted of 25 subjects without exposure to neurotoxic agents. When compared with controls, slowing was noted in the sural nerve (mean = 34.0 vs. 40.1 m/sec, $p<0.02$). All values were then adjusted for age and temperature and transformed to Z values (mean=0, standard deviation=1), whereupon slowing was seen in the sural (-2.21 vs. -0.52, $p<0.0001$) and median motor nerves (0.19 vs. 0.91, $p<0.03$). Duration of employment was significantly correlated with slowing of sural velocity ($r=-0.40$, $p<0.004$). Altogether, 46% of the study group had one or more abnormal nerve conduction velocities, versus 5% of the control group ($p<0.001$).

ACKNOWLEDGEMENTS

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NERVE CONDUCTION VELOCITY STUDY OF WORKERS EXPOSED TO PHENOXY HERBICIDES

by

Raymond Singer, Marion Moses, José Valciukas, Ruth Lilis
and Irving J. Selikoff
Environmental Sciences Laboratory
Mount Sinai School of Medicine
New York, New York

Introduction

The phenoxyaliphatic acid herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) have been widely used in maintenance of right of way and in forest, range and crop management for over 30 years. These compounds are readily metabolized and excreted (Kohli et al., 1973; Saverhoff et al., 1977) and their acute mammalian toxicity is low. The LD50 of 2,4,5-T is reported to be 300 mg/kg and 100 mg/kg in the rat and dog respectively and that of 2,4-D ranges from 300 to 1000 mg/kg in rats, guinea pigs and rabbits (Herbicide Handbook, 1979). However during the manufacture of these herbicides undesirable byproducts such as chlorinated dioxins have been found to contaminate the final products. 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7, 8-TCDD) which contaminates 2,4,5-T is thought to be the most toxic contaminant. 2,3,7,8-TCDD has an LD50 of 0.6 µg/kg in the guinea pig and is an animal carcinogen and teratogen in very low doses (Huff et al., 1980). 2,4-D may also be contaminated with chlorinated dioxins. However, the highly toxic 2,3,7,8-TCDD was not found in 2,4-D (Norstrom et al., 1979). Recently some Canadian formulations of 2,4-D have been found to be contaminated with 1,3,6, 8-TCDD (Cochrane et al., 1980).

A 1:1 combination of the n-butyl esters of 2,4,5-T and 2,4-D comprised Agent Orange, a defoliant widely used by the U.S. military in South Vietnam from 1965 to 1970 (Young et al., 1978). In 1971, the U.S. Environmental Protection Agency suspended most food crop uses of 2,4,5-T and in 1978 most other uses were suspended based on unresolved questions regarding effects of low-level exposures on human health. Proceedings to determine if the suspension should be made permanent continue and as yet no decision has been reached (U.S. Environmental Protection Agency, 1978). 2,4,5-T is no longer being manufactured in the United States.

Human Health Effects

There have been many reports of adverse effects on human health from exposure to 2,3,7,8-TCDD in relation to industrial accidents (Huff et al., 1980). The first known accident occurred in the United States in 1949 when a trichlorophenol reactor exploded. Similar accidents occurred in 1952 and 1953 in Germany, in 1963 in Holland and in 1968 in England. In 1976 an explosion occurred at a chemical plant in northern Italy, resulting in release of a toxic cloud over the surrounding community. Evacuation of the town of Seveso which was heavily contaminated with 2,3,7,8-TCDD was ordered (Homberger, 1979). Health effects are also known to occur from the manufacturing process itself when overexposure occurs unrelated to an explosion, most notably in the United States in 1962 (Bleiberg et al., 1964) and 1964 (May, 1977), and in Czechoslovakia in 1965 to 1968 (Hradecka-Vrbickova, J., 1981). Adverse health effects have also been alleged in Vietnam veterans and in south Vietnamese exposed to Agent Orange

but few data are available for evaluation of the problem and it is not yet known whether 2,3,7,8-TCDD is a factor in the reported effects.

While there is no pathognomonic syndrome related to exposure to 2,3,7,8-TCDD, effects on the skin, liver and nervous system have been widely reported in workers exposed in industrial accidents (Huff et al., 1980). The most consistently reported effect has been chloracne, a refractory follicular dermatosis of varying extent and severity depending on the duration and amount of exposure. Central and peripheral nervous system effects have also been widely reported and will be reviewed below.

Ashe and Suskind (1950) examined four workers who developed chloracne following exposure to the contents of a overheated reactor during the manufacture of trichlorophenol for the production of 2,4,5-T. Irritability, nervousness, insomnia, loss of libido, and impotence were reported. Symptoms of peripheral neuropathy included pain and weakness in the lower extremities. Histopathologic examination of several small cutaneous nerves in one case showed destruction of myelin sheaths and nerve fibers with replacement by connective tissue. In a followup study (Suskind, 1953) of 11 affected workers exposed at the 1949 accident and 25 affected workers from the 2,4,5-T production area, 35 of whom had shown chloracne, aches and pains associated with peripheral neuropathy were found in 27 cases. Fatigue, nervousness, irritability, and decreased libido were also found.

Bauer et al. (1961) studied workers who had developed chloracne in the manufacture of 2,4,5-T. Fatigue, muscle weakness and pain, particularly in the proximal muscles of the lower extremities, were prevalent symptoms. A few cases of paresthesia and hypesthesia--sensory neuropathy--were found as well as inability to concentrate, memory deficits, sleep disturbances including increased somnolence, decreased drive, and intolerance to alcohol.

Poland et al. (1971) studied 73 current employees at a 2,4,5-T and 2,4-D manufacturing plant, 13 of whom had chloracne. An increased prevalence of neurological findings was not reported. A positive correlation between severity of chloracne and score on the hypomania scale of the Minnesota Multiphasic Personality Inventory was found.

Kleu and Goltz (1971) conducted a followup study of ten workers acutely exposed to TCDD 15 years earlier and reported fatigue, muscle weakness, decreased libido, alcohol intolerance, and memory loss.

Goldman (1972) studied 42 workers involved in an accidental venting of steam during the manufacture of trichlorophenol. Seven showed signs or symptoms of central nervous system (CNS) involvement, and 3 of polyneuropathy.

Pazderova-Vejlupkova et al. (1981) examined 55 of 80 workers employed at a plant manufacturing 2,4,5-T who were exposed from 1965 through 1968 and were examined during the years 1967 to 1973. Fatigue, sleep disorders, headache, and sexual dysfunction were reported. Peripheral

nervous system symptoms included muscle weakness and pain in the lower extremities. Electroencephalographic studies were conducted in 34 workers. Wave patterns were abnormal in 27% and borderline in 21%. Motor and sensory electromyographic studies were performed on 55 workers using the upper limb and its contralateral lower limb, with abnormal findings in 31% (17 cases). On the basis of severity of CNS symptoms, a subsample of 36 workers were examined by a psychiatrist, who characterized 35 as "neurasthenic" or "depressive". Mild Schwann cell pathology was found at autopsy in the most severely affected worker.

