CHALLENGES IN THE NEXT GENERATION OF RISK/CONTROL ASSESSMENT – MIXTURES, SUSCEPTIBILITY, AND TOXICOLOGY-EPIDEMIOLOGICAL DISCORDANCE

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Introduction

Emerging science on several fronts presents challenges to existing regulatory risk and control strategy assessments. These formal assessments are largely designed to evaluate individual chemical substance exposures and effects in adult animals. The emerging scientific hypotheses being tested involve evidence that real-world multiple exposures, or mixtures, including metabolites and degradation products, may have additive or greater joint actions, even if the individual substances have little or null effect alone. Another hypothesis is that *in utero* or neonatal exposures to environmental toxicants, or, in general, the timing of exposure, alters susceptibility to disease later in life. This implies that the dose-effect studies underlying risk assessments may be looking at the wrong time frames. Support for these hypotheses also lays in comparisons of epidemiology studies of humans and wildlife, with observed effects at concentrations from nM to low uM, with developmental and other toxicology studies in animals and in vitro systems, showing predicted effects at low to mid uM levels. These comparisons can show both discordance and concurrence[1]. The discrepancy gap, or uncertainty of 2 or 3 orders of magnitude, needs to be accounted for in risk assessment, and may be due to numerous modulating factors not considered at present. This paper will discuss: (a) the discordance/concurrence and comparative results; (b) mixtures and omitted factors review; and (c) explore possible quantitative and theoretical factors of the discordance and an inherent uncertainty.

Material and Methods

The methods used involve an examination of several lines of evidence, including toxicological, methodological, physical-chemical, and epidemiological, in a weight-of-evidence approach. Examples of the discordance/concurrence between toxicology and epidemiology compare predicted effects levels and margins of exposure, with observed effects associations. Molar (M: moles per litre or kg) conversions for tissue assume matrix density of water. Evidence on single and chemical mixture effects is presented succinctly. An example analysis of the effects of mixtures, synergies, variability in exposure, life stage, and other factors is given for purposes of comparing toxicological based margins of exposure with actual. Theoretical and empirical evidence will be used to suggest quantitative estimates of uncertainty, and inherent precision limits to estimates of margins of exposure, safety and risk.

Results and Discussion

Various toxicological models provide, for example, in vitro data on, endocrine disruption and androgen receptor antagonism;[2, 3] PXR and CAR interaction[4]; PKC translocation[5,6]; and aromatase inhibition,[7] and in vivo data for mice, rats, and monkeys [8,9,10,11,12,13,14], including neurotoxicity and enhanced susceptibility. These data show that single compounds, such as DDT, DDE, PCBs, PBDEs, HCBD, TBBPA, and others, and metabolites, have IC50, EC50, Ki, or equivalent potencies of between 100 nM and 100 uM, as a cross summary. E-Screen assays report nM potencies (EC01-05; mostly 1-300 nM) for xenoestrogenic actions, overlapping human body burdens.[15] Experience shows comparisons may be made to single chemical exposures or body burdens of <10 pmole/g to > 2 nmole/g, rather than using the molar M of the toxicology data, which is 1000 times larger. Using the mole/g concentrations, a 3 or 4 order of magnitude margin of exposure or safety may be asserted, although "safety" factors are not included. This statement conflicts with the need to use comparable M data, variability, some human epidemiological studies and animal experiments. The following Molar comparisons show both discordance and concurrence uncertainty.

The Jacobson's Human Children Cohort – Prenatal and Postnatal Exposure[16]

The Jacobson's studies saw developmental effects and cognitive deficits in the highest exposed group. This was associated with PCBs, even though the exposure was a complex mixture in fish. This exposure can be measured as mother's milk > 1250 ng/g lipid, or a weighed average of 3.6 uM (maximum 2600 ng/g or 7.5 uM). Alternatively, the cord blood effect concentrations of >5.0 to> 9.0 ng/ml, ww, or > 14 to > 26 nM, can be considered. Assuming a cord blood lipid concentration of 0.25%, brings these concentrations to >2000 ng/g lw to >3600 ng/g lw, or >5.8 uM to >10.4 uM. Discordance?

