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Concepts and Procedures in Ecotoxicology¹⁾

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A multitude of non-biological substances are being produced at present in an attempt to provide a steadily increasing world population with better living conditions. This industrial activity causes measurable changes in the material quality of the environment. A comprehensive and thorough assessment of the effects of all the chemical compounds which are found widespread in the environment is not possible because of the overwhelming number of such products. In order to detect any undesired effects upon the natural environment as early as possible, it is essential to investigate representative model substances which are suitable for such studies because of their production levels, areas of application, persistence, distribution behaviour, degradability and expected biological activity.

These topics are also the bases for the present world-wide activities as regards environmental chemicals laws. Since a large number of single factors determine the behaviour of chemicals in the environment, it is necessary to use step-wise elucidations for legal purposes. This is achieved by estimating the ecotoxicological behaviour by physico-chemical characteristics and upon feasible test procedures.

At present, globally about 50000 chemicals are produced and used with an integral production level of about 150 million tons annually. Realizing this it is evident that a well structured assessment of their ecotoxicological potential is needed according to appropriate criteria. These are for individual chemicals: production level, use pattern, dispersion tendency, persistence, biotic and abiotic conversion, and biological activity.

In principle, there are different approaches for assessment:

Monitoring, Occurrence and Effects in Ecosystems

This results in real data both for occurrence and changing the physical environment as well as populations of flora and fauna but gives no immediate prospective information.

Assessment of Trends in Occurrence and Effects

(Environmental specimen banking and long-term monitoring)

This approach allows by backward analyses of stored samples estimation of trends in the occurrence of a chemical. Its correlation with long-term ecological monitoring allows for prospective evaluation.

Simulation of the Environment

Simulation experiments give rather good data for the estimation of the fate of chemicals in the environment. With respect to effects on organisms they are limited except the most sensitive indicator organisms or indicatory effects are available.

Screening in Simple Test Procedures¹⁾

This gives indications on the potential for occurrence and effects and should be used mainly on

a comparative basis.

According to the potential of a chemical for ecotoxicological effects derived from production and fate (exposure probability), the approaches have to be selected as appropriate.

Keywords—concepts hazard assessment ; ecol. hazard profile ; accumulation ; degradation ; fish ; algae ; activated sludge ; rats ; photomineralization

Introduction

It took more than one decade to convince all involved parties—government officials, politicians, chemical manufacturers, and the public as a whole as well as even experts in some scientific disciplines—that the increasing use of organic chemicals calls for concern with respect to the long-term effects on environmental quality. Today, there are only a few local examples of serious harm to man or the environment caused by chemicals after application. On the other hand it has to be realized that chemical substances when once dispersed regionally or even globally probably can no more be eliminated in a short period of time from the biosphere. Therefore, with environmental chemicals there is an urgent need to take measures for prospective environmental protection. Thus, it is most appreciated that some countries—including Japan and the U. S. A.—have already

legal regulations aiming in this prospective protection, and that all industrialized countries within the OECD are in the process of taking similar actions. The European Communities have adopted a directive on the frame work for national regulations.

To demonstrate the necessity of an inventory type approach as mentioned above, Table I gives production figures of chemicals. In order to judge how realistic estimates for the future will be it is worthwhile to compare present-day figures with data from 10 or 20 years ago. You see that in 1950 the world production of organic chemicals totalled only 7 million tons. It had grown to 63 million tons by 1970 and the estimate for 1985 is 250 million tons. It seems that despite oil crisis and reduced rate of the annual growth of chemical industry the estimate seems to be realistic.²⁾

For bulk material, Table II shows the

TABLE I. Organic Chemicals—World Production and Release of Organic Compounds in the Environment, 1970

a) World Production

	1950	1970	1985	Release in Env. 1970
Grand total (10 ⁶ t)	7	63	250	20

b) Release in the Environment

Manufactured	(10 ⁶ t)	Natural sources	(10 ⁶ t)
Solvents	10	Methane	1600
Detergents	1.5		
Pesticides	1	Terpene type hydrocarbons	170
Gaseous base chemicals	1		
Miscellaneous	7	Lubricating and industrial oils	2—5

TABLE II. Production of Plastics in Japan

(in 1978/tons)

Thermo Plastics		Thermo Setting	
Polyethylene	1766572	Phenolic resin	267514
Polystyrene	1031770	Urea resin	581746
Polypropylene	744662	Melamine resin	124799
Polybutene	24929	Unsaturated polyester resin	184045
Petroleum resin	66886	Alkyd resin	120120
PVC	1204274	Epoxy resin	47939
Polyvinyl alcohol	106729	Silicone	25587
PVDC	29786	Polyurethane foam	168675
PNMA	95656		
Polyamide	54956	Sub-Total	1526425
Polycarbonate	27061		
Others	68643	Grand Total	6748349
Sub-Total	5221924		

TABLE III. Estimate of Total Additives used for Plastics in Japan (in 1978/tons)

