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Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans: The Risks to Human Health. A Review

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1 PCDDs and PCDFs are ubiquitous and persistent in the environment. They are to be found in body tissues of both humans and animals.
2 The most extensively studied PCDD is 2,3,7,8-TCDD. It has been shown to produce a wide range of effects and is considered to be a (non-genotoxic) carcinogen in animals.
3 Studies into the mechanisms of toxicity so far reveal that there is involvement of a specific receptor (Ah), however further work is required to elucidate the mechanisms of the various effects.
4 Reports on a number of human exposures to PCDDs and PCDFs are described. Results from human epidemiological studies are difficult to interpret: there have been problems in methodology; there has been inadequate information on intake, and exposures have often been to mixtures of PCDDs and/or PCDFs together with other related compounds.
5 Many regulatory authorities faced with the problem of providing an index of risk from exposure to mixtures of PCDDs and PCDFs have employed the concept of 'TCDD equivalents'.
6 Whether or not PCDDs and PCDFs pose a significant human health risk at current levels of exposure they remain of considerable interest to the toxicologist.

Chemistry, sources and environmental fate of PCDDs and PCDFs

Chemistry

The polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two series of tricyclic aromatic compounds. PCDDs and PCDFs can loosely be described as chlorodibenzo derivatives of the basic chemical structures *p*-dioxin and furan, respectively (Figure 1). Related bromine substituted dibenzo-*p*-dioxins and dibenzofurans also exist as contaminants of commercial chemicals, but are not discussed in this paper.

The number of chlorine atoms can vary between one and eight and there are many positional isomers with 75 PCDDs and 135 PCDFs, in the latter case, the large number is due to the asymmetry of the basic dibenzofuran molecule.

The information available on the chemical and physical properties of PCDDs and PCDFs suggests that both series of compounds are extremely stable, in pure form, even on heating up to 700°C. There are some general trends regarding volatility and solubility in polar solutions – both decreasing with the increase in number of chlorine atoms. The most extensively studied isomer is 2,3,7,8-TCDD. This isomer has a melting point of about 305°C and a boiling point of 421°C.[1] 2,3,7,8-TCDD is lipophilic and is almost insoluble in water. The vapour pressure is very low and therefore it tends not to volatilize readily.[2]

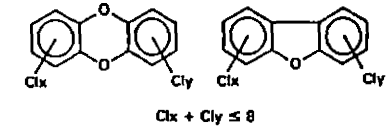

Figure 1 Structures of PCDDs and PCDFs

There are no current technical uses for PCDDs or PCDFs, although they have been synthesized in the laboratory in order to generate analytical standards.[1] PCDDs and PCDFs very rarely occur in the environment as the only products from any one particular source. They often occur with other

related polyhalogenated species such as polychlorinated biphenyls (PCBs) polychlorinated terphenyls (PCTs), chlorinated phenols (e.g. trichlorophenol (TCP) and pentachlorophenol (PCP)), and phenoxy acetic acid herbicides (e.g. 2,4-D and 2,4,5-T).

The advances achieved in analytical techniques over recent years have allowed for much improved detection limits for both PCDDs and PCDFs. Environmental samples can now be analysed down to the parts per trillion level (ppt) or 1 in 10^{12}, or in some cases down to the parts per quadrillion level (ppq) or 1 in 10^{15}.

There are essentially three stages in the analytical determination of PCDDs and PCDFs in environmental and biological samples. Stage 1 involves the extraction of the chemicals from the matrix. The technique used will depend upon the matrix involved. For example adipose tissue or human breast milk samples require a simple solvent extraction procedure; but for municipal incinerator fly-ash, where the chemicals are more strongly bound to the matrix, it is more usual to treat the matrix with acid prior to soxhlet extraction with toluene.[3]

Stage 2 of the procedure is to clean-up the extracts obtained from stage 1. The degree of clean-up required depends upon the sample matrix, the subsequent analytical instrumentation used, and personal preference of the analyst. The efficiency of the extraction and clean up procedures can be determined by the use of ^{13}C internal standards and calculating the percentage recovery of the material to correct for PCDDs and PCDFs which may have been lost during the procedure. The total recovery of both PCDDs and PCDFs depends upon the particular isomer in question. The procedure may however be subject to certain inaccuracies, either because of the PCDD or PCDF isomer used or because of adsorption of the standard onto the matrix, thereby decreasing the extraction efficiency.[3,4]

The final stage in the process is to analyse quantitatively the cleaned-up extract for the presence of PCDDs and PCDFs. The procedure used will depend upon the accuracy required for the final result: for detection of PCDDs and PCDFs down to the ppt concentration, the usual procedure is gas chromatography coupled to a mass spectrometer (GC/MS). The retention time observed on the chromatogram provides the qualitative evidence for the presence of a particular isomer (i.e. specific PCDD/PCDF) or homologue (i.e. total PCDDs/PCDFs with a specified number of chlorine atoms). The results obtained from the mass spectrometer (MS) provide both additional qualitative information (from observation of the mass to charge ratio (m/z) for the mass fragments detected) and quantitative information (from the magnitude of the response).

The use of immunoassay techniques have been described in the literature and although still requiring some further development, they show considerable potential. Fishbein[5] described a radioimmunoassay technique for the determination of 2,3,7,8-TCDF in biological tissues. The detection limits of this assay are reported to range between 20 pg/g (ppt) to 4.0 ng/g (ppb) for this particular isomer. Values for TCDF in various Aroclors (PCBs) and tissues determined by radioimmunoassay are reported to correlate with those obtained by GC/MS.

Because it is necessary to measure PCDDs and PCDFs at extremely low concentrations and because these chemicals often occur in very complex mixtures, the analytical technique often requires extraction and concentration by factors of several thousand in order to bring the concentration of PCDDs and PCDFs up to measurable levels. Other organic compounds that are present (often at much higher concentrations) can interfere with their determination.[6]

The determination of PCDDs and PCDFs in other chlorinated organic samples, such as phenoxyalkanoic herbicides presents a number of analytical problems.[7,8] The presence of 'predioxins' in such chemicals can lead to the formation of PCDDs during the analytical procedure, thus resulting in increases in the apparent amounts present. Special adaptations to the analytical technique are required when chemicals which are structurally related to PCDDs and PCDFs are present, as such chemicals may have similar extraction properties.[7]

The importance of quantitative analysis of PCDCs and PCDFs has increased over recent years. The problems involved are very complex because of the large number of interfering chemicals present; the extremely low detection limits often required; and the large number of isomers, representing a wide range of toxicological properties and potencies. At present quantitative determinations of PCDDs and PCDFs have mostly been conducted on total isomers (homologues) e.g. total TCDDs, TCDFs, PeCDDs, PeCDFs, etc., with only selective isomer analyses conducted on those congeners specifically substituted at the 2,3,7,8 positions. Individual isomer analysis is extremely costly and therefore rarely carried out routinely. However, the results of collective isomer analyses are none the less helpful in 'mapping out' major areas of contamination, although the task of more accurate 'risk assessment' from such data becomes more difficult.

Sources

PCDDs and PCDFs have been detected in a number of emissions resulting from combustion sources. The detection of these chemicals in such a wide range of samples led to the formulation of the 'trace chemistries of fire' hypothesis, which states that numerous products arise in trace amounts during the many chemical reactions that occur during combustion. Identified sources that have aroused a great deal of public concern include emissions from municipal waste incinerators, industrial waste incinerators, in particular PCB waste and also PCB fires. Other identified sources of PCDDs and PCDFs include exhaust emissions from diesel and petrol fueled vehicles.[10,11] The latter is associated with the lead content of fuels (particularly as these contain a dichloroethane scavenger, a precursor of both PCDDs and PCDFs) and is much reduced in vehicles with catalytic converters.[12] Very small amounts have also been detected in cigarette smoke and charcoal-grilled steaks.[13] Research into the formation of PCDDs and PCDFs from combustion sources has indicated that the presence of these chemicals will depend upon the conditions of the combustion process. In particular, the molecular structure and chlorine content of the fuel, the presence of precursors and contaminants, the reaction temperature within the combustion zone and post combustion zone, the residence time of reactant and intermediate products in the high temperature zone, mixing efficiency of air with fuel, air:fuel ratio, fuel-feed size and finally the presence of metals in the process acting as catalysts.[14]

The possibility of formation of PCDDs and PCDFs from municipal waste incineration has been recognized for some years, following their determination in fly ash[15] and subsequently, in emissions from such sources. They may occur as a result of chemical precursors in the fuel supply e.g. PCBs or chlorophenols and/or via *de-novo* synthesis from the pyrolysis of chemically unrelated chlorinated compounds. It has been suggested that chlorine-containing plastic materials such as PVC may provide the largest source of organic chlorine, and this has resulted in concern over other possible PCDD/PCDF sources such as hospital waste incinerators, which burn large quantities of PVC.[16]

The presence of PCDDs and PCDFs as unwanted by-products in a number of commercially available industrial chemicals has added to the general public concern over these substances, in particular their presence as impurities from the manufacture of many industrial and agricultural chemicals based upon chlorophenols and chlorinated aromatic hydrocarbons. Chlorophenols have been used as fungicides, herbicides, slimicides, and were key intermediates in the production of phenoxy acetic acid herbicides (2,4-D and 2,4,5-T). Other chemicals of concern include PCBs, which are known to contain high levels of PCDFs.

Environmental fate of PCDDs and PCDFs

Environmental and biological monitoring has revealed that PCDDs and PCDFs occur fairly ubiquitously. 2,3,7,8-TCDD has tended to be the most widely studied of these chemicals, however because of the chemical and physical similarities within the group of PCDDs and PCDFs, it is likely that a general trend will be seen with the other PCDDs and PCDFs.

Because of their chemical nature, PCDDs and PCDFs are strongly bound to particulate matter, this will influence the way they move through the environment. 2,3,7,8-TCDD is tightly bound to peaty soils high in organic matter and usually remains in the upper layers of such soil types, undergoing very little vertical migration.[17] Therefore transport of 2,3,7,8-TCDD in this environment will generally occur by mobilization of soil particles.

Levels of PCDDs and PCDFs in water *per se* are thought to be very low, since adsorption to particulate sediment seems to predominate. However, suspension of particulate matter may occur. Animals living in this environment may acquire these chemicals directly through the ingestion of sediment or indirectly from the trophic food chain. There are few data on levels of PCDDs/PCDFs in air. Information so far available indicates that the levels in this medium (as for water) are low. It is generally believed that uptake of PCDDs/PCDFs by inhalation may not be an important source of exposure.[18]

It has been suggested that the primary source of exposure to man of PCDDs and PCDFs is from combustion and that uptake occurs from the consumption of foods such as fish, dairy produce and meat.[19] Levels of PCDD and PCDFs in vegetable matter are reported to be generally very low, and where contamination has been found, it is largely on the surfaces, arising from surface deposition rather than by uptake from the soil.[20]

The main degradation process for dioxins and presumably dibenzofurans is by photolysis, involving surface exposure to UV light, in the presence of a hydrogen donor. Such donors include natural substances such as leaf waxes, natural organic films on water surfaces, pesticides or formulating agents as well as the presence of otherwise undesirable pollutants such as spilled oils.[21,22] The half-life of 2,3,7,8-TCDD in soil, in the absence of UV light is estimated at 10 years,[23] thus indicating that any other degradation processes take place at a much slower rate.

Dioxins do gradually volatilize from soil and plant surfaces, but the importance of this route to 'clear' dioxins from the environment is unknown. The rate of volatilization depends upon several environmental properties, as well as on the degree of chlorination of the molecule.[24] A few fungal and bacterial species have been found to be capable of degrading dioxins, although their importance relative to other degradation processes is unknown.[25–27]

Animal studies

Absorption, distribution and excretion

Animal studies have demonstrated that absorption of 2,3,7,8-TCDD by the gastrointestinal tract and through the skin depend upon the vehicle used.[28] For other PCDDs and PCDFs, the isomer and chlorination number will also be important. The bioavailability of these chemicals from soil will depend upon the soil type and has been said to vary between 1 and 70% when contaminated soil was administered to guinea pigs.[29] PCDDs and PCDFs are strongly adsorbed onto incinerator fly ash and they are more readily absorbed by animals administered solvent extracts of fly ash than by those receiving fly ash itself.[30]

The tissue distribution of 2,3,7,8-TCDD varies with species,[28,31] dose[32], time after dosing and previous exposure to 2,3,7,8-TCDD.[33] Monkeys and guinea pigs concentrate 2,3,7,8-TCDD in the fat, muscle and skin, whereas other species concentrate TCDD to a greater extent in the liver.[34] Radio-labelled TCDD administered intravenously to mice and rats[35,36] has been found to concentrate in the nasal turbinates; a site of 2,3,7,8-TCDD-induced tumours in rats.

The majority of 2,3,7,8-TCDD in the liver is associated with the microsomal fraction.[31] The distribution of 2,3,7,8-TCDF differs from 2,3,7,8-TCDD in that it concentrates in fat and skin more than liver, although the distribution varies with time, repeated dosing[37] and size of dose.[38]

The retention of PCDDs and PCDFs by the liver increases with chlorination (between four and six chlorines), with PCDFs being retained longer than PCDDs. The retention in fat however, is longer for PCDDs[39] indicating differing rates of metabolism. Nishizumi & Masuda[40] demonstrated liver accumulation in rats of 1,2,3,4,7,8-HxCDF and 2,3,4,7,8-PeCDF to be dose-dependent, with an increased proportion of the dose retained at low dose levels.

2,3,7,8-TCDD has been detected in the fetuses of dosed female rats[41] although the concentration was lower than in the mothers. Nau & Bass[42] reported the transfer of ^{14}C 2,3,7,8-TCDD from pregnant NMRI mice to the fetus, with the fetuses receiving 0.05-0.1% of the administered intraperitoneal dose. Changing the route of administration to oral or sub-cutaneous apparently decreased the amount in the fetus, but did not alter the distribution pattern, demonstrating the majority of the dose to be in the fetal liver. In a further study 2,3,7,8-TCDD was also shown to transfer to the pup via the milk.[42]

A dietary study with PCDF mixtures demonstrated the ability of these substances to cross the placenta in female ddN mice, but the greatest uptake was shown in the suckling pup, indicating milk to be the major vector.[43] The majority of the absorbed dose in the dam was found in the liver. The relative isomer distributions in the pups were reported to differ from those in the diet, with 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF being preferentially stored.

2,3,7,8-TCDD is metabolized to a very limited extent, although glucuronide conjugates[44,45] and hydroxy derivatives[46] have been identified. The metabolism of 2,3,7,8-TCDD has been related to cytochrome P-450 monooxygenase activity and there is evidence to suggest P-450/*P*-448 inducers, such as phenobarbital may influence the rate of 2,3,7,8-TCDD metabolism. The rate of 2,3,7,8-TCDD metabolism by rat liver microsomes has been reported to be 10^4-fold slower than that of Benzo[a]pyrene.[28] Metabolites of 2,3,7,8-TCDD (2-hydroxy-3,7,8-trichlorodibenzo-*p*-dioxin and 2-hydroxy-1,3,7,8-tetrachlorodibenzo-*p*-dioxin) are considered to be over 100 times less potent than the parent compound.[47] For other dioxins, mono- and di-hydroxy derivatives of dibenzo-*p*-dioxin; 1-CDD; 2-CDD; 2,3,-DCDD; 2,7-DCDD; 1,2,4-Tri-CDD and 1,2,3,4-TCDD have been reported to be excreted by rats,[48] however no metabolites of OCDD have been detected.[49,50] A scheme has been proposed with a 2,3-epoxide intermediate, which cannot be readily formed by compounds substituted at the 2,3,7,8-positions.[44] Unidentified polar metabolites of 2,3,7,8-TCDF have been reported in the urine of monkeys, rats and guinea pigs.[37,38,51] No metabolites of other PCDFs have been reported in the tissues of treated animals, which would indicate metabolites are readily excreted.

The excretory half-life of 2,3,7,8-TCDD has been found to vary with species and is not always related to its relative toxicity for that species.[28] The half-life values in rodents have been found to range between 10 and 36 days[28] and in monkeys a value of 1000 days has been proposed.[52] Biliary excretion has been proposed as a major route of 2,3,7,8-TCDD elimination;[53] 2,3,7,8-TCDD is also excreted in the milk.[42] The excretion of 2,3,7,8-TCDF also varies with species, but is comparatively

rapid, with half-life values of 2 days in rats and 8 days in the monkey.[50,51] Metabolites of 2,3,7,8-TCDF generally appeared in the urine and bile, whilst the faeces contained mainly unaltered 2,3,7,8-TCDF.

Acute toxicity

The acute toxicity of 2,3,7,8-TCDD varies widely both quantitatively and qualitatively with species/strain/sex of the animals, the administration protocol, and even, between research groups. However, certain aspects are common to all animals studied: progressive body weight loss; thymic atrophy (especially of the cortex), a considerable delay from administration to death (up to 45 days) and gastrointestinal haemorrhages. Various signs which have been seen in one or more species administered a single dose of 2,3,7,8-TCDD include: reduction in food consumption (although this is not primarily responsible for the decrease in body weight); rough coat; dehydration; muscular necrosis; loss of fat deposits; hypocellular bone-marrow; haemolysis; changes in overall serum protein levels and their ratios; testicular degeneration, particularly of the seminiferous tubules which develop multinucleated giant cells; necrotic spermatocytes and spermatozoa; distention of the gall bladder; bile duct proliferation; an increase in hepatocyte size together with a temporary proliferation of their rough endoplasmic reticulum and increase in the prominence of the nucleolus (indicative of protein synthesis); fluorescence of liver and bones, indicating excess porphyrin deposition; fatty droplets in the phetatocytes; ascites; hyperplasia of the renal pelvis and inflammatory infiltration of organs.[28]

LD_{50} values for 2,3,7,8-TCDD in animals, when administered by gavage, have been reported to vary between 0.6 $\mu g\ kg^{-1}$ in Hartley guinea pigs (the most susceptible species) to 5051 $\mu g\ kg^{-1}$ in the Golden Syrian hamster.[28] 2,3,7,8-TCDD is undoubtedly the most toxic and most extensively studied of all PCDDs and PCDFs, although the limited information available on the others indicate that the clinico-pathological signs seen following acute administration were very similar[54,55] but the potencies vary considerably, depending upon the isomer in question and also the species tested.[54] The most acutely toxic isomer studied in the PCDF series is 2,3,7,8-TCDF, which has been reported to have an oral LD_{50} of 5 $\mu g\ kg^{-1}$ in the guinea pig,[54] though recent work on 2,3,4,7,8-PeCDF indicates this to be more potent in certain assays.[56]

Sub-acute toxicity

The responses of experimental animals in sub-acute studies indicate that the total dose administered is probably more important than the size of a single dose or frequency of administration.[57] The most extensively reported sub-acute study of 2,3,7,8-TCDD yet published is that of Kociba *et al.*[58] This was a 13-week study in which young adult Sprague Dawley rats were exposed to 2,3,7,8-TCDD at doses of 0.001, 0.01, 0.1 and 1 $\mu g\ kg^{-1}d^{-1}$, for 5 days per week, by gavage. At the end of the treatment some of the rats were killed and autopsied, whilst others were allowed a 13-week recovery period. Female rats were more sensitive than males. A no-effect level was identified at 0.001 $\mu g\ kg^{-1}$ (equivalent to a total dose of 0.065 $\mu g\ kg^{-1}$) with a total dose of 0.65 $\mu g\ kg^{-1}$ producing only small changes in relative liver weights. Effects observed at the highest dose levels for both male and female animals included severe weight loss, haematological changes, increase in urinary porphyrins, increased relative liver weights associated with gross changes, increased lipid content and changes in the size and shape of hepatocytes. Other changes noted were decreased thymus weight, thymic involution and a decrease in food intake and females experienced uterine changes. Some reversal of these effects were shown following termination of treatment, in particular body weight (complete in females and partial in males) but no firm conclusions could be drawn from these findings.