Boeri et al. (1978) utilized nerve conduction velocity in an investigation of neurological dysfunction resulting from the chemical exposure associated with the Seveso accident. They compared two groups which differed in proximity to the site of the accident; 470 from the closer zone and 152 from the zone at lower risk. NCV of the ulnar and peroneal motor nerves was measured. A high prevalence of abnormalities was found in both populations (19.8% versus 13.2%). The authors attributed the increased prevalence in part to the prior exposure of the less heavily exposed population to chemicals associated with the industries of the area: wood-working, metal-working, and chemical industries. Still, a greater prevalence of polyneuropathy was found in the group living closer to the site of the accident.

A followup examination of the Seveso population was recently reported (Boeri, 1980). A subgroup of the exposed population (N=277) was compared with residents of an unexposed town (N=350) for signs and symp-

toms of peripheral neuropathy. Motor nerve conduction velocity was measured on the ulnar and peroneal nerves. A 4.5% frequency of peripheral neuropathy was found in the Seveso population for which no other etiology (such as diabetes) could be found. A significantly greater prevalence of peripheral neuropathy was found in the Seveso population than in the control group. Sensory nerve velocities were not assessed.

A few reports are available concerning the human health effects of 2,4-D. Goldstein (1959) found severe sensory and motor disorders in three cases of percutaneous exposure to 2,4-D used as a spray. Cases of neuropathy following exposure to 2,4-D have also been reported by Todd (1962), Berkeley et al. (1963), Berwick (1970), and Wallis et al., (1970). In all of these cases the exposure was to the already formulated product. Chloracne has not been reported with exposure to 2,4-D. The only reports in which chloracne had been found in 2,4-D workers occurred with concomitant exposure to 2,4,5-T (Bleiberg, 1964; Poland, 1971).

Animal Studies

While there have been a large number of studies of chlorinated benzo-p-dioxins and related compounds in experimental animals, very few have focused on the nervous system. Elovaara et al. (1977) administered a single intragastric dose of 2,3,7,8-TCDD to rats, and found anomalous CNS function in some of the treated rats. Gross et al. (1978) found that 2,3,7,8-TCDD administered intraperitoneally or orally to rats resulted in CNS symptoms of irritability, restless ness, and increased

aggression. Bucher (1946) and Hill (1947) found that mice, cats, rabbits, guinea pigs, and monkeys exposed to 2,4-D could develop myotonia, motor disorders, and paralysis of extremities. Desi et al. (1962) found that parental administration of 2,4-D in rats resulted in electroencephalographic abnormalities and decrements of rat performance, associated with pathology in the spinal cord. Elo and Ylitalo (1979) and Way (1969) presented additional histological evidence of nervous system susceptibility to 2,4-D.

Nerve Conduction Velocity

Nerve conduction velocity (NCV) assessment has been used by many investigators in studies of toxic agents such as lead, mercury, and solvents (Spencer and Schaumberg, 1980). NCV has also been used in studies of workers occupationally exposed to neurotoxic agents (Seppäläinen et al., 1978, 1979, 1980; Buiatti et al., 1978). Buchthal et al. (1975) and Behse and Buchthal (1978) have studied the association of slowed NCV with symptoms of neuropathy and histological findings and provide evidence concerning the importance of electrodiagnosis in the evaluation of toxic neuropathies. NCV measurement is well suited for epidemiological studies since 1) the technique is non-invasive 2) it can be performed quickly and accurately on large numbers of people and 3) the results are metric (in continua) and can be related to other metric, continuous variables such as duration of exposure or biological indicators of exposure when available.

An important aspect of NCV assessment is its sensitivity to toxic changes before other signs or symptoms are present. When rats are injected with a neurotoxic agent such as n-hexane, NCV slowing is found before signs of frank neuropathy such as weakness and paralysis appear (Takeuchi et al., 1980). Pre-symptomatic NCV slowing is also found among diabetics before symptoms or other signs of neuropathy (Thomas, 1980). Since NCV slowing is an early indicator of neuropathy, measurement of NCV is increasingly being used in studies of environmental and occupational toxic exposures.

Present Study

In April 1979, chlorinated dibenzo-p-dioxin contamination was found in locations away from a chemical plant producing 2,4,5-T and 2,4-D in Jacksonville, Arkansas, a small community near Little Rock. The source of the contamination was found to be toxic wastes leaking from drums stored above ground at the 93 acre plant site. Levels of 40 ppm of 2,3,7,8-TCDD were found in the wastes and 2 ppb in a sewer system outlet at the plant that discharged into the city system. The problem was investigated by the Arkansas State Department of Health, and the Governor's office ordered production of 2,4,5-T suspended until the health and safety implications of the findings were investigated and evaluated. 2,4-D, however, continued to be produced.

2,4,5-T and 2,4-D had been produced at this plant since 1957. In the late 1960's more than 14 million pounds of Agent Orange (a 1:1 combination of the n-butyl ester of 2,4,5-T and 2,4-D) were manufactured at this site.

In 1974 there was a reactor accident during the manufacture of trichlorophenol resulting in an outbreak of chloracne in the crew assigned to do the clean-up. Except for this episode which involved fewer than a dozen workers, chloracne had not been a serious problem at this facility.

In July 1979 at the request of the Arkansas State Health Department, a health survey of current and former workers at this plant was conducted by the Environmental Sciences Laboratory of the Mount Sinai School of Medicine. While 2,4,5-T production had ceased at the plant in April nonetheless some workers were involved in redrumming of contaminated wastes in leaking drums. All workers were exposed to 2,4-D which was still being produced.

Methods

Subjects: During July 1979, a health survey was conducted of 190 active, retired, and former workers of the plant. All 83 current workers were invited to participate and 76 did so, representing 86% of the active work force. Workers were included for NCV assessment if no positive history of diabetes, neurological disease, or excess alcohol consumption was found in the screening interview. Because of time limitations, NCV was measured in only 55 of these workers (53 active, 2 retirees).

All workers had been exposed to phenoxy herbicides to some degree. Subjects were interviewed regarding exposure to other toxic chemicals,

since some of these might affect NCV. Concomitant exposure to other neurotoxic agents was not found in any of the workers studied. Nine workers were found to have had possibly significant prior exposure to such agents, which included pesticides, solvents, and--in two workers--2,4-D.

The original protocol included examination of workers who had had minimal or no direct exposure to 2,4,5-T or 2,4-D. However, due to unexpected time limitations, this was not done. The electrophysiologic team remained unaware of the exposure history of the individual subjects.

The control group consisted of 17 Environmental Sciences Laboratory staff and eight brake workers examined as part of a survey of asbestos exposed workers (N=25). All subjects were screened prior to testing for exposure to neurotoxic agents, history of diabetes, stroke, other neurological disease, and alcohol use. The brakeworkers were occupationally exposed to solvents. Control subjects were excluded if alcohol consumption exceeded four drinks per day. Exposed workers were interviewed concerning alcohol use and an index of weekly consumption was computed. Only limbs that had suffered no significant trauma were tested. If the back had been injured, or if disc disease or "sciatica" were reported, the subject's sural nerve velocity was not measured.

