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Combined exposure to low doses of pesticides causes decreased birth weights in rats

Ulla Hass[a,*], Sofie Christiansen[a], Marta Axelstad[a], Martin Scholze[b], Julie Boberg[a]

[a] National Food Institute, Technical University of Denmark, Division of Diet, Disease Prevention and Toxicology, Kemitorvet, Building 202, 2800 Kgs. Lyngby, Denmark
[b] Brunel University London, Institute of Environment, Health and Societies, Dept of Life Sciences, Uxbridge, Middlesex. UB83PH, UK

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ABSTRACT

Decreased birth weight is a common effect of many pesticides in reproductive toxicity studies, but there are no empirical data on how pesticides act in combination on this endpoint. We hypothesized that a mixture of six pesticides (cyromazine, MCPB, pirimicarb, quinoclamine, thiram, and ziram) would decrease birth weight, and that these mixture effects could be predicted by the Dose Addition model. Data for the predictions were obtained from the Draft Assessment Reports of the individual pesticides. A mixture of equi-effective doses of these pesticides was tested in two studies in Wistar rats, showing mixture effects in good agreement with the additivity predictions. Significantly lower birth weights were observed when compounds were present at individual doses below their no-observed adverse effect levels (NOAELs). These results emphasize the need for cumulative risk assessment of pesticides to avoid potentially serious impact of mixed exposure on prenatal development and pregnancy in humans.

1. Introduction

1.1. Background

Human risk assessment of chemicals is largely based on the No Observed Adverse Effect Levels (NOAELs) which are derived from experimental studies of exposure to individual chemicals in animals. However, humans are typically exposed to more than one chemical at a time. For mixtures of endocrine disrupting chemicals including pesticides, there is experimental evidence showing that substantial mixture effects on reproductive development can occur even though each of the individual chemicals is present at doses at or below their NOAELs [1–3]. For example, we have found adverse effects on male sexual development and gestation length after combined developmental exposure of rats to endocrine disrupting pesticides at dose levels below NOAELs of the individual pesticides [4,5].

Low birth weight is a marker for a non-optimal prenatal development in humans and experimental animals. Perturbations to this environment can have detrimental effects on the foetus and lead to persistent pathological consequences later in life [6], manifested in the "Barker hypothesis" on developmental origin of adult disease [7]. Low birth weight is generally considered as a predictor for increased risk of several diseases later in life, including obesity and type 2 diabetes [8]. It is suggested that this is caused by fetal programming, i.e. physiological adaptations in response to changes in the environment to prepare for postnatal life [9]. A recent Danish study on children of women who worked in green houses and thus were likely exposed to mixtures of pesticides has found lower birth weights in exposed children, but increased body fat accumulation from birth to school age [10].

Reduced birth weight is a common effect for many pesticides in experimental studies, but there is no empirical evidence on combined developmental exposure to low doses of pesticides that can decrease birth weight. The so-called component-based mixture approach for the toxicological assessment of pesticides anticipates the effects of a mixture on the basis of the toxicity of its components, and therefore allows quantitative predictions of mixture

* Corresponding author.
E-mail address: ulha@food.dtu.dk (U. Hass).

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toxicities, without the need to test different mixture ratios at different mixture doses. Different concepts exist for the calculation of mixture effects on the basis of the toxicity of its components, and there is no scientifically robust data available for evaluating potential mixture effects on this endpoint and for selecting the best model for predicting the mixture effects. This study aimed to test the hypothesis that a mixture of six pesticides (cyromazine, 4-(4-chloro-2-methylphenoxy) butanoic acid (MCPB), pirimicarb, quinoclamine, thiram, and ziram) can cause decreased birth weight at doses below their individual NOAELs, and that these mixture effects can be predicted by the Dose Addition model.

1.2. Rationale for mixture study

Decreased birth weight after exposure to pesticides is often reported in regulatory studies submitted for approval of pesticides. It is a developmental toxicity effect that may be induced via different and in most cases unknown mechanisms of action. This complicates the choice of the best possible component-based mixture model, namely Independent Action (IA) and Dose Addition (DA). Both models rely on an additivity assumption, which is based on the expectation that all chemicals in the mixture exert their effects without influencing each other's action. DA is based on the idea that all components in the mixture behave as if they are simple dilutions of one another [11]. In contrast, IA is commonly thought to apply in cases where the compound exerts their effects through strictly independent, i.e. dissimilar mechanisms [12]. These competing toxicological assumptions are also reflected in their different data demands: to apply both models for predicting a mixture dose causing a 10% decrease in birth weight (ED_{10}), DA would require from all mixture compounds knowledge about their individual ED_{10}s, a data scenario that is manageable from a risk assessment point of view. In contrast, IA would demand from all compounds effect estimates smaller than a 10% effect change, and the more compounds are present in a mixture, the lower the individual effect estimates become that are required as input values for the calculation of an IA mixture response. These experimental demands for IA are beyond what is technically achievable with the number of animals per dose group normally used in regulatory toxicity studies, and were not achievable in the current study where information about the individual compounds were derived not directly from experimental data, but rather from reports with summarising data descriptors. Consequently, we considered only DA as an option to predict the responses from a mixture of pesticides on birth weight.

