EFFORTS IN CHEMICAL INDUSTRIES

SAFETY ASSESSMENT IN THE RESEARCH AND DEVELOPMENT OF NEW CHEMICALS

Junshi MIYAMOTO

Takarazuka Research Center Sumitomo Chemical Co., Ltd.
4-2-1, Takatsukasa, Takarazuka, Hyogo, Japan

Abstract.....A variety of testings to be incorporated in the research and development program of new chemicals to assess their fates and effects onto man and his environment should be so designed as to duly furnish reliable preliminary information. A rapid in vivo assay for animal carcinogenicity and a model ecosystem for bioaccumulation in aquatic organisms are discussed as examples of these brief testings, while further research needs are mentioned to ultimately assess the impacts of chemicals to the whole environment.

The chemical substance is one of the indispensable components in modern way of life, and more than 60,000 of various types of chemicals are actually used including pharmaceuticals, pesticides, food additives, finishing resins, dyestuffs, plastics and synthetic fibers. Since they are used for betterment of human life, it is quite obvious that they should not endanger humans and their environment through production, use and disposal. And in this respect owing to tremendous progress during recent decades in the area of toxicology and environmental health sciences the detailed procedures to clarify toxicological properties and environmental impacts of chemicals are established, and as a result their possible adverse effects will be largely predictable in many cases. In other words it is now possible to discard those chemicals with no tolerated safety features. Thus for example in the field of pesticide research where the present author has some experience and expertise, a voluminous pile of documents containing every toxicology information guaranteeing safety should be submitted for the registration, with 44 binders of papers weighing 268 kg in a certain case. Thus, the more efficacious and at the same time the less harmful pesticidal products are being eagerly pursued.

It is widely recognized at present that one out of twenty to thirty thousand candidate compounds will be successfully commercialized as pesticide with several ten

million US dollars necessitating eight to ten years of endeavours. Therefore, in the research group of pesticide industries in the world the efficacy testing programs are inextricably combined with another screening program attempting to clarify the toxicological properties of the materials in a hope that through these testings the decision will be made whether the compound in question may be forwarded to the more advanced stages, or whether the original direction of development should be modified or the compound abandoned due to certain unfavorable properties (Fig. 1). Since no extensive and expensive testings like *in vivo* carcinogenicity study or radiotracer metabolism study can be made during early stages of research and development, the testing procedures for every toxicity profile should preferably be well simplified to be incorporated during the respective screening steps, and by which a fairly large number of compounds can be tested. The deepest consideration to be taken into account in these whole toxicological and environmental program will be to select which combinations of testings at which stages, otherwise aside from time and resources spent unefficiently, the toxicity testing may erroneously kill the promising compound, or the hazardous compound may be escaped to reach the next development stage.

The situation underlying development of new general industrial chemicals will be similar, in that safety should anyway be assessed before their extensive production and use. However, every test procedure during early development stages should better be far simpler and less expensive, as compared with pesticides. Thus OECD set up so-called MPD as a preliminary battery system for assessing safety of new chemicals onto man and his environment.

Briefly the MPD is composed of basic physico-chemical properties as well as several rather simple mammalian and eco-toxicity testings. These data will be effectively utilized to predict the fates and effects of the compound in a variety of eco-compartments, if combined with detailed and comprehensive data base already available for similar or analogous compounds.

It should be admitted however that there are ample gaps at present between these simple testings and detailed test procedures. Also sometimes undue extrapolation of simple test results may lead to erroneous conclusion with respect to the eventual effects of chemicals. Thus those scientists engaged in the toxicity program are always most keen in elaborating the better and simpler testing procedures to be used for bridging the gaps, and to be correlated with the more laborious testings currently required by worldwide regulatory agencies. Although the present state-of-the-arts are not necessarily well advanced to propose any testing procedure of these kinds, every effort should be made to develop more rational procedure to be conducted more easily. In the paragraphs that follow, a few representative examples of these efforts will be given in the area of (1) *in vivo* short-term assay for carcinogenicity, and (2) aquatic systems for subacute toxic effects and bioconcentration. They will moreover hopefully constitute subsequent test procedures next to simplest battery like MPD.