Stabilizers		
15150	Antioxidants (incl. synergist)	8600 tons
	Light stabilizers	1050 tons
	PVC stabilizers	5500 tons
Filler		
22500	TiO ₂	8500 tons
	Talk	6000 tons
	CaCO ₃	8000 tons
Pigments		
15200	Organic	1200 tons
	Inorganic	1000 tons
	Carbon black	13000 tons
Nucleating agents		
90		
Plasticizers		
320633	Phtalic acid ester	275400 tons
	TCP	5047 tons
	Aliphatic	1405 tons
	DOA	19626 tons
	Epoxy	11999 tons
	Polyester	7143 tons
Flame retardants for TV		
2000	Organic	1500 tons
	Inorganic	500 tons
Total : 375073 tons (5.6 % on total plastics)		

production of different polymers and the total and broken-down data. The amount of additives used demonstrate that *e. g.* with a world production of 50 million tons of polymers, the total additives used are 2.5 million tons which urgently calls for their

Fig. 2. Estimate of Concentration of Chemicals in 100 Years from Now

environmental assessment (Table II and III).

From another point of view the importance of chemicals production is further emphasized: If we consider a chemical which is today just at the limit of detectability in environmental samples and estimate an annual increase of its production of 2 % it will occur in environmentally significant concentrations in a hundred years from now (Fig. 1). Optimistically seen, this would indicate that we have still about 100 years time to solve the problems with those environmental chemicals which are insignificant in the microgram/kilogram concentration range in organisms today.²⁾

This statement, however, is too optimistic since there are already today some chemicals

occurring all over the world in man or biota in concentrations of mg/kg like the PCB's, HCB, and some other persistent compounds.

During the last decade, it was shown that industrial chemicals occur not only in the areas of their application but also globally or in overregionally distribution. This results in an unexpected and unintended presence of environmental chemicals everywhere, including food and even the human body. Therefore, it was internationally agreed that all-and for the time being-new products should be subjected to a test procedure which includes physico-chemical, ecotoxicological, and toxicological questions. Evaluation procedures in the area of physico-chemistry and toxicology are developed. Corresponding test procedures in the new area of ecotoxicology are still in the stage of development.

Aim of Prospective Measures

There are three areas in the context of concern which determine whether one used chemical has a potential for an toxicological or ecotoxicological impact. These are the amounts used and released into the environment, the fate in the environment, and unintended effects on any living organisms. Despite the importance of the quantitative aspect of release and pathways into the environment and of the effects. I would like to mainly concentrate on the fate assessment since concepts and principles for experimental elucidation are the same as for investigating effects. The problem area of production level, use pattern, and pathways into the environment is not a problem of experimental work but of statistical investigations.

The proper evaluation of a chemical has to be based on an adequate focussing and weighting of the data obtained within the

three problem areas. Today, it is generally accepted that the concept to follow for the prospective assessment of chemicals has to be based on a most feasible initial investigation which is then gradually improved up to so-called simulation experiments or even field experiments if necessary. That means that a gradual security parallels this process of evaluation.

This implies for science that we have to realize in each respect what the data which we have developed really mean for the environment. The concept of a type of step-sequence-testing is economically necessary. But even without considering economic aspects it is scientifically reasonable since it would be nonsense to put large experimental efforts into a compound for which problems can be excluded beforehand. Furthermore, it has to be emphasized that the more sophisticated experimental investigations are and the more they really represent environmental conditions the less is their representativity. This means, correct environmental data apply only for the complete environmental conditions of investigation.

One condition, however, must be fulfilled in any step of testing: it must be excluded that a harmful chemical is not recognized whatever procedure is used.

The necessary scale and proper weighting of investigated factors has to be supplemented by the knowledge of the quantitative relevance of data. When using comparative approaching (yardsticks or trigger values) it is necessary to know how safe is the ranking. There would be one further request namely to correlate for more complex investigations the improvement of the quality of obtained data with the effort.

I would like to emphasize the broad

scope of scientific ecotoxicology. It might be appropriate to recapitulate some descriptions of this term. An early one, of 1974, reads as follows :

"In contrast to classical toxicology, which deals with the toxic effects of chemicals on individual organisms, ecotoxicology is the study of the toxic effects of environmental chemicals on natural populations in various ecosystems.

The goal of such a study is to describe the reciprocal actions of environmental chemicals and natural populations on each other.

Primary emphasis in the research program will be on the following points.

- 1) Selection of suitable model compounds.
- 2) Occurrence and distribution mechanisms of these substances.
- 3) Fate of these chemicals under biotic and abiotic conditions.
- 4) Determination of dose level effects, taking into account the practically encountered concentrations.
- 5) Estimation of synergistic effects.

Ecotoxicology utilizes the results, values and estimations of classical toxicology and extends these conclusion to the ecosystem level."

For the SCOPE (Scientific Committee on Problems of the Environment) Project 4, Ecotoxicology, which resulted in SCOPE Report 12, the following definition was developed :

"Ecotoxicology is concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems ; it includes the transfer pathways of those agents and their interactions with the environment."

