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Some considerations concerning the theory of combined toxicity: A case study of subchronic experimental intoxication with cadmium and lead

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ABSTRACT

Rats were exposed intraperitoneally (3 times a week up to 20 injections) to either Cadmium and Lead salts in doses equivalent to their 0.05 LD_{50} separately or combined in the same or halved doses.

Toxic effects were assessed by more than 40 functional, biochemical and morphometric indices. We analysed the results obtained aiming at determination of the type of combined toxicity using either common sense considerations based on descriptive statistics or two mathematical models based (a) on ANOVA and (b) on Mathematical Theory of Experimental Design, which correspond, respectively, to the widely recognised paradigms of effect additivity and dose additivity. Nevertheless, these approaches have led us unanimously to the following conclusions:

(1) The above paradigms are virtually interchangeable and should be regarded as different methods of modelling the combined toxicity rather than as reflecting fundamentally differing processes.
(2) Within both models there exist not merely three traditionally used types of combined toxicity (additivity, subadditivity and superadditivity) but at least 10 variants of it depending on exactly which effect is considered and on its level, as well as on the dose levels and their ratio.

1. Introduction

Vegetable foodstuffs produced or gathered in areas polluted with toxicants emitted by chemical and metallurgical industries are virtually always chemically contaminated (in particular, with toxic heavy metals) and rank high up among health risk factors for the residents of these areas (e.g. Privalova et al., 2001). Such contamination involves, as a rule, more than one metal – a fact that places emphasis on the issue of combined risk assessment, which should be scientifically underpinned by understanding of the particular type of combined toxicity in each particular case of this kind. It was shown, for instance, that consumption of potatoes and other vegetables grown in kitchen gardens in a town located close to a big copper smelter (Katsnelson et al., 2010a) or of wild-grown edible mushrooms gathered in the woods near another one (Katsnelson et al., 2011) makes a significant input into the exposure of the respective town populations to lead and cadmium. The same combination is characteristic of ambient air pollution in the vicinity of the above industrial enterprises. Meantime, these metals are highly toxic, and some of their adverse effects are quite similar, for instance, on kidneys.

To characterise combined toxicity, modern toxicology usually operates the terms "additivity", "synergism" (or synergy, or potentiation, or superadditivity), and "antagonism" (or subadditivity). However the exact meaning of each of these terms can vary depending on the underlying paradigm of combined adverse action preferred by a researcher (e.g. Goldoni and Johansson, 2007; Yeh et al., 2009; Katsnelson, 2002; Katsnelson et al., 2010b; Howard and Webster, 2013) or, even if not explicitly, by an Agency (e.g. the US EPA or the ACGIH).

The so-called independence paradigm assumes that a similar effect of two or more substances is due to their action at *different* biological sites, and so the effect of one chemical is independent of the presence of another chemical. The most known mathematical expression for this paradigm in case of exposure to two toxics A and B is the so-called Bliss independence assumption (Bliss, 1939):

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$$P_u(A,B) = P_u(A)P_u(B) \quad \text{or} \quad P_a(A,B) = P_a(A) + P_a(B) - P_a(A)P_a(B) \tag{1}$$

where P_u and $P_a = (1 - P_u)$ are the fractions of the system, unaffected and affected respectively, P_u(A,B) and P_a(A,B) are the results of joint action of A and B.

However, expression (1) is applicable only to the indices that have the meaning of the probability of a certain event (for example, the probability of death after an acute impact) or the proportion of objects that are characterised by this or that change in the status (for example, the percentage of animals with a shift in some index beyond a preset reference level). In the epidemiological literature, a change of such proportion under some harmful (specifically, toxic) exposure is commonly called "response"' rather than "effect".

Expression (1) is not applicable in cases where the result of the toxicant's impact is estimated by a quantitative shift in this or that index for the status of the organism compared with the baseline or the control value. In such cases, for estimating the type of combined impact the central assumption is that of additivity of effects. In case of a combination of two toxicants, this assumption is expressed by the equation:

$$E(A,B) - E(0,0) = [E(A,0) - E(0,0)] + [E(0,B) - E(0,0)] \tag{2}$$

where E(A,B) is the value of an index after a combined action of two toxicants, E(A,0) and E(0,B) are the same index's values when but one of the toxicants is acting, while E(0,0) is the same index's value in the absence of both toxicants (Howard and Webster, 2013). If the actually observed effect of this combination (A + B) is higher or lower than the expected effect E(A,B) for zero interaction (Eq. (2)), we deal with "synergy" or "antagonism", respectively.

