The scientific assessment of combined effects of risk factors: different approaches in experimental biosciences and epidemiology

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Abstract (233 words)

The analysis of combined effects of substances or risk factors has been a subject to science for more than a century. With different goals, combined effect analysis was addressed in almost all experimental biosciences. The major theoretical foundation can be traced back to two distinct origins. First, to the work by the pharmacologist Loewe on the concept of concentration additivity and second to the biometrician Bliss and the concept of independent action. In the search for a general solution and a unified terminology the interrelations of the concepts have extensively been studied and experimental findings reviewed. Meanwhile there seems to be consensus in experimental sciences that each concept has its role in predicting combined effect of agents and both are used for hazard und risk management.

In contrast, epidemiologists describe combined effects mainly in terms of interactions in regression models. Although this approach started from a probabilistic model equivalent to the concept of independent action this origin is rarely acknowledged and effect summation is usually the preferred concept nowadays. Obscure biological meaning, the scale dependency of interaction terms as well as unavoidable residual confounding are taken as reasons why no new insights in combined effect analysis are likely to occur from epidemiology.

In this paper we sketch the history of ideas and the state of the arts in combined effect analysis. We point to differences and common grounds in experimental biosciences and epidemiology.

Key Words: combined effect analysis, synergism, antagonism, additivity, independent action

Introduction

The analysis of combination effects has been a subject to science for more than a century. Countless publications were produced in experimental sciences like agricultural sciences, cancer research, enzymology, hygiene, drug research, pharmacology, toxicology and ecotoxicology. These studies had vastly different aims such as exploring mechanisms of action, improving drug therapy, reducing toxic side effects, or proposing risk management strategies. Unfortunately, often neither were references made to earlier work nor was cross-discipline cooperation sought. Hence, fundamental concepts and approaches were reinvented and then applied as novelties in subsequent work without even realizing the conceptual equivalence. Meanwhile several text books and reviews on the topic have been written [e.g. 1,2,3], proposals to unify and standardize methods and terminology were brought forward [4,5], experimental evidence was systematically produced [6,7,8,9], and corresponding improvements of chemical risk management have been suggested (see [10,11,12] for compilations). However, there is still ambiguity with respect to the need of addressing combined effects and what scientific concept should be taken as basis.

Also in epidemiology, the analysis of combined effects is a prevailing topic [13]. In contrast to experimental sciences where combined effect analysis starts from biological reasoning the topic is addressed in epidemiology often in terms of interactions in regression models [14]. Given the scale dependency of interaction terms as well as residual confounding it is debated whether epidemiological approaches in general are sufficient for a reliable assessment of combined effects [15].

This paper was stimulated by a workshop of the German Society of Medical Informatics, Biometry and Epidemiology aiming at an exchange on approaches to combined effect analysis in experimental sciences and epidemiology. In this paper we sketch the history of ideas in order to look for respective differences and common ground in both fields. We follow the ideas in a loose historical way starting from experimental sciences. We retain the terminology used in experimental sciences throughout this paper and will therefore be speaking of “agents” whose effects on organisms are studied in dependence of certain

“concentrations” or “doses”. However, the wording agent-effect-relationship can easily be translated to factor-risk-relationship which is more familiar in epidemiology or public health. The concepts for the assessment of combined effects apply rather generally. In what follows we start by introducing the fundamental concepts used as references in the analysis of combined effects, summarize the confusing terminology, and outline the search for the right concept. After sketching how combined effect analysis is addressed in epidemiology we discuss differences and common grounds.

Concepts for analyzing combination effects

Combined effect analysis is dominated by terms like synergism, potentiation or antagonism. Often without a clear definition, these terms are intuitively used to denote that an observed effect is higher or lower than expected. But what effect size can be expected when organisms or populations are exposed simultaneously to more than one substance or risk factor? The assessment of combined effects therefore relies on biological concepts describing how effects of single substances/factors translate to joint effects.

