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Prediction of the Toxicity of Dimethylformamide, Methyl Ethyl Ketone, and Toluene Mixtures by QSAR Modeling

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In this study, we analyzed the toxicity of mixtures of dimethylformamide (DMF) and methyl ethyl ketone (MEK) or DMF and toluene (TOL) and predicted their toxicity using quantitative structure-activity relationships (QSAR). A QSAR model for single substances and mixtures was analyzed using multiple linear regression (MLR) by taking into account the statistical parameters between the observed and predicted EC_{50}. After preprocessing, the best subsets of descriptors in the learning methods were determined using a 5-fold cross-validation method. Significant differences in physico-chemical properties such as boiling point (BP), specific gravity (SG), Reid vapor pressure (rVP), flash point (FP), low explosion limit (LEL), and octanol/water partition coefficient (Pow) were observed between the single substances and the mixtures. The EC_{50} of the mixture of DMF and TOL was significantly lower than that of DMF. The mixture toxicity was directly related to the mixing ratio of TOL and MEK (MLR EC_{50} equation = 1.76997 – 1.12249 × TOL + 1.21045 × MEK), as well as to SG, VP, and LEL (MLR equation EC_{50} = 15.44388 – 19.84549 × SG + 0.05091 × VP + 1.85846 × LEL). These results show that QSAR-based models can be used to quantitatively predict the toxicity of mixtures used in manufacturing industries.

Key Words : QSAR, Chemical mixtures, Toxicity

Introduction

More than 10 million chemicals are commercially used worldwide, and it is known that hundreds of chemicals are newly introduced each year.[1] As of the writing of this manuscript, roughly 43,000 chemicals are used in Korea.[2] While some chemicals have little effect on human bodies and the environment, many can cause serious damage at low concentrations. For this reason, threshold limits on chemical exposure are recommended by the American Conference of Governmental Industrial Hygienists.[3]

To date, most assessments of toxicity and environmental exposure have been performed for single substances. Studies on mixtures, however, are important because mixed chemicals have greater effects on human bodies and the environment from a toxicological perspective.[4] For this reason, researchers have addressed the issue of toxicity of mixtures.[5-8] However, testing mixtures requires far more experiments than does the testing of single compounds, and thus more requires time and expense. For these reasons, studies of mixtures have not been published at a high rate.

In order to estimate toxicological effects on human bodies and the environment, several researchers have suggested assessment methods utilizing physico-chemical properties such as hydrophobicity (octanol/water partition coefficient) and solubility. However, as previously mentioned, it has also been suggested that there are limits to toxicity evaluations[11] of[12] mixtures by using only physico-chemical properties.[9-12] These methods are limited because characteristics such as functional groups, reactivity, and biological properties of intermediates produced by metabolic processes are not taken into account; therefore, it is necessary to consider multiple types of assessment.[13,14] Quantitative-structure activity relationship (QSAR) analysis, which applies variables including physico-chemical properties and biological information to an equation to estimate and analyze toxicity, has been widely used to estimate the toxicity of materials. QSAR analysis has been used mainly in the fields of medicine, pharmacy, environmental science, toxicology, and biology as a way of predicting physico-chemical and biological properties *via* statistical models, and its use has gradually spread to other areas.[13,16-18] Additionally, QSAR analyses are expected to be valued by national and international organizations from a chemical management perspective, in that toxicity estimates generated by QSAR are allowed in REACH (registration, evaluation, and authorization of chemicals). In particular, hazard estimations in the context of occupational health are needed. Studies to predict hazards for the purpose of protecting worker health are needed because various kinds chemicals are often handled in large quantities, and mixtures are used much more often than single substances.

Therefore, this study aimed to predict the toxicity of mixtures of dimethylformamide (DMF) with methyl ethyl ketone (MEK) and/or toluene (TOL), which are handled

mainly in the synthetic leather manufacturing industry, using a QSAR model with physico-chemical properties and half-maximal effective concentration (EC_{50}) as variables, which vary depending on the ingredients and their mixing ratios.

Materials and Methods

Targeted Materials and Mixing Composition. DMF, MEK, and TOL were mixed in particular ratios that corresponded to products used by synthetic leather manufacturers (Table 1).

Physico-chemical Properties Used. Data on physico-chemical properties of each DMF, MEK, and TOL were obtained from material safety data sheet (MSDS) provided by the Korea Occupational Safety and Health Agency and Sigma-Aldrich. Physico-chemical properties such as boiling point (BP),[19] specific gravity (SG),[20] Reid vapor pressure (rVP),[21] flash point (FP),[22] low explosion limit (LEL),[23] and octanol/water partition coefficient (Pow)[24] were measured.

