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Initial analyses of the relationship between "Thresholds" of toxicity for individual chemicals and "Interaction Thresholds" for chemical mixtures

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Abstract

The inter-relationship of "Thresholds" between chemical mixtures and their respective component single chemicals was studied using three sets of data and two types of analyses. Two *in vitro* data sets involve cytotoxicity in human keratinocytes from treatment of metals and a metal mixture [Bae, D.S., Gennings, C., Carter, Jr., W.H., Yang, R.S.H., Campain, J.A., 2001. Toxicological interactions among arsenic, cadmium, chromium, and lead in human keratinocytes. Toxicol. Sci. 63, 132–142; Gennings, C., Carter, Jr., W.H., Campain, J.A., Bae, D.S., Yang, R.S.H., 2002. Statistical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. J. Agric. Biol. Environ. Stat. 7, 58–73], and induction of estrogen receptor alpha (ER-α) reporter gene in MCF-7 human breast cancer cells by estrogenic xenobiotics [Gennings, C., Carter, Jr., W.H., Carney, E.W., Charles, G.D., Gollapudi, B.B., Carchman, R.A., 2004. A novel flexible approach for evaluating fixed ratio mixtures of full and partial agonists. Toxicol. Sci. 80, 134–150]. The third data set came from PBPK modeling of gasoline and its components in the human. For *in vitro* cellular responses, we employed Benchmark Dose Software (BMDS) to obtain BMD_{01}, BMD_{05}, and BMD_{10}. We then plotted these BMDs against exposure concentrations for the chemical mixture and its components to assess the ranges and slopes of these BMD-concentration lines. In doing so, we consider certain BMDs to be "Interaction Thresholds" or "Thresholds" for mixtures and their component single chemicals and the slope of the line must be a reflection of the potency of the biological effects. For *in vivo* PBPK modeling, we used 0.1× TLVs, TLVs, and 10× TLVs for gasoline and six component markers as input dosing for PBPK modeling. In this case, the venous blood levels under the hypothetical exposure conditions become our designated "Interaction Thresholds" or "Thresholds" for gasoline and its component single chemicals. Our analyses revealed that the mixture "Interaction Thresholds" appear to stay within the bounds of the "Thresholds" of its respective component single chemicals. Although such a trend appears to be emerging, nevertheless, it should be emphasized that our analyses are based on limited data sets and further analyses on data sets, preferably the more comprehensive experimental data sets, are needed before a definitive conclusion can be drawn.
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Keywords: Chemical mixtures; Interaction thresholds; Dose-response relationship; NHEK cells; MCF cells; PBPK modeling; Metals; Endocrine disruptors; Gasoline; BMDS

Introduction

In February 2005, the Society of Toxicology (SOT), jointly with the Agency for Toxic Substances and Disease Registry (ATSDR) and other sponsors, held a workshop in Atlanta, Georgia. The central theme of the workshop was *Contemporary Concepts in Toxicology: Charting the Future: Building the Scientific Foundation for Mixtures Joint Toxicity and Risk Assessment*. This workshop was a follow-up based upon the recommendations of a 2002 SOT Expert Panel that identified specific biologically based hypotheses and experimental approaches to generate data useful to enhance assessments and develop realistic policies for chemical mixtures. One of the nine hypotheses generated by the Expert Panel in the 2002 meeting was "Apparent dose thresholds for interactions are higher than individual chemical thresholds." The present paper, which was part of the program on "Dose to Response" in the abovementioned workshop, was assigned to the first author of this paper to specifically address this hypothesis.

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The idea of "Interaction Thresholds" was introduced in 1996 by El-Masri et al. (1996) as the minimal level of change in tissue dosimetry associated with a significant health effect. That research work was based on PBPK modeling of the competitive inhibition of trichloroethylene metabolism by 1,1-dichloroethylene. To estimate the "Interaction Threshold," the model of El-Masri et al. (1996) included a description of the percentage of enzyme (i.e., CYP2E1) sites occupied by either chemical in the presence or absence of the other.