For the purpose of the Journal Ecotoxi-

cology and Environmental Safety, which is the journal of the International Society of Ecotoxicology and Environmental Safety (SECOTOX) the term has been defined as follows :

"Ecotoxicology can be defined, for the purposes of the new Journal and the Series, as the study of the adverse effects of chemicals, natural products, and physical agents on populations and communities of species of plants, animals, and microorganisms as they occur and are organized in nature. In contrast to classical Toxicology, which deals predominantly with the toxic effects of chemicals on individual organisms, Ecotoxicology is essentially the study of the toxic effects of environmental chemicals on naturally occurring populations in various ecosystems, including Man. The major emphasis of studies in Ecotoxicology is to describe and interpret the reciprocal action of environmental chemicals to natural populations and communities."

Although these three and further existing explanations of the term Ecotoxicology differ in their wording there is no discrepancy in the understanding and the broad scope of this new field of science. The variety of ecosystems, with largely varying abiotic and biotic factors including natural fluctuations of populations are well documented. Data on size of these fluctuations and their correlation to causative factors are known only scarcely. Thus, reliable research programs in ecotoxicology present a tremendous effort. Consequently, for the evaluation of the ecotoxicological potential a few important factors have to be selected and investigated. These are the factors representative for the fate of chemicals in the environment. As regards the assessment of effects, only

some typical or sensitive species or mixed populations can be investigated in addition to the toxicological testing aiming at establishing human safety.

The parameters governing the fate of chemicals in the environment consider both the characteristics of the chemical as well as the environment. They include dispersion and accumulation—that is the mobility, transport processes of a chemical within the environment, and degradation under biotic and abiotic conditions. The term degradation is now frequently used for the disappearance of the chemical species investigated. A final elimination from the environment can however only be proven by elucidation of its mineralization, that is its total break-down to water, CO_2 etc.³⁾

Description of Test Procedures

The determination of persistence and degradability of a chemical contaminant in the environment is one important parameter in the overall evaluation of a component's potential environmental ecotoxicological hazards. Yet any chemical that is released into the environment may be affected by physical transport processes or altered chemically by exposure to various biological and chemical agents in the environment. The physical processes have a tendency to distribute, dilute or concentrate but not to destroy the contaminant, yet these transport processes can have considerable impact on the biological and chemical reactions that may take place. However, until there is an understanding of the transport and behaviour of chemicals in the environment, it is difficult to devise the appropriate methods of preventing environmental exposure. Only with the appropriate understanding, methods of manufacture, handling, use, and disposal can be devised to minimize the problem. Similarly, a knowledge

of transport through food chains or by physical means permits the appropriate avoidance reaction. A number of examples could be cited to illustrate how a prior understanding of chemodynamics of a substance could have been utilized to avoid difficulties such as with DDT, PCBs and the various heavy metals. If one were to consider the path followed by a chemical from its release or escape to the site of action in a single organism or a population of organisms it would be found that there are at least four major steps:

- 1) Interaction of the chemical with the environment during its transport to the boundary of the organism.
- 2) Interaction with the boundary of the organism.
- 3) Passage through the boundary.
- 4) Intercellular action.

It is with the first step that the chemodynamics is primarily concerned with, and this is—for our feeling—the step at which preventive action can be taken. Nevertheless each of the four steps a number of processes or reactions can occur which are common in several or all the steps. Of course these processes are dependant on both: the properties of the substances as well as the property of the particular element of the system with which it may be interacting.

On this basis then, the physical chemical properties of the compound permit some prediction of probable behaviour and—perhaps—an estimate whether the exposure received by man is able to produce serious consequences.

Chemical reactions occurring in the environment may—and have already done so—lead to a serious air pollution problem; on the other hand hazardous substances are known to be altered to substances which may be

inocuous or completely mineralized thus ending the pollution potential of the contaminant.

These alteration and degradation processes can be divided into three categories :

- 1) Biodegradation - effected by living organisms.
- 2) Photochemical degradation - nonmetabolic degradation requiring light energy.
- 3) Degradation by chemical agents-nonmetabolic degradation which does not require sunlight.

Biodegradation and photodegradation of organic compounds appear to be the most desirable because they result in many cases in completely mineralized end products, whereas chemical reactions usually result in modifications in the parent compound. A variety of techniques have been used to determine biodegradation in soil and water. Many of the techniques are generally considered suitable for rapid screening of biodegradability, whereas others have been more commonly used for detailed investigation of the biodegradation process. With all the techniques used, the analytical method provides the greatest spectrum of cost and desirable information. Using radiolabelled compounds is undoubtedly the best way to study a chemical's persistence and breakdown in spite of the cost.

Different test procedures for biodegradability have been adopted or are still in discussion. These include, however, only aquatic biodegradation. The situation is different for soil biodegradation :

- 1) Contrary to water or to a lesser extent air, soil once contaminated can technologically not be cleaned up.
- 2) Soil contamination of a persistent nature could long term (time scale unit : deca-

des) lead to aquafer contamination (drinking water supply).

- 3) Aerobic conditions prevail in soils up to a depth of 50—100 cm only. Once transported to anaerobic layers opportunities for, and the rate of, biodegradation in general decrease and processes involved are of a different nature.
- 4) As the occurrence of compounds will often be of a strictly localized nature, case studies will have to be done.