Experimental Determination of Half-maximal Effective Concentrations (EC_{50}). HepG2 cells (human hepatocytes) were acquired from the Korean Cell Line Bank. The cells were cultivated in DMEM (10% FBS, 100 unit/mL penicillin, and 100 μg/mL streptomycin) and MEM badges in a 5% CO_2 atmosphere at 37 °C. They were seeded onto 96-well plates (Corning Inc., Corning, New York, USA) at a concentration of 5×10^4 cells/well and were mixed with DMF, MEK, and/or TOL after 24 h. The cells were transferred into MEM culture medium to be stabilized for 24 h at a concentration of 5×10^4 cells/well in a 96-well plate. Samples were grouped as shown in Table 1 and treated with the appropriate solutions for 3, 6, 12, and 24 h. After culture, the medium was removed, the cells were diluted by 1/10 with the CCK-8 assay kit (Woongbee, Seoul, Korea) in DMEM culture medium, and then incubated for 1.5 s at 37 °C. EC_{50} values were determined by measuring the absorption of formazan at 450 nm.

Table 1. Classification of experimental groups and mixing ratios of chemicals

Groups	Mixing ratio (Vol./Vol.)
Single	
DMF	1.0
MEK	1.0
TOL	1.0
Mixture [DMF+MEK (DM) and DMF+Tol (DT)]	
DMF+MEK (DM1)	DMF+MEK=1.0:0.5
(DM2)	DMF+MEK=1.0:1.0
(DM3)	DMF+MEK=0.5:1.0
DMF+TOL (DT1)	DMF+TOL=1.0:0.5
(DT2)	DMF+TOL=1.1:1.0
(DT3)	DMF+TOL=0.5:1.0
DMF+MEK+TOL (DMT)	DMF+MEK+TOL=1.0:1.0:1.0

DMF, dimethylformamide; MEK, methyl ethyl ketone; TOL, toluene; DM1, DMF + MEK = 1.0:0.5; DM2, DMF + MEK = 1.0:1.0; DM3, DMF + MEK = 0.5:1.0; DT1, DMF + TOL = 1.0:0.5; DT2, DMF + TOL = 1.0:1.0; DT3, DMF + TOL = 0.5:1.0; DMT, DMF + MEK + TOL = 1.0:1.0:1.0

Statistical Validation of the Model. SAS JMP PRO software (ver. 10.0, SAS Institute Inc., Cary, NC, USA) was used to verify the designed model and the goodness-of-fit with the physico-chemical properties or mixing ratio of DMF, MEK, and TOL, as well as the EC_{50}, as presented in Table 2. Stepwise regression was used to select a subset of variables and 5-fold cross-validated R^2 (Q^2) was used as a criterion to enter and remove variables from the QSAR model. QSAR regression models were evaluated by calculating performances (R^2, RMSE, and Q^2), with the goodness of a model calculated as follows.

$$R^2 = 1 - \frac{\Sigma(y_{obs} - y_{pre})^2}{\Sigma(y_{obs} - y_{mean})^2} \text{ for the training set}$$

$$Q^2 = 1 - \frac{\Sigma(y_{obs} - y_{pre})^2}{\Sigma(y_{obs} - y_{mean})^2} \text{ for the validation set}$$

$$RMSE = \sqrt{\frac{\Sigma(y_{obs} - y_{pre})^2}{N}} \text{ for the training set} \quad (1)$$

The QSAR regression model was analyzed by measuring the degree of relevance between the descriptors used and the estimated EC_{50} using the linear regression Eq. (2) below with a training set divided into the pre-set number of folds following a cross-validation test.

$$y = a_0 + a_1X_1 + a_2X_2 + \cdots a_nX_n \quad (2)$$

ANOVA was performed to assess the differences in physico-chemical properties among the experimental groups, and the QSAR regression model was cross-validated. All results were presented as mean percentages with standard deviation.

Results

Comparison of Physico-chemical Properties by Experimental Groups. EC_{50} values and physico-chemical properties of the treatments were measured and presented in Table 2. The properties of the solutions significantly varied depending on whether they contained a single substance or a mixture, and the manner of mixing. For DMF, the values of EC_{50} were higher in the DMF + MEK (DM) group than in the DMF group, whereas they were lower in the DMF + TOL (DT) group than in the DMF group. BP, SG, and FP were lower in the DM and DT groups than in the DMF group, but rVP was significantly higher in the DM and DT groups than in the DMF group. The mixture of DMF, MEK, and TOL showed higher BP, SG, and FP, and lower rVP. In the mixtures, physico-chemical properties varied depending on the mixing ratios of MEK and TOL.

