Assessment of the Health Effects of Chemicals in Humans: I. QSAR Estimation of the Maximum Recommended Therapeutic Dose (MRTD) and No Effect Level (NOEL) of Organic Chemicals Based on Clinical Trial Data[1]

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ABSTRACT: The primary objective of this investigation was to develop a QSAR model to estimate the no effect level (NOEL) of chemicals in humans using data derived from pharmaceutical clinical trials and the *MCASE* software program. We believe that a NOEL model derived from human data provides a more specific estimate of the toxic dose threshold of chemicals in humans compared to current risk assessment models which extrapolate from animals to humans employing multiple uncertainty safety factors. A database of the maximum recommended therapeutic dose (MRTD) of marketed pharmaceuticals was compiled. Chemicals with low MRTDs were classified as high-toxicity compounds; chemicals with high MRTDs were classified as low-toxicity compounds. Two separate training data sets were constructed to identify specific structural alerts associated with high and low toxicity chemicals. A total of 134 decision alerts correlated with toxicity in humans were identified from 1309 training data set chemicals. An internal validation experiment showed that predictions for high- and low-toxicity chemicals were good (positive predictivity >92%) and differences between experimental and predicted MRTDs were small (0.27–0.70 log-fold). Furthermore, the model exhibited good coverage (89.9–93.6%) for three classes of chemicals (pharmaceuticals, direct food additives, and food contact substances). An additional investigation demonstrated that the maximum tolerated dose (MTD) of chemicals in rodents was poorly correlated with MRTD values in humans ($R^2 = 0.2005$, n = 326). Finally, this report discusses experimental factors which influence the accuracy of test chemical predictions, potential applications of the model, and the advantages of this model over those that rely only on results of animal toxicology studies.

[1]This report is not an official U.S. Food and Drug Administration guidance or policy statement. No official support or endorsement by the U.S. Food and Drug Administration is intended or should be inferred.

Key Words: *MCASE*, computational toxicology, human clinical data, MRTD, NOEL, QSAR, predictive modeling, FDA.

INTRODUCTION

FDA's Center for Drug Evaluation and Research (CDER), Office of Pharmaceutical Science (OPS), Informatics and Computational Safety Analysis Staff (ICSAS) provides a Computational Toxicology Consultancy Service for the Agency. This service develops, validates, and uses computational toxicology software to provide decision support information for Agency regulatory and research decisions. The Service has invested considerable time and effort in the development of new software programs to meet the needs of the Agency because until recently commercial programs have been either unavailable or have provided unreliable estimates of the toxicities of FDA regulated pharmaceuticals [1]. Our non-clinical research is targeting the development of software to predict toxicology endpoints necessary for product marketing and the dose range at which chemicals elicit their toxic effects [1–3]. It is our mission to develop a complete battery of software which corresponds to all of the major toxicology studies recommended by the Agency's Centers. Our clinical research effort is focused on the development of software to predict the potential organ and organ system adverse effects of chemicals in adult humans. These software use data obtained from pharmaceutical clinical trials and post-market surveillance of the adverse effects of pharmaceuticals that were reported in FDA/CDER's Spontaneous Reporting System (SRS) and Adverse Event Reporting System (AERS) databases [4].

The major objective of this investigation was to develop a new quantitative structure activity relationship (QSAR) expert system to estimate the MRTDs and NOELs of organic chemicals in humans. We believed that MRTD and NOEL estimates derived from human data would provide a more relevant, accurate, sensitive, and specific estimate of the toxic dose level of chemicals in humans compared to current risk assessment models which rely upon the extrapolation of data from animal toxicology tests. We have previously demonstrated that *MCASE* can be used to predict the

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potential carcinogenicity of chemicals in male and female rats and mice [1]. We hypothesized that a comparable module set could be constructed to characterize structural alerts for the MRTDs and NOELs of chemicals in humans using a continuous dose concentration data set.

We used MRTD data for pharmaceuticals as a means of estimating MRTD and NOEL doses of chemicals in humans in our model. The MRTD is empirically derived from human clinical trials, thus it is a direct measure of the threshold for dose-related adverse effects of chemicals in humans. Clinical trials for pharmaceuticals are performed to evaluate safety and efficacy of drugs and to identify specific dose levels that elicit beneficial and adverse pharmacologic effects. The MRTD of a pharmaceutical is an estimated upper dose limit beyond which a drug's efficacy is not increased and/or undesirable adverse effects begin to outweigh beneficial effects.

A pharmaceutical's MRTD and NOEL in humans are directly related to one another. Based upon our analyses of the therapeutic dose ranges for pharmacologic effects of drugs in our database, the overwhelming majority of drugs demonstrate efficacy over a small range of treatment doses. An analysis of the MRTD database revealed that most drugs do not demonstrate efficacy or adverse effects at a dose approximately 1/10 the MRTD (data not presented). Based upon this observation, NOEL is defined as MRTD/10 in this study. For a few noteworthy pharmaceutical categories (*e.g.* some chemotherapeutics and immunosuppressants), the clinically effective dose may be a dose that is accompanied by substantial adverse effects. In such cases, the true NOEL value may be less than 1/10 the MRTD. On the other hand, for chemicals that are not pharmaceuticals there is no MRTD and the NOEL can be considered a dose above which any compound related effect is likely to be considered an adverse effect and a manifestation of toxicity.

The clinical MRTD is in some ways analogous to the maximum tolerated dose (MTD) used in rodent carcinogenicity studies. The maximum tolerated dose in rodents is a dose beyond which toxicity may result in an unacceptable effect on survival in a two year carcinogenicity study. The MRTD of a pharmaceutical is an estimated upper dose limit beyond which a drug's efficacy is not increased and/or undesirable adverse effects begin to outweigh beneficial effects. Both experimental values are derived from studies that employ long treatment periods (generally 3–12 months in humans, 18–24 months in rodents), multiple treatment doses, and the use of a placebo (negative) control group. Clinical trials can identify adverse effects of pharmaceuticals in humans that are poorly assessed in animal toxicology studies (*e.g.* cognitive and mood altering effects, *etc*.). Thus, the MRTD can be a more effective measure of the threshold for adverse effects of chemicals in humans.

MATERIALS AND METHODS

Software and Hardware

The software program used in this investigation is a two-component system that includes a utility operating system (*MCASE* version 3.55, obtained from MultiCASE, Inc.) and individual modules for specific toxicology endpoints. The toxicology modules were developed at FDA under a Cooperative Research and Development Agreement (CRADA) between FDA and MultiCASE, Inc. The *MCASE* program was run on a Compaq (formerly DEC) Alpha workstation (500 MHz) that included a DECram and an OpenVMS operating system. The program reduced the simplified molecular input line entry system (SMILES) codes of organic chemicals to all possible 2–10 consecutive atom molecular fragments. Fragments of active and inactive molecules were then compared, and through molecular subtraction those fragments (structural alerts) that were only associated with active (positive) molecules were identified. The program then identified QSAR attributes and/or molecular fragments that were modulators of activity. Modulators are molecular structure parameters that correlate with enhanced or diminished activity of chemicals that share a common structural alert, *e.g.* activating fragments, inactivating fragments, log P, graph index. The combination of these data was used to develop a quantitative estimate of the potential toxicity of test chemicals. The results of QSAR prediction experiments were saved in a summary data file, a comprehensive data file, and a statistical analysis file in ASCII format.

