Dose–Response Analysis in Risk Assessment: Evaluation of Behavioral Specificity

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Several methods of quantitative risk assessment that have been described recently are particularly applicable to neurotoxic end points. These methods can be broadly divided into two types of approaches based on their treatment of dose–response data to estimate risks. Benchmark approaches estimate risks using variability in response to a fixed dose level in comparison with background control variability. Probabilistic approaches estimate risks using the variability in the dose to produce a small effect in the sample population. The current report seeks to extend the development of probabilistic approaches for neurotoxic end points. Because behavioral data are often used to assess therapeutic efficacy as well as toxicity (unwanted effects), this analysis focused on the relative risks of producing these effects with the same agent. The therapeutic potential of GBR 12909 was determined by its ability to decrease cocaine-maintained responding in monkeys. The effects of this agent were also assessed in the same monkeys using food-maintained responding to provide an indication of behavioral toxicity. GBR 12909 decreased both behaviors, with complete decreases on drug-seeking behavior occurring at doses that had minimal effects on food-maintained responding. The difference in the estimates of doses to decrease drug-seeking and food-maintained behavior suggested that specific therapeutic effects could be obtained in the absence of unwanted side effects for a definable proportion of the population. These results also suggest that multiple behavioral end points can be useful for identifying specific effects of chemicals for the purposes of risk assessment. — Environ Health Perspect 104(Suppl 2):391–396 (1996)

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Introduction

Risk assessment is the attempt to estimate the chance of obtaining an adverse effect of exposure to an agent. Although it is possible to predict virtually safe levels if sufficient information is known about doses of an agent that produce adverse effects in humans, risk assessments are typically based on effects obtained over a range of non-toxic-to-toxic doses determined in animals. Such methods were developed initially to predict the chance of producing carcinogenic and mutagenic effects with exposure to ionizing radiation. Higher doses were often associated with an increased incidence of cancers, so the focus of risk assessment shifted toward the attempt to predict effects produced by very low doses. Two problems developed. First, the spontaneous rate of cancer production called into doubt the ability to measure an increase in risks associated with very low doses of an agent. Second, several low-dose extrapolation approaches were developed based on the assumption that any increment in dose would increase the probability of an effect. These issues have questioned the relevance of the low-dose extrapolation approach for other types of adverse effect. For example, few neurotoxic events occur spontaneously, although disease, age, or other processes may exacerbate their effects. Likewise, mechanisms such as cellular repair, plasticity, system redundancy, or tolerance may contribute to the lack of measurable effect following exposure to low doses of neurotoxic agents. Alternatively, some agents have been simply found to lack effects at low doses. As a result, risk assessment methodology for noncancer end points shifted away from the estimation of effects of very low doses toward the attempt to estimate levels of an agent that might be considered safe.

The earliest practice used to predict safe levels was the acceptable daily intake approach (ADI), which attempted to predict a dose (within an order of magnitude of uncertainty) that could be tolerated over the lifetime without producing harm. However, while some argue that it was virtually impossible to determine an entirely safe dose, others pointed to an apparent inappropriateness of using the word "acceptable" in the context of a poison. This led to a revised terminology called the reference dose (RfD) approach. This approach first finds a dose level with no effect (i.e., no observable adverse effect level, or NOAEL) or a minimal effect (i.e., lowest observable adverse effect level, or LOAEL) in animals and then divides this dose by a series of uncertainty factors (invariably 10-fold each) to calculate a RfD for humans. The resulting RfD could be several orders of magnitude smaller than the original NOAEL or LOAEL (*1*). There have been a number of criticisms of the RfD approach (*2–6*), including *a*) its dependence on the actual dose spacing used, *b*) its failure to incorporate the slope of the dose–response function,

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Abbreviations used: ADI, acceptable daily intake; RfD, reference dose; NOAEL, no observable adverse effect level; LOAEL, lowest observable adverse effect level; DA, dopamine; TLVs, threshold limit values; FRF, fixed-ratio 30-response food presentation; FRC, fixed-ratio 30-response cocaine injection; ANOVA, analysis of variance; LD_{50}, median lethal dose; ED_{50}, median effective dose.

