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A quantitative activity-activity relationship model based on covariance structure analysis, and its use to infer the NOEL values of chemical substances

Jun-ichi Takeshita, Masashi Gamo, Koji Kanefuji and Hiroe Tsubaki

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Abstract. Here we prove the usefulness of the quantitative activity-activity relationship (QAAR) approach by giving an example of its practical application. To assess and manage chemical substances, experiments are often done on animals. However, in terms of time and cost efficiencies and the need for animal protection, there is increasing global demand to use statistical methods to reduce the need for animal testing. Although the QAAR approach has been introduced to estimate unknown toxicity values from the relationships between different toxicity endpoints (item for observation of hazardousness), there are few examples of its practical application. We considered the QAAR approach was useful from the viewpoint of effective utilization of existing data. We therefore adopted covariance structure analysis as a statistical method to develop our QAAR model for inferring the missing no-observable-effect level (NOEL) values for each target (e.g. internal organ) in animal testing data from repeated dose toxicity studies and reproductive toxicity studies. We emphasize here that the data were sparse. One of the major advantages of our model is that it enables us to make estimations by using confidence intervals, which means that we not only infer the missing NOEL values but also quantify their uncertainty. As a specific example, we discuss toluene, for which there were 19 missing NOELs out of 48 endpoints. Finally, we discuss the validation of the model in accordance with the Organisation for Economic Co-operation and Development's Principles for Quantitative Structure-Activity Relationship models, and we conclude that the accuracy of this model is high.

Keywords. animal testing data, covariance structure analysis, no-observable-effect-level (NOEL), prediction of missing values, quantitative activity-activity relationship (QAAR)

1. Introduction

Here, we prove the usefulness of a quantitative activity-activity relationship (QAAR) approach by developing a QAAR model. To assess and manage chemical substances, experiments are often done on animals. However, in terms of time and cost efficiencies and the need for animal protection, there is increasing global demand to use statistical methods to reduce the need for animal testing. As an approach to estimating the missing toxicity values at untested endpoints (an endpoint being an outcome measure used to assess hazardousness), the quantitative structure-activity relationship (QSAR) and a QAAR have been introduced; see e.g. OECD [20]. The QSAR is a method for extrapolating from chemical structure or physicochemical properties, or both, to other properties or activities. The QAAR, on the other hand, is a method for extrapolating from one activity to another.

The Organisation for Economic Co-operation and Development (OECD) and some countries have been developing QSAR models and discussing their possible application. The OECD approach can be seen in their work [20, 21]; the EU approach in the work of ECHA [7, 8]; the US approach in the work of OECD [18], USEPA [10], Denison and Florini [6], and Denison [5]; the Canadian approach in the work of Environment Canada [9] and Health Canada [12]; and the Australian approach in the work of Australian Government [2].

In contrast, the QAAR approach has not been systematically investigated, and there are few examples of its practical application. However, we consider that the QAAR approach would work well if we needed to know the toxicity at untested endpoints. As an example, for toluene there are animal testing data on its effects on the liver, kidney, urine, weight, brain and death. If a risk assessor wants to know the effect of toluene on the digestive tract, the use of existing data rather than physicochemical properties seems attractive. However, the QAAR approach enables us to estimate the effects of toluene on the digestive tract from the existing data. In other words, the QAAR approach more effectively utilizes existing data.

In the case of usual statistical inference, the training set is dense, and we predict the missing values in the external data on the basis of their relationship structure. In other

words, the explanatory and explained variables are fixed. However, the training set that we assemble in this paper is very sparse, and the missing values depend on each substance, because the existing animal testing data are limited to fragmentary endpoints reported in the literature. (We describe the training set in detail later.) This implies that we are not able to fix the explanatory and explained variables. Because covariance structure analysis is a method of estimating implied covariance structure from the sample covariance of the training data, we can decide on the explanatory and explained variables on a case-by-case basis. Covariance structure analysis originally introduced by Joreskog and Van Thillo [13], and summarized by Bollen [3] and Kline [14], who complied the classical reference books on the subject. This enables us to create QAAR models. Therefore, here, we used covariance structure analysis as a statistical method.

