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Theoretical risk assessment of lead, cadmium and arsenic mixture linked to consumption of garden products from Cotonou, Benin

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Abstract

Human-beings' exposures, whether simultaneous or sequential, are not just to one chemical but to chemicals' mixture. It is the case of coexposure to lead, cadmium and arsenic through the consumption of vegetables from Cotonou (Benin republic). This survey is a theoretical assessment of risk linked to mixture of these toxic based on assumption of contaminated garden products according to concentrations addition model recommended with regard to the assessment of chemicals mixture. The Daily Exposure Dose calculated in µg/kg/day for lead, cadmium and arsenic is respectively, 9.0, 2.67 and 170.03 for a child and respectively, 2.76; 0.82 and 52.30 for an adult against WHO norms that are 3.6; 1 and 2.14 µg/kg/day. The addition of doses gave respectively, 181.7 µg/kg/day for a child versus 55.88 for an adult against fixed value, 6.74 µg/kg/day. The mixture hazard index (HI_m) calculated according to the model that uses reference value of each component (RfD_i) and the one that uses mixture reference values (RfD_m), is respectively, 84.67 and 132.6 for a child but 26.02 and 40.81 for an adult whereas this $HI_{m/adult}$ calculated without considering arsenic exposure is around 1. These results showed that, chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly.

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Introduction

The previous survey revealed that the vegetables taken from some garden sites in Cotonou, in Benin Republic were contaminated by a mixture of heavy metals, lead, cadmium and arsenic (Koumolou *et al.*, 2012). In fact, in Benin Republic, the poor farming practice regulation, the location of garden sites that are either on old garbage dumps or located near swamps and the sanitary quality of the watering water are responsible for the introduction of traces metal into gardening products (Koumolou *et al.*, 2013). This situation needs a health risk assessment. Certainly, both US EPA and the French Committee on Toxicity recommended the need of an assessment based on toxicological and ecotoxicological experimental data (RECORD, 2011). But, the scientific community also recognizes that an understanding of risk requires consideration of the characteristics of the host population, the environmental chemical or chemical mixture, and the exposure milieu. Consequently, human health risk assessments done by EPA generally follow the paradigm established by the National Academy of Sciences (NRC, 1983) that describes a group of interconnected processes for performing a risk assessment that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. If these factors are not adequately addressed, the real knowledge about environmental chemicals toxicology may be substantially misrepresented (Mumtaz *et al.*, 2010). Otherwise, the legislations about chemicals are predominantly based on assessments carried out on individual substances (Santé Canada, 1995; US-EPA, 1989; US-EPA, 1992; WHO, 2000; Tarantino, 2006). But, since human-beings and their environments are exposed to a wide variety of substances, there is increasing concern in the general public about the potential adverse effects of the interactions between those substances when present simultaneously in a mixture (EU, 2012). The standard definition of a chemical mixture is any set of multiple chemicals regardless of source that may or may not be identifiable, that may contribute to joint toxicity in a target population (EPA, 1986). With regard to the assessment of chemical mixtures, three basic types of action for combinations of chemicals were defined to characterize the risk linked to chemicals mixture (Bliss, 1939, Loewe and Muischnek, 1926, Plackett and Hewlett, 1948, Plackett and Hewlett, 1952). These are similar action, dissimilar action and interactions. For mixtures of similarly acting chemicals, the effects can be estimated according the doses/concentrations model. If chemicals act independently from each other, usually through different modes of action that do not influence each other, response addition is given by the sum of probabilities minus their product for two substances. But, for more than two substances, it is more complicated and better represented by the product of the complementary probabilities. Interactions including antagonism, potentiating, and synergies usually occur at medium or high dose levels. However, most of the scientists working on toxicological or ecotoxicological interactions, recommend the use of models based on doses/concentrations addition because they overestimate the real effect of the mixture (EPA, 1986; NRC, 1983; U.S. EPA, 2000). That is why, before the assessment based on toxicological and experimental data (RECORD, 2011), the aim of this work is to theoretical assess the public health problems linked to the consumption of the contaminated vegetables according to this latest established method called CA model.

Material and methods

Material

Two garden sites in Cotonou have been our study framework: Houeyiho (H) and Godomey (G). 21 Composite samples of eight different vegetables *Amaranthus hybridus* (amaranth), *Daucus carota* (carrot), *Lactuca sativa* (lettuce), *Spinacia oleracea* (spinach), *Allium cepa* (green onion), Brassica oleracea (cabbage), Corchorus olitorius (fiddle), *Solanum macrocarpum* (nightshade) were carried out on two major gardening sites from Cotonou in Benin. The vegetables were analyzed in lead (Pb), cadmium (Cd) and arsenic (As) by atomic absorption spectrophotometry and the health risk assessment was performed using the standardized approach recommended for mixture chemicals.