Lake Ontario Fish Eating Mother's Infant Cohort[18]

Significant linear relationships (with dose-response) were found between the most heavily chlorinated PCBs in cord blood and performance impairments on the Habituation and Autonomic clusters of the Neonatal Behavioral Assessment Scale at 25-48 hours after birth The most highly exposed neonates performed even worse. Cord blood PCBs, DDE, HCB, Mirex, lead, and maternal hair mercury levels were determined, but only PCBs were related to the NBAS performance. Measured concentrations for PCBs were: 25^{th}-ile – 0.174 ng/g ww, or 0.5 nM; 50^{th}-ile – 0.525 ng/g ww, or 1.5 nM; 75^{th}-ile – 1.11 ng/g ww, or 3.2 nM. DDE levels ranged from 0.2 nM to 0.6 nM ww. HCB ranged from 0.07 nM to 0.35 nM ww. Mirex was below detection and reported as zero. Lead ranged from 1.00 ug/dl to 2.00 ug/dl, and maternal hair Hg ranged from 0.40 ng/mg to 0.70 ng/mg. Cord blood lipid was not given, but assuming 0.25% yields 75^{th}-iles for: PCBs – 1.3 uM lw; DDE – 0.24 uM lw; and HCB – 0.14 uM lw. Discordance?

A Spanish Children's Cohort – Prenatal Exposure [19,20]

In a Spanish cohort of preschool children, 75^{th}-ile cord blood DDT > 0.21 ng/ml ww, and DDE > 1.94 ng/ml ww, were associated (DDT signif) with verbal, memory, quantitative, and perceptual performance deficits[19]. This was a mixtures exposed cohort. Molar equivalents for DDT and DDE are 0.6 nM and 6.1 nM respectively. Only wet weights were analyzed. Lipid concentrations in cord blood were 0.26% (pers. comm.) which gives 75^{th}-ile DDT at 81 ng/g lw or 0.23 uM, and for DDE, 746 ng/g lw or 2.35 uM. Maximums were about 10 times higher – 26 uM. In the same cohort, those children with cord serum concentrations of HCB >1.5 ng/ml ww (5 nM) had a significantly increased risk (relative risk (95% CI) of having a poor Social Competence = 4.04 (1.76-9.58) and ADHD = 2.71 (1.05-6.96)) at 4 years of age.[18] Lipid weight is 577 ng/g or 2.0 uM. Maximum HCB was 9.82 ng/ml ww (3,777 lw -13 uM). Discordance?

Age-of -Exposure and Endocrine Studies

High levels of serum p, p'-DDT (blood samples from 1959-1967) predicted a significant five-fold increase risk of breast cancer among women born after 1931.[21] These women were under age 14 in 1945, when DDT came in widespread use and mostly under age 20 as DDT use peaked. Women not exposed before age 14 showed no association. Tertiles of concentration were: tertile 1, < 8.09 ug/L (< 23 nM); tertile 2, >8.09 – 13.9 ug/L (< 23 – 40 nM); tertile 3, > 40 nM (no lipids provided). Concurrence or discordance?

A Chinese human female cohort had T-DDT effects on estrogen and progesterone homeostasis at a median serum concentration of 5922 ng/g lw (18.5 uM) with 5,542 ng/g lw (17.4 uM) for DDE. [22] Serum PCB level and consumption of Great Lakes fish are associated with significantly lower levels of T4 and free thyroxine index in women, and significantly lower levels of T4 in men. Mean PCB serum levels in case men were 4.7 ng/g ww (13 nM), or 822 ng/g lw (2.2 uM). In women these were 2.6 ng/g ww (7 nM), or 305 ng/g lw (0.83 uM). DDE levels were also measured but were not associated with the endpoints measured. These levels were 4.6-ng/g ww (15 nM) for men, and 3.3 ng/g (10 nM) for women. Lipid factors yield DDE levels of 180 nM and 160 nM lw respectively.[23] Concurrence?

