A Challenge to the Ultimate 3R's – *In Silico* Approach to Evaluate Chemical Safety for Humans

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Summary

Safety assessment of chemicals is one of the most important issues in regulatory science. Until recently, at least in Japan, safety has been addressed on a hazard basis for industrial chemicals. The toxicity of chemicals has been evaluated, for the most part, by using rodents and then extrapolating to humans. Because of animal welfare concerns as well as the requirement for high-throughput, in vitro, omics, and in silico systems have been developed and introduced. Our ultimate goal is the assessment of safety for humans. Among the many assay systems, only an in silico system can focus directly on human beings. No single (Q)SAR can do this task as a standalone. A combination of models, however, revealed an acceptable performance in evaluating the mutagenicity of chemicals because of the relative simplicity of correlating chemical structure with mutagenicity. General toxicity, however, is not easy to evaluate because there are many factors that affect the results of toxicity testing. No single SAR or (Q)SAR can evaluate the general toxicity of chemicals. Our project, sponsored by NEDO/METI, aims to carry out safety assessment of chemicals evaluated in silico. The final goal is to assist toxicology experts in making accurate and efficient safety assessments of chemicals for humans. We constructed databases, not only using the results of animal repeated dose tests but also including the metabolism of chemicals and the mechanisms of toxicity of the chemical. We are constructing a platform to integrate these databases that also will include the categories of chemicals that can be assessed, based not only on structure but also on activities. We also plan to build a Bayesian network incorporating experts' knowledge.

Keywords: in silico, chemical safety assessment, genotoxicity, repeated dose toxicity, database

1 Introduction

For the high-throughput safety assessment of chemicals, we have developed (Q)SAR models, especially for genotoxicity, carcinogenicity, and skin irritation as a means of hazard identification. In this field, the (Q)SAR is now used more or less successfully, though many limitations remain. The (Q)SAR for the repeated dose general toxicity of chemicals was not developed until recently because so many factors affect hazard identification. We now have a high quality and expanded database of repeated dose toxicity. The development of computer science is dramatic, and now we have a strong tool for safety assessment, i.e., *in silico* safety assessment, computing toxicology, etc. One of the most important tasks is to construct a good database with the flexibility to apply it to any models and, more importantly, using high quality data from repeated dose toxicity tests. To achieve usable *in silico* systems to evaluate the general toxicity of chemicals, we must have strategies regarding not only what information should be incorporated as part of a good database but also how to use the accumulated information. We aim to evaluate chemical safety by extrapolation from the information based on data of the test results on related chemicals. Moreover, it is important to explain the mechanisms of how the identified toxicity occurs. For this purpose, we need more information on Absorption, Distribution, Metabolism, and Excretion (ADME) for the target chemical.

We have used animal studies as a gold standard to assess chemical safety for human risk. However, we have discovered many hurdles that need to be overcome:

1. Species difference is an important factor for extrapolating from test data using rodents to primarily human risk assessment. Metabolism may play the most important role in determining species differences in the safety of chemicals.
2. Dose setting for animal tests is another important factor in the extrapolation from experimental dose levels to human exposure levels. For example, in the case of industrial and agricultural chemicals, people are exposed through the environment, usually at low dose levels. Sometimes we tested up to extremely high dose levels, and it is questionable whether the findings at such high doses can be extrapolated to actual exposure levels for humans. For human risk assessment, this issue should be discussed more globally.
3. Another hurdle concerns how to evaluate complex mixtures and to understand the target of interest. This is an old but still relevant issue to consider for human risk assessment.

The threshold of genotoxicity and the genotoxic carcinogenicity of chemicals is one of the most difficult issues we face.

4. We also have to consider the target of risk assessment, i.e., the population or individual. Certainly there are high risk groups. Furthermore, the type of chemical makes a difference as, for example, pharmaceutical drugs generally are consumed by individuals, whereas industrial chemicals and food-related chemicals have a more widespread impact, making it more difficult to consider individual responses.
5. Finally, to the extent we consider animal studies the gold standard for human risk assessment, animal welfare becomes the essential concern.

