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Proposing new approaches for the risk characterisation of single chemicals and chemical mixtures: The source related Hazard Quotient (HQ_S) and Hazard Index (HI_S) and the adversity specific Hazard Index (HI_A)

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ABSTRACT

A hazard quotient (HQ) for a single chemical and the hazard index (HI) for a mixture of chemicals were first described as approaches for risk characterisation by the EPA. HQ is defined as the ratio of exposure to an appropriate reference dose such as the ADI. HI is the sum of the HQs of the chemicals in a mixture. HQ and HI have been used to characterise risk after various exposure scenarios. However, both approaches have a significant limitation in the way they are used. The accurate use of HQ or HI requires estimation of aggregate exposure, that is, exposure to a given chemical(s) from all possible relevant sources. In many studies, risk is assessed assuming exposure from a specific source such as, consumption of water or a specific food item, in which chemical(s) concentration(s) have been measured. In this case the classic HQ/HI approach can result in significant underestimation of risk. For this purpose, we developed an alternative approach, named as Source Related HQ (HQs) where HQ_S is the ratio of the exposure from the specific source of interest to the respected reference values. According to our approach the HQ_S, before being compared to the reference dose, should be adjusted by a correction factor, in order to simulate aggregated exposure. A correction factor can be calculated based on the permitted exposure contribution from the specific source to the permitted aggregated exposure. Another important limitation specific to the HI approach is the use of chemical specific ADIs that do not correspond to the same critical effect. In this study, we present an analysis based on the individual critical effects, in order to derive the critical effect and an adversity specific Hazard Index (HI_A) and risk characterisation for the whole mixture.

1. Introduction and problem formulation

Chemicals' safety for human health and the environment is based up today in risk assessment of single substances. However, exposure in one and only chemical, rather than on chemical mixtures and other non-chemical stimuli, is not corresponding to real-life exposure scenarios. [1–5]. Recognising this fact, several regulatory bodies have developed methodologies and guidance for the cumulative risk assessment [6,7] while discussion and research on assessing not only chemical mixtures but to proceed to real-life risk simulation (RLRS), considering realistically low doses and multiple stimuli, is evolving [8–13].

In the present study we propose two new approaches for the risk characterisation of single chemicals and of chemical mixtures: The Source Related Hazard Quotient (HQ_S) and Hazard Index (HI_S) and the Adversity Specific Hazard Index (HI_A). Our study aiming on improving the two classic risk characterisation methodologies of hazard quotient (HQ) and hazard index (HI). HQ and HI were first described by the United Sates Environmental Protection Agency (EPA) National-scale Air Toxics Assessment (NATA) for use in air toxics risk assessment [14]. HQ was defined as the "*The ratio of the potential exposure to a substance and the level at which no adverse effects are expected (calculated as the exposure divided by the appropriate chronic or acute value)*" and HI as the "*The sum of hazard quotients for toxics that affect the same target organ or organ system. Because different air toxics can cause similar adverse health effects, combining hazard quotients from different toxics is often appropriate*" [15].

Aggregate exposure has received only partial consideration in the four NATAs, implemented to date. The scope of the assessment was the estimation of risk from inhalation of air toxins from various sources although a number of the chemicals assessed were also present in food and water (e.g. cadmium, lead, arsenic, mercury, PCBs, and dioxin) which are associated with neurological and not just respiratory effects [16]. HQ and HI methodologies for risk characterisation, whilst considering a single route or a single source of exposure, have since been implemented in a number of studies. This is the case with many dietary studies where the estimation of risk has been associated with the concentrations of chemicals in a specific food item [17–26]. The lack of availability of data and/or the capacity to estimate actual aggregated exposure has likely driven the calculation of a HQ based on exposure to one food item only. Subsequent comparison of the partial exposure to the reference dose (e.g. comparison of a partial EDI to the ADI, equal to comparing the HQ = EDI/ADI to the value of "1") can lead from moderate to high underestimation of the actual risk.

In similar studies assessing the risk to mixtures [19,25], HQ_S were summed-up to derive a HI where the respective ADIs being derived for different adverse endpoints / critical effects. This practise is misleading with regards estimation of the actual risk and should be discouraged. An example of this case is when chemicals are grouped on the basis of their assumed toxicity as in the case for organophosphorus (OP)

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pesticides which are known to cause neurological effects [19]. However, as we demonstrate in one of our case studies [27], the ADIs may be based on different critical effects and different than the generally assumed toxicity, again leading to an unrealistic risk assessment.