The MRTD Database

This investigation began with a list of 2114 pharmaceuticals included in FDA/CDER's toxicology and SRS databases [4]. The compounds included both older and newly marketed pharmaceuticals, pharmaceutical aids, diagnostic agents, and a few non-regulated substances with pharmacologic properties. We identified the MRTD values for 1653 of these compounds using *Martindale: The Extra Pharmacopoeia* (1973, 1983, and 1993) and *The Physicians' Desk Reference* (1995 and 1999) [5, 6]. 344 compounds were ineligible for analysis due to chemical class restrictions (see Chemical Class below), but the remaining 1309 were suitable and their MRTD values used to construct databases and *MCASE* modules. The MRTD values in this set formed a continuous, 8-log distribution (0.00001–1000 mg/kg-bw/day) and there was no clear separation of compounds with high and low MRTD values (**Figure 1, A95** data). A major portion (1235/1309) of our MRTD values were obtained for marketed pharmaceuticals and these data are non-proprietary. The data set used in this investigation, excluding proprietary data, will be made publicly available at the FDA/CDER Website (www.fda.gov/cder/icsas/mrtd.html), and will include the preferred generic name, molecular structure (SMILES code), and MRTD values.

MRTD Values

Routes of Exposure

Most of the MRTD values that were included in our database were determined in pharmaceutical clinical trials that employed an oral route of exposure and daily treatments, usually for 3–12 months. The pharmaceuticals were given as single or divided dose treatment regimens to achieve desired pharmacological effects. In contrast, roughly 5% of the

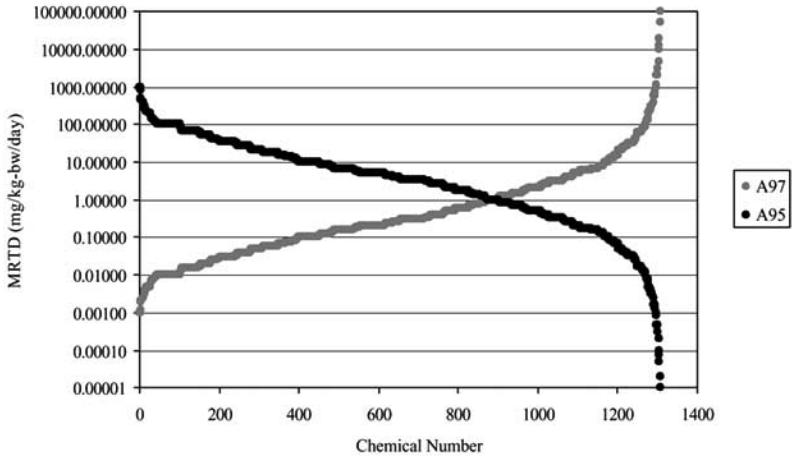

Fig. (1). Plot of chemical number in the database versus the corresponding log-normalized MRTD value in mg/kg-bw/day. Chemical numbers refer to the position of a particular chemical within the entire data set of 1309 chemicals listed in order of increasing activity.

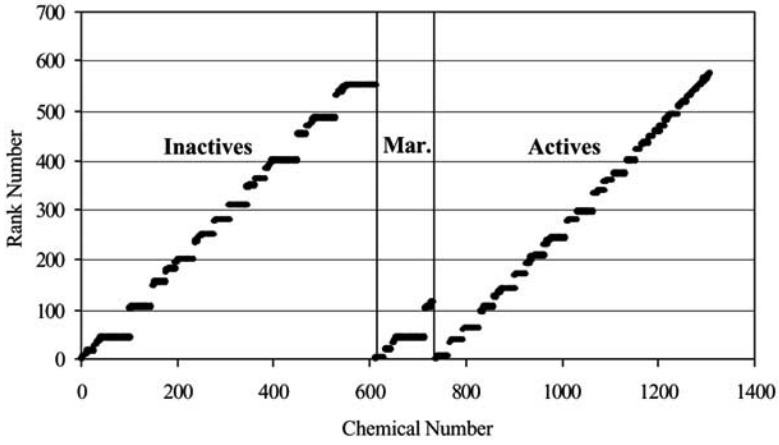

Fig. (2). Plot of chemical number in the database versus corresponding rank number for each subset of inactive, marginal and active chemicals in the **A95** module. Chemical numbers refer to the position of each chemical within the entire data set of 1309 chemicals listed in order of increasing activity.

pharmaceuticals in the MRTD database were antineoplastics and anesthetics and were administered intravenously and/or intramuscularly. If separate MRTDs were reported for different routes of exposure, the oral MRTD was used in our QSAR model. In addition, some pharmaceuticals have different MRTD values recommended for male and female adults, children, and/or elderly patients. In this situation we exclusively used the MRTD values for the average adult patient.

Dose Units

Pharmaceuticals that are administered orally are usually tested over a limited range of doses and have MRTDs reported as mg/day. In this investigation we converted the mg/day unit to mg/kg-body weight (bw)/day based upon an average adult weighing 60 kg. In contrast, the dose unit for most antineoplastic drug MRTDs is reported as mg/m^2, which was converted to mg/kg-bw/day using the formula mg/kg-bw/day = $mg/m^2/37$ for an average adult. Additionally, a few drugs had MRTDs reported in parts per million (ppm), which were converted to mg/kg-bw/day on the basis that 1000 ppm equals 25 mg/kg-bw/day for an average 60 kg adult.

Chemical Class

Although our MRTD database initially included values for many types of chemicals, certain classes could not be included in the model. These were: inorganic chemicals, high molecular weight polymers (>5000 Daltons), fibers, salts, and mixtures of organic chemicals. In addition, small

$$\text{Inactive chemical CASE unit activity} = 10 + 10(a/x) \quad \textbf{(Equation 1)}$$

$$\text{Marginal chemical CASE unit activity} = 20 + 10(b/y) \quad \textbf{(Equation 2)}$$

$$\text{Active chemical CASE unit activity} = 30 + 50(c/z) \quad \textbf{(Equation 3)}$$

Table 1: Distribution of active, marginal and inactive chemicals in the A95 High-Toxicity and A97 Low-Toxicity MRTD/NOEL modules.

	Chemicals in Training Data Set			
Module No.	**Inactive**	**Marginal**	**Active (%)**	**Total**
A95	613	120	576 (44.0)	1309
A97	570	125	614 (46.9)	1309

molecules (<100 Daltons) were excluded as test chemicals due to poor predictivity [1].

Construction of *MCASE* MRTD/NOEL Database Modules

This investigation employed the *MCASE* software program "dose response" utility to construct MRTD/NOEL database modules, where the MRTD values were treated as two separate distributions. The first distribution allowed modeling of "high-toxicity" chemicals, defined as compounds with low MRTDs, and the second allowed modeling of "low-toxicity" chemicals with high MRTDs. This was achieved by creating one distribution that was the inverse of the other (**Figure 1**). Structural alerts for high toxicity chemicals were identified in the first distribution (**A95** module) and, by assigning the highest activity scores to the lowest toxicity chemicals, low-toxicity structural alerts were identified in the second (**A97** module). The experimental data input files for each distribution contained a total of 1309 molecular structures obtained from publicly available sources [7, 8] and corresponding activity scores in CASE units derived from MRTD values in our database. A CASE unit is a standard *MCASE* program unit for representing toxicological activity where inactive chemicals are assigned a score of 10–19.9 CASE units, marginally active chemicals are scored 20–29.9 CASE units, and active chemicals are scored 30–80 CASE units.

