CHEMICAL MIXTURES: VALIDATION OF WEIGHT-OF-EVIDENCE PREDICTIONS FOR INTERACTIONS.

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Abstract
Binary interactions of chemicals can be evaluated based on the toxicological and mechanistic data. Weight-of-evidence (WOE) predictions can be made regarding the direction (i.e., synergism, antagonism) of the interaction. The paper presents examples of validations of such predictions based on *in vitro*, *in vivo*, and epidemiological data.

Introduction
The Agency for Toxic Substances and Disease Registry's (ATSDR) approach to chemical mixtures risk assessment was presented to the audience of the 28th International Symposium on Halogenated Persistent Organic Pollutants in Birmingham last year.
Briefly, if no toxicity data are available on the mixture to be evaluated as a whole entity, approaches to evaluate the toxicity of components of the mixture are commonly used (1,2,3). For most mixtures, component-based approaches such as the hazard index (HI) are recommended. The hazard index approach assumes dose additivity. Exposures or doses for the various components of the mixture are scaled by a defined level of exposure generally regarded as acceptable or safe (i.e., health-based guidance value) by the agency performing the assessment.
The general equation for the hazard index (*HI*) calculation is:

$$HI = \frac{ChemExposure_1}{DR_1} + \frac{ChemExposure_2}{DR_2} + \frac{ChemExposure_n}{DR_n}$$

$ChemExposure_1$ is defined as the level of exposure to the first chemical in the mixture and DR_1 is some defined level of exposure to the first chemical (i.e., health-based guidance value), $Exposure_2$ and DR_2 are the corresponding levels for chemical 2, and the summation can extend to any number of chemicals, signified by the n.

When the hazard index for a mixture exceeds unity (1), a concern for the potential hazard of the mixture increases. Component-based approaches are most useful when augmented with a weight-of-evidence (WOE) evaluation of the potential for non-additive interactions among the components in the mixture. WOE is a qualitative judgment, based on empirical toxicity observations and mechanistic considerations, which categorizes the most plausible nature of any potential influence of one compound on the toxicity of another for a given exposure scenario. The WOE evaluations of the mixtures' components are used to qualitatively adjust the HI. For example, if the component-based analyses indicate that several binary combinations will have more than additive joint toxic action, the HI may underestimate the actual toxicity of the mixture. Conversely, if the component-based analyses indicate that several binary combinations will have less than additive joint toxic action, the HI may overestimate the actual hazard presented by the exposure scenario. Experimental work to validate predictions is needed.

Validation using *in vitro* testing
The latest ATSDR's project in the area of risk assessment of chemical mixtures was to investigate the possible joint actions of chlorinated dibenzo-*p*-dioxins (CDDs), polybrominated diphenyl ethers (PBDEs), and phthalates (also known as phthalate esters) on endocrine, developmental, and neurobehavioral endpoints in humans. CDDs and PBDEs are bio-persistent due to their slow degradation and elimination from the body. Phthalates are rapidly metabolized and eliminated from the body, but as exposure to phthalates is continuous, phthalates and their metabolites are continuously cycling through the body.

The available mechanistic understanding of toxicity caused by each class of these chemicals alone is not sufficient to reliably predict the direction or magnitude of any interaction between all three chemicals or between any two pairs of chemicals, except for PBDEs and TCDD. *In vitro* mechanistic evidence indicates that PBDEs antagonize TCDD activation of the AhR signal transduction pathway (4), but there are no studies that address possible joint action of PBDEs and TCDD on any toxicity endpoint. Furthermore, the mechanistic evidence suggesting possible antagonism is offset by thyroid toxicity data for TCDD alone and PBDEs alone that suggest the possibility of joint additivity on the basis of a common non-AhR-mediated mode of action (i.e., inhibition of T_4 binding by hydroxylated intermediates). Given the co-occurrence of CDDs, PBDEs, and phthalates in humans and the commonality of certain types of effects, ATSDR recommends that the default assumption of joint additivity be employed to assess mixtures of these chemicals using a modified hazard index approach (5).

Validation of the additivity prediction for PBDEs and phthalates is in progress. The very preliminary results are presented in Figure 1.