As mentioned above, many hurdles have yet to be overcome for human risk assessment. The first step is accurate and efficient hazard identification. Here, I will discuss *in silico* evaluation for mutagenicity and repeated dose general toxicity as an acceptable method to clear at least several of these hurdles.

2 Mutagenicity

The Salmonella/microsome mutation assay (Ames assay) and *in vitro* mammalian cell chromosomal aberration assay are used almost exclusively to evaluate chemical mutagenicity. This project was funded by the Ministry of Health, Labour and Welfare (MHLW), Japan, and the project leader was Makoto Hayashi, National Institute of Health Sciences (NIHS). The NIHS members of this project were E. Kamata, M. Honma, A. Hirose, et al.; Lhasa Ltd MultiCASE Incorporated also contributed.

96% of Ames assay positive chemicals, more than half of which were epoxide, had a molecular weight of less than 3000. We used this condition as a pre-filter for *in silico* evaluation of the genotoxicity of chemicals. Figure 1a,b shows the performance of a combination of three models using two kinds of decision tree, i.e., combination 1 and combination 2 (Hayashi et al., 2005). This combination approach also can be applied to the *in vitro* chromosomal aberration assay. For combination 1, we judge chromosomal aberration positive by *in silico* only when two or more models are evaluated as positive. When two or more models have negative results, the final judgment is negative. The sensitivity was 70.8%, specificity 81.8%, and total concordance 76.6% compared to the actual *in vitro* chromosomal aberration assay. The performance is somewhat higher than for an individual model, but the performance of chromosomal aberration prediction is still lower than that of the bacterial mutagenicity assay. Also, the applicability was 10% lower than for the Ames combination 1. For combination 2, we judge chromosomal aberration positive by *in silico* only when all three models are declared positive and negative when all three models have a negative outcome. The sensitivity was 88.6%, specificity 93.2%, and total concordance 91.1%.

Good concordance was obtained when several models were combined. Generally, combination 1 showed less concordance but more chemicals could be evaluated, while combination 2 could evaluate fewer chemicals but showed higher concordance. Selection of the decision tree depends on the purpose of the *in silico* evaluation.