A last remark in view of all the things mentioned above : The complexity of the interactions between substrate and inoculum as well as the diversity of local conditions and requirements should not be underestimated.

Of course a lot of work is done on certain substances (*e.g.* pesticides) in more sophisticated tests. But for our feeling in course of testing new substances first thing to be done is to establish reliable, simple, inexpensive methods. Scientifically it would be desirable to establish the complete transformation or degradation pathways. But that would be a time consuming and costly process. It might be done for environmentally significant bulk products only.⁴⁾

Photochemical Degradation

It is well known, that photochemical reactions - transformations and degradations of these chemicals-occur in the environment. They have led in some cases to a serious air pollution problem ; on the other hand hazardous substances are known to be destroyed (completely) under the influence of light and thus removed from the environment. Of course, the potential mechanisms for photodegradation vary with the medium involved. Of the three media gas, liquid and solid photodegradation in the gas phase is the best understood. The opportunities for

liquid phase photolysis in the environment will be principally those occurring in aqueous solutions.

In many cases chemicals in the atmosphere absorb sunlight directly and undergo photochemical reaction; this may happen in aqueous solution also. Major attention has been given to indirect photolysis, especially to photooxidation, that means processes initiated by the photolysis of ozone. The rate controlling step in this oxidation process is the reaction with OH radicals. Any chemical liberated to the atmosphere will inevitably be converted by this oxidation-cycle at a rate determined by its rate of reaction with OH radicals.

It seems very difficult to perform atmospheric field investigations. As there is no generally applicable and adopted atmospheric simulation method available, supposed tests have to be judged on the basis of their feasibility to the environment. Therefore, feasible tests for photodegradation have been selected within the OECD programme. One of these tests includes the photomineralization. Such a test-carried out in free or absorbed state-supplies further going information on the (final) fate of a chemical than those tests based on the disappearance of the parent compound or the primary reaction step. - Scientifically it would be desirable to establish the complete transformation or degradation pathways. That would be a costly and time consuming process. Therefore, it might be done for environmentally significant bulk products only.

We have to recognize that the knowledge of light induced transformation or degradation is insufficient at present. Nevertheless, there are three degradation tests designed as screening tests available.

- 1) Atmospheric Simulation Test (Fujiki Test)
- 2) Microphotomineralization Test (GSF Test)
- 3) Natural Sunlight Test in Aqueous Solution (EPA Test)

- ad 1) The Fujiki-method measures the disappearance of the test compound from air.
- ad 2) The GSF-method measures the generation of CO₂ from the test compound in the presence of selected particulate matter.
- ad 3) The EPA-method measures the disappearance of the test compound from aqueous solution.

Recently developed data with the Fujiki- and the GSF-test on nine of fifteen chemicals show a good comparability.

Yet the actual state of knowledge is such that at present besides those screening tests no sophisticated environmental simulation test can be proposed.⁴⁾

Ecotoxicological Profile Analysis

Two approaches to the assessment of ecotoxicologic problems have crystallized during recent years. One is based on reducing overall ecologic systems to their simplest form and, from experimental investigations on these, to draw conclusions about the behaviour of chemicals in the environment. The second method consists in an attempt to define parameters which govern the behaviour of chemicals in ecological systems.

These parameters are then processed using experimental designs which come close to conditions encountered in the environment.

A sound and scientifically reliable determination of these factors would be a subject of extensive research for each chemical in question. For a screening, however, feasible methods for investigation are necessary which necessarily have limits in reliability

- Concept : Comparative assessment in a system of tests
- Aim : Ranking of chemical substances
 Screening for ecotox. potential
 Priority list for further studies

Fig. 2. Ecotox. Profile Analysis, Concept/Aims

and interpretability. Therefore, any feasible test systems need either calibration for known environmental data of model substances or should be interpreted as a type of ranking of chemicals, that is as a comparative evaluation of the chemicals.

Thus a test system called Ecotoxicological Profile Analysis has been developed for comparative ecotoxicological screening and the behaviour of tested chemicals in the set of tests is assessed as a whole.

The concept which we have followed in this system is to assess the ecotoxicological potential of a chemical comparative to other chemical products.^{5,6)}

Therefore, during the course of investigations no significant changes in methodology are possible in order to really obtain comparative data from standardized procedures. This concept includes the facility of evaluating individual chemicals relative to environmentally acceptable reference compounds. The use of this test system aims at the establishment of priority lists of chemicals in general, based on a set of experimental data. As *e.g.* for pesticides, it ranks this special

group of chemicals properly within the large family of all chemicals.

The approach which we followed to achieve these aims was first to use environmentally relevant concentrations in all experiments; that is typically an exposure level of 50 $\mu\text{g/kg}$. In order to obtain unequivocal analytical results, all chemicals under investigation are used in ^{14}C -labelled form.

