CHLORINATED ORGANIC CHEMICALS AND HUMAN HEALTH RISK ASSESSMENT: RESEARCH UPDATE

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Introduction: The purpose of this paper is to provide a general overview of the types of research on chlorinated organic chemicals, conducted since the publication of the Expert Panel report[98] on human and environmental risk assessment. The content is restricted to information published between September 1993 and May 1995 with information potentially relevant to the assessment of human health risks. A brief description of recent research in some important areas is provided, but this is not considered to be a comprehensive, critical or interpretive review. Review articles are not discussed. The following topic areas are covered: degradation, environmental concentrations, tissue concentrations and exposure, natural and anthropogenic sources, epidemiology, PCBs and PCDD/Fs (dose-response, mechanisms, TEFs and chemical interactions).

Degradation: Recent data confirm and add strength to the conclusions that degradation of chlorinated organics occurs in natural systems, that microorganisms can evolve capabilities to metabolize xenobiotics, and that degradation of these chemicals in the environment occurs to a significant degree. Microorganisms or cell cultures have been identified that can metabolize chlorophenols, chlorobenzenes, PCBs, chlorobenzoates, chloroguaiacols, chlorotoluenes, chloroanilines, DDT and chlorinated alkanes and alkenes.[3,11,15,21,23,27,36,43,47,55,80,92,96] Higher organisms such as species of white rot fungi have also been reported to degrade chlorinated substances such as PCBs.[121] Many organisms, upon exposure to chlorinated organics, have been found to have evolved mechanisms to metabolize these chemicals.[48,56,93,106]

Studies have shown that PCBs can be broken down in natural systems, including soils close to electrical substations releasing Aroclor 1248[71] and river sediments.[13,14,26,45,107]

As reported in a number of studies, biodegradation of chlorinated organics in the natural environment is a significant process and this may have played a major role in the decrease of concentrations of certain chlorinated organics from the greater concentration of the early 1970s.[13,14,48]

Environmental concentrations: The database on environmental concentrations of chlorinated organics in all media is vast, and rapidly expanding.[22,68,78,95,110] In many cases association of measured concentrations with adverse effects is not possible since environmental exposures involve

several chemicals, including metals and non-chlorinated organics. Where temporal trends have been studied, it is clear that concentrations of persistent chlorinated organics (PCBs and DDT being the most widely studied) have declined over the past 10-15 years and are probably continuing to decline.[14,17,52,54,61,94]

Tissue concentrations and exposure: Tissue concentrations of PCBs, PCDD/Fs, DDT and other persistent pesticides have been measured in humans with known exposure and in humans from specifically exposed groups.[7,16,30,33,65,73,102,103] The area of greatest interest has been that of PCBs and PCDD/Fs in breast milk and the resultant exposure of breast fed babies.[9,33,57,60,67,90] Most investigators do not relate these tissue concentrations to any adverse effects, and exposures through breast milk were reported to be lower than the recommended guideline levels (*e.g.*, WHO) for the protection of health.[33,77,97,108,116] In one study in which effects were reported, a small number of babies in the Netherlands experienced average intakes in excess of the ADI of various countries, and cumulative TCDD intake was related to changes in ALT, ASP and platelet count; however, there was no control group, and the significance and permanence of the changes are not known.[90]

Natural and anthropogenic sources: Several sources of PCDD/F to the environment have been identified and attempts have been made to quantify the amounts of production. EPA[37,38] in a working draft of the dioxin reassessment document estimated that the major contribution was from incineration with wood burning and forest fires making only minor contributions. However, not all researchers agree with the EPA's estimates and other reports suggest that natural sources are more significant than anthropogenic sources.[1,2,49,100] This controversy notwithstanding, the contribution of anthropogenic sources of PCDD/F is on the decline. This has resulted already from process changes at pulp and paper mills, reduction in the manufacture of pesticides associated with inadvertent production of PCDD/Fs, and in the United States, illegalization of leaded fuels and halogenated additives and stringent new emission standards for incinerators.[39,40,41,42,100]

Although there are numerous naturally occurring chlorinated organics, both simple and complex, their production is poorly quantified.[1,49] Nonetheless, some environmentally significant chlorinated organics from methyl chloride to PCDD/Fs are produced in the natural environment, some in excess of anthropogenic sources and others in comparable amounts.