A95 Module

The **A95** module was constructed to identify structural alerts and other properties of toxic chemicals having low MRTD values. The 8-log range of experimental MRTD data values was normalized and converted to a linear scale of 10 to 80 CASE units using **Equations 1–3**, above, and the following method: The data set of 1309 chemicals was rank ordered from highest to lowest MRTD (least to most active; **Figure 1**, **A95** data) and then divided into three subsets, representing inactive, marginal and active chemicals. The term "break-point" was used to describe the point of partition between each of the three subsets, in mg/kg-bw/day. The optimal **A95** break-points for inactive/marginal and marginal/active subsets were determined to be 4.99 and 2.69 mg/kg-bw/day, respectively. This was achieved by systematically adjusting the break-points in both the **A95** an **A97** modules to minimize the number of chemicals in the marginally active subsets and to avoid seeing the same structural alerts in both modules. The resultant **A95** subsets (**Table 1**) contained 613 inactives (MRTDs of 1000–5.00 mg/kg-bw/day), 120 marginals (4.99–2.70 mg/kg-bw/day), and 576 actives (2.69–0.00001 mg/kg-bw/day). The total number of chemicals in the inactive, marginally active and active subsets correspond to values x, y and z, respectively, in **Equations 1–3**. Each chemical within a subset was assigned a "rank number" starting with the highest MRTD, which indicated where a particular chemical appeared in the subset (values a, b and c, in **Equations 1–3**, for inactive, marginal and active subsets, respectively; see also **Figure 2**). This number allowed the position of a chemical within the subset to be expressed as a fraction between 0 and 1 (*e.g.* a/x for inactives). When more than one chemical had the same MRTD value, the same rank number was assigned to those chemicals. For example, the first and second chemicals in the inactive subset share the same MRTD value so both were assigned a rank number of 1 (a = 1); however, the third and fourth chemicals in the inactive subset each have unique MRTD values so were assigned rank numbers of 3 and 4, respectively (a = 3; a = 4). This ensured that when CASE unit activities were calculated using **Equations 1–3**, chemicals with the same MRTD value were assigned the same CASE unit activity.

The three equations were used to confine each subset of MRTDs to the specific ranges of CASE unit activities required by *MCASE*: 10–19.9 CASE units for inactives, 20–29.9 CASE units for marginally actives, and 30–80 CASE units for actives.

A97 Module

The **A97** module was constructed to identify structural alerts and other properties of low-toxicity chemicals that have high MRTD values. The module used an *inverse*-MRTD value that was calculated as the reciprocal of the experimental MRTD value for a given chemical. For example, a MRTD of 0.0100 mg/kg-bw/day for a high-toxicity chemical was converted to an *inverse*-MRTD value of 100 mg/kg-bw/day for the **A97** module (**Figure 1**). Conversely, a MRTD of 100 mg/kg-bw/day for a low-toxicity chemical was converted to an *inverse*-MRTD value of 0.0100 mg/kg-bw/day. Using the procedure described

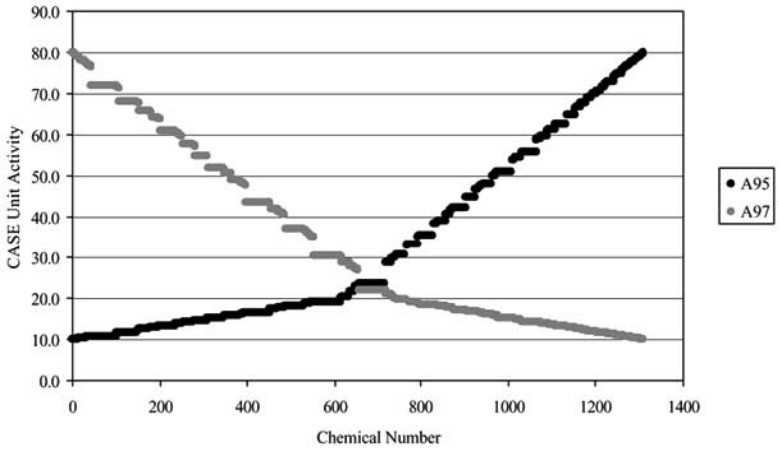

Fig. (3). Plot of chemical number in the database versus corresponding CASE unit activity values.

Table 2: Selected MRTD and *inverse*-MRTD values with their corresponding CASE unit activities for the A95 and A97 modules.

	A95 MRTD Values (mg/kg-bw/day)	CASE Units[a]		A97 *Inverse*-MRTD Values (mg/kg-bw/day)	CASE Units[a]
Inactive	1000.	10.0	***Actives***	0.00100	79.9
	100.	10.7		0.0100	71.9
	10.0	16.5		0.100	43.4
	5.00	19.0		0.200	30.2
Marginals	4.50	20.3	***Marginals***	0.222	29.9
	2.70	29.8		0.370	20.6
Actives	2.00	33.3	***Inactives***	0.500	19.1
	1.00	42.2		1.00	17.2
	0.100	67.8		10.0	12.3
	0.0100	76.6		100.	10.7
	0.00100	79.0		1000.	10.2
	0.000100	79.6		10000.	10.1
	0.0000100	79.9		100000.	10.0

[a]Inactive chemicals are assigned 10–19.9 CASE units, marginally active chemicals are assigned 20–29.9 CASE units, and active chemicals are assigned 30–80 CASE units.

above for the **A95** module, the data set of 1309 chemicals was partitioned into three subsets using optimal *inverse*-MRTD break-points of 0.210 and 0.400 mg/kg-bw/day for active/marginal and marginal/inactive partitions, respectively. The resulting subsets (**Table 1**) contained 570 inactives (*inverse*-MRTD values of 100,000–0.400 mg/kg-bw/day), 125 marginals (0.399–0.210 mg/kg-bw/day) and 614 actives (0.200–0.00100 mg/kg-bw/day). Rank numbers were assigned as described above and **Equations 1–3** were used to calculate CASE unit activities for the 1309 chemicals. Since the data set is inverted in the **A97** module, chemicals scored as active in this module are listed as inactive in the **A95** module.

Figure 3 illustrates the distribution of calculated CASE unit activities for each of the modules and selected values are presented in **Table 2** with their corresponding MRTDs.

Evaluation of MRTD Experimental Data

This investigation used the *MCASE* software program "dose response" utility and two database modules (**A95** and **A97**) to predict the relative toxicity of test chemicals and

Table 3: Results of an internal validation experiment using 120 test chemicals.

Experimental Group[a] No. (Act.)	MRTD Values	Predictions I	M	A	VA	Mean |log (exp./pred.) |[b] ± std.
52 (I)	1000 – 5.00	34	17	1	0	0.34 ± 0.26
11 (M)	4.99 – 2.70	3	6	2	0	0.27 ± 0.11
44 (A)	2.69 – 0.0167	5	8	30	1	0.70 ± 0.69
6 (VA)	0.0166 – 0.00001	0	0	4	2	1.93 ± 1.42
113 (Total)						

[a]Of the 120 chemicals, five chemicals had inadequate coverage and were ineligible for *MCASE* analysis. A further two chemicals displayed high variance MRTD predictions and were considered "no calls." These seven chemicals were excluded from the validation statistics presented above.

[b]Since the values of log(exp./pred.) for each chemical can be either negative or positive, depending on whether the predicted dose is higher or lower than the experimental dose, the absolute value is used for calculating the mean and standard deviation of these data.

their MRTD values. Test chemicals were submitted as ".mol" files accompanied by a data input file ("D-file") in ASCII format. The experimental output data files from the two modules were analyzed using a predetermined set of human expert rules in a three step procedure: (1) Identification of decision alerts in the **A95** and **A97** database modules; (2) analysis of selected portions of *MCASE* experimental output data from an individual module; and (3) a manual combination of the experimental results from the two MRTD modules.