c) a lack of biological justification for uncertainty factors, *d*) its questionable applicability for some types of data, *e*) the fact that the RfD is not a risk figure but rather a point estimate of an exposure at which risk is considered negligible, and *f*) its perverse ability to reward biased risk assessors with less conservative risk figures for using small sample sizes. In response, a new trend in risk assessment that uses more quantitative approaches has emerged. These approaches are of interest because they circumvent many of the problems associated with the RfD and low-dose extrapolation approaches and thus provide true quantitative risk estimates. Given the recent increase in concerns over other end points such as neurotoxicity (*7,8*), and the equivocal performance of the older approaches to estimate risks (*9,10*), the time is ripe to scrutinize these quantitative methods.

Quantitative approaches generally describe dose–effect functions in mathematical terms and specifically incorporate the variability in different parameters of this function to assess risks (*6,11*). One subfamily of these approaches can be characterized by its focus on variability in an effect at a fixed-exposure level (effect-tolerance or benchmark approaches). One advantage of this approach is that statistical techniques to fit functions and characterize variability are readily available. Another subfamily of approaches is characterized by a focus on variability in exposure levels that can be shown to produce a fixed effect (dose-tolerance or probabilistic approaches). The advantage of the dose-tolerance approaches is that they characterize individual differences, thus providing direct information as to the potential adequacy of the use of an uncertainty factor for individual variability (*6*). The current report extends the use of the probabilistic (dose-tolerance) approach to multiple behavioral end points in the same organisms to address the specificity of the effect of the agent.

The agent used for these analyses is a drug that may have therapeutic merit. Though drugs are not commonly thought of as neurotoxicants, any pharmacologist or toxicologist will acknowledge that high doses of therapeutic agents will have adverse effects. It has been a common practice over the years to compare doses expected to produce a therapeutic effect with those expected to produce a toxic effect. The current report departs from this approach by attempting to compare the risk of producing these effects. Another small departure from standard methods is the use of a different end point in the current report. Behavioral toxicology has been primarily concerned with the direct effects of toxic agents on learned or acquired behaviors. Most often, these behaviors are experimentally developed in the laboratory, typically by training animals to respond under schedules of food reinforcement. Stable rates and patterns of behavior provide a baseline from which a dose–effect function can be determined. While almost all of these studies have employed behaviors that lead to the delivery of food as a reinforcer, some others have shown that the event used to maintain behavior can determine different effects for some agents (*12*). It has been known for some time that drugs can be used to maintain responding (e.g., self-administration studies). Interestingly, some toxicants have been shown to serve as reinforcers. For example, toluene has been shown to support responding in monkeys (*13*). Little is known of solvent abuse; however, growing knowledge suggests that, if left unchecked, drug abuse results in changes in behavior that are sufficient to meet the criteria of a psychiatric disorder (*14*). Neurotoxic changes are also seen with prolonged exposure to drugs of abuse (*15*). Thus, drug abuse may be the most prevalent form of behavioral toxicology observable in humans today. One question is whether a therapeutic agent could be developed that would decrease drug abuse but not alter other normal behavior.