There are few published reports of sub-acute studies on other PCDDs. A 13-week study conducted on a mixture of HxCDD isomers in rats and mice at doses of between 2.5 to 100 $\mu g\ kg^{-1}$ body weight per week demonstrated weight loss, hepatotoxicity and thymic atrophy. The effects seen varied between species and between sexes in the rat.[59]

The production of chloracne-like lesions has been reported in hairless mice following dermal application of 2,3,7,8-TCDD.[60] Four weeks following treatment (0.1 $\mu g\ kg^{-1}$ in 0.1 ml acetone, three times per week) the skin became hard and shiny with fine scales. Histologically, there was hyperkeratinization of the stratum corneum, epidermal hyperplasia, keratinization of the dermal cysts, reduction in sebaceous glands and lymphocytic infiltration of the dermis. A number of *in vitro* studies have also been used to study this effect.[61,62]

Schwetz *et al.*[63] studied the effects of several dioxins (2,7-DCDD, 1,2,3,4-TCDD, 2,3,7,8-TCDD, HxCDDs and OCDD) on the production of comedone formation in rabbits' ears. The animals were exposed to the chemical for 5 days per week for 4 weeks. The most reactive of the chemicals was 2,3,7,8-TCDD (at a total dose of 0.8 μg) followed by HxCDD (mixed isomers, at a dose of 200–1000 μg). The other chemicals failed

to elicit a response at the doses tested. However, as different solvents were used, valid comparisons are not possible.

Sub-acute effects of orally administered 2,3,7,8-TCDF in Rhesus monkeys, indicate that although the signs of intoxication of this PCDF are similar to 2,3,7,8-TCDD, it is 10–100 times less toxic than its PCDD equivalent,[64] although a no-effect level (NEL) has yet to be established. Mixtures of TCDFs, PeCDFs and HxCDFs, when administered in the diet to rats for 4 weeks, demonstrated significant changes similar to those reported for 2,3,7,8-TCDD.[65]

Chronic toxicity

Many of the non-carcinogenic effects of chronic exposure of rodents to PCDDs are described in the studies relating to the carcinogenicity of these compounds. Results of these studies have been summarized in Table 1. A number of pathological effects typical of PCDD exposure have been described, although liver appears to be the major target organ. As yet it has not been possible to determine NELs for chronic toxicity of dibenzo-*p*-dioxin, 2,7-DCDD, 2,3,7,8-TCDD and HxCDD. Administration of 0.0014 μg $kg^{-1}d^{-1}$ of 2,3,7,8-TCDD for 2 years caused an increase in centrilobular fatty metamorphosis of the liver in male rats.[66] Kociba *et al.*[67] reported significant ($P<0.05$) changes in the numbers of swollen hepatocytes (increases in females, decreases in males) in rats receiving 0.001 μg $kg^{-1}d^{-1}$ 2,3,7,8-TCDD. The lowest dose of HxCDD mixture (0.18 μg $kg^{-1}d^{-1}$) produced toxic hepatitis in both male and female rats.[59] Dietary administration of 500 ppm of 2,7-DCDD for 2 years in rats produced fatty metamorphosis of the liver and an increase in chronic murine pneumonia[68] and 5000 ppm dibenzo-*p*-dioxin in the diet for 2 years produced nephropathy in male rats and a fatty metamorphosis of the liver in females.[69]

The chronic effects of 2,3,7,8-TCDD and TCDF on non-human primates (*Macaca mullata*) have been described in a number of studies[64,70–72] with a number of similarities reported in the findings. Young Rhesus monkeys exposed to 2,3,7,8-TCDD in the diet (approximately 0.01 μg $kg^{-1}d^{-1}$) demonstrated periorbital oedema, loss of facial hair and eyelashes, accentuated hair follicles and dry scaly skin.[71] After 6 months on this regimen, anaemia was also noted and by 9 months, haemorrhages and thrombocytopenia were detected. Between months 5–12, five of the original eight monkeys died, with death attributed to the effects of pancytopenia. The following lesions were detected at autopsy: hypocellular bone marrow; hyperplasia; hypertrophy and metaplasia of the bronchial mucosa, bile ducts, pancreatic ducts, salivary ducts and most epithelia; hyperplastic gastritis and ulceration of the gastric mucosa; squamous metaplasia and keratinization of the sebaceous glands and hair follicles; keratinization of the meibomian glands; irregular growth of toe and finger nails; haemorrhages in most tissues, dilation of the bile duct; an increase in the number of mucus secreting cells of the gut, at the expense of the parietal and zymogenic cells; cardiac enlargement; haemolysis and ascites. One monkey was essentially unaffected; no changes in serum lipid, cholesterol, protein, globulin: albumin ratios or SGPT were found.

Carcinogenicity

The available data on the carcinogenicity of PCDDs in rodents are summarized in Table 1. There appears to be very little, if any, data relating to carcinogenicity testing of PCDFs.

Most dioxins tested demonstrated a carcinogenic potential, although dibenzo-*p*-dioxin did not and preliminary reports of investigations on OCDD would not be expected to show any tumour excess due to the short period of administration.[73] The most potent rodent carcinogens yet tested appear to be 2,3,7,8-TCDD and an HxCDD mixture. The commonest sites for tumour production are liver[59,67,68,74] and also hard palate/nasal turbinates and tongue for 2,3,7,8-TCDD.[67]

2,3,7,8-TCDD is considered to be a very potent promoter[75] however, other studies on the mechanism of 2,3,7,8-TCDD carcinogenicity have found it also to be a cocarcinogen,[76] a weak carcinogen,[78] a complete carcinogen,[39] and an anticarcinogen;[79] the effects varying with the model used. The current view on the mechanism of carcinogenicity of 2,3,7,8-TCDD indicates that it is a non-genotoxic carcinogen, thereby justifying attempts to identify a NEL. One suggested level comes from the rat feeding study reported by Kociba *et al.*[67] and is estimated at 0.001 μg $kg^{-1}d^{-1}$.

Mutagenicity

2,3,7,8-TCDD has been tested in a variety of mutagenicity test procedures with conflicting results and attempts at repeating positive studies have been unsuccessful. Detailed reviews of the mutagenicity of 2,3,7,8-TCDD have been carried out by Wassom *et al.*[80], EPA[26] and Giri.[81] All concluded that the results were equivocal and further studies were required. In a more recent review by Shu *et al.*[82] it was suggested that 2,3,7,8-TCDD falls into the category of 'non-genotoxic' carcinogens, which includes a number of substances such as oestrogens, asbestos and compounds such as phenobarbital and chlorinated hydrocarbons which have been shown to be carcinogenic in animals. The

Table 1 Chronic toxicity and carcinogenicity of PCDDs

Compound (Ref.)	*Dose*	*Species*	*Tumour type*	*Other observations*
Dibenzo-p-dioxin NCI (1979)[68]	5000 or 10 000 ppm in diet	Mice	None	Decrease in body weight and decrease in survival in top dose females. Interstitial renal inflammation in females.
	5000 or 10 000 ppm in diet	Rats	None	Decrease in body weight and decrease in survival in top dose females. Incidence of chronic murine pneumonia increased and increase in nephropathy in male rats.
2,7-DCDD NCI (1979a)[68]	5000 or 10 000 ppm in diet	Rats	None	Decrease in body weight. Hepatotoxic lesions. Increased chronic murine pneumonia and interstitial renal inflammation. Decrease in survival.
	5000 or 10 000 ppm in diet	Female mice	None	Decrease in body weight. Hepatotoxic lesions (focal necrosis)
	5000 or 10 000 ppm in diet	Male mice	Hepatocellular adenoma at both doses; leukaemia/lymphoma at 5000 ppm	Decrease in body weight.
HxCDD mixture (1,2,3,6,7,8 and 1,2,3,7,8,9) NTP (1980)[69]	5, 2.5, 1.25 μg kg bw^{-1}/week^{-1} by gavage	Rats	Hepatocellular adenoma and carcinoma neoplastic nodules at high dose in males and all doses in females. Follicular cell adenoma of thyroid in low dose males.	Decrease in body weight gain (doses 2.5 and 5 μg kg^{-1}). Lung lesions (adenomatous hyperplasia), toxic hepatitis.
	5, 2.5, 1.5 μg kg bw^{-1}/week^{-1} by gavage	Male mice	Hepatocellular adenoma and carcinoma/neoplastic nodules at high dose	No overt health effects. Fatty metamorphosis and pigmentation of liver (high dose).
	10, 5, 2.5 μg kg bw^{-1} week^{-1} by gavage	Female mice	Hepatocellular adenoma and carcinoma/neoplastic nodules in high dose group.	No overt health effects. Liver haematopoiesis.
	Skin painting	Mice	None	None
1,3,7,8-TCDD NTP (1982)[66]	0.001 μg three times per week skin painting	Male mice	None	Survival rates slightly decreased.
	0.005 μg three times per week skin painting	Female mice	Fibrosarcoma of integumentary system	Survival rates slightly decreased.
	0.01, 0.05, 0.5, μg kg bw^{-1}/week^{-1} by gavage	Male rats	Thyroid follicular cell adenoma (high dose)	Decrease in body weight gain. Toxic hepatitis (high dose). Other liver effects focal cellular change (high dose) and fatty metamorphosis. Adenomatous hyperplasia of lung. Hyperkeratinization of stomach (high dose).
	0.01, 0.05, 0.5 μg kg bw^{-1}/week^{-1} by gavage	Female rats	Liver neoplastic nodule/carcinoma (high dose) pituitary adenoma (low dose only)	Decrease in body weight gain. Toxic hepatitis (high dose). Other liver effects (focal cellular change and fatty metamorphosis [except at high dose level]). Adenomatous hyperplasia of lung. Decrease in hyperplasia of bile duct. Increase in lymphocytic inflammatory infiltration of lachrymal gland. Decrease in mineralization of renal pelvis.

Table 1 Continued

Compound (Ref.)	*Dose*	*Species*	*Tumour type*	*Other observations*
NTP (1982)[80]	0.01, 0.05, 0.5 μg kg bw^{-1}week^{-1} by gavage	Male mice	Hepatocellular adenoma/carcinoma (high dose). Alveolar/bronchiolar adenoma (high dose)	Increased focal calcification of brain, decreased focal calcification of basal ganglia. Toxic hepatitis (high dose). Lymphocytic inflammatory infiltration of lung (including bronchus) salivary glands and kidney.
NTP (1982)[80]	0.06, 0.2, 2 μg kg bw^{-1}week^{-1} by gavage	Female mice	Hepatocellular adenoma/carcinoma (high dose). Thyroid follicular cell adenoma (high dose). Histiocytic lymphoma (high dose).	Increased focal calcification of brain; decreased focal calcification of basal ganglia. Toxic hepatitis (high dose). Lymphocytic inflammatory infiltration of lung (including bronchus) salivary glands and kidney. Decrease in lymphocytic inflammatory infiltration of multiple organs. Increased calcification of renal pelvis.
Kociba *et al.* (1978)[83]	0.001, 0.01, 0.1 μg kg bw^{-1}d^{-1} in the diet	Male rats	Squamous cell carcinoma of hard palate/nasal turbinates and tongue; adenoma of adrenal cortex at 0.1 μ. None at lower doses.	Reduced body weight gain (from 6 months to 2 years) at a high dose. Haematological effects at high dose including anaemia/panecytopenia. Porphyrin and ALA level alterations (high dose). Liver damage (at two highest doses, less extensive than in females). Respiratory effects (less extensive than females [high dose]) and cardiovascular effects; adrenal haematocysts (high dose).
Kociba *et al.* (1978)[83]	0.001, 0.01, 0.1 μg kg bw^{-1}d^{-1} in the diet	Female rats	Hepatocellular hyperplastic nodules, at 0.1 and 0.01 μg. Squamous cell carcinoma of hard palate/nasal turbinates and tongue; hepatocellular carcinoma; keratinising squamous cell carcinoma of lung at 0.1 μg	Reduced body weight gain (from 6 months to 2 years) at high dose. Haematological effects at high dose, including anaemia/ pancytopenia. Porphyrin, ALA and creatinine alterations (high dose). Liver damage (at two highest doses, more extensive than in males). Swollen hepatocytes (low dose). Thymic or splenic atrophy (high dose). Respiratory system effects, (more extensive than males) and cardiovascular effects. Increased adrenal cortical haemorrhage and pancreatic atrophy and fibrosis.

mechanism by which such compounds induce carcinogenesis in animals varies depending upon the chemical).

Mutagenicity assays have been conducted in a number of strains of *Salmonella typhimurium*.[83–87] All studies have demonstrated negative results, with the exception of Hussain *et al.*[85] and Seiller[84] who reported positive results for the induction of revertants in one strain, TA 1532, sensitive to frameshift mutations. Attempts to repeat these studies using either TA 1532 or other frameshift-sensitive strains were unsuccessful.[83,86,87]

A summary of genotoxicity studies with test systems other than *S.typhimurium* has been described by Shu *et al.*[82] and is presented in Table 2.

Table 2 2,3,7,8-TCDD genotoxicity studies in systems other than *S.typhimurium*. Adapted from Shu *et al.*[82]

Test	*Result*	*Reference*
Mutagenicity: *E. coli Sd-4*	+	85
Mutagenicity: *S. cerevisiae*	+	88
Mutagenicity: mouse lymphoma cells	+	89
Viral inactivation: QB virus	–	90
DNA binding: rat liver *in vivo*	–	91
Unscheduled DNA synthesis } hepatocytes	–	92
Unscheduled DNA synthesis } *in vitro*	–	93
Transformation: BHK cells	+	95
Transformation: C3H/10T$_{1/2}$ cells		96
Sex-linked recessive lethal: *Drosophila*	–	97
Dominant lethal: Rat	–	94

A more detailed discussion of some of these findings is summarized below.

Hussain *et al.*[85] reported 2,3,7,8-TCDD to induce a reversion to streptomycin independence in *E. coli* Sd-4 at doses above 2 μg ml^{-1}, however, at these concentrations, survival was below 20%.

Bronzetti *et al.*[86] tested the mutagenic effect of 2,3,7,8-TCDD in a suspension test in *Saccharomyces cerevisiae*. The author reported a positive, dose-related response (over the range of 0.5–10 μg TCDD ml^{-1}) in mitotic gene conversion and reverse point mutation in the presence of mouse liver S10 mix. The maximum increase was approximately fourfold at 10 μg ml^{-1}, with 15% survival; no changes were noted in the absence of S10 mix. This study has been criticized on the grounds that the weak positive effect was observed only at higher doses in the presence of considerable toxicity and may have resulted from the selection of spontaneous mutant colonies, already present in the yeast cell population.[82] Bronzetti *et al.*[88] also reported on an intransanguinous host mediated assay on CD-1 mice, in which a maximal mutational effect was noted in both liver and kidney, 20 days after administration of 25 μg of 2,3,7,8-TCDD by gavage.

Rogers *et al.*[89] found mutation rates to increase in L5178Y mouse lymphoma cells exposed to 2,3,7,8-TCDD when methotrexate, thymidine or thioguanine were used as the selective agents, but not when ouabain or cytosine arabinoside were used. Cell survival was decreased at 2,3,7,8-TCDD concentrations of 0.1 mg ml^{-1} and above. Clone morphology was reported to be changed by 2,3,7,8-TCDD concentrations of 0.01 mg ml^{-1} and above, but growth in TCDD-free medium for 36 h before plating reversed this change. It is not possible to draw conclusions from this study as it has never been repeated, even though equivocal results were obtained. It has been suggested that the response seen may have occurred by a mechanism other than mutagenesis.[82]

A negative dominant lethal effect in male Wistar rats was reported by Khera & Ruddick.[94] Doses of 2,3,7,8-TCDD (4, 8 or 12 μg kg^{-1}) were administered for 7 days before seven sequential mating trials over 35 days and produced toxic effects in some of the animals.

Meyne *et al.*[98] found no evidence of an increase in chromosome aberrations, micronuclei or sister chromatid exchange (SCE) in bone marrow cells of C57BL/6J or DBA/2J mice exposed to 2,3,7,8-TCDD (0, 50, 100, 150 μg kg^{-1} i.p.). It was noted that the doses administered proved to be hepatotoxic to these strains of mice. A number of other studies (reported by EPA[28]) have found no evidence of chromosomal abnormalities in rats treated with 2,3,7,8-TCDD; the administered dose in one study was reported to be 1 μg kg^{-1} week^{-1} by gavage for 45 weeks. Two studies were reported as giving weakly positive results for chromosomal aberrations: Loprieno *et al.*[93] found a significant increase in chromosome aberrations in CD-1 mouse bone marrow cells, when assaying at 96 h, but not at 124 h after an i.p. injection of 10 μg 2,3,7,8-TCDD kg^{-1}. The second study (Green *et al.*, cited by Wassom *et al.*[80]) reported a weakly positive result for chromosomal aberrations in bone marrow cells from Osborne-Mendel rats receiving 4 μg kg^{-1} twice weekly for 13 weeks, no controls were used and comparisons were made with cells from animals receiving lower doses. In addition, no details were given of the aberrations observed. No conclusions can be drawn from this study.