Epidemiology: Recent epidemiology studies do not provide information that would lead to a change of the conclusion made in the Report of an Expert Panel[98], that low environmental concentrations of chlorinated organic chemicals would not be associated with adverse health effects in humans. Several investigators have assessed neurological and developmental parameters in children born to parent(s) of the Yu-Cheng incident (exposure to PCB-contaminated rice oil).[18,19,50,51,69,101,119,120] Reported effects on Yu-Cheng children included depression of some parameters related to cognitive function[18,69] and mildly disordered behaviour and increased activity levels.[19,120] Guo *et al.*[50] concluded that children born 7 to 12 years following maternal exposure displayed delayed development but did not show significant behaviour problems compared to control children. Developmental and neurological effects were not correlated with tissue concentrations in either the mother or her offspring.[101] The biological significance of some of the parameters assessed, for example, in terms of behavioural function as a whole, is uncertain, since only some parameters were different from controls while others were not, and no dose-response relationship was evident.

An increased incidence of mortality from bladder cancer and soft tissue sarcoma was reported among a group of 754 workers exposed to TCDD during an industrial accident in 1949, however incidences were not increased among those workers with exposures high enough to have caused chloracne.[24] Lack of reliable exposure data, the apparent lack of a dose-response relationship and confounding exposure to the bladder carcinogen 4-aminobiphenyl do not allow the assignment of causality with TCDD exposure. In eight of these workers who contracted chloracne and had elevated blood levels of TCDD, serum cholesterol was increased and gamma-globulin was reduced.[59]

Studies of adults with moderately increased body burdens of PCDD/F revealed no effects on various immunological parameters.[83,84,86,87]

The half-life of TCDD was determined to be 11.3 years based on serum concentrations from 1982 and 1987 in veterans of Operation Ranch Hand (exposed to Agent Orange contaminated with TCDD).[117] In an investigation in which reproductive outcomes among these veterans were assessed, no statistically or biologically meaningful increases in spontaneous abortions, stillbirths, birth defects, birth defect severity, developmental delays or hyperkinetic syndrome were associated with paternal exposure to Agent Orange.[118]

PCBs and PCDD/Fs: A vast amount of research has been conducted in an attempt to determine the mechanism of action of PCBs and PCDD/Fs and to ascertain the role of the Ah receptor. A recently developed strain of mice lacking the gene for the Ah receptor may provide a useful research tool.[44] Areas of research having implications for the refinement of the risk assessment for these chemicals include evaluation of the dose-response at low doses, elucidation of mechanisms of toxicity and impact on key metabolic pathways, evaluation of the relationship between toxicity and enzyme induction, development of TEFs, and study of the interactions between mixtures of PCBs and PCDD/Fs and influence on toxicity and enzyme induction. A brief overview of the types of research that have recently been reported in these areas is presented below.

The results of attempts to define the dose-response relationship for TCDD-related effects have been reported. Endpoints studied include liver cell proliferation[74], CYP1A1 and/or CYP1A2 and associated enzyme activities[31,74], CYP1A1 mRNA production[112], immunologic reactions[82,85,86] and tumour promotion.[104] A study relating tissue dosimetry with enzyme induction at low doses in mice provides information on the shape of the dose-response curve that may have implications for high-low dose extrapolations in risk assessment.[31] Low-dose thresholds have been reported for CYP1A1 mRNA production induced by TCDD in rats[112], biochemical and histological changes in rats induced by dietary PCB[20], lipid/lipoprotein changes induced by PCB in monkeys[10], reproductive effects as predicted from kinetic studies[114] and enzyme induction, CYP1A1 and 1A2 mRNA production and immunotoxicity parameters in mice.[82] Data on enzyme kinetics support the existence of a threshold in the TCDD-induced decrease in nuclear estrogen receptors *in vitro*.[114] The evidence for an antipromotional or protective effect of TCDD on liver tumour promotion at very low doses indicates a threshold in this response and is discussed by Kitchin *et al.*[64] In addition, the dose-response curve for replicative DNA synthesis in liver cells of DEN-treated rats has been found to be less steep than

the curve for CYP1A1 and CYP1A2 induction, indicating that the dose-response cannot be defined solely on the basis of enzyme induction and that use of surrogate biomarkers may lead to overestimation of risk.[74,104] The application of current knowledge to dose-response modelling and to risk assessment is discussed by Kohn *et al.*[66]

