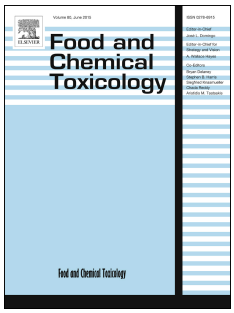

Setting safer exposure limits for toxic substance combinations

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SETTING SAFER EXPOSURE LIMITS FOR TOXIC SUBSTANCE COMBINATIONS

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Keywords:

Exposure Limits

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Synergistic Effects

Cumulative Risk Assessment

Toxic Stimuli

Mixtures

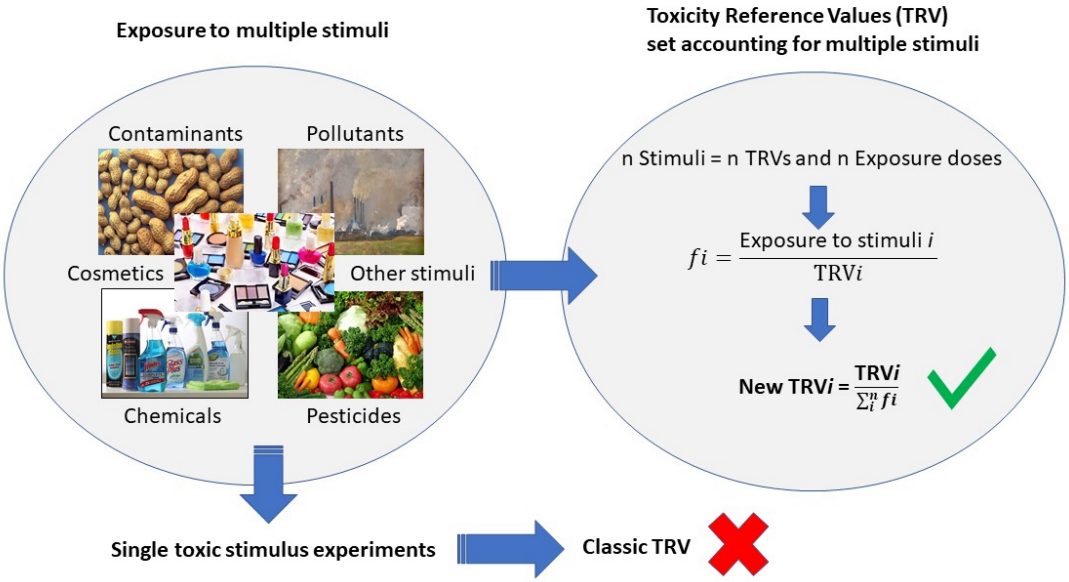
New approach

Toxicity Reference Values

Abstract

Toxic stimuli (stressors) exposure limits are typically based on single toxic stimuli experiments but are presently used for both toxic stimuli in isolation and in combination with other toxic stimuli (simultaneous co-exposure or exposures separated in time). In the combination case, typically less of each constituent of the combination is required to cause damage compared to the amount determined from single stressor experiments. Thus, exposure limits based on single toxic stimulus experiments are inadequate for setting limits for stressor combinations.

This article presents a recommended simplified approach to improving regulatory exposure limits for toxic stimuli combinations, and a more expansive and expensive alternative of the recommended simplified approach. The recommended approach will partially compensate for the enhanced adverse effects of toxic stimuli combinations relative to adverse effects of toxic stimuli in isolation. The approach covers myriad categories of toxic stimuli reflective of real-life exposures due to lifestyle, iatrogenic, biotoxin, occupational/environmental, and psychosocial/socioeconomic conditions. The proposed approach 1) assumes that all potential toxic stimuli to which an individual might be exposed have the same mechanisms/modes of action on biological mechanisms, and are, thus, indistinguishable by the impacted organism; 2) normalizes the myriad stimuli by converting the doses of toxic stimuli exposures to the respective toxicity reference values (TRV) fractions; 3) sum all the TRVs fractions from these toxic stimuli exposures; and 4) divides all the single substance TRVs by the sum of fractions. While it is an additive approach conceptually, it differs from other additive approaches in the breadth of its inter-category coverage, in order to reflect true inter-category real-life simulation. The newly posited approach does not account for hormetic, antagonistic, or synergistic effects of toxic stimuli in combination. It does not adjust for 1) low-dose toxicants with adverse effects that have been under-reported, or 2) exposure limits like the Occupational Safety and Health Administration - Permissible Exposure Limits (OSHA PELs) that are orders of magnitude above levels shown by published single toxic stimuli studies to have caused adverse effects. Practical considerations for the application of this approach are presented.


Exposure to multiple stimuli
Contaminants
Pollutants
Cosmetics
Other stimuli
Chemicals
Pesticides
Toxicity Reference Values (TRV)
set accounting for multiple stimuli
n Stimuli = n TRVs and n Exposure doses
$fi = \frac{\text{Exposure to stimuli } i}{\text{TRV}i}$
New TRVi = $\frac{\text{TRV}i}{\sum_i^n fi}$
Single toxic stimulus experiments
Classic TRV

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Highlights

- Presumable “safe” exposure limits based on single toxic stimulus experiments are inadequate for stressors’ combinations.
- A simplified approach to improving regulatory exposure limits for toxic stimuli combinations is presented.
- The approach proposes conversion of exposures to TRVs fraction and division of single TRVs by the sum of these fractions.
- Practical considerations for the application of this approach are presented.