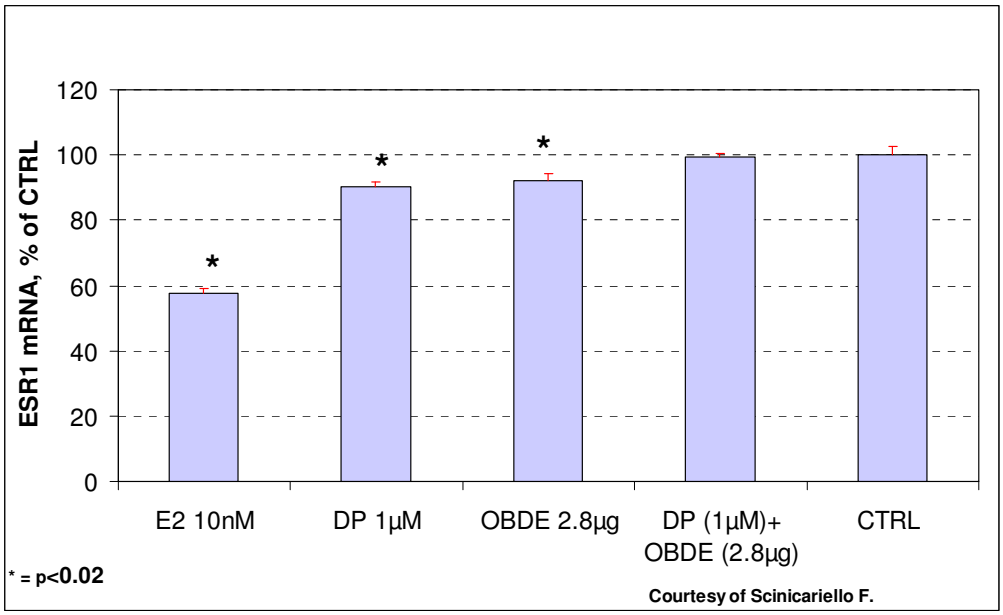


Figure 1. Effect of dioctyl phthalate (DP) and Octa-BDE (OBDE) on the expression of Estrogen Receptor – Alpha (ESR1) mRNA.

MCF-7 cells were grown in phenol red-free IMDM medium and 5% charcoal treated calf serum for 24 hours with either 10nM of Estradiol (E2), or 1µM DP, or 2.8µg OBDE, or a solution containing 1µM DP and 2.8µg OBDE. ESR1 mRNA was determined by real time reverse-transcriptase PCR. mRNA was quantified using the "delta-delta Ct' method. Results are presented as percent of control cells (CTRL) and represent t the mean of 9 experiments ± standard error (t-test used for statistical evaluation). The individual chemicals down regulate the ESR1mRNA. When present together in the medium, there was no d difference in ESR1 mRNA compared to the control. Less-than-additivity is suggested.

Validation using *in vivo* animal testing

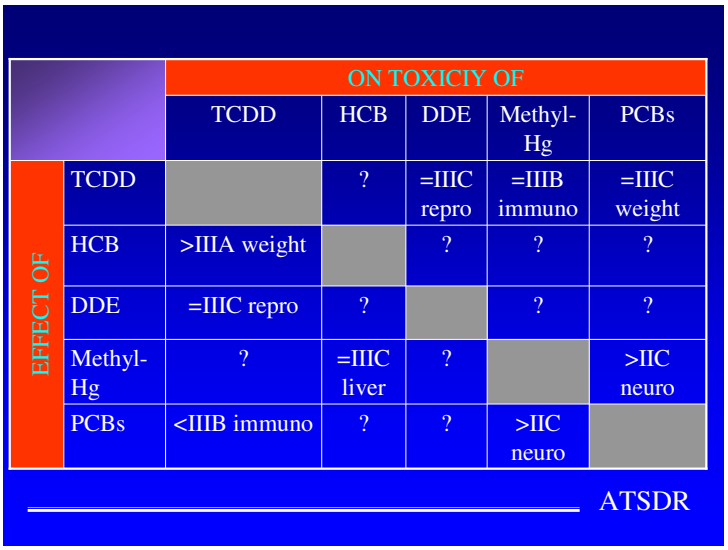
ATSDR in cooperation with TNO in the Netherlands attempted validation of WOE predictions in laboratory animals. In a study of 210 binary combinations of 15 chemicals, WOE was used to evaluate the binary pairs (6). Three combinations—methyl mercury/benzene, methyl mercury/lead, and benzene/trichloroethylene—were predicted to have interactions greater than additive toxicity and were referred to laboratories for toxicity studies. The study showed that WOE methodology works particularly well for chemicals with similar mechanism of action as demonstrated by laboratory confirmation of predictions for some nephrotoxicants.

Validation using epidemiological studies
Validation of WOE predictions using epidemiological data is difficult due to the nature of human exposure. However, obtained data can serve as supporting evidence regarding toxicologic conclusions for certain mixtures.

One of the first ATSDR's interaction profiles dealt with chemical mixtures often found in contaminated fish (7). Hexachlorobenzene, *p,p*=-DDE (the predominant metabolite of *p,p*=-DDT), methylmercury, PCBs and CDDs are persistent chemicals that bioaccumulate in higher food-chain organisms and are part of a set of 11 substances that have been identified as pollutants that may present health hazards with frequent consumption of contaminated fish from the North American Great Lakes. In addition, these chemicals are found, to varying degrees, in other dietary components including fish from other parts of the world (e.g., the Baltic Sea), human milk, dairy products, and meat.

The WOE analysis indicates that only a limited amount of evidence is available to support the possible interactions of a few pairs of the components: (a) hexachlorobenzene potentiation of 2,3,7,8-TCDD reduction of body and thymus weights ; (b) PCB antagonism of TCDD immunotoxicity and TCDD developmental toxicity ; and (c) synergism between PCBs and methylmercury in disrupting regulation of brain levels of dopamine that may influence neurological function and development. For the remaining pairs, additive joint action at shared targets of toxicity is either supported by data or is recommended as a precautionary measure. The WOE results are presented in Table 1.

There have been several studies published regarding research in populations around the Great Lakes since the release of the ATSDR's interaction profile. Some of them may support the ATSDR's evaluations, but cannot by itself make predictions about the interactions.



Children exposed prenatally to PCBs and methylmercury scored poorly on the McCarthy Performance Test at 38 months of age but no effect was seen at 54 months of age (8). Children exposed to PCBs, methylmercury and lead showed impaired performance on behavioral tests (9). PCBs and DDE were markedly elevated in an adult fisheating cohort (10). Exposure to PCBs, not DDE, was associated with lower scores on several measures of memory and learning. Parents exposed to PCBs and DDE had a higher than expected proportion of male children than female children if the father had elevated PCB levels (11).

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