Previous studies have shown that operant responding can be maintained by cocaine delivery (self-administration), which provides an animal model of drug abuse (*16*). Left unrestrained, cocaine self-administration can occur to the point of producing serious harm in animals (*17*). Such excessive behavior may result in neurotoxic effects in central mesolimbic dopamine (DA) pathways associated with reinforcement (*18*). In contrast to some forms of drug abuse, there is no currently accepted strategy to treat cocaine abuse. However, clues from successful treatment approaches suggest that targeting mechanisms associated with the abuse potential of a drug may lead to a treatment drug. Recently, a specific neuronal site (the DA reuptake or transporter) has been identified as a receptor that is strongly associated with the reinforcing effects of cocaine (*19*). Studies have shown that drugs that bind to this site need not exhibit reinforcing effects in humans (*20*) or in animals (*21*). These observations have led to the suggestion that long-acting DA reuptake inhibitors may attenuate cocaine-seeking behavior in a manner similar to the ability of methadone to decrease opiate-seeking behavior or nicotine patches to decrease smoking (*22*). However, there is no clear consensus as to the type of effect a medication should exhibit on cocaine self-administration in animals that would predict its efficacy to decrease cocaine-seeking behavior in humans. The current research is meant to address this issue from a therapeutic point of view; however, it is obvious that some concern should be directed at the potential toxicity of such an agent. Thus, the current studies are designed to compare the relative therapeutic actions of an agent being developed to treat cocaine abuse with other unwanted effects. The current studies focus on one compound, GBR 12909, which has previously been shown to exhibit a number of effects similar to those of cocaine and has a high affinity for the DA reuptake site. In contrast, because GBR 12909 is long acting and has a chemical structure unlike cocaine, it may block the effects of cocaine. Interestingly, this drug has been reported to exhibit a nonstimulant profile of action in normal human volunteers following oral administration (*23*). Thus, this agent could serve as a prototype from which even more therapeutic and less toxic analogs could be developed. The primary focus of the current work was to separate those effects. For these purposes, the effects of GBR 12909 on food-maintained behavior were taken to represent a behaviorally toxic effect, and its effects on cocaine self-administration were used as an indication of its therapeutic potential.

To accomplish the risk assessment analysis of these data, methods previously developed to assess the risks of exposure to agents using a single behavioral end point were used (*24*). This approach was originally described by Dews (*3,4*). Data from individual subjects are first described by means of a mathematical function, and the dose resulting in a small but measurable effect is estimated from each function. Through replications in different subjects, a distribution of individual point estimates is obtained. The variability in these estimates is used to predict population tolerances, from which the proportion of the population that would be expected to be affected at lower levels can be determined. This method has been used extensively to assess the risks associated with exposure to a variety of organic solvents (*25–27*). These estimates of a small decrement in neurobehavioral functioning in relatively small

sample sizes (n = 10–12) agreed well with established threshold limit values (TLVs). A subsequent report (*28*) compared estimates from small (n = 10) and large (n = 40) sample sizes to illustrate the effectiveness of these relatively small experiments, as well as to compare estimates obtained with this approach to those of others. This approach has also been compared with other approaches in terms of its use of the dose–effect data (*6,10*), applicability to cross-species extrapolation (*29*), and ability to characterize the neurotoxic effects of the pesticide carbaryl (*6*). The potential advantage of using two end points in the present studies is that the nonspecific (toxic) effects of the agent can be directly compared to its intended therapeutic effects, thereby producing a therapeutic ratio.

Methods

This report uses methods that were previously published (*30,31*). Briefly, rhesus monkeys were surgically implanted with a subcutaneous port/catheter system (*32*) and trained to respond under a multiple fixed-ratio 30-response food presentation (FRF), fixed-ratio 30-response cocaine injection (FRC) schedule to establish a baseline where approximately equal rates of responding were maintained by food and cocaine. The acute effects of GBR 12909 were then determined on these performances using eight monkeys. Individual control rates of responding for both performances were obtained for several noninjection control sessions and served as a baseline for each monkey. The effects of pretreatment with GBR 12909 were assessed on this baseline by comparing the mean overall rates of responding during drug sessions with rates during control sessions. Two advantages of these data are that the effects of the agent were well characterized (doses were assessed 2–3 times per monkey) and the individual differences in effect occurring among the monkeys could serve to model individual differences in the human population.

GBR 12909 (1–1.7 mg/kg) decreased responding maintained by cocaine almost completely without affecting food-maintained responding. This type of effect showed that cocaine-maintained responding could be decreased by low doses of this drug. With higher doses of GBR 12909, food-maintained responding decreased. This effect may have been associated with a drug-induced disruption of behavior or because some aspect of food-maintained responding provided a greater resistance to the drug effect than that seen with cocaine-maintained responding. A mean dose–effect function for each of these behavioral end points was then constructed using the individual data from the eight animals. While there was no overlap of these curves (i.e., GBR 12909 always decreased cocaine-maintained responding at lower doses than food-maintained responding), some animals were more sensitive to the effects of GBR 12909 than others. However, traditional statistical approaches confirmed that GBR 12909 had different effects on these two behaviors. For example, single factor repeated measures analysis of variance (ANOVA) indicated a significant main effect of maintaining event (F = 18.428; df = 1,14; p < 0.0007), dose (p < 0.0001), and an interaction.