The major criteria of concern in this context are accumulation potential and degradability which are to a certain extent correlated to dispersion tendency. The experimental design to provide data for these criteria includes standardized tests for excretion and metabolism by rats, accumulation by fish and by algae, the conversion upon waste composting as well as the rate of mineralization with sunlight, and activated sludge. Due to the correlations within the mentioned criteria, the obtained data-set might also contribute to a rough forecast of exposure. According to this approach the conclusions to be drawn from the data must recognize the complete system of tests.

The reason being that the evidence to be presented is not to cover just one specific problem such as bio-accumulation. Rather, the factors embraced in the system should respond to the most varied possible properties of the chemical undergoing investigation and represent the most varied possible environ-

Criteria Tests Data		
Accumulation potential	Mammals	excretion, metabolism
	Fish	slope of uptake
	Algae	slope of uptake
Degradability	waste composting	conversion rate
	photochemical	mineralization rate
	activated sludge	mineralization rate
	waste incineration	mineralization rate

Fig. 3. Ecotox. Profile Analysis—Approach

mental effects on the chemical. Individual data are to be indications for a relative estimate of overall environmental behaviour on comparing them with those of a reference chemical whose environmental properties are well known from extensive long-term tests and analytical data. It is then possible to designate the tested substance as being more or less environmentally compatible than the reference substance. Based on results of research to date, the reference substance should be just environmentally compatible.

It is necessary to select and design individual test systems in such a way that the results and conclusions are as close a match as possible, at least qualitatively, to the results of long-term tests with complex systems and to analytical environmental investigations.

In their nature the tests substantially fulfil the requirements of simplicity, rapid procedure, capacity for standardisation and low cost.

In this scheme, individual environmental conditions are simulated and controlled but no attempt is made to simulate ecosystems. A series of tests is being designed to supply information regarding the behaviour of chemical substances under various environmental conditions.

These tests comprise :

- a) simulated technological processes (aerobic degradation with activated sludge, aerobic degradation in garbage pits).
- b) simulated biological processes (excretion, biodegradation/metabolism, retention/bioaccumulation)
- c) complete degradation under simulated atmospheric conditions.
- d) simulated transport processes (evaporation from soil and water, solubility, distri-

bution in standardized water/soil systems and water/organic solvent systems, see-page in standardized soils).

While the used tests are in part related to more complex long-term investigations, the experimental procedure has been suitably simplified for the requirements of the profile analysis. Since the chemicals being investigated are assessed in relation to each other by the testing system, those requirements of individual tests which are normally needed to safeguard absolute results can be left out of consideration. For example, where it seemed feasible, aeration of the test apparatus was waived and the tests were performed in a closed system with an adequate reserve of air.

The substances being tested are employed as ^{14}C -labelled compounds. As a result, there is no need for working out trace-analytical methods and (polar) transformation products are embraced without additional resources ; working up and analysis are simple, rapid and reliable. Observing the biological activity of the organisms in some of the biological tests serves merely for controlling the progress of the tests and is not the object of the investigation. If the substances produce severe disturbances in the organisms (toxicity, inhibition) then these are eliminated by reducing the concentration.

The chemicals investigated so far were selected for covering different classes of chemicals, different use patterns, a variety of physico-chemical characteristics, and also some homologous series.

Results of the Ecotoxicological Profile Analysis

In the following set of tables some of the more recent results are presented.¹⁾

For an attempt to correlate structural

TABLE IV. Bioaccumulation of Organic Chemicals in Fish (Golden orfe)
after 3 Days of Exposure

Chemical	Medium concentration in water ($\mu\text{g/l}$)	Bioaccumulation factor BF_3^a
Aldrin	2	3,890
2, 5, 4'-Trichlorobiphenyl	36	2,620
2, 2'-Dichlorobiphenyl	50	2,420
2, 4, 6, 2', 4'-Pentachlorobiphenyl	34	2,320
Hexachlorocyclopentadiene	48	1,230
Hexachlorobenzene	39	1,200
Pentachloronitrobenzene (Quintozen)	0.4	1,140
Pentachlorophenol	42	1,050
Anthracene	26	910
2, 6-Di- <i>tert</i> -butylphenol	37	660
Biphenyl	33	280
Di (2-ethylhexyl) phthalate	20	460
2, 4, 6-Trichlorophenol	30	310
<i>n</i> -Dodecylbenzene sulfonate (Na-salt)	48	130
4- <i>tert</i> -Butylphenol	46	120
<i>p</i> -Nitrophenol	50	57
Hexadecanol	48	56
Dodecane	37	52
Chlorhexidine	50	42
Hydroquinone	80	40
Carbaryl	43	34
Metribuzin (Sencor)	54	11
Cortisone acetate	49	10
2, 6-Dichlorobenzamide	50	10
<i>p</i> -Bromobenzoic acid	50	< 10
Monolinuron	45	< 10
<i>p</i> -Chloroaniline	52	< 10
2, 4-Dichlorophenoxyacetic acid	50	< 10
Aniline	55	< 10
Maleic acid	31	< 10
<i>p</i> -Chlorobenzoic acid	53	< 10
Nitrobenzene	50	< 10
HPMA	45	< 10
2, 4-Dichlorobenzoic acid	50	< 10
Coumarin	46	< 10

(Chemicals are listed with decreasing accumulation)

^a) Bioaccumulation factor after 3 days:

$$\text{BF}_3 = \frac{\text{Chemical concentration in fish } (\mu\text{g/g})}{\text{Medium chemical concentration in water } (\mu\text{g/ml})}$$

Balance = $91.9 \pm 2\%$ (s_x), except hexachlorocyclopentadiene 37% and dodecane 51% (s_x = standard deviation of mean).

characteristics or the type of use pattern with test data, the investigated chemicals have been combined into groups in these tables.