When two or more interactive chemicals are studied together, theoretically, there could be infinite interaction thresholds depending on the dose levels used for the individual chemicals in the studies. However, if we specify certain occupational or environmental exposure concentrations for all the other component chemicals in the mixture except one, we may obtain an interaction threshold for that set of specific exposure conditions. Even though the concept sounds quite straightforward, there are very few such dose–response data sets for chemical mixtures. It is therefore even more of a challenge to attempt to analyze and compare interaction thresholds of chemical mixtures with their respective dose thresholds of individual component chemicals. In addition to the very limited availability of experimental data sets for such analyses and comparisons, two other issues arose in carrying out this assignment from the SOT. First, how do we define "Interaction Threshold"? Do we simply follow our earlier definition by El-Masri et al. (1996)? Given some of the data sets to be utilized are from cell culture systems and PBPK modeling was not carried out in these studies, the dose metrics are different. What do we do in this instance? The second issue relates to "interaction." How do we know that toxiõcological interactions indeed happened in the mixtures that we selected, particularly at the low dose region? These issues are discussed, respectively, in the Methods and Discussion sections.

To explore this relatively new area of inter-relationship of "Thresholds" between chemical mixtures and their respective component single chemicals, we used three different sets of data and two different types of data analyses. Two of the data sets were from our own laboratory: one on cytotoxicity in normal human epidermal keratinocytes (NHEK) from treatment of metals and a metal mixture (Bae et al., 2001; Gennings et al., 2002), the other on venous blood levels from physiologically based pharmacokinetic (PBPK) modeling of gasoline and its components in the human based on a human PBPK model extrapolated from an earlier published rat PBPK model (Dennison et al., 2003, 2004). The other data set came from Gennings et al. (2004) on induction of estrogen receptor alpha (ER-α) reporter gene in MCF-7 human breast cancer cells by estrogenic xenobiotics and mixture. We employed Benchmark Dose Software (BMDS) (USEPA, NCEA; http://cfpub1.epa.gov/ncea/cfm/bnchmrk/versions.cfm?ActType=default) and PBPK modeling to assess the ranges and slopes related to "Interaction Thresholds" or "Thresholds" for mixtures and their component single chemicals. The experimental details, as well as our thoughts on the significance of the findings are given below.

Methods

Origins and contents of the data sets. The first data set was from the normal human epidermal keratinocytes (NHEK) studies reported by Bae et al. (2001) and Gennings et al. (2002). The cells were treated with As, Cr, Cd, Pb, or a mixture of the four metals for a 24-h period, re-fed with fresh metal-free medium for 3 days prior to viability (cytotoxicity) analysis by the MTT assay (Mossman, 1983). At least three replicate experiments were conducted and five to seven dose levels covering a concentration range of about 100- to 1000-fold plus control were used. The lowest concentrations studied for single metals were from a fraction of μM to a few μM range whereas those for the metal mixture were in the nM range. The mixing ratios for the mixture were based on LD_{50} for cytotoxicity for each individual metal except lead where no cell killing was observed even at concentrations as high as 100 μM. Thus, for the highest mixture concentration, 1×, the mixing ratio was approximately 7.7:4.9:6.1:100 (in μM) for As:Cr:Cd:Pb. This mixture solution was diluted serially 1:3 to get 0.333×, 0.111×, 0.037×, 0.0123×, 0.004×, and 0.0014× mixture solutions for dose–response studies. Table 1 provides a summary of the composition of the metal mixture. Responses of NHEK cells to the metal mixture were highly dose-dependent. A growth stimulatory effect (hormesis) was observed at the very low concentrations. As the mixture concentration increased, a trend of additivity changed to synergistic cytotoxicity. At the highest concentrations tested, however, antagonistic interactions were observed (Bae et al., 2001; Gennings et al., 2002). Since these studies were from our own laboratories, we have the original raw data for the present analyses.