Methods of risk assessment of lead, cadmium and arsenic mixture

Human health risk assessments done by EPA generally follow the paradigm established by the National Academy of Sciences (NRC, 1983) that describes a group of interconnected processes for performing a risk assessment that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. The meanly of the four steps are:

- Hazard identification: what health problems are caused by the pollutants?

- Dose-response evaluation: what are the health problems at different exposures?

- Exposure assessment: how much heavy metal do people consume through vegetable during a specific time period? How many people are exposed?

- Risk characterization: what is the extra risk of health problems in the exposed population?

Method to calculate the hazard index (HI) using reference value of each chemical

The first method of dose/concentration addition approaches most frequently used is the hazard index (HI). The hazard index (HI) is the sum of the hazard quotients (HQ), i.e. the ratios between exposure and the reference value (RfD) for each component to be evaluated according to ATSDR formula (ATSDR, 2004).

$$HI = \sum_{i=1}^{n} \frac{EstimatedIntake_i}{RfD_i} = \sum HQ_i = \sum DED_i / RfD_i \qquad (1)$$

$$HQ_i = DED_i / RfD_i \qquad (2)$$

$$DED_i = (Q \times D_i) / BW \qquad (3)$$

$$DED_{adult} = (DED_{child} \times BW_{child}) / BW_{adult} \qquad (4)$$

Then: $$HI = \sum \frac{(Q \times D_i)}{(BW \times RfD_i)} \qquad (5)$$

With:

Q: average quantity of vegetables consumed (kg)

D_i: average concentration measured of i^{th} chemical in vegetables (µg/kg)

BW: body Weight of the consumer (kg)

DED_i: total daily exposure dose of i^{th} chemical

RfD_i = reference value of i^{th} chemical or daily allowed dose (µg/kg/day)

HQ: hazard quotient = ratio between the observed average of DDE_i and corresponding tolerable daily dose (RfD_i)

HI: hazard index = ∑ HQi

DED_{adult} = daily exposure dose in adult (µg/kg/day)

DED_{child} = daily exposure dose in child (µg/kg/day)

BW_{child} = average weight of a child (20 kg)

BW_{adult} = average weight of an adult (65 kg)

Method to calculate the hazard index (HI) using reference value of total mixture

With the heavy metals, as lead, cadmium and arsenic mixture, the RfD of a each compound is based on an effect that is not the group effect, so the HQ can be refined by identifying the RfD_m for the group effect and adjusting the HI_m accordingly. In fact, when only component toxicity data are available and dose addition can be assumed, knowledge of individual chemical RfD_i can be used to determine the mixture RfD_m (Svendsgaard and Hertzberg, 1994). Assuming stable exposure conditions, the mixture intake is then determined by the amount of food (vegetables) eaten (total mixture dose D_m), while the relative proportions (f_i) of mixture components are constant and each dose is scaled according to doses isoeffective (doses producing negligible risk of adverse effects) with the combination (Berenbaum, 1989):

$$\frac{1}{D_m} = \frac{f_1}{D_1} + \frac{f_2}{D_2} + \frac{f_3}{D_3} \qquad (6)$$

$$RfD_m = \frac{1}{\frac{f_1}{RfD_1} + \frac{f_2}{RfD_2} + \frac{f_3}{RfD_3}} \qquad (7)$$

$$HQ_m = \frac{DED_m}{RfD_m} \qquad (8)$$

$$HQ_m = \frac{(D_m \times Q)/BW}{RfD_m} = HI_m \qquad (9)$$

Risks regarding the adult consumer can be deduced from these values (Ricoux and Gasztowtt, 2005):

$$HQ_m/adult = \left(\frac{HQ_m}{child} \times BW_{child}\right)/BW_{adult} \qquad (10)$$

With:

RfD_m = reference value of total mixture (µg/kg/day)

f_i = fraction of each chemical in the total mixture ($\sum f_i = 1$)

Q: average quantity of vegetables consumed (kg)

D_i: average concentration of i^{th} chemical in vegetables (µg/kg)

D_m: dose of total chemicals mixture that produce the same response (µg/kg)

BW: body Weight of the consumer (kg)

DED_m: daily exposure dose of total chemicals mixture (µg/kg)

HQ_m: mixture hazard quotient

HI_m: mixture hazard index

BW_{child} = average weight in adult of 20 kg

BW_{adult} = average weight in adult of 65 kg

In one as in the other method, when the HI is less than 1, the combined risk is considered acceptable but values higher than 1 would indicate potential health concern to be considered.