Animal Studies

The Rice monkey study noted effects at 50 ng/ml ww, or 1650 ng/g lw PCBs in milk, or 0.14 or 4.7 uM [24] Discordance? In a captive Kestrel exposure study, the egg stage was exposed to an equivalent 1500 ng/g ww, or about 3 uM of a PBDE (mainly penta) mixture, and then the nestlings were exposed to day 29 by gavage, before first meal, to an average 15.6 ng/g/bird/day, or 0.21 uM [25]. These exposures resulted in changes in thyroid, vitamin A, and glutathione homeostasis, and induced oxidative stress. Concurrence?

An Occupational Exposure to DecaBB and DecaBDE Study[26]

The exposed workers were employed in a plant that manufactured only the brominated products – decabromobiphenyl (PBB, from Jan. 1, 1973 to March 31, 1977) and decabromobiphenyl oxide (PBBO, from March 31, 1977 to May, 1978) Serum samples were taken in August, 1978. PBB serum levels were significantly higher among the exposed group than the unexposed group. Notably, no positive identification of the DecaBB or DecaBDE was made. PCBs were also detected. An unexpectedly high prevalence (4/35) of primary hypothyroidism was found among the PBB workers. Sensory and motor nerve velocities were also significantly reduced compared to controls. Serum PBB levels ranged from 0.5 ng/ml to 1340 ng/ml ww – mono through nona, except tetra, was detected. Most of the PBB concentration was made up of nona, octa, and hepta. Assuming mean MW of 835 makes above serum PBB levels approximate 0.6 nM to 1.6

uM. Only the mono and tri PBBOs were detected, ranging from 0.2 ng/ml (<1 nM) to 4.7-ng/ml (12 nM) ww. T-PCBs ranged from 1.0 ng/ml to 33,000 ng/ml ww. The dominant homologues contained 4, 5, and 6 chlorines. Assuming mean MW of 326, T-PCBs range from 3 nM to 101 uM. Concurrence or discordance?

Discrepancy Summary

Mostly, and obviously, whether ww or lw were reported in the associative analysis was determining. Concurrence occurred more for endocrine effects, and discordance more for DNT. Higher clinical effect concentrations (lw) did approach and overlap the predicted potencies of the toxicological models in the <1 – 20 uM range. Generally, the ww results were low nM. These data generally supported the clinical effects and time of exposure hypotheses, Large discordant results originally hypothesized came from measurement errors using moles/g instead of the molar M, and this may be common. There still remains a 1 to 3 order of magnitude uncertainty in effects levels, and a large number of sources of variation and uncertainty that evidence shows enter into the in vivo exposure-effects domain, and modulate the metric. These include: lipid estimation and standardization; mixtures additivity and synergism; gender, age at exposure and numerous susceptibility aspects; inherent uncertainty in the theoretical foundation, physical-chemical properties, and organism complexity; relative tissue sensitivity; uptake, clearance and general metabolic and control mechanisms variability; other polymorphisms or gene variances; chronic and variable exposure; complexity of multiple biochemical interactions, endpoints and mechanisms; and existing health conditions. Overall, this puts an inherent limit on the precision with which predictions of risk can be made.

Selected Quantitative and Theoretical Factors in the Uncertainty

The major physical and chemical properties of compounds arise from the electron configurations in each compound and their interactions. These are in the domain of quantum mechanics where it makes no sense to talk in terms more precise than statistical relationships and probabilities. In this domain, it is probability distributions that are causally determined, not specific events. For example, chemical partitioning space maps represent neutral organic compounds using ellipses or lines. The lines/ellipses encircle the chemical space the respective compounds are most likely to occupy, and represents the range of uncertainty in measured and estimated physical-chemical property data.[27, 28] These uncertainties can be 4 to 6 orders of magnitude for chemicals with many congeners, or as few as 1 or 2 orders. Adding in the biological complexity, and complex SARs with wide and varied chemical domains, provides additional factors, large variations, and multiple steps, all of which acting together lead to contingencies and chance, or inherent uncertainty. Taken together, it makes no sense to talk with certainty about a point estimate of risk, a single event in isolation, in one specific context, that is abstracted from all the real uncertainties of joint occurrences of multiple events. This uncertainty could range from 100 to 1 million.