Abernethy *et al.*[96] demonstrated concentrations of 2,3,7,8-TCDD above 4 pM to promote the transformation of mouse embryo fibroblasts (C3H/10T$_{\frac{1}{2}}$) which had been pretreated with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (0.5 μg ml^{-1}). Comparison of maximal response with the promoting agent TPA (12-0-tetradecanoylphorbol-13-acetate), indicated that the dose required for 2,3,7,8-TCDD was 10^4 less than for TPA and suggested 2,3,7,8-TCDD was the most potent promoter yet tested with C3H-10T$_{\frac{1}{2}}$ cells.

The binding of [^{3}H] 2,3,7,8-TCDD to liver protein, RNA and DNA when administered i.p. to Sprague Dawley rats at a dose of 7.5 μg kg^{-1} was determined by Poland & Glover.[91] The results demonstrated the majority of bound 2,3,7,8-TCDD to be associated with protein (0.01% of administered dose) with virtually none binding to nucleic acids.

There is some evidence, from studies on yeasts, to suggest that 2,3,7,8-TCDD has mutagenic potential. However, the data from bacterial gene mutation assays, clastogenicity studies and DNA damage assays were negative. Negative results were also obtained in a sex-linked recessive lethal assay in *Drosophila* and in a dominant-lethal assay in rats. Whilst the balance of results are negative, there is evidence from cell transformation assays that 2,3,7,8-TCDD has tumour promoting activity.

Mutagenicity studies on other PCDDs have only been carried out on 2,7-DCDD and OCDD. Dibenzo-*p*-dioxin has also been studied. Green & Moreland[99] reported (in abstract form only) no increase in chromosome aberrations in rat bone marrow cells following treatment with 10 μg kg^{-1} dibenzo-*p*-dioxin or 2,7-DCDD, by intubation for 5 days, however, it should be noted that no dose range was used. Furthermore, it has been reported[80] that using the same protocol, a negative result was also obtained for 2,3,7,8-TCDD, but

using a different protocol Green & Moreland obtained a positive result for 2,3,7,8-TCDD. No other dioxins were tested in this revised system.

Commoner[100] reported that dibenzo-*p*-dioxin failed to demonstrate an increase in revertants when tested at doses between 0 and 100 μg per plate in assays using *S. typhimurium* strains TA 100, 1535, 1537 and 1538 with and without S9 mix.

Finally, OCDD has been tested in a number of strains of *S. typhimurium*.[84] The results indicated a negative response with strains TA 1530, 1531 and G46 and questionable results when tested in TA 1532 and 1534 strains. No details of dosing or activation system were provided.

There appears to be little information on the mutagenicity of PCDFs. The mutagenic effects of four PCDFs (2,8-DCDF; 3,6-DCDF; 2,3,7,8-TCDF and OCDF) and dibenzofuran have been studied in various strains of *S. typhimurium* with and without S9 mix. None of these dibenzofurans were found to be mutagenic.[101]

Cytotoxicity

A number of *in vitro* studies have been carried out on 2,3,7,8-TCDD.[102-105] In the majority of cell lines challenged, no evidence of toxic effects were noted. This suggests either that the systems used may be unsuitable or that the *in vivo* effects of 2,3,7,8-TCDD may only be seen in highly organized systems (possibly signifying a requirement for the Ah receptor). *In vitro* systems have however been used successfully in the investigations of hyperkeratinization.[61,62]

In humans the most common effect of exposure to PCDDs and PCDFs is chloracne/epidermal hyperplasia. Two cell culture systems, XB cells (epithelial cells, derived from a mouse teratoma) or neonatal human foreskin cells, have been found to exhibit hyperkeratinization when challenged with 2,3,7,8-TCDD.[61,62] It is reported that XB cells possess the Ah receptor protein and keratinization response was proportional to the binding affinity of the test compound for the receptor.[61]

Reproductive toxicology

2,3,7,8-TCDD is generally accepted as being one of the most potent teratogens/fetotoxins in all the common laboratory species studied. Mice present teratological lesions, mainly cleft palate and kidney anomalies/hydronephrosis.[106-109] A number of fetotoxic effects have been reported in rats including intestinal haemorrhages and subcutaneous oedema, as well as kidney abnormalities.[93,106,110-112] Other effects noted include decreased fetal weight[111] and also increased fetal death.[94] Pups born to mothers receiving 2,3,7,8-TCDD in the diet also had

Table 3 Reproductive effects of 2,3,7,8-TCDD

		Dose required (ng kg bw^{-1}d^{-1}) to produce					
		Effect		*Minimal effect*		*No effect*	
Parameter	*Species and ref.*						
Male fertility	Rat[86,102]	4000	[7d]	100*	[12 mo]	100*	[12 mo]
Female fertility	Rat[113]	10	[MGS]	–		1	[MGS]
	Rat[114a]	2000	[14d]	–		500	[14d]
	Monkey[72,112a,112]	10	[6 mo]	2	[7 mo]	0.72	[4y]
General reproductive performance	Rat[113]	10	[MGS]	1*	[MGS]	1*	[MGS]
	Monkey[72,110]	2	[7 mo]	–		0.72*	[4y]
Teratogenicity/ embryolethality	Mouse[108]	1000	[6–15d of pregnancy]	100	[6–15d of pregnancy]	10*	[6–15d of pregnancy]
	Rat[109]	500		–		125	
	Rabbit[111]	100		–		–	
	Monkey[72,110]	2	[7 mo before + all pregnancy]	–		0.72	[15–30 mo before + all pregnancy]
Fetotoxicity	Mouse[107,108]	50000	[6–15d of pregnancy]			3000	[6–15d of pregnancy]
	Rat[109]	125		–		30	
	Rabbit[111]	100				–	
	Monkey[110]	0.72	[15–30 mo before + all pregnancy]	–		0.12	[15–33 mo before + all pregnancy]
Postnatal development	Monkey[110]	0.12	[45 mo but not for 10 mo before or during pregnancy]	–		–	

* Indicates the basis for the figure is tentative.
– Indicates not known.
[ ] Indicates duration of dosing.
MGS Multigeneration study.

reduced survival rate and the transfer of pups at birth to control foster mothers did not increase survival, indicating the effects were probably due to *in utero* exposure rather than exposure via mother's milk.[94] Table 3 summarizes the data.

Maternal responses have included increased liver/body weight ratios in some mice studies and vaginal haemorrhage, decreased fertility and body weight gain in some of the rat studies. Fetuses of New Zealand rabbits, demonstrated increases in extra ribs and soft tissue abnormalities, in addition to maternal toxicity at doses of 0.25 $\mu g\ kg^{-1}$ and above and malformations at 0.1 $\mu g\ kg^{-1}$.[115]

There are few studies on the reproductive effects of 2,3,7,8-TCDD in the male. 2,3,7,8-TCDD (4 $\mu g\ kg^{-1}d^{-1}$ for 7 weeks) can cause inflammation of the testes which may be secondary to general toxicity in rats[94] and possible modifications of testosterone hydroxylase activities following single oral doses of 0.2, 1 or 5 $\mu g\ kg^{-1}$.[115]

A number of authors have suggested NELs for teratogenic effects of 2,3,7,8-TCDD, but many of these estimates are uncertain (Table 3). The lowest dose used in rodent reproductive toxicity studies was 0.001 $\mu g\ kg^{-1}d^{-1}$, which produced increases in the incidence of cleft palate in a CF-1 mouse teratology study, although such effects were not evident at the next higher dosage.[108]

A recent review of the reproductive toxicology of 2,3,7,8-TCDD suggests that the range of toxic effects of TCDD on the reproductive system is consistent with its toxicokinetics, distribution of the Ah-receptor (and AHH enzyme induction) and also suppression of immune function.[114] 2,3,7,8-TCDD mostly occurs in the environment as part of chemical mixtures (as indeed do other PCDDs and PCDFs). There is evidence to suggest that in the presence of some other polychlorinated compounds, the teratogenic effects of 2,3,7,8-TCDD may be enhanced. A combination of 2,3,7,8-TCDD and 2,3,4,5,3',4'-hexachlorobiphenyl (HCB) administered by gavage to C57BL/6N mice on days 10–13 of gestation produce a tenfold increase in the incidence of cleft palate in pups compared to the level produced by a dose of 2,3,7,8-TCDD alone.[115] Hassoun & Denker,[116] reported that co-administration of β-napthoflavone, a microsomal mono-oxygenase inducer with no teratogenic action itself, was found to increase the incidence of 2,3,7,8-TCDD induced cleft palate in mice. Finally a mixture of 2,3,7,8-TCDD and 2,3,7,8-TCDF administered by gavage by C57BL/6N mice on day 10 of gestation produced a more than additive increase in cleft palate and small increases in kidney malformations.[117]

A few studies have been reported in non-human primates. McNulty[70] exposed pregnant Rhesus monkeys to a total of 0.2, 1 or 5 $\mu g\ kg^{-1}$ of 2,3,7,8-TCDD given in nine doses, by gavage, between days 20–40 of gestation. The two animals receiving the 5 $\mu g\ kg^{-1}$ dose aborted on days 47 and 48 of gestation and died on days 85 and 136. Of the four animals receiving 1 $\mu g\ kg^{-1}$ three aborted, with only one animal showing overt signs of maternal toxicity. None of the four animals receiving the 0.2 $\mu g\ kg^{-1}$ dose demonstrated signs of maternal toxicity, although one may have aborted. The signs of maternal toxicity found were an initial thickening and reddening of the eyelids, followed by weight loss, dry skin, loss of hair, anaemia, purpura and bleeding from the nose and mouth. At autopsy, squamous metaplasia of the sebaceous glands, hyperplasia of the bile duct, gingiva and hypoplasia of the bone marrow were noted. A parallel study giving 1 $\mu g\ kg^{-1}$ TCDD as a single dose on days 25, 30, 35 or 40 of gestation produced similar results to the divided dose regimen. Of the three live fetuses from both experiments in the 1 $\mu g\ kg^{-1}$ groups minor abnormalities of the palate and uvula were noted. No abnormalities were noted in three fetuses from the 0.2 $\mu g\ kg^{-1}$ group. Although the 0.2 $\mu g\ kg^{-1}$ group (with an average intake of approximately 0.01 μg per kg body weight per day) did not differ significantly from control animals, it is not possible to draw any firm conclusions from such small group sizes. Animals from higher dose groups exhibited signs of toxicity but aborted prior to these symptoms becoming obvious. No conclusions could be drawn on whether abortions were due to maternal toxicity or effects of 2,3,7,8-TCDD on the fetus.

The direct administration of 2,3,7,8-TCDD (or indeed any other of the PCDDs or PCDFs) during gestation is not necessarily required to produce adverse effects on the offspring. Since 2,3,7,8-TCDD is lipophilic and accumulates in the adipose tissue, is poorly metabolized and has a long half-life in most species, particularly in primates, maternal exposure before conception can lead to ultimate mobilization from adipose stores and thereby transfer to the developing offspring during gestation and also lactation. In a study by Schantz *et al.*[118] subtle effects on maternal/infant behaviour were noted when the animals were mated one year after withdrawal of 2,3,7,8-TCDD administration (5 ppt and 25 ppt) in the maternal diet for about 4 years. There were no obvious toxicological effects of TCDD exposure on the offspring or the mother. The main finding was that TCDD-exposed mother–infants dyads spent more time in close social contact than did controls. The authors suggest that the infant exposure to TCDD may have been the determining factor and that the effects were

probably mediated via the mother's milk. The authors noted little difference from control infants with regard to social or environmental exploration, self-directed behaviour, stereotypic behaviour or play behaviour and suggest the findings from this study indicate mothers were responding to subtle, physical or behavioural cues in modifying their care of TCDD offspring. Further reports of this study, seen as abstracts only, noted changes (both positive and negative) in learning tasks with increasing levels of TCDD in the infants' fat. The authors calculated a NEL of 0.05 ng TCDD kg bw^{-1}d^{-1} based on transfer from maternal fat to infant via milk.[52,119]

The effects of various PCDDs, other than 2,3,7,8-TCDD have been studied for teratogenic potential in Wistar rats by Khera & Ruddick[94] and in Sprague Dawley rats by Schwetz *et al.*[63] Isomers studied were 2-CDD, 2,3-DCDD, 2,7-DCDD, 1,2,3,4-TCDD, OCDD and mixed isomers (unspecified) of HxCDD. Of these PCDDs only the HxCDD was truly fetotoxic and teratogenic, with effects found at the lowest dose used i.e. 0.1 μg kg^{-1}d^{1}.[63] Some effects were noted with the other PCDDs, but these occurred at much higher dosage levels. Khera & Ruddick[94] found 2,7-DCDD to produce myocardial lesions in the fetus at 1000 and 2000 μg kg^{-1} d^{-1} levels, although Schwetz *et al.*[63] found no such effects at 100 000 μg kg^{-1} dose; 1,2,3,4-TCDD produced non-significant increase in skeletal abnormalities at 100 and 200 μg kg^{-1} dose, but not at higher dose levels;[94] OCDD produced subcutaneous oedema at 500 000 μg kg^{-1} dose.[63] Finally, negative results were obtained when 1,2,3,4-TCDD and 2,7-DCDD were tested in dominant lethal/fertility studies in Wistar rats.[94] Nagayama *et al.*[43] have shown certain PCDFs can cross the placenta. The limited information available from 2,3,7,8-TCDF teratogenicity studies indicate it to be teratogenic in mice producing cleft palate and hydronephrosis. However, the dosing periods and observations were limited in most studies and would not necessary have picked up all susceptible organs.[17,120-122] Hassoun *et al.*[120] demonstrated a strain difference in the mice tested in their study, this was thought to be dependent upon the presence of the responsive Ah locus.

Immunotoxicology

One of the most consistent pathological changes following administration of 2,3,7,8-TCDD and related compounds, has been involution/atrophy of the thymus, particularly the cortex, with an associated depletion of lymphocytes. Both humoral and cell mediated immune effects have been demonstrated following 2,3,7,8-TCDD (and 2,3,7,8-TCDF) treatment, although the actual response varies with animal species/strain/age and the experimental protocol. In common with other effects produced by PCDDs and PCDFs, the alterations in immune system functioning appear in general to parallel the levels of Ah receptor and arylhydrocarbon hydroxylase (AHH) inducibility. Vecchi *et al.*[123] determined the number of haemolytic plaque forming cells (PFC) produced in different strains of mice (differing in AHH responsiveness) after administration of 2,3,7,8-TCDD or TCDF followed by i.p. injection of sheep erythyrocytes. In the AHH responsive mice (C3H/HeN and C57BL/6) the number of PFC produced after prior administration of 1.2 μg kg^{-1}d^{-1} for 7 days of 2,3,7,8-TCDD was significantly reduced. In non-responsive mice (DBA/2) a very much greater dose was required (30 μg kg^{-1}d^{-1}) in order to elicit the same response. For 2,3,7,8-TCDF, 180 μg kg^{-1} reduced humoral response in C57BL/6 mice, but not in DBA/2 strain mice, again demonstrating a relationship with AHH responsiveness.

A study on 2,3,7,8-TCDF in Hartley guinea pigs indicated the effects are similar to those of 2,3,7,8-TCDD, although doses required were increased by approximately tenfold.[124] Alterations in immune responses have been detected in the absence of overt signs of toxicity.

A number of immunological effects have been studied in mice by Clark *et al.*[17] Groups of C57BL/6 male mice received four weekly i.p. injections of 2,3,7,8-TCDD at doses of between 0.001 μg kg^{-1} week^{-1} and 100 μg kg^{-1} week^{-1} and were studied 1 week after the fourth dose. The results indicated the cellular content of the thymus was lowered by 50% by doses of 1 μg kg^{-1} week^{-1} and doses of 25 μg kg^{-1} week^{-1} were shown to reduce dramatically the cellularity of the spleen and peripheral lymphocytes. The number of plaque-forming cells in the spleen produced in response to sheep erythrocytes (administered i.p.) or trinitrophenol conjugated *Brucella abortus* was considerably decreased at 6 days after immunization in animals receiving 10 μg kg^{-1} week^{-1}; IgM, IgG and IgA responses were also affected. Further investigations indicated that depletion of T-helper cells was not the cause of the decreased immune competence. The production of cytotoxic T-lymphocytes in response to the injection of 2×10^7 P-815 irradiated tumour cells (a source of the H-2^d alloantigen) when measured 6 or 7 days after immunization, was reduced in suspensions of spleen cells from animals receiving 0.1 μg kg^{-1} week^{-1} or higher doses. The generation of cytotoxic T-cells *in vitro* was reduced when cultures were made from the peripheral lymph nodes of

mice which had received 0.001 μg kg^{-1} week^{-1}. The addition of 2,3,7,8-TCDD (25 ng) directly to control cultures produced no suppression, whereas the mixing of cultures from control and test animals (0.1 μg kg^{-1} .week^{-1}) produced the response associated with the test culture. The level of precursors to cytotoxic T-cells was not reduced suggesting the response was due to an increase in suppression, possibly via an activation of T-suppressor cells.

Subsequent work by the same group found that the immunosuppressive effects of 2,3,7,8-TCDD were dependent on the Ah gene, not the H-2 locus.[126] By transplanting lymphomyeloid cells from one strain of mouse into irradiated recipient mice, the Ah genotype of the recipient was found to be dominant. It was suggested that this was due to an Ah receptor present in the thymus; the thymic receptor would be expected to have a lower dissociation constant (*Kd*) than the liver Ah receptor.

The developing immune system in animals has been found to be particularly susceptible to the effects of 2,3,7,8-TCDD exposure. The effects can be mediated either through *in utero* exposure or postnatal exposure from mother's milk. Pre- and postnatal exposure to 2,3,7,8-TCDD in F344 rats has been shown to produce prolonged reduction in certain immune functions: delayed hypersensitivity and lymphoproliferative response.[127] The doses received by the mothers to elicit a response in the offspring were 5 μg kg^{-1} by gavage on days 0, 7 and 14 after birth, with some groups receiving an additional prenatal dose on day 18 of gestation. Some indication of the exact dose received by the pups is required however before one can extrapolate these findings to man. A similar study conducted in 5-week-old Swiss-Webster mice whose mothers had received 2,3,7,8-TCDD in the diet (1, 2.5 or 5 ppb) from 4 weeks before mating to 3 weeks after birth (total dose approximately 4 μg kg^{-1} for the 1 ppb group) has been reported.[128] The results were not entirely consistent with those reported by Faith & Moore,[127] as no alterations were detected in delayed hypersensitivity, lymphoproliferation or antibody response to sheep erythrocytes although a significant dose-related increase in mortality due to *S.typhimurium* endotoxin was demonstrated together with a reduction in PFC at 2.5 and 5 ppb.