Results

Hazard identification and dose-response evaluation

Individually, the chronic toxic effects of lead include anemia, neuropathy, chronic renal disease and reproductive impairment; cadmium causes emphysema, chronic renal disease, and cancer of the prostate and possibly of the lung; arsenic causes dermatitis, skin cancer, sensory neuropathy, and cirrhosis, angiosarcoma of the liver, lung cancer and possibly lymphatic cancer. In kidney, the combination exposure of metals such as lead, cadmium and arsenic results in increased urinary excretion of porphyrins and this have been suggested as a good biomarker for the combined or mixed exposure of metals (Watson and Muti, 2004; Wang *et al.,* 2008). Exposure to these metals mixtures has also been shown to result in clastogenic and aneugenic effects in peripheral lymphocytes (Wang *et al.,* 2008). For the reference toxicological values (RfD), the AFSSA has recommended 3.6 mg/kg and 1 mg/kg of body weight for lead and cadmium values respectively, as acceptable daily dose (AFSSA, 2003). WHO establishes the temporary admissible daily dose to 2.14 bodily weight g/kg per day (Santé Canada, 2006).

Report of vegetables consumption data

Due to missing data on the consumption of vegetables in the general population, the principle of making a food survey of a target population of children, has been retained to estimate the risk according to a pessimistic scenario. Risks regarding the adult consumer can be deduced from these values. The collection of dietary data has been fully informed. The quantities of the consumed vegetables were estimated according to the images of vegetables presented on props to 100 parents who gave these vegetables at least once a day to their children in Cotonou (Table 1).

Table 1. Results of vegetables consumption done with 250 children in Cotonou, of age 5 to 12 years old.

Quantity of vegetables		Number of Children	% Children
At least, once a day	100g	45	18
	50g	130	52
	30g	35	14
	20g	30	12
	10g	10	4
Average/child	51.00g	250	100%

The minimum average of vegetables consumed per child in a day was the mean M:

$$M = \Sigma\,(\text{Quantity (g)} \times \%\ \text{Children})/100$$

With:

M: Mean (The minimum average Amount of Vegetables Consumed per child in a day)

Quantity (g): minimum amount of vegetables consumed by children in a day (g)

% Children: percentage of children corresponding to different amounts

$$M = [(100\times 18) + (50 \times 52) + (30 \times 14) + (20 \times 12) + (10 \times 4)]/100 = 51.00\ g$$

Exposure assessment and risk characterization

Measurement of lead, cadmium and arsenic in vegetables samples is reported in Table 2.

Table 2. Contamination of vegetables by toxic metals on the two sites in Cotonou

	Lead (ppm)		Cadmium (ppm)		Arsenic (ppm)	
RfD	0.3 (INERIS, 2006)		0.05 (CE/466/2001)		0.1 (CE/466/2001)	
Sites	G	H	G	H	G	H
L. sativa	4.84	3.10	0.89	0.63	237.62	260.48
A. hybridus	5.01	3.73	0.93	5.13	171.63	259.92
S. macrocarpum	2.52	3.38	0.82	0.64	282.73	316.28
B. oleracea	6.69	3.12	1.73	0.55	230.81	325.30
D. carota	1.06	1.14	1.22	0.72	300.51	251.45
C. olitorius	3.5	4.76	0.91	1.17	271.76	241.41
A. cepa	2.85	3.36	0.43	0.26	358.67	323.52
S. oleracea	4.08	3.46	0.34	0.52	231.84	204.01
Average ± SD/site	3.82 ±1.73	3.25 ±1.00	0.91 ±0.43	1.20 ±1.60	260.70 ±56.04	272.80 ±44.25
Average± SD/metal	**3.53±0.40**		**1.05±0.20**		**266.75±8.55**	

NB: 1 to 70% of arsenic is in inorganic form (EFSA, 2009). But, we estimated it in this assessment at 25%. For t-test, p > 0.05 for all values. H = Houéyiho; G = Godomey.

For each metal, $p > 0.05$ after comparison of all values amongst garden sites. Therefore, we summed up over all consumed garden products. This explains why the consumption data (Table 1) was recorded for all vegetable without consideration for a specific species, although analyses in heavy metals were done for each vegetable separately.

Calculation of HI

First method

In this first method, we have summed the hazard indexes of each substance, without taking mechanisms of action or target organs similarity into account.

The results are reported in Table 3.