Conduction velocities of the median motor, median sensory, and sural nerves were assessed. Median motor velocity was measured in 53 cases

and 25 controls; median sensory velocity in 54 cases and 21 controls; and sural sensory velocity in 50 cases and 20 controls. All stimulation was supramaximal. Latency was measured in the motor nerves at the first point of negative deflection, and for the sensory at the peak negative deflection. Waveform measurement was conducted without knowledge of the subject's employment history.

The recording electrode for the median motor nerve was affixed at the center of the thenar eminence over the adductor pollicis brevis, and the reference electrode ringed its tendon of insertion at the thumb. The nerve was stimulated proximally medial to the aponeurosis musculi bicipitis brachii, and distally at the wrist between the palmaris longus and the flexor carpi radialis tendons. The median sensory evoked potential was recorded using a ring electrode around the middle interphalangeal joint of the index finger, and the reference electrode was affixed around the distal interphalangeal joint. The nerve was stimulated at the distal median motor stimulation point. The recording electrode for the sural was a ring electrode shaped into a strip, and taped so that one end touched the lateral malleolus and the distal end pointed towards the posterior tip of the calcaneus. The reference electrode was placed approximately two centimeters distal and parallel to the recording electrode. The stimulation site for the sural varied somewhat, since occasionally the evoked potential could not be detected without varying the stimulation site. The initial site chosen for each subject was slightly distal to the midsection of the two heads of the gastrocnemius. If the evoked potential was inadequately detected, the nerve was stimulated more distally in a line extending from the

initial stimulation site towards the recording electrode. Stimulation and recording procedures were rechecked if the sural evoked response was not elicited. The distance between the stimulation site and the recording electrode was never less than 11 centimeters.

A Teca TE42 Electromyograph with digital averager was used in the field research. The unit had been calibrated by the manufacturer prior to the study. The calibration trace was printed on each EMG trace. The unit remained in calibration throughout the study.

Skin temperature of the limb was measured using a Rochester Electro-Medical thermistor probe applied to the skin surface successively at three points on the arm (the proximal and distal stimulation sites, and at the thenar eminence of the palm), and at two points on the leg (over the stimulation and recording electrode sites). The temperature values were averaged for each limb.

The distance of the nerve segment was assessed using flexible mylar measuring tape. The shortest distance between the points were followed, and the tape measure was in contact with the skin at all points along the surface of the tape measure. Care was taken not to stretch the skin by pressing too hard on the tape measure. Nor was the distance shortened by pulling the tape measure so that contact with the skin was lost. (Relatively short distances can have a large effect on the actual calculation of the NCV for a given nerve). A standard range of one millimeter was acceptable for inter-rater reliability, a procedure conducted on somewhat less than half the cases.

A Disa 1500 Electromyographic System was used for the examination of some of the control subjects. Since it was possible that the two units might not be measuring in concordance, both units were compared using volunteer subjects at the Environmental Sciences Laboratory. Nine sural, two median motor, and one median sensory nerves were used. Each nerve was examined using both the Teca and Disa units, utilizing their respective recording electrodes. The units had been recently calibrated by the manufacturer, and the units were frequently checked. The Teca stimulating electrode was used throughout the study, since indentations remain temporarily on the surface of the skin - this is more convenient for marking the points of stimulation. The correlation between the measurement of the two units, using the same points of stimulation and pickup, was calculated to be +0.97, $p<0.0001$. Therefore, we concluded that the two units were measuring evoked potentials in concordance.

Analysis

In this group, chloracne was infrequent and could not serve as a variable in the analysis.

Limb temperature. Nerve conduction velocity is affected by temperature at the approximate rate of 2 meters per second per degree centigrade (Goodgold and Eberstein, 1978; de Jesus et al., 1973; Halar et al., 1980). Limb temperature is a function of many variables not related to exposure to neurotoxic agents, such as room temperature, outdoor temperature, the amount of clothing worn, blood circulation,

and the subject's age. Limb temperature can vary to the extent that interpretation of significant differences between two groups can be compromised; a mean temperature difference of only two degrees centigrade could lead to false conclusions concerning true group differences.

To reduce the variance produced by temperature on nerve conduction velocity, some investigators warm the limb to a standard temperature. However this is not a practical technique in the field when many subjects must be examined and time is limited. An approach currently used by some investigators (Halar et al., 1980; de Jesus et al., 1973) is the mathematical adjustment of all velocities to that expected at a standard temperature. The equations were derived from experimental manipulation of limb temperature in the laboratory. The equations presented by de Jesus et al. (1973) were used in this study to adjust each velocity to 36 degrees centigrade (Appendix A). This temperature was chosen following the approach of Rosenfalk (1975) who reported norms based upon the heating of limbs to 35-37 degrees centigrade.

Age: Nerve conduction velocity declines with age at the approximate rate of 1.5 meters/second/decade depending upon the nerve examined (Behse and Buchthal, 1971; Nielsen, 1973; Rosenfalk, 1975; Buchthal et al., 1975). Investigators who have considered the effect of age upon nerve conduction velocity usually attempt to match each subject with an appropriate control when comparing two groups. However, under some circumstances the appropriate control data may not be available. An additional shortcoming of the matching procedure is that the control data are based upon a single observation. Assuming linearity of

the age-effect upon conduction velocity, a regression equation can be constructed which is based upon many data points; that is, all subjects in the data pool.

In a recent study of lead-exposed workers, Buchthal and Behse (1979) used regression equations to determine the expected nerve conduction velocity of a theoretical matched control." The mean difference in velocity between the subjects and the regression-produced 'matched control' was calculated. The significance of the difference between the groups was evaluated with the t-test for correlated means.

Z-score standardization: The Z-score transformation is widely used when comparing values from distributions with different means and standard deviations, since after transformation the distributions have a mean of zero and standard deviation of one (Anastasi, 1976). Since the most common distribution of biological parameters follows the normal distribution, the Z - score transformation of biological data has many advantages (Valciukas and Lilis, 1980). The departure from the mean of a measure can be immediately ascertained, since probability of a given Z value is known, assuming a normal distribution. This transformation may also be used to assess the significance of data from individual cases.

All conduction velocity values were converted to Z - values in this study using the following equation:

$$Z = \frac{\text{(observed velocity - velocity predicted on the basis of age)}}{\text{the standard error of estimate}}$$

The velocity predicted on the basis of age is derived from the regression equations presented by Buchthal et al. (1975) for the sensory nerves and Nielsen (1973) for the median motor. Velocity was adjusted to that expected at 36 degrees centigrade, as discussed above (Appendix A).

The study and control groups were compared in two ways: prevalence of abnormalities and comparison of means. The study group was further analyzed by examining velocity as a function of duration of employment. A Z value corresponding with the 0.01 (one-tailed test) or less probability level was designated as an abnormal value.

