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An Overview of the Effects of Dioxins and Dioxin-like Compounds on Vertebrates, as Documented in Human and Ecological Epidemiology

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Abstract

Dioxins and dioxin-like compounds are primary examples of persistent organic pollutants that induce toxicity in both wildlife and humans. Over the past 200 years these compounds have been almost exclusively generated by human activity and have left a string of disasters in the wake of their accidental release. Most recently, the contamination of the Irish pork supply with dioxins resulted in an international recall of all Irish pork products. Epidemiologic data on human and ecological dioxin exposures have revealed a common pattern of biological response among vertebrate species, which is mediated through activation of the Aryl hydrocarbon Receptor (AhR). These AhR-mediated effects include profound consequences on the vertebrate individual exposed in early-life, with respect to myriad developmental endpoints including neurologic, immunologic, and reproductive parameters. Humans appear to be susceptible to these effects, in a manner similar to that of the laboratory and wildlife species which have demonstrated such outcomes. Furthermore, epidemiologic data suggest that there is little or no margin of exposure for humans, with respect to these developmental effects. Given these concerns, prudent public health policy should include the continued reduction of exposures.

INTRODUCTION

TCDD – or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin – is the prototype for a family of persistent, structurally-related compounds that induce toxicity via a common mechanism of action, resulting in a common spectrum of biological responses. The term “dioxins” may be used to refer to polyhalogenated dibenzo-*p*-dioxins and furans – compounds which are not intentionally generated, but rather are byproducts of industrial or combustion processes – and sometimes polyhalogenated biphenyls, naphthalenes, and azo/azoxybenzenes – which are, or have been, commercially produced for industrial usage. Of these, however, only a limited number of congeners in fact exhibit dioxin-like properties, including those with lateral

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halogenation at greater than three sites. These may be chlorinated, brominated, or combined chloro-bromo congeners.

Polychlorinated biphenyls, or PCBs, are a large family of chemicals, comprised of 209 possible congeners, only a small subset of which are dioxin-like. There are multiple, overlapping structural classes of PCBs, but PCBs are inherently found as mixtures, and never exist in the absence of dioxin-like PCBs in the ambient environment. Similarly, TCDD and PCBs are seldom found in the absence of one another. Irrespective of this, the majority of individual PCBs possess their own intrinsic toxicities, and furthermore can interact with dioxins and other PCB congeners additively, synergistically, and/or antagonistically, imparting high variability to the activity of the observed mixtures [1].

In total, there are 7 polychlorinated dibenzo-p-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 12 PCBs which are considered dioxins or dioxin-like compounds by the World Health Organization [2]. Because such individual dioxin and dioxin-like molecules exhibit variable toxic potency, but via a common mechanism of action, a relative potency ranking scheme has been devised that assigns a Toxic Equivalency Factor (TEF) to each compound, as compared to the most toxic dioxin, TCDD. This relative potency of dioxin-like chemicals to TCDD is often calculated as the ratio of the half maximal effective dose (ED₅₀) for TCDD to the ED₅₀ for the dioxin or dioxin-like compound of interest. TEFs are assigned based on all the available data for a dioxin-like chemical compared to TCDD [2]. Thus, the TEF assigned for TCDD is 1, and it is less than 1 for all other compounds, except 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), for which the TEF is also 1. This system allows for the quantitative expression of the toxicity of a single chemical in terms of an equivalent concentration of TCDD. In the case of a dioxin mixture -- as found in the environment or biological tissues -- known TEFs for the individual dioxins or dioxin-like components, and their concentrations in the mixture, can be utilized to determine a total toxic equivalency (TEQ) for the mixture. The TEQ is calculated as the sum of the individual products of the TEF and the concentration of each compound [3]. For the purposes of this discussion, dioxins -- including PCDDs and PCDFs -- and dioxin-like compounds which have been included in the World Health Organization's TEF scheme will be heretofore referred to jointly as "dioxin(s)" [2].