Table 3. Toxicological data about exposure of lead, cadmium and arsenic mixture for child and adult

Chemical	Exposure dose D_i of i^{th} chemical (mg)	Amount of vegetables consumed by a child by day (kg)	RfD (µg/kg)	DDE_i (µg /kg/day)		HQ		HI	
				Adult	Child	Adult	Child	Adult	Child
Lead	3,53	0.051	3.6	2.76	9.0	0.76	2.55	26.02	84.67
Cadmium	1,05		1	0.82	2.67	0.82	2.67		
Arsenic	66.68		2,14	52.30	170.03	24.43	79.45		

$HI_{child} = 84.67$

$HI_{adult} = 26.02$

Second method

In this second method, the risks' assessment has been secondly refined by using the mixture's reference value according to the formula of Berenbaum (1989). Let us calculate, first, the relative proportions (f_i) of each component in the mixture.

$$\frac{m_{Pb}}{f_{1Pb}} = \frac{m_{Cd}}{f_{2Cd}} = \frac{m_{As}}{f_{3As}} = \frac{m_{Pb} + m_{Cd} + m_{A}}{100}$$

So:

$f_1 = 4.95\% = 0.0495$

$f_2 = 1.48\% = 0.0148$

$f_3 = 93.57\% = 0.9357$

Inserting the values of D_i from Table 3 in the formula (6) gives:

$1/D_m = f_1/D1 + f_2/D2 + f_3/D3$

So, $D_m = 23.72$ mg/kg

Then, let us calculate RfD_m. Inserting the values of RfD_i from Table 3 in the formula (7) gives $RfD_m = 0.456$ µg/kg

Inserting the values of RfDm in the formula (9) gives:

For a child: $HQ_{m/child} = HI_{m/child} = 132.6$

Risks regarding the adult consumer can be deduced from these values (Ricoux and Gasztowtt, 2005). So according formula 10: $HQ_{m/adult} = HI_{m/adult} = 40.81$

All these results are reported in Table 4.

Table 4. Toxicological data while using mixture reference value

Chemical	Exposure dose of mixture D_m (mg)	Quantity of vegetables consumed by day by a child (kg)	RfD_m (µg/kg)	HQ_m		HI_m	
				Adult	Child	Adult	Child
Mixture of lead, cadmium and arsenic	23.72	0.051	0.456	40.81	132.6	40.81	132.6