Abbreviations

AD	Alzheimer's Disease
ADI	Acceptable daily intake
ATSDR	Agency for Toxic Substances and Disease Registry
CIT	Citrin
CKD	Chronic Kidney Disease
EMF	Electromagnetic field
EPA	Environmental Protection Agency
MF	Magnetic fields
MRLs	Minimal Risk Levels
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
OTA	Ochratoxin A
PAD	Peripheral Arterial Disease
PEL	Permissible Exposure Limits
PN	Peripheral Neuropathy
REL	Recommended Exposure Limits
RFC	Inhalation Reference Concentration
RFD	Oral Reference Dose
TRV	Toxicity reference values

Abstract

Toxic stimuli (stressors) exposure limits are typically based on single toxic stimuli experiments but are presently used for both toxic stimuli in isolation and in combination with other toxic stimuli (simultaneous co-exposure or exposures separated in time). In the combination case, typically less of each constituent of the combination is required to cause damage compared to the amount determined from single stressor experiments. Thus, exposure limits based on single toxic stimulus experiments are inadequate for setting limits for stressor combinations.

This article presents a recommended simplified approach to improving regulatory exposure limits for toxic stimuli combinations, and a more expansive and expensive alternative of the recommended simplified approach. The recommended approach will partially compensate for the enhanced adverse effects of toxic stimuli combinations relative to adverse effects of toxic stimuli in isolation. The approach covers myriad categories of toxic stimuli reflective of real-life exposures due to lifestyle, iatrogenic, biotoxin, occupational/environmental, and psychosocial/socioeconomic conditions. The proposed approach 1) assumes that all potential toxic stimuli to which an individual might be exposed have the same mechanisms/modes of action on biological mechanisms, and are, thus, indistinguishable by the impacted organism; 2) normalizes the myriad stimuli by converting the doses of toxic stimuli exposures to the respective toxicity reference values (TRV) fractions; 3) sum all the TRVs fractions from these toxic stimuli exposures; and 4) divides all the single substance TRVs by the sum of fractions. While it is an additive approach conceptually, it differs from other additive approaches in the breadth of its inter-category coverage, in order to reflect true inter-category real-life simulation. The newly posited approach does not account for hormetic, antagonistic, or synergistic effects of toxic stimuli in combination. It does not adjust for 1) low-dose toxicants with adverse effects that have been under-reported, or 2) exposure limits like the Occupational Safety and Health Administration - Permissible Exposure Limits (OSHA PELs) that are orders of magnitude above levels shown by published single toxic stimuli studies to have caused adverse effects. Practical considerations for the application of this approach are presented.

1. Introduction

1A. Overview

Myriad of regulatory agencies throughout the world have responsibility for protecting their constituents from the adverse effects of potential toxic stimuli (in this article, these stressors will be referred to as ***toxic stimuli***, since they can cover more than the ***toxic substances*** usually mentioned in the toxicology literature). The data sources used by these agencies to determine toxic stimuli exposure safety have been of two main types: laboratory experiments (mainly on animals) and epidemiology studies (mainly on humans). By far, the dominant source for safety determination of potentially toxic stimuli has been single stressor studies, mainly in laboratory animals (Hernandez and Tsatsakis, 2017; Maffini and Neltner, 2015; Sexton, 2012; Tsatsakis et al., 2016).

While these single toxic stimuli studies are designed to elucidate mechanisms that will allow credible attribution of adverse effects to the toxic stimuli being tested, they are poor simulations of real-

life human experience (Agathokleous et al. 2019; Hernández et al., 2020; Tsatsakis et al., 2017). Humans 1) are exposed to multiple disparate categories of myriad toxic stimuli in parallel and/or over time and 2) do not live in the typical pristine caged animal laboratory environment of no sun, no wind, no rain, no temperature variations, no enrichment, etc. These multiple multi-category human toxic stimuli exposures can result in combined effects that are additive, synergistic, potentiative, or antagonistic.

As will be shown later, much research has been published on accounting for increased toxicity of toxic stimuli combinations, especially on relating mixture toxicity effects to the constituent single stressor characteristics and/or toxicity effects. Most of these combination toxicity studies focus on chemicals and tend to focus further on narrow sub-categories (e.g. metals, pesticides, solvents, etc). They can be viewed mainly as intra-category. They show reasonable agreement with the mechanistic theories of combination effects. However. the voluminous amounts of individual constituent data and constituent interaction data required to incorporate inter-category interaction effects accurately exclude these intra-category approaches from applying to the real-life situation of accounting for inter-category (e.g. chemicals, materials, radiations, noise, etc.) toxic stimuli combination effects to influence regulatory exposure limits.

Typically, the effects of toxic stimuli combinations are not taken into account in setting regulatory exposure limits. This results in regulatory exposure limits that are potentially orders-of-magnitude greater than those exposures shown in the biomedical literature to have caused damage (Docea et al., 2018; Fountoucidou et al., 2019; Tsatsakis et al., 2019a, b, c). The current study presents a recommended simple approach for modifying regulatory exposure limits to partially compensate for effects resulting from the full spectrum of inter-category toxic stimuli combinations, as well as a more expensive and detailed alternative approach.