The major theoretical foundation of the scientific assessment of combined effects can be traced back to two distinct historical origins [16]. First, there is the well known and frequently cited work by Loewe and Muischnek [17] leading to the isobologram method and founding what later was termed the concept of concentration additivity. Second, the statistical background of combined effect analysis was introduced by Bliss [18] whose ideas were elaborated in a series of publications [19,20,21] and were communicated as the concepts of simple similar action and independent action.

Concentration Addition (CA) is given by

$$c_1/EC_{x,1} + c_2/EC_{x,2} = 1 \qquad (1)$$

with c_i denoting the applied concentrations (of substance/factor 1 and 2, respectively) and EC_x their individual concentration that provokes a certain effect x., e.g. the effect concentration 50% (EC_{50}). CA is based on the assumption that any constituent of a mixture

or exposure can be replaced totally or in part by the same "toxic unit" (e.g $c_1/EC_{x,1}$) of another with the effect of the mixture remaining constant. So in the simplest case, according to CA the individual mixture components behave as if they were dilutions of each other. When interpreted in view of the mechanism of action, concentration addition is taken to be applicable if the substances have an identical molecular mechanism and hence display a similar mode of action. In case the concentration effect relationships of the agents are parallel (in a certain sense) equation 1 takes a simple explicit form which is known as simple similar action. Several indexes have been introduced to assess deviation from additivity. The so called additivity index [22] and the mixture toxicity index [23] have been found especially useful.

The concept of Independent Action (IA) is given by

$$P_{1,2} = P_1 + P_2 - P_1P_2 \qquad (2)$$

with P_i denoting the effects/risks of substances/factors 1 and 2, respectively, caused when present singly at the concentration at which they are present in the mixture. IA is therefore given explicitly and the combined effect is directly calculated from the effects of the single agents. From a biological point of view IA is based on the idea that agents contribute to a common endpoint but act upon different subsystems within the same organism, in short: having different sites and modes of action. A plethora of different names has been used for IA such as simple independent action [19], response addition [24], multiplicative survival model [25] effect multiplication [26], response multiplication [27] and even effect summation [28].

A third reasoning has greatly influenced and at the same time confused the discussion on the analysis of combined effects. As a naïve approach combination effects were often expected to equal the sum of the individual effects, say

$$P_{1,2} = P_1 + P_2 \qquad (3)$$

Effect summation appears as a special case of IA and according to Plackett & Hewlett [20] applies as such to target organisms with negatively correlated susceptibilities (see below). In

general however, a closer inspection of Eq. 3 reveals disturbing short-comings of this approach which furthermore lacks pharmacological plausibility. Nowadays, most researchers in experimental sciences seem to agree that combined effects do not simply equal the sum of single effects and that therefore this model does not provide a reasonable reference line although the equation may appear intuitively plausible and is easy to handle.

The concepts are not limited to two agents only and the equations 1, 2 can easily be generalized to n substances or factors [7].

Consequences from co-existence of concepts - confusion of terminology

According to many reviews of the field the non-uniform and inconsistent use of terms used to label combination effects has been a main reason for confusion and misunderstandings [1-5]. Table 1 gives an overview on terms that have been used in the literature. Not only were various terms used to address the same type of combined effect but also were the same terms used for different understandings. Not surprisingly, re-analysis of the very same data even led to apparently conflicting results by different authors [16]. The terms synergism, antagonism and potentiation are particularly prone to misunderstanding. For example, up to seven different types of antagonisms and synergisms can be found in literature [29,30]. Whereas synergism usually is meant to denote a greater than additive effect, some authors consider this to be a special case of additivity [16]. Also no clear distinction is made between synergism and potentiation. While it is widely accepted that potentiation refers to a combined effect that is greater than a synergistic effect, both terms are also used synonymously [31]. Based on pharmacological considerations, it was even proposed to regard potentiation a special case of antagonism [32]. Also the term ‘interaction’ is used with numerous different meanings. Given the different use in pharmacokinetics and -dynamics and in biostatistics it is unlikely that it can be used in a clear and meaningful way for general use. It was therefore repeatedly proposed to refrain from using the term interaction at all in combination effects analysis. This proposal has also been made for epidemiology [15].