Discussion

For instance, 143,000 chemicals were notified and needed to be assessed (RECORD, 2011). This survey only valued three of them: lead, cadmium and arsenic through consumption of vegetables from two garden sites in Cotonou according the doses/concentrations addition models recommended. Dose addition is the default approach in situations where the dose for each individual component is at a level at which effects are not expected to occur, be observable, or be of concern; however, when the doses are combined, effects of concern may be expected or observed in response to the higher dose level of the mixture. First, we have summed the hazard indexes of each substance, without taking mechanisms of action or target organs similarity into account (Table 2). The risks' assessment has been secondly refined by considering the mixture's reference value (Table 3). We have to point out that, for these cases study, the chemical composition of mixtures must be well characterized. According to the measurements of lead, cadmium and arsenic in vegetables samples (Table 1), an analysis of the results revealed that multiple sources of contamination by toxic pollutants affect the sanitary quality of the grown vegetables. Comparison of the contamination level of all vegetables on the gardening sites showed no significant difference ($P > 0.05$) from one site to the other (Table 2). So, for the risk's assessment, we can consider only the average of the concentration of each heavy metal in all the vegetables coming from the same garden site. The WHO standards (1998) for the limit of concentrations were all passed. The information provided by questionnaire on consumption (Table 1) and the results of the risk's assessment revealed that Daily Exposure Dose (DED in ug/kg/day) of lead, cadmium and arsenic mixture are respectively, 9.0; 2.67 and 170.03 for a child of 20 kg body weight and respectively, 2.76; 0.82 and 52.30 for an adult of 65 kg body weight (Table 3) against WHO's norms that are 3.6; 1 and 2.14 µg/kg/day (WHO, 1998). With regard to the assessment of chemicals mixture, it is recommended the use of models based on doses/concentrations addition. This addition of doses gave respectively, 181.7 µg/kg/day for a child versus 55.88 for an adult against fixed value, 6.74 µg/kg/day. These results showed that, chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly and whose dose is lower than its DED. The mixture hazard index (HI_m) calculated according to the model that uses reference value of each component (RfD_i) and the one that uses mixture reference values (RfD_m), is respectively, 84.67 and 132.6 for a child but 26.02 and 40.81 for an adult (Table 3 and 4). The values of DDE obtained for children were higher than the limits permitted by WHO (1998) and were more alarming than those obtained with adults. Toxic metals, lead, cadmium and arsenic are ubiquitous environmental contaminants in an industrialized society. Although all of these metals are natural constituents of the earth's crust, their distribution has been radically altered by human activity, and they are now dispersed widely in air, food, soil and water (NAS, 1972; NAS, 1977; NAS, 1979). This justifies their presence into the vegetables. These toxic levels show that absorption of heavy metals has become the norm rather than the exception. The population of Benin increases, and these are more than 8 million of Beninese, of which about 50% that are less than 20 years old are supposed to consume these products without forgetting the exports toward the others regions in west Africa. Moreover, the DDE obtained must be added to the daily dose provided by the line feed, as much as children were exposed to the same heavy metals through other foods in the same way as the general population (Ricoux and Gasztowtt, 2005). In every case, the HI was very much greater than 1 either for children or adults. While considering metals in the mixture, substance by substance, the risk is least for the adults. For example, in Table 3, there is no risk for adult who consumes vegetables with lead and cadmium (HQ_i <1) contrarily to child (HQ_i >1). First, these HI results confirm that, as reviewed by Kortenkamp *et al.* (2009), there is evidence that dose/concentration addition can produce reliable estimates of combined effects, if the components share either a strictly identical molecular mechanism of action. Feron and Groten (2002) concluded in their review on mixture toxicity that dose addition is indeed appropriate for risk's assessment of a mixture of chemicals with simple similar action. The addition of doses implies that toxicity can be expected if the summed dose is high enough to exceed the threshold of toxicity of the mixture, even when the dose level of each individual chemical is below its own effect threshold. A dose-additive approach was, also, used by Wolansky *et al.* (2009) who showed that sub-threshold doses of individual pyrethroids, when combined in a mixture, produced measurable neurotoxicity in rats. For heavy metals, the combined exposure to metals such as lead, cadmium and arsenic may lead to both additive and synergistic effects, but also antagonistic effects have been described. Among many organs affected by metals, the kidney is one important target organ, which relates to the kidney's ability to reabsorb and accumulate divalent metals (Wang *et al.,* 2008). These results also show that contamination and exposure are serious risks to human health and especially for children. Therefore, children are more exposed since they consume, with relation to their weight, at least twice as much food as adults and that the contaminants are more easily absorbed in their bodies (RCAP, 1996). In this chemicals mixture risk's assessment, the estimated fraction of arsenic in the mixture, is more than 90%, otherwise, the adults would not be seriously in danger, for, HI_m calculated for the adults, without exposure to As, is around 1 (1.58), contrarily for children that is 5.22. However, the inorganic proportion of the arsenic varies from 1 to 70%. This big variation in the evaluation of the inorganic arsenic proportions in food makes difficult this mixture assessment. Yet, it is arsenic in the mixture that creates the risk. This means that, besides the differences associated with age, the proportion of each substance in the mixture (ratios of doses) may affect the response. The difference between HI_m calculated according to method of relative proportions and HI_m calculated without consideration to these fractions confirms this observation. The literature (RECORD, 2011) indicates that other factors may affect the response such as, for example, the order in which the substances are administered; the frequency of exposure; the existence of environmental exposures (alcohol, tobacco); the individual susceptibility (genetic polymorphism, inter-ethnic differences). Otherwise, this difference points out some limits in the use of such models. Also, if these methods effectively predict the toxicity of the mixture in a high proportion, it should remain into minds that cases of synergism or antagonism also exist. So doubts persist on the validity of the models 'results (RECORD, 2011). Aware of that, both US EPA and the French Committee on Toxicity already recommended the need of an assessment based on toxicological and ecotoxicological experimental data on the mixture itself (RECORD, 2011).

Conclusion

Mixtures are one of the unknown forms of toxicity. For this vein, the survey attempted to theoretically assess a sanitary risk of lead, cadmium and arsenic mixture linked to consumption of vegetables from some garden sites in Cotonou (Benin) according to doses/concentrations addition models. The results showed that, chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. The results also revealed that, an assessment of chemicals mixture is depended on the risk assessment model used the composition of the mixture, the dose of each component in the mixture and the characteristics of exposed individuals. About the models, in summary, the hazard index gives the closest results to those obtained by the methods on the mixture, it presents also the advantage to be simple, fast and recognized by the International agencies. But care must thus be taken when using these methods. Nevertheless, the, the conclusion about HI calculated is that, there is a risk of public health linked to consumption of vegetables contaminated by toxics metals in Cotonou. Thus, the adoption of reasonable behavior is needed to associate food security and public health.

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