The use of DA as a pragmatic approximation for the prediction of mixture effects of also non-similarly acting chemicals seems to be justified, as there is no current empirical example of a situation in which the concept of IA provides an accurate prediction that is also more conservative (i.e. cautious) than DA, supporting the use of DA as a conservative default in cumulative risk assessment [13,14]. Furthermore, an analysis of the quantitative difference between predictions based on DA or IA suggested that the differences that might be expected in practice in this study are small.

For this mixture study, the following steps were applied to test the hypothesis that the joint effects of the pesticides on birth weight can be predicted by DA:

1) Selection of six pesticides for which clear decreases of pup birth weight has been reported at dose ranges without any signs of maternal or other toxicity. All data were obtained from regulatory studies (Draft Assessment Reports).
2) Dose-response data from each pesticide were analysed by nonlinear regression modelling.
3) For a mixture composed of pesticides in equi-effective doses, the expected mixture responses were predicted by DA, and then used for the experimental planning of the mixture studies.
4) Two mixture studies (range-finding and main study) were performed, both in a fixed-ratio design. The range-finding study was conducted to identify and avoid mixture doses in the main study that would cause marked maternal toxicity or marked effects on pregnancy parameters, such as litter size and pup survival.
5) Comparison of the predicted and observed mixture effects and evaluation of DA as tool for the cumulative risk assessment of pesticides.

2. Materials and methods

2.1. Selection of pesticides

Relevant data on birth weight were available from prenatal developmental toxicity studies [15] or one- or two generation studies [16,17] as described in risk assessment reports for regulatory use. This data selection was considered ideal for investigating the application of DA for regulatory purposes while limiting the number of test animals. In an evaluation based on the Draft Assessment Reports (DARs) of 224 approved pesticides in EU around 175 caused decreased fetal or birth weight [18]. Pesticides were selected for inclusion in the mixture studies only if their reported decreased birth weight was observed at dose ranges without clear signs of maternal toxicity. This was done to avoid combination toxicity on the dams or reductions in the number of offspring. It was kept in mind that decreased maternal weight gain during pregnancy can often be listed as indicative for maternal toxicity, but in reality may be due to decreased fetal weight. Other reported toxicity in dams, e.g. liver toxicity, was also avoided. Pesticides showing effects on birth weight at more than one dose with a clear dose-response relationship were prioritized to improve the robustness of regression modelling. The six pesticides selected for the mixture testing were cyromazine, MCPB, pirimicarb, quinoclamine, thiram, and ziram.

2.2. Test compounds

The vehicle used were corn oil (product no. C8267-2.5L from Sigma/Aldrich. The test compounds were Cyromazine (97%) CAS: 66215-27-8 (product no.:551295-25G from Sigma/Aldrich), MCPB (99.8%) CAS: 94-81-5 (product no: 36145-20G from Sigma/Fluka), Pirimicarb (98.7%) CAS: 23103-98-2 (product no: 45627-15G from Sigma/Fluka), Qinoclamine (99.9%) CAS: 2797-51-5 (product no: 32719-3G from Sigma/Fluka), Thiram (99.9%) CAS: 137-26-8 (product no 45689-5G from Sigma/Fluka), Ziram (98.2%) CAS: 137-30-4 (product no: 45708-5G from Sigma/Fluka).

The same batch of substances was used in both the range-finding and the main study.

2.3. Dose-response modelling and benchmark dose estimation

Only mean values for birth weight changes have been reported, consequently only mean birth weight changes were used for data analysis. When data were available from more than one study on the same pesticide, the reported absolute weight values data were normalized by their control mean to a relative effect scale and pooled. If results were reported for both genders separately, an overall mean from both were used. Nonlinear regression analyses were performed using the best-fit approach [19], i.e. a variety of nonlinear regression functions were fitted independently to the same data set and the best-fitting model was selected using a statistical goodness-of-fit criterion. Dose-response data and regression curves are shown in Fig. 1. Five percent effect doses (ED_5) were derived from these regression fits in order to establish the mixture