Figure 1 shows the essential elements of the method of risk assessment applied to these data. First, the effect of GBR 12909 on each type of response was determined relative to each monkey's baseline rate for each response by dividing rates during drug sessions by rates during control sessions for each component. This represents a normal dose–effect function for a single animal, only two different end points could be described. Next, a straight line was fit to the linear portion (only those doses that produced greater than a 20% decrement but less than an 80% decrement in responding) of each curve using linear regression. Effect (percent of control) was plotted against the natural logarithm (ln) of the dose. The figure shows only the mean circumscribed descending limb, from 90 to 10% of control. The variability (SD) in the 90 and 10% of control was determined from the individual functions (horizontal lines). For example, the mean ln dose of GBR 12909 to produce a 10% decrement in cocaine-maintained responding was –1, with a SD of about 0.5. As is apparent from Figure 1, the doses of GBR 12909 required to decrease food-maintained responding were high. In fact, for three animals complete decreases in food-maintained responding were not produced by the highest dose of GBR 12909 studied. Since previous studies suggested that a higher dose (5.6 mg/kg) would completely abolish responding, it was conservatively assumed that similar effects would be obtained in these animals and that it would be in the best interest of the animals to not test higher doses.

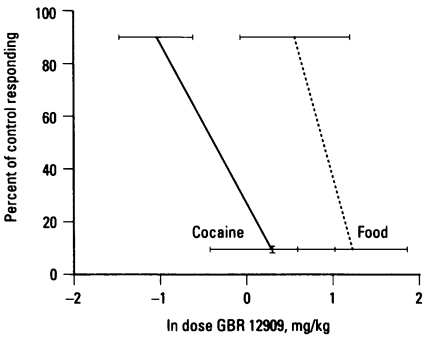

Figure 1. Transformed dose–effect summaries of the rate-decreasing effects of GBR 12909 on responding maintained under multiple FR30 schedules of cocaine delivery (solid line) and food-pellet delivery (dotted line). Effects are the mean of double-determined observations of the rate-decreasing effects (see text for a description of the restriction of the range) in eight monkeys, expressed as a percentage of the individual baseline rate of responding. Doses were transformed to the natural logarithm (ln). Variability (1 z-score) in the dose to produce 90 and 10% effects, based on differences in the individual curves, is indicated by the horizontal bars.

The estimates of risk were then obtained by calculating the successive probabilities (i.e., p = 0.1, p = 0.01, p = 0.001) of obtaining a 10% decrement in this group of animals using a z-score approach. In the past, only estimates from the low end of these distributions have been assessed because of the specific interest in the effects of low doses. In this study, the overlap of the effects of similar doses of GBR 12909 on both cocaine- and food-maintained responding could be directly compared. Transformation then allowed determination of doses (in milligram per kilogram) expected to decrease responding by 10% in the specified proportion (i.e., 1 out of 10, 1 out of 100, etc.) of the population (*24*). Figure 1 characterizes the descending limbs of the dose–effect curve for each end point. (A mean curve was never used for the analysis; it simply represents the mean slope joining the mean 90 and 10% point estimates.) The mean parameters (a=intercept; b=slope) for the individual curves, the 90% effect (in ln dose and in milligram per kilogram), and the SD of ln dose for cocaine-maintained responding were

	FRC, mg/kg			
	a	b	90% (ln)	90%
Mean	18.103	–74.52	–1.0538	0.37
(SD)			(0.4226)	

The resulting risk figures for estimation of a 90% effect in successively smaller proportions (1/10 [p = 0.1], 1/100 [p = 0.01], and 1/1000 [p = 0.001]) of the population based on cocaine-maintained responding were