In Table IV, the different accumulation data of the fish test are compiled. Ranking

for accumulative properties is identical with the data of the fish and algae test, there is, however, a significant difference in the activated sludge test. This might be due to different mechanisms: biotic accumulation versus sorption to the living and dead sludge

TABLE V. Excretion and Retention of Organic Chemicals by Rats
(% of the applied doses)

Chemical	Elimination (%)		Retention (%)			
	Faeces	Urine	Adipose tissue %/g	Liver	Lung	Whole body
Hexadecanol	13.9	13.3	1.02	0.90	0.65	65.4
Hexachlorobenzene	30.2	0.4	1.89	1.33	0.20	57.2
2,6-Dichlorobenzamide	21.9	46.5	0.15	3.83	0.03	47.3
2,4,6,2'-4'-Pentachlorobiphenyl	30.0	1.4	0.80	0.82	0.12	40.7
Aldrin	67.3	0.9	0.66	0.75	1.86	29.1
<i>n</i> -Dodecane	12.6	6.4	0.10	0.60	0.09	12.1
2,4,6-Trichlorophenol	22.2	82.3	n.d.	n.d.	n.d.	7.8
Di(2-ethylhexyl)phthalate	47.7	52.7	0.01	0.03	0.03	7.1
Maleic acid	17.3	7.7	0.04	0.45	0.04	6.7
<i>p</i> -Chloroaniline	43.6	41.5	n.d.	n.d.	n.d.	4.9
2,5,4'-Trichlorobiphenyl	101.1	6.8	0.06	0.18	0.07	3.2
2,6-Di- <i>tert</i> -butylphenol	72.4	10.9	0.03	0.10	0.01	2.9
Nitrobenzene	15.4	59.3	n.d.	0.43	0.06	2.3
Hexachlorocyclopentadiene	78.5	17.6	0.05	n.d.	n.d.	1.9
Pentachlorophenol	30.9	56.6	n.d.	0.60	n.d.	1.6
Monolinuron	17.2	81.0	n.d.	0.24	0.02	1.5
Carbaryl	24.5	54.5	n.d.	n.d.	0.03	1.4
2,4-Dichlorophenoxyacetic acid	3.0	99.5	n.d.	n.d.	n.d.	1.1
<i>p</i> -Bromobenzoic acid	6.1	94.5	0.01	n.d.	n.d.	0.9
Cortison acetate	79.7	24.0	n.d.	n.d.	n.d.	0.8
Anthracene	50.2	43.5	n.d.	0.14	n.d.	0.8
ADPA	91.7	1.0	n.d.	0.04	n.d.	0.7
Coumarin	46.3	68.6	0.01	0.24	n.d.	0.6
Metribuzin (Sencor)	57.8	32.7	0.01	n.d.	n.d.	0.6
Aniline	21.5	61.8	n.d.	0.05	n.d.	0.5
Pentachloronitrobenzene	89.9	8.7	n.d.	n.d.	n.d.	0.4
Biphenyl	17.7	60.7	0.01	0.04	n.d.	0.4
<i>p</i> -Nitrophenol	6.5	71.0	n.d.	0.02	n.d.	0.4
Hydroquinone	81.4	18.6	n.d.	n.d.	n.d.	0.4
2,2'-Dichlorobiphenyl	65.1	34.0	n.d.	0.11	0.01	0.2
<i>p</i> -Chlorobenzoic acid	5.8	83.4	n.d.	0.01	n.d.	0.1
<i>p-tert</i> -Butylphenol	30.1	82.2	n.d.	0.02	n.d.	0.1
Na- <i>n</i> -Dodecylbenzene sulfonate	51.8	33.5	n.d.	0.01	n.d.	n.d.

(Chemicals are listed with decreasing retention)

Balance = $92.8 \pm 2.7\%$ (s.e.), except *n*-dodecane 31% and maleic acid 32%
n.d. = not detectable ($<0.01\%$).

biomass.

Table V shows the complete set of data obtained in the animal elimination and degradation test.

Table VI shows the data obtained in the activated sludge test. The natural products included in this table are easily biodegradable.

The data of photomineralization shows in

Table VII give a clear ranking of the tested chemicals. For all chemicals only little volatile organic fragments have been detected. This indicates that the higher molecular weights within the photodegradation pathways are the more persistent ones.