Regression Model for Predicting EC_{50} in Human Liver Cells.

Regression Model by Mixing Ratios: The multiple linear regression (MLR) model for predicting the toxicity of mixtures based on the ratios of DMF, MEK, and/or TOL is

Table 2. Results of the EC_{50} and physico-chemical properties experiment

Groups	HepG2 cell	Physico-chemical properties				
	EC_{50} (mg/100 μL)	BP (°C)	SG (g/mL)	rVP (kPa)	FP (°C)	LEL (%)
DMF	1.785	153.0	0.948	0.93 ± 0.15	58.0	2.2
MEK	3.068	80.0	0.805	21.63 ± 0.15	3.0	1.8
TOL	0.396	110.0	0.864	6.87 ± 0.21	4.0	1.1
DM1	2.887	114.4 ± 0.44	0.9042 ± 0.0001	8.40 ± 0.100	12.29 ± 0.298	2.627 ± 0.040
DM2	2.765	102.9 ± 0.07	0.8813 ± 0.0001	10.90 ± 0.173	6.14 ± 0.298	2.310 ± 0.046
DM3	2.686	93.8 ± 0.15	0.8565 ± 0.0006	14.57 ± 0.115	1.64 ± 0.271	2.027 ± 0.025
DT1	0.837	127.9 ± 0.10	0.9247 ± 0.0004	4.07 ± 0.058	21.09 ± 0.012	2.033 ± 0.042
DT2	0.854	123.7 ± 0.35	0.9129 ± 0.0002	4.87 ± 0.058	18.07 ± 0.465	1.733 ± 0.015
DT3	0.873	118.4 ± 0.32	0.8979 ± 0.0004	5.80 ± 0.100	13.32 ± 0.321	1.470 ± 0.010
DMT	1.882	103.5 ± 0.10	0.8780 ± 0.0003	10.43 ± 0.153	6.02 ± 0.515	1.810 ± 0.017
F value	–	26454.790	53988.549	5771.546	9986.224	836.403
p value	–	0.000	0.000	0.000	0.000	0.000

BP, boiling point; SG, specific gravity; rVP, Reid vapor pressure; FP, flash point; LEL, low explosion limit; DMF, dimethylformamide; MEK, methyl ethyl ketone; TOL, toluene; DM1, DMF + MEK = 1.0:0.5; DM2, DMF + MEK = 1.0:1.0; DM3, DMF + MEK = 0.5:1.0; DT1, DMF + TOL = 1.0:0.5; DT2, DMF + TOL = 1.0:1.0; DT3, DMF + TOL = 0.5:1.0; DMT, DMF + MEK + TOL = 1.0:1.0:1.0.

Table 3. QSAR model according to chemical mixing ratio by multiple linear regression (MLR)

Chemicals	No. of parameter	Training		5 fold Cross-validation
		R^2	RMSE	Q^2
DMF	1	0.0007	1.0791	–
MEK	1	0.7124	0.5798	–
TOL	1	0.6738	0.6165	–
DMF, MEK	2	0.7212	0.6093	0.2495
DMF, TOL	2	0.6753	0.6575	0.2458
MEK, TOL	2	0.9493	0.2598	0.8581
DMF, MEK, TOL	3	0.9502	0.2781	0.8191

DMF, dimethylformamide; MEK, methyl ethyl ketone; TOL, toluene; RMSE, root mean square error; R^2 and Q^2, coefficient.

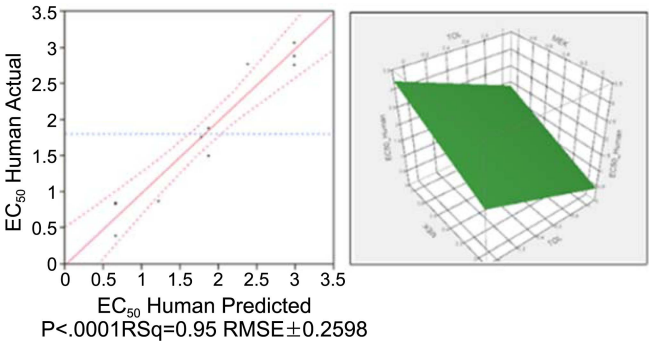

Figure 1. Plot of experimental and predicted values of EC_{50} by linear regression (according to chemical mixture). y = 1.76997 – 1.12249 × TOL + 1.21045 × MEK.

presented in Table 3. The goodness of fit (R^2, RMSE) for all solutions, whether a single compound or a mixture, showed goodness of fit (R^2) ranging from 0.6738 to 0.9502 except, with the exception of the solution with DMF as a single substance.

Five-fold cross-validation in a leave-many-out (LMO) manner was performed to identify the stability and predictability of the models. MLR models by mixing ratios of MEK + TOL (Q^2 = 0.8581) and DMF + MEK + TOL (Q^2 = 0.8191) showed Q^2 over 0.5, which is generally considered an acceptance level, other mixing ratios had little impact on the toxicity with low acceptance values. With an optimized model, experimental and predicted values of EC_{50} by MEK and TOL were compared and presented in Figure 1.

R^2 and RMSE were 0.9493 and 0.2598, respectively. The linear regression equation of EC_{50} = 1.76997 – 1.12249 × TOL + 1.21045 × MEK, indicated that EC_{50} decreased as the concentration of TOL increased, and EC_{50} was proportional to the concentration of MEK.

Regression Model by Physico-chemical Properties. An optimized regression model, which was optimized *via* a linear regression equation with the physico-chemical properties of B, SG, rVP, FP and LEL, is presented in Table 3. Compared with the R^2 value for the training set, goodness showed R^2 values ranging from 0.8850 to 0.9846 when 2 or more properties were selected. When 5-fold cross-validation in a leave-many-out (LMO) manner was performed, Q^2 had its maximum value of 0.9349 when SG, rVP, and LEL were selected (Table 4).

With an optimized model, the experimental and predicted values of EC_{50} by SG, rVP, and LEL were compared and presented in Figure 2. R^2 and RMSE were 0.9840 and 0.1574, respectively. The linear regression equation of EC_{50} = 15.44388 – 19.84549 × SG + 0.05091 × rVP + 1.85846 × LEL indicated that EC_{50} decreased as SG increased, and that EC_{50} was proportional to rVP and LEL.

Discussion

Despite its toxicity to the liver, DMF is widely used in many manufacturing industries owing to its convenient miscibility.[25] In particular, DMF is used in the form of a mixture containing MEK and/or TOL.[8] Thus, in this study,

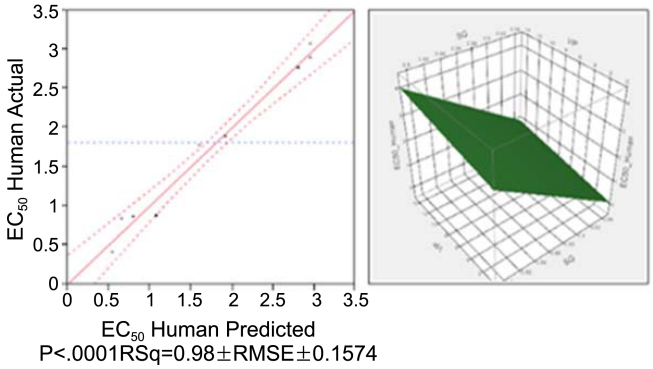

Figure 2. Plot of experimental and predicted values of EC_{50} by linear regression (according to physico-chemical properties). y = 15.44388 – 19.84549 × SG + 0.05091 × rVP + 1.85846 × LEL.

mixtures of DMF, MEK, and/or TOL were split into several groups depending on their mixing ratios and their physico-chemical properties (BP, SG, rVP, FP, LEL, and Pow) were measured. Influential mixing ratios and physico-chemical properties were identified *via* a 5-fold cross-validation in a LMO manner, and the MEK + TOL ($Q^2 = 0.8581$) and DMF + MEK + TOL ($Q^2 = 0.8191$) groups showed Q^2 over 0.5, which is generally considered an acceptance level.[26] For the physico-chemical properties, a combination of SG, rVP, and LEL had the highest Q^2 value (0.9349). The experimentally determined EC_{50} values estimated from mixing ratios were used to generate following linear regression equations. The equation $EC_{50} = 1.76997 – 1.12249 \times TOL + 1.21045 \times MEK$ indicated that EC_{50} decreased as the concentration of TOL increased, and that EC_{50} was proportional to the concentration of MEK. $EC_{50} = 15.44388 – 19.84549 \times SG + 0.05091 \times rVP + 1.85846 \times LEL$ is a linear regression equation containing the terms SG + rVP + LEL, suggesting that EC_{50} was proportional to SG and inversely proportional to rVP and LEL.

It was reported that a linear solvation energy relationship (LSER) analysis was very useful for determining optimized descriptors and predicting a QSAR model. Variables used in an LSER analysis include the physico-chemical properties of molecular weight, solubility, and Pow. Although a chemical substance maintains its own physico-chemical properties without external influences, interactions between ingredients change the properties when they are mixed, due to a variety of interactions caused by differences in structure, branched chains, hydrophobicity, and solubility. It seems that the significant differences in physico-chemical properties between a single substance and a mixture resulted from the alteration of physico-chemical properties by chemical reactions among bipolar DMF, polar MEK, and/or non-polar TOL.

Table 4. QSAR model according to physico-chemical properties by multiple linear regression (MLR)

Parameter	No. of parameter	Training		5 fold Cross-validation
		R^2	RMSE	Q^2
BP	1	0.2975	0.9047	–
SG	1	0.2141	0.9570	–
rVP	1	0.1465	0.9973	–
LEL	1	0.4317	0.8137	–
BP, LEL	2	0.8850	0.3913	0.6191
SG, LEL	2	0.9485	0.2619	0.8681
BP, FP, LEL	3	0.9846	0.1547	0.7359
SG, rVP, LEL	3	0.9840	0.1574	0.9349

BP, boiling point; SG, specific gravity; rVP, Reid vapor pressure; FP, flash point; LEL, low explosion limit; RMSE, root mean square error; R^2 and Q^2, coefficient.

Altered physico-chemical properties of mixtures lead to changes in metabolic processes, and thus in their effects on health (synergistic or antagonistic)[27] and the degree of health impairment caused by them.[28] Of the physico-chemical properties, hydrophobicity has the most significant impact on the division of an aqueous phase at the cell surface. Non-polar materials interact with lipid membranes to affect microorganisms; it was reported that a logPow between 2.0 and 4.0 is predictive of human toxicity, while a value higher than 4.0 has little, if any, effect.[29] Kim *et al.*[30] reported that TOL with a Pow of 2.45 had possible toxicity while the Pow values of DMF (1.01) and MEK (0.31) were low. In a study in which the liver toxicity of DMF and TOL was compared in rats, Kim & Chung[8] reported that DMF showed more noticeable toxicity when injected with TOL than when injected alone.

This study used human hepatocytes (HepG2) to determine EC_{50} values in experimental groups. EC_{50} values in the TOL (0.396 mg/100 μL) and DT groups were much lower than those in the DMF (1.785 mg/100 μL), MEK (3.068 mg/100 μL), and DM groups. Furthermore, linear regression analysis indicated that EC_{50} tended to decrease as the concentration of TOL increased. These results seem to be due to the Pow, as described above. Pow is closely related to BP, SG, rVP, FP, and LEL. Thus, a change in Pow changes physico-chemical properties including volatility, thus exposure possibility.

In this study, the derived linear regression equation ($EC_{50} = 15.44388 – 19.84549 \times SG + 0.05091 \times rVP + 1.85846 \times LEL$) with descriptors of physico-chemical properties indicates that, in mixtures, EC_{50} decreases as SG increases and EC_{50} increases as rVP and LEL increases. This result suggests that Pow is influenced by SG and that the higher rVP of MEK as compared with DMF and TOL indicates a higher volatility.

As a result of our studies of the toxicity of mixtures of DMF, MEK, and/or TOL, we found that the presence of TOL and the SG are the physico-chemical properties that are most influential on toxicity. Although this study was confined to predicting the toxicity of the mixtures used in the synthetic leather manufacturing industry, it proposes possibilities for future studies on chemical mixtures used in industry and provides a base of data from which to work.

Conclusion

This study was predicted the toxicity of mixtures of DMF,

MEK, and/or TOL, which are used mainly in synthetic leather manufacture, using QSAR, with physico-chemical properties and EC_{50} as variables, which varied depending on the ingredients and their mixing ratios.

Goodness-of-fit (R^2, RMSE) and 5-fold cross-validation were applied to evaluate the quality of MLR models. Result showed there was a significant difference in experimentally determined EC_{50} values for a single substance and a mixture, and the toxicity in humans of a mixture of DMF, MEK, and TOL depends mainly on the ratio of TOL and SG.

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