Researchers continue to work to try to elucidate the mechanism of action of PCDD/F. Recent areas of interest have been the influence of these chemicals on oxidative stress, intercellular communication and on normal metabolic pathways, particularly those involved in glucose metabolism and protein phosphorylation. Oxidative stress resulting from superoxide production in macrophages has been suggested as a possible mechanism of TCDD-induced toxicity[5,6], as has disruption of calcium homeostasis.[63] Interference with intercellular communication may be related to PCB- and TCDD-induced effects in liver cells *in vitro*.[8,28] Other data suggest a relationship between modulation of protein phosphorylation and TCDD-induced immunosuppression.[79] *In vitro*, a potent protein kinase inhibitor was found to prevent TCDD-induced CYP1A1 transcription and to reduce Ah receptor concentrations in Hepa1 cells, indicating a link between protein phosphorylation and Ah receptor activity.[105] In addition it appears that the Ah receptor is phosphorylated prior to ligand binding.[113] The available data support the possibility that protein kinase activity stimulated by TCDD is related to Ah receptor-dependent and independent events, although the mechanism by which these could occur is not known.[75,115] Recent work indicates that the pathway for induction of protein kinases by TCDD is distinct from the mode of action involving nuclear translocation and gene transcription.[35] A detailed description of work done in this area and a theory to explain the findings is presented by Matsumura.[75] It has also been proposed that interaction of TCDD with the Ah receptor influences glucose transport through reduction in concentrations of glucose transporter proteins.[89] In guinea pigs, the most sensitive species to the toxic effects of PCB and PCDD/F, PEPCK (a key enzyme in gluconeogenesis) was not reduced after exposure to a coplanar PCB, indicating that interference with gluconeogenesis is not part of the mode of toxic action of PCB in the guinea pig.[53,88] Glucose uptake was inhibited in mice, rats and guinea pigs and this effect was considered to be mediated by the Ah receptor.[34]

While it is generally agreed that a primary mode of action of PCBs and PCDD/Fs is associated with Ah receptor binding, some effects of these chemicals cannot be explained on this basis alone. Some data indicate that immunotoxicity of PCBs and PCDD/Fs does not appear to be related to Ah induction.[46,70] Quantification of Ah receptor mRNA content in various tissues from TCDD-sensitive and relatively insensitive mouse strains revealed no significant differences in mRNA content, suggesting that factors other than Ah receptor binding are associated with strain differences in toxicity.[72] The results of comparative *in vitro* studies of Ah receptor binding using TCDD and methylcholanthrene led to the conclusion that the extreme toxicity of TCDD cannot be explained solely on the basis of its affinity for the Ah receptor.[99]

It is becoming clear that repeated exposure to mixtures of PCBs and PCDD/Fs may not induce toxicity in the way that would be predicted based solely on data from acute, single chemical exposures and that pharmacokinetics and dosing regimes need to be considered in using laboratory data for risk assessment purposes. In a study of the potential of mixtures of PCDFs and PCDDs (free of 2,3,7,8-TCDD) to cause cleft palate in mice and comparison of predicted toxicity based on International TEFs, it was found that the predicted teratogenicity based on relative potencies was

higher than the actual teratogenicity.[81] This is consistent with the finding that the teratogenicity of TCDD in the strains of rat most and least sensitive to the acute toxic effect was similar.[58] Other data also suggest that the interaction of mixtures of PCBs and PCDD/Fs may be antagonistic rather than additive, and it is likely that alterations in toxicokinetics caused by exposure to mixtures of these chemicals and by repeated dosing explain the non-additive toxicity of mixtures.[25,29,76,104,109,111] These data point to the fact that TEFs derived from short-term bioassays using single chemical exposures cannot be used reliably in risk assessments involving multiple chemical and/or repeated exposure, and would most likely lead to an overestimate of risk. This is consistent with the observation that the susceptibility of rats to acute toxic effects is not correlated with susceptibility to other toxic effects such as fetotoxicity.[91]

Evaluation of TEFs continues, and approaches to improvements in the derivation of TEFs have been suggested. A method of predicting the order of magnitude of TEFs for PCBs based on receptor binding affinity as indicated by the free energies of binding, lipophilicities, electron affinities and entropies has been proposed.[62] The WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), have begun to create a data base containing information relevant to the setting of TEFs and based on the available information, to assess the relative potencies and to derive consensus TEFs for PCDDs, PCDFs and dioxin-like PCBs.[4] TEFs were recommended for 3 non-ortho-, 8 mono- ortho- and 2 di-ortho-substituted PCBs.

Conclusions: Overall the information published since the Report of an Expert Panel[98] supports the conclusions made by the panel and adds additional weight of evidence to these conclusions. A large amount of new information supports the conclusion that environmental concentrations of chlorinated organics are declining and that degradation by natural systems is a significant process. An area of controversy in which conflicting data have been reported is that of sources of PCDD/F, and further research is required to determine whether there are significant sources that can be reduced. New information in the area of mechanism of action of PCBs and PCDD/Fs adds support to the conclusion that these act via receptor-dependent mechanisms; however, further research is required to precisely define the operative mechanisms and the relevance these may have to human health risk assessment.

Acknowledgement

Funding was provided by the Chlorine Chemistry Council.

References will be distributed at the presentation.