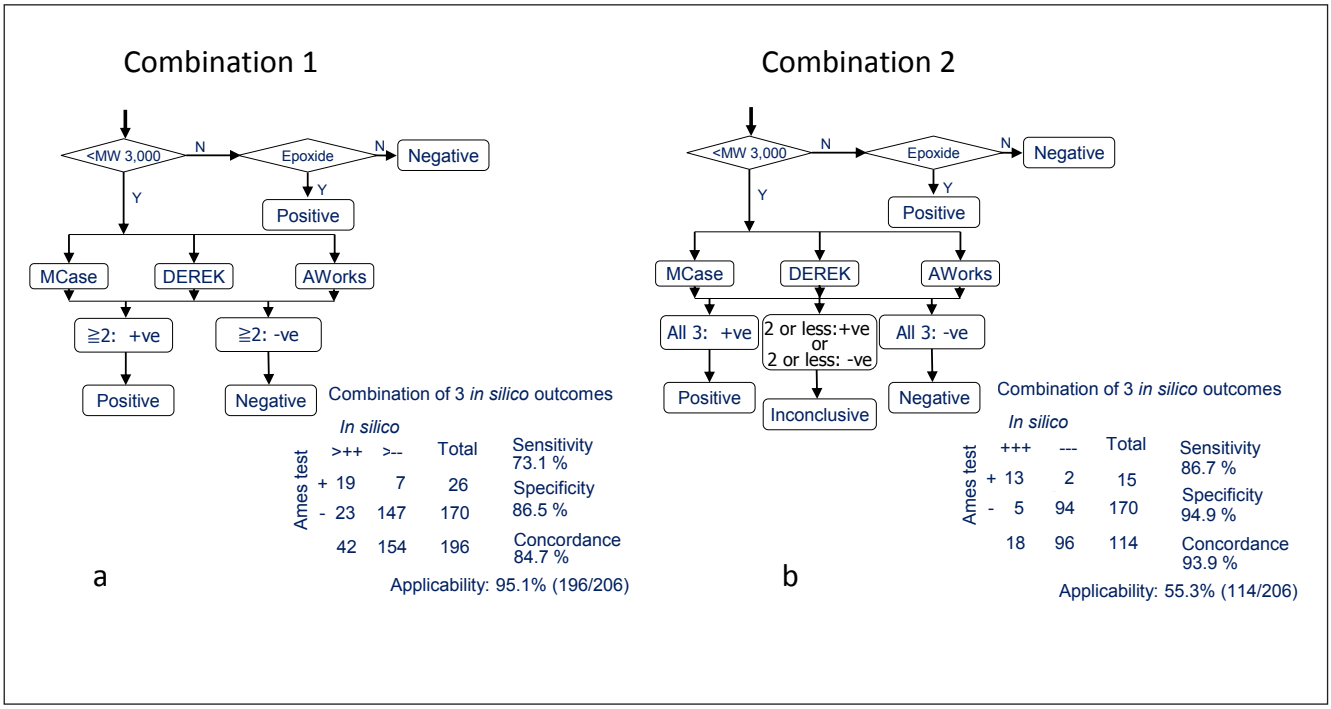

Fig. 1: Decision trees to evaluate bacterial mutagenicity by the combination of (Q)SAR models and their performance

3 Hazard Evaluation Support System (HESS) Integrated Platform

3.1 Rodent repeated dose toxicity

We initiated the project to construct "Hazard Evaluation Support System (HESS) Integrated Platform" under the auspices of the New Energy and Industrial Technology Development Organization (NEDO) and the Ministry of Economy, Trade and Industry (METI). Table 1 shows the organization and main members. HESS is designed to support the toxicologist/assessor for efficient and accurate assessment of chemicals based on knowledge of the chemicals with similar structures and/or activities (Hayashi and Sakuratani, 2011). This system is designed to support expert judgment and not to judge by itself. The system has been developed primarily by toxicologists, pathologists, and risk assessors, with substantial support from IT people. The system is designed to be fully compatible with the OECD (Q)SAR Application Toolbox. To realize this, the main part of the platform was designed by LMC, Bourgas University, where the Application Toolbox was developed.

Figure 2 shows the outline of the platform. The main database is the repeated dose toxicity test report database, and the majority of data are from 28-day repeated dose toxicity (RDT) GLP studies on existing chemicals in Japan, under the auspices of MHLW/NIHS. We also are constructing the database of toxicological mechanisms/Adverse Outcomes Pathway. We understand the importance of metabolism, which may be a key factor in determining species differences. We also are developing a rat metabolism map database and metabolism simulator, as well as a human/rat ADME database. Our final aim is to make the platform support expert judgment for as-

Tab. 1: Organization of the HESS project

Sponsor:
New Energy and Industrial Technology Development Organization (NEDO)/Ministry of Economy, Trade and Industry (METI)
Project Leader:
M. Hayashi BioSafety Research Center, Foods, Drugs and Pesticides
Members:
National Institute of Health Sciences (NIHS) *A. Hirose, M. Honma, M. Yoshida, A. Kamata, M. Sunouchi*
National Institute of Technology and Evaluation (NITE) *Y. Sakuratani, J. Yamada, T. Yamada, A. Maekawa, T. Abe, S. Nishikawa, K. Kobayashi, HQ. Zhang, T. Tanaka, R. Hasegawa*
Fujitsu Limited *T. Yamashita, K. Sakai*
Bourges "Prof. Assen Zlatarov" Univ. *Prof. O. Mekenyan, Prof. S. Dimitrov*
Tohoku University *Prof. Y. Yamazoe, Prof. K. Yoshinari*
Kwansei Gakuin University *Prof. T. Okada*