Mixtures and susceptibility are other realities that must be in the conceptual framework of risk assessment if it is to be sensible. Seegal proposed an “environmental hypothesis”, that the neurochemical effects of developmental exposure in rats to contaminated salmon are not due solely to PCB exposure.[29] He found that dietary exposure of rats exposed to Great Lakes salmon (contaminated with many chemicals) containing as little as 13.9 ug/kg/day (40 nM) of PCBs, resulted in significant decreases in regional brain dopamine concentrations compared to controls. A similar experiment, using only the PCB congener 2,4,2’,4’-TCB (PCB 47), also induced significant dopamine reductions, but only at levels > 100-fold higher (>4 uM) than the fish-eating rats, exposed to a mixture. This suggests interactions or synergism in the fish mixture.

Co-exposure of mice to PCB 52 and PBDE 99 showed a five-fold dose-based enhancement or synergism of developmental neurobehavioral effects at low doses where the individual compounds do not have an effect. Co-exposure to PCB 153 and MeHg, or PBDE 99 and MeHg both showed a ten-fold synergism at low doses. Co-exposure to PCB 126 and MeHg also showed possible additive interaction effects at some doses. In some cases the effects worsened with age, and in others, were sustained.[30] Neonatal exposure to PFOS and PFOA causes neurobehavioural defects in adult mice.[31] Co-exposure to PBDE 209 with PFCs (PFOA) during neonatal brain development enhances neurobehavioural defects in mice.[31] In vitro co-exposure to PCBs and MeHg act synergistically to reduce rat brain dopamine content.[32] Other reports show at least additive interactions for estrogenic, thyroid active, androgen antagonists, and neurotoxic mixtures.[33,34,35,36] Taken together, joint exposure entails a joint probability, or multiplication, of the individual synergistic factors to account for the uncertainty introduced by co-exposure. This could be 100 to 1000 for just the noted compounds, which would be applied to margin of exposure (MOE) estimates for single compounds.

For present purposes, the susceptibility discussion will focus on selected quantitative factors involved in assessing risk to the fetus as compared to considering the newborn or first year of life. There is evidence of close correspondence between maternal organochlorine concentrations across all trimesters and soon after delivery, and between maternal and fetal blood at delivery.[21] Equilibrium distribution of lipophilic chemicals between the human maternal and fetal compartments at full haemochorial development of the placenta (12-13 weeks gestation) greatly increases the fetal dose per kg/bw, however, risk assessment may consider only the single chemical dose at birth or later. At 12 to 13 weeks gestation, the fetus weighs 14 -23 grams average, compared to 3500 grams, for example, at birth, or an average of 7,500 or 10,000 grams averaged over 6 or 12 months of age, respectively, sometimes used in risk assessments. With the fetus as target, the dose per unit body weight is 152 to 714 times the infant weight range given. This is another uncertainty factor that logically enters into the discordance and should be included in the MOE calculation.

In summary, initial estimates of inherent uncertainty in predictions of margins of exposure or safety for single compounds in isolation range over several orders of magnitude. Empirical estimates for PBDEs, PCBs, and MeHg suggest a factor of 500 for joint exposure. Adding PFOS could increase this to 1000. Accounting for at-least dose – additive mixtures will increase this factor in concentration proportion (compounds such as T-DDT, BPA, Triclosan, perchlorate, HBCCD, TBBPA, dioxins, metabolites, degradation products, and so into the hundreds). Taking the fetus as target can involve another multiplier of 152 to 714, bringing the uncertainty estimate to 6 orders of magnitude or more. In conclusion, risk assessment of individual chemicals in isolation should divide MOE calculations by at least 6 orders of magnitude to protect the fetus, and therefore, eventually, the infant, child, and adult individual.

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