1B. Single Toxic Stimuli Studies

The data evaluation component of a 2018 monograph addressing the viability of OSHA's Permissible Exposure Limits (Kostoff, 2018) showed that the overwhelming majority of the studies used by regulatory agencies (mainly in USA) for determining harmful exposure levels were focused on a single toxic stimulus. These included studies for determining the:

a) Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs) (OSHA, 1970; 1988a, 1988b, 2020a),

b) National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Limits (RELs) (OSHA, 2020b; NIOSH, 2003a; 2003b; 2003c),

c. American Conference of Government Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) (OSHA, 2020c; ACHIH, 2001a; 2001b; 2013) and Biological Exposure Indices (BEIs) (OSHA, 2020c, ACGIH, 2006a; 2006b),

d. Environmental Protection Agency (EPA) Inhalation Reference Concentration (RFC) (EPA, 1994; Dorman, 2008; EPA, 1987; EPA, 2003; EPA, 2003b; EPA, 2010) and Oral Reference Dose (RFD) (EPA, 1993; 1994; 2005),

e. Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs) (ATSDR, 2018; 2019; 2019b), and

f. German Ad-hoc Working Group on Indoor Guidelines of the Indoor Air Hygiene Committee Health Precaution Guide Value (RWI) and Health Hazard Guide Value (RWII) (IAHC-SSHA, 2013; 2013b; 2013c; 2014; 2015).

The 2018 monograph also showed that, for the sample of PELs examined, the PELs ranged from one-to-four orders-of-magnitude above exposures shown to cause adverse health effects in the biomedical literature.

1C. Toxic Stimuli Combination Studies

Numerous biomedical studies have shown that combinations of toxic stimuli can enhance the adverse effects of any one of their constituents (relative to its effects when acting in isolation) (Hernandez, 2013; Vardavas, 2016; Docea, 2017; Kalogeraki, 2017; Kostoff, 2017; Kostoff, 2018b). Section B of the Bibliography in Kostoff monography (Kostoff, 2018b) provides a comprehensive listing of articles focused on effects from combinations of toxic stimuli. These toxic stimuli are not limited to chemicals, but encompass multiple categories including radiation (ionizing and non-ionizing; visible and non-visible), heat, force, sound, biotoxins, iatrogenic, lifestyle choices, socioeconomic factors, physical and emotional stress, just to name a few. Only few combinations of potentially toxic stimuli decrease the adverse effects of any constituent; some of these may occur at low toxic stimuli doses to produce hormetic responses.

In the case of disease contributions, toxic stimuli combinations typically allow less of each component to cause damage compared to the levels obtained when examining the toxicity of each component in isolation (single toxic stimulus experiment, for assessing damage from the potentially toxic stimulus), as the following three examples show. A study entitled "Synergistic toxicity produced by mixtures of biocompatible gold nanoparticles and widely used surfactants" (Ginzburg, 2018) showed that these mixtures produced synergistic toxicity at concentrations where the individual components were benign. A second study entitled "Synergistic action of the nephrotoxic mycotoxins ochratoxin A and citrinin at nanomolar concentrations in human proximal tubule-derived cells" (Schulz, 2018) showed that only concurrent (but not individual) exposure to ochratoxin A (OTA) and citrin (CIT) at nanomolar concentrations led to (i) an increase of TNF protein and mRNA, (ii) a decrease of COX-2 protein and mRNA, (iii) a decrease of E-cadherin protein and (iv) an increase of vimentin and alpha-SMA protein. Finally, a third study entitled "DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 Mt magnetic fields (Static Or 50 Hz)" (Zmyslony, 2000) showed that lymphocyte exposure to magnetic fields (MF) at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 mug/ml FeCl2 did not produce a detectable damage of DNA either. However, when the FeCl2-incubated lymphocytes were simultaneously exposed to 7 mT MF the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF.

1D. Impact of toxic stimuli combinations on setting safe exposure limits

Thus, when setting safe exposure limits for a constituent of a given combination based on results from experiments involving that constituent in isolation (single toxic stimulus), and considering uncertainty factors only for inter-and intra-species differences, the exposure limit values will be substantially higher than those at which the constituent might cause damage when used in combination. Stated differently for the case of a single pathway, "when multiple components in a sample affect the

same pathway, their combined toxicity can usually be described by the concentration addition concept and may induce significant toxicity to aquatic organisms" (Gustavsson, 2017). It should be emphasized this quoted statement holds strictly when it is known that the same pathway is affected by the multiple constituents. The same concept could also be applicable to the case of different pathways being affected.

Several approaches have been proposed to assess the effects of multiple toxic stimuli (EFSA, 2019; Sarigiannis, 2012, Goumenou, 2019). A survey of the history of methods for "evaluation of cumulative health risks from the combined effects of multiple environmental stressors" (Sexton, 2012) provides some idea of the progress made towards this goal until 2012. Section A of the Bibliography in Kostoff monography (Kostoff, 2018b) provides a comprehensive listing of articles focused on the general topic of Cumulative Risk Assessment to date. A number of mixture assessment methodologies relevant to the approach proposed/recommended in this article, with special focus on additivity approaches are available (Goumenou and Tsatsakis, 2019; Lambert and Dawson, 2019; Lasch et al., 2020; Sarigiannis, 2012).

The more important question is how can these demonstrated enhanced adverse effects (resulting from combinations of toxic stimuli) be exploited to improve the regulatory process for setting exposure limits? Given the numbers of potentially toxic stimuli in the environment, in occupational settings, and in daily life, the numbers of potential toxic stimuli combinations are essentially infinite (Kostoff, 2018b). The dose levels of each combination constituent capable of causing damage can vary depending on the numbers, types, timing, and dosages of other constituents of the combination (Kostoff, 2018b).