	Probability		
	0.1	0.01	0.001
ln dose	–0.652	–2.321	–3.076
Dose, mg/kg	0.19	0.10	0.05

The mean parameters for food-maintained responding were

	FRF			
	a	b	90% (ln)	90%, mg/kg
Mean	170.45	–134.6	0.5579	2.02
(SD)			(0.6258)	

The resulting risk figures for estimation of a 90% effect in successively smaller proportions (1/10 [$p = 0.1$], 1/100 [$p = 0.01$], and 1/1000 [$p = 0.001$]) of the population based on food-maintained responding were

	Probability		
	0.1	0.01	0.001
ln dose	–0.328	–1.318	2.437
Dose, mg/kg	0.72	0.27	0.09

Comparisons of the distributions of doses to decrease each type of responding 10% by t-tests indicated ($p < 0.001$) the behavioral specificity of the effects of GBR 12909 (i.e., the two behaviors were affected by different doses).

Conclusions

The current report is not intended to exhaustively explore the relationships between different effects of an agent on different end points but rather to extend the development of a risk assessment technique using a well-defined data set. The current methods are of interest because they directly measure sources of variability that have been assumed in other risk assessment processes. For example, the use of the sample variance to estimate risks provides data directly relevant to one source of uncertainty in risk assessment (individual differences). In the RfD approach this uncertainty factor is typically assumed to be an order of magnitude. The current studies also suggested that, while there was a clear difference in the effects of GBR 12909 on these two types of behavior, the variability in effects on each provided relevant data from which risks could be assessed.

The current methods established dose–effect relationships for GBR 12909 on two different behaviors. These dose–effect functions suggested that each behavior was affected differently by this drug, as expected. GBR 12909 decreased drug-seeking behavior. These effects may be similar effects to the use of methadone to treat heroin or nicotine patches to treat tobacco abuse (*28,29*). In addition, the effects of GBR 12909 on cocaine-maintained responding may also be similar to those of delivering free food on behavior maintained by food delivery. However, in contrast to the decrease in cocaine-maintained behavior produced by GBR 12909, the decrease in food-maintained behavior by this drug was considered adverse (i.e., an effect that might interfere with the normal behavioral functioning). The variability in the likelihood of producing each effect in individual animals was then used to specify the probability of seeing that effects at doses that would be expected to affect certain proportions of the population.

Further analysis of the current data was used to explore potential therapeutic indices for GBR 12909. Traditional therapeutic ratios have been constructed using comparable levels of effect on different end points (e.g., LD_{50}/ED_{50}). The current approach could extend these methods to compare the extent to which similar levels of effect on both behaviors overlapped. For example, the low end of the distribution of effects for food-maintained responding suggests that 1 out of 10 individuals would exhibit a 10% decrease in responding when given 0.72 mg/kg GBR 12909. Likewise, 1 out of 100 individuals would be expected to exhibit this effect when given 0.27 mg/kg. For cocaine-maintained responding, the high end of the distribution suggests that 9 out of 10 individuals would exhibit at least a 10% decrease in drug-seeking behavior at 0.45 mg/kg. Thus, a dose could be conceived that diminished drug-seeking in at least 90% of the population and without producing adverse effects in 90 to 99% of the population .

In contrast, it may be of interest to compare the overlap of a highly therapeutic dose (i.e., the ED_{90} for cocaine-maintained responding) with a minimally toxic dose (i.e., ED_{10} for food-maintained responding) in order to assess the probabilities of obtaining the most benefit at the least cost. Alternatively, it might be argued that only a single dose of GBR 12909 could be given at one time, focusing the emphasis of such analyses on distributions of two different levels of effect (e.g., a dose of 1.7 mg/kg decreased drug-maintained responding almost completely while having little effect on food-maintained responding). Obviously, the extent to which there is no apparent overlap between the mean desired and undesired effects suggests the drug may be relatively safe, although a 1/10 chance of an adverse effect may warrant concern. The present data suggest that there is some overlap of effects with GBR 12909, although it is by no means complete. It may be constructive to compare the degree of overlap with that of other therapeutic agents (e.g., diazepam) when assessed on relevant behaviors (e.g., punished and nonpunished responding). In the present case, some of this overlap may have resulted from the treatment of the data. The slope of the function for food-maintained responding was steeper than that for cocaine-maintained responding, suggesting that the artificial decreases imposed for monkeys that did not exhibit complete decreases in food-maintained responding may have inflated variability.