Results

Forty-six percent of the study group had one or more abnormal nerve conduction velocities, versus 5% of the control group (chi square= 17.1, $p<0.001$). Twenty-four of the 50 sural nerves measured fell below the first percentile, versus 2 of the 20 control sural nerves, using the equation presented by Buchthal et al. (1975). One subject's sural nerve evoked potential was absent.

The effect of alcohol on nerve conduction velocity within the study group was examined. In general, alcohol consumption was low, averaging 2.68 drinks per week. There was no difference ($p<0.39$) in the alcohol consumption of those subjects with an abnormal velocity when compared with those subjects with normal velocities. The correlation of reported alcohol consumption and nerve conduction velocity in the three nerves was not significantly different from zero. Therefore, the effect of alcohol consumption is unlikely to have produced the observed group differences.

Table 1 presents the t-test analysis of the Z-scores of the study versus control populations.

INSERT TABLE 1 HERE

The probability of a one-tailed t value is shown, since the study group is hypothesized to have slower velocities than the control group. The mean velocities of the median motor and sural sensory nerves were significantly slower in the study group than those found in the control group. (The nerve conduction velocity values before the adjustment for age and temperature are presented in Appendix B).

The mean sural velocity Z value of the study group is approximately 2 standard deviation units below that of the control group. This difference corresponds with a mean slowing of approximately 8 meters/second. For the median motor nerve, the mean velocity slowing is approximately 3 meters/second.

An additional t-test analysis was conducted on the study population reduced by removing eight individuals who reported consuming more than 28 alcoholic drinks per week. Also removed was the one case of mild diabetes. The four cases who had reported back injury were included in the cases removed. The results of the t-test are presented in Table 2.

INSERT TABLE 2 HERE

The mean velocities of the median motor and sural sensory nerves were still significantly slower in the study group than that found in the control group, further reducing the likelihood that the velocities were slowed due to consumption of alcoholic beverages.

A correlation coefficient was computed to assess the association of velocity (age and temperature adjusted) with duration of employment. Sural nerve

INSERT TABLE 3 HERE

conduction velocity was highly correlated with duration of employment ($r=-0.40$, $p<0.004$), while the velocities measured in the median motor and median sensory were not correlated with duration of employment.

Further t-test analaysis was performed after removing the nine workers with possibly significant prior exposure to neurotoxic agents. Removal of these cases did not affect the prior findings (Table 4). The correlation of sural NCV with duration also was essentially the same ($r=-0.42$, $p=0.005$).

It was possible that a residual effect of age upon sural NCV remained after the standardization process. Such an effect could have spuriously increased the correlation between NCV and duration of employment. This possibility was explored by computing the partial correlation of sural NCV (standardized) with duration of employment, controlling for age. A significant relationship was still found ($r=-0.32$, $p<0.03$), supporting the previously described negative correlation of exposure with sural NCV.

In theory, the variance of sural velocity can be modelled with greater verisimilitude as a function of age, skin temperature, and duration of exposure, without using statistical parameters derived from calculations based upon other samples. Such a model is expected to reflect more accurately the actions and interplay of the dependent variables within this sample since the parameters are based upon variation within the study sample. This model was tested by computing the multiple correlation of age, skin temperature, and duration of employment with unstandardized sural velocity.

The multiple correlation coefficient was computed to be 0.52 ($p<0.002$), which exceeded the correlation of duration of employment and standardized sural NCV (as described above, $r=-0.40$, $p<0.004$). The t-test for difference between correlated r's showed that the correlation coefficients differed significantly ($t=2.08$, $p<0.05$). As expected, the model using unstandardized sural NCV provided better fit for this data set. When comparing the relative contribution of age, skin temperature, and duration of employment to the multiple correlation coefficient, it was found that the variable contributing most to

the correlation coefficient was duration of employment (accounting for 83% of the total sum of squares). The partial correlation of sural NCV and duration of employment in this model was essentially the same as found with the model using standardized sural NCV.

DISCUSSION

An increased prevalence of abnormal nerve conduction velocities was found among chemical workers exposed to the phenoxy herbicides 2,4,5-T and 2,4-D. Conduction velocity of the median motor and sural sensory was significantly slower in the study versus control group. Slowed sural sensory velocity was significantly correlated with duration of employment.

Neurons are believed to be particularly vulnerable to toxic insults (Thomas, 1980), due in part to their unusual morphology which enable them to transmit precise information over considerable distances. The center of neuron metabolism can reside over a meter from an axon site, resulting in vulnerability to disruption of sustaining processes and measurable decrements of function.

Why would the sural nerve be more affected by exposure to the phenoxy herbicides than the median motor or median sensory nerve? An explanation may lie in the observation that the sural nerve is located farthest from the source of tissue nutrition, the fibers are fewer, have a smaller diameter and less myelination. The sural is thus perhaps more likely to suffer from disruption of normal nervous tissue metabo-

lism and function. Toxic susceptibility of neurons has been linked with both increased fiber length and smaller nerve diameter (Thomas 1980).

This explanation is supported by findings of Behse and Buchthal (1978) who studied 167 patients with polyneuropathy of varied etiology. They determined that the sural nerve is more likely to have abnormal electrophysiological findings than the median sensory, while the superficial peroneal and sural tended to have similar electrophysiological findings.

Boeri et al. (1980) and Pazderova-Vejlupkova et al. (1981) reported that nerve conduction velocity decreased over time in exposed subjects, although it was thought that there was no continuing environmental exposure. Possibly the lipid-soluble TCDD is released over time and continues to affect the nervous system. Therefore, the correlation of nerve conduction velocity and duration of employment would reflect the effects of extended environmental and intraindividual exposure.

In laboratory animals, exposure to 2,3,7,8-TCDD has been associated with pathological changes in the liver, immune, and reproductive systems (Huff et al., 1980) although no reports are available concerning effects on the peripheral nervous system. Since slowed nerve conduction velocity can be an important early indicator of toxic exposure, the relationship of such neurological impairment to adverse effects in other body systems, either concomitant or delayed, is an important clinical and scientific question.

It is of interest that this group of workers examined by us was also exposed to 2,4-D as well as 2,4,5-T and its associated dioxin contaminant. Since 2,4-D is known to be neurotoxic it is possible that some of the abnormal findings here described might have been influenced by this exposure. This possibility is being further investigated by studies of another group of workers who had been exposed to 2,4,5-T (contaminated with dioxin) but not exposed to 2,4-D.

In addition to occupational exposure, important public health questions are also raised regarding environmental exposure to these chemicals. Much public debate and concern was evident among the citizens of Jacksonville in response to the toxic waste problem at the plant, while worldwide concern was stimulated by the episode in Seveso, Italy. In such situations, early detection of adverse health effects can help to provide guidance concerning implementation of preventive and control measures.

REFERENCES

Anastasi, A. (1976). Psychological Testing. (4th edition) MacMillan, New York.

Ashe, W. and Suskind, R.R. (1950). Reports on chloracne cases, Monsanto Chemical Co., Nitro, West Virginia. Dept. of Env. Health, Univ. of Cinn. College of Medicine.