While dioxins are generated naturally, through such processes as forest fires and volcanic eruptions, it is human activity which has been primarily responsible for the generation of this class of chemicals over the past two centuries. Historically, industrial activities were the largest sources of dioxins, but many responsible processes have since been regulated, and today uncontrolled combustion -- such as burning of household waste in open containers -- is the greater concern. In addition to their infrequent natural occurrence, dioxins are of interest in their toxicological potency, and their varied and extensive adverse health consequences. Dioxins have a long and blemished history, and the persistent and novel nature of this class of compounds continues to generate scientific and regulatory interest.

HISTORY OF DIOXIN PRODUCTION AND EXPOSURES

The history of anthropogenic dioxin production and dioxin poisoning is nearly 200-years old and persists today. The earliest evidence of man-made dioxin molecules comes from a German chemical production plant in Lampertheim, South Hesse that was manufacturing washing soda (sodium carbonate, also known as sal soda or soda ash) by the *LeBlanc* process as early as 1827, and until at least around 1890, when it was replaced with chloralkali electrolysis -- both of which processes generated dioxin [4]. It was not until the 1980s, when a playground and a facility intended for children's use were slated to be built on the site where the plant had stood,

that the extensive dioxin soil contamination was identified. It was many decades after the polluting of this site began, that chloracne was first characterized in 1897. This persistent cystic and hyperkeratotic skin condition, first identified in German industrial workers, remains a hallmark of dioxin exposure [5].

The use of PCBs began in the early 20th century, with commercial production --inevitably of mixtures that contained dioxin-like PCBs -- being initiated in 1929. PCBs were widely used as cooling fluids in electrical transformers, as well as in hydraulic fluids, lubricants, and plasticizers until 1978, when the Congressional ban on their sale and use in the US first took effect, as part of the Toxic Substances Control Act of 1976. In 1947, X-disease, a hyperkeratotic condition akin to chloracne, but which could culminate to death, was first reported in American cattle, and was later demonstrated to arise from exposure to dioxin-like compounds [6]. A decade after X-disease was observed in cattle, in 1957, a massive die-off of commercially raised chickens in the U.S. occurred due to a pathology referred to as chick edema disease, later found to be caused by TCDD-contaminated feed supplies [7]. In the interim between these two animal poisonings, in 1949, an explosion in a Monsanto chemical plant in Nitro, West Virginia resulted in the exposure of workers to the dioxin-contaminated herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and persistent chloracne was observed in the exposed individuals [8]. Later studies on these and other occupationally dioxin-exposed workers demonstrated an increase in all cancers combined, in the most highly exposed workers [9].

Between 1962 and 1970 Agent Orange, the American military code name for an herbicide containing 2,4,5-T and contaminated again with TCDD – similar to the contaminated 2,4,5-T in the 1949 Monsanto explosion – was used as a defoliant in Vietnam to reduce enemy ground cover, as part of Operation Ranch Hand. Recent studies of the Ranch Hand cohort have revealed that American military exposures to Agent Orange were associated with an increased risk of diabetes and an increased risk of multiple cancers, with increased duration of potential exposure conditions [10]. The Institute of Medicine found sufficient evidence of association between Agent Orange exposure and soft-tissue sarcoma, non-Hodgkin lymphoma, Hodgkin disease, and chronic lymphocytic leukemia [11].

In 1968 in Kyushu, Japan, a rice bran oil company's supplies became contaminated with PCBs and PCDFs, and the contaminated oil was sold and fed to livestock and humans, resulting in the deaths of hundreds of thousands of birds. In humans, skin lesions, fatigue, and altered reproductive and immunologic function were symptoms of what was referred to as "Yusho" (literally, "oil") disease, and developmental delays were also observed in children [12]. Sadly, this incident was essentially repeated in Taiwan in 1979, but was referred to in Chinese as "Yucheng" disease, also meaning "oil." In this event, the association between gestational or lactational exposure was more clearly associated with impaired cognitive development and behavioral problems, and recent studies have also demonstrated persistent effects, with respect to altered reproduction parameters post-pubertally among the exposed males [13].