Thus, there are effectively an infinite number of dose levels of any constituent that could be used as a threshold for initiating damage, since there are essentially an infinite number of combinations of toxic stimuli. This reality is orthogonal to the present exposure limit regulatory process, where each constituent is given one exposure limit (for each of two or three pre-specified exposure durations) based on single toxic stimulus experiments, and this exposure limit is expected to be relevant under all conditions.

This article presents a recommended regulatory-enhancing approach that is: 1) credible; 2) exact for the limiting case when the myriad toxic stimuli are indistinguishable by the organism; 3) relatively simple to apply to all toxic stimuli; and, 4) capable of adjusting regulatory exposure limits to partially compensate for the added damage of toxic stimuli combinations.

2. Methods, Results, and Discussion

2A. Intrinsic Flaw in Regulatory Process

Single toxic stimulus experiments used as a basis for setting regulatory exposure limits have an intrinsic flaw not emphasized sufficiently by researchers and regulators alike. For illustrative purposes, consider the following simple hypothetical examples. Assume we have a country of five million people.

Exemple 1. Assume: 1) we divide its population into five equal groups of one million people per group; 2) we have five different toxic chemicals; 3) each group is exposed to one chemical only, at the

TRV dose for that chemical; 4) all five TRVs are identical. By TRV definition, there should be no adverse effects among any of the five million people from exposure to these five chemicals.

Exemple 2. Assume: 1) we re-divide the same population into two groups, where Group A has four million people and Group B has one million people; 2) Group A is not exposed to any of the five chemicals, and Group B is exposed to all the five chemicals, again at the TRV dose level for each chemical; 3) each of the five chemicals exerts identical pathological effects through the same mode of action. As a result, each of the one million members of Group B will be exposed to an effective dose of 5xTRV. This means that one million people will (potentially) experience adverse effects, since the TRV dose level has been exceeded. The four million people in Group A will experience no adverse effects, since they have not been exposed to these chemicals.

Thus, the total amount of the five chemicals used has remained the same in both cases, but the number of people experiencing adverse effects has increased from zero to (potentially) one million because the chemicals were used in combination (Figure 1). These adverse effects from the combination would not have been predicted from the single toxic stimuli tests, since the single toxic stimuli tests for each constituent showed no adverse effects at the TRV level. This limiting case (where the chemicals are effectively the same from the perspective of the test subjects) shows the necessity of testing potentially toxic stimuli in combinations as a basis for setting regulations on exposure limits. There are further potential benefits from the hypothetical limiting case assumption that the toxic stimuli in a combination are indistinguishable from the perspective of the organism. This concept is known as hormesis and it has been extensively reviewed elsewhere (Agathokleous, E, 2019; Agathokleous, E., 2019a; Agathokleous, E., 2019b.; Agathokleous, E., 2019c; Calabrese, E.J., 2019).

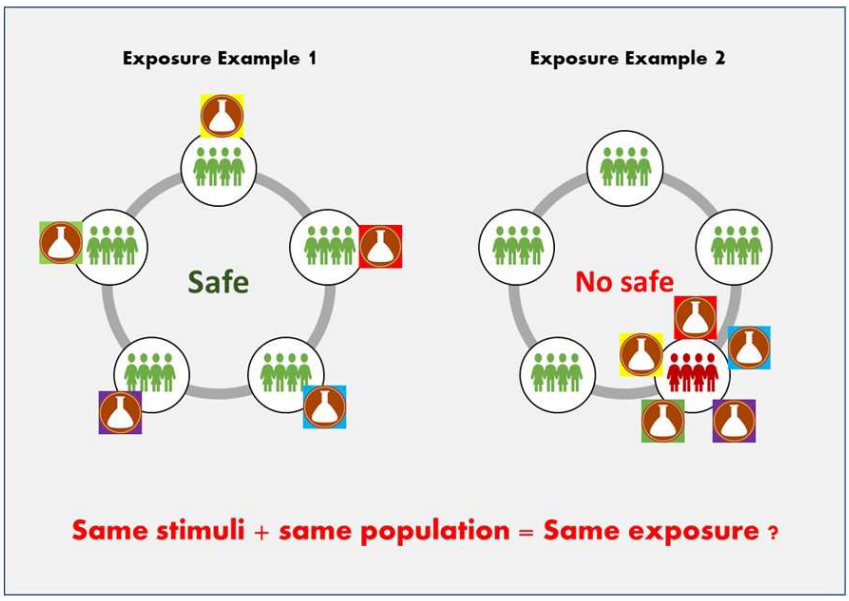

Figure 1. Different exposure scenarios for the same amount of chemicals in the same population

2B. Role of combinations in correlation-causation nexus

Many epidemiological studies attempt to extract correlations from data to serve as a starting point for showing causation. A study may show that substance X increased at a given rate over some period of time, and disease Y increased at the same rate over the same period of time. Therefore, the correlation between substance X and disease Y could serve as a starting point for identifying the mechanisms by which substance X could contribute to disease Y.

However, “absence of evidence is not evidence of absence”! As the hypothetical example in Section 2A above shows, the increase in adverse effects between Case 1 and Case 2 was not correlated with any change in the amount of each of the five toxic constituents or the total amount of the five constituents in the whole polulation. The lack of correlation did not exclude causation. One needs to ensure that the appropriate variables, or, in the present case, combinations of variables, are selected for evaluating correlation. Trends in combinations of potential toxic stimuli need to be considered when evaluating a correlation-causation nexus.