Bauer, H., Schulz, K.H. and Spiegelberg, U. (1961). Berufliche Vergif-tungen bei der Herstellung von Chlorphenol - Verbindungen. Arch. Gewerbephathol. Gewerbehyg. 18:538-555. Reported in IARC (1978). Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans.

Behse, F. and Buchthal, F. (1978). Sensory action potentials and biopsy of the sural nerve in neuropathy. Brain, 101:473-493.

Behse, F. and Buchthal, F. (1971) Normal sensory conduction in the nerves of the leg in man. J. Neurol. Neurosurg. Psychiat. 34: 404-414.

Berkeley, H. and Magee, K. (1963). Neuropathy following exposure to a dimethyline salt of 2,4-D. Arch. Int. Med. 111:351-353.

Berwick, P. (1970). 2,4 - dichlorophenoxyacetic acid poisoning in man. J. Am. Med. Assoc. 214(6):1114-1117.

Bleiberg, J., Wallen, M., Brodkin, R., et al. (1964). Industrially acquired porphyria. Arch. Dermatol. 89:793-797.

Boeri, E. (1980). Cross-sectional neurological study of a TCDD-exposed population in the Seveso area compared with an unexposed population. Unpublished report, Instituto Neurologico, Milano, Italy.

Boeri, E., Bordo, B., Crenna, P., et al. (1978). Preliminary results of a neurological investigation of the population exposed to TCDD in the Seveso region. Riv. Pat. Nerv. Ment. 99:111-128.

Buchthal, F. and Behse, F. (1979). Electrophysiology and nerve biopsy in men exposed to lead. Brit. J. Ind. Med. 36:135-147.

Buchthal, F., Rosenfalck, A., and Behse, F. (1975). Sensory potentials of normal and diseased nerves. In: Peripheral Neuropathy. Edited by P.J. Dyck, P.K. Thomas, and E.H. Lambert. Philadelphia: W. B Saunders 1:442-464.

Buiatti, E., Cecchini, S., Konchi, O., Botara, P., & Bulgarelli, G. (1978). Relationship between clinical and electromyographic findings and exposure to solvents, in shoe and leather workers. British Journal of Industrial Medicine 35:168-173.

Cochrane, W., Singh, J., Miles, W., Wakeford, B., Scott, T. (1980). Analysis of technical and formulated products of 2,4-dichlorophenoxy acetic acid for the presence of chlorinated dibenzo-p-dioxins. Presented at the workshop, Impact of Chlorinated Dioxins and Related Compounds on the Environment, Rome, October 22-24, 1980.

Creso, E., De Marino, V., Donatelli, L., & Pagini, G. (1978). Effette neuropsicofarmacologici della TCDD. Boll. Soc. It. Sper., 54:1592-1596.

Crow, K.D. (1970). Chloracne. A critical review including a comparison of two series of cases of acne from chlornaphthalene and pitch fumes. Trans. St. Johns Hosp. Dermatol. Soc. 56:79-99.9

de Jesus, P.V. Jr., Hausmanowa-Petrusewicz, I. and Barchi, R.L. (1973). The effect of cold on nerve conduction of human slow and fast nerve fibers. Neurology 23:1182-1189.

Desi, I., Sos,J., Sule, F., and Markus, V. (1962). Nervous system effects of a chemical herbicide. Arch. Env. Health 4:101-108.

Elo, H.A. and Ylitalo, P. (1979). Distribution of 2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid in male rats: Evidence for the involvement of the central nervous system in their toxicity. Tox. App. Pharm. 51:439-446.

Elovaara, E., Savolainen, H., Parkki, M.G., Aitio, A., and Vainio, H. (1977). Neurochemical effects of 2,3,7,8 -Tetrachlorodibenzo-p-dioxin in Wistar and Gunn rats. Res. Comm. Chem. Path. and Pharm. 18:3.

Goldmann, P.J. (1972). Schwerste akute Chloracne durch Trichlorphenol-zersetzungsprodukte. Arbeitsmed. Sozialmed. Arbeitshyg. 7:12-18.

Goldstein, N., Jones, P., and Brown, J. (1959). Peripheral neuropathy after exposure to an ester of dichloro-phenoxy acetic acid. J.A.M.A., 171(10):1306-1309.

Goodgold, Joseph and Eberstein, Arthur. (1978). Electrodiagnosis of Disease, 2nd edition. Williams & Wilkins, Baltimore, 1978.

Halar, E.M., DeLisa, J.A., & Bronzovich, F.V. (1980). Nerve conduction velocity: Relationship of skin, subcutaneous and intramuscular temperatures. Arch. Phys. Med. Rehabil. 61:199-203.

Hay, A.W.M. (1977). Tetrachlorodibenzo-p-dioxin release at Seveso Disasters, 1:289-308.

Herbicide Handbook (1979). Weed Science Society of America, 4th Ed. Champaign, Illinois.

Seppäläinen, A., Lindstrom, K., and Martelin, T. (1980). Neurophysiological and psychological picture of solvent poisoning. Am. J. Ind. Med. 1:31-42.

Seppäläinen, A. M., Hernberg, S., & Kock, B. (1979). Relationship between blood lead levels and nerve conduction velocities. Neurotoxicology 1(2):313-332.

Seppäläinen, A. M., Husman, K., and Martenson, C. (1978). Neurophysiological effects of long-term exposure to a mixture of organic solvents. Scand. J. Work Environ. & Health 4:304-314.

Suskind, R.R. (1953). Reports on Clinical and Environmental Surveys. Monsanto Chemical Co., Nitro, West Virginia. Department of Environmental Health, Univ. of Cinn. College of Medicine.

Takeuchi, Y., Ono, Y., Hisanaga, N., Kitoh, J., and Sugiura, Y. (1980). A comparative study on the neurotoxicity of n-pentane, n-hexane, and n-heptane in the rat. Brit. J. Ind. Med. 37: 241-247.

Todd, R. (1962). A case study of 2-4-D intoxication. J. Iowa Med. Soc. 52:663-664.

Thomas, P. (1980). The peripheral nervous system as a target for toxic substances. Chapter in Spencer, P., and Schaumberg, H. (1980). Experimental and Clinical Neurotoxicology, Williams and Wilkins, Baltimore.

U.S. Environmental Protection Agency (1978). Rebuttal presumption against registration and continued registration of pesticide products containing 2,4,5-T. Federal Register 43(78):17116-17157, April 21, 1978.

Valciukas, J. and Lilis, R. (1980). Psychometric techniques in environmental research. Environ. Res. 21:275-297.

Wallis, W. E., Van Poznak, A., and Plum, Fred. (1970). Generalized muscle stiffness, fasciculations, and myokymia of peripheral nerve origin. Arch. Neurol. 22:430-439.

Way, J. M. (1969). Toxicity and hazards to man, domestic animals, and wildlife from some commonly used auxin herbicides. Residue Reviews 26:37-62.