Identification of Decision Structural Alerts in A95 and A97 Modules

A large molecular library of 2–10 atom fragments is generated from chemicals in a training data set and a small set of fragments that are present primarily in active chemicals (*i.e.* structural alerts) are identified by *MCASE*. The program determines the statistical significance of the structural alerts based upon the numbers of active chemicals that possess the fragments and the results of pairwise *t*-tests. After generating structural alerts for the **A95** and **A97** modules, human expert rules were used to identify a subset of these alerts that were biologically significant, which we classified as "decision alerts." The relative biological significance (biologic potency) of each structural alert was calculated by multiplying its average CASE unit activity by its frequency of appearance in the database module, and was expressed as the alert's total CASE unit activity ($CASE_{TOT}$). For example, a MRTD/NOEL structural alert that appeared in 3 chemicals having an average of 45.0 CASE units would have a $CASE_{TOT}$ of 135 units ($3 \times 45 = 135$). We defined a decision alert as having ≥150 $CASE_{TOT}$ and a possible decision alert as having 100–149 $CASE_{TOT}$. Alerts with <100 $CASE_{TOT}$ were defined as not significant. In practice these rules evaluate a structural alert that occurs in only one chemical and nearly all two-copy alerts as not biologically significant.

Analyses of Data from a Single MRTD/NOEL Module

MCASE output data from a prediction experiment is exportable as a comprehensive data file ("R-file") and a summary data file ("J-file"). In our investigation we discarded substantial portions of the *MCASE*-generated experimental data, including the conclusion statements from the R- and J-files for the test chemicals. We also rejected predictions for all test chemicals with poor structural coverage, defined as having two or more unknown fragments; all structural alerts with "highly degenerate" or "different environment" warnings; and all structural alerts having <100 $CASE_{TOT}$. Our MRTD/NOEL prediction was based upon the "TOTAL Projected QSAR Activity x, (x = response)" for the decision alert, which is given in the R-file and expressed in CASE units. This activity value is calculated by the program following analysis of potential QSAR attributes (*e.g.* log P, HOMO/LUMO constants, *etc*.) and molecular fragment (*e.g.* activating fragments, inactivating fragments) modulators of activity of the decision alert. We then performed a reverse conversion of the CASE unit activities of individual decision alerts into MRTD values using the closest CASE unit activity listed in our MRTD database and its corresponding MRTD value.

Prediction of MRTD Values using Two MRTD/NOEL Modules

Since our investigation employed a model estimating chemical toxicity using two different MRTD/NOEL database modules (**A95** and **A97**), it was necessary to combine the experimental output data from each of the two modules to obtain the final MRTD and NOEL predictions. The following four human expert rules were used to combine the data:

(1) If one decision alert was identified in a test chemical using either the **A95** or the **A97** module, the MRTD/NOEL estimate was based upon the R-file CASE unit activity of that decision alert. When the test chemical had two or more decision alerts for toxicity in one module, the median CASE unit activity of these alerts was used to calculate the MRTD. The NOEL dose was defined as 1/10 the MRTD.

(2) If only a *possible* decision alert was identified in a test chemical using either the **A95** or the **A97** module data, the MRTD/NOEL estimates were based upon the CASE unit activity of that possible alert.

(3) If no decision alerts were identified in either the **A95** or the **A97** module, the test chemical was assigned a default MRTD value of 4.0 mg/kg-bw/day. This value is the median MRTD value in the training data set chemical range (see Discussion).

Table 4: Statistical evaluation of test chemical predictions.

		Predicted		
		I+M	**A+VA**	**Total**
Experimental	**I+M**	60	3	63
	A+VA	13	37	50
Total		73	40	113
Positive Predictivity	= 37/40 = 92.5%			
Specificity	= 60/63 = 95.2%			
False Positives	= 3/63 = 4.8%			
Sensitivity	= 37/50 = 74.0%			
False Negatives	= 13/50 = 26.0 %			
Coverage	= 115/120 = 95.8%			

(4) On rare occasions when one decision alert was identified in one module, but a different alert was identified in the other module, the two resulting MRTD/NOEL estimates were averaged. However, if the two MRTD/NOEL estimates exhibited a variance of more than 100-fold, the test chemical was evaluated as ineligible for a prediction.

Validation Studies

There were two different types of validation experiments performed in this investigation: an internal cross-validation experiment and an experiment to estimate the structural coverage of the MRTD modules for different types of test chemicals.

Internal Validation Study

An internal validation study was conducted to assess the accuracy of MRTD predictions using the model. The study included three separate experiments in which 40 chemicals were removed from the training data set (for a total of 120 test chemicals), and their MRTD values were predicted using the remaining portion of the training data set (1269 chemicals for each experiment). The 40 chemicals were selected from the training data set that was rank-ordered based upon the magnitude of the MRTD values so that the test set represented a balanced group of high- and low-toxicity chemicals. The accuracy of the predictions for the 120 chemicals were estimated using two different methods. The first method evaluated the ability of the model to place a test chemical into one of four activity ranges: Very active (VA) chemicals had MRTD values of 0.0000100 to 0.0166 mg/kg-bw/day (75–80 CASE units); active (A) chemicals had MRTD values of 0.0167–2.69 mg/kg-bw/day (30–74.9 CASE units); marginally active (M) chemicals had MRTD values of 2.70–4.99 mg/kg-bw/day (20–29.9 CASE units); and inactive (I) chemicals had MRTD values of 5.00–1000 mg/kg-bw/day (10–19.9 CASE units). These ranges were chosen because they correspond to the standard designations for active, marginally active and inactive chemicals on the CASE unit activity scale, with the addition of the "very active" category for the high end of the active range. The resulting 4 × 4 confusion matrix (**Table 3**), containing predicted versus experimental values for the four ranges, was converted to a 2 × 2 matrix for the purpose of evaluating the predictive performance of the model for MRTD (**Table 4**). Statistical comparisons of MRTD predictions and experimental results were performed using the method of Cooper *et al.* [9].

The second method evaluated the magnitude of the log-fold difference between the reported MRTD values and the predicted values. For example, the log-fold difference between reported (experimental) and predicted MRTDs of 25.0 and 2.50 mg/kg-bw/day respectively, is 1 (log (25.0/2.5) = 1). Similarly, a 2-fold and 100-fold difference are equivalent to a 0.301- and a 2-log-fold difference. The overall log-fold differences in sets of reported and predicted MRTDs were expressed as the mean and standard deviation from the mean.

Coverage Study

We evaluated the coverage of the **A95** and **A97** database modules for four types of FDA-regulated substances: direct food additives, food contact substances, and pharmaceuticals described in either new drug applications (NDAs) or investigational new drug applications (INDs). The structures for the direct food additives and food contact substances used in this experiment were from CFSAN's Priority-Based Assessment of Food Additives (PAFA) database [10]; the structures for the drugs were obtained from our own internal chemical structure database. Food contact substances are chemicals that might be present in food from processing, equipment, packaging, *etc*.

RESULTS

Experimental Attributes of the MRTD/NOEL Modules

We constructed two different database modules to estimate the MRTD/NOEL values of test chemicals: The **A95** module for high-toxicity chemicals and the **A97** module for low-toxicity chemicals. The modules used a training data set of MRTD values for 1309 chemicals with many different clinical indications and pharmacological properties, which were partitioned into inactive, marginal and active subsets,

Table 5: Distribution of structural alerts in the A95 High-Toxicity and A97 Low-Toxicity MRTD/NOEL modules.