For example, consider the study by Nevison (Nevison, 2014) which aimed to identify potential autism environmental contributing factors by correlating those contributing factors whose temporal increases parallel the observed temporal increases in autism. The author started with "a list of the top ten environmental compounds suspected of causing autism and learning disabilities". She found that "Most of the suspected environmental toxins examined have flat or decreasing temporal trends that correlate poorly to the rise in autism. Some, including lead, organochlorine pesticides and vehicular emissions, have strongly decreasing trends. Among the suspected toxins surveyed, polybrominated diphenyl ethers, aluminum adjuvants, and the herbicide glyphosate have increasing trends that correlate positively to the rise in autism." She did not look at the trends of toxic stimuli combinations within the ten (including those toxicants whose trends were flat or decreased over time), but rather concentrated on trends of specific toxic stimuli. There remains the possibility that the toxic stimuli combinations could potentially account for some/much of the increase in autism.

2C. Numerical Difficulties in Setting Exposure Limits based on each Toxic Stimuli combination

In the USA, there are about 85,000 chemicals in commerce registered with the US Environmental Protection Agency (EPA) through the Toxic Substances Control Act inventory (EPA, 2019). An analysis of the PUBMED MeSH Tree showed the existence of c.a. 4,000 described diseases (Kostoff, 2015). Thus, there are about 340,000,000 potential one-to-one chemical-disease combinations that would have to be examined to assess which of the chemicals are contributing factors to which diseases. Most of these 340,000,000 potential chemical-disease combinations have not been tested for chemicals applied in isolation (the contribution of one chemical to one disease), much less so in combinations reflective of real-life exposures (Kostoff, 2015; 2018b).

In the USA, federal legally enforceable occupational exposure limit regulations are governed by the Occupational Safety and Health Administration (OSHA). Of the c.a. 85,000 chemicals referenced above, perhaps c.a. 500 are regulated by OSHA through their issuance of Permissible Exposure Limits (PELs). The 2018 monograph of Kostoff (Kostoff, 2018a) shows that, based on sampling ten of these c.a. 500 OSHA 'regulated' chemicals, the OSHA PELs are typically one-to-four orders-of-magnitude higher than exposures shown in the biomedical literature to cause biological damage/adverse health effects.

Section 1B above and companion studies show that most of the biomedical literature-based exposures on which the OSHA PEL regulations (as well as other Federal Agency regulations and guidelines) depend are exposures in isolation (one chemical used in the experiment) (Kostoff, 2015; 2018b). When exposures to these chemicals are combined as in real-life, synergistic effects that magnify their damage are often the result. Thus, the one-to-four orders-of-magnitude gap between OSHA PELs and biomedical literature findings mentioned in the previous paragraph would be increased when toxic stimuli are combined. In many respects, ***the USA workplace is effectively unregulated for chemical exposures***, given the orders-of-magnitude difference between 1) the regulated OSHA PEL exposure limits, and 2) the levels of exposures shown in the biomedical literature to cause damage.

Furthermore, a 2016 paper (Kostoff, 2016) provided examples of several studies reported in the biomedical literature that may have been manipulated to underestimate the severity of the results. The topics of these studies tended to have significant political/commercial/military sensitivity. Because of the difficulty in obtaining these types of data, it is unknown how widespread this phenomenon of data manipulation is in the biomedical literature. Underestimation of the severity of the results in the reported biomedical literature would increase further the gap between Federal exposure limit regulations and exposures that cause damage in the real world.

In the eBook entitled Pervasive Causes of Disease (Kostoff, 2015), c.a. 8,000 factors (chemicals, radiations, drugs, biotoxins, etc) that contribute to c.a. 4,000 diseases were identified. These c.a. 8,000 contributing factors were obtained from analyses of the existing biomedical literature and should be viewed as a very low floor. Only a miniscule fraction of the potential 340,000,000 chemical-disease pairs mentioned at the beginning of Section 2B have been tested (much less the other non-chemical toxicant-disease pairs) and published in the biomedical literature. About 800 of these c.a. 8,000 potential disease contributing factors (e.g., smoking, pesticides, high-fat diets, heavy metals, advanced glycation end products, etc) were judged to be 'pervasive' (Kostoff, 2015), i.e., they contributed to at least a threshold number (many tens) of diseases.

How many of these potential disease contributing factors might contribute to a specific disease? In the past five years, the first author has identified potential contributing factors (existing and discovered) to three major chronic diseases (Chronic Kidney Disease (CKD) (Kostoff, 2015b), Alzheimer's Disease (AD) (Kostoff, 2018d), and Peripheral Neuropathy (PN)/Peripheral Arterial Disease (PAD) (Kostoff, 2019) as part of protocol development to prevent and reverse these diseases. For CKD, AD, and PN/PAD, on the order of c.a. 500-800 potential contributing factors were identified for each disease. Again, this number should be viewed as a floor, given 1) the limitations on full inclusion of contributing factors that have been reported in the literature for these three chronic diseases, and 2) the text mining limitations/difficulties on extracting potential contributing factors from these text databases. Many of these potential contributing factors were pervasive based on the eBook findings, but many were not.

Many potential contributing factors (pervasive or not) to any given disease did not surface in the eBook because 1) the research had not been done on the linkage between the potential toxic stimulus and the disease, or 2) the research had been done but not reported, or 3) the research had been reported improperly. These research/reporting deficiencies resulted in severe limitations on the total number of potential contributing factors to disease reported, and the number reported that could pass the numerical threshold of pervasiveness.