The second data set was from a publication by Gennings et al. (2004) in which an estrogen receptor alpha (ER-α) reporter gene assay was studied using MCF-7 human breast cancer cells. The cells were first transfected using Lipofectin™ and 16 to 18 h later treated with one of the six endocrine active compounds [methoxychlor (MXC), *o,p*-DDT (DDT), beta-hexachlorocyclohexane (β-HCH), bisphenol A (BPA), octylphenol (OCT), and 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN)] or the mixture of these six chemicals. The test concentration ranges were 0–10 μM for MXC, DDT, β-HCH, and OCT; 0–1 μM for BPA; 0–0.1 μM for DPN; and 0–8 μM (total concentration) for the mixture. The mixing ratios for the six chemicals were based on no-observable-effect concentrations from preliminary concentration range-finding studies; thus, the resulting ratio is approximately 0.4715:0.0047:0.4715:0.471:0.0047:0.0005 for MXC:DPN:DDT:β-HCH:OCT:BPA, respectively (see Table 2). The resulting data were assessed as units of luciferase activity normalized to the β-galactosidase activity from individual reaction wells. The endpoint of interest in these studies was the average fold of induction of ER-α reporter gene. The maximal fold of induction for the single chemicals ranges 3.193 to 11.637 at or near the highest concentration whereas that of the mixture is at 7.337-fold at the highest concentration. Gennings et al. (2004) concluded that the mixture exhibited antagonistic interactions. In this paper, one of the tables provided invaluable information in that dose–response data were presented with standard deviation (SD) reported. Since BMDS analysis can be done with data points containing average±SD and the respective sample size, the information presented in this table was very helpful. It is hoped that in future publications on mixture toxicology such essential dose–response information and sample size, even the model code and details, will be routinely included to aid in probable further analyses.

The third data set was specifically generated for this paper from our own laboratory on the PBPK modeling in humans of gasoline and five component

Table 1
The composition of equal toxic metal mixture

Metals	Conc. μM "LD_{50}"	Proportion for 1× Mixture
Arsenic	7.7	6.5%
Chromium	4.9	4.1%
Cadmium	6.1	5.1%
Lead	100[a]	84.3%

[a] We were never able to determine a LD_{50} for lead in human keratinocytes because of its low cytotoxicity. We chose to use a high tested concentration of 100 μM to represent LD_{50} for lead.

Table 2
The composition of a mixture of endocrine active compounds for estrogen receptor alpha reporter gene assays

Chemicals	Proportion in the mixture
Methoxychlor	47.15%
DPN	0.47%
o,p-DDT	47.15%
β-HCH	4.71%
Octylphenol	0.47%
Bisphenol A	0.05%

marker chemicals (benzene, ethylbenzene, toluene, *o*-xylene, *n*-hexane) and a lumped "chemical" containing the remaining hydrocarbons in the gasoline. This human PBPK model was an extrapolation from a published rat PBPK model that was verified with experimental inhalation pharmacokinetic studies on gasoline fractions and component chemical markers. The details of experimental information can be found in Dennison et al. (2003, 2004). Briefly, this PBPK model for rats, consisting of four compartments (liver, fat, rapidly perfused tissues, and slowly perfused tissues), was developed based on pharmacokinetic experiments. The PBPK model tracks selected target components [benzene, ethylbenzene, toluene, *o*-xylene (BTEX), *n*-hexane] and a lumped chemical group representing all non-target components. In the liver compartment of this PBPK model, competitive metabolic inhibition was incorporated to describe the inhibition effect of the lumped "chemicals" on any individual and combined target compounds. Pharmacokinetic data were obtained by conducting gas uptake pharmacokinetic studies with male Fischer 344 rats in a closed chamber. Chamber air samples, which reflect pharmacokinetic fates of the marker chemicals studied, were analyzed every 10–20 min by gas chromatography and all non-target chemicals were co-integrated. Rather than systematically going through numerous pharmacokinetic experiments involving single chemicals, two-, three-, four-, and five-components in combination, Dennison et al. (2003) took the approach of performing a simple suite of single-chemical and five-chemical mixture experiments and then went straight to the full mixture, the gasoline. Judging on the comparisons of numerous sets of model simulations and the corresponding experimental data, this rat PBPK model appeared to have done a consistently good job of describing the kinetic behaviors of the selected chemical markers. For the present paper, we extrapolated the rat PBPK model to a human PBPK model by incorporating all the parameters relevant to human. We then carried out computer simulations using 0.1×, 1×, and 10× of threshold limit values (TLVs) for the marker chemicals (i.e., target chemicals and gasolines) as input exposure dose levels (see Table 3 for specific concentrations). The resulting venous blood concentrations at various time points from PBPK modeling simulation are considered as "Thresholds" for individual chemicals and mixtures (see discussion in the following section).