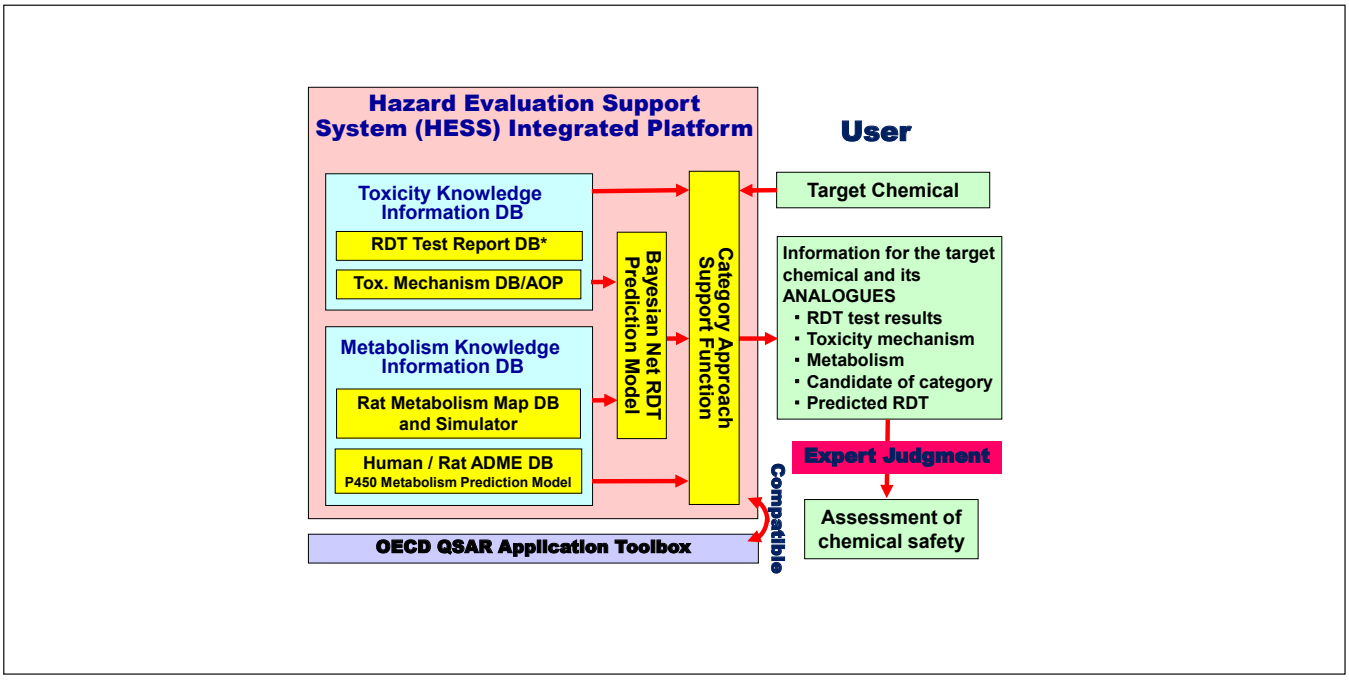

Fig. 2: The outline of the Hazard Evaluation Support System (HESS) Integrated Platform

sessment of chemical safety for humans, and therefore we are accumulating information on the metabolism of both humans and rodents. Figure 3 shows the status of the database as of March, 2011.

3.2 Toxicity knowledge information database

Repeated dose toxicity test report database

We collected mainly GLP test data on 28-day repeated dose toxicity using rats. The database contains data, possibly numerical data, on hematological examination, blood biochemical examination, histopathological examination, etc. To standardize the histopathological findings, we will construct a thesaurus for 4300 findings in 56 organs (Nishikawa et al., 2010).