We develop a QAAR model for inferring the missing no-observable-effect level (NOEL) values in animal testing data from repeated dose toxicity studies and reproductive toxicity studies. (We describe these toxicological terms in detail in the next paragraph.) One of the major advantages of our model is that it enables us to make estimations by using confidence intervals, which means that we can not only infer the missing NOEL values but also quantify their uncertainty.

Here, we give a brief description of toxicological terms; see also Chapters 6.4 and 6.5 in the work of van Leeuwen and Vermeire [11]. A repeated dose toxicity study deals with the general toxicological effects of repeated daily exposure via different routes for various fractions of the expected lifespan, up to a complete lifespan. The usual regulatory requirement is at least a 14- or 28-day test in rats. The aim of this test is to provide information on the likely effects of repeated exposure on target organs. Furthermore, this test should provide information on dose-response relationships, leading to identification of the NOEL value. The NOEL value is the highest dose at which no effects have been observed in available toxicity studies. A reproductive toxicity study deals with all phases of the reproductive cycle, including impairment of male or female reproductive function or capacity and the induction of non-heritable effects in the progeny from conception to sexual maturity. Such effects include death, growth retardation, and structural and functional effects. The general aim of this test is to resolve the following four issues: 1) whether administration of the substance to males or females, or both, before conception and during pregnancy and lactation affects reproductive function or capacity; 2) whether administration of the substance during pre- or post-natal development induces non-heritable effects in the progeny; 3) whether the pregnant female is potentially more susceptible to general toxicity; and 4) the dose-response relationship for any effects on reproduction.

To create our QAAR model, we used the following procedure. First, we assembled a training set from the Initial Risk Assessment Report published by Japan's National Institute of Technology and Evaluation (NITE) [17]. The set has 165 substances and 48 endpoints. However, the training set is characterized by very few observed values because of the very limited nature of the available animal testing data. Second, we designed a covariance structure model based on the correlations among the NOEL values of each endpoint. Third, we developed an expression for the optimal predictive equation with the implied covariance structure. Finally, we discuss the validation of the model in conformity with the OECD Principles for QSAR models [21]. It should be noted that our approach depends on only the statistical correlations of the training data.

2. Database

In this section we describe a defined endpoint in conformity with OECD Principle 1 of QSAR [19]. We assembled endpoint data from the Initial Risk Assessment Report, one of the research achievements of a project on "Chemical Risk Assessment and Development of Risk Assessment Methods in the Program for Comprehensive Assessment and Management of Chemicals," which was run by the Chemicals Evaluation and Research Institute and NITE and was funded by the New Energy and Industrial Technology Development Organization (FY 2001–2006). This report can be accessed on the NITE website [17].

The Initial Risk Assessment Report included results for 167 substances. These substances are listed in the Japan Pollutant Release and Transfer Register as Class I Designated Chemical Substances. The target substances are among 354 chemicals with high emission rates and hazardousness. In other words, these substances are important in terms of risk assessment and management of chemicals. Therefore, we considered these substances to be suitably representative for assembling a training data set for assessing risk.

We arranged the following dose toxicity and reproductive toxicity report items: animal (species, number, sex, and age in weeks), administration method (route, medium, and grade), dosage, target (e.g. internal organ), existence or non-existence of effects, and literature information. The database comprised 165 substances and a total of roughly 66,000 and 17,000 records of repeated dose and reproductive toxicity, respectively (i.e. number of tests $\times$ number of targets per test $\times$ number of doses).

We chose 10 representative targets for the repeated dose toxicity studies and four types of experiment for the reproductive toxicity studies (Table 1), and we fixed the NOEL values, classified by animal species (rat or mouse), target, and exposure route, as multivariate data. In the case of the reproductive toxicity studies we ascribed the types of experiment to the targets. In addition, if there was more than one study using the same animal species, target, and route, we used the geometric mean of the results. (Because a NOEL value is a positive number, positively skewed, and in orders of magnitude, throughout this paper we analyze the NOEL value data by using log transformation. We therefore use geometric means.) The resulting endpoints are summarized in Table 1. Throughout the paper, we de-

Table 1: Endpoint used in this study.