The last table (Table VIII) of results of the profile analysis shows most recent results

TABLE VI. Volatilization, Mineralization, Conversion (% of the applied amount), Non-Extractable Residues (% of retained amount in Sludge, dry weight basis), and Accumulation of Chemicals by Activated Sludge

Chemical	Volatilization ^{a)} (%)	Mineralization (% CO ₂)	Metabolites		Non-Extractable Residues (%)	BF ₅ ^{b)}
			Sludge (%)	Water (%)		
Hexachlorobenzene	1.8	n. n.	n. n.	n. n.	3.7	35,000
2,5,4-Trichlorobiphenyl	0.5	—	11.4	n. d.	2.9	32,000
Pentachlorobiphenyl	1.2	0.2	1	—	5.1	27,800
Chlorhexidin	0.2	0.1	—	—	94.2	26,700
Aldrin	1.5	—	11.1	n. d.	9.5	18,000
Anthracene	1.7	—	—	—	6.6	6,700
2,2'-Dichlorobiphenyl	6.6	0.1	2.3	0.8	13.1	6,300
Pentachloronitrobenzen	7.0	—	39.2	—	27.5	4,500
Hexadecanol	28.5	28.0	30	—	80.3	3,170
Di-(2-ethylhexyl)-phthalate	1.0	—	1	n. d.	12.4	3,000
Maleic acid	26.9	26.3	41	—	96.8	2,800
2,6-Di- <i>tert</i> -butylphenol	7.7	1.1	29.8	15.1	31.9	2,600
Hexachlorocyclopentadiene	2.0	—	49	26.4	89.1	2,350
Coumarin	30.0	29.5	29	n. d.	96.2	1,400
<i>n</i> -Dodecane	38.0	37.0	18.8	—	95.3	1,300
<i>p</i> -Chloraniline	37.0	17.0	23.7	14	87.4	1,300
HPMA	3.3	3.1	—	—	97.2	1,260
Pentachlorophenol	0.5	—	10.6	5	33.4	1,100
Aniline	37.0	37.0	21.4	11.7	93.8	1,030
Hydroquinone	7.6	7.5	19.7	24.4	93.9	520
Biphenyl	9.1	8.5	7.8	8.5	50.0	430
Cortisone acetate	31.0	31.0	15	20	91.4	400
<i>p-tert</i> -Butylphenol	4.9	0.2	3.7	n. d.	48.8	240
<i>p</i> -Chlorobenzoic acid	5.4	5.1	—	n. d.	95.3	170
Na- <i>n</i> -Dodecylbenzenesulfonate	3.5	3.0	3.7	—	43.6	140
Carbaryl	32.2	29.7	1.6	n. d.	82.1	90
Monolinuron	0.5	—	n. d.	8	31.7	70
2,4,6-Trichlorophenol	0.1	—	n. d.	55	48.0	40
Nitrobenzene	1.7	0.4	n. d.	n. d.	72.2	40
<i>p</i> -Nitrophenol	1.4	1.1	0.5	34.7	75.0	30
Metribuzin (Sencor)	2.5	—	n. d.	n. d.	13.9	30
2,6-Dichlorobenzamide	0.5	—	2.0	n. d.	14.3	30
<i>p</i> -Bromobenzoic acid	1.1	—	—	n. d.	52.5	19
2,4-Dichlorophenoxyacetic acid	0.1	—	0.1	n. d.	39.5	17
2,4-Dichlorobenzoic acid	0.3	n. d.	—	n. d.	79.7	9

a) Organic compounds and CO₂.

b) Bioaccumulation factor.

$$BF_5 = \frac{\text{Chemical concentration in sludge } (\mu\text{g/g D.W.}) \text{ after 5 days}}{\text{Final concentration of chemical in water phase } (\mu\text{g/ml})}$$

n. d. = not detectable (<0.1%).

— = not determined.

Balance = 91.8 ± 1% (s_x), except 2,2'-dichlorobiphenyl 64.2% and biphenyl 44.6%.

TABLE VII. Photomineralization (% of the applied amount) of Organic Chemicals after 17 Hours

Chemical	Applied concentration (ng/g silica gel)	Organic fragments (%)	Photomineralization (% CO ₂)
Hexachlorobenzene	125	< 0.1	1.5
Di-(2-ethylhexyl)-phthalate	4,490	< 0.1	1.6
2,4-Dichlorobenzoic acid	30	< 0.1	2.8
Dodecane	240	~ 0.7	2.9
Hexadecanol	1,680	~ 0.5	3.1
<i>n</i> -Dodecylbenzene sulfonate (Na-salt)	97	< 0.1	3.2
2,2'-Dichlorobiphenyl	77	< 0.1	3.5
2,4,6,2',4'-Pentachlorobiphenyl	60	< 0.1	5.2
2,5,4'-Trichlorobiphenyl	130	~ 0.1	5.9
<i>p</i> -Chlorobenzoic acid	30	< 0.1	6.3
Nitrobenzene	60	< 0.1	6.7
Aldrin	45	< 0.1	8.8
Biphenyl	80	< 0.1	9.5
HPMA	2,500	< 0.1	12.1
Maleic acid	165	< 0.1	17.0
2,4-Dichlorophenoxyacetic acid	115	~ 0.1	26.2
<i>p</i> -Chloroaniline	163	< 0.1	27.7
2,6-Di- <i>tert</i> -butylphenol	30	< 0.1	29.5
<i>p</i> -Bromobenzoic acid	33	< 0.1	36.4
Monolinuron	65	~ 0.1	38.3
<i>p</i> -Nitrophenol	80	~ 0.2	39.1
Carbaryl	3	< 0.1	40.3
Pentachloronitrobenzene	40	< 0.1	43.0
Hexachlorocyclopentadiene	64	< 0.1	46.0
Aniline	39	~ 0.8	46.5
<i>p-tert</i> -Butylphenol	100	< 0.1	46.8
Metribuzin (Sencor)	25	< 0.1	56.9
Cortisone acetate	49	< 0.1	57.3
Hydroquinone	62	< 0.1	57.4
Coumarin	250	< 0.1	59.2
Pentachlorophenol	80	< 0.1	62.0
2,4,6-Trichlorophenol	80	< 0.1	65.8