To improve the way the classic HQ and HI methodologies are used, we propose two new approaches for the risk characterisation of single chemicals and chemical mixtures: The Source Related Hazard Quotient (HQ_S) and the Adversity Specific Hazard Index (HI_A), described below.

2. Methodology

2.1. The Source Related Hazard Quotient (HQ_S) and Hazard Index (HI_S)

HQ_S is the ratio of the exposure from the specific source of interest to the respected reference values. Speaking for dietary exposure the comparison of a HQ_S directly with a reference dose such as the Acceptable daily intake (ADI), disregards the reality that a consumer is exposed to a chemical from many different sources and not only the specific food item. In order to overcome the difficulty of estimating real aggregated dietary exposure we propose an extrapolation from the specific source exposure to the aggregated, by using the legally permitted exposures.

Considering that:

EXP aggregated	should correspond to	*Sum of permitted exposure from all related food items*
EXP from specific food item	should correspond to	*Permitted exposure from the specific food item*

Aggregate exposure should be:

$$EXP\ aggregated = EXP\ from\ specific\ food\ item\ X \frac{Sum\ of\ permitted\ exposure\ from\ all\ related\ food\ items}{Permitted\ exposure\ from\ the\ specific\ food\ item} \quad (1)$$

We named as source specific correction factor (CF) the fraction of exposure to the chemical present at the maximum permitted concentration in the food item in relation to total exposure from all relevant food items which are also contaminated at the maximum permitted concentration of chemical. In other words, the CF is the ratio of the permitted contribution to exposure of the specific food item to overall dietary exposure (Eq. (2)).

$$CF = \frac{Permitted\ exposure\ from\ the\ specific\ food\ item}{Sum\ of\ permitted\ exposure\ from\ all\ related\ food\ items} \quad (2)$$

Based on Eqs. (1) and (2) we conclude that:

$$EXP\ aggregated = \frac{EXP\ from\ specific\ food\ item}{CF} \quad (3)$$

As the criterion to be fulfilled for considering non-risk is that:

$$EXP\ aggregated < ADI \quad (4)$$

and combining Eqs. (3) and (4) then:

$$\frac{EXP\ from\ specific\ food\ item}{CF} < \mathrm{ADI} \quad (5)$$

which is equal to:

$$\frac{(EXP\ from\ specific\ food\ item)/CF}{ADI} < 1 \quad (6)$$

which is equal to:

$$\frac{HQs}{CF} < 1 \quad (7)$$

and,

$$\frac{EXP\ from\ specific\ food\ item}{ADI} < \mathrm{CF} \quad (8)$$

meaning, eventually that for no-risk we should have:

$$HQ_S < \mathrm{CF} \quad (9)$$

In our case studies, we applied this methodology to contaminants and pesticides using existing European Union (EU) Maximum Levels (standing for the maximum permitted occurrence) [28] and the Maximum Residue Limits (MRLs), respectively. For example, for pesticides, the CF is given by the following equation (Eq. (2)):

$$CF = \frac{(Consumption\ of\ specific\ food\ X\ MRL\ in\ the\ specific\ food)}{\sum_{i}^{n} (Consumption\ of\ food\ i\ X\ MRL\ in\ the\ food\ i)} \quad (10)$$

As can be see above the CF can be used in two mathematically equivalent ways: a) using Eq. (6) (to extrapolate source specific exposure to aggregate exposure), then to produce the classic HQ, dividing with the exposure with the ADI, and finally compare with the value of one or b) using Eq. (8) and directly compare the HQ_S with the CF where HQ < CF