Methods of analyses. For *in vitro* cellular responses (i.e., NHEK cytotoxicity and induction of ER-α reporter gene), we employed Benchmark Dose Software (BMDS; Version 1.3.2) (USEPA, NCEA) to assess the ranges and slopes of BMD_{01}, BMD_{05}, and BMD_{10}. The modeling was carried out using polynomial model at the 0.95 confidence level. In these cases, we consider BMDs to be "Interaction Thresholds" or "Thresholds" for mixtures and their component single chemicals, respectively. The resulting three "Thresholds" (i.e., BMD_{01}, BMD_{05}, and BMD_{10}) were then plotted, using Microsoft Excel, against their respective exposure concentrations to obtain dose–response lines. We use the term "lines" because they all appear to be linear. In all the cases, the slopes of the lines can be easily discerned visually among single chemical dose–response lines vs. the corresponding chemical mixture dose–response line although precise quantitative information on slopes of the line can be obtained from the Excel files.

For *in vivo* PBPK modeling of human venous blood levels of gasoline and its components, we used 0.1× TLVs, TLVs, and 10× TLVs for benzene, toluene, ethylbenzene, *o*-xylene, *n*-hexane, and gasoline as input dosing for PBPK modeling (Table 3). The gasoline PBPK modeling was carried out as a mixture of the above marker chemicals plus a lumped chemical representing whole gasoline. In this case, the venous blood levels at the end of the exposure period under the hypothetical exposure conditions of 0.1×, 1×, and 10× TLVs become our designated "Interaction Thresholds" or "Thresholds" for gasoline and its component single chemicals. The rationale for using venous blood concentrations for "Thresholds" was primarily due to the CNS narcosis effects of the volatile organics and gasoline *per se*. The interaction, as reported earlier by Dennison et al. (2003, 2004), was from competitive enzyme inhibition among BTEX, *n*-hexane, and the lumped chemicals. Similarly as described above, we then plotted the venous blood concentrations against their respective exposure concentrations at 0.1×, 1×, and 10× TLVs for the chemical markers (i.e., benzene, toluene, ethylbenzene, *o*-xylene, *n*-hexane) and gasoline samples. For this effort, we did PBPK modeling for two types of gasoline samples.

Rationale and significance for using the terminology of "Interaction Threshold" or "Threshold". Throughout this paper, we use the terms of "Interaction Threshold" or "Threshold" for chemical mixtures or single chemicals, respectively. These terms were used in quotation marks to emphasize that their respective meanings are for this paper and under the conditions of the analyses conducted in this paper. Since BMD_{01}, BMD_{05}, and BMD_{10} were derived from the dose–response curves for cytotoxicity or folds of induction of ER-α reporter gene, they are at the lower end of the experimental range for the respective toxic/biological effects. Since in risk assessment BMD_{10} is often used as a "point of departure" for linear extrapolation to zero and for further dose–response assessment, an "Interaction Threshold" (for a chemical mixture) or "Threshold" (for a single chemical), if ever present, would likely be within or near the range of BMD_{01} to BMD_{10} on the BMD vs. dose line if these BMDs are plotted against the dose levels. Furthermore, the slope of such a plot reflects the potency of the toxic/biological activity for the chemical mixture and its component single chemicals. Thus, it is our belief that a family of such BMD vs. dose level lines from a chemical mixture and its corresponding component single chemicals, plotted together in the same graph, would offer insight on the relationship between "Interaction Threshold" of the chemical mixture and "Thresholds" of component chemicals. Similarly, 0.1×, 1×, and 10× TLVs represent threshold values for worker safety based on expert opinion following evaluation of scientific information at the time. Even though at 1× or below, single chemical exposure at such a level is supposed to be free from adverse health effects, at or near 10× TLV, minimal adverse health effect(s) may be expected. Furthermore, in the case of chemical mixtures, whether exposure at or slightly lower than the levels of TLVs (or BEIs) for individual component chemicals would offer protection has been questioned (Thomas et al., 1996; Dobrev et al., 2002). Thus, we believe that the "Interaction Threshold" and "Threshold" are within or near the ranges of the plotted internal dose metrics (CV) vs. concentration lines. Once again, the slopes of these lines should reflect the potency of the toxic/biological activity of the chemical mixture and the component chemicals.