One interesting feature of the current approach is the variability of an effect (and hence, risks estimates) that was almost constant over the range of the dose–effect function. For example, examination of the variability at different effect levels (i.e., 90, 50, 10%) on each curve demonstrated a remarkable similarity at different points for food-maintained responding (from 0.639 at 90% to 0.626 at 10%). Variability increased slightly with the increasing effect of GBR 12909 on cocaine-maintained responding (from 0.423 at 90% to 0.724 at 10%). These observations illustrate that dose-tolerance and benchmark approaches characterize variability in a completely different manner. Confidence intervals invariably flare at the high and low ends of the dose–effect function, even if the observed variability is greatest at the intermediate doses. One feature of these hyperboliclike functions is that, with sufficient variability in the dose–effect data, this flare may preclude the determination of a benchmark dose because the entire lower confidence interval may never intercept the 10% effect level.

A feature of quantitative approaches that appears to be gaining acceptance is the use of logarithmic conversions of dose. While log-normal distributions have been used and accepted in pharmacology for many years (*33*) primarily because receptor interactions can span orders or magnitude, their use in risk assessment has received less attention. Previous studies have pointed to the advantage of preventing negative numbers in risk assessment (*3,4*) and to the validity of log-normal distributions of individual effects when sufficient numbers of subjects are used (*27*). Standard benchmark approaches may also convert doses to log doses in unexposed algorithms (*2*). In the current data this conversion had an unusual effect on the upper end of the ED estimates. When the normally distributed ln point estimates were

converted back to real numbers, the converted distribution dispersed at a logarithmic rate of change. The result should be that observed effects rarely exceed those predicted at either end of the distribution, although in practice predicted effects at the low end seem to occur with more regularity than at the high end. This could diminish some concern of the predicted overlap of these two effects described above.

Another feature of the current method that deserves consideration is the use of linear functions to fit individual dose–effect data. There may be several issues including the likelihood that other types of functions may fit the data better. However, previous authors have noted that the type of the function used to fit the linear region usually has little effect on the risk estimate (*24,34*). In fact, traditional statistical techniques (ANOVA, etc.) may bias the slope of the function by incorporating excessive determinations of 0 and 100% effects. Eliminating excessive determination of doses without effect seems desirable, but it requires an arbitrary rule for data exclusion. The use of functions that can effectively use all the data to more accurately fix the linear portion (e.g., logistic curves) should be explored. Linear functions are preferred, of course, because they strongly suggest that a direct relationship exists between dose and effect. While it is obvious that agents may have many effects, the most parsimonious conclusion upon observation of a linear function is that the one that is being measured is directly affected by the agent. Unfortunately, not all dose–effect data can be accurately characterized by a linear function. For example, there is often an increase in responding with some agents when assessed on behavioral measures. These increases inevitably give way to decreases, creating an inverted U-shaped function. Wood and Cox (*35*) recently reported risk assessments using data in which low doses of toluene increased rates and higher doses decreased rates. They fit a linear function to the ascending limb (rate-increasing effects) alone. While this approach clearly avoids the issues of the interpretation of a complex function and uses the most sensitive effect of toluene, it questions the issue of whether any effect of a toxicant is adverse. In the current studies, effects of GBR 12909 on drug-seeking behavior were the most sensitive but were not considered toxic.