Without a precise definition many terms are used rather intuitively so newcomers are inclined to understand ‘additivity’ as equivalent to simple summation of effects while others misinterpret ‘independence’ or ‘non-interaction’ as toxicologically irrelevant. A standardisation of terminology is still not achieved despite continuous discussions and several proposals [5,16,33,34,35,36]. Nowadays it is held that statements on the type of combination effects need to answer first to the question what effect is expected by the combination of agents, namely which biological concepts is taken as the reference. It has therefore been proposed to refer to the reference model by using the terms “Loewe additivity” when equation 1 and “Bliss independence” when equation 2 is meant [5].

The quest for the right concept

None of the concepts introduced for the analysis of combination effect found support by all researchers. There is a still ongoing debate on which of the two concepts is the “better” or even the “correct” concept. In this debate several touchstones are addressed: 1. the role of mechanisms and modes of action of agents, 2. the case of a sham combination, and 3. notions of causality.

Since the concept of concentration addition as well as independent action is derived from basic pharmacological reasoning it has been questioned what role pharmacological similarity or dissimilarity plays in order to select the most suitable concept. On the one hand, it is not clear from the concepts whether an identical or different site of action is a characteristic of the agents or of the biological system. Can there be two agents that do not interfere with each other at all but still contribute to an integral effect such as death? Do agents acting at the same site always show similar (e.g. parallel) concentration-response-curves? Neither concept provides mechanistic explanations for the joint action in complex systems. On the other hand the terms ‘similar’ and ‘dissimilar’ effect are used in the literature with various degrees of stringency. In pharmacology, strictly speaking, an identical molecular mechanism of action at the same substructure of an acceptor has been proposed as a prerequisite for a

similar effect of different substances, and correspondingly dissimilar effects would involve different mechanisms of action [2]. Other authors require that a similar effect is localized at least in the “same site of primary action”, while a dissimilar effect is distinguished from this by the criterion of differences in a substances’ fundamental structure (different “parent compounds”) [37]. However, recent research has shown that mixtures which were designed of components known to act either by identical or by completely different molecular mechanisms of action were in fact better predicted by concentration addition or independent action respectively [7,9,38,39].

The idea of a “sham combination” has repeatedly been used as a touchstone in the search for the right concept. A sham combination describes a thought experiment in which an agent is combined with itself, e.g. a dilution of the same substance. As the sham combination is literally the agent simply in another concentration the expected combined effect should equal the effect of that agent in the total concentration, say show concentration additivity. However, based on the concepts of independent action or effect summation the sham combination will often result in a synergistic or antagonistic effect. This counterintuitive result was taken as proof for the inconsistency of the concept of independent action [5,40].

In epidemiology, notions of causality derived from the sufficient cause model have been introduced as another touchstone. We will discuss this later.

Interrelationships and the importance of concentration-response-curves

All arguments for and against each concept for the analysis of combined effects have been exchanged for quite some time. Given that the two concepts of concentration additivity and independent action are considered legitimate reference standards it is hence straight forward to study the interrelation of the concepts.

Plackett & Hewlett [20] introduced a generalised model of correlated independent action (eg 4). For a combination of two agents with concentrations c_1, c_2 the response surface $P_{1,2}$ was modelled by the bivariate normal distribution f. The correlation coefficient Φ was used to

differentiate 3 subtypes of independent action.

$$P_{1,2} = 1 - \int_{y_2}^{\infty} \int_{y_1}^{\infty} f(c_1, c_2, \phi) dc_1 dc_2$$

(eq. 4)

with	$\phi = 0$	$P_{1,2} = P_1 + P_2 - P_1 \cdot P_2$	Independent Action
	$\phi = 1$	$P_{1,2} = \max(P_1, P_2)$	„no addition“
	$\phi = -1$	$P_{1,2} = P_1 + P_2$	effect summation

Within this model uncorrelated susceptibility of organisms leads to the (simple) independent action of eq. 2, whereas in case of a total correlation of susceptibilities the effect of the combination equals the effect of most potent agent. The latter case has also been termed “no addition” by Könemann [13] and bridges as part of his mixture toxicity index to the concept of concentration additivity. The subtype of total negative correlation is especially interesting as it is one of the rare theoretical foundations of the model of effect summation.