Results

NHEK cytotoxicity from individual metals and a metal mixture

Of the numerous BMDS dose–response plots, we chose to present three plots (Figs. 1–3) from the metal mixture experiments as an illustration. Fig. 1 shows the BMDS plot mean response of NHEK cytotoxicity as a result of exposure to

Table 3
PBPK modeling of gasoline and components in humans at 0.1× TLVs, TLVs, and 10× TLVs

Entities for modeling	Modeling input exposure doses
Benzene	0.05, 0.5, 5 ppm
Toluene	5, 50, 500 ppm
Ethylbenzene	10, 100, 1000 ppm
Xylene	10, 100, 1000 ppm
Hexane	5, 50, 500 ppm
Lumped chemicals [a]	30, 300, 3000 ppm

[a] We use gasoline TLVs for this group of chemicals.

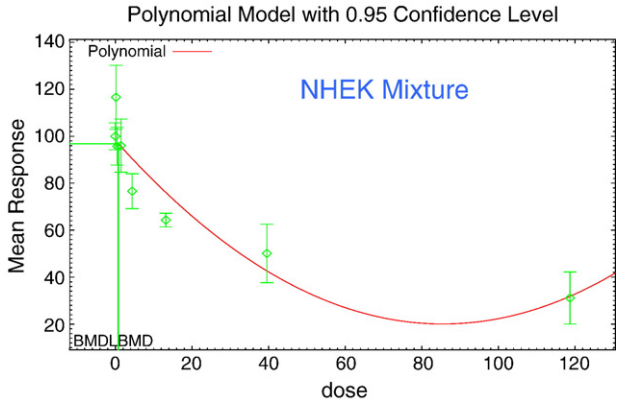

Fig. 1. The BMDS analysis (Gnuplot Graph) for NHEK cytotoxicity from the metal mixture treatment.

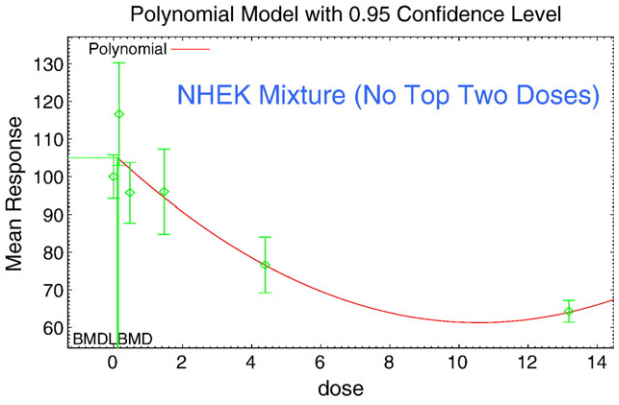

Fig. 3. The BMDS analysis (Gnuplot Graph) for NHEK cytotoxicity from the metal mixture treatment excluding the data from the two highest mixture concentrations.

the metal mixture. The dose–response curve is U-shaped because NHEK cytotoxic interaction from the metal mixture is dose-dependent with the highest concentrations causing antagonistic interactive cytotoxicity (Bae et al., 2001; Gennings et al., 2002). To explore the dose–response relationship without the highest concentrations, we present Figs. 2 and 3 where the top one or top two doses were eliminated from analyses, respectively. Since the BMDs obtained from these three BMDS analyses were different, we created the respective plots of BMD_{01}, BMD_{05}, and BMD_{10} against their respective exposure concentrations to demonstrate the resulting linear relationship for the metal mixtures in relation to its individual component metals (Figs. 4–6). It should be noted that, in order to keep the line for Pb in the same graph, we transformed the BMDs for Pb to log BMD values for plotting in Figs. 4–6. Two important observations can be made with the data presented in Figs. 4–6. First, when the regression lines and slopes of the respective BMDs were compared between the mixture and its respective component single chemicals, our analyses revealed that the mixture "Interaction Thresholds" appear to stay within the bounds of the "Thresholds" of its respective component single chemicals. Second, the foregoing relationship does not seem to hold true anymore (Fig. 6) as certain data points (e.g., the highest one or two concentrations from the mixture and the single component chemicals) were eliminated from the analysis.

