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BIOLOGICALLY-BASED DOSE-RESPONSE MODEL FOR NEUROTOXICITY RISK ASSESSMENT

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ABSTRACT: *The regulation of neurotoxicants has usually been based upon setting reference doses by dividing a no observed adverse effect level (NOAEL) by uncertainty factors that theoretically account for interspecies and intraspecies extrapolation of experimental results in animals to humans. Recently, we have proposed a four-step alternative procedure which provides quantitative estimates of risk as a function of dose. The first step is to establish a mathematical relationship between a biological effect or biomarker and the dose of chemical administered. The second step is to determine the distribution (variability) of individual measurements of biological effects or their biomarkers about the dose response curve. The third step is to define an adverse or abnormal level of a biological effect or biomarker in an untreated population. The fourth and final step is to combine the information from the first three steps to estimate the risk (proportion of individuals exceeding an adverse or abnormal level of a biological effect or biomarker) as a function of dose. The primary purpose of this report is to enhance the certainty of the first step of this procedure by improving our understanding of the relationship between a biomarker and dose of administered chemical. Several factors which need to be considered include: 1) the pharmacokinetics of the parent chemical, 2) the target tissue concentrations of the parent chemical or its bioactivated proximate toxicant, 3) the uptake kinetics of the parent chemical or metabolite into the target cell(s) and/or membrane interactions, and 4) the interaction of the chemical or metabolite with presumed receptor site(s). Because these theoretical factors each contain a saturable step due to definitive amounts of required enzyme, reuptake or receptor site(s), a nonlinear, saturable dose-response curve would be predicted. In order to exemplify this process, effects of the neurotoxicant, methylenedioxymethamphetamine (MDMA),*

were reviewed and analyzed. Our results and those of others indicate that: 1) peak concentrations of MDMA and metabolites are achieved in rat brain by 30 min and are negligible by 24 hr, 2) a metabolite of MDMA is probably responsible for its neurotoxic effects, and 3) pretreatment with monoamine uptake blockers prevents MDMA neurotoxicity. When data generated from rats administered MDMA were plotted as biological effect (decreases in hippocampal serotonin concentrations) versus dose, a saturation curve best described the observed relationship. These results support the hypothesis that at least one saturable step is involved in MDMA neurotoxicity. We conclude that the mathematical relationship between biological effect and dose of MDMA, the first step of our quantitative neurotoxicity risk assessment procedure, should reflect this biological model information generated from the whole of the dose-response curve.
Keywords: *Neurotoxicity risk assessment*

INTRODUCTION

Risk assessment is the analytical process by which the nature and magnitude of risks are determined (OTA, 1990). There are four steps or components of the risk assessment process: hazard identification, dose-response assessment, exposure assessment, and risk characterization (National Research Council, 1983). Hazard identification entails a qualitative evaluation of toxicity produced by an agent and the relevancy of these data to the human situation. The dose-response assessment seeks to determine a quantitative relationship between exposure to an agent and the extent of toxicity. The third component, exposure assessment, is concerned with the numbers of individuals exposed and the magnitude and duration of the exposure. Finally, the combination of the results from the first three steps provides the risk characterization.

It is the second step of the risk assessment process, dose-response assessment, on which this report focuses. Evaluation of neurotoxicants and other noncarcinogens is generally derived from observations of a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) in people or animals exposed to a particular agent. The NOAEL and LOAEL are thought to approximate the theoretical threshold below which no effect is observed. In the final risk characterization step, these NOAEL or LOAEL values are usually divided by safety or uncertainty factors to produce a reference dose (RfD). It is assumed that if human exposure is below the RfD, then little health risk exists.

There are several features of this RfD or safety factor approach which deserve consideration. First, the method assumes a theoretical threshold dose below which no biological effects of any type are observed. Not only is the determination of a threshold dose influenced by the sensitivity of the analytical methods employed, but the theoretical bases of a threshold dose may be questioned. If due to normal variation in cellular function an adverse effect can occur in untreated control subjects, then endogenous or exogenous factors may already be supplying a stimulus which is

equivalent to a dose above the threshold dose. If exposure to an agent augments this stimulus, then an additional risk is expected and no threshold dose exists for that agent (Gaylor and Slikker, 1990). Secondly, the magnitudes of the safety factors used to determine RfDs [interspecies extrapolation (10), intraspecies extrapolation (10) and acute vs chronic exposure (10) = 1000] are based more on best estimates than actual data (Sheehan *et al.*, 1989; McMillan, 1987) and have been questioned for empirical reasons (Gaylor *et al.*, 1990). Finally, the RfD approach uses a single observation, the NOAEL or LOAEL instead of a complete dose-response curve to calculate risk estimations. Chemical interactions with biological systems are often specific, stereoselective and saturable, such as enzyme-substrate binding leading to metabolism, transport and/or receptor-binding, any or all of which may be a requirement of an agent's effect or toxicity. Therefore, a chemical's dose-response curve may not be linear. The certainty of low dose extrapolation is markedly effected by the shape of the dose-response curve (Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation, 1971). Therefore, the appropriate use of a dose-response curve may enhance the certainty of risk estimations.