With regard to the risk characterisation a mixture of n chemicals in a specific food item the source related HI_S should be calculated as follows:

$$HI_S = \sum_{i}^{n} (HQs)i \quad (11)$$

Combing equations 9 and 11:

$$HIs < \sum_{i}^{n} (CFs)i \quad (12)$$

In addition, as the criterion for the mixtures is HI to be < 1, we have:

$$HI = \sum_{i}^{n} \left(\frac{Exp\ agr\ i}{ADIi}\right) < 1 \quad (13)$$

Which is equal to:

$$\sum_{i}^{n} \left(\frac{HQs\ i}{CFi}\right) < 1 \quad (14)$$

2.2. The adversity specific Hazard Index for mixtures (HI_A)

As discussed above the HI is the sum of the HQ of the chemicals in a mixture (Eq. (13)). It is generally agreed that the sum should be refer to chemicals that cause adverse effects at least in the same target organ or system, and ideally with the same Mode of Action (MoA). However, in many instances the criterion used for grouping chemicals is a broad toxicity such as neurotoxicity for OPs. This is not always correct because even among chemicals which belong to the same chemical group (based on their structure) and that may cause the same adverse effect in some dose, their ADIs might be derived based on dissimilar critical effects. In addition, a number of endpoints may be used for setting the ADI which may not be tissue or organ specific, e.g. changes in body weight. Examples include the OP ethion, whose critical effect is embryotoxicity and fenpyroximate, another OP, whose critical endpoint is body weight change. To address these inconsistencies, we propose the use of the critical effects used to derive ADIs as a criterion for grouping and the derivation of the adversity specific HI_A (where "adversity" here is the specific critical effect) (Eq. 15).

$$HI_A = \sum_{i}^{n} HQi = \sum_{i}^{n} \left(\frac{Exp\ i}{ADI\ i}\right) \quad (15)$$

Where, n is the number of chemicals in the mixture and all ADIs refer to the same critical effect / endpoint.

This criterion is easy to apply and is closer to the ideal situation of considering the MoA, which however, in most cases is unknown.

HI_A methodology can be also be used for assessing the risk for a specific adversity of interest after exposure to a mixture of chemicals. In order to do this one should derive the HQ for each chemical by replacing ADIs with NOAELs/UF for a specific adverse effect (Eq. 16).

$$HI_A = \sum_{i}^{n} HQi = \sum_{i}^{n} \left(\frac{Exp\ i}{NOAEL/UF\ i}\right) \quad (16)$$

For example, in assessing the risk of inhibition of cholinesterase (ChE) for a group of OPs the respective NOAELs for this effect should be considered to set the HI_A. The same methodology can be used for any kind of adverse effect and endpoint; even those not included in existing testing protocols designed for regulatory use, for example, inflammation, oxidative stress, etc.

3. Case studies

3.1. HQ_S for PCBs in fish

In our first case study the six "indicator" non-dioxin-like polychlorinated biphenyls (NDL-PCBs) set from various international bodies such as the European Commission [28] for sea monitoring, were measured in fish from Greek seas [29]. Our aim was to evaluate the risk to the Greek population from exposure to these NDL-PCBs through fish consumption only, excluding all other food items. These 6 NDL-PCBs share a common MoA and we regarded it as an assessment group (Σ(PCB-6)), following a whole-mixture approach.

As described above, the CF was defined and calculated as the ratio of the maximum permitted daily intake through fish consumption (fish consumption * maximum level in fish) to the maximum permitted daily intake through the whole diet (SUM ($food_i$ consumption * maximum level in the food*i*), where i represents each food commodity / group considered for the dietary intake) meaning cheese, eggs, demersal fish (FAOSTAT), pelagic fish (FAOSTAT), fish (DAFNE-ANEMOS), meat and products, milk and products and total added lipids. The calculated CF was 0.43 and 0.72 for the FAOSTAT and DAFNE DBs respectively.

The calculation of the HQs (equal to a HI_S as in this case we have a whole mixture approach and named for this reason as HI_f) was calculated dividing the estimated daily intake (EDI) with the respective reference value. The EDI from fish consumption (EDIf) was calculated based on a) the daily fish consumption for the Greek population (g/person), as indicated from two different databases FAOSTAT (http://www.fao.org/faostat/en/) and DAFNE-ANEMOS (http://www.hhfgreece.gr/DafnesoftWebV2/), and b) The Σ(PCB-6) occurrence (contamination) determined in the study of fish tissue, expressed as the 95th percentile (ng contam/g fish), and c) the mean body weight for an adult consumer (70 kg). As an ADI value is not yet available for the Σ(PCB-6) a proposed guidance value of 10 ng/kw bw per day was used [30].