Young, A.L., Calcagni, J.A., Thalken, C.E., et al. (1978). The toxicology, environmental fate, and human risk of Herbicide Orange and its associated dioxin. OEHL TR-78-92, USAF Occupational and Environmental Health Laboratory, Brooks Air Force Base, Texas, October, 1978.

Table 1. Mean nerve conduction velocities of study versus control group (*)

NERVE	GROUP	N	MEAN Z - VALUE	STD. DEV.	T	PROB T (ONE-TAILED)
Median Motor						
	Study	53	0.20	1.5	-1.89	0.03
	Control	25	0.91	1.70		
Median Sensory						
	Study	54	-0.16	1.15	-0.52	0.30
	Control	23	-0.04	0.81		
Sural						
	Study	50	-2.21	1.09	-4.28	0.0001
	Control	20	-0.52	1.62		

*Based upon Z values which represent the subtraction of the temperature corrected observed velocity from the velocity expected on the basis of age, divided by the standard error of estimate.

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Table 2. Mean nerve conduction velocities of study versus control group (*) (**)

NERVE	GROUP	N	MEAN Z - VALUE	STD. DEV.	T	PROB T (ONE-TAILED)
Median Motor						
	Study	44	0.28	1.26	-1.77	0.04
	Control	25	0.91	1.02		
Median Sensory						
	Study	45	-0.10	1.15	-0.22	0.41
	Control	23	-0.04	0.81		
Sural						
	Study	41	-2.17	1.04	-4.13	0.0002
	Control	20	-0.52	1.62		

---

* Based upon Z values which represent the subtraction of the temperature corrected observed velocity from the velocity expected on the basis of age, divided by the standard error of estimate.

** All cases who consumed more than 28 alcoholic beverages per week (N=8) and the one case with slight diabetes have been removed from this analysis.

Table 3. Years of Employment vs. Nerve Conduction Velocity

Adjusted for Age and Temperature

	Median Motor	Median Sensory	Sural
r	-0.04	-0.10	-0.40
p	0.80	0.48	0.004
N	53	54	50

Mean years of employment = 7.0, standard deviation = 6.2, range = 1-28 years.

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Table 4: Mean nerve conduction velocities of study versus control groups.(*,**)

NERVE	N	MEAN Z-VALUE	STD. DEV.	T	PROB T (ONE-TAILED)
Median Motor					
Study	44	0.11	1.17	-2.29	0.01
Controls	25	0.92	1.02		
Median Sensory					
Study	44	-0.23	1.06	-1.01	0.15
Controls	23	0.04	0.81		
Sural					
Study	41	-2.11	1.17	-4.58	0.0001
Controls	20	-0.52	1.02		

---

* Based upon Z values which represent the observed velocity deviated from the velocity expected on the basis of age and limb temperature.

** Nine cases with possibly significant prior exposure to neurotoxic agents removed.

Appendix A. Equations for Temperature and Age.

$$VNMT = VNM \times (10^{(0.018 \times (36-TEMP\_ARM))});$$

$$VMST = VMS \times (10^{(0.018 \times (36-TEMP\_ARM))});$$

$$VSST = VSS \times (10^{(0.018 \times (36-TEMP\_LEG))});$$

$$VNMP = 69.5 - (0.18 \times AGE);$$

$$VMSP = 59.5 - (0.15 \times AGE);$$

$$VSSP = 57.4 - (0.05 \times AGE);$$

$$ZNM = (VNMT-VNMP)/3.4;$$

$$ZMS = (VMST-VMST)/4.6;$$

$$ZSS = (VSST-VSSP)/3.7;$$

where,

VNMT = Velocity median motor temperature adjusted

VMST = Velocity median sensory temperature adjusted

VSST = Velocity sural temperature adjusted

VNM = Velocity median motor observed

VMS = Velocity median sensory observed

VSS = Velocity sural observed

VNMP = Velocity median motor predicted on the basis of age

VMSP = Velocity median sensory predicted on the basis of age

VSSP = Velocity sural predicted on the basis of age

ZNM = Standardized median motor velocity, age and temperature adjusted

ZMS = Standardized median sensory velocity, age and temperature adjusted

ZSS = Standardized sural velocity, age and temperature adjusted

Appendix B. Mean nerve conduction velocities (m/sec) of study versus control group

NERVE	GROUP	N	MEAN AGE	MEAN VELOCITY	STD. DEV.	T	p (*)
MEDIAN MOTOR	Study	53	34.3	57.0	3.26	-0.56	0.29
	Control	25	32.8	57.6	5.20		
MEDIAN SENSORY	Study	54	34.4	47.8	5.36	0.81	0.21
	Control	23	37.2	46.8	4.47		
SURAL (**)	Study	50	34.0	40.3	3.48	-2.05	0.02
	Control	20	40.1	42.8	4.90		

(*) One tailed test

(**) The controls are significantly older for the sural comparison

BOX 69 • JACKSONVILLE, ARKANSAS 72076 • PHONE 501-982-2132

September 13, 1976

Mr. Jarrell E. Southall
State of Arkansas
Department of Pollution Control
and Ecology
8001 National Drive
Little Rock, AR 72209

Dear Mr. Southall:

The method used by Transvaal, Inc. for the determination of levels of TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) in phenoxy-herbicidal acids and esters was provided to you together with other information and a cover letter dated March 15, 1976.

The method of separation of TCDD from waste streams and soil samples depends upon exhaustive extraction of appropriate quantities of the sample with diethyl ether-hexane mixture (2:1) prior to treatment of the concentrated extract as under an ester preparation.

For example:

A. Aqueous waste stream samples

A portion of the waste stream sample, say 2 liters, is rendered alkaline to pH 11.5 and continuously extracted via liquid-liquid extraction for a period of 24 hours using ether-hexane as the extractant. The extract is reduced in volume to near dryness by evaporation in a stream of nitrogen on a water bath and then treated as if it were an ester preparation.

B. Soil samples

A well-mixed portion of the soil sample, say 250 grams, is weighed in a tared dish and permitted to dry to constant weight at 55-60° C. The loss in weight is noted and recorded as percent loss in weight. The dry sample is slurried in ether-hexane mixture and transferred quantitatively to a

(Continued)

Soxhlet cone within an extractor. The extract receiver is charged with more of the ether-hexane mixture and the sample is permitted to extract for a period of 24 hours. The extract is then treated as if it had been an ester sample after reduction in volume as mentioned under Item A above.

Sincerely,

TRANSVAAL, INC.

A. E. Sidwell
Director of Research

AES:ew

HISTORY OF TRICHLOROPHENOL AND 2,4,5-T ACID MANUFACTURE
AT
JACKSONVILLE, ARKANSAS

Trichlorophenol and 2,4,5-T acid have been manufactured in Jacksonville, Arkansas, for the past nineteen years. Another phenoxy-herbicide, 2,4-D acid has been made for a longer period at the same location. However, this is written to provide a history of the production of 2,4,5-trichlorophenol and 2,4,5-T acid. Both of these materials have been the subject of recent and continuing public interest.