An often cited quantitative relation of eq. 4 was derived by Wahrendorf and Brown [41] showing that effect summation always predicts equal or greater combined effects than independent action which in turn predicts a mixture effect that equals or is greater than the effect of the most potent agent.

Interrelations between independent action and concentration addition can not be derived generally but depend on several factors. Both concepts for combined effect analysis assume that concentration-effect-relationships can be modelled and effects (in case of eq. 2) or effect concentrations (in eq. 1) can be extrapolated from monotonous concentration-response-curves fitted to the experimental data. Combined effects analysis in experimental sciences is very much centred on the study of these concentration-effect-relationships. To this end, it is aimed at an experimentally complete description of these relations, meaning that concentrations of single agents as well as the combinations are chosen to produce effects

covering the full response surface well spaced between no and 100% response. The better the relation is described the better justified is the choice of a concentration-response curve to extrapolate. Standard regression models make use of e.g. the functions of the normal distribution (Probit analysis), the logistic distribution (Logit analysis) or the Weibull distribution (Weibit or Gompertz analysis). Several additional functions are available to fit concentration-response data even when standard models are not suitable e.g. when special focus is on the estimation of low or high effect concentrations [42]. These functions have the common feature of being applicable to a monotonic sigmoid or hyperbolic concentration-effect relationship, i.e., when effects increase with increasing concentrations or doses. Although the fundamental curve types (say linear, exponential or sigmoid) are easy to detect it is sometimes ambitious to differentiate functions within the types [42]. Finally, concentration response curves are different for different outcomes as well as for different risk measures, so changing scope from risk to risk ratios would mean changing the shape of the factor-effect curve.

In combined effect analysis it was discovered early that the quantitative relations between the concepts for the assessment of combined effects depend on features of the concentration response curves. Berenbaum [4] showed that the concept of concentration addition equals the concept of effect summation for linear and the concept of independent action for exponential dose-response-curves leading him to the judgement that concentration addition is the general solution in combined effect analysis. Christensen and Chen [27] gave proof that for Weibull-type dose-response curves concentration addition always predicts greater combined effects than independent action in case of steep curves (steeper than exponential) and vice versa for flat curves. Drescher and Boedeker [43] provided more general relations for normal and logistic curves showing that the concepts interrelations not only depend on the steepness of dose-response curves but also on the concentrations and effect levels. By making use of these known relationships worst case scenarios for risk assessment based on a prediction concept can be derived [44]. If detailed information on concentration response curves is not available at least an overall assessment of the

deviations is possible. The maximum factor by which predictions of a combined effect based on CA compared to IA differ equals the number of agents combined [45].

Combined effect analysis in epidemiology

In epidemiology, there is also quite a history of combined effect analysis. This discussion is centred on the term interaction and usually addresses the case when effects of an exposure to one agent differ across strata of another exposure. In a regression model a product term will be needed to address this heterogeneity. Unfortunately, interaction is dependent on the scale and a “no interaction” in a multiplicative model as used e.g. for calculating risk ratios will at the same time show up as an interaction in an additive model used e.g. for calculating risk differences. Furthermore, various understandings of interaction among statisticians and epidemiologists have added to the confusion of terminology in combined effect analysis [13]. Despite the broad coverage of the topic of interaction in textbooks the usage is still rather unsatisfactory in present publications. Knol et al [14] found in a recent study that in a majority of articles interaction between exposures were explicitly addressed but in about half of these papers information provided was insufficient for a valid assessment.

However, there seems to be no longer any controversial discussion in epidemiology on the general concepts of interaction as coined in the writings of Rothman and Greenland. This was different in former times. Rothman [46,47] derived from the idea of independence of causes that a combined effect can only be considered to be synergistic, when two factors jointly have an effect which is greater than the sum of the effects of each factor separately. This reasoning that effect summation is the only concept for adequate evaluation of the causal nature of combined effects was later questioned by several authors [48,49]. In these publications different and more flexible reference models were considered and the importance of the chosen effect measures was pointed out. It was argued to not restrict considerations to a single model only, but to fit data by the most plausible biologic models. Basically, the struggle was about whether there is a scale which is generally more suitable to

assess biologic interaction [13]. Rothman et al. [50] proposed to settle controversies by specifying the context in which interactions are studied. According to this proposal there is no need for any biologic foundation of the models in statistical contexts that mainly aim at prediction. Furthermore, in biologic contexts a definition for interaction or synergism is deemed not necessary as these terms do not provide information on the mechanism of agents. In public health context, it was finally suggested that synergy should be interpreted as departure from additivity of rate differences whereas for individual decision making departure from additivity of risk differences should be the reference.