Some studies have been reported on the effects of other PCDDs on the immune system. 2,7-DCDD has been shown to produce a number of decreased immune responses, including a dose-related decrease in IgM-producing PFCs from splenic cells of young female B6C3F1 mice.[130] The effects of 2,7-DCDD were not as potent as 2,3,7,8-TCDD (used as a positive control). This finding is of interest as 2,7-DCDD has very low or no binding affinity for the Ah receptor and may indicate some aspect of PCDD/PCDF toxicity to be independent of the Ah receptor, although further work in this area is required. The same study also reported a negative effect on the immune system by OCDD in one test system, although it was not studied as comprehensively as 2,7-DCDD.

The immunosuppressive effects of pentachlorophenol contaminants, including OCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF were studied in mice by Kerkvleit *et al.*[129] Mice (C57BL/6 strain) were given a single gavage dose of the compounds 2 days prior to i.p. inoculation with 2.5 × 10^8 SRBC (T-dependent antigens). Mice were killed 5 days after inoculation. The IgM response of isolated splenic cells was not reduced by OCDD at doses of up to 500 mg kg^{-1}, however a 50% inhibitory dose (ID_{50}) of 7.1, 85 and 208 μg kg^{-1} was shown for 1,2,3,6,7,8-HxCDD; 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF respectively. In a similar study, Vecchi *et al.*[123] reported 2,3,7,8-TCDD to have an ID_{50} of 0.65 μg kg^{-1}.

Proposed mechanisms of toxicity of PCDDs and PCDFs

Ah receptor and enzyme induction

The similarities between the effects produced in experimental systems by PCDDs, PCDFs and other halogenated hydrocarbons such as PCBs and PBBs (polybrominated biphenyls) suggests the possibility of a common mechanism.[54] Such a mechanism was proposed by Poland & Glover[131] and was based upon studies on 2,3,7,8-TCDD following the identification of the 'Ah-receptor'. The studies involved the use of mice strains having a high sensitivity for AHH induction (C57BL/6) a low sensitivitity (DBA/2) or crosses having intermediate sensitivity. The sensitivity to AHH induction was found to relate to levels of a so called 'Ah-receptor'.

The Ah (aromatic hydrocarbon) receptor is a soluble intracellular protein that binds specific halogenated aromatic compounds such as 2,3,7,8-TCDD and certain polycyclic aromatic hydrocarbons such as 3-methylcholanthrene. A major function of the Ah-receptor is to regulate the induction of specific forms of cytochrome P-450. In animals exposed to aryl hydrocarbon hydroxylase (AHH) inducers. It has been demonstrated that the binding affinities of compounds for the Ah-receptor correlate well with their toxicity and ability to induce AHH[132] and also δ-aminolevulinic acid (ALA) synthetase.[133] The induction of these

enzyme activities is long lived and occurs following the administration of very low levels of dioxins. The activity of AHH in hepatic microsomes in female rats was seen to be significantly increased following an oral administration of a 0.002 μg kg^{-1} dose of 2,3,7,8-TCDD.[130]

Compounds capable of inducing both AHH and ALA synthetase (e.g. PCDDs, PCBs, PCDFs and other halogenated aromatics) have two common properties: the first is halogen atoms occupying a minimum of three (or four for maximal activity) of the lateral ring position (i.e. 2,3,7,8) and secondly, at least one ring position must be unsubstituted. Therefore the binding affinities of various PCDDs and related compounds for the Ah-receptor can in general be explained by the shape and electronic configuration of the molecule. However, the precise mechanism of PCDD interactions with the Ah-receptor has not yet been determined.

The ligand–receptor complex is transported into the nucleus, where it is thought to bind to DNA. Evidence for this step has been provided by the analysis of radio-labelled 2,3,7,8-TCDD in preparations of cell nuclei.[132] The interaction of the complex with DNA is thought to rely on both electrostatic and configurational effects.[125] The binding of the complex to DNA alters the rate of translation of a battery of genes coding for enzymes and other macromolecules, possibly via promotor and inhibitor domains[136] which are ultimately responsible for the range of biological effects seen.

The requirement for a receptor to mediate the action of PCDDs on DNA would explain the low level of 2,3,7,8-TCDD binding to isolated DNA compared to many known genotoxic carcinogens and the lack of mutagenic potential in test systems. Several species (both vertebrate and invertebrate) including humans[114] possess a TCDD-binding receptor, homologous to the Ah-receptor identified in the mouse and it has been suggested that this protein has been conserved during evolution to perform a role in these species.[137] It has also been suggested that the Ah-receptor complex could have once served in evolution as a hormone or growth factor receptor for a variety of ectodermally-derived tissues.[138] The Ah-receptor has been isolated from a number of organs[114] and it is conceivable that it exists in such tissues without a natural ligand and is activated only when tissues are exposed to potent toxins such as 2,3,7,8-TCDD.[138] There are both species and strain differences in the response to 2,3,7,8-TCDD and it is possible that not only concentrations of the receptor but also variations in the binding affinities, translocation into the nucleus and response of the affected genes could explain such differences.

The Ah-receptor has many physicochemical properties that are similar to the properties of receptors for steroid hormones and it appears that the Ah-receptor and steroid receptors belong to a common family. Kester & Gasiewicz[137] reported that studies from several laboratories have shown the Ah-receptor to be an oligomeric protein, comparable to steroid receptors in Stokes radius, sedimentation coefficient, molecular weight and frictional ratio, with an equilibrium dissociation constant for 2,3,7,8-TCDD within the range characteristic of steroids. The Ah-receptor is more hydrophobic than steroid receptors, however, it interacts with polyanions (including DNA) in a similar manner. It has been reported that the Ah-receptor, like receptors for steroid hormones contain sulphydryl groups which are essential for ligand binding function, although there exist subtle differences between the two receptor types in response to a spectrum of sulphydryl-modifying agents.[134] In spite of the number of similarities between the two receptor types, the Ah-receptor is apparently not identical to any known steroid receptor.[137] It has been suggested that there is slight evidence that oestradiol levels may affect the action of 2,3,7,8-TCDD and its receptor at the genomic level.[139] However, Kester & Gasiewicz[137] reported that oestradiol, hydrocortisone, progesterone, testosterone (and also the non-steroidal oestrogen diethylstilboestrol) did not enhance Ah-recovery or stability and furthermore TCDD binding was not inhibited by any of these steroids, indicating that they do not compete with TCDD for specific binding to the Ah-receptor.

In a study by Gasiewicz & Bauman[141] evidence had been presented for the existence of at least three forms of the Ah-receptor prepared from rat hepatic cytosol *in vitro* and identified as: an unoccupied form, an occupied form showing some affinity toward DNA and an additional occupied form that is less negatively charged and has greater affinity for DNA. The transformation to the latter was dependent upon the presence of 2,3,7,8-TCDD and the duration of the incubation and temperature. As assumed for the transformation of steroid hormone receptors, this transformation may be due to exposure of positively charged groups on the receptor molecule. The precise mechanism that mediates the transformation of the Ah-receptor to DNA binding species is unknown. However, the finding that the fully transformed receptor can be isolated from hepatic nuclei of rats pre-treated with TCDD seem to indicate that the above process may occur *in vivo*.

There is therefore evidence to suggest that Ah-receptor binding is necessary for many aspects of PCDD/PCDF toxicity and it is likely that a definite structure–activity relationship exists. 2,3,7,8-TCDD

has a very high affinity for the receptor and also has greatest toxic potency. The role of the Ah-receptor binding and receptor-mediated events in the tumour promoting activity of 2,3,7,8-TCDD has yet to be fully determined.[142]

Lipid peroxidation

Lipid peroxidation of membrane components, as measured by levels of malonaldehyde and conjugated dienes, has been reported to increase following the administration of 2,3,7,8-TCDD to the rat.[143,144] Other authors have disputed these findings as in another study there was found to be no increase in ethane exhalation (another marker for lipid peroxidation) after administration of 2,3,7,8-TCDD.[145]

An increase in the level of free-radicals formed due to lipid peroxidation, has been suggested as a mechanism for some of the non-specific effects of PCDDs.[143] It has been suggested that a correlation exists between species susceptibility to TCDD-induced hepatic lipid peroxidation, AHH inducibility, inhibition of GSH-PX (selenium-dependent glutathione peroxidase), thymic atrophy and body weight loss.[131] The administration of the antioxidant butylated hydroxyanisole (BHA) or reduced glutathione to female Sprague-Dawley rats which received 2,3,7,8-TCDD demonstrated a decrease in the level of lipid peroxidation (as malonaldehyde formation) and the BHA treatment protected against the mortality produced by 2,3,7,8-TCDD.[144] These results suggest that the levels of oxidizing molecules are increased by 2,3,7,8-TCDD administration, by either a primary effect, e.g. due to direct inhibition of glutathione peroxidase or a secondary effect due to 2,3,7,8-TCDD being transported into the nucleus and inducing oxidizing enzyme synthesis.

Vitamin A

Some of the effects of TCDD intoxication resemble those of vitamin A deficiency e.g. keratosis and immunosuppression and, it has been suggested that depletion of vitamin A stores in the liver produced by 2,3,7,8-TCDD administration may be responsible for some of the signs of 2,3,7,8-TCDD toxicity.[146] An oral dose of 2,3,7,8-TCDD (10 $\mu g\ kg^{-1}$) has been found to modify the metabolism of vitamin A, when administered to Sprague Dawley rats.[147] The administration of vitamin A was shown to extend the survival time of female Sprague Dawley rats when given 2,3,7,8-TCDD.[147] Whilst these studies demonstrated vitamin A metabolism to be altered by 2,3,7,8-TCDD, it is not known whether this is a primary or secondary effect.

Other mechanisms

Other mechanisms proposed to explain the multifarious effects of 2,3,7,8-TCDD include modifications in hormone levels, changes in metabolism due to enzyme induction and modifying the response of the epidermal growth factor receptor.

Interactions

Since 'dioxins' bind to the Ah-receptor and are inducers of hepatic enzyme activity, it is possible that they may modify the toxicity of other xenobiotics. Furthermore, the modification of 'dioxin' toxicity by other components of an environmental mixture, is also possible. Studies on these interactions are not extensive at present, but those which have been published to date indicate that the combined effects may be additive/synergistic[79,115,148–151] or antagonistic.[79,152] The major factors in determining any interactive effect are the genetic make-up of the exposed animal and the exact chemical nature of the mixture.

TCDD equivalents

There is at present no reliable, rapid test for assessing the biological activity of environmental samples likely to contain PCDDs and PCDFs. The assessment of potential risk is based on individual isomer analysis and relating these results to the toxicity (where known) of the individual isomers.' In order to simplify the risk assessment procedure and to permit some form of regulatory control, the concept of 'TCDD equivalents' has been introduced. This concept is derived from relating the toxicities of groups of isomers to the toxicity of 2,3,7,8-TCDD to produce a 'toxic equivalence factor' with a summation procedure to give a 'TCDD equivalent' for the mixture. The major drawback of the TCDD equivalents concept is that it is assumed all isomers in a defined group have the same toxicity and that all interactions are simply additive.

Whilst TCDD equivalents are of use for screening mixtures, there are not enough data on the toxicities of many of the isomers to give a definite estimate of risk. Furthermore, where equivalence factors are derived, they are often based upon *in vitro* studies. The lack of reliable toxicity data is highlighted by the different equivalence factors given to the same groups of isomers by different authorities.[153]

Although the concept cannot be used as a tool for accurately predicting human health effects, it is useful in assessing priorities in terms of potential risk to human health.

Mortality and morbidity in humans

There has been a considerable number of reports of

'non-experimental' human exposure to both PCDDs and PCDFs. In virtually all' instances however, exposures were to mixtures of PCDDs and/or PCDFs as well as to a range of other chemicals sharing similar properties. Furthermore it has not been possible to ascertain accurately the uptake by individuals. Dose–response relationships cannot therefore be established for any of the adverse effects observed. Commonly the design and evolution of the reported studies detract from the conclusions drawn by the authors. Nonetheless the human epidemiological data need to be evaluated carefully as animal data have demonstrated considerable variation in species sensitivity and toxicokinetics.

Circumstances under which human exposures to PCDDs and PCDFs have taken place fall into a number of categories. Exposure to 2,3,7,8-TCDD is the prominent feature in most occupational studies, and has been associated with: the synthesis of trichlorophenol and trichlorophenate; the synthesis, compounding and use of trichlorophenoxyacetic acid herbicides and accidental releases to the environment in both types of industrial processes. Exposure to PCDFs has been studied in the follow-up of incidents involving PCBs: this includes the 'Yusho' incidents (associated with ingestion of rice oil contaminated with PCBs) and following accidental fires involving electrical transformers containing PCBs.

Exposures associated with the synthesis of trichlorophenol and trichlorophenate have resulted in outbreaks of chloracne and malaise. Dugois *et al.*[154] reported chloracne in workers at a French factory synthesizing 2,4,5-trichlorophenol from tetrachlorobenzene. Other complaints by the workforce included loss of weight, anorexia, weakness, headache, digestive disturbances and conjunctivitis. These observations were not necessarily related to the presence or severity of chloracne. The authors were unable to identify the agent or agents responsible for the outbreak, although it is conceivable that PCDDs could at least have been partly responsible for some of the effects seen.

DOW Chemicals mortality data

Investigations into mortality of trichlorphenol workers at DOW Chemicals have been reported by Cook *et al.*[155,156] The first report[155] referred to only a few workers and the most that could be concluded from the results (apart from the development of chloracne in exposed workers) was that the TCDD contaminant was unlikely to be a very powerful human carcinogen. The second report[156] involved a far more detailed analysis of the Company data. A mortality study was carried out on a total of 2189 men involved in the manufacture of higher chlorinated phenols between 1940 and 1979. An attempt was made to provide estimates of 2,3,7,8-TCDD exposure potential for each job. Up to the end of 1979 death certificates were obtained for a total of 298 men known to have died. For cancer sites of particular interest (such as soft tissue tumours and non-Hodgkin's lymphoma) there were no significant increases in standard mortality ratios nor were dose–response relationships observed.

A study of workers synthesizing penachlorophenol (PCP) and sodium pentachlorophenate (Santobrite)

A group of workers at a plant synthesizing PCP and Santobrite between 1950 and 1978 were reviewed in 1979, 1980 and 1982.[157] Exposures had been to PCP and intermediate lower chlorophenols, and a number of PCDDs and PCDFs. The PCP contained hexa-, hepta- and octa-homologues of PCDDs at levels of 5–15 ppm, 23–250 ppm and 1900 ppm, respectively. No 2,3,7,8-TCDD was detected in the PCP product at the then detection limit of 10–15 ppb. The Santobrite product contained hexa-CDDs and OCDD.

The results of the general health study among the workers were stated to show a non-significant elevation of serum triglycerides compared with controls. Those with chloracne had greater disturbances than those exposed without chloracne.

The authors reported that exposure to PCP had probably varied over the years, with higher exposures in the earlier years and lower exposure latterly following considerable improvements in conditions. Because of the limitations in the design of the study, little can be said about the true incidence or natural history of the observed effects. However, it does support observations noted in other studies of the association between' dioxin' exposure, chloracne and disturbance of lipid metabolism.

The Philips Duphar accident

An explosion occurred in March 1963 at a plant synthesizing sodium trichlorophenate from 1,2,4,5-tetrachlorobenzene, causing widespread contamination of the containing building. A study was conducted to look at the effects of exposure during cleaning-up activities in 1963.[158] The results revealed normal liver function tests in the workers exposed and a number of cases of chloracne. A follow-up report on morbidity was subsequently published.[159] However, details of the studies are limited and to date no significant differences have been attributed to exposure.

The Coalite episode

A number of studies have been reported following an incident in 1968 at a UK plant (Coalite)

synthesizing 2,4,5-trichlorophenol. As a result of unscheduled conditions a higher than normal yield of 2,3,7,8-TCDD occurred followed by an accidental release of reagents.[160] Initially, a study was conducted on 14 workers identified as having been involved in the accident.[160] Some slight complaints were reported plus a variety of abnormal biochemical and haematological results, which all returned to normal in 10 days. A follow-up of 79 workers who had suffered from chloracne up to the end of 1968[161] found that the condition persisted in a quarter. There were however, no acceptable biochemical indicators of toxicity, before, during or after chloracne developed and off-spring of these workers were reported to be normal and healthy. Unfortunately, in common with a number of other reports, the lack of epidemiological expertise, and inadequate study design leave it open to a number of criticisms. Further *ad hoc* investigations on various subsets of these exposed workers have been reported by other authors. Jensen *et al.*[162] reported chloracne in two fitters, and also in a son of one and wife of another. No other adverse effects were noted (liver function and lipids were normal). Walker & Martin[163] independently conducted a study of eight men with chloracne 10 years after cessation of exposure. Five had raised serum triglyceride levels and gamma glutamyl transpeptidase (GGT) levels, and all had reduced high density lipoproteins (HDL) and increased total cholesterol. Martin[164] compared exposed Coalite workers with controls for biochemical effects, and found higher cholesterol and triglyceride levels in the exposed group together with higher D-glucaric acid/creatinine ratio. Finally, Blank *et al.*[165] carried out a study of genotoxic effects 10 years after exposure, and found no statistically significant differences between exposed and non-exposed groups.

The BASF explosion

In 1953 an explosive release took place at a BASF plant from a vessel in a process synthesizing 2,4,5-TCP from 1,2,4,5-tetrachlorobenzene. Hoffmann[166] and Goldmann[167] gave accounts of the incident and Goldmann[168] reviewed the conditions observed in the 42 chloracne-affected workers and described the natural history of their development. Subsequent to these reports, Theiss *et al.*[169] reported a mortality study of the total number of people involved in the incident (70 exposed initially and four for short periods during the next 2 years). In this population, 66 cases of chloracne and dermatitis were recorded and overall the mortality for the 'dioxin exposed' group was less than external controls, but slightly higher than for the internal controls. It was reported that mortality for 'all malignancies' was above expectation, but individually only stomach cancer reached statistical significance. The small size of the population and the small numbers of deaths do not permit definitive conclusions to be reached from this study. The authors refer to reports of stomach cancer excess in other studies.

The exposure to trichlorophenoxyacetic acid-based herbicides and their 'dioxin' contaminants has perhaps led to the most widely studied aspect of human health effects relating to dioxins. A brief account is provided of some of these studies, with an indication of the effects noted in the populations studied.