Gaylor and Slikker (1990) proposed a procedure for calculating health risk from exposure to a neurotoxicant. Risk is defined as the proportion of a population whose levels of a measure of toxicity, *e.g.*, low levels of serotonin (5-HT), indicate an adverse level of the measure. In the absence of a well-established adverse level, an abnormal level may be used which is based upon the variation of levels observed among control animals.

The risk assessment process for neurotoxicants described by Gaylor and Slikker (1990) requires the use of a dose-response curve. A quadratic interpolation between doses of the logarithms of various measures of neurotoxicity was used. That procedure may be adequate within the experimental dose range but it is not recommended for extrapolation for low doses below the experimental dose range. The main purpose of this report is to consider a dose-response model which is biologically plausible in order to increase the validity of estimates of risk. The procedure will be demonstrated with examples of alterations in 5-HT neurotransmitter concentrations and concentrations of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus or frontal cortex of male rats exposed to methylenedioxymethamphetamine (MDMA). Doses corresponding to low levels of risk estimated from the proposed risk assessment procedure will be compared with doses obtained from the NOAEL or LOAEL divided by safety factors.

The rationale for the use of the saturation-type model is based on the biological effects produced by MDMA in the rat as reported in the literature. Our studies with ^{3}H-labeled MDMA indicate that peak concentrations of total radioactivity are evenly distributed in the rat brain after oral administration by 30 min and are eliminated by 24 hr (Ali *et al.*, 1990). MDMA is not thought to be the proximate toxicant because direct injection of MDMA into regional brain areas fails to result in neuronal (Molliver *et al.*, 1986) or neurochemical (Ali *et al.*, 1990) alterations. Therefore, a metabolite of MDMA, probably other than methylenedioxyamphetamine (Schmidt *et al.*, 1987; Gollamudi *et al.*, 1989), is thought to be responsible for the neurotoxicity. Several

metabolites have been described for MDMA (Lim and Foltz, 1988). Although not all the enzymes necessary for the metabolism of MDMA have been characterized, we have demonstrated that some are stereoselective and likely to be saturable (Gollamudi *et al.*, 1989). Therefore, the apparent requirement that MDMA be enzymatically bioactivated to produce toxicity provides at least one basis for a saturable dose-response model.

Another possible basis of a saturation-type biological model emanates from studies of the antagonism of MDMA effects by the 5-HT uptake inhibitor, citalopram (Schmidt *et al.*, 1987). Although carrier-mediated transport of MDMA into the nerve terminal or carrier-mediated transport of monoamines out of the nerve terminal could explain the protective effects of citalopram on MDMA-induced alterations, either mechanism could provide a rationale for a saturable dose-effect relationship.

Currently, the exact mechanism whereby MDMA produces its neurotoxic effects is not known. It is known, however, that the stereoisomers of MDMA possess different behavioral, neurochemical and metabolic potentials (Anderson *et al.*, 1978; Schmidt *et al.*, 1987; Gollamudi *et al.*, 1989). Even though a "receptor" site for MDMA has yet to be determined, these reported stereoselective interactions strongly suggest that MDMA does not produce its effects via nonspecific mechanisms. Therefore, the possibility of a further specific interaction of MDMA or metablite with a saturable "receptor" also supports the need for a saturable biological model.

METHODS

Based on these experimental and theoretical biological considerations, a saturation model was used to describe the relationship between the regional brain concentrations of an endogenous neurotransmitter (expressed as % of the normal average) and dose of a neurotoxicant.

$$\% = \frac{1+A\cdot Dose}{1+B\cdot Dose} \times 100\%$$

At dose = 0, the 5-HT concentration is 100% of the normal average. A and B are constants estimated from the data where A/B is the minimum % obtained at large doses and (A-B) 100% is the % reduction from the normal average per unit of dose for low doses approaching zero. A and B are estimated by nonlinear regression. The properties of the saturation curve are illustrated in Figure 1 for levels of 5-HT in the hippocampus of male rats.