Type of toxicity study	Animal species	Testing method	Target
Repeated dose toxicity	Rat Mouse	Inhalation exposure experiment Oral exposure experiment	Liver Kidney Blood Urine Weight Death Spleen Digestive tract Respiratory organ Brain
Reproductive toxicity	Rat Mouse	Inhalation exposure experiment Oral exposure experiment	Effect, on a male parent, of exposure of the male parent (F0_M) Effect, on a female parent, of exposure of the female parent (F0_F) Effect, on the offspring, of exposure of the female parent (F1_100) Effect, on the offspring, of exposure of the both the parents and the offspring (F1_111)

fine endpoints by using a combination of target, animal species, and route. For example, the effect of oral exposure on the liver of the rat is an endpoint. Note that we used only four endpoints related to the reproductive toxicity studies, for which there were comparatively large amounts of data. We denoted the endpoints of the effects of reproductive toxicity studies as F0_M (effects on a male parent with exposure of the male parent), F0_F (effects on a female parent with exposure of the female parent), F1_100 (effects on the offspring with exposure of the female parent), and F1_111 (effect on the offspring with exposure of both the parents and the offspring).

Because in the repeated dose toxicity studies we did not use the results of inhalation exposure experiments on the digestive tract or of oral exposure experiments on the respiratory organs, and in the reproductive toxicity study we did not use the results of the mouse inhalation exposure experiments, the total number of endpoints was 48. The endpoints of the first two above-mentioned experiments were not used because the respective organs were not related to the exposure route; in the case of the last experiment mentioned above there were no experimental studies available that could provide endpoints.

3. Covariance Structure Analysis

In this section, we describe the unambiguous algorithm used in the study, in accordance with OECD Principle 2 for QSAR models.

3.1. Preliminary analysis

Here, we discuss the database used as the training set for our covariance structure modeling. More precisely, we demonstrate the observed ratio of each endpoint in the database (i.e. the number of substances with observed NOEL values as a ratio of the total number of substances; for example we calculated $0.3515 = 58/165$, where 58 is the number of substances for which we had observation data from inhalation exposure experiments involving the liver in rats). We also demonstrate the concurrently singular and interrelated structure of the database. Table 2 lists the observed ratios for each endpoint. The total number of endpoint-observed ratios was very small; this usually makes it difficult to infer missing values. However, in the database we assembled, there were high levels of correlation between the endpoint values. We illustrated this with a scatter-plot matrix using the liver and kidney toxicological data (Figure 1). Hence, we inferred the missing NOEL values for each endpoint by designing covariance structure modeling among endpoints for targets and animal species.

3.2. Covariance structure modeling

We describe here how we designed covariance structure modeling and estimated the implied covariance structure from the database constructed in the preceding section. We created the model as follows, using IBM SPSS AMOS 19 (`http://www-03.ibm.com/software/product/us/en/spss-amos/`) as statistical software.

Step 1 : We set exposure paths as latent variables.

Step 2 : We explored causal relations exhaustively by us-

Table 2: Observed ratios for each endpoint (the number of substances with observed NOEL values as a ratio of the total number of substances [i.e. 165]), where "inhalation" and "oral" denote "inhalation exposure experiment" and "oral exposure experiment," respectively, and − means that the respective endpoint is not used. For example, we calculated 0.3515 = 58/165, where 58 is the number of substances for which we had observation data from inhalation exposure experiments involving the liver in rats.

	Rat		Mouse	
Target	inhalation	oral	inhalation	oral
Liver	0.3515	0.6182	0.1939	0.2848
Kidney	0.2121	0.5879	0.1091	0.1758
Blood	0.3879	0.6434	0.1697	0.1879
Urine	0.2606	0.3758	0.08485	0.09897
Weight	0.4606	0.7091	0.2000	0.3273
Death	0.2727	0.4727	0.1576	0.2364
Spleen	0.1091	0.2727	0.04848	0.1576
Digestive tract	−	0.2364	−	0.08485
Respiratory organ	0.3576	−	0.1576	−
Brain	0.04848	0.04848	0.04196	0.1030
F0_M	0.9091	0.2121	−	0.4848
F0_F	0.1576	0.3394	−	0.09091
F1_100	0.3152	0.4121	−	0.1879
F1_111	0.05455	0.2303	−	0.0661