The Danish phenoxy herbicide industry experience

In Denmark, the use of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), which contains 2,3,7,8-TCDD has always been limited, and it has not been marketed since 1980. The principal production has been of D-type phenoxy herbicides (based upon 2,4-dichlorophenol) and M-type (based upon 4-chloro-ortho-cresol). While these herbicides contain PCDDs, they are thought to be of the less toxic variety and 2,3,7,8-TCDD is apparently not present.[170] Lynge[171] attempted to study all persons in Denmark involved in the manufacture of phenoxy herbicides before 1982. However, of the four factories investigated, only two had suitable data for analysis. The study was undertaken in the hope of evaluating the potential carcinogenic effects of the DM phenoxy herbicides. A number of analyses were conducted looking at employees from the two factories separately and also in combination. While the conclusions seem to add support for an excess risk of soft tissue sarcoma to be associated with phenoxy herbicide exposure, the authors were uncertain about the validity of their findings, since various biases may have operated.

A UK phenoxy herbicide study

A mortality study was conducted by Coggan *et al.*[172] on 5754 people of a total workforce of 5784 employed between 1947 and 1975, classified according to whether their exposure to phenoxy acids was 'high', 'low' or 'background'. 2-Methyl-4-chlorophenoxyacetic acid (MCPA), considered not to contain 2,3,7,8-TCDD was the principal product manufactured and contract sprayed by the company between 1947 and 1982. A range of other compounds were manufactured, bought in and formulated including 2,4,5-T and other phenoxy acids, though on a smaller scale. Mortality statistics were compared with those expected for England and

and the regions of Lombardy. An attempt was made to follow-up all children born in the contaminated zones. The study involved the complete examination of 2989 infants at birth and a periodic examination of physical and behavioural developments subsequently. No clear association was demonstrated between health [illegible]

thus making the task of determining exposure of these subjects to 2,3,7,8-TCDD very difficult.

Greenwald et al.[168] investigated the association of soft tissue sarcoma with service in Vietnam and herbicide exposure, using the New York Cancer Registry. No [illegible]

Wales and separately for aggregated rural areas for the period 1968–1978. The results did not suggest that these chemicals were powerful carcinogens. There were difficulties in defining an adequate control group, and the study was of insufficient power to identify low orders of carcinogenicity.

The West Virginia 2,4,5-T factory experience

In 1949 during the synthesis of 2,4,5-T, a reaction vessel overheated and the explosion which followed resulted in the contamination of the local environment. Four workers exposed in 1949 in the clean-up after the accident were examined. Subsequently an investigation was carried out on a larger number of exposed plant workers.[173,174] A mixture of signs and symptoms was reported including chloracne, hepatic enlargement, peripheral neuritis, delayed prothrombin time and elevated serum lipids. Of some 200 workers thought to have been exposed, 86% developed chloracne. There was said to be an increase in upper gastrointestinal tract ulcers as well as a decrease in pulmonary function following exposure. There were no increased risks detected for cardiovascular, hepatic, renal or nervous system diseases and no adverse effects regarding reproductive function or birth defects were recorded.

Moses & Prioleau[175] using a population of workers exposed following this incident, studied the incidence of skin lesions present up to 30 years after the onset of disease. Of 37 workers found still to have chloracne, 21 were reported to have had the condition for 30 years. In a further study conducted in 1979 on both current and retired workers,[176] a variety of non-specific symptoms were shown to be equally distributed among those with and without chloracne. The results demonstrated that acute effects of 2,3,7,8-TCDD were largely reversible with the exception perhaps of skin effects, changes in peripheral nerves and possibly changes in liver metabolism. No reproductive effects were detected. In addition to TCDD exposure, it was also reported that 50% had also been exposed to the known human carcinogen 4-amino biphenyl.

A Czechoslovakian herbicide factory study

Between 1965 and 1968, 80 out of 400 production workers became ill in a Czech factory synthesizing trichlorophenoxyacetic acid-based herbicides. The illness was attributed to the TCDD liberated in the process.[177] Although environmental levels were not measured at the time, TCDD was detected in the product and was detected several years later in the building and on painted surfaces. Of the 80 workers who had reported adverse effects, 55 were followed-up in hospital, where the natural history of development of complaints were studied. Disorders reported were immediate or delayed and included porphyria, lipid disturbance, hepatic diseases with plasma protein disturbances, abnormal glucose tolerance tests and central and peripheral nervous system disease. No cardiac, renal or blood diseases were reported. When workers were reinvestigated, some 15 years later, lipid metabolism effects and glucose tolerance abnormalities were still observable. The authors also reported severe general atherosclerosis associated with some psychiatric disorders.

A follow-up study of workers synthesising 2,4-D and 2,4,5-T

Poland et al.[178] followed-up 73 workers previously studied by Bleiberg et al.[179] at a factory synthesizing 2,4,-D and 2,4,5-T, containing 2,3,7,8-TCDD. In the original study, 11 workers had uroporphyrinuria, several had hirsutism, hyperpigmentation or skin fragility, or all three. Two had vesicular eruptions on 'exposed' areas, two had hepatic dysfunction with liver biopsies showing UVR fluorescence. Three workers were considered to have fulfilled the criteria for the diagnosis of porphyria cutanea tarda (PCT). It was shown that neither chloracne nor PCT were related the to degree of exposure to 2,4-D and 2,4,5-T and furthermore 6 years later there were no cases of PCT. However, over this period the TCDD content of the products would have been markedly reduced as would other porphyriagenic agents. The study demonstrated the prevalence of liver dysfunction to be minimal. Personality disturbances seemed to be related to chloracne. However, no cardiovascular system, respiratory system, gastrointestinal or central nervous system associated diseases could be linked with exposure. In conclusion, the results do seem to indicate a link with TCDD exposure and interference with liver porphyrin metabolism, although some authors[180] have disputed this finding, linking the raised porphyrin levels with hexachlorobenzene exposure.

The Seveso accident

This incident occurred at a 2,4,5-trichlorophenol manufacturing plant causing the release of about 2–3 kg of 2,3,7,8-TCDD, as well as TCP and other substances. The toxic cloud drifted downwind, settling over fields and houses in the vicinity. Three major zones were identified according to the level of TCDD soil contamination. A medical surveillance programme was set up, but it was not designed as a sensitive epidemiological study. Chloracne was first reported some 6 weeks after the accident, the frequency of chloracne correlated to some degree with the level of TCDD soil contamination. It was reported that by the end of 1978 the

ence of soft tissue sarcoma in exposed agricultural and forestry workers in Sweden[193,195] have not been confirmed by later studies. However, some studies have indicated ... cystitis and a further case study[206] reported an unverified skin condition, nose bleeds, [illegible]

chloracne effect had disappeared.

Biochemical effects were studied between 1976 and 1981 in a group of 700 children, subdivided according to zone of residence. Parameters studied included liver function tests, lipid metabolism and porphyrins. There appeared to be no significant difference between groups when related to degree of exposure or incidence of chloracne. In the study of porphyria, although a few cases of 'hereditary' chronic hepatic prophyria (CHP) were reported, a number of confounding causes were involved. In the 'non-hereditary' cases, porphyria was reported, but its clinical manifestation – PCT, was lacking. Goldstein *et al.*[181] reported 2,3,7,8-TCDD to be the most potent porphyrogenic agent known. It inhibits uroporphyrinogen decarboxylase (UD). In chronic TCDD poisoning, this results in an increase in liver uro- and heptacarboxyporphyrins. It appears that a single dose does not produce porphyria, although it has been suggested that such an exposure will lead to PCT if there is a predisposition in the form of an inherited UD defect.

The long-term effects of TCDD in children, aged 6–10 years at the time of the incident were investigated by Mocarelli *et al.*[182] Children were examined annually from 1976 to 1982. Various subsets were identified according to TCDD contamination of soil at residence. A slight increase in gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) was found in boys in the 'highest contamination zone' (where levels of up to 54 ppb had been recorded). The results seemed to indicate that males were more susceptible than females to this effect. However, it was noted that even the most 'exposed' children did not have alteration in cholesterol and triglycerides.

Biochemical determinations of liver function were carried out in 427 of the most heavily exposed persons during the year following the incident. No untoward effects were recorded in this population; paradoxically the 'control' group chosen seemed to exhibit a marginally greater abnormality than the exposed group.

Neurological effects in the exposed populations were followed-up.[183] Peripheral neuropathy was found in 733 residents, who were evacuated 15–20 days after the accident from the most polluted area. The observations made were the result of screening only a proportion of the total number, at 9 months and 21 months after the incident. The authors identified some cases of mild polyneuropathy.

In view of the animal evidence for a teratogenic effect of 2,3,7,8-TCDD, a Seveso Birth Defects register was set up in 1977. This included all live and stillbirths in the various Seveso zones and in 11 municipalities, covering data from hospitals, local health authorities and paediatric services.[184] The available data showed no single birth defect to be unequivocally linked with 2,3,7,8-TCDD exposure. It should be noted that due to the limited number of births in the high exposure zone, the likelihood of detecting anything less than an enormous effect was minimal.

Spontaneous abortion incidence was reported to the health authorities before 1978, and after this time the only available data were from hospital records. The information gathered indicated no difference between polluted and unpolluted zones, although it was noted that a wide variation in rates within each zone occurred, possibly attributable to the small numbers involved. A further study was reported by Remotti *et al.*[185] stating that there was no increase in abortion rate in the 6 months after the incident.

At the time of the Seveso incident, a number of abortions were carried out. Chromosome analyses were performed on aborted tissues, as well as maternal peripheral blood.[186] Although higher frequencies of chromosomal lesions were found in fetal tissues when compared to adult fibroblasts, they were comparable to those observed in amniotic fluid cells of 'normal pregnancies' at corresponding gestation periods. In 1979, aborted tissues and specimens of maternal blood became available from control (unexposed) pregnancies. There appeared to be no statistically significant differences between maternal blood and placental cells between the 'exposed' and 'unexposed' groups. However, a higher frequency of chromosomal abnormalities was noted in the 'exposed' fetal tissues, indicating the possibility of an effect linked to maternal exposure to 2,3,7,8-TCDD. Although to date there is no evidence for an increase in birth defects in this population, it is still not possible to draw definitive conclusions about a lack of genetic consequence associated with the chromosomal aberrations in the exposed population. Remotti *et al.*[185] reported on a study of placental tissues from a number of women who were 8–12 weeks pregnant at the time of the incident, and who had subsequently been exposed to environmental levels of 2,3,7,8-TCDD. Of the 22 placentae studied, all showed normal histology, but seven of these showed unusual ultrastructural changes. The women were asymptomatic and examination of the fetuses showed no abnormalities. Therefore the significance of these findings remains to be determined. The lesions identified could have resulted from factors other than TCDD exposure.

Investigations into the incidence of stillbirths and neonatal deaths indicated that in general, the rates were below those reported for Italy overall and were within the ranges for the province of Milan

and the regions of Lombardy. An attempt was made to follow-up all children born in the contaminated zones. The study involved the complete examination of 2989 infants at birth and a periodic examination of physical and behavioural developments subsequently. No clear association was demonstrated between health problems and exposure, but compliance rates were reported to be as low as 30–50%. Any biochemical abnormalities noted were always associated with chronic hepatitis B virus infection.

Immunocompetence was studied in children with and without chloracne, in workers at the chemical factory and military personnel. No immunological impairment was detected. A further series of studies have been cited in which immune function in exposed children was investigated.[182] The subjects demonstrated higher titres of total haemolytic complement activity in serum, lymphocyte response to phytohaemagglutinin and pokeweed mitogen in the early part of the series of studies and a tendency toward higher values of peripheral blood lymphocytes. These changes did not correlate with any clinical disease.

Finally, studies conducted on mortality of persons living around the area of Seveso at the time of the incident have to date failed to reveal any excess in overall mortality for particular causes such as cardiovascular disease, hypertension and liver disease. A report by the Seveso Cancer Registry indicated a higher incidence of soft tissue sarcoma but no other cancers in exposed subjects, though causality is not yet demonstrable.[187]

The health experiences of civil and military personnel potentially exposed to phenoxyacid herbicides have been investigated in a number of studies. Conditions studied in these populations including soft tissue sarcoma, other malignant disease, non-malignant physical disease and psychological disease and the development of birth defects in the offspring of exposed persons, are discussed below.

Agent Orange and the study of defects in Vietnam Veterans

Between 1962 and 1971 a range of herbicides/defoliants was sprayed in Vietnam. The most widely used was 'Agent Orange', which was reported to be contaminated by up to 30 mg kg^{-1} 2,3,7,8-TCDD. Among the 2.6 million Americans in Vietnam, only 1200 were crew involved in aerial spraying and 200 were civilians disposing of the surplus. It is reported that in addition to Agent Orange other herbicides based on Picloram and insecticides based on Malathion were also used, thus making the task of determining exposure of these subjects to 2,3,7,8-TCDD very difficult.

Greenwald *et al.*[188] investigated the association of soft tissue sarcoma with service in Vietnam and herbicide exposure, using the New York Cancer Registry. No such association could be identified.

Erickson *et al.*[189] used the Atlanta Congenital Defects Program in a case-control study to determine if children of Vietnam Veterans had any excess risk of birth defects. Among the problems involved in the study was that of evaluating actual exposure to Agent Orange. No evidence could be found for an excess risk of birth defects. Donovan *et al.*[190] reported on a study in Australian Vietnam Veterans, in which similar problems were encountered regarding assessment of actual exposure. Once more, no association between exposure and risk of malformations in the offspring were found.

A study of the general health of the US Air Force herbicide sprayer Veterans was reported in which the results were compared with a control group not involved in spraying.[191] The study revealed no increase in the incidence of chloracne or soft tissue sarcoma. However, the exposed Veterans did show higher incidence of non-melanoma skin cancer, minor birth defects in offspring, neonatal deaths, liver enlargement, porphyria cutanea tarda and psychological disorders. The methods used to establish measurement of exposure in this study are open to criticism on the grounds of errors of misclassification and an alternative approach to the use of the data has been proposed.[192]

A further study has been reported on US Air Force herbicide spraying personnel during 1962–1971, with controls chosen as crew from cargo aircraft.[193] The study now includes a planned follow-up to investigate mortality and morbidity until 2002. The results so far have not revealed any abnormal herbicide-associated mortality experience. However, it has disclosed a number of medical findings in the exposed group whose significance is of a minor or undetermined nature at present. The extended detailed follow-up will indicate their health significance. The investigators considered there to be insufficient evidence at present to support a cause and effect relationship between herbicide exposure and adverse health in the exposed group, and the data do seem reassuring in so far as none of the reported symptoms are common to those of dioxin exposure.

Studies to identify a link between the use of phenoxyacid herbicides and the development of soft tissue sarcoma and malignant lymphoma have been conducted in Sweden,[194–197] New Zealand[171,198–201] and United Kingdom.[172] Earlier reports of an association with an increased incid-

ence of soft tissue sarcoma in exposed agricultural and forestry workers in Sweden[194,195] have not been confirmed by later studies. However, some studies have indicated an excess in risk of non-Hodgkin's lymphoma in exposed workers. In all cases the studies are unable to reveal a link specifically with 2,3,7,8-TCDD, since the exposures are always confounded by the presence of the phenoxyacid herbicides.

Missouri waste oil contamination – exposed population studies

In 1971, 29 kg of 2,3,7,8-TCDD-contaminated sludge was mixed with waste oils and used as a dust suppressant over ten counties of Missouri.[202] By 1983 some 241 residential, work and recreational areas were suspected of having become contaminated.

A number of studies were reported on the populations thought to be at risk from the exposures. In a pilot epidemiological study, 130 people out of some 800 considered to be at risk were examined for detailed physical, biochemical, immunological and haematological defects. The results demonstrated no abnormalities of porphyrin metabolism, liver function or lipid metabolism. There may be a number of reasons why no specific effect was seen, possibly resulting from the limited scope of the study.

Part of the pilot study involved the study of immunity,[203] while there were no statistically significant differences between 'high' and 'low' exposure groups in terms of delayed hypersensitivity on skin test (indeed there are questions about the technique) within the 'high' exposure group there was a small percentage of individuals with diminished T-helper to T-suppressor cell ratios, suggesting that 2,3,7,8-TCDD exposure may affect human cellular immunity. Further work would be required to confirm these findings. Results of this pilot study have been published in greater detail and the significance of the findings further discussed.[204]

A further study on residents exposed whilst living on a contaminated caravan site in Missouri was reported by Hoffman *et al.*.[205] None of the studies on residential exposure provide any evidence to suggest any adverse effects on liver function or lipid metabolism disturbance in relatively high risk groups overall.

An outbreak of poisoning resulting from three horse-riding arenas having been sprayed with the contaminated dust suppressant has been reported.[206] Several incomplete case reports were provided of children and adults with varied symptoms, including skin rashes. One child was reported to have suffered nose bleeds and haemorrhagic cystitis and a further case study[206a] reported an unverified skin condition, nose bleeds, headaches and diarrhoea. Exposed horses, dogs, cats, birds and mice were seriously affected and died. Animals were more affected than humans; this could result from marked differences in species sensitivity and/or from differences in intake.

As part of the general Missouri study, levels of 2,3,7,8-TCDD were measured in adipose tissue taken from groups of exposed and non-exposed subjects.[207] The cases were persons exposed to 2,3,7,8-TCDD either at work, residentially or recreationally. All persons in both groups had detectable levels of TCDD in their adipose tissues. These results indicate the possibility of other background exposures. Of those subjects demonstrating adipose tissue levels greater than 100 ppt, five came from persons exposed during production of hexachlorophene and one came from a man who had been horse-riding in a contaminated arena.

Some 600 workers at a trucking terminal in Missouri were investigated in 1983 to evaluate the potential health effects of exposure to TCDD-contaminated waste oil used as a dust suppressant.[208] A former worker who had become grossly contaminated, subsequently (some 10 years after the first exposure) developed porphyria cutanea tarda and later developed tumours in the right femur and pelvis. Angiosarcoma was diagnosed, but the site of origin (bone or soft tissue) was not determined. The causal association remains uncertain. The study of workers at the contaminated terminals is continuing and further studies are being carried out on workers exposed at several chemical plants, but to date nothing significant has been reported.

