A Dosimetric Analysis of Behavioral Effects of Acute Toluene Exposure in Rats and Humans

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The literature on behavioral effects of exposure to toluene is difficult to assess due, in part, to a wide variety of exposure conditions employed and outcomes measured. This study investigated whether previous experiments would be more consistent with each other if toluene exposure parameters were expressed not as concentration and duration, but as estimated amount of toluene in tissues. A physiologically based pharmacokinetic (PBPK) model was used to estimate concentration of toluene in arterial blood (C_a{TOL}) from published studies in rats and humans exposed acutely to toluene vapor. Data for rats were selected from studies of avoidance behavior using both rate of responding and measures of successful responding. Data for humans were from studies of choice reaction time (CRT).

Behavioral measures were converted to proportion of baseline to place them on a common scale across experiments. A meta-analysis was done to fit dose-effect curves using C_a{TOL} and the rescaled effects. Results demonstrated that effects were an orderly function of C_a{TOL} and were not influenced by concentration or duration of exposure, except as exposure influenced C_a{TOL}. In rats, response rates first increased, reached a peak, and then declined as C_a{TOL} increased. Successful avoidance in rats and CRT in humans always declined as C_a{TOL} increased. In rats, response rates were increased by 10% at C_a{TOL} ~ 13 ml/L. In humans, reaction times increased by 10% at C_a{TOL} ~ 3 ml/L. Cross-species comparisons were made with the following caveats: PBPK uncertainties, few human data, and poor task comparability.

Toluene is a volatile organic compound which is produced worldwide in large quantities for use in a variety of industrial and commercial applications. It is among the largest in volume of compounds released into the United States atmosphere (United States Environmental Protection Agency, 1989), and human exposures may occur in a variety of occupational and environmental settings. There is relatively rich scientific literature on the effects of acute exposure to toluene on some functions of the central nervous system (CNS). Inhalation of toluene vapors (and a number of other organic solvents) by human and nonhuman species is frequently reported to produce acute effects on behavior that are complex functions of concentration and duration of exposure. However, this literature is difficult to interpret, in part because of the large variety of exposure scenarios employed, including differences in concentration and/or duration of exposure to toluene. When comparing results within or across species, the inconsistencies in the literature become more troublesome. It is possible that comparing toluene body burdens (rather than exposure parameters) across species and across experiments will help to compare and interpret the scientific literature on the acute effects of toluene.

Another complication in the literature on the acute effects of toluene is that the course of effects or the effective dose may differ depending on the behavioral test used. Some indices of behavior uniformly deteriorate as concentration or duration of exposure increases, while other measures form an inverted U-shaped curve as concentration or duration increases. Again, comparison of different behavioral procedures is complicated by the large variety of exposure scenarios used. It may help to interpret and compare behavioral outcomes if a common internal dose metric were used instead of exposure conditions.

The present paper has three purposes: (a) to review the literature on behavioral effects of toluene in nonhuman and human species, (b) to subject tractable parts of the literature to meta-analysis after estimation of toluene blood concentrations via physiologically based pharmacokinetic (PBPK) models, and (c) to discuss and synthesize information about behavioral effects in terms of internal dose of toluene.

NONHUMAN BEHAVIORAL STUDIES

Dose—Effect Function for Successful Performance

When the dependent variable in toluene exposure studies was some measure of successful performance, e.g., successful avoidance, correct discrimination, and accurate recall, the dose—effect functions appear, in general, to reflect monophasic decrements with increasing toluene exposure. This seems true...
in rats performing avoidance tasks (Harabuchi et al., 1993; Kishi et al., 1988; Mullin and Krivanek, 1982; Wada et al., 1989), although some studies have demonstrated slight improvements in performance at small exposure values (Shigeta et al., 1978, 1980; Wada et al., 1986). Animals performing appetitively controlled tasks usually also show monophasic decrements in reinforcement rate. This was true of tasks controlled by fixed-consecutive-number schedule in rats (Wood et al., 1983) and VI schedules in mice (Glowa, 1987; Moser and Balster, 1981). Similar results have been obtained with pigeons (Wada et al., 1993), monkeys (Taylor and Evans, 1985), and baboons (Geller et al., 1985). As an exception to this pattern, Bushnell et al. (1994) observed a slight facilitation of signal detection by rats early in test sessions in toluene vapor, followed by concentration-related decrements.

**Dose–Effect Function for General Activity and Rate of Responding**

*General activity.* Simple motor activity studies in rodents have demonstrated an increase in general activity when the administered amount of toluene is sufficiently small or the duration of administration is sufficiently short (Bushnell et al., 1985; De Ceaurriz et al., 1983; Gause et al., 1985; Kjellstrand et al., 1985; Paksey et al., 1982). With increasing concentration and/or duration of toluene exposure, the initial increases in activity reached a peak and then declined, eventually reaching values below baseline in rodents (Bushnell et al., 1985; Hinman, 1987; Nakagagi et al., 1983; Wada et al., 1989; Wood and Colotla, 1990). All of the above studies used inhaled toluene except Paksey et al. (1982) and Nakagagi et al. (1983) who used ip injection.

*Rate of operant responses.* Many experiments measured operant response rate (as opposed to reinforcement rate) in the same study. Response rates were also observed to increase as the lowest-concentration effect of toluene administration, just as observed for general activity. Increased response rate was seen during avoidance behavior in rats (Bushnell et al., 1978, 1980) and during matching-to-sample performance in baboons (Geller et al., 1985). As with general activity measures, when the administered amount of toluene in operant studies became sufficiently large, biphasic dose–effect curves were found for response rate in mice (Glowa, 1987; Glowa et al., 1986; Moser and Balster, 1981), pigeons (Weiss et al., 1979; Wada et al., 1993) and monkeys (Taylor and Evans, 1985). All of the above were inhalation exposures except for Wada et al. (1993), who used im injection. As exceptions to the above generalization, Moser and Balster (1985, 1986) reported a monophasic dose–effect function, with no increased rate in fixed-ratio and fixed-interval responding.

*Simultaneously measured response rates and successful performance.* Some experimenters reported simple response rate or activity measures in addition to success of performance, measured in the same subjects during the same session. In most cases, the simple response rate formed the familiar biphasic dose–effect curve while successful performance in the same subjects formed a monophasic declining curve (Geller et al., 1985; Glowa, 1987; Harabuchi et al., 1993; Kishi et al., 1988; Moser and Balster, 1981; Wada et al., 1989). Exceptions to these results (Shigeta et al., 1978, 1980) demonstrated improvement in quality of performance along with increased response rate.

Most of the studies containing both response rate and successful performance measures have found both biphasic and monophasic dose–effect functions in the same subjects for the two kinds of measurement respectively. These findings indicate that the shape of the dose–effect function is determined by the type of dependent variable measured and not the different exposure parameters used in different studies. Equating the internal dose across studies may, nonetheless, facilitate comparisons of studies using similar outcome measures.

**Potential Role of Dosimetry**

It is plausible that the behavioral results reviewed above are a simple function of blood toluene concentration and that the actual duration and concentration of exposure only matter in that they produce the particular blood toluene concentrations. None of the experimenters measured the blood concentrations before, during, or after task performance. Occasionally blood concentrations were measured in resting rats which were identically exposed to toluene as the behaving rats. While it is technically simpler to use nonperforming animals for the dosimetry, this technique probably underestimates the tissue concentrations of toluene in the performing animals, because of elevated pulmonary ventilation and cardiac output and hence the toluene uptake rate in subjects performing the tasks. However, estimates are lacking of such differences in activity and metabolic rate and the consequent effects on the uptake on inhaled toluene.

**HUMAN BEHAVIORAL STUDIES**

With few exceptions, experimental studies in humans employ exposure concentrations not greatly exceeding the various standards for industrial protection, the effects of which are expected to be small. This makes estimation of dose–effect functions difficult. For toluene, the time-weighted average threshold limit value is 100 ppm (American Conference of Governmental Industrial Hygienists, 1991). Another difficulty with the human literature is that few experimenters study more than a few concentrations of exposure. Thus, it is difficult to estimate a behavioral dose–effect function from individual studies in the literature.

When significant acute effects of low-level toluene on the ability of human subjects to perform tasks have been reported, they were always decrements in the quality of performance. Experimenters frequently measured multiple dependent variables in a given experiment, including various measures of
To address the issues regarding the observed complexity of the effects of exposure duration and concentration, data in the literature were subjected to a dosimetric meta-analysis. For studies in which sufficiently detailed reporting made it possible, exposure data were used to estimate the blood toluene concentration using a PBPK model of the form of Ramsey and Anderson (1984). Behavioral dependent variables were converted to a common metameasure so that data could be pooled across studies. Empirical functions were fitted to the dependent metameasures (Benignus, 1994) as predicted from the estimated toluene blood concentrations. The same procedure was followed for rat and human studies with appropriately scaled PBPK model parameters.

A META-ANALYSIS USING A PBPK MODEL

The most appropriate dose metric for toluene is arterial blood, because venous blood, especially before equilibrium, has been depleted of toluene by tissue uptake and its toluene concentration does not represent what is being supplied to the brain by arterial blood (Baelum, 1991; Carlsson, 1982; Wallen et al., 1984). Arterial blood concentrations of solvents have occasionally been estimated from properly collected alveolar breath samples (Kelman, 1982). When dose measures appeared in human behavioral experiments, however, they were usually venous blood. Even when blood or breath samples were collected, the samples were usually taken infrequently and not during task performance. The appropriate independent variable would be the blood toluene during performance.

METHOD

Procedure. To evaluate the hypothesis that the behavioral effects of acute toluene inhalation are related to internal dose, arterial toluene concentration ($C_{\text{Ar}}$) was estimated from the exposure data in selected reports in the literature using a PBPK model. Behavioral data were converted to numerical values from figures in the reports using a graphical analog-to-digital converter (SummaSketch Model III). Dependent variables were expressed as proportion of baseline or control in cases where it was sensible to do so (see Appendix I). Descriptive curves were then fitted to behavioral vs estimated dose data using nonlinear procedures (Gallant, 1975) with PROC NLIN from the Statistical Analysis System (SAS, 1996). The nonlinear fits were made by pooling all of the selected studies into one data base and fitting one curve per dependent variable.

Selecting a report from the literature for analysis required that (a) sufficient quantification of exposure and behavioral data was given, (b) a wide range of exposure concentrations and/or durations was used, (c) a number of data points were given, and (d) rats or humans were used as subjects (because of the absence reliable estimates for some PBPK parameters for other species). For human subjects, requirements of wide-range exposures and many data points could not be met and consequently the meta-analysis is less general. Appendix I gives the reasons for excluding articles in the analysis. The studies selected for successful performance and for response rate in rats are given in Appendix 2 with particulars and rationale. Successful performance and response rate data from rats all came from studies of avoidance experiments. Appendix 3 gives the studies and particulars for the meta-analysis of human performance. In this analysis, CRT with visual pattern stimuli were selected and all dependent variables were converted to reciprocals of CRT to provide a scale which descends as performance is impaired and these inverse scores were then converted to percent of baseline.

The PBPK model. A PBPK model (Ramsey and Anderson, 1984) was encoded in a BASIC language program (Reitz, 1991) and converted to a FORTRAN batch-mode program for use in the present work. To assure that programming errors were not made, the final program was extensively tested using comparisons of solutions to the original model. Parameters (see Appendices 4 and 5) were selected from the literature for rats and humans, respectively.

Determination of $C_{\text{Ar}}$ via a PBPK model requires knowledge of physiological parameters which are frequently not available for a particular situation. For example, pulmonary ventilation and cardiac output increase with physical work as well as with anxiety and these increases will increase the rate at which toluene is taken up. In each of the rat studies, subjects were performing a behavioral task involving physical work and stress. Pulmonary ventilation and cardiac output were first determined from the individual experiments by scaling these parameters to body weight. Laboratory rats will approximately double their ventilation during maximal exercise and it seems that a task such as avoidance behavior does not elevate exercise levels to nearly maximal. It was arbitrarily decided to simulate a slight amount of exercise by multiplying estimated pulmonary ventilation and cardiac output by 1.2. If the value is wrong, the $C_{\text{Ar}}$ will be wrong by some amount, especially during the early
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part of acquisition and elimination of toluene. Other errors in determining \( \text{Ca}_{\text{TOL}} \) are also possible because of other erroneous physiological parameters. The effect of such error would be to reduce the degree with which a curve fits the dose–effect data and to increase error variance. For the human studies pulmonary ventilation and cardiac output were rounded to 7 L per minute, a typical value for resting conditions. Resting conditions are appropriate to humans performing a CRT task which involves little physical work and no threat of aversive stimulation.

Typically, behavioral data are collected continuously over a segment of time and the means reported for each of several segments during the acquisition or elimination of toluene. \( \text{Ca}_{\text{TOL}} \) frequently was changing during a segment of performance. In these cases \( \text{Ca}_{\text{TOL}} \) was estimated repeatedly over time by the PBPK model during each segment of performance and the values were integrated and normalized as a mean. The mean \( \text{Ca}_{\text{TOL}} \) for each particular segment was then used for the corresponding reported mean behavioral measure in fitting the dose–effect curve.

The dose–effect equations. The dose–effect functions were fitted using the logistic model (Ashford, 1958). This model is frequently used in behavioral work and has a number of advantages (Corso, 1967). Other approximately ogive-shaped curves could have been selected, but for a meta-analysis, it is doubtful that the exact curve shape is critical. The equation fit to those data in which baseline was scaled to a value of 1.0 and decrements were expressed as fractions of baseline is

\[
F_1 = \frac{1}{1 + e^{ \beta_1 \text{Est} \text{ar} \text{t}_{\text{B}}}}
\]

in which \( F_1 \) is the behavioral measure, \( e \) is the base of natural logarithms (\( e \approx 2.71828 \)), \( \beta_1 \) and \( \beta_2 \) are empirical coefficients, and \( \text{Est} \text{ar} \text{t}_{\text{B}} \) is the estimated arterial blood toluene.

The equation fit to data which increase over a baseline of 1.0 in a dose–effect function is

\[
F_2 = \frac{1}{1 + e^{ -\beta_2 \text{Est} \text{ar} \text{t}_{\text{B}}}} + 1
\]

in which \( F_2 \) is the behavioral measure. A biphasic effect in which the behavioral measure increases as dose rises, reaches a maximum, and is followed by a decrease may be described by the combination of an increasing function of dose and a decreasing function of dose. The product of Eqs. (1) and (2) provides such a function, as follows

\[
F_3 = F_1(F_2)
\]

in which \( F_3 \) is the biphasic behavioral measure. Note that Eq. (3) has four parameters, two for Eq. (1) and two for Eq. (2). Equation (1) was used to fit quality of performance data and Eq. (3) was used for the biphasic response-rate data.

No claim may be made that Eqs. (1) or (3) represent known physiological processes. Equation (1) is frequently used to fit dose–effect functions and Eq. (3) follows a similar process as is frequently hypothesized for mechanisms of action in which an excitatory neural effect is eventually overcome at higher concentrations by a different inhibitory neural effect.

Statistics. After a curve was fitted to the rat data, an attempt was made to predict the residuals of the fitted curve from exposure concentration and duration. A statistically significant prediction of the residuals by either concentration or duration would indicate that not all of the results were accounted for by \( \text{Ca}_{\text{TOL}} \). The prediction was made and tested with a mixed model using SAS PROC MIXED in which the prediction model was

\[
R_i = C_i + D_i
\]

in which \( R_i \) is the residual at observation \( i \) and \( C \) and \( D \) represent the corresponding concentration and duration of exposure. All observations from a particular study were considered to be repeated measures on the same sampling unit. An unstructured covariance matrix was fitted using restricted/residual maximum likelihood methods with the scoring algorithm for iteration. Type III \( F \) statistics are given under Results. Because of the few studies available and the lack of wide-range dose–effect data, the human curve fit was not statistically tested.

RESULTS

Rat Avoidance Performance

The proportion of successful avoidance responses in rats exposed to toluene is plotted in Fig. 1 as a function of \( \text{Ca}_{\text{TOL}} \). Data collected during uptake (filled points) and during elimination of toluene (open points) were pooled across four studies. The plotted line in Fig. 1 was given by Eq. (1) which was used to fit the data, yielding \( \beta_1 = -15.7 \) and \( \beta_2 = 3.14 \). The mixed-model regression revealed that neither concentration \( (F(1,2) = 10.94, p = 0.080) \) nor duration of exposure \( (F(1,3) = 0.18, p = 0.702) \) accounted for a significant amount of the variance in the residuals from the fitted logit curve. The fitted curve with the empirical points from both uptake and elimination, plus the statistics from the mixed-model residuals analysis, demonstrate that the effect of toluene on quality of performance is a monotonic orderly function of \( \text{Ca}_{\text{TOL}} \) and that the only significant factor for predicting behavioral effect was \( \text{Ca}_{\text{TOL}} \).

To illustrate the relationship between exposure concentra-
TABLE 1
Estimated Exposure Durations and Concentrations Required to Reduce Avoidance Performance in Rats by 10%

<table>
<thead>
<tr>
<th>Duration (min)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>4330</td>
</tr>
<tr>
<td>60</td>
<td>3120</td>
</tr>
<tr>
<td>120</td>
<td>2370</td>
</tr>
</tbody>
</table>

*Estimates from PBPK model using a 350-g rat with $C_{\text{TOL}} = 73$ ml/L. The $C_{\text{TOL}}$ was estimated from the fitted logit curve evaluated at 10% point (see arrow in Fig. 1).

The concentration and duration, the PBPK model was used to estimate the concentration needed to produce a value of $C_{\text{TOL}} = 73$ ml/L in 30, 60, or 120 min. The latter value of $C_{\text{TOL}}$ was associated with the 10% deficit in quality of performance (see the arrow in Fig. 1). The concentration and time combinations are given in Table 1.

Rat Biphasic Response Rate Curves

Proportional response rates in rats exposed to toluene are plotted in Fig. 2 as a function of $C_{\text{TOL}}$. Data collected during uptake (filled points) and during elimination of toluene (open points) were pooled across four studies. The plotted line in Fig. 1 was given by Eq. (3) which was used to fit the data, yielding $\beta_1 = -29.2$, $\beta_2 = 5.71$, $\beta_3 = -3.71$, and $\beta_4 = 0.689$. The mixed-model regression revealed that neither concentration ($F(1,4) = 0.18$, $p = 0.692$) nor duration of exposure ($F(1,4) = 0.50$, $p = 0.551$) accounted for a significant amount of the variance in the residuals from the fitted curve. As above, the fitted curve with empirical points from both uptake and elimination, plus the statistics from the mixed-model residuals analysis, demonstrate that the effect of toluene on rate of responding is a biphasic orderly function of $C_{\text{TOL}}$ and that the only significant factor for predicting this behavioral effect was $C_{\text{TOL}}$.

For illustrative purposes, the PBPK model was used to estimate the toluene exposure concentrations needed to produce three levels of $C_{\text{TOL}}$ (see points A, B, and C in Fig. 2) in 30, 60, and 120 min. The results are given in Table 2.

Human CRT Performance Dose-Effect Curves

The proportional decrement in human CRT performance is plotted in Fig. 3 as a function of $C_{\text{TOL}}$. Due to the low doses of $C_{\text{TOL}}$, the model of Eq. (1) was fitted in a range of the dose-effect curve which appears to be linear, despite the model's nonlinear form. The fitted parameters were $\beta_1 = -3.22$ and $\beta_2 = 0.941$. Extrapolation beyond the dose range observed would be especially inappropriate in this case. The arrow indicates that a 10% impairment should occur at an $C_{\text{TOL}}$ of nearly 3 ml/L. The inhaled air concentrations at 30, 60, and 120 min which have been estimated via the PBPK model to produce the $C_{\text{TOL}}$ at the 10% impairment dose are given in Table 3.

DISCUSSION

Use of Estimated Blood Concentration as the Predictor

From the above review and limited meta-analyses, it appears that dosimetric information can greatly improve and simplify

TABLE 2
Estimated Exposure Durations and Concentrations Required to Produce Various Effects on Rate of Responding in Rats

<table>
<thead>
<tr>
<th>ppm Required to Produce Listed Effect</th>
<th>Rising limb 10% effect</th>
<th>Peak effect 10% effect</th>
<th>Falling limb 10% effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (min)</td>
<td>($C_{\text{TOL}} \sim 13$ ml/L)</td>
<td>($C_{\text{TOL}} \sim 85$ ml/L)</td>
<td>($C_{\text{TOL}} \sim 152$ ml/L)</td>
</tr>
<tr>
<td>30</td>
<td>970</td>
<td>5000</td>
<td>8720</td>
</tr>
<tr>
<td>60</td>
<td>770</td>
<td>3580</td>
<td>6200</td>
</tr>
<tr>
<td>120</td>
<td>640</td>
<td>2710</td>
<td>4640</td>
</tr>
</tbody>
</table>

*Estimates from PBPK model using a 350-g rat with $C_{\text{TOL}}$ values as stated in the table. The $C_{\text{TOL}}$ was from the fitted curve evaluated at points A, B, and C in Fig. 2.
predictions of the behavioral effects of acute inhaled toluene. A variety of effects poorly related to the concentration and duration of inhaled toluene become clearly ordered by converting the exposure parameters to $\text{Ca}_{\text{TOL}}$, an index of internal dose. Similarly estimated arterial blood concentrations were found to be good predictors of behavioral and neurophysiological changes occurring during acute inhalation of trichloroethane (Boyes et al., 1997).

Using data from the available sufficiently documented publications, it appears that $\text{Ca}_{\text{TOL}}$ was the only important independent variable. This was indicated by the fact that neither concentration or duration of exposure to toluene accounted for a significant amount of residual variance after the effects of $\text{Ca}_{\text{TOL}}$ were considered in either of the two dependent variables measured in rats. Inspection of Figs. 1 and 2 also illustrates that responding during exposure and after exposure (filled vs open points) followed the same functions. This was also true for individual studies (not illustrated with figures) in which both during and after exposure data were collected. It is possible, however, that concentration or duration of exposure exert an effect independent of $\text{Ca}_{\text{TOL}}$, but that individual-subject data would be required to detect such effects.

The findings and conclusions of the present report should be taken with caution, however, because only summary data from other reported experiments were used. Pharmacokinetic prediction of $\text{Ca}_{\text{TOL}}$ from exposure data is fraught with possible error, even for physiologically based models. Unless, e.g., alveolar ventilation and cardiac output are known (and they almost never are) the predictions can be erroneous by an important amount. The same may be said for other variables affecting the PBPK model.

Even if important errors in $\text{Ca}_{\text{TOL}}$ were made in the present analysis, the effects of such error would have been either to bias the estimate of the internal dose or to increase the variability of the internal dose across individuals or studies. In the case of biased estimation, the effect on the analysis would be to shift critical features of the dose–effect curve to the right or left and/or to distort the shape of the curve. Such error would not affect the conclusion that $\text{Ca}_{\text{TOL}}$ is a more appropriate independent variable than concentration and duration of exposure. One should not, however, consider the $\text{Ca}_{\text{TOL}}$ values as correct in an absolute sense from the PBPK estimation procedure. If the errors in $\text{Ca}_{\text{TOL}}$ increased variability, the effect would have been to make the dose–effect curve fit more poorly than otherwise. Thus, more accurate estimates of internal dose should produce a better fit to the behavioral data.

**Dose–Effect Curves**

Possible problems with meta-analyses. In all cases, dependent variables were transformed to a common scale (percentage of baseline). This transformation works well when baseline values are not near zero, but seriously distorts shape and interpretation when small baseline values occur. For open field activity level, baselines are frequently small (e.g., Hinman, 1987). The method of analysis, therefore, required the exclusion of this kind of data.

Interpretational problems are common when different types of dependent variables are measured across studies. To merge heterogeneous studies risks inappropriate transforms and distortions; to exclude them reduces generality of quantitative analyses. In the present article, the case is made that several behavioral dependent variables are systematically related to internal dose. Qualitative inspection of the data and the assumption of parsimony leads to the hypothesis that other dependent variables are similarly related to internal dose, even though the particulars may be different.

**Nonhuman data.** The present meta-analyses clearly indicate that the shape of the dose–effect function for toluene

### Table 3

<table>
<thead>
<tr>
<th>Duration (min)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>120</td>
<td>27</td>
</tr>
</tbody>
</table>

$a$ Estimates from PBPK model using parameters of Appendix 5 $\text{Ca}_{\text{TOL}} = 3$ ml/L. The $\text{Ca}_{\text{TOL}}$ was from the fitted logit curve evaluated at 10% point (see arrow in Fig. 3).
differs depending upon the behavioral endpoint measured. Measures of "activation" (e.g., response rate or motor activity) appear to respond to toluene in a biphasic fashion, with increases in activity at low or moderate concentrations and decreases in activity at high concentrations. This biphasic function may reflect a general property of CNS depressant substances (Evans and Balster, 1991) and has been attributed to suppression of the inhibitory influence of cortical structures on behavior at low tissue concentrations followed by inhibition of motor pathways at higher concentrations (Bushnell et al., 1975; Bushnell and Crofton, 1996). The monophasic dose–effect function obtained for measures of successful performance is consistent with the scant literature in which accuracy, rather than rate of responding, was measured (but see Bushnell et al., 1994). The usual lack of facilitation at low concentrations suggests that disinhibition of motor responses does not increase choice accuracy, whereas it may increase response rate.

The scale factor for dose–effect curves may differ according to experimental designs. For example, Wood and Cox (1995) studied a large number of subjects and obtained extensive repeated measures on appetitively conditioned rats. Careful analysis of this extensive data base revealed effects that appeared to be larger and to commence at lower exposure levels than for other similar studies. It was not clear whether this was due to reduced variance in the experiment or to actual increased sensitivity of the particular version of the task.

**Human data.** All measures of behavior assessing quality of performance in humans appear to be affected by acute toluene exposure in a monophasic, detrimental manner. In many instances, performance was not declared to be significantly affected by the authors of individual studies, but when data were combined across studies and a curve fitted, the trends were clear even for lower concentrations of exposure (see Fig. 3). It appears that human performance is impaired by 10% when \( C_{a_{TOL}} \approx 3 \text{ ml/L} \) (see Table 3 for exposure equivalent exposure conditions). The estimated exposure concentrations of Table 3 are lower than have been reported as statistically significant for any single study and result from a meta analysis of combined data. Such low values of exposure should be treated with considerable suspicion until dose–effect curves can be experimentally verified.

**Comparisons of human and nonhuman findings.** Comparisons across species when using \( C_{a_{TOL}} \) from PBPK models should be viewed with great caution. There are several possible explanations for the apparent difference in sensitivity to toluene across species. The difference between species could be due to some methodological differences, which when understood, could change the conclusion. Such possible differences are (a) inaccuracies in the predictions of \( C_{a_{TOL}} \) due to erroneously selected model parameters or (b) differences in the difficulty or sensitivity of behavioral tasks across the two species.

With rate of responding or activity level (Fig. 2) in nonhuman subjects, effects of toluene (increased rates) began at a considerably lower concentration (\( C_{a_{TOL}} \approx 13 \text{ ml/L} \)) than did changes in quality of performance (\( C_{a_{TOL}} \approx 70 \text{ ml/L} \)) (Fig. 1). The value of 13 ml/L is not much greater than the \( C_{a_{TOL}} \) that impaired quality of performance in humans (ca. 3 ml/L arterial blood). The interpretation of the increased activity and rates of responding in nonhumans is difficult because it is not demonstrably detrimental to quality of performance in these tasks. No objective activity or comparable response-rate data have been collected for human subjects exposed to toluene.

If humans and rats could be demonstrated to differ in sensitivity even if the methodological issues were resolved, a number of possible explanations for the difference in sensitivity would remain, including differences in (a) the partitioning of toluene between blood and brain, (b) the (possible) metabolism of toluene to a more potent metabolite in humans, (c) the (possible) metabolism of toluene in the brain, (d) sensitivity of brain tissue to toluene or its active metabolite, and (e) neurological substrates governing performance of the tasks. Possibilities (b) and (c) are not supported by the present results because \( C_{a_{TOL}} \) was a good predictor of behavioral outcomes during both uptake and elimination phases of the curve. This pattern suggests that toluene itself is the active compound because it seems unlikely that toluene and a more water soluble metabolite would have parallel time courses for both uptake and elimination.

**SUMMARY**

The results reported in the present article have implications for the risk assessment of toluene exposure and, probably, other volatile organic compounds. The principal points are as follows.

1. Expressing the independent variable as internal tissue concentration rather than as external exposure parameters improved the concordance across studies, reduced the number of dose parameters, and made it possible to display data from both toluene uptake and elimination phases on one plot.

2. When maximum environmental concentrations are given in a risk assessment, they should be determined from a dosimetric analysis much as is already done for carbon monoxide (United States Environmental Protection Agency, 1991) and as implicitly suggested in Tables 1–3.

3. The shape of the dose–effect curve depends on the kind of behavior studied. For successful performance (e.g., number of correct responses) the curve shows a monotonic decrease as \( C_{a_{TOL}} \) increases. For activity or rate of responding the curve is biphasic, first increasing and then reaching a peak and eventually decreasing as \( C_{a_{TOL}} \) increases.

4. Although there is a high probability of bias in estimation, it appears that humans are more sensitive to the effects of increases in \( C_{a_{TOL}} \). Before such a conclusion can be supported, however, much more human and some more rat data must be collected in experiments designed to resolve the issue.
APPENDIX 1

Reasons for Exclusion of Studies from the Meta-analysis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. (1983)</td>
<td>No CRT data</td>
</tr>
<tr>
<td>Baelum et al. (1990)</td>
<td>No CRT collected with visual pattern stimuli</td>
</tr>
<tr>
<td>Bushnell et al. (1994)</td>
<td>Unable to convert reported data to percentage</td>
</tr>
<tr>
<td>Cherry et al. (1983)</td>
<td>Uncertain about start of exposure; only two points</td>
</tr>
<tr>
<td>Hinman (1987)</td>
<td>Difficult to scale dependent variable*</td>
</tr>
<tr>
<td>Horvath et al. (1981)</td>
<td>No CRT data</td>
</tr>
<tr>
<td>Nakagagi et al. (1983)</td>
<td>Noninhalation exposure</td>
</tr>
<tr>
<td>Paksy et al. (1982)</td>
<td>Noninhalation exposure</td>
</tr>
<tr>
<td>Wada et al. (1986)</td>
<td>Latency data only</td>
</tr>
<tr>
<td>Wood and Cox (1995)</td>
<td>Raw data given on one animal; means for low ppm only</td>
</tr>
<tr>
<td>Wood et al. (1983)</td>
<td>Only three rats</td>
</tr>
</tbody>
</table>

* Dependent variable was activity rate. Baseline activity was near zero.
Thus, the percentage-of-baseline transformation would have yielded extremely large (and not meaningful) values for increased activity. Studies of this nature would require a more appropriate transformation for meta-analyses.

APPENDIX 2

Sources of Rat Data

Figures 1 and 2

Harabuchi et al. (1993). Mean avoidance responses (lever press) and simple response rate from a total of 16 rats repeatedly measured during the dark phase, collected before, during 240 min of exposure, and for up to 180 min after exposure. Data taken from their Figs. 3B and 4B, respectively.

Kishi et al. (1988). Mean avoidance responses (lever press) and simple response rate data from a total of eight rats repeatedly measured before, during 240 min of exposure, and for up to 140 min after exposure. Data taken from their Figs. 8 and 7, respectively.

Figure 1 Only

Mullin and Krivanek (1982). Mean avoidance responses (lever press) from a total of six rats in three exposure levels (including control), each rat measured repeatedly during 240 min of exposure. Data taken from their Table 5.

Wada et al. (1989). Mean avoidance responses (shuttle box) from a total of 40 rats (8 rats in five exposure levels, including control), measured repeatedly during 240 min exposure and for 3 days after exposure (only up to 180 min postexposure data used). Data taken from their Fig. 1.

Figure 2 Only

Shigeta et al. (1980). Mean response rate (lever press) in an avoidance experiment from a total of 15 rats exposed to all toluene levels and repeatedly measured 60, 120, and 240 min after exposure for 240 min to 350, 750, or 1500 ppm toluene.

APPENDIX 3

Sources of Human Data (Fig. 3)

Dick et al. (1984). Mean CRT from a total of 30 subjects collected for 20 min after 100 and 220 min of exposure to 100 ppm toluene and after 60 min of postexposure air breathing. Data taken from their Table 4, Panel B, "Choice Reaction: Response Time." A total of four points, including preexposure.

Echeverria et al. (1989). Mean pattern recognition latency from a total of 21 subjects collected for 2 min after 210 and 240 min of exposure to either 75 or 150 ppm toluene. Data taken from their Fig. 2C. A total of three points including air control.

Gamberale and Hultengren (1972). Mean spokes test reaction time from a total of 12 subjects during a stepwise exposure schedule (20 min per step) of nominally 100, 300, 500, and 714 ppm toluene. Data taken from their Table 2, "Spokes." A total of four points.

Iregren et al. (1986). Mean CRT from a total of 12 subjects collected for 10 min after 120 and 210 min of exposure to 80 ppm toluene. Data taken from their Table 2, "Choice Reaction Time." A total of three points including air control.

Olson et al. (1985). Mean CRT from a total of 16 subjects, collected for 10 min after 110 min and 230 min exposure to 80 ppm toluene. Data from the two measurements were averaged by the authors. Data taken from their Fig. 5, first two points. A total of two points including air control.

Rahill et al. (1996). Mean response times from a total of six subjects, collected at three measurement times during a 6-h exposure to 100 ppm toluene. Data from the five subsets of the ANAM computerized test battery which were significantly affected by toluene (their Table II) were averaged. A total of six points, three from air control and three from exposure condition. The gradual increase of toluene concentration in the chamber was simulated by four step segments of increasing concentration. The exercise portion of exposure was handled by a commercially available whole-body human biological simulator, QCP3 (Biological Simulators, Inc.). Simulated exercise level was 33W. QCP3 provided estimates of alveolar ventilation, cardiac output, and altered perfusion of fat and liver compartment. For the exercise portion of the exposure,
the following parameters (from QCP3) were used in the PBPK model; alveolar ventilation, 960 L/h; cardiac output, 554 L/h; fraction of blood to fat, 0.0208; and fraction of blood to liver, 0.112.

APPENDIX 4

Parameters Used in the Rat PBPK Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>From individual reports</td>
</tr>
<tr>
<td>Alveolar ventilation rate (L/h)</td>
<td>Scaled to body weight</td>
</tr>
<tr>
<td>Cardiac output (L/h)</td>
<td>Scaled to body weight</td>
</tr>
<tr>
<td>Proportion of fat tissue</td>
<td>0.090 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Proportion of liver tissue</td>
<td>0.049 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Fraction of blood to fat</td>
<td>0.090 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Fraction of blood to liver</td>
<td>0.250 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Fraction of blood in body</td>
<td>0.064 (Wang, 1959)</td>
</tr>
<tr>
<td>Liver/blood partition coefficient</td>
<td>4.640 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Fat/blood partition coefficient</td>
<td>28.000 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Tissue/blood part coefficient</td>
<td>1.540 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Blood/air partition coefficient</td>
<td>18.000 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Maximum metabolic rate (ml/h)</td>
<td>7.500 (Purcell et al., 1990)</td>
</tr>
<tr>
<td>Michaelis-Menten constant (ml/L)</td>
<td>0.300 (Purcell et al., 1990)</td>
</tr>
</tbody>
</table>

* Predicted values were arbitrarily multiplied by 1.2 to approximate their elevation due to task performance.

APPENDIX 5

Parameters Used in the Human PBPK Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>70 kg fixed</td>
</tr>
<tr>
<td>Alveolar ventilation rate (L/h)</td>
<td>415 (Carlsson, 1982)</td>
</tr>
<tr>
<td>Cardiac output (L/h)</td>
<td>Matched to alveolar ventilation</td>
</tr>
<tr>
<td>Proportion of fat tissue</td>
<td>0.190 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Proportion of liver tissue</td>
<td>0.026 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Fraction of blood to fat</td>
<td>0.050 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Fraction of blood to liver</td>
<td>0.260 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Fraction of blood in body</td>
<td>0.071 (Benignus et al., 1994)</td>
</tr>
<tr>
<td>Liver/blood partition coefficient</td>
<td>2.980 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Fat/blood partition coefficient</td>
<td>65.800 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Tissue/blood part coefficient</td>
<td>1.370 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Blood/air partition coefficient</td>
<td>15.600 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Maximum metabolic rate (ml/h)</td>
<td>336.0 (Tardif et al., 1995)</td>
</tr>
<tr>
<td>Michaelis-Menten constant (ml/L)</td>
<td>0.550 (Tardif et al., 1995)</td>
</tr>
</tbody>
</table>

REFERENCES


Biological Simulators, Inc. Thomas Coleman, Ph.D., P.O. Box 14014, Jackson, MS. 39226-4014. Internet address: BIOSIM@NETBOX.COM.


DOSIMETRICS OF ACUTE TOLUENE EXPOSURE