The current report applies a quantitative approach to assess the risks of obtaining two different effects with the same agent. Drug-seeking behavior was targeted for selective elimination by doses of GBR 12909 that were not expected to produce toxic effects. It should be clear from these results that a decrease (or increase) in one behavior alone may not be the best indication of behavioral toxicity. Likewise, selective decreases in food-seeking behavior produced by other agents [e.g., appetite suppressants (*36*)] may be an intended effect. Literature is incomplete on the different behavioral effects that agents can have on behaviors maintained by different events. In the absence of specific knowledge of the effect of an agent on behaviors maintained by certain events, complete decreases in responding may warrant concern. When a single behavior is studied, it must be considered that doses which decrease responding completely might decrease all types of responding completely, a clear reason for concern. However, when one behavior is more affected than another, it could be because the susceptible behavior is less well maintained. This possibility points to the importance of reinforcement processes in the assessment of behavioral toxicology. In the current case, a decrease in the relatively well-maintained food-presentation behavior is clear reason for concern.

The dose-tolerance method was easily adapted to demonstrate a selective effect of GBR 12909 on two different behaviors, as well as to estimate the risks of obtaining those effects in a population. This method should be considered positively over other approaches since it directly employs individual differences in sensitivity to an agent as a metric of risk. However, not all types of end points can be studied using single-subject, repeated-exposure designs. Recently, we have developed a variant of the dose-tolerance approach for use with groups-design single-exposure data (Bogdan et al., unpublished data). This approach uses an iterating line-fitting program to calculate all possible dose–effect combinations that could be created from individual effects when different groups of animals are treated with different doses (e.g., three dose groups with six animals each would produce a 6×6×6 matrix yielding 216 possible dose–effect functions). This approach was very effective when applied to a data set addressing the effects of carbaryl on motor activity of rats (*6*). The risk figures it produced were less conservative than those of the current (within-subject) approach but were more conservative than those produced by the maximal likelihood estimation/confidence-interval approach. Future studies will continue to compare the results of these approaches to provide the practitioner a range of options for quantitative risk assessment.

REFERENCES

1. Barnes G, Dourson M. Reference dose (RfD): description and use in health risk assessments. Reg Toxicol Pharmacol 8:471–486 (1988).
2. Crump KS. A new method for determining allowable daily intakes. Fundam Appl Toxicol 4:854–871 (1984).
3. Dews PB. On the assessment of risk. In: Developmental Behavioral Pharmacology (Krasnegor N, Gray J, Thompson T, eds). Hillsdale, NJ:Lawrence Erlbaum, 1986;53–65.
4. Dews PB. Some general problems in behavioral toxicology. In: Neurobehavioral Toxicology (Annau Z, ed). Baltimore:The Johns Hopkins University Press, 1986;424–434.
5. Dourson ML. New approaches in the derivation of acceptable daily intake (ADI). Comments Toxicol 1:35–48 (1986).
6. Glowa JR, MacPhail RC. Dose-response models for quantitative risk assessment in neurotoxicology. In: Handbook of Neurotoxicology, Vol 3: Approaches and Methodologies (Chang L, Tilson H, eds). New York:Raven Press, 1995; 777–787.
7. McMillan DE. Risk assessment for neurobehavioral toxicity. Environ Health Perspect 76:155–161 (1987).
8. Raffaele KC, Rees DC. Neurotoxicology dose/response assessment for several cholinesterase inhibitors: use of uncertainty factors. Neurotoxicology 11:237–256 (1990).
9. Weiss B. Neurobehavioral toxicity as a basis for risk assessment. Trends Pharmacol Sci 91:59–62 (1988).
10. National Research Council. Environmental Neurotoxicology. Washington:National Academy Press (1992).
11. Cote I, Rees DC, Glowa JR. An introduction to the principles of risk assessment. In: The Vulnerable Brain and Environmental Risks, Vol 3: Toxins in Air and Water (Isaacson R, Jensen K, eds). New York:Plenum Press, 1994;183–207.
12. Barrett JE. Effects of alcohol, chlordiazepoxide, cocaine and pentobarbital on responding maintained under fixed-interval schedules of food or shock presentation. J Pharmacol Exp Ther 196:605–615 (1976).