Analytical chemists exposed to a TCDD standard

Oliver[209] reported on the clinical progress of three scientists who had become exposed to 2,3,7,8-TCDD standards whilst developing analytical methods for assessing the dioxin content in 2,4,5-T. The author considered that effects seen in the analysts resulted solely from exposure to 2,3,7,8-TCDD, since there had been no previous reports of adverse effects in colleagues exposed to similar reagents in the absence of dioxin contamination. After limited exposure on few occasions there were varying latent periods before the onset of skin effects and signs and symptoms of disease. The effects seen varied between the individuals, although liver function tests were normal in all three. On the whole signs and symptoms resolved after about a year following exposure and subsequently follow-up was abandoned because of negative findings (personal communication).

if used thoughtfully.

PCDDs and PCDFs therefore present a great challenge to both regulator and investigative toxicologist. At present the chemic-

20: 401–4.

[9] Arsenault RD. Pentachlorophenol and contai chlorinated dibenzo-

Transformer oil: exposure of maintenance workers and exposure after fires

A study was conducted on transformer maintenance workers who had been exposed to the mixed polychlorinated biphenyl product Aroclor 1260, and occasionally Aroclors 1255 and 1242.[210] The chemical contaminants of PCBs tend to be PCDFs rather than PCDDs. Examination of bulk oils have revealed TCDF concentrations of 13–116 ppb by weight. The results indicated that apart from statistically significant differences for adipose PCBs, 24-hour urinary 17-hydroxycorticosteroid excretion, serum PCB concentration and serum gamma GT, there were no significant biochemical differences found between currently exposed, past and non-exposed groups.

Following an electrical transformer fire in an office building in Binghamton (USA), the building became widely contaminated with soot, containing PCBs (28–23 000 ppm) and PCDFs (1–1200 ppm). Air sampling at the final stages of decontamination showed maximum total PCDF and specifically 2,3,7,8-TCDF concentrations of 264 and 23 pgm^{-3}, respectively. Low concentrations of PCDDs and polychlorinated biphenylenes were also detected.[211]

Two years after the incident, chemical analyses were conducted on liver and fat biopsies of subjects 'exposed' to the soot and the results were compared to fat measurements from 'non-exposed' persons.[212] Although levels of PCDDs and PCDFs were higher in the exposed subjects, they were also detected in non-exposed persons at a lower level, thus indicating a 'background' body burden of these substances. The signs observed in one exposed subject included hypertension and raised serum cholesterol and triglycerides; whether the association was causal or incidental is not established. In addition this subject and two others studied had mild portal fibrosis. However in all cases, exposure to a range of compounds had been involved.

Toxic rice oil (Yusho) outbreaks

In 1968 in south-west Japan and in 1979 in Taiwan, two extensive groups were poisoned by rice bran oil contaminated with PCB heat exchange fluid. The poisoning resulted in thousands of people being affected by a wide range of signs and symptoms referrable to most organ systems, and including swelling of the upper eyelids with discharge and acneiform eruptions with skin pigmentation.[213] Babies born to both affected and non-affected females were found to have abnormal pigmentation of skin, eyelids, nails and gums as well as increased conjunctival discharge. The incident was originally thought to be due to PCBs. The PCBs however, were found to contain high levels of PCDFs and polychlorinated quaterphenyls and more recently, the presence of a further compound, 3,4,3',4'-tetrachlorobiphenyl has also been detected.[214] Levels of PCBs and PCDFs measured in blood, adipose tissue and liver of the two groups were determined[211,212] and a number of estimates of amounts of contaminants ingested were assessed.[217] In general it was found that the clinical severity correlated more closely with total amount of oil consumed rather than amount of oil $kg^{-1}d^{-1}$; this is consistent with results from animal studies with 2,3,7,8-TCDD.[37]

The health status of affected persons in Japan and Taiwan was reviewed some years later.[218] Although a number of signs and symptoms had reduced considerably, some persisted in a substantial proportion of those re-examined. By the end of 1982, 112 of the affected Japanese had died (of whom in only 31 cases was cause of death confirmed). The small numbers, variety of conditions and the very mixed exposure did not permit any causal association to be established between death and PCDF exposure. A further analysis[219] up to 1983 of 487 females and 552 males, of whom 28 females and 42 males had died, presumably the same population reported by Masuda,[218] concluded that there was no significant excess of liver cancer in males and mortality for ischaemic heart disease in females. Other authors[220] reviewing 79 male and 41 female deaths in this population found a significant overall cancer death excess only for males (33 observed versus 15.5 expected) accounted for largely by liver cancer (9O:1.6 E) and lung cancer (8O:2.5 E). A significant excess for heart disease was not found in females in this analysis.

Background 'dioxin' exposure in man

Monitoring of human tissues for the presence of PCDDs and PCDFs indicates that there are 'background' sources of exposure to these compounds.[221] The primary source of exposure is largely thought to arise from environmental pollution from waste disposal, incineration and other combustion operations.[222]

PCDDs and PCDFs tend to concentrate mainly in lipid-rich tissues, i.e. adipose tissue and human milk-fat, and to a lesser extent in liver, muscle, kidney[223] and blood.[224] Most of these compounds remain in the body for some time after exposure. Estimates of half-life values of PCDDs and PCDFs have been reported in the literature.[207,225] The values seem to depend both on the chlorination number of the chemical as well as the specific isomer in question. Patterson *et al.*[207] estimated the half-life of 2,3,7,8-TCDD in adipose tissue to be between 5–8 years, commenting that previous

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estimates of one year were not consistent with the results they had obtained in their studies. Another study conducted by Poiger & Schlatter[226] indicated a half-life figure of approximately 5.5 years for 2,3,7,8-TCDD in a single male volunteer, although a more recent presentation indicates the figure to be nearer 10 years.[228]

Since samples of liver, kidney, muscle and adipose tissue can normally only be taken during surgery or autopsy, the favoured approach for more general monitoring studies has been the measurement of PCDDs and PCDFs in human milk and blood. Hitherto the latter has only been of use when major exposures to these chemicals are expected, since the levels are generally very low in such samples because of the limited fat content of blood. However more recently, blood lipid estimations of 2,3,7,8-TCDD have been found to correlate well with adipose tissue levels and lipo-suction has replaced more conventional biopsy. Jensen[225] carried out a review of results obtained from determinations of PCDDs, PCDFs and PCBs in human milk, blood and adipose tissue. He concluded that the levels associated with 'background' exposure to PCDDs and PCDFs may be 50–300 times lower than levels associated with adverse effects from occupational or accidental exposure. Comparing the results from PCB analyses, it was demonstrated that these occur at levels of about 10 000 times higher than the sum of levels of PCDDs and PCDFs although these substances are [illegible] than PCBs. Because these organics have similar sites of concentration in the body, and because of the potential for toxicological interactions, Jensen suggests it would be wrong to look at only one contaminant at a time. There is experimental evidence for antagonism between certain PCBs and 2,3,7,8-TCDD in effects on enzyme induction (aryl hydrocarbon hydroxylase, ethoxy resorufin O-deethylase) and immunotoxicity (plaque-forming cell response) varying with the ratio of the two agents[227] and, also, evidence for potentiation of teratogenic effects with other PCBs (HCB).[115]

Discussion

PCDDs and PCDFs are both ubiquitous and persistent in the environment. They are generated by natural and man-made combustion processes as well as being present as unwanted by-products in a range of commercially important compounds. They have been found in body tissues of apparently unaffected animals and humans and it appears that their presence in both occurs largely as a result of the ingestion of food. Even if at the prevailing levels of environmental contamination it were determined that no significant human health hazard existed, they are of considerable interest to the toxicologist.

The most extensively studied of the PCDDs and PCDFs is 2,3,7,8-TCDD. This isomer in certain species has been shown to be one of the most toxic compounds known. The effects produced in animals are wide ranging and include thymic atrophy, immune system dysfunction, teratogenicity, fetotoxicity and carcinogenicity. Of the PCDDs and PCDFs so far studied it appears that the toxic effects seen tend to vary quantitatively rather than qualitatively, with different species, strains and genders differing in their responses. Whilst some insight has been achieved into some of the mechanisms of action at the molecular level, much remains to be determined. The involvement of the Ah-receptor and the necessity for chlorine substitution at positions 2,3,7 and 8 is generally accepted, however further investigation is required into other possible mechanisms of action. Until the mechanisms of toxicity are clearly understood it will be difficult to extrapolate the results of animal studies to man.

The human data on 'dioxins' are limited and difficult to interpret. Human exposure studies have virtually all been 'non-experimental' and on that ground alone present difficulty in interpretation. Too often the quality of investigation and the time lag between exposure and investigation have adversely affected the outcome of the study. To varying degrees all exposures studied have been to mixtures of PCDDs or PCDFs as well as to other compounds. In the epidemiological studies, it is rarely possible to distinguish the effects of the 'dioxins' from other associated compounds. Neither has it been possible to distinguish between the relative human toxicity of different 'dioxins' present in mixtures.

Attempts to estimate the carcinogenic risk to man from exposure to 2,3,7,8-TCDD have failed to generate consistent results and are therefore unreliable. As 2,3,7,8-TCDD is considered a tumour promoter and non-genotoxic it is reasonable to propose the use of safety factors to assess the 'acceptability' of a particular level of exposure. Since PCDDs and PCDFs mostly occur as chemical mixtures, many Regulatory Authorities have adopted the use of the 'TCDD Equivalents' scheme. This approach relies on the simple addition of toxicological potencies of individual chemicals. There are obvious drawbacks to this approach as chemicals in mixtures may also act synergistically or even antagonistically. However, until biological assays to predict the overall potency of 'dioxin' containing mixtures are available the 'equivalents' approach seems to be the best option

if used thoughtfully.

PCDDs and PCDFs therefore present a great challenge to both regulator and investigative toxicologist. At present the ubiquitous nature of these chemicals in the environment, the lack of proven no-effect levels in animal studies and the paucity of data which can be related to man present a number of problems. In the future, with the elucidation of the mechanisms by which these chemicals produce their multitude of effects, the task of establishing 'acceptable levels' in the environment will be made easier. Furthermore, it may be possible to gain some insight into the mechanisms of certain natural disorders.

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References

[1] IARC. Some fumigants, the herbicides 2,4-D and 2,4,5-T, chlorinated dibenzodioxins and miscellaneous industrial chemicals. *IARC Monograph* 1977; 15: 41–102.

[2] Schreiber R. The clean up begins. *Bulletin of Environmental Contamination and Toxicology* 1984; 33: 726–34.

[3] Barnes DG. Assessing TCDD emissions from municipal waste combustors. *Chemosphere* 1983; 12: 645–55.

[4] Stanley JS & Sack TM. Protocol for the analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by high resolution gas chromatography/high resolution mass spectrometry. EPA 600/4-86-004; January 1986.

[5] Fishbein L. Analysis of aromatic amines, organochlorine compounds and 'Dioxin' in biological samples. In *Proceedings of an International Workshop*. Environ Specimen Banking Monit Relat Banking, 1984.

[6] Tong HY, Shore DL, Karasek FW, Helland P & Jellum E. Identification of organic compounds obtained from incineration of municipal waste by HPLC fractionation and GC-MS. *Journal of Chromatography* 1984; 285: 423–41.

[7] Ryan JJ, Lizotte R & Newsome WH. Study of chlorinated diphenylethers and chlorinated 2-phenoxyphenols as interferences in the determination of chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans in biological samples. *Journal of Chromatography* 1984; 303: 351–60.

[8] Bottomley P. Chromatographic analysis of hazardous impurities in pesticides. *Analytical Proceedings* 1983; 20: 401–4.

[9] Arsenault RD. Pentachlorophenol and contained chlorinated dibenzodioxins in the environment. A study of environmental fate, stability and significance when used in wood preservation. *American Wood-preserver's Association* 1976; 122–48.

[10] Crummett WB. Environmental chlorinated dioxins from combustion – the trace chemistries of fire hypothesis. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, ed. O. Hutzinger pp. 253–63. Pergamon Series on Environmental Science, no. 5. Oxford: Pergamon.

[11] Ballschimiter K, Buchert H, Niemczyk R, Munder A & Swerev M. Automobile exhausts versus municipal-waste incineration as sources of the polychlorodibenzodioxins (PCDDs) and furans (PCDFs) in the environment. *Chemosphere* 1986; 15: 901–15.

[12] Marklund S, Rappe C, Tysklind M & Egeback K. Identification of polychlorinated dibenzofurans and dioxins in exhausts from cars run on leaded gasoline. *Chemosphere* 1987; 16: 29–36.

[13] Esposito MP & Watkins DR. Airborne dioxins: The problem in review. Presentation given at the 73rd Annual Meeting of the Air Pollution Control Association, June 22-27, 1980, Montreal, Quebec.

[14] Crummett WB & Townsend DI. The trace chemistries of fire hypothesis: review and update. *Chemosphere* 1984; 13: 777–88.

[15] Olie K, Vermeula PL & Hutzinger O. Chloro-*p*-dioxins and chlorodibenzofurans are trace components of fly ash and flue gas of some municipal incinerators in the Netherlands. *Chemosphere* 1977; 8: 455–9.

[16] Doyle BW, Drum DA & Lauber JD. The smouldering question of hospital wastes. *Pollution Engineering* (Jul, 1955); 35–9.

[17] Young AL. Long-term studies on the persistence and movement of TCDD in a natural ecosystem. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds g eds RE Tucker, AL Young & AP Gray, pp. 173–90. Environmental Science Research 26, 1983.

[18] Oehme M, Mano S, Mikalsen A & Kirschmer P. Quantitative method for the determination of femtogram amounts of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in outdoor air. *Chemosphere* 1986; 15: 607–17.

[19] Rappe C, Nygren M, Lindstrom G & Hansson M. Dioxins and dibenzofurans in biological samples of European origin. *Chemosphere* 1986; 15: 1635–9.

[20] Facchetti S, Balasso A, Fichner C. Studies on the absorption of TCDD by some plant species. *Chemosphere* 1986; 15: 1387–8.

[21] Crosby DG. Conquering the monster – the photochemical destruction of chlorodioxins. *American Chemical Society* 1978; 173: 1–12.

[22] Liberti A, Brocco D, Allergrini I & Bertoni G. Field photodegradation of TCDD by ultraviolet radiations. In: Dioxin: Toxicological and Chemical Aspects eds F Cattabeni *et al.*, vol. 1, 1978.

[23] Tschirley FH. Dioxin. *Scientific American* 1986; 254: 21–7.

[24] Hutzinger O, Blumich MJ, Berg M & Olie K. Sources and fate of PCDDs and PCDFs: An overview.

Chemosphere 1985; 14: 581–600.

[25] Bumpus JA, Tien M, Wright D & Aust SD. Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 1985; 228: 1434–6.

[26] Hutter R & Philippi M. Studies on microbial metabolism of TCDD under laboratory conditions. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment ed. O Hutzinger, pp. 87–93. Pergamon Series on Environmental Science no. 5. Oxford: Pergamon, 1982.

[27] Matsumura F, Quensen J & Tsushimoto O. Microbial degradation of TCDD in a model ecosystem. In: Human and Enviromental Risks of Chlorinated Dioxins and Related Compounds eds RE Tucker, AL Young & AP Gray, pp. 191–219, Environmental Science Research vol. 26. 1983.

[28] EPA (US Environmental Protection Agency). Health assessment document for PCDDs, parts 1 and 2: External Review Draft 1984; PB 84-220268.

[29] Gallo M. Cited in *Pesticide and Toxic Chemical News* 1985; 13: 13.

[30] Van den Berg M, deVroom E & Olie K. Bioavailability of PCDDs and PCDFs on fly ash after semichronic oral ingestion by guinea pig and Syrian golden hamster. *Chemosphere* 1986; 15: 519–33.

[31] Gasiewicz TA, Geiger LE, Rucci G & Neal RA. Distribution, excretion and metabolism of 2,3,7,8-TCDD in C57BL/6J, DBA/2J and B6D2F_1/J mice. *Drug Metabolism and Disposition* 1983; 11: 397–403.

[32] Gasiewicz TA & Neal RA. 2,3,7,8-TCDD tissue distribution, excretion and effects on clinical chemical parameters in guinea pigs. *Toxicology and Applied Pharmacology* 1979; 51: 329–39.

[33] Allen JR, Van Miller JP & Nor[illegible] Tissue distribution, excretion and [illegible] of (^{14}C)-TCDD in rats. *Food and Cosm[illegible]xicology* 1975; 13: 501–5.

[34] Van Miller JP, Matlar RJ & Allen JR. Tissue distribution and excretion of tritiated TCDD in non-human primates and rats. *Food and Cosmetics Toxicology* 1976; 14: 31–4.

[35] Applelgren LE, Brandt I, Bittebo EB, Gillner M & Gustafsson JA. Autoradiography of 2,3,7,8-tetrachloro (^{14}C) – dibenzo-*p*-dioxin: accumulation in the nasal mucosa. *Chemosphere* 1983; 12: 545–8.

[36] Gillner M, Bittebo EB & Brandt I. Uptake and specific binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the olfactory mucosa of mice and rats. *Cancer Research* 1987; 47: 4150–9.

[37] Decad GM, Birnbaum LS & Matthews HB. 2,3,7,8-TCDF tissue distribution and excretion in guinea pigs. *Toxicology and Applied Pharmacology* 1981; 57: 231–40.

[38] Birnbaum LS, Decad GM & Matthews HB. Disposition and excretion of 2,3,7,8-TCDF in the rat. *Toxicology and Applied Pharmacology* 1980; 55: 342–52.

[39] Van den Berg M, Olie K & Hutzinger O. Uptake and selective retention in rats of orally administered chlorinated dioxins and dibenzofurans from fly ash and fly ash extract. *Chemosphere* 1983; 12: 537–44.

[40] Nishizumi M & Masuda Y. Enhancing effect of 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran on diethyl nitrosamine hepatocarcinogenesis in rats. *Cancer Letters* 1986; 33: 333–9.

[41] Moore JA, Harris MW & Albro PW. Tissue distribution of ^{14}C-tetrachlorodibenzo-*p*-dioxin in pregnant and neonatal rats. *Toxicology and Applied Pharmacology* 1976; 37: 146–7.

[42] Nau H & Bass R. Transfer of 2,3,7,8-TCDD to mouse embryo and foetus. *Toxicology* 1981; 20: 299–308.

[43] Nagayama J, Tokudome S, Kutatsune M & Masuda Y. Transfer of PCDFs to the foetuses and offspring of mice. *Food and Cosmetics Toxicology* 1980; 18: 153–8.

[44] Ramsey JC, Hefner JG, Karbowski RJ, Braun WH & Gehring PJ. The *in vivo* biotransformation of 2,3,7,8-TCDD in the rat. *Toxicology and Applied Pharmacology* 1983; 69: 180–4.

