FOUR DIFFERENT DOSE-RESPONSE CURVE SHAPES FOR ENDOCRINE-DISRUPTING CHEMICALS- CONSEQUENCES FOR RISK ASSESSMENT

Daniel M. Sheehan

Daniel M. Sheehan and Associates, 1422 Scott St. Little Rock, AR 72202

Introduction

Risk assessments for non-genotoxic chemicals, including endocrine disruptors, make traditional assumptions in assessing dose-response data. These assumptions include: 1) a threshold exists, below which there are no adverse effects in exposed populations, and 2) there is a monotonic dose-response curve [1]. In practice, experimental studies use a limited number of animals and dose levels. Generally, a narrow range of high doses is used in order to maximize detection of adverse outcomes. Similar guidelines have been in place for almost a half-century. However, evidence from wildlife populations exposed to low concentrations of a mixture of chemicals demonstrates a wide variety of endocrine-related abnormalities, many of which are developmental in nature [2]. These findings, together with laboratory studies [1], show that chemicals can be active at doses orders of magnitude below the accepted safe dose derived from high dose studies. Four prototypical dose-response curve shapes (no-threshold Michaelis-Menten, no-threshold linear, non-monotonic, and threshold) have been found in the literature [3].

This paper examines some consequences for risk assessment of using standard dose-response analysis procedures on the first three curve shapes.

Materials and Methods

Details of the curve shapes can be found in the cited literature.

Results

No-Threshold Curves

A. Michaelis- Menten Curves

We conducted a very large study of estrogen-induced sex-reversal in developing red-eared slider turtles, in order to clearly test the threshold hypothesis [4]. In these animals, egg incubation temperature determines sex; males develop at relatively low temperatures and females at higher temperatures; intermediate temperatures produce both sexes. Estradiol administered to the eggshell at a male-determining temperature generates females; males resulted when eggs were treated with two different aromatase inhibitors at a female-determining temperature [4]. These

chemicals inhibit estradiol production. This model allowed a test of Hoels' finding that if an endogenous chemical causes an effect, there can be no threshold when the same chemical is administered; the threshold has been exceeded by the endogenous chemical [5]. Seven estradiol dose groups and a control, each with 300 eggs, were incubated at a temperature providing a small fraction of females. The lowest dose, 40 ng/Kg, sex-reversed 14.4% of the animals. The smooth curve fit a modified Michaelis equation and regressed through the Y-axis, striking the negative X-axis at an endogenous dose of 170 ng/Kg of estradiol equivalents. To our knowledge, this was the first test of the no-threshold hypothesis in which all three necessary elements can be demonstrated. These are: 1) the presence of an endogenous chemical which 2) controls an irreversible event with 3) a dose-response curve that regresses to the origin after accounting for the effect of the endogenous dose. The findings provide direct evidence that no threshold exists for this effect, and provide as well as an estimate of the dose of the endogenous chemical. Subsequently we found 26 dose-response curves in the literature that also fit a Michaelis-Menten equation, which was further modified to account for background not controlled by hormone [1]. A form of this equation was derived that normalized dose to the ED_{50} (X-axis)and response to the maximal response (Y-axis) for each data set. This allowed all 178 data points from the reviewed studies to be plotted on the same graph. The Michaelis-Menten dose-response line fit to the aggregated data set displayed good statistics and regressed to the origin [1]. Several other examples of published no-threshold dose-response models were also discussed, among them dioxin [1].

B. No-Threshold Linear Dose-Responses

A number of linear dose-response curves that regress to the origin have been recovered from the literature (Sheehan and Blair, unpublished observations). For example, di-(n-butyl) phthalate decreases ano-genital distance in prepubertal rats, regressing to the control with an $r^2 = 0.99$ [6]. This dose-response curve shape for non-genotoxic effects is not frequently reported as such, although it is not infrequent in the literature.

Non-Monotonic Dose-Response Curves

Non-monotonic dose-response curves have also been reported in the literature. We ovariectomized rats and implanted them with a wide range of estradiol doses in Silastic tubes. We then measured uterine estrogen receptor (ER) levels [7]. ER was increased by 33% over controls at low doses, and decreased to 50% of controls at high doses, providing an inverted-U dose-response curve. The up-regulation of the ER occurred at doses below those that increase uterine weight. Additionally, low maternal doses of estrogens increase fetal prostate weight while high doses decreases it; permanent changes in morphology also were defined [8] (see also the paper by vom Saal in this volume). It has long been recognized that Vitamin A, in either deficiency or excess, induces malformations in the same seven organ systems [9,10]. Non-monotonic curves are not unusual in the endocrine system, and may be the result of two regulatory events, each with a separate dose-response curve, which in sum provide a non-monotonic curve.

Discussion

Risk Assessment Consequences of No-Threshold Dose-Response Relationships

Dose-response curves that smoothly regress to the control value are expected if an endogenous chemical is already active. The explanation is that the administered chemical possesses hormonal activity that adds to an endogenous background of that activity; as shown mathematically by Hoel, every dose, no matter how low, will exhibit activity [5]. One mechanism is via binding to a receptor that mediates the activity; clearly, other mechanisms, e.g., enzyme inhibition, could lead to the same lack of an apparent threshold. Each chemical that binds to such a receptor and acts as an agonist will increase activity in a manner dependent on, among other things: 1) the endogenous chemical concentration (endogenous dose) in the target cell; 2) the response elicited by the endogenous dose 3) the receptor concentration; 4) the exogenous chemical concentration; 5) the chemicals' affinities for the receptor; 6) the response intensities due to the endogenous and exogenous chemicals; and 7) the shape of the dose-response curve.

In risk assessments for non-genotoxic chemicals, a threshold is assumed to exist. This is the justification for the use of the statistically limited NOAEL. The NOAEL is then divided by an uncertainty factor (often 100) to obtain a dose that is assumed to be without risk. Only one point, and not the curve, is used in this procedure. More recently, a benchmark approach has been developed that utilizes all the data points [11]. Nonetheless, this procedure assumes that a safe dose exists and that application of factors to account for intra- and inter- species variability results in a dose that carries no added risk. On the other hand, no-threshold models assume a risk exists at every dose, no matter how low. Responses occurring in the lowest 10% response range of the Michaelis-Menten curve are virtually proportional to dose. The second form of dose-response curves are those that regress linearly to the origin. It is possible that some linear dose-response curves may be the low dose linear section of a Michaelis-Menten curve. Regardless of origin, risk is expected at any dose for Michaelis-Menten or linear responses, and chemicals acting through the same mechanism will add to the risk, with the incremental increase in risk being dependent on where the dose falls on the dose-response curve.

Because exposures to mixtures are common, dose additivity of agonists is expected. Thus, no dose is without effect under these conditions, and chemicals with similar activity will display additive effects. This conclusion is a direct challenge to current policies that 1) assume a safe dose exists and 2) that current risk assessment procedures are adequate to define the safe dose.

Confidence in the concept of a threshold is so strong in the Toxicology community that testing the presumed safe dose range is not required or considered. This confidence is of particular concern, as an assumption is a very weak scientific statement: a hypothesis is a stronger statement than an assumption because there is a presumption that a hypothesis should be tested. Because of the crucial role of the threshold assumption for the validity of risk assessments of non-genotoxic chemicals, this assumption should be treated with great skepticism and further tested as a hypothesis.

In the most thorough review of the threshold assumption, Daston [12] concluded "If an endogenous chemical is already driving a response, any additional dose will have an effect. The concept of a "practical threshold" and/or a NOAEL should not be applied under these circumstances. Rather, actual risks should be calculated and expressed quantitatively".

Risk Assessment Consequences of Non-Monotonic Curves

Vitamin A causes major malformations in both excess and deficiency conditions [9,10]. Populations will exhibit some distribution of Vitamin A levels, and risk will exist at both tails of the curve.

Furthermore, it appears unlikely that there can be any threshold for Vitamin A toxicity in the human population due to population heterogeneity in Vitamin A levels [12]. Non-monotonic curves are not unusual in endocrine system responses. For example, we found that uterine estrogen receptor content was increased ~30% in ovariectomized rats treated with a constant low dose of estradiol [7]. In animals receiving high doses, ER content was reduced 50%. While these compensatory mechanisms may be important for an outcome such as reproduction, they could also fail to protect against adverse outcomes. Up- and down-regulation of the estrogen receptor as a function of estrogen levels should be investigated as a possible cause of monotonic curves, such as seen in estrogen regulation of prostate weight and morphology.

Using current risk assessment procedures, a proclaimed safe dose may fall on the ascending or descending arm of the dose-response curve. Although the consequences of exposure to the assumed safe dose depend on where this dose lies on the curve and the nature of the response, the accepted model is wrong, and adverse effects can be expected at a presumably safe dose.

Given the diversity of recognizable dose-response curve shapes, and the adverse risk implications of using a single policy-driven model, a much-expanded range of doses, endpoints, and models should be incorporated into risk assessment practice. Justification by assumption needs to be replaced with specific guidelines to detect and evaluate risk for each of the prototypical dose-response curves.

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