RDT Test report DB

Total 500 chemicals (526 reports)
- **OECD TG407 (Japanese GLP Test Data): 169 reports**
- **OECD TG422 (Japanese GLP Test Data): 145 reports**
- **Other (USA NTP Studies, Journal papers, etc): 212 reports**

***Thesaurus for 4300 histopathological findings in 56 organs was defined.**

Toxicity Mechanism DB

Mechanism information for the critical toxicity observed in RDT: 117 chemicals.

Rat Metabolism Map DB

Metabolism maps of chemicals mainly in the rat liver: 675 chemicals (420 maps).

Human / Rat ADME DB

ADME information for discussing the species differences in toxicity: 60 chemicals.

Fig. 3: The states of each database in the HESS (March, 2011)

3.3 Toxicity mechanisms database

Mechanism information for critical toxicity such as the necrosis of hepatocytes observed in the repeated dose test was collected on about 130 chemicals from 150 references. The outline of the database is shown in Figure 4 and an example in Figure 5.

3.4 Metabolism knowledge information database

Rat metabolism map database and simulator

The database of the metabolism map, primarily in the rat liver, was constructed with more than 600 maps on 400 chemicals. The metabolism simulator was based on the metabolism of rat liver microsome.

<Mechanism Information>
- Toxicity
- Possible Chemistry Reaction /Metabolism
- Possible Toxicant
- Possible Interaction with Target Molecule
- Possible Effects
- Target Organ/Tissue/Cell etc.

<Mechanism Summary>
- Possible Mechanism Summary

<Experimental Information>
- Species
- Experimental Design
- *in vitro / in vivo / ex vivo*
- Dose / Concentration Employed
- Effective Dose / Concentration

<Other information>
- Other Compounds studied
- Additional Information
- Authors' Suggestion

Fig. 4: Toxicity mechanism database

Mechanism information for the critical toxicity such as necrosis of hepatocytes observed in RDT was built in (about 130 chemicals, 150 references)