13. Wood RW. Stimulus properties of inhaled substances: an update. In: Nervous System Toxicology (Mitchell CL, ed). New York:Raven Press 1982;199–212.
14. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 3rd ed rev. Washington:APA Press, 1987.
15. Nestler EJ. Molecular mechanisms of drug addiction. J Neurosci 12:2439–2450 (1992) .
16. Balster RL. Drug abuse potential evaluation in animals. Br J Addict 86:1549–1558 (1991).
17. Aigner T, Balster RL. Choice behavior in rhesus monkeys: cocaine versus food. Science 201:534–535 (1978).
18. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 242:715–723 (1988).
19. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223 (1987).
20. Peck AW, Hamilton M. Psychopharmacology of bupropion in normal volunteers. J Clin Psychiat 44:202–205 (1983).
21. Bergman J, Madras BK, Johnson SE, Spealman RD. Effects of cocaine and related drugs in nonhuman primates: III. Self-administration by squirrel monkeys. J Pharmacol Exp Ther 251:150–155 (1989).
22. Rothman RB. High affinity dopamine reuptake blockers as potential cocaine antagonists: a strategy for drug development. Life Sci 46:PL17–21 (1990).
23. Søgaard U, Michalow J, Butler B, Lund Laursen A, Ingersen SH, Skrumsager BK, Rafaelsen O. A tolerance study of single and multiple dosing of the selective dopamine uptake inhibitor GBR 12909 in healthy subjects. J Int Clin Psychopharmacol 5:237–251 (1990).
24. Glowa JR, DeWeese J, Natale ME, Holland JJ, Dews PB. Behavioral toxicology of volatile organic solvents: I. Methods: acute effects of toluene. J Environ Pathol Toxicol Oncol 6:153–168 (1986).
25. Glowa JR. Behavioral toxicity of *n*-octane. In: Advances in Modern Environmental Toxicology, Vol 6 (Mehlman MA, ed). Princeton, NJ:Princeton Scientific Publishers, 1984;245–253.
26. Glowa JR, Dews PB. Behavioral toxicology of volatile organic solvents: IV. Comparisons of the behavioral effects of acetone, methyl ethyl ketone, ethyl acetate, carbon disulfide, and toluene of the responding of mice. J Am Coll Toxicol 6:461–469 (1987).
27. Glowa JR. Behavioral toxicology of volatile organic solvents: V. Comparisons of the behavioral and neuroendocrine effects among *n*-alkanes. J Am Coll Toxicol 10:639–646 (1992).
28. Glowa JR. A comparison of dose-effect based risk assessment methods. Neurosci Biobehav Rev 15:153–158 (1991).
29. Rees DC, Glowa JR. Extrapolations to humans for neurotoxicants. In: The Vulnerable Brain and Environmental Risks, Vol 3: Toxins in Air and Water (Isaacson R, Jensen K, eds). New York:Plenum Press, 1994;207–230.
30. Glowa JR, Wojnicki FHE, Matecka D, Bacher J, Mansbach RS, Balster RL, Rice KC. Effects of dopamine reuptake inhibitors on food and cocaine maintained responding: I. Dependence on unit dose of cocaine. Exp Clin Psychopharmacol 3:219–231 (1995).
31. Glowa JR, Wojnicki FH, Matecka D, Rice K, Rothman RB. Effects of dopamine reuptake inhibitors on food and cocaine maintained responding: II. Comparisons of effects of psychomotor stimulants and subchronic effects. Exp Clin Psychopharmacol 3:231–239 (1995).
32. Wojnicki FHE, Bacher JD, Glowa JR. The use of subcutaneous vascular access port in rhesus monkeys. Lab Anim Med 44:491–494 (1994).
33. Gaddum JH. Lognornal distributions. Nature 156:463–466 (1945).
34. Crump KS, Allen BC, Faustman EM. The Use of the Benchmark Dose Approach in Health Risk Assessment. Final report. Washington:U.S. Environmental Protection Agency, 1992.
35. Wood RW, Cox CC. A repeated-measures approach to the detection of the minimal acute effects of toluene. Toxicology 6:221 (1986).
36. Corwin R, Schuster CR. Anorectic specificity as measured in a choice paradigm in rhesus monkeys. Pharmacol Biochem Behav 45:131–141 (1993).