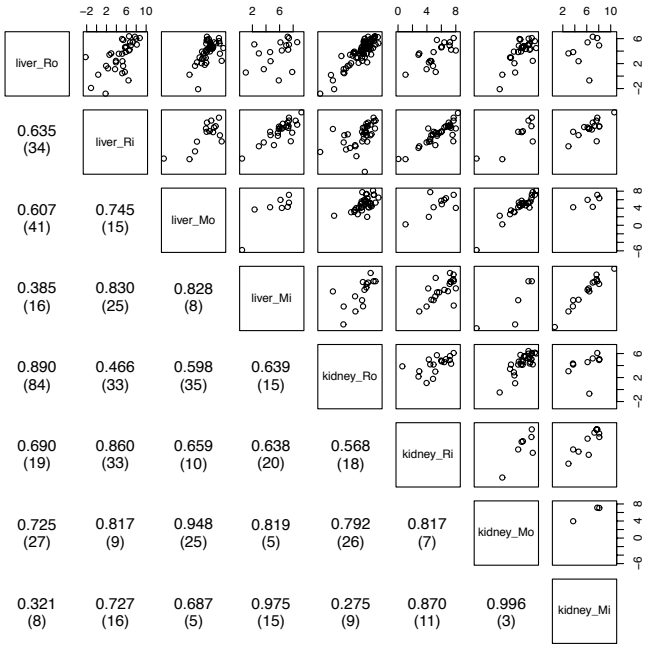

Figure 1: Scatter-plot matrix of logarithmic NOEL values for the effects of substances on the liver and kidney. R and M denote "rat" and "mouse," respectively, and i and o denote "inhalation exposure experiment" and "oral exposure experiment," respectively. In the triangle forming the bottom-left half of the matrix we list the Pearson correlation coefficients (pair-wise deletion of missing values); sample sizes are in parentheses. There were strong correlations among the endpoint NOEL values or rats and mice with respect to liver and kidney effects using the same delivery routes.

ing kidney and liver toxicological data as endpoints, because these data covered major organs. We then chose the model that had the lowest Browne-Cudeck criterion value (BCC). The BCC is a measure of the relative quality of a statistical model. It was developed for covariance structure modeling and is equivalent to the Akaike information criterion in the case of normal models; see the work of Browne and Cudeck [4] for more details.

Step 3 : We drew lines between strongly correlated endpoints in order of decreasing observed ratios and Pearson correlation coefficients (pair-wise deletion of missing values).

The modeling is illustrated in Figure 2 (also see e.g. Path Diagrams, Chapter 4 in the work of Mulaik [15]). The variables framed by squares, ellipses, and circles are observed variables, latent variables, and error variables, respectively. A latent variable is a variable that cannot be observed directly. An error variable is attached to every observed variable. One- and two-ended arrows denote linear and non-directional (correlational) relationships, respectively.

We inferred the NOEL values and standard deviations of each endpoint from the above model (Table 3). We also determined the covariances among all of the endpoints in the supporting document.

4. Inference of Missing Values

Here, we describe the optimal linear predictor by using the implied covariance structure. Let x and y be vectors of the observed and missing values of a particular substance, respectively; and let $\hat{\mu}_x$ and $\hat{\mu}_y$ be vectors of the means of the observed and missing values, respectively, of all of the substances appearing in the database. If we describe the implied covariance matrix as

$$\begin{pmatrix} \Sigma_{xx} & \Sigma_{xy} \\ \Sigma_{xy}^t & \Sigma_{yy} \end{pmatrix},$$

then the optimal linear predictor is:

$$\begin{cases} \hat{y} = \hat{\mu}_y + \Sigma_{xy}^t \Sigma_{xx}^{-1} (x - \hat{\mu}_x), \\ \mathrm{Cov}(e) = \Sigma_{yy} - \Sigma_{xy}^t \Sigma_{xx}^{-1} \Sigma_{xy}; \end{cases}$$

where t and e denote the transposed operator and error vector, respectively, of the linear regression model; see Section 2.5 in the work of Anderson [1] for more details. The first equation enables us to infer the missing NOEL values, and the second one provides the confidence interval of the estimation.

