RESEARCH REPORT - No. 1602 FY 2016-2017

Title of research project	Construction of the database of in vivo toxicity tests and its application to the in silico prediction and evaluation of in vivo toxicity
Research project no.	1602
Research period	FY 2016–2017
Name of principal research Investigator（PI）	Kouichi YOSHINARI

【Abstract】

The aims of this study were 1) the expansion of the HESS database by using risk assessment reports from Food Safety Commission, Cabinet Office, Government of Japan, 2) the analysis of the mechanism and species differences of toxicities, 3) the development of categories based on toxicity mechanism and chemical properties, 4) the evaluation of threshold of toxicological concern (TTC) values using HESS database, and 5) the development of in silico toxicity prediction models, for the safety assessment of the chemical compounds in food.

Using the available reports of risk assessments, we built the database of the repeated dose toxicity (RDT) test data of 224 substances (674 tests), which can be imported to HESS. This has increased the chemical space of the HESS database. The custom database also can be imported to OECD QSAR Toolbox.

In addition, we collected the data on reproductive and developmental toxicity tests from the risk assessment reports and literature and summarized the data of 91 tests as Microsoft Excel data. We further constructed a FileMaker database of 6 kinds of phthalates (DIDP: 5 tests, DINP: 4 tests, DBP: 29 tests, DEHP: 22 tests, BBP: 17 tests, DNOP: 2 tests). To our knowledge, these databases are the first systematic and detailed database for reproductive and developmental toxicity and might be valuable for the safety assessment of the reproductive and developmental toxicity.

We next evaluated the usefulness of these databases. At first, using the database of the reproductive and developmental toxicity tests, we investigated the toxicological characteristics of the phthalates and found that the reproductive toxicities of phthalates depended on their side chain length, and that those with shorter side chains, such as DBP, DEHP, and BBP, were more toxic at low doses than those with longer side chains, such as DIDP, DINP and DNOP. We also found that the side chain length was not related to the liver toxicity of the phthalates.

Using the RDT data of pesticides, we analyzed the species differences between rats and dogs and between rats and mice. As the results, we found that some toxicity endpoints observed in rats are often observed in dogs rather than in mice, but some observed in rats are frequently observed in mice rather than in dogs, depending on the type of toxicity. In addition, these differences and similarities depended on the test periods and sexes.

In the evaluation of TTC values using the HESS database, we found that the 5%ile values calculated for the Cramer's class I, class II and class III were 800, 348 and 166 μg/person/day,

respectively. The value for Class III is comparable to that for the Munro's dataset, whereas the value for class I is lower than those for Munro's (1996) and EU's COSMOS (2017) datasets. In addition, the comparison of these datasets revealed that the HESS database has different chemical space from those of the Munro's and COSMOS, suggesting that combining these databases is useful for TTC analysis.

Next, we investigated the relationships between chemical structures and in vivo toxicity using the databases and found following relationships: a certain group of compounds containing a naphthalene moiety with ALT level increase in rat 28-day RDT tests; a pyrazole ring with liver dysfunction-related endpoints, a nitrile moiety with abnormal lipid metabolism in the liver, a triazole ring with liver injury/cell death in rat 90-day RDT tests, and a pyrazole ring with abnormal lipid metabolism in the liver, and a triazole ring with fatty changes in the liver in rat 2-year RDT tests. Furthermore, we constructed a category for the compounds showing toxic effects in central nervous system (CNS), based on both the physicochemical properties important for the permeability to blood brain barrier and reaction mechanisms for the interaction with macromolecules leading to CNS toxicity.

We also constructed quantitative structure-activity relationship (QSAR) models that predict hepatotoxicity from chemical structure information using the databases including HESS. In the classification models of hepatocellular hypertrophy using three kinds of machine learning methods (deep learning, random forest, support vector machine), the support vector machine using HESS as a training set showed the best prediction performance with prediction accuracy of 0.76 and sensitivity of 0.90. Moreover, the results suggest that it is important to use a consensus model that combines the prediction results of multiple models for chemical safety evaluation.

Finally, to understand the role of drug metabolism in the toxicity of chemical compounds, we assessed the reactivity to cytochrome P450s (P450s) of chemical compounds in vitro and investigated the relationship between the reactivity and in vivo toxicity using the pesticides' RDT database. As the results, we found statistically significant relationships between the reactivity to certain P450 enzymes and some RDT endpoints, such as increases in γ-GTP, in liver relative weight, in liver absolute weight, and in thyroid relative weight, and decreases in blood total protein and in blood globulin level, suggesting a possible involvement of reactions with P450s in these toxic endpoints.

In conclusion, we have expanded the HESS database by use of risk assessment reports from Food Safety Commission and demonstrated the usefulness of those toxicity test data and importance of the construction of toxicity databases, through the various types of analyses and investigations described above using the datasets collected. We believe that these results are useful and helpful to establish a novel system(s) based on in silico methods using those toxicity databases for the assessment of influences of chemical compounds on food safety.