Estrogen receptor alpha reporter gene assay

The results of our BMDS analyses on the ER-α reporter gene assays published by Gennings et al. (2004) are summarized in Fig. 7. Similar to the results obtained with the NHEK cytotoxicity studies, the "Interaction Threshold" of the mixture of endocrine active compounds is within bounds of the "Thresholds" of its components chemicals.

PBPK modeling of gasoline and its component chemical markers in humans

Fig. 8 shows that the "Thresholds" of gasoline component chemical markers cover a wide range from baseline near *x*-axis to all the way near *y*-axis. It is not surprising, therefore, to see that the "Interaction Thresholds" for the gasoline and the

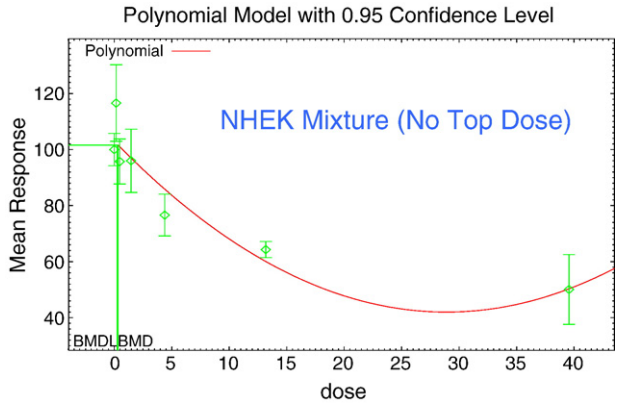

Fig. 2. The BMDS analysis (Gnuplot Graph) for NHEK cytotoxicity from the metal mixture treatment excluding the data from the highest mixture concentration.

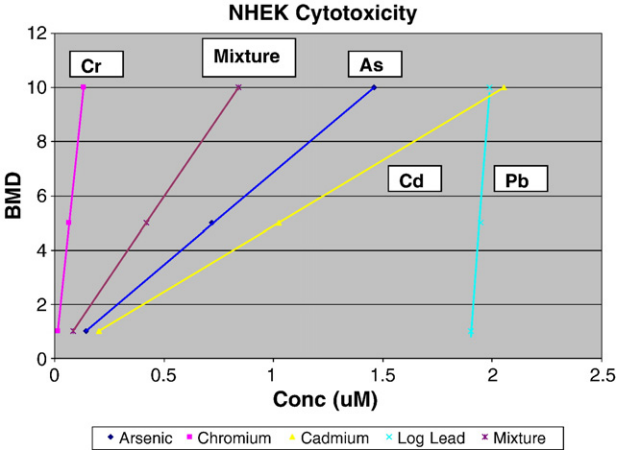

Fig. 4. Linear relationship between BMD_{01}, BMD_{05}, and BMD_{10} and their respective exposure concentrations from metals and the metal mixture in NHEK cytotoxicity studies.

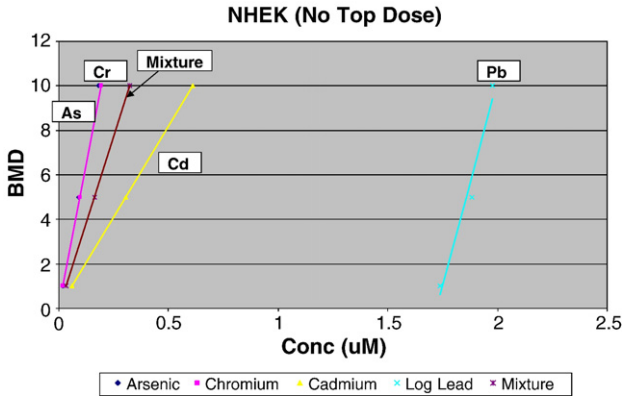

Fig. 5. Linear relationship between BMD_{01}, BMD_{05}, and BMD_{10} and their respective exposure concentrations from metals and the metal mixture in NHEK cytotoxicity studies (excluding the data from the highest concentration).