Interestingly, the preference for the concept of effect summation was concluded from the model of independent action (eq. 2) only by making the assumption that small effects are studied [47]. Nowadays, the appropriateness of the concept of effect summation is concluded from the sufficient cause model [51]. VanderWeele [52] defined a sufficient cause interaction to be present between two factors if there is a sufficient cause in which both factors are present and derived rules to conclude the presence of sufficient cause interaction from statistical interaction.

Basis for theoretical analysis in epidemiology is the simple setting of a dichotomous effect which is studied as provoked by dichotomous factors. From that scenario all possible response types are derived and reflected with respect to interaction types [51,53]. However, although the response type approach helps to lighten interaction phenomena it does not provide a reference for a 'true' concept for analysis. E.g. the so called "causal synergism" response type of a combination effect of two factors showing no effect singly (at the cut-off for dichotomisation) could also follow from concentration additivity and this response type would then be regarded as a classical example for no interaction. So, for translating response types in combined effect terminology a definition of a reference model is still needed.

In epidemiologic publications, only little notice has been taken of the previous work in experimental biosciences. Kupper and Hogan [54] did one of the rare acknowledgements of the dose additivity concept by a hypothetical example. The example is in fact that of a sham

combination showing that summation of effects can give highly implausible results in the case of non linear dose response relations. Kodell & Gaylor [55] compared independent action to dose additivity and noted that both concepts agree for linear dose-response-relations. Miettinen [49] acknowledged the more general concept of independence (eq. 4) [20] and the relations given by Wahrendorf & Brown [41]. He pointed out that the ideas of correlated susceptibilities ask for another reference model than simple effect summation.

Discussion

The sketch of ideas brought forward in the scientific analysis of combined effects in experimental sciences and in epidemiology shows that very different lines were followed with little cross-referencing and so far resulted in very different approvals of the state of art. Experimental sciences claim their potential in disclosing principles of combined effects in a degree of confidence that is sufficient to encourage regulations [12]. In contrast, from an epidemiologic point of view the scientific means are considered rather modest. It might therefore be interesting to see whether there is common ground.

First, touchstones like models of mechanism of action, the sham combination or causal models seem to have no convincing strengths to opponents as all of them start with an arbitrary definition. Touchstones are derived from an idea and are designed to prove agreement with that idea which itself remains untested and untestable. Concluding effect summation to be a reference model from sufficient cause models might therefore be considered the same tautological approach as requiring sham combination qualities for agents that act independently. However, lack of a touchstone for the right concept is a problem only if synergism is considered a biological phenomenon whose identification must not be dependent on models and scales. However, we prefer to judge synergism as a scientific construct related to a defined concept of combined effects. These concepts can be expressed in different mathematical models which make use of different concentration-response-curves. Scale and model dependency in this view follows from interaction concepts

rather than limits the knowledge process. Given the complexity of agents-effects relations on the different, interlinked levels of biological complexity, from molecules to individual organisms, and finally to whole populations, the search for natural laws of interaction finds little comfort in simple models of causality.