(Chemicals are listed with increasing photomineralization)

TABLE VIII. Photomineralization in Twin-reactor

Chemical	CO ₂ (%)	$M = \frac{K_1}{K_2}$
<i>K</i> ₁ <i>K</i> ₂	<i>K</i> ₁ <i>K</i> ₂	
Lindane(1.V)/PCP	6.4/61.5	0.10
Lindane(2.V)/PCP	5.3/49.6	0.10
Nitrobenzene /PCP	6.6/65.0	0.10
HCB /PCP	2.2/67.0	0.03
PCP /PCP	65.0/68.6	0.94

(ref. PCP in *K*₂ ; irradiation time 17 hrs)

with the twin-sector photomineralization reactor.

In the two experiments with lindane two irradiation lamps—a fairly new one and an old, burned-out one were used. Without determining the light intensity, the differences are easily corrected for. Thus, the remodeling of the reactor resulted in increased reproducibility and reliability of obtained results.

The complete system of ecotoxicological profile analysis is to recognize chemicals

TABLE IX. Dynamics of Organic Xenobiotics in Cultivated Soil

Chemical	Duration of Experiment (years)	Total Residues after 1 Season (mg/kg)	Total Residues after total Duration of Experiment (mg/kg)
Aldrin	10	1.88	0.35
<i>p</i> -Cl-Aniline	7	0.40	0.22
HCB	4	0.68	0.22
Trichloroethylene	2	0.22	0.17

which have a potential for ecotoxicological significance. With the selection of data presented, this was to be shown, Compounds which have been recognized should be subject to more sophisticated investigation or extensive testing. The evaluation, weighting, and focussing of data has to be done—despite the comparative approach—for each compound separately recognizing all relevant factors involved. Therefore, we do not intend to calculate environmental significance figures by complex formula, attributing factors to data and add them to an overall figure. When many compounds will have been investigated and correlations reliably established, such a mathematical model based on test data may be possible in the future.

Limitations of Testing

What is the state of the art with respect to the interpretation of test results? We know definitively that physico-chemical characteristics are determining factors for the fate of chemical substances in the environment. Thus, their determination, any method to assess degradation, mobility gives relevant results, but what is their significance, that means quantitative relevance?

Many attempts have been made to establish the “predictability” of physico-chemical characteristics by correlating them with laboratory standardized test data. This graph is just one example to correlate water solubility with fish-accumulation. In a log-log plot this

seems to be feasible, indicating that the correlation is correct in the “order of magnitude” range. However, data from standardized fish accumulation tests are no environmental data. To demonstrate the problem I use some data of chemicals in soil.

Table IX summarizes some data of long-term open-air experiments with ¹⁴C-chemicals in soil. It had been shown that these data are predictive to the environment within the range of variation caused by climate and soil properties. The data for the four chemicals—which really have a wide range of physico-chemical characteristics—show that they lead to comparable residual radioactivity in soil:

Aldrin derived radioactivity is reduced within 9 years by a factor of 5

p-Cl-aniline derived radioactivity is reduced within 6 years by a factor of 2

HCB derived radioactivity is reduced within 3 years by a factor of 3

Trichloroethylene derived radioactivity is reduced within 1 year by a factor of 1.3

Conclusion

The present status of testing chemicals for ecotoxicological behaviour can be summarized as follows:

- 1) Test data lead to a roughly proper ranking for comparative evaluation. Since we can rely on model chemicals with known environmental behaviour, the comparative approach is appropriate.
- 2) The physico-chemical characteristics and

behaviour of chemicals in tests show a greater variety than their respective behaviour in the environment since in the environment competitive factors are effective. Therefore, physico-chemical characteristics and test data are to be determined only with the appropriate accuracy.

- 3) Due to feasibility reasons, biological activity can only principally be assessed

by elucidating effects on certain selected species. It cannot be excluded that for a given chemical other species are more sensitive.

- 4) There will be a continuous need for research to improve the whole set of test procedures, especially with respect to reliability, feasibility, and establishment of test significance.

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