The reversal protocol for these three chronic diseases requires that their major contributing factors be eliminated before reversal is even remotely possible. Therefore, minimizing toxic stimuli exposures becomes central to healing. Minimizing these exposures can be accomplished in at least two ways: avoidance and regulation. Toxicant exposures can (in theory) be avoided, although this may not be realistically possible in many cases. Additionally, government can regulate exposure ceilings, to minimize those exposures that cannot (in practice) be avoided. If government regulations are inadequate, then the likelihood of developing chronic diseases will be increased and the likelihood of reversing (or preventing) these diseases will be decreased.

2D. Proposed Regulatory Modification to Partially Account for Toxic Stimuli Combinations

The end goal of this article is to propose a revision to existing regulatory exposure limits for potentially toxic stimuli to account for combinations. Such a revision would have to be credible, simple, relatively inexpensive to implement, and not overly harsh or restrictive, or it would have no chance of implementation.

Two concept options will be proposed: a bottom-up approach and a top-down approach. Most of the narrative will concentrate on the preferred top-down approach.

2D1. Bottom-Up Approach

2D1a. All Binary Combinations from Most Common Toxic Stimuli

The main existing approach used for setting regulatory exposure limits is based on laboratory results from single toxic stimuli animal experiments. The obvious first step in incorporating mixture effects would be to run the simplest mixture experiments based on the most widely used toxic stimuli, and generate regulatory exposure limits based on those mixture results. How would this be accomplished in practice?

There are about 85,000 chemicals registered with the EPA, as stated previously. Assume there are at least 15,000 non-chemical potentially toxic stimuli, to give a total number of c.a. 100,000 potentially toxic stimuli to consider for mixture and regulatory purposes. The number of potential combinations of r items in a pool of n items is given by the Binomial Coefficient $C(n,r)=n!/(r!*(n-r)!)$, where [!] denotes the factorial function, n is the total number of items, and r is the number of items in each combination. For large n, and $r/n \ll 1$, $C(n,r)c.a. = n^r/r!$

Including binary interactions among all these c.a. 100,000 potentially toxic stimuli would require $10^{10}/2$ mixture experiments, plus 10^5 single toxic stimulus experiments to generate a baseline for comparison. This is orders-of-magnitude too many experiments to be practical; a sub-set must be selected. One possibility is to start with the present set of c.a. 500 OSHA (mainly) chemicals that are regulated for occupational environments. Perform a sampling of the population and identify the top c.a. 1,000 toxic stimuli to which people are exposed. Identify the c.a. 500 most common non-OSHA-regulated substances in that sampling, add that to the c.a. 500 substances presently regulated by OSHA, to arrive at a total of c.a. 1,000 toxic stimuli to include in the regulatory pool. Approximately 500,000 binary mixture experiments would be required to cover the pool, plus 1,000 single toxic stimulus experiments to serve as the baseline (if mixtures of three constituents were desired, then c.a. 167,000,000 additional experiments would be required). Obviously, myriad selection procedures are possible to arrive at the number of single toxic stimuli and their binary combinations to be tested.

How would specific regulation targets be set from the results of these binary experiments, since the doses of each constituent of the binary combination would be inter-dependent in establishing toxicity? For each binary experiment, one constituent would serve as the primary, and the other as the secondary, to minimize the parametric variation range. The secondary constituent's dose would be set at ½ its single stressor TRV, and the primary constituent's dose would be varied until adverse effects for the mixture are shown. By the end of the experimental process, for each constituent in its primary role, there would be 1,000 dose values at which toxicity begins to be observed. A conservative selection might take the median of the highest quartile as a regulatory exposure limit for the primary constituent. A harsher restriction might take the median of the lowest quartile as a regulatory exposure limit for the primary constituent.

2D1b. Most Common Binary Combinations from Most Common Toxic Stimuli

While the bottom-up approach in 2D1a offers a reasonable compromise between numbers of toxic stimuli regulated and numbers of combinations examined, it does require an expansive and expensive testing program. For example, binary combination laboratory studies in which EMF is one constituent are estimated to range in cost from c.a. one million dollars to multiples of that (private conversations with EMF study managers). And, these costs are for one test species, typically one strain. As one EMF binary combination study using two different strains of Sprague-Dawley rats showed (Fedrowitz, 2004), the presence of adverse effects from the combination depended on the strain of rat selected. One strain showed adverse effects, the other did not! Which strain would be more representative of the human population? Would the results have been different for species other than rats, and for strains/sub-sets within those other species? For optimal human simulation from non-human experiments, should multiple species/strains be tested for each toxic stimulus combination?

Different categories of substance combinations would present different levels of complexity and cost, and these costs would also vary by where and by whom the experiments were conducted. Assuming assembly-line testing of binary combinations, and an average cost of one million dollars per experiment with one species, the above scenario would cost about $500 billion!

A scaled-down approach that focused on the most common combinations could reduce costs considerably, albeit with further reduced coverage. Under this scenario, a representative population sampling would be conducted to identify the most common toxic stimuli and the most common toxic stimuli combinations (Kapraun et al, 2017). Assume the same c.a. 1,000 toxic stimuli as in 2D1a are selected, and the most common c.a. 5,000 binary combinations of these toxic stimuli are identified. A constraint might be added such that each of the 1,000 toxic stimuli is present in at least one of the c.a. 5,000 combinations, to insure full-spectrum representation. Then, the number of binary experiments would be reduced by c.a. two orders-of-magnitude from the number suggested in 2D1a, and the costs reduced commensurately. The same procedures as in 2D1a would be used to arrive at regulatory exposure limits. Again, this is a compromise between cost/practicality of degree of coverage.