Secondly, experimental sciences and epidemiology find common ground also in the request to explicitly specify the aim of combined effect analysis. In experimental sciences two main aims can be differentiated: (i) the study of mechanisms of combined action and (ii) the description and prediction of combined effects. Addressing the first aim means that experimental data are checked for agreement or disagreement with specific mathematical models and agreement e.g. with a model of simple similar action would be taken as evidence that agents interfere with the same site of action and have a similar mode of action. Although this approach is rather common in drug-receptor studies it is limited as many mechanisms can lead to experimental data which can be fitted by the same mathematical model. In combined effect analysis it is even worse as agreement with a model could also mean that interaction deterred the effects from another - correct - reference model, and vice versa. Kupper and Hogan [54] phrased this assessment dilemma as ".. any observed significant interaction (or synergic) effect may be nothing more than an indication that the wrong (null) model was assumed in the analysis". So, agreeing with Rothman et al. [50] who addressed the aim of mechanism of action studies as the context of biologic interaction nothing can be learned from terms like synergism in this situation.

In contrast, the description and prediction of combined effect is primarily not interested in how combined agents act but whether the combined effects are more/less than expected from the effects of the single components. The focus is on optimizing wanted effects or safeguarding against unwanted effects. Describing combined effects is often done on a case-by-case basis. For example, in Germany, lung cancer from the combined exposure against asbestos and polycyclical aromatic hydrocarbons is now legally considered an occupational disease when individual exposures exceed limits derived from an additive model. In epidemiology, this approach might also be useful for scoring risk profiles. The prediction of

combined effects focuses on the expectation rather than on the deviations from the expectation. Prediction typically calls for simple instruments to enable prospective risk management and regulations taking combined effects into account. A prospective model should have been proven suitable to cover a broad range of substances. At the same time the model should be sufficiently simple for being included in legal procedures and indicators should be available to characterize respective agents [9]. A suitable concept for addressing combined effect in risk management might as well be based on worst case scenarios [44]. This approach is different from the prediction in the context of statistical interaction as laid out by Rothman et al. [50] because mathematically demanding models might not be used in regulatory practise – even if they have a high predictive power.

Thirdly, experimental sciences and epidemiology share common problems in combined effect analysis. It is usually taken that in epidemiology effects of single agents can never be observed because there is always a background exposure which might modify or confound. From this perspective, epidemiological studies are always combined effect studies. In contrast, in experimental sciences it is idealized that a causally homogenous background is provided e.g. by genotypic standards of test organism, random allocation of treatment groups, and high standards in the physical or chemical properties of agents. Hence, introducing a factor into this experimental setting is considered the only variation and observed effects are completely attributed to this factor. However, e.g. test chemicals can never be applied without impurities, galenic supplements or solvents, physical exposures are provoked by tools and measures have to be taken to account for avoidance strategies of organisms. In ecotoxicological field studies confounding factors may be present when parts of natural ecosystems (e.g. ponds, streams) are chosen as experimental platforms. After all, the epistemiological framework for the component based analysis of combined effects does not seem to be so much different in experimental sciences and in epidemiology. Experimenters however, tend to ignore these restraints.

A clear distinction between approaches of combined effect analysis in experimental sciences and epidemiology lies in the study design. Whereas in the former knowledge of the whole

concentration-response surface of single agents as well as their combination is thought to be indispensable this is rarely attempted in epidemiological studies. Even theoretical analysis is typically constrained to dichotomous factors. From a concentration-response perspective little can be learned from this design as the responses can not be followed on the concentration–response curve. Furthermore, concentration additive joint effects can result even from no effect concentrations of single agents [8,56]. Hence, making use of elements of dose-response analysis might add to the tools of combined effect analysis in epidemiology.

Finally, little use has been made from quantitative interrelations of the concepts in epidemiology so far. The preferred use of effect summation as the reference line may be a handy approximation, beyond any reflection on the true nature of combined effect. In case of linear concentration response curves which are assumed e.g. for the estimation of risk differences the concept of effect summation would lead to identical results as concentration addition. However, as has been pointed out already, effect summation may lead to strong underestimation of combined effects for low doses and therefore gives no worst-case-estimation.

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Table 1: Commonly used terms for combination effects

Term	Combined effect meant to be
augmentation, enhancement, potentiation, sensitation, superadditivity, supraadditivism, synergism, synergy	greater than expected
additivity, additivism, independence, indifference, non-interaction, summation, zero-interaction	as expected
antagonism, antergism, depotentiation, desensitation, infraadditivity, negative synergism, non-interaction, potentiation, subadditivity, zero-interaction, no addition	less than expected