Figure 1 Development of a New Chemical (in a Biological Field)

A number of short-term assays, mostly mutagenicity tests, have hitherto been used to assess carcinogenicity of chemicals because of their relative simplicity. Actually some of these test procedures are proposed to have increased correlation with rodent in vivo assays. However based on the critical evaluation of these cumulative data, the correlation appears not to be so high, as shown in Table 1 (Yamazaki 1983).

Table 1 Correlation between Animal Carcinogenicity and Short-term Tests^{a)}

Animal Carcinogenicity ^{b)}	Short-term Tests ^{b)}
+	+ 28
	± 7
	— 21
±	+ 13
	± 4
	— 21
—	+ 5
	± 3
	— 18

a) Compiled from data by H. Yamazaki ; "Toxicology Forum"
6 (2) 170 (1983)

b) + ; Sufficient evidence

± ; Limited evidence

— ; Inadequate evidence (definition based on IARC)

Sometimes the Ames assay gives false positive result for certain reasons including microbial nitroreductase activity (Table 2, Kogiso et al. 1986), while as shown in Table 3, different mutagenicity assays give discrepant results due to inherent nature of test procedures (Yoshitake et al. 1986). It is the opinion of several researchers including the present author that the apparent discrepancy found in Table 1 is derived from the very characteristics of these short-term assays originally intended to investigate mutagenicity, thus difficult to judge potential for carcinogenicity, since the mechanism leading to carcinogenesis is very complicated and has not been fully clarified yet. Therefore although these shortterm assays will be useful in obtaining preliminary insight into carcinogenicity of compounds, they should not be regarded a priori as reliable simple methods to replace long-term rodent studies.

Another simplified assay system for carcinogenicity is proposed by Ogiso et al. (1985). Briefly the rat is given 200 mg/kg ip of diethylnitrosamine (DEN), as shown in Fig. 2, and 2 weeks thereafter the compound to be tested is given for 6 consecutive weeks, during which partial hepatectomy is carried out. Eight weeks after initiation of DEN treatment the liver is dissected out, and glutathione S-transferase (placental

Table 2 Mutagenicity & Carcinogenicity of Some Nitro-compounds

		1-Nitro-naphthalene	2-Nitro-naphthalene	Fenitro-thion
Carcinogenicity		—	+	—
Mutagenicity				
TA 100,	— S 9 mix	+	+	—
	+ S 9 mix	+	+	+
TA 100NR*	— S 9 mix	—	—	—
	+ S 9 mix	—	+	—

* nitroreductase deficient

Table 3 Mutagenic Potentials of Miloxacin and Nalidixic Acid

System	Mutagenicity	
	Miloxacin	Nalidixic Acid
Rec-assay	+	+
Ames test (TA102)	+	+
DNA gyrase (Bacteria)	+*	+*
Micronucleus test	—	—
Dominant lethal test	—	—
Topoisomerase-II (Eukaryote)	—*	—*

+* : Inhibition

—* : No inhibition

Miloxacin

Nalidixic acid

Fig. 2 In-vivo Short-term Assay for Hepatocarcinogenesis

type) positive foci are determined. Table 4 shows results of the assay. Out of 30 known hepatocarcinogens 27 give positive results, with 29 non-carcinogens giving negative response, regardless of their mutagenic potential (Ito, 1986). Thus the correlation with the long-term animal study is quite high. One may argue against very limited (single) organ specificity of the test, but it appears this system is much more desirable as a screening test for carcinogenicity than other mutagenicity-based short-term assays, since the testing reflects early in vivo histological changes in the process of carcinogenesis, and moreover that the correlation is quite satisfactory. And it is hoped that similar in vivo short-term assay systems with multi-organ responses will be to be elaborated.

Table 4 Results of In Vivo Short-term Assay for Hepatocarcinogen

Compound	Mutagenicity	
	+	-
Hepatocarcinogen	9/10 (90%)*	18/20 (90%)** ; 27/30 (90%)
Non-carcinogen	0/6 (—)	0/23 (—) ; 0/29 (—)

* o-toluidine

** chlofibrate, di (2-ethylhexyl) phthalate

Although elucidation of environmental impact sometimes requires much more efforts than those stipulated in the present regulations, the present state of the arts is already sufficient to detect by detailed examination some adverse effects of chemicals, such as persistency and chronic toxic effects of DDT and PCB. Therefore if a few considerations and trials are added to the simple routine testings, much more useful information will be forthcoming in some cases. For example, when additional parameters are incorporated in a bioaccumulation testing with dynamic flow system, subacute toxic effects will be detected more clearly, e.g. with soft X-ray scanning skeletal curvature by diazinon, and with histopathological examination anemia by molinate.