	Structural Alerts[a]				
Module No.	**Non-Signif.**	**Poss. Dec. Al.**	**Decision Alerts**	**Total**	
A95 High-Toxicity	100	25	56	181	
A97 Low-Toxicity	158	37	78	273	
	258	62	134	454	Total

[a]Structural alerts are automatically determined from the input molecular library, in this case of 1309 chemicals. Using human expert rules, decision alerts are defined as having a $CASE_{TOT}$ of ≥150 CASE Units; possible decision alerts (Poss. Dec. Al.) have 100–149 CASE Units and non-significant alerts (Non-Signif.) have <100 CASE Units.

as described above (see also **Table 1**). Identification of the optimal break-points to define each subset of chemicals was critical for the generation of well-defined clusters of high- and low-toxicity structural alerts in each of the modules. After processing the 1309 chemicals, the modules contained a fragment library of 903,274 2–10 atom fragments. In the **A95** module, a small subset of 181 fragments (**Table 5**) were correlated with low MRTD values in humans and were subsequently identified as structural alerts. By applying our human expert rules, 56 were identified as decision alerts and a further 25 were identified as possible decision alerts. In the **A97** module, 273 fragments that were correlated with high MRTD values in humans were identified as structural alerts. 78 of these structural alerts were identified as decision alerts and a further 37 were identified as possible decision alerts. The decision alerts were the primary basis of MRTD/NOEL predictions in this investigation.

Decision Alerts in Structurally Similar Chemicals

We examined the types of chemicals in the training data set that had specific decision alerts in the **A95** high toxicity training data set and the **A97** low toxicity data set. We discovered that these decision alerts were often derived from clusters of chemicals that were structurally similar, and that many of the chemicals within a cluster had similar profiles of toxic effects. Five examples of decision alerts identified using the **A95** module are presented in **Table 6**; each of these alerts was derived from a cluster of almost exclusively active chemicals. Furthermore, the chemicals contained in each cluster are the only examples in the training data set which contain the specific alert with an identical number of hydrogens on each atom. The decision alert "CH_2–CH–NH–CH–" was identified in a cluster of 11 active chemicals, 8 of which were inhibitors of the angiotensin-converting enzyme (ACE inhibitors) and are used to treat hypertension. The alert "N^+–CH–" was found in a larger cluster of 20 active chemicals, 18 of which are either antimuscarinics or muscle relaxants. Another large cluster of 19 chemicals, 17 of which are active, was identified to contain the decision alert "CH_2-CH-c= <2-OH>", where the hydroxyl substituent is bonded to the second atom of the fragment. 15 of the 19 compounds in this cluster were categorized as sympathomimetics. The decision alert "n=c-CH-" was found in 8 active chemicals, 6 of which were identified as sedating antihistamines; and finally the decision alert "n=c–c.=<2–NH_2>" was identified in 13 chemicals, where 5 of the 11 active compounds are antineoplastics and a further 3 are used to treat hypertension. **Figure 4** illustrates the location of each structural alert in a molecule from its respective cluster.

Accuracy of MRTD Predictions

An internal cross-validation experiment was performed in order to estimate the accuracy of MRTD predictions using our model. The study involved a total of 120 test chemicals that represented a balanced set of high and low toxicity chemicals. The accuracy of the predictions was first estimated by evaluating the ability of the model to correctly assign a test chemical to one of four possible dose ranges, and secondly by comparing the predicted MRTD value of a test chemical to its known reported value. We also examined the log-fold difference between the known MRTD value and the predicted value for each test chemical.

The results of this study demonstrated that the model made good predictions of toxicity for the test chemicals (**Tables 3** and **4**). When evaluating the ability of the model to assign a test chemical to the correct dose range, the positive predictivity and specificity were high at 92.5% and 95.2%, respectively. Sensitivity of the model was lower but still acceptable at 74.0%. Finally, the coverage of the 120 test chemicals was high, with 95.8% of chemicals having fewer than two unknown fragments.

The results also showed that the model was able to estimate the MRTD value of most types of test chemicals to within 10-fold the experimental value. For example, the average log-fold difference between the MRTD values reported in labeling and the predicted values was only 0.34, 0.27, and 0.70 for test chemicals that were inactive, marginally active, or active, respectively (**Table 3**). In contrast, the average log-fold difference between labeling and predicted MRTD values for six very toxic test chemicals was large (1.93 ± 1.42, **Table 3**).

Coverage of MRTD Database Modules

In the next series of experiments we investigated the structural coverage of the **A95** and **A97** database modules for 6882 chemicals, divided into three different types as regulated by the Agency: pharmaceuticals, direct food additives, and food contact substances. Test compounds containing two or more fragments that are not represented in the training data set are considered uncovered and unsuitable for analysis. The experiments demonstrated that all of the modules exhibited excellent coverage for all three types of substances (**Table 7**). The highest coverage was obtained for NDA pharmaceuticals (93.6%) and the lowest coverage was achieved for food contact substances (89.9%).

Table 6: Examples of clusters of chemicals which share decision alerts identified in the A95 High-Toxicity database module.

CH_2–CH–NH–CH–

CASE Act.	MRTD (mg/kg-bw/day)	Chemical Name	Effect[a]
78	0.00167	(proprietary)	sympathomimetic; bronchodilator; beta-adrenergic agonist
68	0.0833	(proprietary)	antihypertensive; ACE inhibitor
66	0.133	Perindopril	antihypertensive; ACE Inhibitor
56	0.333	Ramipril	antihypertensive; ACE inhibitor
51	0.533	Trandolapril	antihypertensive; ACE inhibitor
49	0.667	Enalapril	antihypertensive; ACE inhibitor
48	0.800	Nylidrin	sympathomimetic; vasodilator; tocolytic
46	0.833	Moexipril	antihypertensive; ACE inhibitor; angiotensin II receptor agonist
39	1.33	Quinapril	antihypertensive; ACE inhibitor
39	1.33	Isoxsuprine	vasodilator
39	1.33	Benazepril	antihypertensive; ACE inhibitor

N^+–CH–

CASE Act.	MRTD (mg/kg-bw/day)	Chemical Name	Effect[a]
76	0.0100	Oxitropium bromide	antimuscarinic; bronchodilator
70	0.0500	Doxacurium chloride	muscle relaxant; neuromuscular blocker
70	0.0500	Atropine N-oxide	anticholinergic
67	0.100	Vecuronium bromide	muscle relaxant; neuromuscular blocker
67	0.100	Pancuronium bromide	muscle relaxant; neuromuscular blocker
62	0.167	Isopropamide	antimuscarinic; antispasmodic
61	0.200	Trospium chloride	antimuscarinic; antispasmodic
59	0.250	Mivacurium chloride	muscle relaxant; neuromuscular blocker
54	0.400	Dimethyltubocurarine	muscle relaxant; neuromuscular blocker
51	0.500	Atracurium	muscle relaxant; neuromuscular blocker
51	0.500	Butropium bromide	antimuscarinic; antispasmodic
50	0.600	Cisatracurium	muscle relaxant; neuromuscular blocker
49	0.667	Homatropine	antimuscarinic; cycloplegic; mydriatic
49	0.667	Tubocurarine	muscle relaxant; neuromuscular blocker
42	1.20	Rocuronium bromide	muscle relaxant; neuromuscular blocker
41	1.25	Propantheline bromide	antimuscarinic; antispasmodic
39	1.33	Prajmalium	antiarrhythmic
39	1.33	Butylscopolammonium bromide	antimuscarinic; cycloplegic; mydriatic; antiemetic
33	2.00	Prifinium bromide	antimuscarinic; antispasmodic
30	2.50	Anisotropine methylbromide	antimuscarinic; antispasmodic

(Table 6) contd…..