As a result of the unfortunate accident in Italy, all manufacturers of 2,4,5-TCP have been reviewing the safety aspects of their operations. The facility in Jacksonville, Arkansas, has safely produced TCP for many years. The chemistry design and operation at this facility is considerably different from that in Italy. Although safety controls and operations here are demonstrably believed to be adequate, additional controls will be installed. These controls will totally contain all safety pressure releases from the TCB reactors and water wash all reaction materials from the released vapors. The added control measures will consist of an additional automatic pressure relief on each reactor, a high pressure blowdown or catch tank, and a water scrubbing tower.

REASOR-HILL CORPORATION

The plant in which these materials are produced in Arkansas was originally a part of the Jacksonville Ordnance plant which was built and operated for the Government in the 30's and, early 40's. The Reasor-Hill Corporation, established in late 1946 by Dr. Lyle O. Hill, a native of Russellville, Arkansas, and the late Mr. Gerald Reasor, then a Chicago business executive, purchased the site from the Government. The corporation first began production of cotton insecticide dust, it later expanded to other types of insecticide production and

finally began making the herbicide, 2,4-D acid and esters, in a well isolated portion of the plant to avoid cross contamination.

Production of 2,4,5-T acid was begun in October 1957 after about a year of laboratory work on the method. Although both 2,4,5-T and 2,4-D are made by an essentially similar process from monochloroacetic acid and an appropriate chlorophenol, 2,4,5-trichlorophenol, from which 2,4,5-T is made, is more difficult to prepare than 2,4-dichlorophenol used in making 2,4-D.

LABORATORY STUDY

Laboratory work by the author and co-workers was concentrated on the preparation of 2,4,5-trichlorophenol as its sodium salt. Though other companies already were engaged in the manufacture of 2,4,5-T acid at that time, relatively little information was available in the form of published chemical literature. However, since one reasonably economical, direct route to prepare the needed trichlorophenol involves the treatment of symmetrical tetrachlorobenzene (TCB) with caustic (NaOH) in methyl alcohol (CH_3OH), this reaction was studied extensively.

Most of the work was done using a Parr laboratory pressure reactor. The reaction needs heat to get started, then requires cooling to control the heat and pressure generated while the primary reaction takes place. Various weight ratios of TCB, NaOH and methyl alcohol were mixed and heated to start the reaction in the Parr reactor. The resulting temperatures and pressures developed during the reaction by the different mixtures were noted. Various lengths of time were employed in ageing after the reaction had subsided to ensure completion. The information obtained was then used to design a method for larger scale manufacture which would minimize potential danger due to temperature and pressure and maximize safe operating conditions and useful product.

It was decided to limit the amount of material to be produced in a plant reactor to a quantity which could be heated to start the reaction but which

also could be cooled successfully in controlling the heat produced during the reaction. Essentially this meant that the reaction vessel must have a volume to internal surface ratio sized to either add or remove heat rapidly in order to insure safety.

INSTALLATION

As a result of the experimental work and consideration of safe operation, Reasor-Hill purchased a 500 gallon vessel designed at 350 PSI and actually tested at 525 PSI. The normal operation proceeded at pressures well below the design pressure. The vessel was fitted with a relief valve to release small amounts of vapor should the pressure exceed the normal working pressure. After the heat generated by the reaction has subsided, which can be controlled by cooling, the material must be aged about five hours. During aging the compound first formed (a methyl ether of TCP) reacts slowly with the alkaline methyl alcohol to form the sodium salt of trichlorophenol, at the same time producing volatile dimethyl ether. Most of the dimethyl ether dissolves in the liquid mixture under pressure, but it has a low boiling point and slowly exerts increased pressure at the temperature employed during the aging process. (Currently the pressures are controlled well below 350 PSI during the aging process, and the dimethyl ether vapors are absorbed in a water scrubber and discharged to waste during distillation, being decomposed by bacteria in the city sewage treatment plant.)

The vessel was also provided with a rupture disc designed to relieve well below the vessel test pressure. This permitted sudden reduction of pressure within the reactor should the pressure during initial reaction rise unduly above the normal working pressure. The pressure reduction allowed rapid evaporation of methyl alcohol which has the effect of sudden cooling of the reactor mass. Such sudden cooling in addition to forced external cooling by water immediately drops the temperature of the reacting mass. The drop in tem-

In addition to the necessary tanks, pumps and piping the vessel was fitted with recording temperature and pressure measuring devices. These recorders were placed so that the operator could know what conditions were present within the reactor at all times after the reaction mixture had been charged to the vessel and fully prepared by checking all valves and bolted closures. The reactor was situated within one of three bays having reinforced concrete walls. The recorders and all operating steam and water valves were placed in the bay furthest away from the reactor.

After a reaction was complete and ageing finished, the reactor was allowed to cool down from the ageing temperature. The pressure thus was reduced so that dissolved dimethyl ether could be vented. The reactor product then could be pumped to a still for the removal of methyl alcohol while adding an equivalent mass of water. When the methyl alcohol had been completely removed, the water solution of sodium trichlorophenate together with sodium chloride and excess caustic was permitted to cool to a temperature that would allow it to remain liquid. This liquid was then pumped to a heated storage tank.

HERCULES INCORPORATED

This reactor and equipment was used by Reasor-Hill without trouble and by Hercules Powder Company (now Hercules Incorporated) who purchased the plant in December, 1961, for several years thereafter. After Hercules bought the plant all insecticide production was stopped at the site. Production was limited to phenoxy-herbicides and various production improvements were made.

Sometime in 1965 Hercules made a decision to increase the capacity of the plant for the production of 2,4,5-T acid. This meant that the capacity for making trichlorophenol had to be increased.

Hercules reviewed the production history of safety and efficacy of the existing procedure for making trichlorophenol. After consideration by their Engineering and Safety Departments two additional reactors of the same design

as the original one were installed. These reactors often were operated round the clock especially during the period that the Government was requiring the material called 'Orange'. ('Orange' was a mixture of about 50-50% by weight of the butyl esters of 2,4-D and 2,4,5-T used by the military to effectively defoliate areas around their installations in Vietnam.) Since that period, as the demand for 2,4,5-T has dropped to that of the domestic and foreign markets, all three of the reactors have been continued in use as required.

TRANSVAAL, INC.

Transvaal, Inc. purchased the plant on October 1, 1971. Under these owners the original method of processing has been continued, with improvements in the handling of potential air or water pollution.

DIOXIN ANALYSIS

The discovery in 1970 that formulations of 2,4,5-T from various sources contain variable amounts of a highly toxic contaminant, TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), prompted the cessation of its use in Vietnam. The use of 2,4,5-T was restricted and a total ban on the use of 2,4,5-T having a "high" dioxin content has been imposed by the U. S. Environmental Protection Agency.

Fortunately investigations by the Aerospace Research Laboratories, U. S. Air Force, published in May 1975, indicate that the dioxin content of 'Orange' supplied to the military from this plant show an average of less than 0.05 parts per million of dioxin. Averaged analyses of samples of 'Orange' from other manufacturers mentioned in the ARL report ranged from 0.12 ppm to 14.2 ppm dioxin. The work done by Aerospace Research employed gas chromatograph-mass spectrum analysis capable of lower concentration determinations than by chromatographic method alone.