Bioaccumulation of a compound is largely dependent on its Pow, and the dynamic flow system is a useful tool in demonstrating bioaccumulation potential. However, because of too simple nature neglecting various environmental circumstances, it often gives artificially high BCF. DDT and fenvalerate both are intrincically highly lipophilic. Metabolism of DDT in aquatic organisms leads to formation of another lipophilic DDE, whereas fenvalerate is subject to extensive metabolic attack, forming polar, biodegradable compounds. As a result when these two are tested in a simple aquatic model ecosystem composed of algae, daphnids, snails and fish with soil sediment, fenvalerate proves to be least bioaccumulative, different from DDT (Fig. 3, Table 5, Ohkawa et al. 1980).

Table 5 Bioaccumulation Ratio of Fenvalerate and DDT in aquatic model eco-system

	Bioaccumulation ratio			
	Fenvalerate	DDT	DDE	DDT-R*
Carp	122	1330	155000	2690
Daphnia	683	2550	—	833
Snail	617	2670	23700	4530
Algae	477	5420	4000	3690

* DDT-R ; sum of DDT, DDE and DDD

Thus the above examples illustrate that during research and development where a large number of candidate compounds will be tested by as simple procedures as possible, with significant results (more reliable information with less expenditure), the current testing schemes are not necessarily well formulated. Therefore, the following research needs will be stressed to get more accurate relevant information and to have sounder assessment.

Fig. 3 An aquatic Model Ecosystem

1. Validated mammalian metabolism and toxicity testings in relation to extrapolation to man
2. Simplified short-term assays for long-term studies including mutagenicity, carcinogenicity, teratogenicity and neurotoxicity
3. Integrated laboratory and field studies on environmental fate of chemicals and their mathematical modelling
4. Selected test species for environmental effects of chemicals and their incorporation to model ecosystems
5. Computer analysis of metabolic sites for chemicals, as well as QSAR studies for biotic and abiotic degradations
6. Refined environmental monitoring and surveillance systems for adverse effects of chemicals
7. Computer-assisted quantitative risk assessment including more acceptable VSD determination
8. Presumed biproduct formation mechanism by abnormal, uncontrollable reaction processes

In the course of these investigations it is evident that international cooperation will be essential in a way similar to pesticide problems which are being pursued jointly through JMPR and CCPR, including recently issued "Code of Conduct" to deal with pesticide matters in developing countries. It is also borne in mind that chemical industry responsible for marketing new materials should play a key role in these undertakings in collaboration with university and government institutions.

REFERENCE

- Ito, N. (1986) : Private Communication.
- Kogiso, M., Yoshitake, A., and Miyamoto, J. (1986) : Unpublished Observation.
- Ogiso, T., Tatematsu, M., Tamano, S., and et al. (1985) : Comparative Effects of Carcinogens on the Induction of Placental Glutathione S-transferase-Positive Liver Nodules in a Short-term Assay and of Hepatocellular Carcinomas in a Long-term Assay. *Toxicol. Pathol.*, **13**, 257.
- Ohkawa, H., Kikuchi, R., and Miyamoto, J. (1980) : Bioaccumulation and Biodegradation of the (S)-isomer of Fenvalerate in an Aquatic Model Ecosystem. *J. Pestic. Sci.*, **5**, 11. For detail of model ecosystems, see ; Miyamoto, J., Klein, W., Takimoto, Y. and T. R. Roberts (1985) : Critical Evaluation of Model Ecosystems. *Pure and Applied Chemistry*, **57**, 1523.
- Yoshitake, A., Kogiso, M. and Miyamoto, J (1986) : Unpublished Observation.
- Yamazaki, H. (1983) : Assessment of Environmental Chemicals for Carcinogenicity to Man. *Toxicology Forum*, **6**(2) 170 (in Japanese).