A key deficiency is that combinations of greater than two toxic stimuli are not included in either of the above proposals. For the approach of 2D1b, this could be corrected in part from the analysis of the population sampling results. The most common combinations of unrestricted size could be identified by pattern analysis, and these combinations used for testing/regulatory setting purposes. The downside of this approach is that the most prevalent toxic stimuli might dominate the combinations, and lesser

but still important toxic stimuli would be ignored for regulatory purposes. The top-down approach in the next section overcomes this problem to some degree.

2D2. Top-Down Approach (Recommended)

The overall regulatory approaches discussed in this article reflect a tradeoff between level of mixture constituent interaction details incorporated vs breadth of coverage. Because myriad categories and doses of toxic stimuli can contribute to toxic stimuli mixtures, many different mechanisms are possible in determining final toxicity of any specific mixture. The bottom-up approach of the previous section incorporated these different mechanisms in its operational mode, mainly through the conduct of expensive experiments. The top-down approach moves to the other end of the spectrum. It reflects minimum interaction detail, but encompasses a much wider collection of toxic stimuli, and does not require the extremely expensive toxic mixture testing program of the bottom-up approach.

The essence of the top-down approach is normalization of the toxicity of each toxic stimulus, then using an additive approach to sum the effects of each normalized toxicity in the combination. The central operating principle is that the cumulative effects are accurate for the hypothetical limiting case where each normalized toxicity looks identical to the organism from all perspectives. Specifically, the top-down approach proposed in this article requires 1) assuming that all potential toxic stimuli to which an individual might be exposed have the same mechanisms/modes of action on the biological mechanisms, 2) normalizing the myriad stimuli exposures by converting the doses of toxic stimuli exposures to the respective toxicological reference value (TRV e.g. ADI) fractions, 3) adding all the TRVs' fractions from these toxic stimuli exposures, and 4) dividing all the single substance TRVs by the sum of fractions.

Since, in the foreseeable future, for a given regulation-setting Agency, one exposure limit value (or perhaps an acute and chronic value) is set for a given toxic stimulus, the individual-oriented concept of the previous sentence needs to be converted to a population-oriented concept. This would require generating a distribution function of exposures for a representative sample of myriad individuals to myriad potential toxic stimuli and performing statistical analyses on the resulting population-based distribution functions. This integrating process would arrive at the requisite TRV fractions to which the sample population is exposed for each of the selected toxic stimuli exposures, which would then be summed over all the selected potential toxic stimuli to provide the total number of TRVs to be used for existing exposure limit reduction.

For example, suppose there were five potentially toxic stimuli to which the greater population might be exposed to. Suppose further, after a representative sample of people had been selected, measurements of their exposures to these toxic stimuli showed that median exposure (in a preset percentage) to Toxic Stimulus 1 (TS1) was 0.5 times present TRV1, TS2 was 0.6 TRV2, TS3 was 0.7 TRV3, TS4 was 0.8 TRV4, and TS5 was 0.9 TRV5. Then, the sum of these five TRV coefficients (fractional TRV exposures, assuming equal importance of all five TRVs), would be 3.5, and the present-day exposure limits for ALL toxic stimuli would be reduced by a factor of 3.5. Obviously, this excludes any hormetic (Agathokleous and Calabrese, 2019), antagonistic, or synergistic effects among combination members, and thus would be 'exact' (for the five toxic stimuli sampled) only for the hypothetical limiting case of identical mechanisms/modes of action among the five potentially toxic stimuli.

Based on the approximately 500-800 potential contributing factors for each of the three chronic diseases identified above (with the very real possibility that hundreds or thousands more contributing factors could be identified if all the e.g. 85,000 chemicals in use were evaluated for their contributions to diseases in isolation or in combination), how many of these c.a. 500-800 could any one person be expected to encounter? That question cannot be answered with any degree of credibility at present, since there are extremely limited data on all the myriad exposures to which people are subjected over their lifetimes. To obtain such cumulative exposure data, people would need to be instrumented for many years with myriad devices to measure temporal exposures to 1) myriad radiation fields, 2) thousands or tens of thousands of chemicals, 3) thousands of non-chemical substances, etc. Given the effectively infinite number of combinations of toxicants possible from the many thousands of toxic stimuli already shown to contribute to myriad diseases (c.a. 8,000, based on the limited results shown in Kostoff, 2015), there is little hope of identifying effective TRVs for all the constituents in each combination within the foreseeable future.

A credible process that would provide a first-order approximation to the full-scale challenge outlined above is as follows. The pervasive causes of disease approach presented earlier by Kostoff (Kostoff, 2015) would be updated and upgraded, to both include the most recent information and cover a broader expanse of biomedical literature using more powerful computer capabilities. Let's assume that 1,000 pervasive causes of disease would result (in (Kostoff, 2015), the causes/contributing factors were classified as foundational, which meant they were tangible primary causes of disease over which potential victims had some control, such as smoking, high-fat-diet, iatrogenic surgical procedures, iatrogenic drugs, radiation exposures, heavy metals, food additives, insufficient exercise, brominated fire retardants, etc). These c.a. 1,000 pervasive causes of disease would serve as the 'pool' from which toxic stimuli would be selected for purposes of identifying average fractional TRV levels of exposure. A sample of the population would be selected, and their exposures to selected toxic stimuli from this 'pool' would be ascertained. To ensure adequate protection for the most vulnerable members of our population, this sample could be weighted towards people who serve on the front-lines of deploying much of, and living nearby, the advanced technologies whose consequences are responsible for major contributions to disease. These groups would include farmers/gardeners (pesticides), cell tower service personnel (wireless radiation), miners (particles, heavy metals), radiotherapy technicians/radiopharmaceutical diagnosticians (ionizing radiation), etc.