This appears to confirm work done in our laboratory covering analysis of pooled retained samples of 2,4,5-T acid produced during the period January 1966 through May 1970. Our findings reported in a letter to Hercules Management dated

January 27, 1971 (copy attached) were to the effect that all samples examined for dioxin by a gas-liquid chromatograph method contained less than 0.1 part per million. However, as a precaution we have continued routine examination of pooled batch samples of 2,4,5-T acid and have never found dioxin present in amounts greater than 0.1 ppm. (After the acid has been converted to an ester the dioxin content should drop because of the increase in mass of the ester.)

Samples of our 2,4,5-T acid examined as a courtesy by a competitive company have also been reported to have TCDD content below 0.1 ppm.

DIOXIN SOURCE

It is the writers firm belief that the source of the dioxin or related dioxins present in the 2,4,5-T acid is the reaction by means of which the trichlorophenol used is formed. Most experts and the Report of the Panel on Herbicides of the President's Science Advisory Committee confirm this view. Our method does not appear to produce dioxin in significant quantity. However we have under way tests of various waste materials and recycled liquors to determine if these might concentrate dixoin and thus be a hazard.

EFFECTS OF EXPOSURE

The writer has worked for twenty years with various phenoxy herbicides both in the laboratory and in the plant. No ill effects have ever been noted. Occasional instances where accidental skin contact with the alkaline sodium trichlorophenate solution, or with raw trichlorophenol dissolved in recycle liquors, has produced local chemical burns on arms or hands. These tend to heal within a short time and the skin appears to be quite normal after healing. This is also true of many plant workers who have from time to time over the years been 'burned'.

A. E. Sidwell

Dr. A. E. Sidwell
Director of Research

18840

September 17, 1976

BOX 69 • JACKSONVILLE, ARKANSAS 72076 • PHONE 501-982-2132

PRESSURE RELIEF FOR TCP

The making of 2,4,5-Trichlorophenol from 1,2,3,4-Tetrachlorobenzene and Methanol is a highly exothermic process requiring temperatures in excess of 100° C. for initiation. To control temperatures and the resulting pressures several knock-downs (application of water to the reactor jacket for cooling) are required. If the knock-downs are not made at the proper time temperature and pressure continue to build further increasing the rate of reaction.

To prevent the loss of reaction control an automatic backup will be used that will make the knock-down if the operator misses. The automatic system will continue to function in the event of electrical failure with an auxiliary battery pack.

If the reactor pressure continues to increase uncontrollably, two, two inch valves can be opened for pressure relief to the scrubber. If the pressure climbs to the maximum working pressure of the reactor, a two inch relief valve will open. At five percent above work pressure the two, two inch valves will open automatically if they have not been opened by the operator previously. At ten percent above working pressure a rupture disc will open emptying the reactor.

The two vent valves and the relief valves empty to the atmospheric scrubber for contacting with water. The rupture discs vent into a blow-down pot with a water quench. If maximum working pressure is exceeded in the blow-down pot a rupture disc opens into the scrubber. Early in the reaction, when it is most likely a pressure runaway will occur, all volital components are extremely soluable in water. Because of methanol use the pressure relief will control the temperature to minimize any undesireable side reactions.

Norman F. Hopkins

NFH:en

18841

State watches Vertac sale for effect on toxin cleanup

JAN 3 1986

BY BOBBI RIDLEHOOVER
Democrat Staff Writer

The state Department of Pollution Control and Ecology expects to be given details within the next few days about the proposed sale of properties owned by the Memphis-based Vertac Chemical Corp., Phillip Deisch, a department attorney said Thursday.

Deisch said PC&E is interested in the proposed sale of Vertac's West Helena plant to Fermenta A.B. of Sweden. The state wants to ensure that the Memphis corporation will be left with enough total assets to clean up and monitor the Vertac Chemical Inc. plant in Jacksonville, which it also owns.

"They indicated they are going to give us the details ... within the next few days," Deisch said of Vertac's proposed sale which will include some product lines and manufacturing plants.

Vertac doesn't have a "specific obligation" to inform the department about sale of its properties, except for the sale of the Jacksonville plant, he said.

"We haven't been apprised of exactly what is happening," he said, adding that the state wants to ensure that the public's interest and the state's interest are protected.

Dioxin wastes have been buried below the ground at Vertac's Jacksonville plant and hazardous chemicals contaminate the shallow groundwater there.

Vertac previously notified PC&E of a proposed sale that did involve the Jacksonville plant. However, after the department filed a motion to stop the sale, it was informed by Vertac that the Jacksonville plant wouldn't be a part of the sale. The department's motion was then withdrawn.

Under state and federal law, Vertac must have insurance and a "hazardous waste closure trust fund" must be set up to make sure that the plant, if closed in the future, is properly closed and monitoring of pollution continues, he said.

Vertac does not have the required insurance nor is the amount of the trust fund up to the level required, Deisch said.

Deisch said that PC&E has instructed Vertac to continue making a "good faith effort" to look for an insurance firm willing to sell them a "non-sudden accident" policy.

He said that Vertac is to report back to PC&E periodically about any progress in finding insurance.

In a related matter, an agreement among state and federal agencies, Vertac and Ensco Inc. over the proposed burn of dioxin wastes states that incineration is planned to begin at Jacksonville no later than March 1986. (It will take several months to burn the waste.)

The disposal of dioxin wastes that have been stored above ground at the plant for about 11 years has generated much controversy since May, when it was announced Vertac planned to burn the waste in a mobile incinerator owned by a Tennessee subsidiary of Ensco Inc. of Little Rock.

An agreement between Vertac and Ensco, which was attached to the first agreement filed in federal court, states that the two companies planned to ship 1,200 drums of Vertac's acutely hazardous waste to El Dorado for tests needed to get Ensco's incinerator certified to burn the waste in Jacksonville.

PC&E and the federal Environmental Protection Agency, however, approved a burn of "surrogate material" at El Dorado. Test burns of dioxin waste are not planned until the incinerator is moved to Jacksonville.

AFLRL TR 75-010

Volume I

ANALYTICAL METHODOLOGY FOR HERBICIDE ORANGE

Volume I: Determination of Chemical Composition

CHEMISTRY RESEARCH LABORATORY/LJ
AEROSPACE RESEARCH LABORATORY

SYSTEMS RESEARCH LABORATORIES, INC.
DAYTON, OHIO 45440

MAY 1975

FINAL REPORT: DECEMBER 1972 — DECEMBER 1974

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AEROSPACE RESEARCH LABORATORIES
Building 450 — Area B
Wright-Patterson Air Force Base, Ohio 45433

AIR FORCE SYSTEMS COMMAND
United States Air Force

NOTICES

[redacted]

[redacted]

[redacted]

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National Technical Information Service
Springfield, VA 22151

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER