Characterizing Potential Human Health Risks from Dioxin-Like Compounds

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After more than three years of intensive effort, the U.S. Environmental Protection Agency (EPA) on 13 September 1994 officially released draft documents describing its reassessment of the potentially adverse effects on human health that may result from exposure to dioxin and related chemical compounds (USEPA 1994). Concerns over such effects have been raised primarily because exposure to these persistent toxic substances is so widespread; virtually everyone carries minute but detectable amounts of dioxin in their body fat. The most alarming of EPA's conclusions is that significant impacts on human health, such as immunotoxic effects, birth and developmental defects, reproductive and metabolic disorders such as diabetes, as well as an increased risk of developing cancer, all may be occurring at or within one order of magnitude of typical background levels of these compounds in the human body.

While much has been learned in recent years about the toxicity of these compounds, major gaps in understanding remain that can be filled only by additional careful scientific study. As a result, EPA's new risk characterization has relied heavily upon unproved assumptions and untested hypotheses, many of which are controversial and lack widespread support in the scientific community. For example, one untested hypothesis states that any exposure, no matter how small, even to just one dioxin molecule, may pose real health risks to humans. Another states that the toxicity of any mixture of dioxin-like substances is the simple sum of the toxicities of the individual components of the mixture. These two assumptions, together with the comparatively long residence times of dioxins in the human body, lie at the heart of EPA's concerns about human health risks from dioxin exposure.

As we discuss in more detail below, these and other questionable assumptions upon which the reassessment's conclusions are based must be clearly distinguished from objective, scientifically proved facts. We do not believe that there is sufficient evidence to conclude that adverse health effects should be expected at or near current human body burdens. Unless the EPA makes a clearer distinction between scientific facts and regulatory policy, these hypothetical risks are likely to be misinterpreted as real.

I. Mechanism of Action and Implications for Low-Dose Risk

Exposure to dioxin-like compounds clearly causes a variety of toxicologic effects in laboratory animals, and scientists generally agree that most of these effects are mediated through the binding of these substances to a protein known as the Ah receptor. However, the daily exposures estimated to give rise to the human background body burden are far smaller, usually thousands to millions of times smaller, than those known to cause various forms of toxicity. The pivotal issue is thus whether adverse

effects on human health can reasonably be expected to occur at or near the current background body burdens of these materials.

Receptor theory predicts that the binding of dioxins to the Ah receptor may be linear at very low exposures, which implies that some binding, in amounts proportional to the dose, may occur even at vanishingly small doses. However, this theory does not predict the shape of the dose-response curve for any biochemical or toxic response; in particular, it does not predict, as EPA's reassessment has assumed, that responses will result whenever binding occurs. Thus, responses may exhibit thresholds, and EPA has failed to demonstrate that any of the responses observed at high doses of dioxin-like compounds lack thresholds. In fact, the existence of thresholds for adverse effects from chemical exposure has been a key element in the science of toxicology at least since the time of Paracelsus, who, in the sixteenth century, wrote that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."

II. High Dose Effects

Cancer

Although dioxin has been shown to cause cancer in laboratory animals, the linkage between exposure and increased human cancer risk is at most inconclusive. Where positive associations have been observed, they are quite weak, even among persons with presumed heavy exposure arising from certain chemical manufacturing processes or industrial accidents (Bertazzi et al. 1993; Fingerhut et al. 1991; Manz et al. 1991; Saracci et al.1991; Zober et al. 1990). Attributing the modest cancer excesses observed among workers to their presumed dioxin exposures is questionable given the workers' known exposures to known carcinogens such as asbestos and 4-aminobiphenyl (*c.f.*, Collins et al. 1993; Asp et al. 1994). Furthermore, control for the influences of cigarette smoking and other significant cancer risk factors has been inadequate. Despite EPA's emphasis on the positive associations that have been reported, there is a substantial body of conflicting evidence that cannot be dismissed, and alternative explanations for the excesses observed in some studies have not been ruled out.

Developmental/Reproductive/Endocrine Effects

When considered carefully, the reports of adverse developmental effects in rodents and humans show fundamental inconsistencies. Because the male offspring of highly exposed pregnant female rats have exhibited altered adult mating behavior (Mably et al. 1992a-c), EPA has argued that similar effects can be expected in humans. However, sexual behavior patterning in rodents is qualitatively different from what is observed in humans; this endpoint is therefore of questionable relevance for potential human health risks, even at high exposure levels. Although other developmental effects have been noted in humans (Rogan et al. 1988) and other primates (Bowman et al. 1989), they have occurred only at doses dioxin-like compounds so high as to cause significant maternal toxicity.

Inclusion of endometriosis as a potential adverse human health effect is also premature. The association of endometriosis with dioxin exposure has been reported in one study of monkeys (Rier et al. 1993), but only *a posteriori,* and alternative causes (e.g., previous exposures to other substances and surgical treatments) have not been ruled out. Furthermore, a contradictory report (Arnold et al.) showing no association between body burdens of dioxin-like compounds and the incidence of endometriosis in monkeys has not received attention in EPA's current draft documents. EPA's selective emphasis on unsubstantiated positive findings does not accurately portray the full weight of the scientific evidence regarding this endpoint.

Certain endocrine disorders have also been cited as adverse outcomes of dioxin exposure, but the evidence is again for high doses and even then is very weak. For example, one study reported a significant increase in the prevalence among men of low total serum testosterone in the third exposure quartile, but this finding was not confirmed in the highest quartile, and the test for trend was not significant (Egeland et al. 1994). Moreover, the most recent Air Force study of Ranch Hands (Wolfe et

al. 1995) found no significant male reproductive effects. Although a possible relationship between decreased glucose tolerance and elevated serum dioxin levels has been reported (Egeland et al. 1992), this association has not been studied sufficiently to determine its mechanistic basis or biological plausibility.

Although these limited observations are intriguing, they are altogether insufficient to conclude that people are at risk of reproductive/endocrine or developmental effects at or near current body burdens.

Immunological Effects

There is no evidence that dioxin exposure compromises immune function in humans. In animals, effects have been noted only at high doses or with protocols that do not evaluate normal immune function (e.g., challenges of rodents with injections of sheep red blood or tumor cells, *c.f.* Vos et al. 1991). Effects on surrogate markers for immune function (e.g., distribution of T-cell subsets and surface marker expression) have been reported in some laboratory animal experiments, but these markers have no known significance for the immune competence of the animals, or humans.. In exposed humans, such effects have not been found at all.

Biochemical Changes

If, as EPA suggests, the "average" human body burden is currently within one order of magnitude of the level required to elicit adverse effects, then sensitive biochemical markers should already be elevated in the general population. One sensitive marker that has been detected in most tissues, including human placenta, is the induction of enzyme cytochrome P4501A1. Induction of this enzyme was markedly evident in placentas from highly exposed (Yusho/YuCheng) or smoking mothers, but it has not been detected in the general non-smoking population (Wong et al. 1985; Wong et al. 1986; Manchester et al. 1992). It seems implausible that adverse health effects would occur at background body burdens if these subtle and sensitive biochemical changes have not been observed.

III. What Is the Correct Dose Metric?

EPA has equated acute and chronic exposure patterns on the basis of projected body burdens even though the corresponding daily doses can differ by factors as large as 10,000-100,000. Body fat is the major storage organ for dioxin. Because it equilibrates very slowly with the daily intake of dioxin, acute administration would be expected to produce far higher peak concentrations in the blood serum and specific target organs, such as the liver, than would administration of the same total dose in small daily increments. Use of body burden as the solely measure of exposure may thus be very misleading.

To illustrate the problem, consider that the LOAEL in the studies of dioxin-induced developmental effects on reproductive organs of the male rat offspring of dams exposed to a single TCDD dose on gestation day 15 was 64,000 pg/kg (Mably et al. 1992a-c). This is virtually identical to the estimated human background body burden of 30-60 ng/kg TEQ, but it is approximately 10,000-20,000 times greater than the 3-6 pg/kg TEQ daily intake rate required to sustain that burden in steady-state. Surely, peak target organ concentrations will be far greater with a single bolus dose of 64,000 pg/kg on one day than they would be with a 6 pg/kg daily intake, and the peak concentration at specific target sites is likely to be critical for target organ toxicity.

Body burden becomes even more problematic as a dose metric when it is comprised of a complex and variable mixture of congeners with marked half-life differentials, and for which only 10% of the TEQ is directly attributable to TCDD (as is typical of human background body burdens). TEQ body burdens appropriate for episodic or bolus dose conditions cannot possibly be appropriate for steady-state exposures to such mixtures, even in the same species and target organ. EPA has failed to make a convincing scientific argument that the body burden of dioxin-like compounds, most of which is likely to be bound up in body fat, and not to Ah receptors, is the correct dose metric for extrapolation

from high dose laboratory animal effects to potential human effects at or near current human background body burdens.

IV. Toxicologic Equivalency Factors (TEFs)

Compounds that bind to the Ah receptor include many chemicals, such as the dioxin-like PCDFs and PCBs, that are present in vastly greater concentrations than dioxin itself. In fact, as was noted above, most of the human background body burden (90%) is due to these other chemicals. The validity of using TEFs to quantify the toxicity of mixtures of these chemicals hinges on the assumption that all of them act in a simple linear, additive manner. EPA's assertion regarding the likelihood of adverse effects at or near current human background body burdens depends on the validity of the additivity assumption. However, this assumption runs contrary to theoretical expectations regarding the effects produced by full agonists in the presence of plentiful but weak partial agonists. It is also contradicted by experimental evidence of antagonism among dioxin-like PCBs, and between dioxin and indole-3-carbinol (Safe 1995), a naturally occurring Ah receptor ligand found in most cruciferous vegetables (e.g., broccoli, brussel sprouts, and cabbage). EPA has furthermore ignored this and other natural Ah ligands such as the PAHs and aromatic amines that can and apparently do compete effectively for receptor occupancy despite their low affinity due to their presence in the human diet at markedly higher concentrations (see, e.g., Safe 1995, Bjeldanes et al. 1991).

The additivity assumption also implicitly presumes that dose-response curves for all endpoints are not only parallel across all congers, but are also all linear in relation to fractional receptor occupancy, despite compelling evidence of nonparallelism for a variety of endpoints (USEPA 1994) and nonlinearity, even for the simplest and most sensitive biological response yet measured, namely, mRNA for CYP1A1 (van den Heuvel et al. 1994). "International agreement" regarding TEF values is clearly not synonomous with "scientifically convincing evidence." Indeed, very little progress has yet been made to the extraordinarily complex problem of developing an experimental design to evaluate the plausibility of EPA's linear additivity assumption. We urge extreme caution regarding the use of highly uncertain TEFs, and recommend far more clarity regarding the mixed nature of exposures, and their potential health implications, particularly in human populations.

V. The Critical Need for Adequate Experimental Designs

For cancer, all of the dose-response models employed by EPA to extrapolate to low-dose human risk incorporate linear components that dominate the predicted responses at low doses. Indeed, the models developed with human data, either for relative or additive risk, are entirely linear, at all exposure levels (USEPA 1994). Yet, the available data are altogether inadequate to provide precise estimates of even the simplest model's parameters. Even if the correct model were low-dose linear, the data are nevertheless inadequate to determine the low-dose slope. They are inadequate to distinguish between linear and alternative nonlinear models. They are inadequate to rule out the possibility of threshold-like responses to exposure. The fact of the matter is that the available data, although voluminous, were never designed to be used for the purpose of human health risk assessment at doses anywhere near the exceedingly low human background exposure levels. They are simply inadequate to this formidable task. There is a critical need to identify experimental designs that are adequate for collecting data that are useful for risk assessment purposes.

VI. Overarching Issues

We, and other independent scientists as well, (see Bradfield et al.1995), have concluded that EPA's risk characterization of dioxin-like compounds relies heavily on many unproved assumptions and untested hypotheses. We have further concluded that there is not a sufficient scientific basis for EPA's assertion that adverse human health effects should be expected at or near background body burdens of these chemicals. Indeed, this EPA assertion appears to have been reached as a

consequence of the mixing of regulatory policy assumptions with science-based inferences in the reassessment documents, particularly in Chapter 9, the Risk Characterization. The blurring of science with policy confuses risk assessment and risk management issues, and it should be avoided at all costs; failure to avoid such confusion does a serious disservice to EPA, and to the scientific and risk assessment enterprises.

Richard Feynman (1984) may have said it best when he described the extraordinary integrity that is always associated with scientific activities of the highest qualtiy:

> "In summary, the idea is to try to give *all* of the information to help others judge the value of your contribution; not just the information that leads to judgment in one particular direction or another... I'm talking about a specific, extra type of integrity that is not lying, but *bending over backwards to show how you're maybe wrong*, that you ought to have when acting as a scientist. And this is our responsibility as scientists, certainly to other scientists, and I think to laymen."

To paraphrase Feynman, EPA must go the extra mile to show clearly the many ways that its provocative conclusions regarding dioxin-like compounds could be wrong.

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