CH_2–CH–c= <2–OH>

CASE Act.	MRTD (mg/kg-bw/day)	Chemical Name	Effect[a]
79	0.00100	Clenbuterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
78	0.00140	Salmeterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
77	0.00667	(proprietary)	sympathomimetic; bronchodilator; beta-adrenergic agonist
74	0.0167	Epinephrine	sympathomimetic
74	0.0267	Fenoterol	sympathomimetic; bronchodilator; beta-adrenergic agonist tocolytic
72	0.0370	Bitolterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
69	0.0667	Norepinephrine	sympathomimetic
62	0.167	Lobeline	central stimulant
62	0.167	Isoproterenol	sympathomimetic; beta-adrenergic agonist
59	0.250	Terbutaline	sympathomimetic; bronchodilator; beta-adrenergic agonist tocolytic
59	0.250	Midodrine	sympathomimetic; alpha-adrenergic agonist
51	0.533	Albuterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
46	0.833	Etilefrine	sympathomimetic
46	0.833	Phenylephrine	sympathomimetic; vasopressor; decongestant; mydriatic
43	1.00	Reproterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
33	2.00	Terfenadine	non-sedating antihistaminic
30	2.67	Formoterol	sympathomimetic; bronchodilator; beta-adrenergic agonist
18	5.33	Sotalol	beta-adrenergic blocker; antiarrhythmic
13	40.0	Labetalol	beta-adrenergic blocker

n=c–CH–

CASE Act.	MRTD (mg/kg-bw/day)	Chemical Name	Effect[a]
77	0.00500	Cerivastatin	statin lipid regulator
67	0.100	Dimethindene	sedating antihistaminic
66	0.133	Chlorpheniramine	sedating antihistaminic
59	0.250	Bisacodyl	stimulant laxative
54	0.400	Carbinoxamine	sedating antihistaminic
54	0.400	Brompheniramine	sedating antihistaminic
50	0.625	Pheniramine	sedating antihistaminic
48	0.800	Dexbrompheniramine	sedating antihistaminic

n=c–c.= <2–NH_2>

CASE Act.	MRTD (mg/kg-bw/day)	Chemical Name	Effect[a]
76	0.00833	Aminopterin	antineoplastic
70	0.0500	Bunazosin	antihypertensive; alpha-adrenergic blocker

68	0.0900	Cladribine	antineoplastic; antimetabolite
61	0.200	Adenosine triphosphate	antiarrhythmic
59	0.267	Doxazosin	antihypertensive; alpha-adrenergic blocker
56	0.333	Terazosin	antihypertensive; alpha-adrenergic blocker
56	0.333	Adenosine-5-phosphate	vasodilator
51	0.500	Methotrexate	antineoplastic; antimetabolite; folate antagonist; immunosuppressant; antirheumatic
49	0.676	Fludarabine	antineoplastic; antimetabolite
42	1.22	Trimetrexate	antineoplastic; antimetabolite
33	2.00	Adenosine	antiarrhythmic
23	3.33	Triamterene	diuretic (potassium sparing)
15	15.0	Adenine	vitamin

[a] Obtained from reference [5] (d) and [8].

Coverage of MRTD Database Modules

In the next series of experiments we investigated the structural coverage of the **A95** and **A97** database modules for 6882 chemicals, divided into three different types as regulated by the Agency: pharmaceuticals, direct food additives, and food contact substances. Test compounds containing two or more fragments that are not represented in the training data set are considered uncovered and unsuitable for analysis. The experiments demonstrated that all of the modules exhibited excellent coverage for all three types of substances (**Table 7**). The highest coverage was obtained for NDA pharmaceuticals (93.6%) and the lowest coverage was achieved for food contact substances (89.9%).

Comparison of Chemical MTD Responses in Rodents and MRTD Responses in Humans

The final study in this investigation compared the long-term toxicities of the same chemicals in rodents and humans. A set of 326 pharmaceuticals was compiled which had human MRTD values reported in labeling [5, 6] and rodent MTDs derived from public sources [11, 12] and reported in FDA/CDER archives. The MRTD value is determined in 3–12 month clinical trial and the MTD is measured in a two year (lifetime) study in rodents. This experiment compares the relative concordance between the human MRTD values with MTD values in rodents for the same pharmaceutical test chemicals, and revealed that the dose values were poorly correlated with one another, having an R^2 coefficient of only 0.2005 (**Figure 5**).

DISCUSSION

Findings

Perhaps the most interesting observation in this investigation was that the model was able to identify a small set of decision alerts that were highly correlated with low MRTD values and the toxicity of the pharmaceutical chemicals in humans. Using the molecular library of the **A95** database module, the program identified 56 decision alerts and 25 possible decision alerts that were present almost exclusively in 576 drugs with low MRTD values in humans (**Table 5**). Furthermore, we discovered that many of the decision alerts were composed of clusters of chemicals that were structurally similar and often shared a similar profile of pharmacological and toxicological effects (**Table 6**). Using the molecular library of the **A97** database module, the program identified 78 decision alerts and 27 possible decision alerts that were present in 614 drugs with high MRTD values in humans. These decision alerts were also composed of clusters of chemicals that were structurally similar, which when used in combination with the **A95** compound clusters provided a rational basis for predictions of both high- and low-toxicity test compounds. The presence of numerous well-defined clusters of compounds within our training data set leads us to believe that our model covers a wide area of structural space and could be utilized to make predictions on test compounds within this area. While we acknowledge that a truly novel molecular structure might be poorly represented in our training data set, our model is able to identify this limitation and allow the user to avoid making a prediction based upon inadequate information.

The high positive predictivity (92.5%) and low false positive rate (4.8%) of the MRTD/NOEL predictions (**Tables 3** and **4**) exceeded all of the toxicological endpoints we have thus far evaluated in our laboratory. We feel the sensitivity of 74.0% was also good, and is a reflection of the relatively large size of the training data set. (However, by enhancing the training data set with a greater number of active chemicals, the level of sensitivity for this model should become even better.) Taken together, these data suggest that the model has identified a set of decision alerts that are highly correlated and predictive of the toxicity of chemicals in humans. The investigation also demonstrated that the model can provide a reasonably good estimate of a test chemical's MRTD. The average log-fold difference between the MRTD values reported in labeling and the predicted values was only 0.34, 0.27, and 0.70 for test chemicals that were inactive, marginally active, or active, respectively (**Table 3**). The only subset of test chemicals with poor MRTD estimates were the six very active test chemicals with a log-fold difference of 1.93 ± 1.42 (**Table 3**). Within this very toxic subset, the MRTD values were significantly overestimated (their toxicity was underestimated) suggesting that the error may have been caused by limited representation of some very toxic drugs in the training database (*e.g.* cardiotoxins, hormones, *etc.*). These data demonstrated that the majority of the test

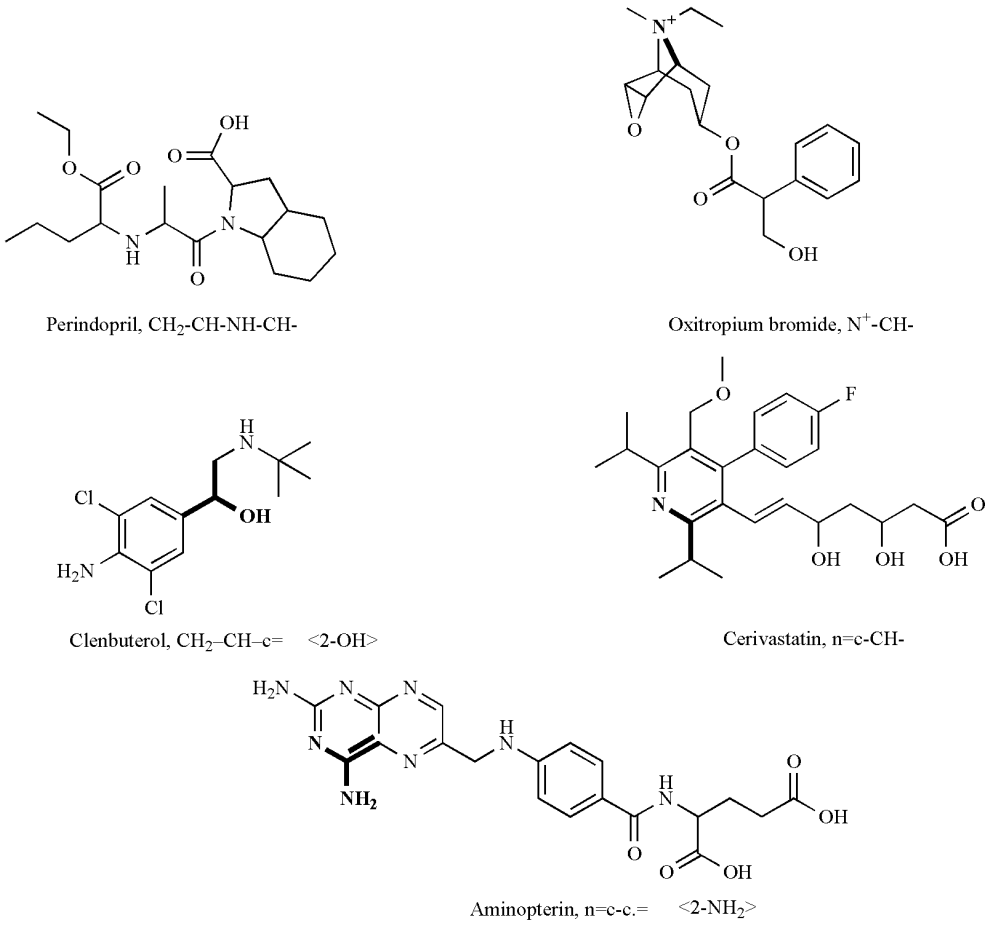

Fig. (4). Selected chemical structures from training data set indicating location of decision alerts.

chemicals had MRTDs predicted within ±10-fold of their reported value.

During the course of this investigation we explored two additional experimental methods to predict MRTD values but were unsuccessful with either of these approaches. One method used the *MCASE* "dose concentration" utility, which treats the MRTD values as a continuous distribution of values and creates a single MRTD module. The second method involved the construction of three MRTD modules for high-toxicity, intermediate-toxicity, and low-toxicity MRTD values. Both of these methods had diminished predictive performance compared to our current model using two modules. The single module failed because it was unable to identify molecular and QSAR properties of low-toxicity chemicals. Furthermore, the dose concentration utility employed certain logic statements that were not helpful and difficult to circumvent. The three module method failed because intermediate toxicity MRTD decision alerts were poorly defined and overlapped with the decision alerts for high- and low-toxicity chemicals.

The **A95** and **A97** MRTD/NOEL modules exhibited good coverage for several different types of substances that are regulated by the FDA, such as NDA and IND pharmaceuticals, direct food additives, and food contact substances (also known as indirect food additives, **Table 7**). Many of the direct food additives are natural substances derived from plants. The good coverage for pharmaceuticals and direct food additives suggests our model may be effective for predicting the toxicity of natural substances. The good coverage for the food contact substances was unexpected because these substances are very unlike pharmaceuticals, being mostly industrial type chemicals used in the manufacture of food packaging materials and food processing equipment [13]. We also observed that enhancement of the training data set improved coverage for these same substances, which suggests that future enhancements of the MRTD database with other marketed drugs and new IND drugs submitted to CDER will make the predictive performance of the MRTD/NOEL modules even better.

Limitations of the Model and Other Considerations

Routes of Exposure

Although the overwhelming majority of the control database MRTD data are based upon the use of an oral route of administration, a small number of MRTD values were based upon non-oral routes of administration of the chemical. We observed that when drugs are reported as

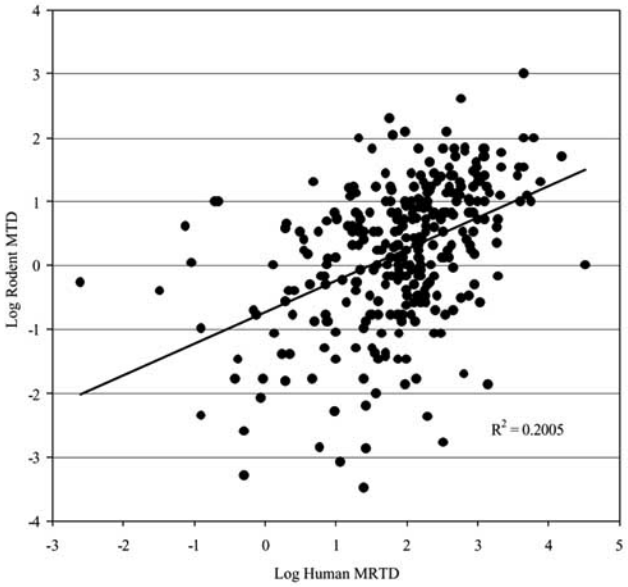

Fig. (5). Plot of the MRTD values versus the rodent MTD values expressed as logarithms for a set of 326 chemicals.

Table 7: Comparison of coverage for four types of test chemicals in A95 and A97 modules

Chemical Type	Number of Test Chemicals	Coverage (%)
NDA Pharmaceuticals	2117	93.6
Direct Food Additives	3039	92.4
IND Pharmaceuticals	92	91.3
Food Contact Substances	1634	89.9

having different MRTDs for oral and non-oral routes of administration the difference in the total daily dose-concentration of the MRTD is limited to about a 2-fold difference. This 2-fold difference is minimal compared to the range of MRTDs within drug clinical indication categories, and the 8-log range of MRTDs across all categories.

Patient Gender and Age

Different MRTDs are sometimes recommended for male, female, elderly, adult, child (<12 years), or infant patients. This investigation collected the MRTD data for these different types of patients but only the average adult MRTD values were used to construct the modules. The amount of uncertainty generated by this decision is thought to be relatively small. For example, the MRTD for children or elderly patients is sometimes lower than the adult MRTD, but the lower MRTD is usually within half of the adult MRTD. Furthermore, the more common recommendation for children and elderly patients is that their MRTD can be gradually increased to the adult MRTD, if needed. Similarly, certain clinical indications can be treated with pharmaceutical doses slightly above the MRTD, if needed; however, these patients are at risk for toxic effects of the drugs. Patients treated with drugs at dose-concentrations above the MRTD might include young patients treated with some antibacterials, adult patients with severe psychoses treated with antipsychotics, and patients with life-threatening disease.

Validation Data Set

The validation test in this investigation used a set of 120 test chemicals, where the total training data set were rank ordered and then a subset were randomly selected. It is possible that our results may have been slightly different if a larger test chemical set or a truly random set of test chemicals had been used.

Optimal Break-point

Optimal break-points are characterized by well-defined clusters of high- and low-toxicity structural alerts, and the absence of the same alert in both the **A95** module and its inverse, **A97**. This investigation used two break-points for each of the **A95** and **A97** modules to divide the active,

marginally active and inactive chemicals. It is possible that this break-point would shift if the database were enhanced with a large number of new MRTD values. Therefore, any enhancements to the database would need to be accompanied by re-evaluation of the break-points and by performing appropriate validation experiments.

Marginally Active Chemicals

There are technical limitations in identifying molecular properties and structural alerts for test chemicals with marginal toxicity, resulting in the absence of structural alerts for many of these chemicals. In cases where only a rejected decision alert is present, it can be used as the basis for a MRTD prediction. However, marginally active test chemicals frequently have no structural alerts, leaving two options on how they should be treated: (1) refrain from predicting test chemicals without structural alerts, or (2) classify all test chemicals without structural alerts as marginally active and having a default MRTD. We elected the latter option and chose 4.0 mg/kg-bw/day, the value in the middle of the training data set chemical range, as the default MRTD. In the validation study a total of 24/120 (20.0%) test chemicals did not have any structural alerts. Of these 24 test chemicals, 5 were marginally active, 6 were active, and 13 were inactive. The average log-fold difference between the measured MRTD value and the predicted value was only 0.504 ± 0.378 (n = 24) leading us to believe that the human expert rule of using 4.0 mg/kg-bw/day provided an adequate estimate of the MRTD of test chemicals without decision alerts.

Estimate of Uncertainty in MRTD/NOEL Predictions

We believe that if the MRTD/NOEL database modules were used to provide an estimated MRTD, this dose should be accompanied by an estimate of the error associated with the predicted value. A comparison of reported versus predicted MRTD values in our internal cross-validation test set, summarized in **Table 3**, revealed that 83.2% (94/113) of predicted values fall within a range of 0.1–10 times the reported value (± 1-log-fold). For practical purposes, if a predicted MRTD was reduced by a factor of 10 this would provide a conservative estimated dose, which accounts for the uncertainty in the prediction. A conservative NOEL estimate would be one tenth of this value.

MRTD Pharmacophores/Toxicophores

Many of the MRTD/NOEL decision alerts were highly correlated with clusters of drugs with specific pharmaceutical clinical indications. It is possible that some of the decision alerts may be associated with pharmacological activity (pharmacophores) and/or associated with undesirable adverse toxicological adverse effects (toxicophores). We believe this issue is very complex and might involve multiple mechanisms which could be difficult to resolve using our MRTD/NOEL methodology alone. We are currently investigating the possibility that the MRTD database could be modeled with other (Q)SAR software platforms and believe that the MRTD database should be included in a battery of human health effect endpoints, including models utilizing methodologies from multiple software applications.

Advantages of the Model

Our MRTD/NOEL model for estimating potential human health effects of chemicals has four major advantages over the current risk-assessment methods, which rely upon extrapolations from data derived from animal toxicology studies.

Reduced Need for Uncertainty Factors

The estimation of NOEL doses of chemicals in humans is presently most commonly undertaken using a variety of methodologies that extrapolate relative risk from the results of animal toxicity studies. These methods usually rely upon a multitude of uncertainty (safety) factor corrections of the animal study data to compensate for inadequate information on, and representation of, the effects of chemicals in humans. The correction factors include those for: (1) route of administration (non-oral to oral), (2) duration of exposure (short-term to chronic), (3) adsorption, distribution, metabolism and excretion (ADME) differences, and (4) animal-to-human extrapolation (*e.g.* body weight surface area scaling factors) [14-28]. Our MRTD/NOEL model eliminates the necessity of using uncertainty factor corrections for animal study data because our model directly predicts chemical toxicity in humans.

Increased Sensitivity and Specificity

Many of the adverse effects of pharmaceuticals that are noted in clinical trials do not have a counterpart in animal studies (*e.g.* cognitive effects, dysphoria, myalgia, mental disturbances, headache, nausea, *etc.*). Estimation of a NOEL based on human MRTD should be more sensitive and specific than a NOEL based on animal extrapolation. The clinical MRTD is in some ways analogous to the maximum tolerated dose (MTD) used in rodent carcinogenicity studies, if it is assumed that the MRTD represents a threshold above which adverse effects of a drug would be observed. Indeed, very few drugs are observed to have no adverse effects noted during clinical trials, suggesting that the threshold for toxicity has been reached in most cases. An experiment was performed to determine whether a measurement related to a toxicity threshold in humans (MRTD) is correlated to a toxicity threshold criterion in rodents (MTD). In this investigation we showed that the human MRTD and rodent MTD values for a data set of 326 pharmaceuticals were very poorly correlated to one another ($R^2 = 0.2005$, **Figure 5**).

Reduced Cost

There are millions of chemicals with little or no animal toxicity study data and finite fiscal and laboratory resources for testing these chemicals. An alternative cost-effective, fast and reliable screening methodology is warranted and urgently needed to help prioritize the potential risk of untested or poorly tested chemicals. Our MRTD/NOEL model offers a feasible, cost effective, and innovative *in silico* solution to this problem. Our model is: (1) automated (designed to process thousands of test chemicals in batch mode), (2) inexpensive (costs are limited to software,

hardware, and trained investigators), and (3) fast (experimental data for thousands of chemicals can be generated in minutes).

Applications

We feel that the development of a QSAR model that can predict the MRTD and NOEL of chemicals in humans based on human data is an important achievement and may have broad research and regulatory applications. These predictions only require a knowledge of the chemical structure and a training data set of pharmaceutical MRTDs. The MRTD database is based upon information reported in labeling for pharmaceuticals and is available at our FDA Website. Because our MRTD database is relatively large (1309 chemicals) and contains a diverse molecular library (903,274 molecular fragments) we believe our model can be applied to many organic chemicals that are not pharmaceuticals. The model also identifies three classes of compounds that cannot be predicted: (1) chemicals that are not covered, having molecular structure fragments which are not represented in the database; (2) chemicals with high variance MRTD predictions; (3) and very small chemicals (<100 Daltons).

Our human data based MRTD/NOEL model could be an important research and regulatory tool in academia, industry and government in situations when chemical toxicity data in animals and humans is limited or unavailable. The model can provide the basis for a possible alternative risk assessment paradigm for the estimation of acceptable exposure in humans. Likewise, it may provide a useful tool to estimate the initial doses of pharmaceuticals in human subjects during Phase I clinical trials. The MRTD/NOEL model could also be useful in setting the exposure safety margin and to prioritize the concern with untested contaminants or degradents that are recognized late in the drug development process. The model may also be used to evaluate the exposure safety margin of food contact substances or the major constituents of dietary and nutritional supplements, direct food additive flavors and spices, and herbal medicines. Finally, we believe the decision alerts that were identified in clusters of chemicals in this study could provide research insights for common mechanisms of chemical toxicity in humans, and these alerts could be linked to extensive, pharmacologic and toxicologic drug studies in both humans and animals.

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ABBREVIATIONS

ADME	=	Adsorption, distribution, metabolism and excretion
AERS	=	Adverse Event Reporting System
CAS	=	Chemical Abstract Service
CASE Unit	=	Standard *MCASE* program toxicological activity unit(s)
$CASE_{TOT}$	=	Total CASE unit activity
CDER	=	Center for Drug Evaluation and Research
CFSAN	=	Center for Food Safety and Applied Nutrition
CRADA	=	Cooperative Research and Development Agreement
FDA	=	U.S. Food and Drug Administration
HOMO	=	Highest occupied molecular orbital
ICSAS	=	Informatics and Computational Safety Analysis Staff
IND	=	Investigational new drug application
LUMO	=	Lowest unoccupied molecular orbital
MCASE	=	Multiple computer automated structure evaluation program
MRTD	=	Maximum recommended therapeutic dose
MTD	=	Maximum tolerated dose
NTP	=	National Toxicology Program
NDA	=	New drug application
NOEL	=	No effect level
ppm	=	Parts per million
PAFA	=	FDA/CFSAN Priority-Based Assessment of Food Additives database
(Q)SAR	=	(Quantitative) structure activity relationships
SMILES	=	Simplified molecular input line entry system
SRS	=	FDA/CDER Spontaneous Reporting System

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