[45] Olson JR & Bitner WE. Comparative metabolism and elimination of 2,3,7,8-TCDD. *Toxicologist* 1983; 3: 103–8.

[46] Weber H, Poiger H & Schlatter C. Acute oral toxicity of TCDD-metabolites in male guinea pigs. *Toxicology Letters* 1982; 14: 117–22.

[47] Mason G & Safe S. Synthesis, biologic and toxic effects of the major 2,3,7,8-tetrachlorodibenzo-*p*-dioxin metabolites in the rat. *Toxicology* 1986; 41: 153–9.

[48] Tulp MTM & Hutzinger O. Rat metabolism of polychlorinated dibenzo-*p*-dioxins. *Chemosphere* 1978; 7: 761–8.

[49] Williams DT, Cunningham HM & Blanchfield BJ. Distribution and excretion studies of octachlorodibenzo-*p*-dioxin in the rat *Bulletin of Environmental Contamination and Toxicology* 1972; 7: 57–62.

[50] Nerback DH, Engblom JF & Allen JR. Tissue distribution and excretion of octachlorodibenzo-*p*-dioxin in the rat. *Toxicology and Applied Pharmacology* 1975; 32: 330–8.

[51] Birnbaum LS, Decad GM, Matthews HB & McConnell EE. Fate of 2,3,7,8-TCDF in the monkey. *Toxicology and Applied Pharmacology* 1981; 57: 189–96.

[52] Bowman RE, Schantz SL, Weerasinghe NCA, Gross ML & Barsotti DA. Abstract from Dioxin '87 Symposium, USA, 4–9 October 1987.

[53] Vinopal JH & Casida JE. Metabolic study of 2,3,7,8-TCDD in mammalian liver microsomal systems and living mice. *Archives of Environmental Contamination and Toxicology* 1973; 1: 122–32.

[54] McConnell EE, Moore JA, Haseman JK & Harris MW. The comparative toxicity of chlorinated dibenzo-*p*-dioxins in mice and guinea pigs. *Toxicology and Applied Pharmacology* 1978; 44: 335–56.

[55] Nishizumi M. Acute toxicity of polychlorinated dibenzofurans in CF-1 mice. *Toxicology and Applied Pharmacology* 1978; 45: 209–12.

[56] Pluss N, Poiger H, Hohbach C, Suter C & Schlatter C. Subchronic toxicity of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) in rats. *Chemosphere* 1988; 17: 1099–110.

[57] Harris MW, Moore JA, Vos JG & Gupta BN. General biological effects of TCDD in laboratory animals. *Environmental Health Perspectives* 1973; 5:

101–9.
[58] Kociba RJ, Keeler PA, Park CN & Gehring PJ. 2,3,7,8-TCDD: results of a 13 week oral toxicity study in rats. *Toxicology and Applied Pharmacology* 1976; 35: 553–74.
[59] NTP (National Toxicology Program). Bioassay of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (gavage) for possible carcinogenicity. NTP-80-12 1980; PB 81-124844.
[60] Puhvel SM, Sakamoto M, Erth DC & Reisner RM. Hairless mice as models for chloracne: study of cutaneous changes induced by topical application of established chloracnegens. *Toxicology and Applied Pharmacology* 1982; 64: 492–503.
[61] Knutson JC & Poland A. Keratinisation of mouse teratoma cell line XB produced by 2,3,7,8-TCDD: an *in vitro* model of toxicity. *Cell* 1980; 22: 27–36.
[62] Milstone LM & Lavigne JF. 2,3,7,8-TCDD induces hyperplasia in confluent cultures of human keratinocytes. *Journal of Investigative Dermatology* 1984; 82: 532–4.
[63] Schwetz BA, Norris JM, Sparschu GL, *et al.* Toxicology of chlorinated dibenzo-*p*-dioxins. *Environmental Health Perspectives* 1973; 5: 87–99.
[64] McNulty WP, Pomerantz I & Farrell T. Chronic toxicity of 2,3,7,8-TCDF for Rhesus macaques. *Food and Cosmetics Toxicology* 1981; 19: 57–65.
[65] Oithi S, Morita M & Fukada H. Comparative toxicity of PCBs and dibenzofurans in rats. *Toxicology and Applied Pharmacology* 1978; 43: 13–22.
[66] NTP (National Toxicology Program). Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (CAS No 1746-01-6) in Osborne-Mendel rats and B6C3F1 mice (gavage study). NTP/80-31 1982; PB 82-163445.
[67] Kociba RJ, Keyes DG, Beyer JE *et al.* Results of a 2 year chronic toxicity and oncogenicity study of 2,3,7,8-TCDD in rats. *Toxicology and Applied Pharmacology* 1978; 46: 279–303.
[68] NCI (National Cancer Institute). Bioassay of 2,7-dichlorodibenzo-*p*-dioxin (DCDD) for possible carcinogenicity. NCI Technical Report 1979a; No. 123. PB 290-570.
[69] NCI (National Cancer Institute). Bioassay of Dichloro-*p*-dioxin for possible carcinogenicity. NCI Technical Report 1979; No. 122. PB 288-475.
[70] McNulty WP. Fetotoxicity of 2,3,7,8-TCDD for Rhesus Macaques (*Macaca mulatta*). *American Journal of Primatology* 1984; 6: 41–7.
[71] Allen JR, Barsotti DA, Van Miller JP, Abrahamson LJ & Lalich JJ. Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-TCDD. *Food and Cosmetics Toxicology* 1977; 15: 401–10.
[72] Schantz SL, Barsotti DA & Allen JR. Toxicological effects in non-human primates chronically exposed to 50 ppt 2,3,7,8-TCDD. *Toxicology and Applied Pharmacology* 1979; 48: A180.
[73] King ME, Shefner AM & Bates RR. Carcinogenesis bioassay of chlorinated dibenzodioxins and related chemicals. *Environmental Health Perspectives* 1973; 5: 163–70.
[74] Toth K, Somfai-Relle S, Sugar J & Bence J. Carcinogenicity testing of herbicides 2,4,5- trichlorophenoxyethanol containing dioxin and pure dioxin in Swiss mice. *Nature* 1979; 278: 548–9.
[75] Pitot HC, Goldsworthy T, Campbell HA & Poland A. Quantitative evaluation of the promotion by 2,3,7,8-TCDD of hepatocarcinogenesis from diethylnitrosamine. *Cancer Research* 1980; 40: 3616–20.
[76] Kouri RE, Rude TH, Joglekar R, *et al.* 2,3,7,8-TCDD as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumours in mice genetically 'non-responsive' at Ah locus. *Cancer Research* 1978; 38: 2777–83.
[77] Nishizumi M & Masuda Y. Enhancing effect of 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran on diethylnitrosamine hepatocarcinogenesis in rats. *Cancer Letters* 1986; 33: 333–9.
[78] Digiovanni J, Viaje A, Berry DL, Slaga T & Juchau MR. Tumour initiating ability of 2,3,7,8-TCDD and Aroclor 1254 in the two stage system of mouse skin carcinogenesis. *Bulletin of Environmental Contamination and Toxicology* 1977; 18: 552–7.
[79] Digiovanni J, Berry DL, Juchau MR & Slaga TJ. 2,3,7,8-TCDD: Potent anticarcinogenic activity in CD-1 mice. *Biochemical and Biophysical Research Communications* 1979; 86: 577–84.
[80] Wassom JS, Huff JE & Loprieno N. A review of the genetic toxicology of chlorinated dibenzo-*p*-dioxins. *Mutation Research* 1978; 47: 141–60.
[81] Giti AK. Mutagenic and genotoxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. a review. *Mutation Research* 1986; 168: 241–8.
[82] Shu HP, Paustenbach DJ & Murray FJ. A critical evaluation of the use of mutagenesis, carcinogenesis and tumour promotion data in a cancer risk assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Regulatory Toxicology and Pharmacology* 1987; 7: 57–88.
[83] McCann. Personal communications cited by Wassom et al, 1978 (No. 80).
[84] Seiller JP. A survey of the mutagenicity of various pesticides. *Experientia* 1973; 29: 622–3.
[85] Hussain S, Ehrenberg L, Lofroth G & Gejuall T. Mutagenic effects of TCDD on bacterial systems. *Ambio* 1972; 1: 32–3.
[86] Geiger LE & Neal RA. Mutagenicity testing of 2,3,7,8-TCDD in histidine auxotrophs of *Salmonella typhimurium*. *Toxicology and Applied Pharmacology* 1981; 59: 125–9.
[87] Gilbert P, Saint-Ruf G, Poncelet F & Mercier M. Genetic effects of chlorinated anilines and azobenzenes on *Salmonella typhimurium*. *Archives of Environmental Contamination and Toxicology* 1980; 9: 533–41.
[88] Bronzetti G, Zelger E, Lee I, Suzuki K & Malling HV. Genetic effects of 2,3,7,8-TCDD in yeast, *in vivo* and *in vitro*. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, ed. O Hutzinger, Pergamon Series on Environmental Science no. 5. Oxford: Pergamon, 1982.
[89] Rogers AM, Anderson ME & Back KC. Mutagenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and perfluoro-*n*-decanoic acid in L5178Y mouse-lymphoma cells. *Mutation Research* 1982; 105: 445–9.

[90] Kondorosi A, Fedorcsak I, Solymosy F, Ehrenberg L & Osterman-Golkar S. Inactivation of QBRNA by electrophiles. *Mutation Research* 1973; 17: 149–61.

[91] Poland A & Glover E. An estimate of the maximum *in vivo* covalent binding of 2,3,7,8-TCDD to rat liver protein, ribosomal RNA and DNA. *Cancer Research* 1979; 39: 3341–4.

[92] Althus FR, Lawrence SD, Sattler GL, Longfellow DG & Pitot HC. Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential carcinogens. *Cancer Research* 1982; 42: 3010.

[93] Loprieno N, Shrana L, Rusciano D, Lascialfari D & Lari T. *In vivo* cytogenetic studies on mice and rats exposed to 2,3,7,8-TCDD. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, ed. O Hutzinger, pp. 419–28, Pergamon Series on Environmental Science no. 5. Oxford: Pergamon Press.

[94] Khera KS & Ruddick JA. PCDDs: Perinatal effects and the dominant lethal test in Wistar rats. *Advances in Chemistry Series* 1973; 120: 70–84.

[95] Hay A, Ashby J, Styles JA & Elliott B. The mutagenic properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *American Chemical Society* 1983; 186: 6 (abstract).

[96] Abernethy D J, Greenlee WF, Huband JC & Boreiko CJ. 2,3,7,8-TCDD promotes the transformation of C3H/10T½ cells. *Carcinogenesis* 1985; 6: 651–3.

[97] Zimmering S, Mason JM, Valencia R & Woodruff RL. Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environmental Mutagenesis* 1985; 7: 87–100.

[98] Meyne J, Allison DC, Bose K, Jordan SW, Ridolpho PF & Smith J. Hepatotoxic doses of dioxin do not damage mouse bone-marrow chromosomes. *Mutation Research* 1985; 157: 63–9.

[99] Green S & Moreland FS. Cytogenetic evaluation of several dioxins in the rat. *Toxicology and Applied Pharmacology* 1975; 33: 161 (abstract).

[100] Commoner B. Reliability of bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 1976; 600/1 – 76 – 022 PB 259–934.

[101] Schoeny R. Mutagenicity testing of chlorinated biphenyls and chlorinated dibenzofurans. *Mutation Research* 1982; 101: 43–56.

[102] Knutson JC & Poland A. 2,3,7,8-TCDD: failure to demonstrate toxicity in twenty three cultured cell types. *Toxicology and Applied Pharmacology* 1980; 54: 377–83.

[103] Rice RH & Cline PR. Opposing effects of 2,3,7,8-TCDD and hydrocortisone on growth and differentiation of cultured malignant ketatinocytes. *Carcinogenesis* 1984; 5: 367–71.

[104] Nelson JO, Menzel RE, Kearney PC, Plimmer JR. 2,3,7,8-TCDD: *in vitro* binding to rat liver microsomes. *Bulletin of Environmental Contamination and Toxicology* 1977; 18: 9–13.

[105] Eaton DL & Klassen CD. Effects of 2,3,7,8-TCDD, kepone and polybrominated biphenyls on transport systems in isolated rat hepatocytes. *Toxicology and Applied Pharmacology* 1979; 51: 137–44.

[106] Courtney KD & Moore JA. Teratogenicity studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-TCDD. *Toxicology and Applied Pharmacology* 1971; 20: 396–403.

[107] Courtney KD, Copeland MF & Robbins A. The effects of pentachloronitrobenzene, hexachlorobenzene and related compounds on fetal development. *Toxicology and Applied Pharmacology* 1976; 35: 239–56.

[108] Smith FA, Schwetz BA and Nitschke KD. Teratogenicity of 2,3,7,8-TCDD in CF-1 mice. *Toxicology and Applied Pharmacology* 1976; 38: 517–23.

[109] Neubert D & Dillman I. Embryotoxic effects in mice treated with 2,4,5-T and 2,3,7,8-TCDD. *Naunyn Schmeiderberg's Archives of Pharmacology* 1972; 272: 243–64.

[110] Sparschu GL, Dunn FL & Rowe VK. Study of the teratogenicity of 2,3,7,8-TCDD in the rat. *Food and Cosmetics Toxicology* 1971; 9: 405–12.

[111] Giavini E, Prati M & Vismara C. Rabbit teratology study with 2,3,7,8-TCDD. *Environmental Research* 1982; 27: 74–8.

[111a] Giavini E, Prati M & Vismara C. Embryotoxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administered to female rats before mating. *Environmental Research* 1983; 31: 104–10.

[112] Murray FJ, Smith FA, Nitschke KD, Humiston CG & Kociba RJ. Three generation reproduction study of rats given 2,3,7,8-TCDD in the diet. *Toxicology and Applied Pharmacology* 1979; 50: 241–52.

[112a] Barsotti DA, Abrahamson LJ & Allen JR. Hormonal alterations in Female Rhesus monkeys fed a diet containing 2,3,7,8-TCDD. *Bulletin of Environmental Contamination and Toxicology* 1979; 21: 463–9.

[113] Mittler JC, Ertel NH, Peng RX, Yang CS & Kiernan T. Changes in testosterone hydroxylase activity in the rat testis following administration of 2,3,7,8-TCDD. *Annals of the New York Academy of Science* 1984; 438: 645–8.

[114] Silbergeld EK & Mattison DR. Experimental and clinical studies on the reproductive toxicology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *American Journal of Industrial Medicine* 1987; 11: 131–44

[115] Birnbaum LS, Weber H, Harris MW, Lamb JC & McKinney JD. Toxic interaction of specific chlorinated biphenyls and 2,3,7,8-TCDD: increased incidence of cleft palate in mice. *Toxicology and Applied Pharmacology* 1985; 77: 292–302.

[116] Hassoun EM & Denkert L. TCDD embryotoxicity in the mouse may be enhanced by β-naphthoflavone, another ligand of the Ah receptor. *Toxicology Letters* 1982; 12: 191–8.

[117] Weber H, Harris MW, Haseman JK & Birnbaum LS. Teratogenic potency of TCDD, TCDF and TCDD-TCDF combination in C57BL/6N mice. *Toxicology Letters* 1985; 26: 159–67.

[118] Schantz SL, Laughlin WK, Van Valkenberg HC & Bowman RE. Maternal care by Rhesus monkeys of infant monkeys exposed to either lead or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Neurotoxicology* 1986; 7: 637–50.

[119] Bowman RE, Schantz SL, Gross ML & Ferguson S. Abstract from Dioxin '87 Symposium, USA, 4–9 October 1987.
[120] Hassoun E, D'Argy R, Denker L, Lundin LG & Borwell P. Teratogenicity of 2,3,7,8-TCDF in BXD recombinant inbred strains. *Toxicology Letters* 1984; 23: 37–42.
[121] Hassoun E, D'Argy R & Denker L. Teratogenicity of 2,3,7,8-TCDF in the mouse. *Journal of Toxicology and Environmental Health* 1984; 14: 337–51.
[122] Weber H, Lamb JC, Harris MW & Moore JA. Teratogenicity of 2,3,7,8-TCDF in mice. *Toxicology Letters* 1984; 20: 183–8.
[123] Vecchi A, Sironi M, Canegrati MA, Recchia M & Garattini S. Immunosuppressive effects of 2,3,7,8-TCDD in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. *Toxicology and Applied Pharmacology* 1983; 68: 434–41.
[124] Luster MI, Faith RE & Lawson LD. Effects of 2,3,7,8-TCDF on the immune system in guinea pigs. *Drug and Chemical Toxicology* 1979; 2: 49–60.
[125] Clark DA, Gauldie J, Szewczuk MR & Sweeney G. Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-TCDD. *Proceedings of the Society for Experimental Biology and Medicine* 1981; 168: 290–9.
[126] Nagarkatti PS, Sweeney GD, Gauldie J & Clark DA. Sensitivity to supression of cytotoxic T-cell generation by 2,3,7,8-TCDD is dependent on the Ah genotype of the murine host. *Toxicology and Applied Pharmacology* 1984; 72: 169–76.
[127] Faith RE & Moore JA. Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-TCDD. *Journal of Toxicology and Environmental Health* 1977; 3: 451–64.
[128] Thomas PT & Hindsill RD. The effect of perinatal exposure to TCDD on the immune response of young mice. *Drug and Chemical Toxicology* 1979; 2: 77–98.
[129] Kerkvleit NI, Braunet JA & Matlock JP. Humoral immunotoxicity of polychlorinated diphenyl ethers, phenoxyphenols, dioxins and furans present as contaminants of technical grade pentachlorophenol. *Toxicology* 1985; 36: 307–24.
[130] Holtzapple MP, McCay JA & Barnes DW. Immunosuppression without liver induction by subchronic exposure to 2,7-dichlorodibenzo-*p*-dioxin in adult female B6C3F1 mice. *Toxicology and Applied Pharmacology* 1986; 83: 445–55.
[131] Poland A & Glover E. A model for the mechanism of action of 2,3,7,8-TCDD. Estrogens in the Environment, ed. McClachan. Netherlands: Elsevier, 1980.
[132] Poland A, Greenlee WF & Kende AS. Studies on the mechanism of action of the chlorinated dibenzo-*p*-dioxins and related compounds. *New York Academy of Science* 1979; 320: 214–30.
[133] Shara MA & Stohs SJ. Biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) congeners in female rats. *Archives of Environmental Contamination and Toxicology* 1987; 16: 599–605.
[134] Kitchin KT & Woods JS. 2,3,7,8-TCDD induction of AHH in hepatic microsomes from female rats. *Toxicology and Applied Pharmacology* 1978; 45: 297.
[135] Hannah RR, Lund J, Poellinger L, Gillner M & Gustafsson JA. Characterisation of the DNA-binding properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *European Journal of Biochemistry* 1986; 156: 237–42.
[136] Jones PBC, Galeazzi DR, Fisher JM & Whitlock JP. Control of cytochrome P_1-450 gene expression by dioxin. *Science* 1985; 227: 1499–1502.
[137] Kester JE & Gasiewicz TA. Characterisation of the *in vitro* stability of the rat hepatic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Archives of Biochemistry and Biophysics* 1987; 252: 606–25.
[138] Pratt RM, Dencker L & Diewert ML. 2,3,7,8-TCDD-induced cleft palate in the mouse: evidence for alterations in palatal shelf fusion. *Teratogenesis Carcinogenesis and Mutagenesis* 1984; 4: 427–36.
[139] Denison MS, Vella LM & Okey AB. Structure and function of the Ah receptor: sulphydryl groups required for binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to cytosolic receptor from rodent livers. *Archives of Biochemistry and Biophysics* 1987; 252: 388–95.
[140] Gallo MA, Hesse EJ, MacDonald GJ & Umbreit TH. Interactive effects of estradiol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic cytochrome P450 and mouse uterus. *Toxicology Letters* 1986; 32: 123–32.
[141] Gasiewicz TA & Bauman PA. Heterogeneity of the rat hepatic Ah receptor and evidence for transformation *in vitro* and *in vivo*. *Journal of Biological Chemistry* 1987; 262: 2116–20.
[142] Sloop TC & Lucier GW. Dose-dependent elevation of Ah receptor binding by TCDD in rat liver. *Toxicology and Applied Pharmacology* 1987; 88: 329–37.
[143] Stohs SJ, Hassan MQ & Murray WJ. Lipid peroxidation as a possible cause of TCDD toxicity. *Biochemical and Biophysical Research Communications* 1983; 111: 854–9.
[144] Hassan MQ, Stohs SJ & Murray WJ. Effects of vitamins E and A on 2,3,7,8-TCDD induced lipid peroxidation and other biochemical changes in the rat. *Archives of Environmental Contamination and Toxicology* 1985; 14: 437–42.
[145] Robertson LW, Regel U, Filser JG & Oesch F. Absence of lipid peroxidation as determined by ethane exhalation in rats treated with 2,3,7,8-TCDD. *Archives of Toxicology* 1985; 57: 13–16.
[146] Thunberg T, Ahlborg UG & Johnsson H. Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Archives of Toxicology* 1979; 42: 265–74.
[147] Hakansson H & Ahlborg UG. The effect of 2,3,7,8-TCDD on the uptake, distribution and excretion of a single dose of (11, 12-^{3}H) – retinyl acetate and on the Vitamin A status in the rat. *Journal of Nutrition* 1985; 115: 759–71.
[148] Rozman K. Hexadecane increases the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): is brown adipose tissue the primary target in TCDD-induced wasting syndrome? *Biochemical and Biophysical Research Communications* 1984; 125: 996–1004.
[149] Bannister R & Safe S. Synergistic interactions of 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl

in C57BL/6J and DBA/2J mice: Role of the Ah receptor. *Toxicology* 1987; 44: 159–69.

[151] Neubert D, Zens P, Rothenwalner A & Merker NJ. A survey of the embryotoxic effects of TCDD in mammalian species. *Environmental Health Perspectives* 1973; 5: 67–79.

[152] Hassoun EA. *In vivo* and *in vitro* interactions of TCDD and other ligands of the Ah receptor: effect on embryonic and fetal tissues. *Archives of Toxicology* 1987; 61: 145–9.

[153] Bannister R & Safe S. The effects of receptor antagonists on the AHH induction activity of 2,3,7,8-TCDD in C57BL/6 and DBA/2 mice: 1,3,6,8-TCDF. *Chemosphere* 1987; 16: 1739–42.

[154] USEPA (United States Environmental Protection Agency). Health assessment document for polychlorinated dibenzo-*p*-dioxins. EPA-600/8-84-014F. National Technical Information Service, Springfield 1985; VA PB86-122546/AS.

[155] Dugois P, Marechal J & Colomb L. Acne chorique au 2,4,5-trichlorophenol. *Archives Maladies Professionelles* 1958; 19: 626–7.

[156] Cook RR, Townsend JC, Ott MG & Silverstein LG. Mortality experience of employees exposed to 2,3,7,8-tetrachloridibenzo-*p*-dioxin. *Journal of Occupational Medicine* 1980; 22: 530–2.

[156] Cook RR, Bond GG, Olson RA, Ott MG & Gondek MR. Evaluation of the mortality experience of workers exposed to the chlorinated dioxins. *Chemosphere* 1986; 15: 1769–76.

[157] Baxter RA. Biochemical study of pentachlorophenol workers. *Annals of Occupational hygiene* 1984; 28: 429–38.

[158] Holmstedt B. Prolegomena to Seveso. *Archives of Toxicology* 1980; 44: 211–30.

[159] Dalderup LM & Zellenrath D. Dioxin exposure: 20 year follow-up. (letter). *Lancet* 1983; II: 1134–5.

[160] May G. Chloracne from the accidental production of tetrachlorodibenzodioxin. *British Journal of Industrial Medicine* 1973; 30: 276–83.

[161] May G. Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. *British Journal of Industrial Medicine* 1982; 39: 128–35.

[162] Jensen NE, Sneddon IB & Walker AE. Tetrachlorobenzodioxin and chloracne. *Transactions of St John's Hospital Dermatology Society* 1972; 58: 172–7.

[163] Walker AE & Martin JV. Lipid profiles in dioxin-exposed workers. *Lancet* 1979; I: 446–7.

[164] Martin JV. Lipid abnormalities in workers exposed to dioxin. *British Journal of Industrial Medicine* 1984; 41: 254–6.

[165] Blank CE, Cooke P & Potter AM. Investigations for genotoxic effects after exposure to crude 2,4,5-trichlorophenol. *British Journal of Industrial Medicine* 1983; 40: 87–9.

[166] Hoffman H Th. Neure erfahrungen mit Hochtoxischen Chlorkohlenwasserstoffen. *Archives of Experimental Pathology and Pharmocology* 1957; 232: 228–30.

[167] Goldmann PJ. Schwerste akute chloracne durch Trichlorphenolzersetzungs-Produkte. *Arbeitsmedizin Sozial Medizin und Arbeitshygeine* 1972; 7: 12–18.

[168] Goldmann PJ. Schwerste akute chloracne eine Massenintoxikation durch. 2,3,6,7-tetrachlorodibenzo-dioxin. *Der Hautarz* 1973; 24: 149–52.

[169] Theiss 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. *American Journal of Industrial Medicine* 1982; 3: 179–89.

[170] Sorup P. In: Chlorophenols and Phenoxy-herbicides. Arbejdstilsynet. Copenhagen 1982. (Cited by Lynge, 1985 [No 171]).

[171] Lynge E. A follow-up of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. *British Journal of Cancer* 1985; 52: 259–70.

[172] Coggan D, Pannett B, Winter PD, Acheson ED & Bonsal J. Mortality of workers exposed to MCPA. *Scandinavian Journal of Work and Environmental Health* 1986; 12: 448–54.

[173] Suskind RR & Hertzberg VS. Human health effects of 2,4,5-T and its toxic contaminants. *Journal of the American Medical Association* 1984; 251: 2372–80.

[174] Zack JA & Suskind RR. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. *Journal of Occupational Medicine* 1980; 22: 11–14.

[175] Moses M & Prioleau PG. Cutaneous histologic findings in chemical workers with and without chloracne with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Journal of the American Academy of Dermatology* 1985; 12: 497–506.

[176] Moses M, Lilis R, Crow KD, *et al.* Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. *American Journal of Industrial Medicine* 1984; 5: 161–82.

[177] Pazderova-Vejlupkova J, Nemcova M, Pichnova J, Jirasek L & Lukas E. The development and prognosis of chronic intoxication by tetrachlorodibenzo-*p*-dioxin in men. *Archives of Environmental Health* 1981; 36: 5–11.

[178] Poland AP, Smith D, Metter G & Possick P. A health study of workers in a 2,4-D and 2,4,5-T plant. *Archives of Environmental Health* 22: 316–27.

[179] Bleiberg J, Wallen K, Brodkin R & Applebaum I. Industrially acquired porphyria. *Archives of Dermatology* 1964; 89: 793–7.

[180] Jones RE & Chelsky M. Further discussion concerning porphyria cutanea tarda and TCDD exposure. *Archives of Environmental Health* 1986; 41: 100–3.

[181] Goldstein JA, Hickman P, Bergman H & Vos JG. Hepatic porphyria induced by 2,3,7,8-TCDD in the mouse. *Research Communications in Chemical Pathology and Pharmacology* 1973; 6: 919–28.

[182] Mocarelli P, Marocchi A, Brambilla P, *et al.* Clinical laboratory manifestations of exposure to dioxin in children: a six-year study of the effects of an environmental disaster near Seveso, Italy. *Journal of the American Medical Association* 1986; 256: 2687–95.

[183] Filippini G, Bordo B, Crenna P, Massetto N, Musicco M & Boeri R. Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzo-dioxin. *Scandinavian Journal of Work and Environmental*

Health 1981; 7: 257–62.

[184] Fara GM & Del Corno G. Pregnancy outcome in the Seveso area after TCDD contamination. In: Prevention of Physical and Mental Congenital Defects. Part B: Epidemiology, Early Detection and Therapy, and Environmental Factors, ed. M. Marois. Progress in Clinical and Biological Research Series, vol. 163B, pp. 279–85. New York: Alan R Liss Inc., 1985.

[185] Remotti G, DeVirgilis G, Bianco V & Candiani GB. The morphology of early trophoblast after dioxin poisoning in the Seveso area. *Placenta* 1981; 2: 53–62.

[186] Tenchini ML, Crimaudo C, Pacchetti G, Mottura A, Agosti S & De Carli L. A comparative cytogenetic study on cases of induced abortions in TCDD-exposed and non-exposed women. *Environmental Mutagenesis* 1983; 5: 73–85.

[187] Puntoni R, Merlo F, Fini A, Meazza L & Sant L. Soft tissue sarcomas in Seveso. *Lancet* 1986; ii: 525.

[188] Greenwald P, Kovasznay B, Collins DN & Therriault G. Sarcoma of soft tissues after Vietnam service. *Journal of the National Cancer Institute* 1984; 73: 1107–9.

[189] Erickson JD, Mulinare J, McClain PW, *et al.* Vietnam Veterans risk for fathering babies with birth defects. *Journal of the American Medical Association* 1984; 252: 903–12.

[190] Donovan JW, Adena MA, Rose G & Battistutta D. Case Control Study of Congenital Anomalies and Vietnam Service (Birth Defects Study). Canberra: Australian Government Publishing Service, 1983.

[191] Lathrop GD, Wolfe WH, Albanese RA & Moynahan PM. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Baseline morbidity study results. US Air Force Report 1983.

[192] Stellman SD & Stellman JM. Estimation of exposure to Agent Orange and other defoliants among American troops in Vietnam: a methodological approach. *American Journal of Industrial Medicine* 1986; 9: 305–21.

[193] Wolfe WH, Lathrop GD, Albanese RA & Moynahan PM. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides and associated dioxins. *Chemosphere* 1985; 14: 707–16.

[194] Hardell L & Sanstrom A. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. *British Journal of Cancer* 1979; 39: 711–17.

[195] Hardell L, Eriksson M, Lenner P, Lundgren E. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy-acids. A case-control study. *British Journal of Cancer* 1981; 43: 169–76.

[196] Eriksson M, Hardell L, Berg NO, Moller T & Axelson O. Soft tissue sarcoma and exposure to chemical substances: a case-reference study. *British Journal of Industrial Medicine* 1981; 38: 27–33.

[197] Wiklund K & Holm LE. Soft tissue sarcoma risk in Swedish agriculture and forestry workers. *Journal of the National Cancer Institute* 1986; 76: 229–34.

[198] Smith AH, Fisher DO, Giles HJ & Pearce NE. The New Zealand soft tissue sarcoma case-control study. Interview findings concerning phenoxy acid exposure. *Chemosphere* 1983; 12: 565–71.

[199] Smith AH, Pearce NI, Fisher DO, Giles HJ, Teague CA & Howard JK. Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. *Journal of the National Cancer Institute* 1984; 73: 1111–17.

[200] Pearce NE, Smith AH & Fisher DO. Malignant lymphoma and multiple myeloma linked with agricultural occupations in a New Zealand Cancer Registry based study. *American Journal of Epidemiology* 1985; 121: 225–37.

[201] Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ & Teague CA. Non-Hodgkin's lymphoma and exposure to phenoxy herbicides, chlorophenols, fencing work, and meat works employment: a case-control study. *British Journal of Industrial Medicine* 1986; 43: 75–83.

[202] Webb KB. The pilot Missouri health effect study. *Bulletin of Environmental Contamination and Toxicology* 1984; 33: 662–72.

[203] Knutsen AP, Roodmen ST, Slavin RG, *et al.* Missouri Dioxin Health Studies Progress Report: Characterisation of Immune Parameters in Humans Potentially exposed to Dioxin. St Louis University Medical School, Missouri Department of Health, Centres for Disease Control, 1983.

[204] Stehr P, Sten G, Falk H, *et al.* A pilot epidemiological study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contaminations in Missouri. *Archives of Environmental Health* 1986; 41: 16–22.

[205] Hoffman RE, Stehr-Green PA, Webb KB, *et al.* Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Journal of the American Medical Association* 1986; 255: 2031–8.

[206] Kimbrough RD, Carter CD, Liddle JA, Oline RE & Phillips PE. Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. *Archives of Environmental Health* 1977; 32: 77–86.

[206a] Kimbrough RD, Falk H & Stehr P. Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil *Journal of Toxicology and Environmental Health* 1984; 14: 47–93.

[207] Patterson DG, Hoffman RE, Needham LL, *et al.* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in adipose tissue of exposed and control persons in Missouri. An interim report. *Journal of the American Medical Association* 1986; 256: 2683–6.

[208] Hope W, Lischwe D, Russell W & Weis S. Porphyria cutanea tarda and sarcoma in a worker exposed to 2,3,7,8-tetrachlorodibenzodioxin-Missouri. *Mortality and Morbidity Weekly Returns* 1984; 33: 113–14.

[209] Oliver RM. Toxic effects of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin in laboratory workers. *British Journal of Industrial Medicine* 1975; 32: 49–53.

[210] Albro PW, Crummett WB, Dupuy AEJ, *et al.* Methods for the quantitative determination of multiple, specific polychlorinated dibenzo-*p*-dioxins and dibenzofuran isomers in human adipose tissue in the parts-per-trillion range. An interlaboratory study. *Analytical Chemistry* 1985; 57: 2717–25.

[211] O'Keefe PW, Silkworth JB, Gierthy JF, *et al.*

Chemical and biological investigations of a transformer accident at Binghamton NY. *Environmental Health Perspectives* 1985; 60: 201–9.

[212] Schecter A, Tiernan T, Schaffner F, *et al.* Patient fat biopsies for chemical analysis and liver biopsies for ultra-structural characteristics after exposure to polychlorinated dioxins, furans and PCBs. *Environmental Health Perspectives* 1985; 60: 241–54.

[213] Karatsune M, Yoshimura T, Matsuzaka J & Yamaguchi A. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environmental Health Perspectives* 1972; 1: 119–28.

[214] Chen PH-S, Luo, M-L, Wong C-K & Chen C-J. Polychlorinated biphenyls, dibenzofurans and quaterphenyls in the toxic rice-bran oil and PCBs in the blood of patients with PCB poisoning in Taiwan. *American Journal of Industrial Medicine* 1984; 5: 133–45.

[215] Bandiera S, Farrell K, Mason G, *et al.* Comparative toxicities of the polychlorinated dibenzofuran (PCDF) and biphenyl (PCB) mixtures which persist in Yusho victims. *Chemosphere* 1984; 13: 507–12.

[216] Kashimoto E, Miyata H, Kunita S, *et al.* Role of polychlorinated dibenzofurans in Yusho (PCB) poisoning. *Archives of Environmental Health* 1981; 36: 321–6.

[217] Hayabuchi H, Yoshimura T & Kuratsune M. Consumption of toxic oil by Yusho patients and its relation to the clinical response and latent period. *Food and Cosmetics Toxicology* 1979; 17: 455–61.

[218] Masuda Y. Health status of Japanese and Taiwanese after exposure to contaminated rice oil. *Environmental Health Perspectives* 1985; 60: 321–5.

[219] Amano M, Yatsuki K & Umeda G. Cohort study on the causes of death in Yusho (PCB poisoning) patients with special reference to death from liver cancer. *Igaku to Seibutsugaku* 1985; 110: 11–16.

[220] Kuratsune M, Nakamura Y, Ikeda M & Hirohata T. Analysis of deaths seen among patients with Yusho: a preliminary report. *Chemosphere* (in press).

[221] Schecter A, Ryan JJ & Lizotte R, *et al.* Chlorinated dibenzodioxins and dibenzofurans in human adipose tissue. *Chemosphere* 1985; 14: 933–7.

[222] Jensen AA. Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in human milk, blood and adipose tissue. *Science of the Total Environment* 1987; 64: 259–93.

[223] Ryan JJ, Schecter A, Lizotte R, Sun Wing F & Miller L. Tissue distribution of dioxins and furans from the general population. *Chemosphere* 1985; 14: 929–32.

[224] Rappe, C & Nygren M. Chemical analysis of human samples: identification and quantification of polychlorinated dioxins and dibenzofurans. *Environmental Science Research* 1984; 30: 305–14.

[225] Gorski T, Konopka L & Brodzki M. Persistence of some polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans of pentachlorophenol in human adipose tissue. *Roczn Pzh* 1985; 36: 297–301.

[226] Poiger H & Schlatter C. Pharmacokinetics of TCDD in man. Presented at the Dioxin Symposium, Bayreuth, West Germany.

[227] Di[illegible]aski T, Tuzzeo I & [illegible] 2,3,7,8-tetrachlorodibenzo-[illegible] antagonistic effects on enzyme induction and immunotoxicity. *Toxicology* 1987; 46: 29–42.

[228] Poiger H & Schlatter C. Presentation at the Dioxin '88 Symposium, UMEA, Sweden, September 1988.

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