Such events were not isolated to Asia. In the interim between the Yusho and Yucheng poisonings, in 1971 an entire town in the United States was exposed to high levels of dioxins when contaminated waste oil was spread on the dirt roads of Times Beach, Missouri, in order to control dust. In the very same year, TCDD was identified as a teratogen [14]. Ultimately, all properties in Times Beach were bought out by the US EPA for a total of \$32M in 1983, the inhabitants were relocated, the town was demolished, and 265,000 tons of regional soil were incinerated. This paved the way for the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, or the Superfund law, as it is more commonly known. During this same period of time, and also in the US, the Great Lakes region

began to witness greatly diminished reproduction among lake trout and mink, which has persisted to the present [15]. Epidemiologic studies in the same geographic region assessed human breast milk collected from nursing mothers across Michigan in the late 1970s, and revealed extensive exposure to PCBs, with milk PCB concentrations as high as 5,100 ppm, ostensibly resulting from high consumption of fish from polluted waterways [16].

In 1976, in Seveso, Italy, an explosion occurred at an Italian chemical plant producing 2,4,5-trichlorophenol, an intermediate in 2,4,5-T synthesis. Because of the nature of the uncontrolled reaction that produced the explosion, not only was TCDD released into the outside environment when the facility was breached, but its levels were far higher than the normal range of 1 ppm for contamination of 2,4,5-T, and may have approached 100 ppm [17]. Within several weeks of the accident, some of the exposed community members exhibited skin lesions consistent with chloracne. In the years that followed, continuing studies of the exposed population supported the potential for TCDD to act as a carcinogen in humans and to increase risk for diabetes, adverse cardiovascular effects, and altered endocrine function [18,19]. Shortly after the Seveso disaster, laboratory cancer studies in rats revealed that chronic, two-year exposure to TCDD at as low a dose as 0.01 µg/kg/day (equivalent to 210 ppt) resulted in increased risk of hepatocellular carcinomas and various squamous cell carcinomas [20,21].

In Binghamton, NY in 1981, a PCB dielectric fluid-filled transformer caught fire in the basement of the Binghamton State Office Building. The fire deposited an oily soot throughout the 18-story building, most notably on the horizontal surfaces, where PCDDs and PCDFs were deposited at levels that varied within the building, but were measured as high as 1200 ppm [22]. As a result of the extensive contamination throughout the structure, millions of dollars were spent on cleaning and remediation, and the building was shuttered for 13 years.

Seven years later, following all the press received by dioxins and PCBs, a massive die-off event in 1988 of about 20,000 harbor seals in the Baltic Sea set off concerns that these persistent organic pollutants were responsible. While the underlying cause of death was found to result from infection with Phocine distemper virus, it is possible that PCB exposure contributed to viral infection and subsequent death. That is, the fish stocks upon which these seals subsisted were known to be extensively contaminated with PCBs, known immunosuppressants. Furthermore, feeding studies utilizing these contaminated stocks produced measurable suppression of immune response among captive harbor seals [23].

Following these numerous accidental human and wildlife exposures to dioxins and dioxin-like compounds, the US EPA issued its first health assessment of the most toxic dioxin molecule, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), in 1985. Shortly thereafter, in 1991, the National Institute of Occupational Health and Safety reported its cancer mortality study of US workers exposed to TCDD, and found that mortality was increased from all cancers combined, particularly among those with the longest occupational exposure and the greatest latency from exposure [24]. While frank poisonings have largely stopped since these documents were published, occasional contamination events have continued in the more than 20 years since the first health assessment. For example, in Belgium in 1999, when an overwhelming percentage of national livestock were given PCB-contaminated feed that resulted in the massive international recall of products containing meat or dairy from the affected animals, including Belgian chocolate, a popular export.