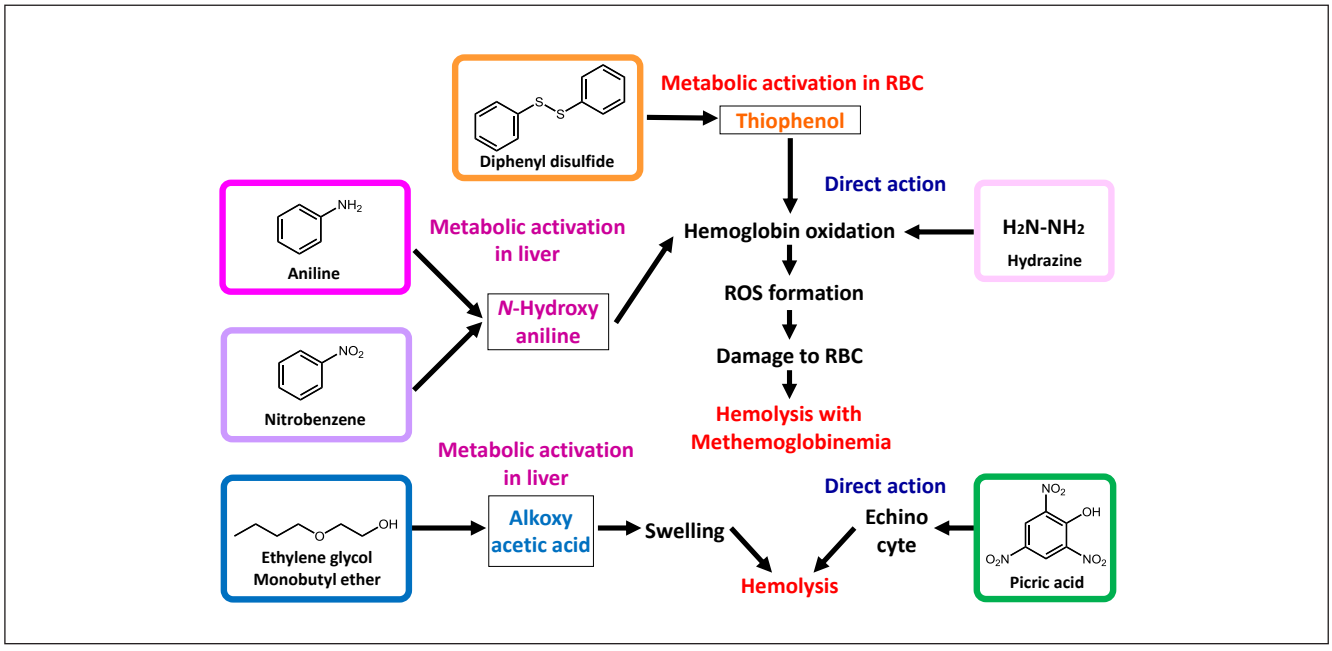

Fig. 5: Mechanism-based categories of hemolytic chemicals

54 hemolytic chemicals were analyzed for their mechanism to induce hemolysis, which is one of the major targets of industrial chemicals

Human/rat ADME database

The ADME is important for evaluating the safety of chemicals. This information is essential for the extrapolation of data obtained from rodent repeated dose tests to the actual human responses. The data in the database are absorption rate, C_{max}, T_{max}, apparent volume of distribution, organs with higher concentration of chemicals than blood, involvement of transporter, related enzyme and molecular information, metabolite species differences, excretion rate, etc. on about 60 chemicals taken from 130 references.

The P450 metabolism prediction model based on ligand structure has been developed. Steps to CYP-based prediction are as follows: 1) To find an initial step of biological reaction for detoxification, although CYP reactions are the most common rate limiting-process, 2) chemical properties such as log P, molecular size, specific functional groups (like sulfonic acids), and instability in aqueous solution often limit the availability of CYP for the metabolism, and 3) logP range (0.7-3) are preferred to CYPs. The templates of 6 CYPs have been established already, and another 6 CYPs are under construction. These cover major human CYPs involved in the metabolism of xenobiotics. The model using these templates can predict not only metabolites but also their order of likelihood.

3.5 Bayesian net repeated dose toxicity prediction model

One important characteristic of the HESS model is to incorporate knowledge and experience from toxicologists who are working in the field of regulatory science. To realize this, we incorporated the module of basic active structure knowledge base through analysis by the Cascade model followed by a Bayesian net prediction model that is supported by the knowledge of specialists, as well as data from our database, i.e., the toxicity knowledge database and the metabolism knowledge database.

3.6 Categorization of chemicals for repeated dose toxicity based on adverse outcome pathways (AOP)

We believe that the categorical approach is the only possible method to evaluate repeated dose toxicity of chemicals *in silico* (Sakuratani et al., 2008). Chemical structure is not enough to make a category, and it is essential to include the similarity of effect(s) that should be supported by the mechanism(s) and metabolism when the chemical is absorbed into the body. Therefore, we have made a high quality database that includes not only findings from the repeated dose toxicity tests but also metabolism, including human metabolism, of xenobiotic chemicals, as well as mechanisms to explain toxicity, together with structure and other information on the physical chemistry of chemicals.

Based on all this information, we constructed several AOP's. Figure 6 shows an example of an AOP for nitrobenzene. Starting from available evidence of the target chemical at hand, we may reach the known pathway through possible mechanisms. Then we can estimate (or even evaluate) the toxicity of the