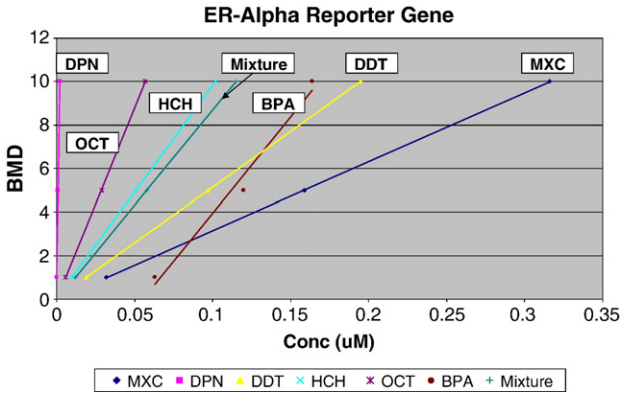

Fig. 7. Linear relationship between BMD_{01}, BMD_{05}, and BMD_{10} and their respective exposure concentrations from endocrine active chemicals and the mixture in estrogen receptor alpha reporter gene assays.

lumped "non-target" chemicals fall within the bounds of the "Thresholds" of the individual component chemical markers.

Discussion

This work represents a first attempt to explore the relationship between "Thresholds" of toxicity for individual chemicals and "Interaction Thresholds" for chemical mixtures and the SOT Expert Panel provided the impetus for this endeavor. The approach we took is different from the treatment of the subject related to NOAELs/LOAELs of mixtures and corresponding single chemicals (Groten et al., 1997). It is interesting to note that despite the three entirely different types of studies (i.e., cytotoxicities, ER-α reporter gene assay, and PBPK modeling in humans), initial analyses of three sets of data appear to provide similar results.

"Thresholds" and "Interaction Thresholds" for chemicals and chemical mixtures

Two aspects warrant further discussion here. First, a range of BMDs (i.e., BMD_{01}, BMD_{05}, and BMD_{10}) or TLV-derived tissue dose metrics (CV from 0.1×, 1×, and 10× TLV) were considered in this paper for "Thresholds" of single chemicals and their corresponding chemical mixtures. This was for the purpose of exploring the relationship between BMDs or TLV-derived CVs with different doses. As it turned out, linear relationship appears to be the rule. Another purpose to choose multiple BMDs or TLV-derived CVs was to provide flexibility. Thus, researchers may use their own judgment as to what constitute a "Threshold" for either a single chemical or a chemical mixture for the specific toxic endpoint studied. We believe that the true thresholds for the single chemicals and the true interaction threshold for the chemical mixture must be on the corresponding BMD (or CV) vs. dose level lines. Furthermore, the slopes of such lines which reflect potency for toxic/biological activities must also be taken into considera-

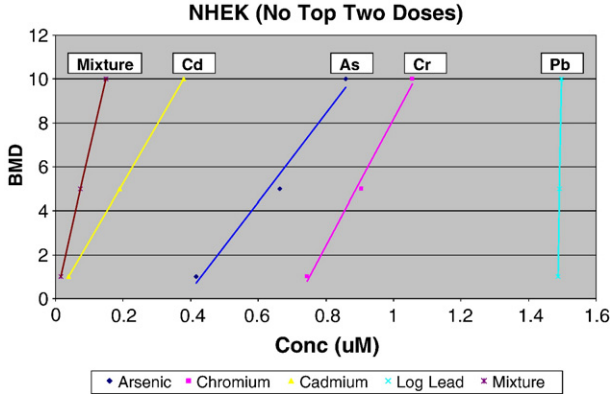

Fig. 6. Linear relationship between BMD_{01}, BMD_{05}, and BMD_{10} and their respective exposure concentrations from metals and the metal mixture in NHEK cytotoxicity studies (excluding the data from the two highest concentrations).