Risk is defined as the proportion of a population whose levels of a measure of toxicity indicate an adverse level. In the absence of a well-established adverse level, an abnormal level may be used which is based upon the variation of levels observed among control animals. For example, a level of 5-HT may be considered abnormal it is lower than the level observed at the 0.1 percentile or above the 99.9 percentile of the background levels observed in an untreated control population. This is, a level of a neurochemical may be considered abnormal if that level is less than that of 1 per 1000 individuals in a normal untreated population. In general, measurements are not

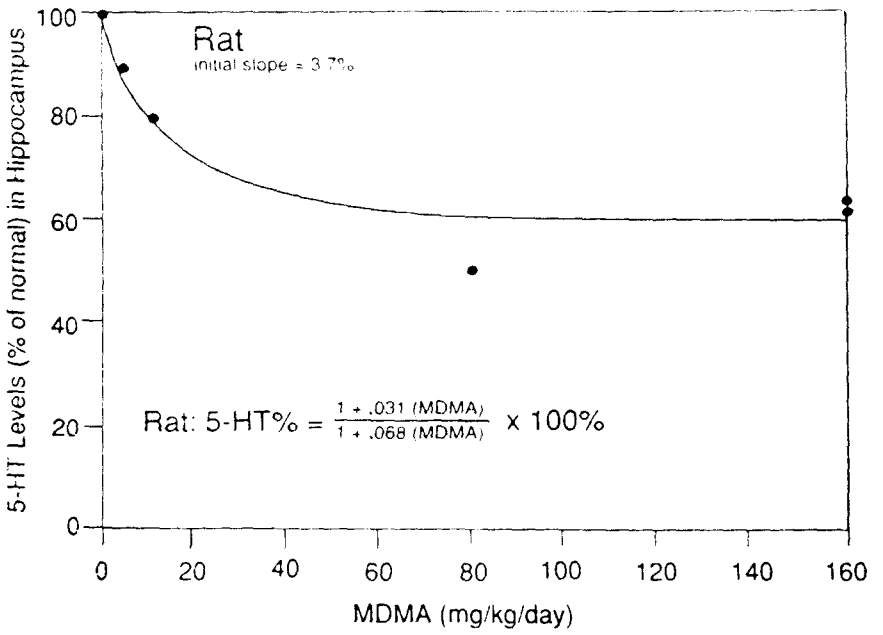

Fig. 1. Dose-response curve describing the hippocampal levels of 5-HT as % of normal controls one month after various doses of MDMA in the male Sprague Dawley rat. The MDMA was administered orally either once or twice a day for four consecutive days and the rats were sacrificed one month later to determine regional brain concentrations of 5-HT. The bold filled circles represent the experimental data and the solid line was determined by the saturation-model equation shown in the middle of the figure where (MDMA) = dose of MDMA in mg/kg/day. The correlation coefficient was $r > 0.98$.

available on 1000 or more individuals. Thus, the 0.1 percentile or 99.9 percentile cannot be observed directly. However, Gaylor and Slikker (1990) noted that many measures of neurotoxicity can be described by a log-Normal distribution, *i.e.*, the logarithms of the measures are described by a Normal (Gaussian) distribution. In such cases, the levels corresponding to any percentile can be estimated.

The numerical procedure for estimating risk as a function of dose is given by Gaylor and Slikker (1990). In the present report the saturation curve described above is used for the dose-response relationship.

RESULTS

The saturation model was fit to the geometric mean levels of 5-HT or 5-HIAA in the hippocampus of frontal cortex of male rats which had been administered MDMA (Slikker *et al.*, 1988; Gollamudi *et al.*, 1989; Slikker *et al.*, 1989; Scallet *et al.*, 1988). These rats were dosed once or twice per day for four consecutive days with various doses of MDMA and sacrificed one month later for regional brain neurochemical analyses. The Statistical Analysis System, (SAS) Procedure NLIN, which is a nonlinear least squares regressions procedure, was used to fit the models (Table 1). The correlation coefficient between the observed and predicted values was high ($r > 0.98$). The

Table 1. Summary of Selected Neurochemical Levels from Exposures to MDMA

	5-HT Hippocampus	5-HT Frontal Cortex	5-HIAA Hippocampus	5-HIAA Frontal Cortex
Saturation Model[a]	$\frac{1+.031D^{b}}{1+.068}\times 100\%$	$\frac{1+.043D}{1+.114D}\times 100\%$	$\frac{1+.069D}{1+.1790}\times 100\%$	$\frac{1+.078D}{1+.1290}\times 100\%$
Mean Minimum[c]	46%	38%	39%	60%
Initial Slope[d]	3.7%	7.1%	11.0%	5.1%
Standard Deviation[e]	0.318	0.312	0.354	.250
Abnormal Level[f]	37%	38%	33%	46%
Reference Dose[g]	0.005 or 0.05 mg/kg/d	0.005 or 0.05 mg/kg/d	0.005 or 0.05 mg/kg/d	0.005 or 0.05 mg/kg/d
Dose for additional risk of 0.0001[h]	0.28 mg/kg/d	0.14 mg/kg/d	0.10 mg/kg/d	0.16 mg/kg/d
Dose for additional risk of 0.001[h]	2.0 mg/kg/d	1.0 mg/kg/d	0.74 mg/kg/d	1.2 mg/kg/d

[a]Percent of mean control level $= \frac{1+AD}{1+BD}\times 100\%$.

[b]D = MDMA dose in mg/kg/day given orally on four consecutive days.

[c]Mean (minimum) asymptote $= \frac{A}{B}\times 100\%$.

[d](B-A) × 100% per mg/kg/day of MDMA.

[e]Standard deviation of $\log_e$ (neurochemical level).

[f]Risk = 0.001, i.e., 0.1 percentile of control levels.

[g]Lowest observed effect level at 5 mg/kg/d ÷ 1000 or no observed effect level at 5 mg/kg/d ÷ 100.

[h]Additional proportion abnormal estimated from expected saturation dose-response and standard deviation.

saturation model was used to estimate the mean response as a function of dose. The levels of 5-HIAA in the frontal cortex showed the least change at high doses, *i.e.*, the largest asymptote. At low doses the levels of 5-HT changed almost at twice the rate in the frontal cortex as in the hippocampus, as indicated by the initial slope (percent change per mg/kg/d of MDMA). Levels of 5-HIAA at low doses of MDMA, however, changed more rapidly in the hippocampus than in the frontal cortex. Since the standard deviations were similar in the four examples, the abnormal levels (expressed as a percent of the normal control level) corresponding to the 0.1 percentile are similar. Only 1 out of 1000 control animals would be predicted to have neurochemical levels below the abnormal level.

Since all four examples showed a dose-response without any apparent threshold dose, it is not clear if investigators would consider the lowest dose tested (5 mg/kg/d) the lowest observed effect level or the highest no observed adverse effect level, as the mean effect at this dose was within one standard deviation of the mean control level. Thus, it is not clear whether a reference dose would be set at 5/1000 = 0.005 or 5/100 = 0.05 mg/kg/d of MDMA. In either case, the reference doses are well below doses that are estimated to produce additional risks above background of 0.0001. The

probability (risk) of an abnormal in control animals was set to equal 0.001. Using the saturation model and the standard deviation, the doses estimated to produce additional risks of 1 in 10,000 and 1 in 1000 animals were calculated using the technique described by Gaylor and Slikker (1990).

DISCUSSION

Use of a dose-response model based upon plausible biological mechanisms provide more validity to prediction than purely empirical models. In the cases investigated here, saturation mechanisms were hypothesized and indeed saturation curves provided relatively good fits to the experimental results ($r < 0.98$). The saturation curve provides an estimate of the asymptote (minimum level) of a neurochemical at high doses. Also, the saturation model provides an estimate of the initial slope (percent change in the neurochemical level per mg/kg/d of MDMA) at low doses. For purposes of illustration, abnormal levels were defined as the 0.1 percentile. That is, only 1 out of 1000 animals would be expected to have neurochemical levels this low in normal control animals. Since these levels are described by a log-Normal distribution (*i.e.*, the logarithms of neurochemical levels are described by a Normal/Gaussian distribution), other percentiles could readily be used for the abnormal level.

For these data it is not clear whether the lowest dose tested (5 mg/kg/d) would be considered a NOAEL or LOAEL. The subjectivity of setting reference doses with safety factors is one of the shortcomings of the RFD approach of risk assessment. On the other hand, use of a plausible dose-response curve in combination with the distribution of neurochemical levels about the dose-response curve provides a procedure to estimate the proportion of abnormal animals as a function of dose. Conversely, doses corresponding to specified levels of risk can be calculated. Hence, quantities with precise scientific definitions can be estimated and provide a rationale basis for decisions regarding risks from exposures to neurotoxicants.

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