The calculated values for the HQs of 0.22 and 0.34 (for FAOSTAT and DAFNE respectively) were well below the respective values of 0.43

Table 1
EU-ADI, critical effects and HIA for the 18 pesticides.

Pesticides	Chemical Category	EU- ADI (mg/kg bw/day)	Critical Effect	HQs	HI_A
Group A- Neurotoxicity					
Chlorpyrifos	OP	0.001	A1. Inhibition of ChE (RBC)	71	4934
Diazinon	OP	0.000		4862	
Aldicarb	CB	0.003		0.6	
Fenthion	OP	0.007	A2. Inhibition of ChE (Plasma)	30	30
Phosalone	OP	0.01	A3. Inhibition of ChE (Brain)	13	355
Metasystox 1	OP	0.0003		342	
Deltamethrin	PY	0.01	A4. Various clinical signs (e.g. salivation, tremors, hypersensitivity, impaired locomotor activity	7	7
Acetamiprid	NC	0.025	A5. Reduced auditory startle responses in pups	5	5
SUM					**5331**
Group B- Reproductive and Developmental Toxicity					
Glyphosate	OP	0.5	B1. Maternal toxicity (mortality), post- implantation losses	199	199
Cypermethrin	PY	0.005	B2. Systemic toxicity, changes in kidneys (weight) and testes (tubular atrophy and calcification)	74	74
Ethion	OP	0.002	B3. Embryotoxicity/ fetotoxicity	360	360
SUM					**632**
Group C- Systemic Toxicity					
Cypermethrin	PY	0.005	C1. Systemic toxicity in rats included increased urea, changes in kidneys (weight) and testes (tubular atrophy and calcification)	74	74
Fenpyroximate	OP	0.01	C2. Body weight changes	27	27
Fenvalerate	PY	0.0125	C3. Body weight gain of the F2b parents	7	7
Permethrin	PY	0.01	C4. Effects on liver weight	2	2
SUM					**110**
Group D- Haematotoxicity					
Chloropropham	CB	0.05	Methaemoglobin changes / Increased thyroid weight and hormones decrease	37	37
SUM					**37**
Group E- Thyroid Effects					
Chloropropham	CB	0.05	E1. Methemoglobin changes / Increased thyroid weight and hormones decrease	37	37
Thiophanate- methyl	CB	0.08	E2. Increased thyroid weight and preneoplastic and neoplastic lesion	0.03	0.03
Imidacloprid	NC	0.06	E3. Increased incidence of mineralisation in the colloid of the thyroid gland follicles	97	97
SUM					**134**
Group F- Carcinogenicity					
Fenoxycarb	CB	0.053	Lungs and liver tumors	0.79	0.79
SUM					**0.79**

OP: organophosphorus, CB: carbamate, PY: pyrethroid and NC: nicotinoid.

and 0.72, indicating no risk for the Greek population from the Σ(PCB-6) through fish consumption.

3.2. HQ_S and HI_A for PPPs in pistachio

In this study 18 pesticides were quantified in pistachios from Iran [27]. The CF was calculated for each of the 18 pesticides by dividing the permitted exposure estimated from pistachio consumption (consumption * current EU MRLs for pistachio) to the overall permitted exposure from the consumption of the foods where the pesticide might be used (Sum of consumption * MRL for each relevant food). Consumption data were retrieved for the EFSA tool PRIMO version 3. In this case we have a component-based approach with 18 CFs (one per each pesticide). The CFs applied to the respective EDIs (calculated for median occurrence and consumption at 97.5%) used for the derivation of the 18 HQ_S.

In order to calculate the HI_A and to group the 18 pesticides we considered the critical effect used for the derivation of the ADIs. The 18 pesticides were placed into groups depending on the critical effects and then into 6 "super-groups" depending on the target organ / system the critical effect belongs (Table 1).

For the 18 pesticides 17 different critical effects were identified, creating 17 groups and 6 super-groups. It should be noted that some of the pesticides had more than one critical effect and consequently were placed into more than one group. Only two of the groups had more than one pesticide: group A1 for ChE inhibitors in RBCs (chlorpyrifos, diazinon, and aldicarb) and group A3 for ChE inhibition in brain (phosalone and metasystox 1). As can be seen in Table 1 for almost all groups of pesticides with the same critical effect the HI_A values were higher than one, with the exception of preneoplastic and neoplastic lesions in thyroid and lungs and liver tumours. In group A1 it is clear that the main contributor is diazinon (HQs = 4862) followed by chlorpyriphos (HQs = 71), and aldicarb (HQs = 0.6) which in isolation poses no-risk. In group A3 the main contributor is metasystox 1 followed by phosalone (HQs 342 and 13 respectively).