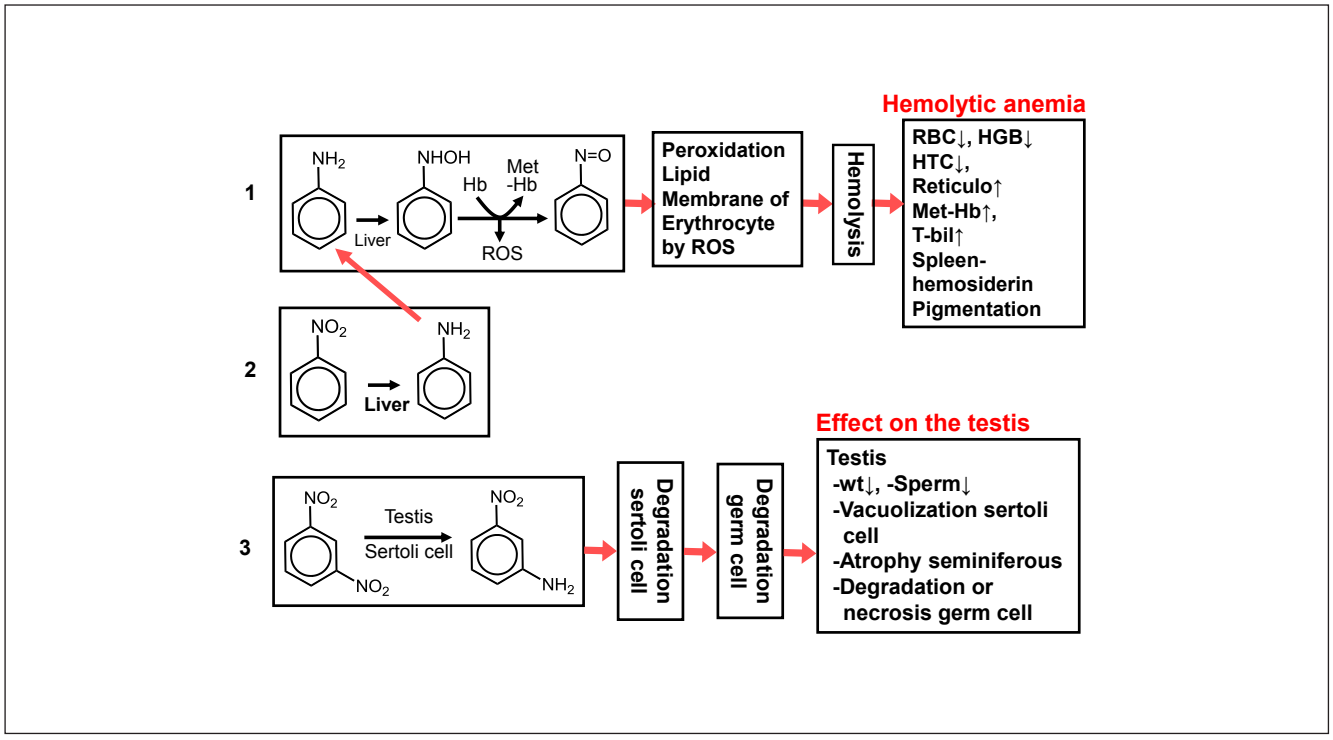

Fig. 6: Example of AOP on nitrobenzene inducing hemolytic anemia and adverse effect on the testis

target chemical with high accuracy (Fig. 7). Such information is sure to help experts in the field judge chemical safety.

3.7 OECD (Q)SAR application toolbox and HESS

As mentioned earlier, we designed our databases and platform to be compatible, and we provide data and other information. An RDT report includes various findings (e.g., histopathological findings, blood test data). To make categories, it is necessary to classify those findings accurately. Therefore, it is necessary to incorporate not only NO(A)EL and LO(A)EL but also observed findings and their dose level information into the Application Toolbox. Also, laboratories use various terms for the histopathological findings, and so it is necessary to develop a thesaurus for the histopathological findings to avoid confusion. The thesaurus will be incorporated into the Toolbox.

4 Conclusion

Currently, it is premature to make a safety assessment of chemicals without animal studies. Therefore, *in silico* systems should be used to make such assessments. For example, *in silico* systems can be used in some specific fields and also for prioritization of chemical selection for tests using animals. For example, the field of chemical mutagenicity already looks quite promising for the elimination of very strong mutagens but not for possible mutagens. In this field, we desire negative evaluation (prediction) models in addition to positive prediction of chemical mutagenicity. At the moment, no standalone SAR/QSAR can evaluate repeated dose toxicity. To achieve the evaluation of repeated dose toxicity by *in silico* systems, it is essential that metabolism and mechanism information be incorporated. Categorical approaches, including mechanistic and Adverse-Outcome-Pathway approaches, are the only way to realize *in silico* evaluation of the general toxicity of chemicals.

Lastly, I would like to emphasize that the *in silico* evaluation is high-throughput, and it covers all 3R's. Moreover, only an *in silico* approach can hope to evaluate chemical safety for humans directly.

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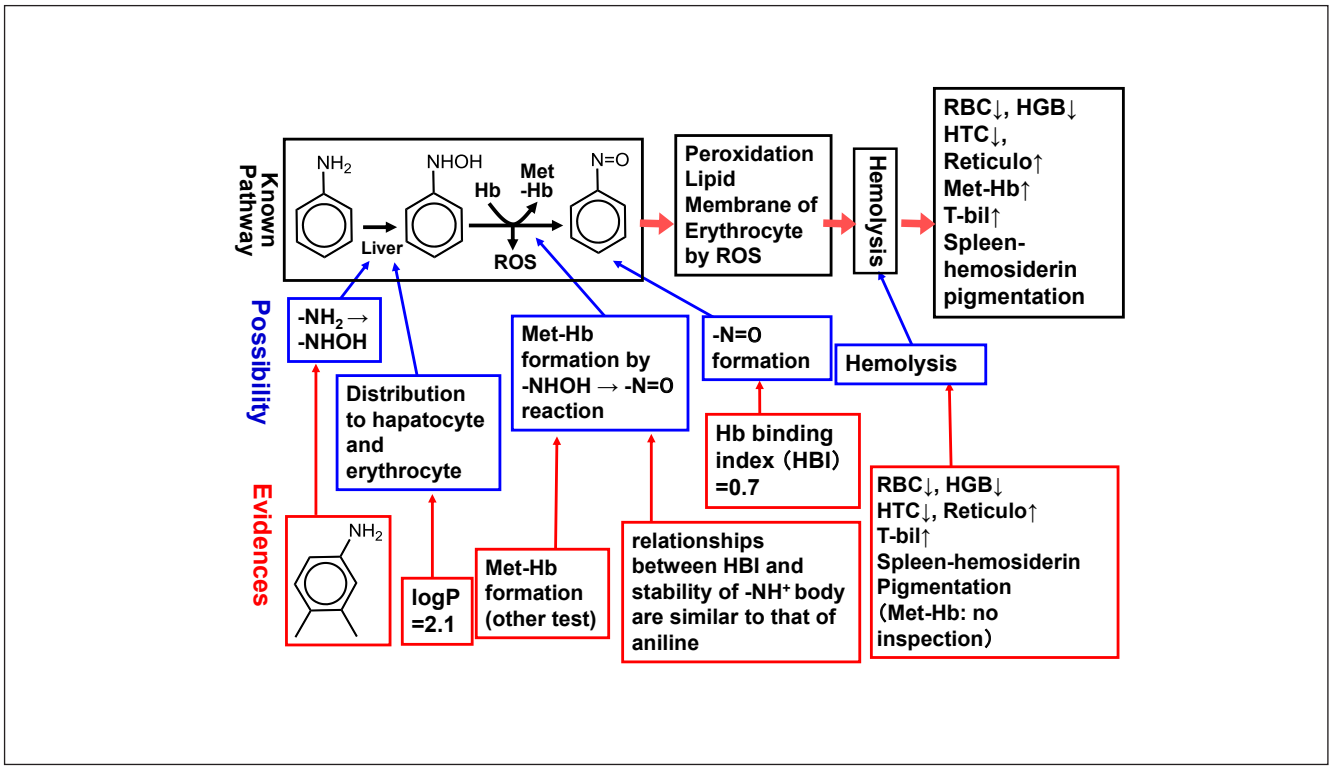

Fig. 7: Estimation of AOP by evidence that was observed on 3,4-dimethyl aniline

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