Most recent in people's memories, of course, is the dioxin poisoning of Viktor Yushchenko in 2004. The then-candidate and now president of Ukraine may have been intentionally poisoned with TCDD, as part of measures to weaken his political influence and potentially remove him from the campaign. Two months prior to the presidential election, Yushchenko was

hospitalized for pancreatitis, which was followed by profound facial acne and edema. The diagnosis of chloracne was confirmed when dioxin blood levels were found to be three orders of magnitude above average. His was not the first case of intentional dioxin poisoning, however. In Vienna in 1997, five people working in secretarial positions at a textile institute were seemingly poisoned with extremely high levels of dioxin, one of whom exhibited the highest serum dioxin levels ever measured in an individual -- 144,000 pg TCDD/g blood fat, or a calculated body burden of 1.6 mg TCDD [25].

Finally, as 2008 drew to a close, all Irish pork products were removed from international shelves due to the known contamination of these animals resulting from ingestion of dioxin-contaminated feed supplies. Disturbingly, this event was essentially a repeat of the Belgian contamination nearly ten years prior.

COMMON MOLECULAR MECHANISM OF ACTION

The mechanism of action by which dioxins exercise their biochemical effects on vertebrate species is through activation of the Aryl hydrocarbon Receptor (AhR), a ligand-activated basic helix-loop-helix transcription factor, and a member of the PER-ARNT-SIM (PAS) superfamily of transcription factors [26]. The AhR is a highly conserved protein across vertebrate species, and related proteins have been identified in invertebrates, such as *C. elegans* and *Drosophila* species [27]. A member of the growing PAS family of key regulatory proteins --which are often thought of as “biological sensors” -- the AhR is believed to play key roles in development, aging, hypoxia, and circadian rhythms [28].

In the cell, in its non-ligand bound state, AhR exists as a cytosolic complex with chaperones, including two molecules of heat shock protein (Hsp) 90, and one molecule each of prostaglandin E synthase 3 (p23) and immunophilin-like protein hepatitis B virus X-associated protein 2 (XAP2, also known as AIP or ARA9) [29]. TCDD enters the cell via diffusion and when it binds AhR, one molecule of Hsp 90, as well as both p23 and XAP2 dissociate from AhR, and the AhR-TCDD complex translocates into the nucleus. Once in the nucleus, AhR must heterodimerize with the AhR nuclear translocator (Arnt), at their respective PAS domains, releasing the remaining molecule of Hsp 90, before transcription can occur. Binding of the AhR-TCDD-Arnt complex to the DNA at dioxin response elements (DRE; or xenobiotic response elements, XRE) in the promoter regions of target genes may then occur -- depending upon the co-activators or co-repressors present -- and transcription may proceed. Examples of other transcriptional proteins that may be recruited to the AhR-TCDD-Arnt complex prior to DNA-binding and subsequently modulate the transcriptional activity of the complex include co-activators such as thyroid hormone receptor/retinoblastoma protein-interacting protein 230 (TRIP230) [30], and co-repressors such as the estrogen receptor alpha isoform (ER α) [31]. AhR is also believed to engage in cross-talk with other key regulatory molecules including nuclear steroid receptors and cell cycle control molecules [32]. Many of the metabolic enzymes which exhibit altered expression after TCDD exposure, are directly regulated by the actions of the AhR-TCDD-Arnt complex on upstream DREs.

By these means, dioxin may hijack the AhR, disrupting its endogenous function. That is, the physiological activator of the AhR likely induces rapid on/off signaling through the receptor. Dioxins, however, are believed to induce toxicity through the persistent activation of the AhR, thereby preventing the AhR from functioning in the maintenance of homeostasis. Furthermore, the persistent activation of the AhR likely results in increased competition for Arnt, and thus other signaling partners for Arnt that may also act as environmental sensors, such as hypoxia-inducible factor-1 (HIF-1), might have greatly reduced opportunities to dimerize with Arnt. The endogenous role played by AhR, in the absence of dioxin signaling, may be involved in

cell-cycle control, and tumor suppression in the stomach, colon, prostate, and hematopoietic tissues [33,34]. AhR also plays an evolutionarily conserved role in development, as drosophila homologs have illustrated. With reference to the actions of dioxins, the AhR is necessary but not sufficient for most dioxin-associated toxicity. That is, irrespective of the machinery associated with the translocation and transcriptional modulation of the AhR, successful dimerization of AhR with Arnt and subsequent binding to the DRE appears necessary for dioxin-mediated induction of many developmental, thyroid, and hepatic effects [35].

Unlike other endpoints for dioxin-mediated toxicity, some observations of TCDD-induced inflammation appear to arise via a non-genomic pathway. This pathway is mediated by ligand-activated AhR, but lacks the requirement for nuclear translocation or for the dimerization partner of AhR, Arnt, thus it also lacks DRE binding and transcription, both primary elements of the classical pathway. Studies into this non-genomic pathway suggest that the early events triggering inflammation, including a rise in intracellular Ca^{2+} concentration potentially from both mitochondrial stores and extracellular sources, may occur within the first 1 minute of TCDD exposure, thus before transcription could proceed, and across multiple cell types [36]. The events subsequent to this intracellular Ca^{2+} concentration rise, which ultimately result in inflammation, are dominated by the activation of protein kinases and phosphatases. This non-genomic pathway is clearly critical in the development of certain adverse health outcomes that historically were believed to result from the only identified means, the classical pathway of transcriptional activity. For example, mice null for Src kinase – which is activated via the non-genomic pathway by TCDD in multiple cell types within 5–15 minutes of exposure, and results in activation of the epidermal growth factor receptor (EGFR) – are less susceptible to the classical wasting syndrome than their wild-type counterparts [36].

The AhR and all associated members of the signaling complex are expressed by both humans and the various laboratory species studied, and human cell and tissue culture studies have illustrated human responsiveness to dioxins consistent with that observed in other species. In addition, exposed human populations have exhibited measurable biochemical responses, with respect to genes involved in drug metabolism as well as other known target genes for AhR [37,38], and adverse health effects have been extensively observed in highly exposed populations.

BIOCHEMICAL AND HEALTH EFFECTS IN VERTEBRATE SPECIES

As previously discussed, the adverse effects of dioxin exposure have been well-established following accidental releases of dioxins into human and natural environments or food supplies. In addition, studies of wildlife, as well as domestic and laboratory animals, have furthered the understanding of potential adverse outcomes of exposure [39].

In the Great Lakes area, which has been extensively polluted with dioxin and dioxin-like compounds, multiple species of birds, fish, reptiles, and mammals have exhibited developmental toxicity, reproductive impairment, compromised immunologic function, and other adverse effects correlated with these exposures. Specific observations correlated with dioxin or dioxin-like compound levels in multiple vertebrate species included hyperplasia of the thyroid and adrenal glands, porphyria, suppressed T-cell-mediated immunity, mammary and ovarian pathologies, reduced viability of offspring, congenital malformations, growth retardation, and an edematous syndrome among the offspring of fish-eating birds comparable to chick edema disease [15]. Terminal members of the Great Lakes food web engage in fish-consumption almost exclusively, and bioaccumulative pollutants such as dioxins are usually found in these species at higher concentrations than those of lower trophic levels. Because humans in this area also consume much local fish, there is concern about the levels of human

exposure and the potential for health effects akin to those observed in wildlife. Similar to the Great Lakes region, wildlife species in the Baltic Sea region, including cetaceans, have exhibited signs of immunotoxicity correlated with exposure to dioxins [23,40]. In addition, humans living in the surrounding area have shown signs of both developmental and immunologic consequences of exposure to these persistent organic pollutants, via dietary fish intake [41,42].