4. Discussion

The HIs approach is a relatively easy way to assess the risk from exposure to chemicals through the consumption of a specific food. This is of particular importance in the risk assessment of the exposure after consumption of a single food item contaminated with a mixture of chemicals, when the aggregate dietary exposure is not known (mainly due to lack of occurrence data for all foods). The direct comparison of the pesticides concentrations with the legally set MRLs in the specific food item, although easier, has the limitation that MRLs are not always derived based on risk assessment. Most MRLs are not safety limits but are the limit of detection of the analytical method considered for their setting. This is because there should not be any chemical in question present (due to no authorised use) in the food item and because they were not identified before the MRL was set. In our approach we consider MRLs for setting the CF that is, the legally permitted contribution of a food item to the aggregated exposure, but in addition, we consider the consumption of the targeted food item and all the other relevant food items. An interesting finding from this study was the difference between the direct comparison approach and the use of the HIs approach in the second case study where the level of fenoxycarb was higher than the respective MRL, yet had HQs < 1, indicating no risk. On the contrary, chlorpropham and cypermethrin, both with HQs > 10, and deltamethrin, fenvalerate, and permethrin, with HQs between 1 and 10, were found in concentrations below the respective MRLs. It should be noted that all these MRLs were set based on the limits of detection (LOD) and that our method was based on chemical contamination levels well below these used for setting the MRLs.

The HI_A methodology is a refinement of the classic approach of considering adverse effects for grouping at the level of target organ or system, it is closer in the ideal situation of considering MoAs for grouping (which in most cases are unknown) and is easy to apply. Our approach considers the known part of the MoA, meaning the adverse outcome (AO). When using an ADI, the AO considered is the critical effect. Different critical effects are different AO and they cannot correspond to the same MoA. Apparently, having the same critical effect doesn't necessarily means that the full MoA is the same. This approach is scientifically consistent with EFSA approach for the formation of cumulative assessment groups (CAGs) [31]. However, in our approach we consider each toxicological endpoint observed and used for setting the ADI (named in some instances as "indicator" in EFSA's report) as a separate AO. The reason for this is that even if an endpoint in isolation might not be considered by all scientists and risk assessors as "adverse", indicates some difference in the underline MoA or mechanism of action and in any case it was considered as adverse when used for setting the ADI. However, in several cases and based on experts' judgment, a dose might be considered as creating adversity due to the disturbance of a set of endpoints which in isolation cannot be considered as adverse or due to their nature or due to the size of disturbance [32,33]. Further effort is needed to reach consensus among regulatory toxicologists regarding in which level each effect should be consider adverse [34–37]. This long-avoided discussion is now necessary also in order to set the critical effect sizes for the benchmark dose [38].

As demonstrated in the case study with the 18 pesticides, grouping based on target organ or system might lead in an overestimation of the risk as based on the critical effects of the super-grouped chemicals which do not have the same MoA (the critical effect may change with dose). However, it remains a possibility that the same organ or system may be affected due to an interaction, for example a potentiation of deltamethrin from diazinon [39]. The issue of interaction needs further investigation. In case study two further steps can be taken to increase the accuracy of the estimated risk, 1) investigate whether the 18 pesticides cause other effects in addition to the known critical effect at other doses so as to procced with adding them to a group with the use of the respective NOAEL/UF and 2) to investigate the existence of data for interactions between the 18 chemicals and how they could be taken into account in the risk assessment process.

In conclusion, we propose that the assessment of risk from exposure to single chemicals and their mixtures can be improved with the development of more sensitive analytical chemistry methods, the increased availability of data, and the refinement of risk assessment methodologies.

Declaration of Competing Interest

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.

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Marina Goumenou*, Aristidis Tsatsakis
Centre of Toxicology Science and Research, University of Crete, School of Medicine, Crete, Greece
E-mail address: marina.goumenou@gmail.com (M. Goumenou).

* Corresponding author.