Another paradigm, the so-called "Loewe additivity", assumes that two or more chemicals act on the same biological site by the same mechanisms of action, and differ in their potency only (Loewe, 1953). Thus A and B assumingly act as one and the same substance and, consequently, as not entering into interaction. If D_A and D_B are isoeffective doses of these chemicals (e.g. their LD_{50}), the same effect of their combination in doses d_A and d_B can be obtained if

$$(d_A/D_A) + (d_B/D_B) = 1.0 \tag{3}$$

When this sum proves to be >1.0 or <1.0, it points to antagonism or synergism, respectively. It is very popular to represent this paradigm with a graphic analogue called Loewe isobole or isobologram (e.g. Sühnel, 1992; Greco et al., 1995; Katsnelson, 2002; Yeh et al., 2009; Katsnelson et al., 2010b).

The definitions of «additive», «more than additive (potentiation, synergy)» and «less than additive (antagonism)» for combined action developed by a special Expert Committee (WHO, 1981), fully comply with the above paradigm of effect additivity. However, later on the so-called Saariselkä Agreement recommended the use of both (effect additivity and dose additivity) models (Greco et al., 1992). More recently, a report of a WHO/IPCS International workshop on "Assessment of combined exposures to chemicals" (Meek et al., 2009) virtually repeated this duality and reproduced the widespread opinion concerning the mechanistic difference between these two models.[1]

[1] "Chemicals that act by the same mode of action and/or at the same target cell or tissue often act in a potency-corrected "Dose Additive" manner. Where chemicals act independently, by discrete modes of action or at different target cells or tissues, the effects may be additive ("Effects Additive" or "Response Additive"). Alternatively, chemicals may interact to produce an effect, such that their combined effect "Departs from dose additivity". Such departures comprise "Synergy", where the effect is greater than that predicted on the basis of additivity, and "Antagonism", where the effect is less than that predicted on the basis of additivity". It is easy to see, however, that the concept of *departure of dose additivity* is explained here based on the paradigm of *effects additivity*!

Meantime, it was demonstrated that the conformability of experimental data with this or that mathematical model of combined toxicity depends essentially on the shape of the dose–effect (or dose–response) curve for an isolated effect of each substance and on which segment of this curve the added effect of the second substance is considered (Sühnel, 1992; Katsnelson, 2002; Yeh et al., 2009). Moreover, the type of combined toxicity may essentially differ depending on which of the components prevails in the combination quantitatively. In particular, this dependence gives biphasic Loewe isoboles, an example of which (for combined LD_{50} of sodium fluoride and manganese chloride in both mice and rats) was presented by Katsnelson (2002) and Katsnelson et al. (2010b). In that case, the combination proved subadditive when fluoride prevailed in it but superadditive when manganese did. Tajima et al. (2002) also came to the conclusion that the type of combined action of two toxicants depends on their doses ratio. Rozman et al. (1996) evaluated the complex interaction between different doses and time–response using equations showing sigmoid dose–response at constant time and sigmoid and sigmoid time–response at constant dose.

It was postulated also that the type of combined action can depend on the organ or the system of the organism to which the effect considered pertains, as well as on the character of the effect (Katsnelson, 2002). This important aspect of the combined toxicity problem is not often paid due attention to, mainly because the majority of experimental work in this field has involved acute *in vivo* intoxications or *in vitro* models, and only one definite effect has been registered rather than many different effects. One of the examples of another kind is our own study in which subchronic lead–fluoride intoxication of rats was evaluated by about 50 functional, biochemical and morphometric indices pertaining to different systems (Katsnelson et al., 2012). That study confirmed the above-mentioned postulate but the authors assessed the type of combined toxicity only speculatively, without any mathematical modelling. The same may be said about the first presentation by Kireyeva et al. (2006) of the results of an experiment with subchronic lead–cadmium intoxication.

Rai et al. (2010) studied combined neuro-developmental toxicity of lead, cadmium and arsenic in a really chronic experiment using a lot of indices, but their conclusion that these metals act *synergistically* is not based on an explicitly presented mathematical analysis. They only mention that "a combination index (CI) was calculated using the software Calcusyn (Biosoft, Manchester, United Kingdom)" and that "CI values less than 1.0 indicated synergism (Zhao et al., 2004)". However just in that paper (dealing with *in vitro* effects of combination chemotherapy) Zhao et al. (2004) stressed that different ways of data transformation (e.g. log transformation) could diminish accuracy of the combined toxicity assessment or even distort it and therefore objected to using any CI-based software, but strongly recommended non-linear regression models of combined toxicity.

An earlier comprehensive review of the toxicological research into same combination (Wang and Fowler, 2008) underlines that combined "effects were found to be mediated by dose, duration of exposure and genetic factors" but also gives no examples of mathematical modelling corroborating this important statement.

More to the point is the following summarising statement given in the ATSDR (2004) overview document: "The predicted direction of interaction for the effects of these mixtures [Pb–As and Pb–Cd] is not consistent across endpoints. **This observation is most striking for the effects of cadmium on the toxicity of lead.** The predicted direction is greater than additive for the neurological effects (the critical effect) and testicular effects (a less sensitive effect), less than additive for renal and hematological effects, and additive for cardiovascular effects".