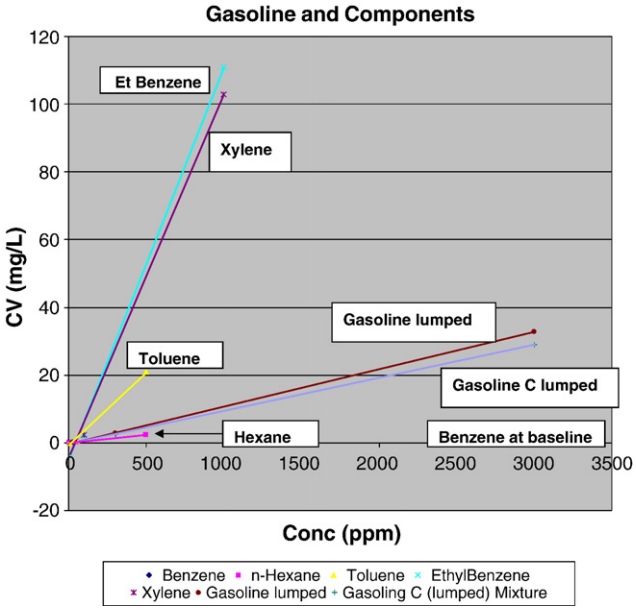

Fig. 8. Linear relationship between BMD_{01}, BMD_{05}, and BMD_{10} and their respective exposure concentrations from gasoline samples and the component chemical markers in PBPK modeling studies. "Gasoline lumped" and "Gasoline C lumped" refer to a gasoline sample and the lumped "non-target" chemicals other than BTEX and *n*-hexane.

tion in assessing the potential to cause adverse health effects by a chemical mixture in comparison to its components single chemicals.

The second aspect is the consideration of BMDs or TLV-derived CVs for "Interaction Thresholds" for chemical mixtures. Implicit in such an approach is the suggestion that interactive toxicities indeed occur. In all three examples presented in this paper, there were evidence for interactive toxicities deviating from additivity under certain experimental conditions. As to whether or not these toxicological interactions will continue to present at the very low dose region, no one really knows for sure. However, at least in the first example, "hormesis" was observed at very low dose levels albeit for a different endpoint all together. As indicated earlier, we do believe that the true interaction threshold is somewhere on the BMD (or CV) vs. dose level lines.

Emerging trend of the relationship between "Thresholds" of toxicity for individual chemicals and "Interaction Thresholds" for chemical mixtures

A trend appears to be emerging where the "Interaction Thresholds" of chemical mixtures appear to be within the bounds of the "Thresholds" of their respective component chemicals in most of our analyses. Furthermore, the slopes of the BMD (or TLV-derived CV) vs. dose levels for chemical mixtures were also within the bounds of those for the corresponding component single chemicals. Thus, our preliminary conclusion would seem to reject the original hypothesis by the Expert Panel of SOT "Apparent dose thresholds for interactions are higher than individual chemical thresholds." However, we would like to offer a word of caution in that the conclusion reached in this paper should be considered tentative. We would like to emphasize that: (1) our analyses are based on limited data sets; (2) as indicated in Figs. 4–6, when certain data points are eliminated from analyses, the relationship between "Interaction Thresholds" of the chemical mixture and "Thresholds" of its component chemicals changes; (3) it is important to consider and define the "Interaction Thresholds" and "Thresholds" carefully; (4) we should always consider the *TREND* and *SLOPE* in this type of analyses; and (5) further analyses using more data sets are needed before a definitive conclusion can be drawn.

As a final remark, we would like to urge that scientists in the toxicology community try their best to share their experimental data, as well as mathematical and statistical model structure and code in sufficient details such that other investigators may either repeat their work or attempt new analyses for the common good. In this regard, we would like to recognize role models such as Melvin Andersen and Harvey Clewell for sharing PBPK models and knowledge, USEPA, with Jeff Gift as the lead scientist, for sharing BMDS, Frederic Bois and colleagues for sharing MCSim software, and undoubtedly others.

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