The ecological consequences of environmental dioxin releases have shed some light on the differential response to dioxins across species. While many wildlife species have illustrated the toxicity of dioxins, the rank order of individual congener toxicity, and thus the TEF rank order, varies across species. For example, some of the PCB congeners have TEF values in fish that are multiple orders of magnitude lower than they are in humans or other mammals [43]. However, some of the same congeners, such as PCB 114, 156, and 157, have higher TEF values in birds than in humans and other mammals. This congener-specific sensitivity suggests that for certain dioxins, not all exposed species will serve as sentinels for the health of humans or other species, including invertebrates, such as the eastern oyster, which may be the most dioxin-sensitive species with respect to developmental endpoints [44]. Furthermore, the matter is complicated by the fact that different congeners dominate different environments and trophic levels,

Poisonings of domesticated species including cows and chickens – such as those which resulted in epidemics of X-disease and chick edema disease, respectively – as well as horses and sheep, have further demonstrated the profound pathologies caused by explicit dioxin exposures at concentrations many fold higher than coincident environmental levels. More recently, events of agricultural contamination such as the Belgian contamination of chicken and cattle and the very recent contamination of Irish pork [45] have also illustrated the political and social repercussions of contamination of the human food supply. While these observations on uncontrolled populations of domesticated species or wildlife provide little information about dose-response relationships, or the precise mechanism of action for particular endpoints, they provide an invaluable picture of potential biological and ecological outcomes of exposures to environmentally relevant concentrations and mixtures of dioxins and other pollutants. In this way, such species and ecological endpoints may serve as sentinels for the health of humans and the environment. Research utilizing extensive laboratory species – including fishes, amphibians, turtles, birds, mice, rats, hamsters, guinea pigs, rabbits, dogs, and non-human primates -- has reiterated the observations made in the wild and in human epidemiologic studies, and demonstrated that the effects of this class of chemicals are not limited to specific species or unusually sensitive strains.

The specific mammalian toxicity of dioxins, which these many species and events have borne out, is extensive [46]. Dioxins have proven to be developmentally toxic, immunotoxic, neurotoxic, and hepatotoxic to mammals. Other adverse health effects include gonadal and lymphoid atrophy, disruption of endocrine signaling, cardiovascular toxicity, bone, skin, and tooth toxicity, carcinogenesis, wasting, and lethality. Specific biochemical events resulting from adult dioxin exposure include upregulated hepatic gene expression of phase 1 and phase 2 drug-metabolizing enzymes including cytochrome P450 (Cyp) 1A1, Cyp 1A2 (which may bind dioxins, resulting in sequestration), Cyp 1B1, glutathione S-transferases (GST), uridine diphosphate glucuronosyltransferase (UDPGT), aldehyde dehydrogenase (ALDH), and a number of other enzymes, resulting in generally altered patterns of metabolism. These metabolic gene expression changes occur across multiple species, however, not all these genes exhibit altered expression in all species, and altered expression patterns may vary between strains within a species. The cytokines, TNF α , IL-6, and IL-1B, as well as many other molecular markers of inflammation may also be induced, potentially via a non-genomic

pathway. Expression of genes whose products are involved in oxidative stress, such as metallothionein [47], cell proliferation, and growth factors and receptors, such as TGFs and EGFR, may all be increased as a result of dioxin exposure. Even circulating hormones and hormone receptor populations may be modulated by dioxins. However, such biochemical changes may be detectable at levels below which the overt toxicity previously described is observed.

HEALTH EFFECTS IN HUMANS

Adverse health effects of dioxin exposure in humans may include cardiovascular disease, diabetes, cancer, porphyria, endometriosis, early menopause, reduced testosterone and thyroid hormones, altered immunologic response, skin, tooth, and nail abnormalities, altered growth factor signaling, and altered metabolism [48]. These are consistent with adverse health outcomes documented in laboratory and wildlife species. In addition, some of the biochemical changes described above in other species, including altered expression of growth factors and their receptors, and metabolic enzymes, have been observed in humans [37,38].

The effects of dioxin exposure during development are also many, and include altered thyroid and immune status, altered neurobehavior at the level of hearing, psychomotor function, and gender-related behaviors, altered cognition, dentition, and development of reproductive organs, and delays in breast development, in addition to altered sex ratios among the exposed offspring. Recent studies have revealed that thyroid-stimulating hormone was elevated in neonates born from mothers with presently elevated plasma dioxin levels, nearly 30 years after their exposures during the 1976 Seveso dioxin disaster [49]. Another study on men who were exposed to dioxin in their youth in Seveso, found that those who were exposed prior to puberty exhibited reduced sperm count and motility as adults, 22 years later, while those who were exposed during adolescence exhibited increased sperm counts and motility [19]. This further illustrates the significance of the timing of dioxin exposure with respect to the outcome. The most notable of these findings, though consistent with findings from animal studies, is the permanence of the effect, and the dose at which the effect occurred -- <68 ppt TCDD in serum lipid, in 1976, shortly after their exposure. This total dioxin load was equivalent to a TEQ of ~150 ppt at that time. This concentration is within only an order of magnitude of present-day means, which approximates 15 ppt on a serum lipid basis. By far, it is these developmental exposures to dioxin that pose the greatest concern, in part because these effects occur at the high-end of the background range for the general population. Further, the alterations in cognition, behavior, immune function, hormonal signaling, and growth illustrate the extent of the sensitivity of the developing individual to dioxins.

CONSIDERATIONS FOR THE FUTURE

Numerous epidemiologic studies of dioxins have been conducted -- many resulting from the accidental dioxin releases described prior -- but these efforts inherently examine exposures to mixtures of multiple compounds, due to the nature of dioxin production resulting from uncontrolled combustion and the resultant mix of congeners. Furthermore, as a consequence of the environmental ubiquity of dioxins, all humans have acquired some exposure, and thus epidemiologic studies are challenged to provide true controls. One of the most useful approaches is the measurement of TEQ in cohort participants, given that it allows for consistent potency determination that can be utilized for comparisons of findings between studies. In addition, careful consideration of timing may play a critical role in epidemiologic studies, with respect to identification of effects. That is, as the studies in the Seveso population have demonstrated, the timing of an exposure with respect to life stage, as well as the timing with respect to the assessment of an effect, may greatly affect the findings of a study on human

dioxin exposures. In fact, recent studies support temporal considerations and suggest the Seveso accident may have even resulted in consequences to a second generation -- children born to exposed parents, with increased risk for developmental neurologic, reproductive, or immune effects [50].

The classical toxicologic paradigm is that the dose makes the poison. In the case of dioxin -- as with many compounds that contemporary toxicology has identified -- it is the timing of exposure in conjunction with the dose which determines the outcome in the exposed individual. Furthermore, the relationship between the level of exposure and the received dose varies across species, and thus for the purposes of investigating the consequences of exposure, an internal measure of dose must be used [51].

Observation of body burden in animal studies, as compared to the current average American body burden of 5 ng TEQ/kg body weight (estimated from blood lipid levels) [48], [52], has allowed for the determination of margins of exposure for specific toxicologic outcomes. For example, developmental reproductive toxicity has been observed in association with a body burden range of 0.7–42 ng/kg, thus suggesting the margin of exposure for humans falls in the range of 0.1–8 (animal body burden at effect level divided by 5 ng/kg). This is quite a broad range, and other endpoints of adversity fall at either the upper or lower ends of this spectrum. Because general population body burdens are at or near the point where adverse health effects may be occurring, there is little or no margin of exposure. This illustrates that, although mean body burdens have declined, continued efforts should be taken to decrease human exposures to dioxins, through the reduction of sources of exposure, as well as the reduction of environmental levels.

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