Consider toluene as an example. In this case, there are 19 missing NOELs out of 48 endpoints; that is, the sufficiency level of the data is about 40%. From oral experiments in rats, there are animal testing data on the effects on the liver, kidney, urine, weight, death, and brain. Figure 3 shows the results of estimations of NOEL values to these oral experiments.

5. Validation of the Model

Here we describe the validation of the model, which conforms with OECD Principle 4 for QSAR models. (In the absence of any specific principle governing QAAR models, we consider this QSAR principle to be fundamentally applicable.) OECD Principle 4 requires the assessment of three attributes: goodness-of-fit, robustness, and predictivity. As indicators of our model's reliability, we calculated the correlation coefficient and the root mean-square error between the logarithmic estimated and observed values for each validation item.

For goodness-of-fit, we used the leave-one-out cross-validation for each endpoint. Note that we also call the leave-one-out cross-validation method the n-fold cross-validation, where n is the sample size. Assume that the structure of the path diagram, and the mean vector and covariance matrix calculated in the previous section, are fixed. The resulting correlation coefficient and the root mean-square error between the logarithmic estimated and observed values were 0.894 and 1.06, respectively. Predictions regarding chemical substances in the training set are used to assess the goodness-of-fit the model, which is a measure of how well the model accounts for variance of response in the training set.

For robustness, we used leave-one-out cross-validation for each substance. Assume that the structure of the path diagram is fixed, but the mean vectors and covariance matrices are recalculated. The resulting correlation coefficient and the root mean-square error between the logarithmic estimated and observed values were 0.872 and 1.17, respectively. Robustness refers to the stability of the model's parameters (which are predictor coefficients)—that is, the stability of its predictions when a perturbation is applied to the training set.

Finally, for predictivity, we used lead, copper, cadmium, bisphenol-A bis(diphenyl phosphate), and triphenyl phosphate as exterior (i.e. non-training set) substances for which NOEL values were obtainable from case studies, thus including both organic chelates and metals. We then estimated the NOEL values. The resulting correlation coefficient and the root mean-square error between the estimated and observed values were 0.901 and 1.26, respectively. Predictions regarding chemical substances in the test set were used to assess the predictive ability of the model, which is measure of how well the model can predict new data. Note that even though we have only inferred the missing values in the training set we can estimate the NOEL values of new chemical substances for all endpoints if the value for at least one endpoint is available.

Verification of conformity with OECD Principle for QSAR models revealed that the accuracy of this model is high.

6. Discussion and Conclusions

We provided a QAAR model based on covariance structure analysis to infer the missing values in animal testing data

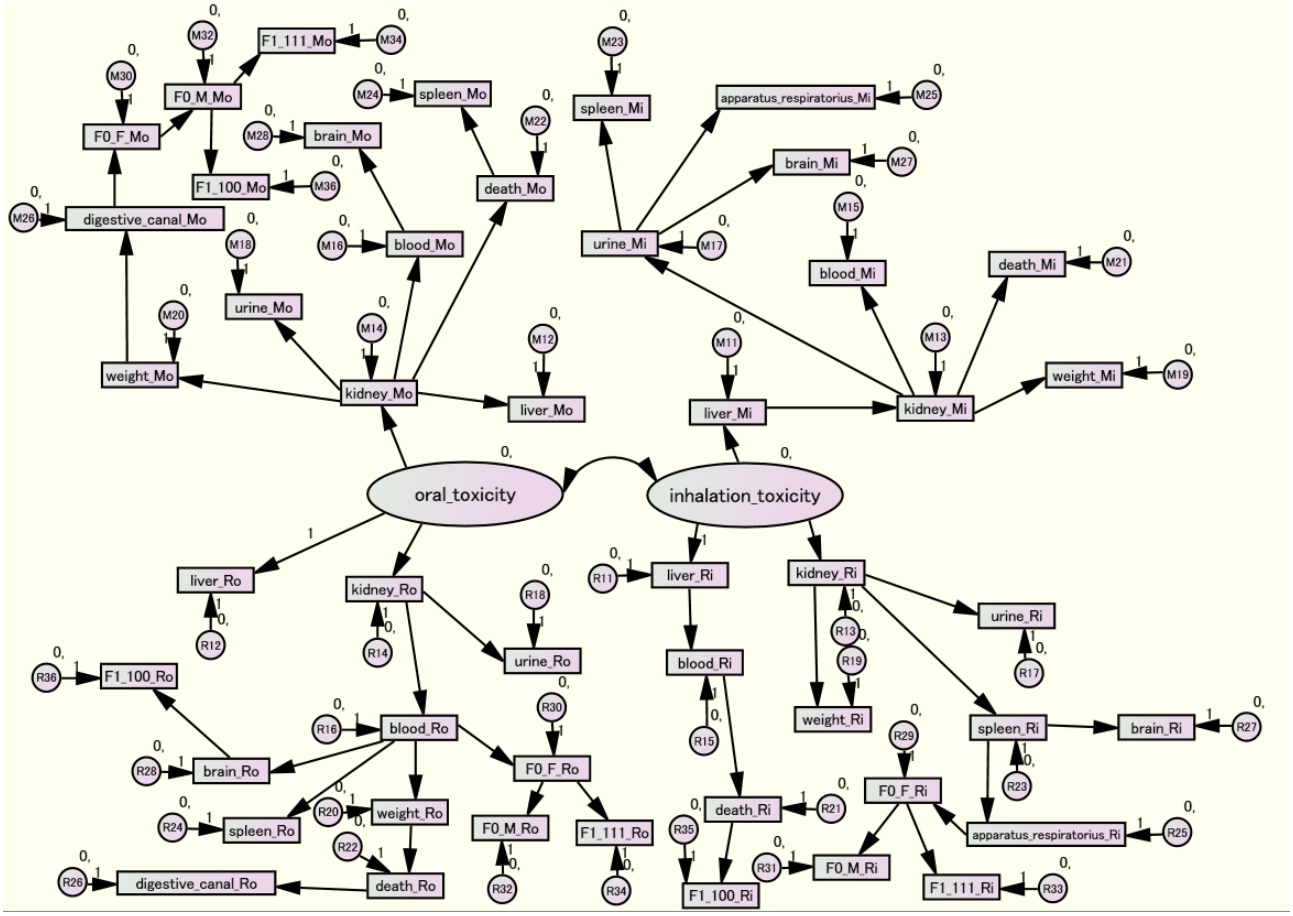

Figure 2: Model with latent variables as exposure path (oral toxicity and inhalation toxicity) (in ellipses). Endpoint names are denoted by "target_animal species_exposure path," e.g. 'liver_Ri' stands for inhalation exposure experiments involving the liver in rats. Letters R and M denote "rat" and "mouse," respectively; and i and o denote "inhalation exposure experiment" and "oral exposure experiment," respectively.

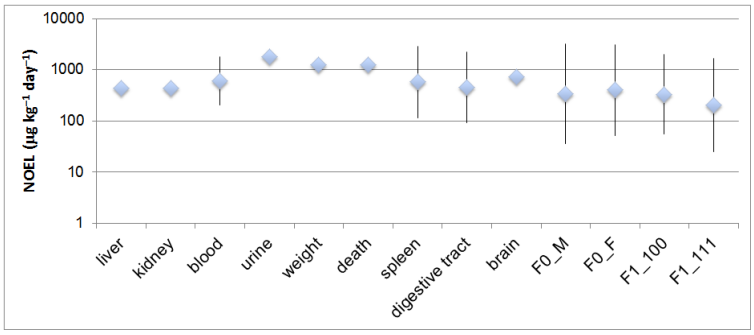

Figure 3: Results for toluene NOEL values related to oral experiments in rats, as calculated by using the optimal linear predictor. Diamonds and error bars are original units of the estimated NOEL values and their 95% confidence intervals, respectively. Endpoints with no error bars are observed values.

Table 3: Logarithmic NOEL values (mean and standard deviations) derived by using the implied covariance structure and estimated from the model. − indicates unused endpoints. The unit used for NOEL values is $\mathrm{mg} \cdot \mathrm{kg}^{-1} \cdot \mathrm{day}^{-1}$.

		Rat		Mouse	
		Inhalation	Oral	Inhalation	Oral
Liver	mean	4.681	3.826	4.769	4.552
	sigma	2.807	2.115	2.466	2.327
Kidney	mean	4.843	4.052	5.025	4.577
	sigma	2.494	2.011	2.573	2.602
Blood	mean	4.438	4.131	4.883	4.557
	sigma	1.939	2.029	2.559	2.828
Urine	mean	4.516	4.155	4.850	4.511
	sigma	3.063	2.063	2.869	2.433
Weight	mean	4.848	4.158	4.912	4.883
	sigma	2.882	1.906	2.664	1.976
Death	mean	5.265	4.584	5.273	5.300
	sigma	2.910	2.138	2.756	2.083
Spleen	mean	4.908	4.083	5.115	4.677
	sigma	2.894	2.246	3.145	2.384
Digestive tract	mean	−	4.218	−	5.319
	sigma	−	1.782	−	1.457
Respiratory organ	mean	4.451	−	4.771	−
	sigma	2.895	−	2.695	−
Brain	mean	3.397	4.546	3.901	5.055
	sigma	1.939	2.261	3.751	2.828
F0_M	mean	5.093	4.251	−	4.613
	sigma	2.316	1.757	−	2.146
F0_F	mean	5.248	4.307	−	5.083
	sigma	2.501	1.769	−	2.146
F1_100	mean	5.501	4.749	−	5.512
	sigma	2.397	1.475	−	1.252
F1_111	mean	5.055	3.987	−	5.200
	sigma	2.451	1.551	−	1.110

for chemical substances. We then discussed OECD Principles 1, 2, and 4 regarding QSAR models as validation of our QAAR model.

Our QAAR model has several debatable characteristics. First, we assembled the data set from the NITE Initial Risk Assessment Report, and we therefore did not use primary data. Thus, the reliability of the database may be questionable. Second, we did not reflect toxicological relevance in constructing the model, but rather, data structure. In other words, we needed to assign the highest priority to the convergence of the model because of the sparseness of the database. However, if adequate toxicological relevance can be obtained, then the reliability of our QAAR approach might increase. This is a consideration for the future. Finally, we were not able to produce a clear statement on OECD Principle 3 regarding QSAR models, which is associated with defining the domain of applicability. However, because the training set was assembled from the Initial Risk Assessment Report, and the substances included in the report are listed in the Japan Pollutant Release and Transfer Register as Class 1 Designated Chemical Substances, we qualitatively determined that the model was suitable for assessing the risks of chemical substances.

Finally, we described how to handle missing values in the respective training sets. We did not treat missing nontoxicity data. Basically, our model implied that all targets were in some way affected by exposure to chemical substances. The existence of missing data might impose inferential bias to an undefined extent. Also, in analyzing human health studies, missing-value estimation has become a standard tool, as in multiple imputation methods. Here, we did not adopt any imputation methods but instead calculated the marginal likelihood of a data set in which missing data existed, under the assumption of data being missing at random. This approach yields results fundamentally equivalent to inferences drawn from multiple imputations assuming infinite imputation.

Although the method we proposed in this paper in the first trial of its kind among QAAR approaches, we consider it a promising approach, because the model was sufficiently validated despite the data limitations.

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Jun-ichi Takeshita and Masashi Gamo
Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, 16-1, Onogawa, Tsukuba, Ibaraki, 305-8569, Japan
E-mail: jun-takeshita(at)aist.go.jp
masashi-gamo(at)aist.go.jp

Koji Kanefuji and Hiroe Tsubaki
The Institute of Statistical Mathematics, 10-3, Midori-cho, Tachikawa, Tokyo, 190-8562, Japan
E-mail: kanefuji(at)ism.ac.jp
tsubaki(at)ism.ac.jp