These exposures to selected toxic stimuli could be ascertained in at least three ways. First, if the exposure data is already in the literature, the literature could be scanned for population distributions of exposures to these selected toxic stimuli. Second, the sample members could be queried for their exposure levels to these selected toxic stimuli. This would be relatively credible for contributing factors related to lifestyle (e.g. diet, exercise, sleep, recreational substances, etc.), iatrogenic (e.g. surgeries, drugs, diagnostics, etc.), and some occupational/environmental exposures (e.g. noise, air pollution, some types of ionizing radiation, etc.). It would not be credible for many of the occupational/environmental exposures, since most people have little or no knowledge of their exposures to specific toxic stimuli at their workplace, in their environment, and in their home. Third, the population sample could be instrumented, and some of the unknown occupational/environmental/home exposures to toxic stimuli could be measured. Measuring exposure trajectories over the long-term is probably not realistic given today's technologies, but acute (perhaps

one or two day) exposure trajectories might be doable. In the future, imbedded chip technologies might allow much longer-term exposure measurements to be made.

Once these exposures to selected toxic stimuli have been ascertained for the sample population by the above three (and perhaps other) approaches, then the distributions can be generated, and the statistical assessments can be performed. The fractional or integer TRV coefficient levels would be summed over all toxic stimuli selected, and the result used as the denominator for reducing the present regulatory exposure limits to partially account for exposures to combinations of toxic stimuli. Myriad rules could be added to the numerical process, such as 1) assuming exposures <0.25xTRV could be hormetic and setting their contribution to the reduction factor to zero, and/or 2) assuming exposures >0.5xTRV could be synergistic and setting their contribution to the reduction factor to unity.

What levels of exposure limit reduction should we expect to partially account for effects of toxic stimuli combinations? Over the past four years, the first author has reviewed many thousands of potential contributing factors to disease identified in (Kostoff, 2015). It would not be unreasonable to expect many people to be exposed to at least tens (perhaps many tens) of these potential contributing factors at near-TRV or greater levels, especially those contributing factors that have been characterized as 'pervasive' (Kostoff, 2015). Then, in this hypothetical limiting case of identical mechanisms/modes of action, the cumulative exposure would be on the order of tens or hundreds of TRVs. This means that present regulatory exposure limits would need to be set one or more orders-of-magnitude lower across the board to avoid increasing the risk of major chronic diseases from large combinations of contributing factors.

However, based on the results from our previous reports (Kostoff, 2018a; 2018b; 2018c), the actual exposure limit reduction numbers computed with the above process (without constraints imposed by the two rules suggested) may be far larger. Lower damage limits from toxic stimuli may be under-reported; synergistic effects may be important in some/many cases; existing regulatory limits for toxic stimuli may be far higher than exposure limits shown to cause harm in the biomedical literature. For example, as was shown in (Kostoff, 2018a) for the ten sampled substances that OSHA regulates, the PELs could range anywhere from about one-to-four orders-of-magnitude above an effective TRV. People who worked in an environment where they were exposed to all ten of these sampled substances in (Kostoff, 2018a) could have an exposure limit reduction on the scale of three orders-of-magnitude based on these ten toxic stimuli alone! Much of that reduction is not due to the effect of combinations, but rather to the present-day PELs not reflecting the exposure limits shown by the biomedical literature to cause damage.

To compensate for this non-combination effects' influence on the computation of the combination reduction factor, the value of any toxic stimulus TRV fraction/coefficient could be limited to unity (i.e., any TRV fraction that was greater than one would be set equal to one). Thus, for the ten sampled substances from (Kostoff, 2018a), the total of their TRV coefficients used for computing reduction in present exposure limits to account for combinations would be ten. Hopefully, when the PELs are reduced to reflect biomedical literature results, the limit on TRV coefficient values to unity for any toxic stimulus would be exact for the hypothetical limiting case for identical mechanisms/modes of action. Thus, if e.g. the sampling results show that the total fractional TRV exposure coefficients sum to 100, then the TRVs would be reduced to one percent of their single toxic stimulus values across the

board. The results could always be weighted for different levels of exposure relative to TRV values, or for different levels of importance for the different toxic stimuli.

3. Conclusions

This article advances a recommended simplified approach (and a more expensive alternative) that will partially compensate for the enhanced adverse effects of combinations of toxic insults if enacted in the regulatory process. The recommended approach essentially divides all TRVs by the sum of fractional TRVs of toxic stimuli to which a representative sample of people are exposed. It would probably reduce present single-toxic stimulus-based TRVs by an order-of-magnitude or more across the board.

It does not account for hormetic, antagonistic, or synergistic effects of toxic stimuli in combination. It does not adjust for low-dose toxicants with adverse effects that have been under-reported, nor does it adjust for exposure limits like the OSHA PELs that are orders of magnitude above levels shown by published single toxic stimulus studies to have caused adverse effects. However, it would be a substantial first step in requiring the regulatory process to account for the real-life enhanced adverse effects of toxic stimuli combinations.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: