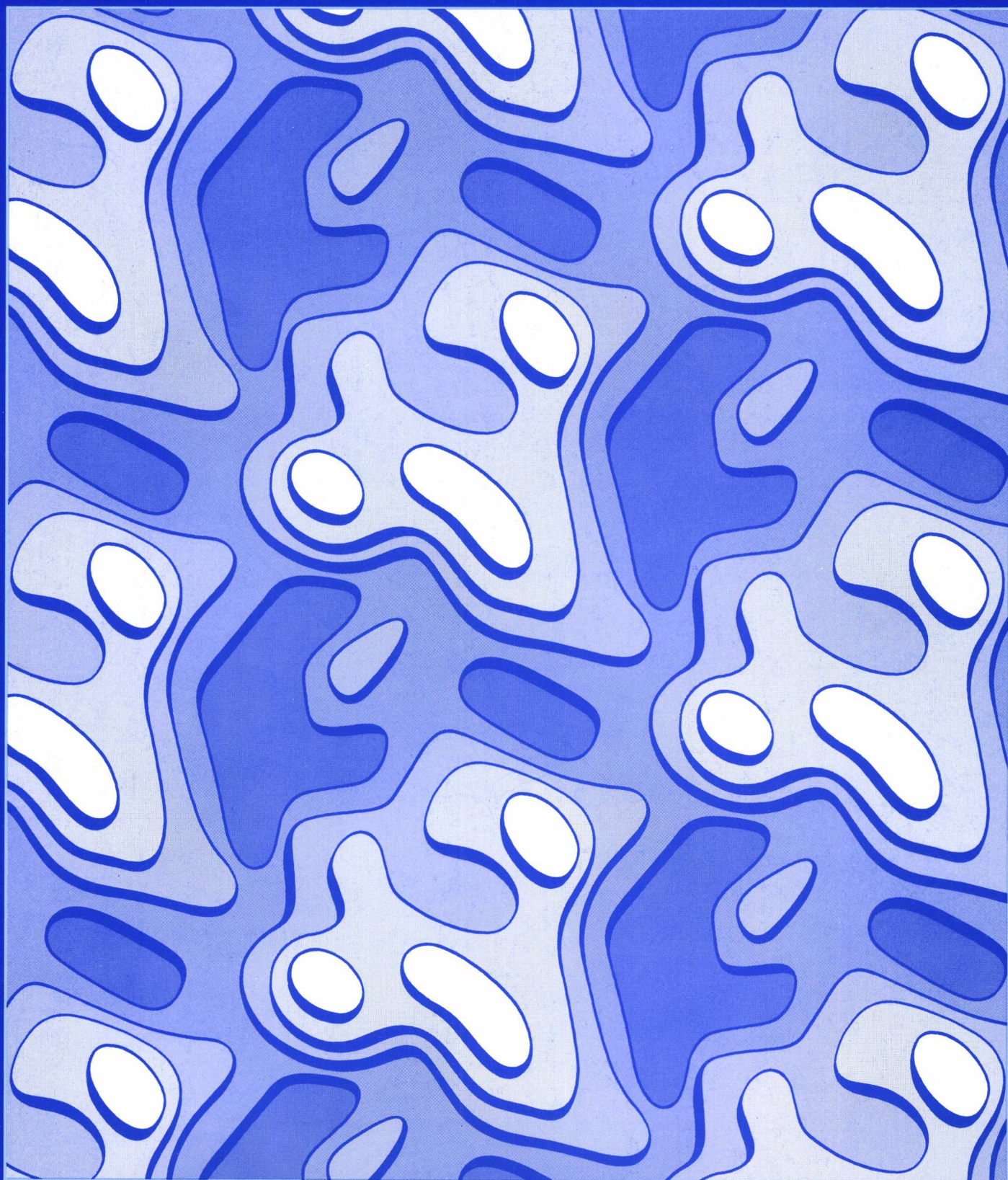




Introduction

In this brochure the work of the Netherlands Organization for Applied Research (TNO) in the field of toxicological studies and screening tests is described. TNO has almost 5000 specialists engaged in scientific research in the industrial, environmental, health-oriented, nutritional and other fields. A great potential is available for contract research in toxicology; for this type of research a total of 283 persons, including 82 with an academic degree, are available.

Since this contract research is performed in a number of institutes or laboratories situated at different localities, those not acquainted with the structure of the Organization may have difficulty in locating the relevant toxicologist to deal with his particular problem.

In this brochure, therefore, you will find a compact survey of the types of studies that can be performed by TNO laboratories in the fields of mammalian toxicology and ecotoxicology, followed by short descriptions of the relevant institutes.

For further information and general enquiries:

Toxicology TNO (TOX-TNO)

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Secretary: Ms Flora de Vrijer

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Mammalian toxicology and related studies



Mammalian toxicology is that branch of toxicology which describes and evaluates the possible effect of chemicals or other products on human health. Health risks may arise from ingestion of substances (oral route), from contact with the skin (dermal route) or from inhalation (respiratory route).

Depending on the uses of the substance under test, one or more of these three routes of administration may be required for a complete evaluation of the possible health risks.

Methods and protocols followed by TNO in the various types of toxicity studies are based on the more or less standardized procedures in use in various countries.

The tests are performed, where necessary, according to the FDA-rules for Good Laboratory Practice (GLP).

The criteria used to examine the effect of substances on experimental animals include among others:

- general behaviour
- growth rate
- intake of food and water
- clinical-chemical parameters of blood and urine
- organ weights
- health condition
- food efficiency
- haematological values
- organ function tests
- mortality
- gross and microscopic pathology

Related studies which are required for certain compounds are mutagenicity and metabolic studies.

For each type of study, detailed protocols are available on request.

A necessary requirement for the performance of many tests is the availability of a suitable analytical method for the test product.

Such methods can be developed on request. (see also page 11: Determination of relevant substance-specific properties).

Oral toxicity tests

These tests are generally carried out with rodents, rabbits or dogs. Special tests may be performed in monkeys and non mammalian systems such as quails.

Test materials in oral toxicity studies are administered with the diet, in the drinking water or by gavage.

The following types of tests are routinely performed:

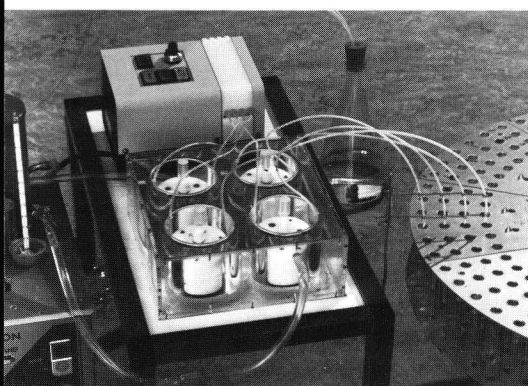
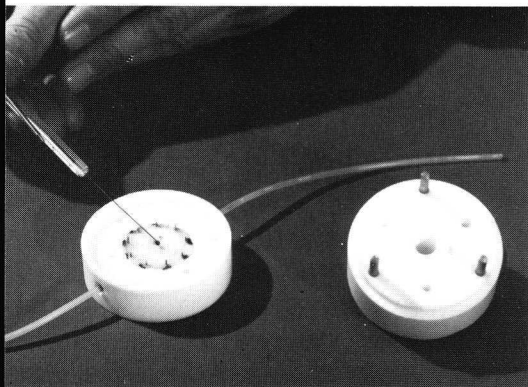
acute*	2 weeks	* For acute toxicity studies (LD ₅₀) the test material can also be administered: intraperitoneally intravenously sub-cutaneously intraventricularly
sub-acute	4 weeks	
sub-chronic	13 weeks	
chronic	104 weeks	
teratogenicity	21 days	
carcinogenicity	2 years or longer	
reproduction	3 generations	

Left
Autoanalyzer
Above
Mammalian toxicity studies with rats

Above
Opened diffusion cell with a skin sample
during application of a toxic substance
Below
Water-jacketed cell container with four
diffusion cells, pump and fraction collector

Right
Technical facilities for short-term inhalation
toxicity studies

Right below
Teratogenicity studies



Dermal toxicity and eye irritation tests

In these toxicity studies of short duration the test material is usually applied to the skin or the eye of rabbits or guinea pigs.

Several types of tests are routinely performed:

acute	2 weeks (rabbit)
sub-acute	3 weeks (rabbit)
sensitization:	
Landsteiner test	6 weeks (guinea pig)
maximization test	3-4 weeks (guinea pig)

More specific measurements of percutaneous absorption are carried out on isolated animal- or human-skin in a diffusion chamber.

These *in vitro* measurements are complementary to *in vivo* tests, because of, for example, exclusion of biotransformation of the penetrating substance.

Inhalation toxicity test

In inhalation toxicity test materials are dosed as vapour, gas, dust or as an aerosol into the air inhaled by the experimental animals.

The tests are generally carried out with rats, mice, hamsters or dogs.

Several types of tests are routinely performed:

acute	2 weeks
inhalation risk test	2 weeks
sub-acute	4 weeks
sub-chronic	13 weeks
chronic	78 weeks (hamster)
	104 weeks (rat)
carcinogenicity	2 years or longer

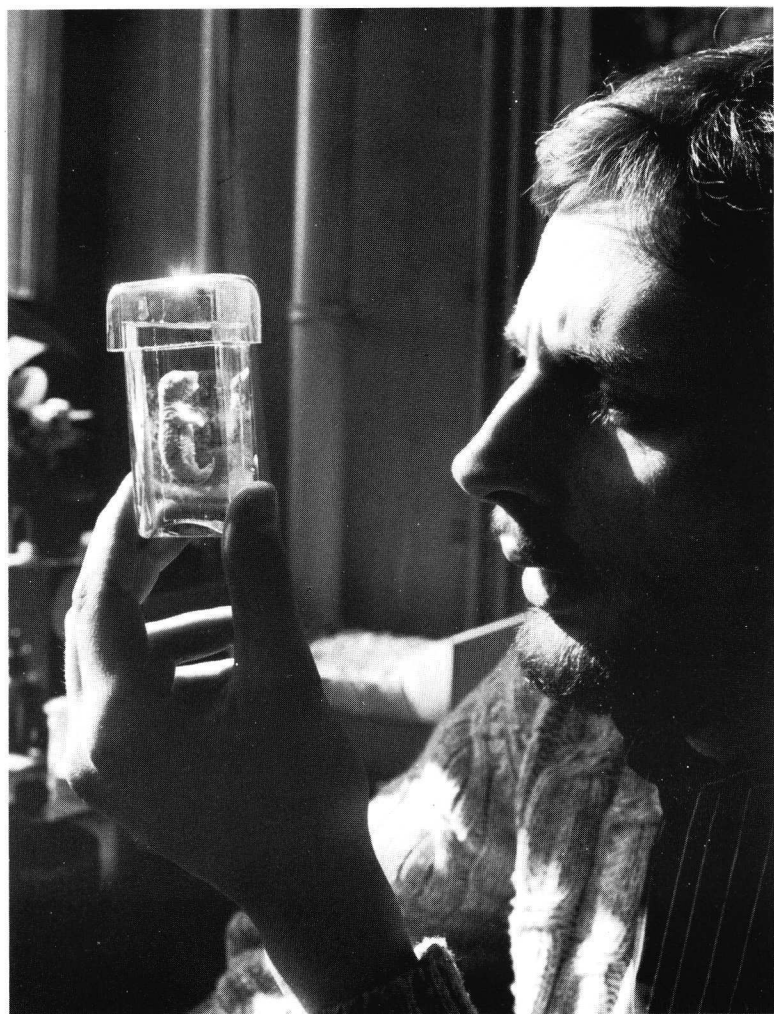
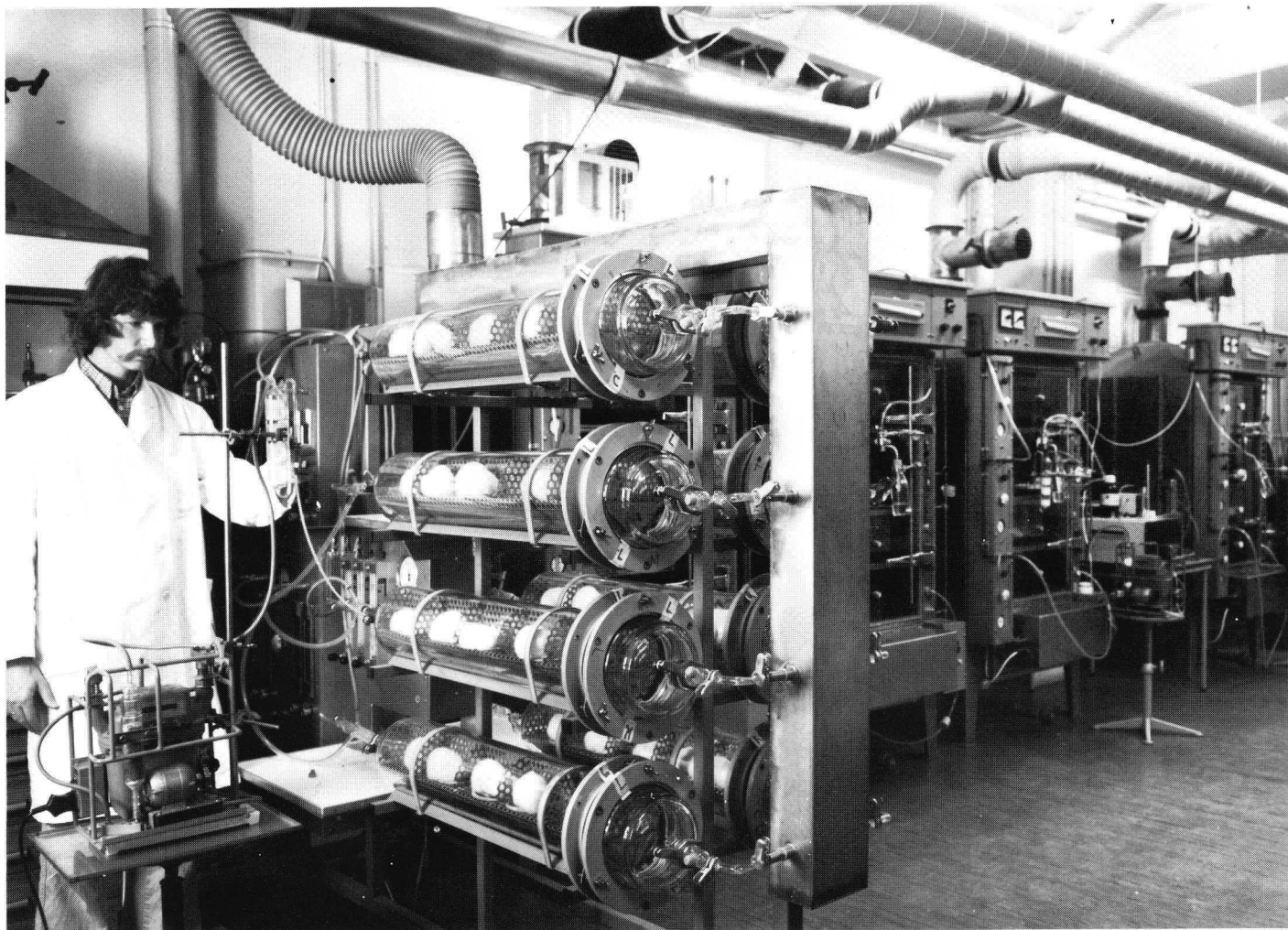
Teratogenicity and reproduction studies are performed if required.

Mutagenicity and DNA-damage studies

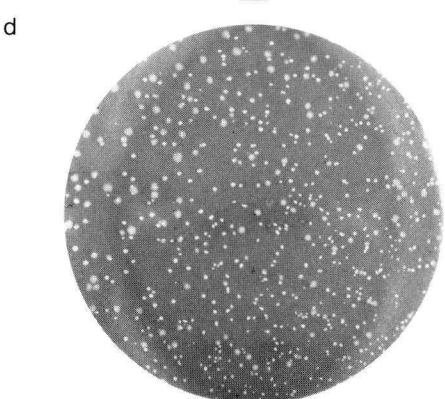
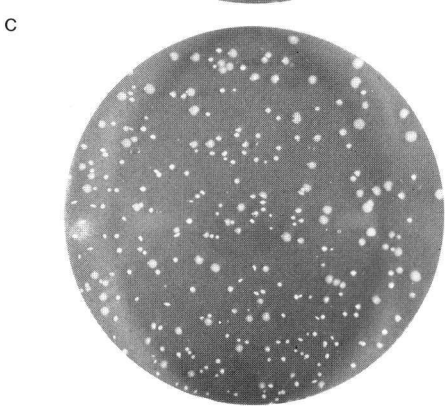
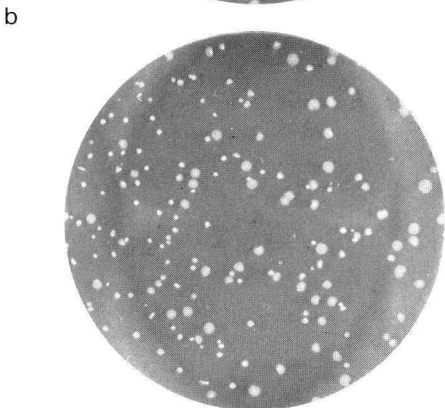
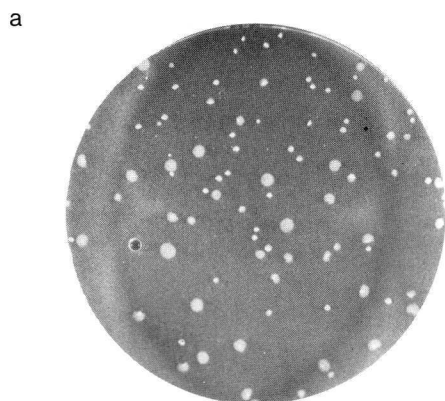
These tests are performed to establish possible mutagenicity or indications for carcinogenicity.

dominant lethal test	rat, mouse <i>in vivo</i>
host mediated assay	
micronucleus test	
sperm morphology	

repair test	micro-organisms
Ames-test (several modifications)	
fluctuation test	
liquid-incubation test	



Mutagenicity studies
 Induction of His⁺ mutants in *S. typhimurium*
 TA 100 by increasing amounts of a 1000-
 fold dilution of waste water
 a: 0 μ l; b: 25 μ l; c: 50 μ l; d: 200 μ l



mutation induction (thioguanine-
 resistance) in Chinese hamster
 cells (CHO)*

induction of DNA-repair replication
 in various human or Chinese hamster
 cells (detection of ³H-thymidine con-
 taining segments of DNA by ultracen-
 trifugation*

induction of unscheduled DNA synthe-
 sis (UDS) in human fibroblasts (autora-
 diographic detection of UDS)*

Painter-test (determination of the
 temporary post-treatment inhibition
 of semi-conservative DNA replication
 in a number of cell lines)*

induction of Sister Chromatid Ex-
 changes (SCE) in Chinese hamster cells
 (CHO) or in rat hepatoma cells*

in vitro cell transformation with baby
 hamster kidney (BHK) cells

cultured mammalian cells

All procedures can be carried out with and without metabolic activation.

induction of Sister Chromatid Ex-
 changes (SCE)
 metaphase test

fish, *in vivo*

* Metabolic activation in developmental stage.

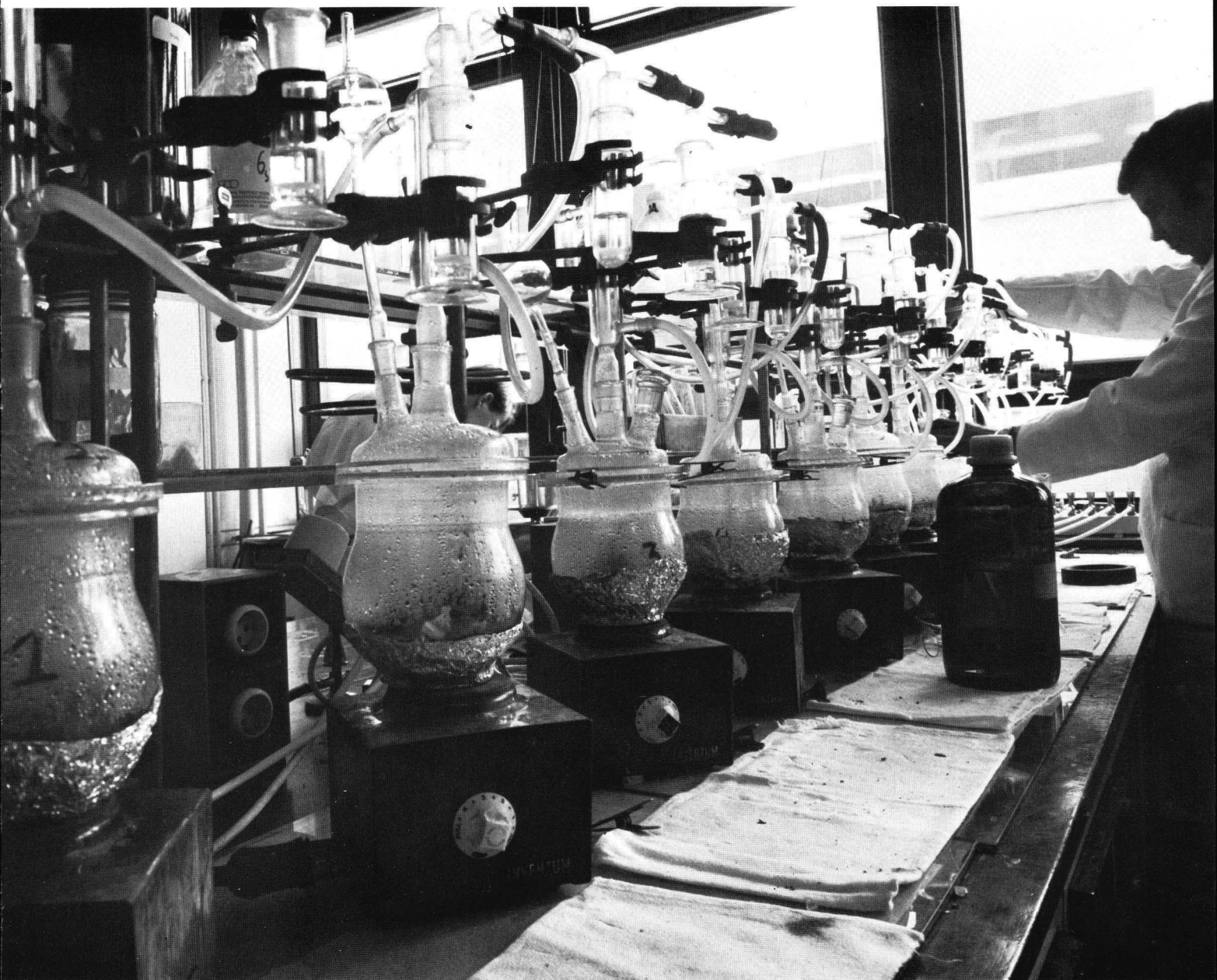
Metabolic studies

Information may be obtained concerning excretion (also in exhaled air), retention,
 biotransformation, identification of metabolites, distribution and pharmaco-kinetics
 of (labeled) * compounds in the whole animal, isolated organs (liver), cultured (liver)
 cells and homogenates of liver and other tissues.

* Synthesis of labeled compounds using primarily ³²P, ³⁵S and ¹⁴C isotopes can be
 performed.

Residues and metabolites of plant protecting agents in vegetable food and feed

Plant protecting agents may leave residues on or in crops. In order to evaluate
 health risks for the consumer, the persistence of the residue and the identity of
 metabolites formed must be established. This work is closely linked to mammalian
 toxicity studies. In cases where other metabolites are formed in plants than in
 mammals, the mammalian toxicity of these metabolites must also be investigated.



The following types of studies may be performed for this purpose:

- residue determinations in food crops
- development of suitable methods for determinations of residues
- identification of metabolites in plants*
- study of the distribution in various plant parts of parent compound and metabolites.
- quantitative aspects of metabolism, including bound residues

* Synthesis of labeled compounds using primarily ^{32}P , ^{35}S and ^{14}C isotopes can be performed.

Toxic residues in foodstuffs of animal origin

As a result of pesticide or drug administration to farm animals, residues may be present in their edible products. In order to evaluate health risks for the consumer, the identity and persistence of the residue must be established. This analytical work is closely linked to mammalian toxicity studies. The following types of analytical work may be performed for this purpose:

- identification of residual compounds, including metabolites of toxicological importance
- residue determinations in meat, milk, eggs etc.

Analytical facilities are available to check the absence of contaminants in diets for test animals, and to check the dosages of test substance administered.

Special studies

- toxicity studies in germfree and gnotobiotic animals (mainly rats and mice)
- immune response studies (mainly rats and mice)
- irritation studies in mucous membranes of the human eye (eye-blink registration)
- study of the cause of death in anaesthetized rats using 'polygraph' observation
- the effects of ionizing radiation on man and animals (incl. radio-isotope toxicity)
- the effects of microwaves on man and animals
- study of the biochemistry of acetylcholinesterase and its inhibition and reactivation by cholinesterase inhibitors
- study of the biochemical mode of action of fungicides and bactericides

In addition more specific investigations can be carried out using the following models or systems:

- neuromuscular function in isolated preparations from rats, guinea pig and man
- heart function in Langendorff perfusion set-up
- passage through stomach and intestines
- resistance to infections (rats, mice)
- animal behaviour (acute and chronic)
 - automatic recording of spontaneous activity
 - operant and instrumental behaviour
- neurotoxicity of compounds for nerve cells or muscle cells in tissue culture
- determination of toxicity of chemicals for various cultured cells (including human cells)

Ecotoxicology and related studies

Ecotoxicology is that branch of toxicology which describes and evaluates the impact and the effect of chemical and/or physical agents on organisms representative for the biosphere. Although in principle influences on human beings are included in the definition, in practice mammalian toxicology, as far as it directly concerns human well-being, is excluded.

The test-program covers 4 different fields of study:

determination of relevant substance-specific properties of chemicals of environmental importance

degradation and transformation of chemical compounds in the environment (air, water, soil)

bioaccumulation and biotransformation in organisms (plants, animals)

toxic effects of chemical compounds in non-human organisms and biotic systems

For each type of study, detailed protocols are available on request.

A necessary requirement for the performance of many tests is the availability of a suitable analytical method in water, soil or biological tissues; such methods can be developed on request. In addition, synthesis of labeled compounds can be performed.

Determination of relevant substance-specific properties

For the performance of tests and the safe handling of compounds the following techniques are required and can be made available. The use of labeled compounds is often required.

Analytical procedures

extraction

clean up

quantitative determination

Physico-chemical properties

volatility

solubility

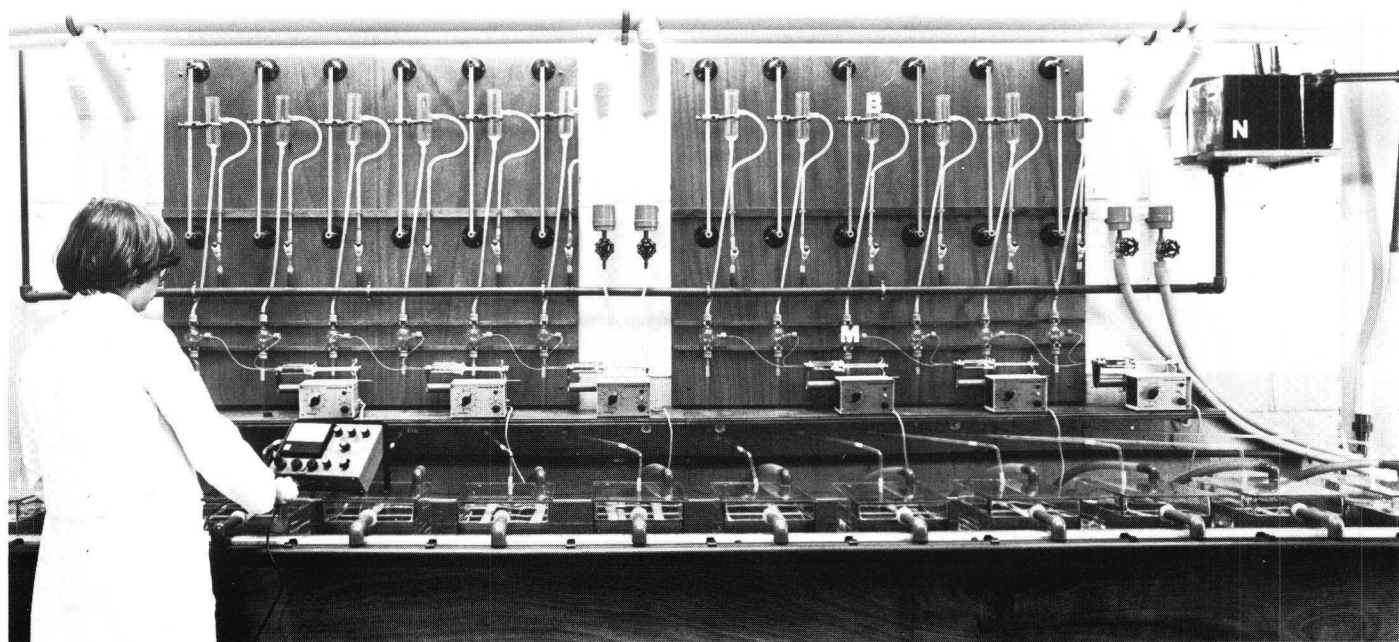
partition coefficient in n-octanol/water

total organic carbon (TOC), chemical oxygen demand (COD), biological oxygen demand (BOD) and related properties

hydrolytic stability at different pH values

Occupational hygiene

in general some elementary human-toxic properties should be known for the safe handling of compounds by laboratory personnel. These include LD₅₀ rat, dermal toxicity and irritant properties etc. as described in the preceding sections on mammalian toxicity.



Left
Ecotoxicity field studies
Left below
Apparatus for constant flow toxicity tests
with aquatic organisms

Degradation and transformation of chemical compounds in the environment

The fate of environmentally relevant compounds is determined by their distribution and possible conversion in the environment by organisms.

Methods for the study of the fate of compounds in air, water and soil are generally different.

Air

UV-stability of chemical compounds

photo-chemical reactivity

thermo-chemical properties

Water

chemical stability at different pH values

photo-chemical degradation

biodegradation

screening tests

The inoculum used in the screening tests is adapted to each specific problem and may be surface water, sea water, active sludge, sewage etc.

die-away tests, with specific analysis of the compound under different conditions (pH, temperature, light); with measurement of total organic carbon (TOC)

Sapromat tests, with measurement of O₂-consumption

OECD-screening tests for detergents

Trickling-filter tests on laboratory scale

continuous-flow tests (confirmatory tests)

OECD-confirmatory test; a laboratory scale active sludge plant with recirculation of the inoculum

Chemostat-test; a continuous flow test without recirculation of inoculum

simulating natural waters

identification of metabolites

supporting methods; a necessary requirement for the performance of biodegradation tests is the previous screening of the toxicity of the compound for the inoculum used

Soil

mobility of chemical compounds in soil

leaching behaviour on soil columns or under field conditions

determination of adsorption isotherms

degradation and transformation

rate of disappearance under field conditions

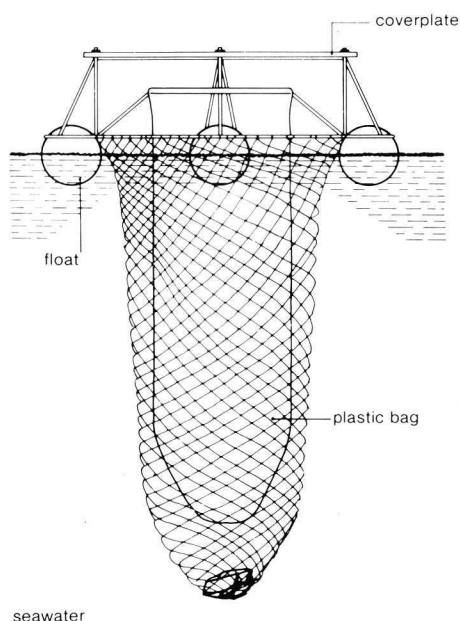
identification of metabolites

quantitative aspects of metabolism, including determination of bound residues

photodegradation on solid surfaces

supporting methods

influence on soil respiration



General methods available for the study of degradation in the environmental area:
metabolism of compounds by pure cultures of bacteria and fungi
development of sampling methods for environmental emissions

Bioaccumulation and biotransformation in organisms

higher plants

bioaccumulation in various plant species

algae

bioaccumulation in aquatic algae

animals

in principle bioaccumulation tests can be carried out with all animals mentioned in the following section, such tests are routinely carried out with

Poecilia reticulata (guppy)

Mytilus edulis (common mussel)

Toxicological studies with non-human organisms

In this field a distinction must be made between the direct establishment of effects in specific tests and mode of action studies.

Specific tests for single effects

In general acute, sub-acute and chronic tests are carried out with single species chosen to represent different ecological systems.

The criteria used to examine the state of health of the animals include: mortality, growth, embryo development and reproduction

Several types of tests are routinely performed:

acute

Poecilia reticulata (guppy)

Daphnia magna

Crustaceans, molluscs

Algae, fungi, bacteria

sub-chronic and chronic

Daphnia magna

Artemia salina (brine shrimp)

Chactogammarus marinus (gammarids)

Ophryotrocha diadema (polychete worms)

embryotoxicity

Lymnaea stagnalis (common pond snail)

plaice

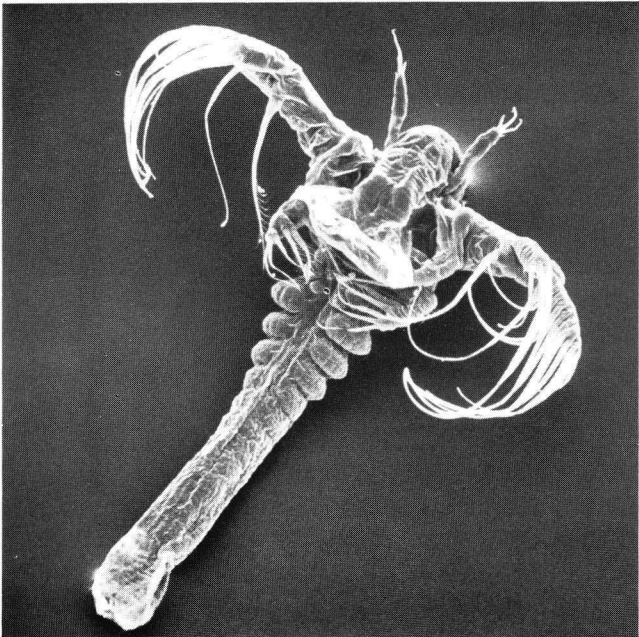
trout

mutagenicity

Ames-test using *Salmonella typhimurium* and several other organisms, with or without bioactivation

sister chromatid exchange test (SCE) using Chinese hamster ovary cells with or without bioactivation

Umbra pygmaea



DNA breaks using Chinese hamster cells
induction of base analog resistance using Chinese hamster cells
in vitro cell transformation tests with BHK cells
tests with multi species systems, and field studies
marine plankton community tests
soil respiration tests
biological monitoring with fresh water or marine mussels, or other animals
distribution of pollutants can be studied by chemical analysis of water or mud
samples and/or by analysis of suitable indicator organisms

Mode of action studies

No specific tests are mentioned under this heading, as such studies usually combine the results of several tests adapted to the specific problem under investigation. Results of specific test recorded in previous sections may be included in such investigations. Special facilities are available for the study of the mode of action of antimicrobial agents.

Technical facilities

Cup-shaped mitochondria in rats pancreas
fed 4 weeks with 1,000 ppm tin 40,000X

Apart from the usual analytical techniques the following advanced facilities are available:

GC-MS, including data-systems

high pressure liquid chromatography

neutron activation analysis

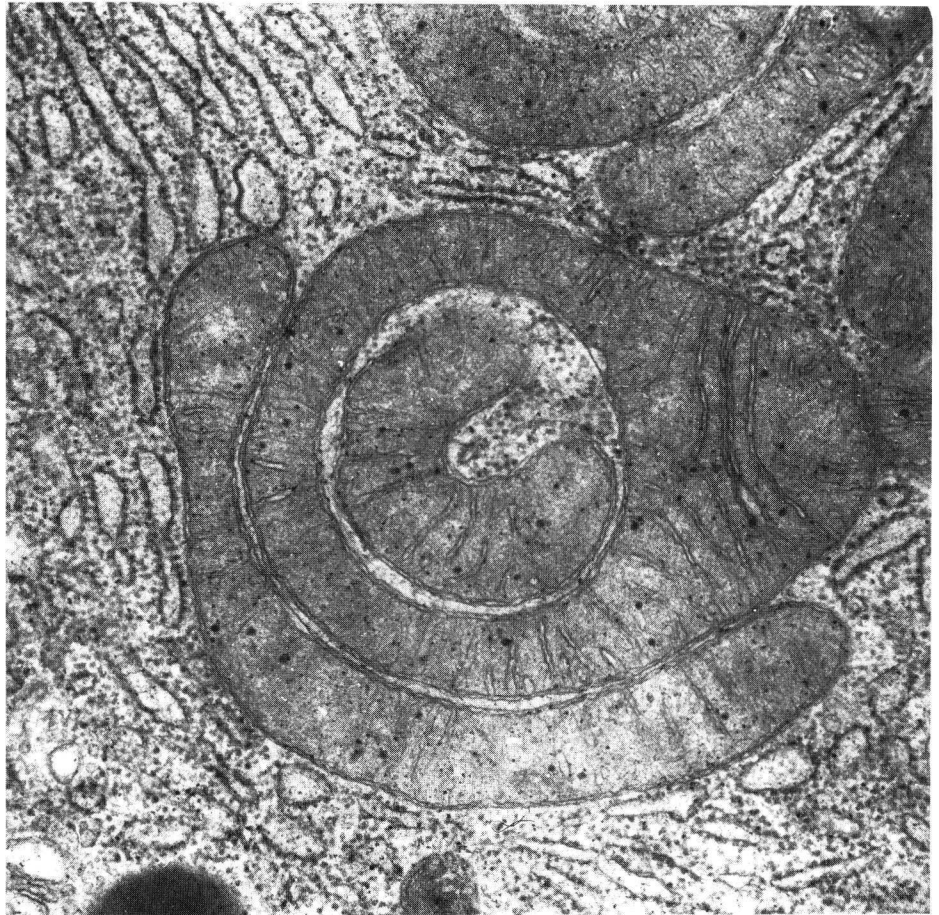
nuclear magnetic resonance spectroscopy

electron microscopy (REM and SEM)

automatic amino acid analyzers

raman spectroscopy

atomic absorption spectrophotometer with heated graphite tube



Products or kind of products usually tested for toxic effects

Some types of products to be tested

new foods (proteins, modified starches, etc.)
food additives and food contaminants
packages and consumer goods (in contact with foods)
cosmetic and toilet articles
household chemicals
feed additives
disinfectants
insecticides and other pesticides
drugs for human use
drugs for veterinary use
polymers for biological applications
new industrial chemicals
chemical products for human use
raw materials
semi-manufactured articles
end products
paints
colouring matters
building materials
chemical wastes from industrial and agricultural production



Institutes

The experimental work in the field of toxicity is carried out in the following institutes each having their own specific scientific know how.

Central Institute for Nutrition and Food Research (CIVO)

Department of biological toxicology

In the period 1940–1960, animal experiments in the CIVO were restricted to the field of foods and nutrition. Growing need for toxicological information, however, stimulated TNO to enter the field of toxicity testing and this work has been carried out in the department of biological toxicology since 1961. More than 15 years of experience have shown that the reports from this department are accepted in many different countries as a basis for approving the use of chemicals and other products. The introduction of toxicity testing has been accompanied by a rapidly increasing demand for toxicity studies from industry, both in The Netherlands and abroad. The background in nutrition remains a special characteristic of the present toxicology programme and this has turned out to be of major advantage in the safety evaluation of industrially treated or new foods since such testing requires knowledge and experience in experimental nutrition and facilities for the preparation of special diets.

Department of toxicological analysis

This department is engaged in analytical studies of toxic contaminants of food and feed, where contaminants are defined as substances leaving residues in food after use in production processes, or by migration from packaging materials.

The department has wide experience in residue studies on pesticides in food and feed. Measurements of test substances in organs of animals are carried out as a component of mammalian toxicity tests. Concentrations of these substances and of contaminants in animal diets are routinely checked.

Monitoring of persistent organic chemicals in wildlife samples (birds, eggs, fish) has also taken place in this department since 1966.

Department of general food chemistry

In this department a variety of chemical analyses are carried out in feeds and physiological fluids as part of toxicological investigations. In addition, analyses of poisonous trace elements and toxic compounds (such as nitrosamines) formed during processing of foodstuffs are carried out.

Location: Utrechtseweg 48, 3704 HE Zeist, The Netherlands
Postal address: P. O. Box 360, 3700 AJ Zeist, The Netherlands
Contact persons:
Biological toxicology: Dr A. P. de Groot
Toxicological analysis: Ir R. H. de Vos
General food chemistry: Dr P. Slump

Division of Technology for Society (MT)

Department of Biology

The biology department of the division of Technology for Society is located in the 'Zuidpolder' complex, Delft.

In its activities it is supported by a wide range of facilities in other disciplines (e. g. analytical chemistry) available within the same division. The activities include both large projects for the evaluation of environmental impact, mainly of chemical agents, and routine testing according to official or industrial specifications. In addition, contributions are made to integrated environmental research projects (pollution abatement, monitoring, waste problems).

The department carries out both laboratory tests and field-studies; for the latter it has a field station in Den Helder at its disposal.

The main effort is dedicated to aquatic tests, but ecotoxicological tests in relation with soil-, air and water pollution are also carried out in this institute.

Details of these tests are described in the handbook 'De bepaling van potentiële milieu-effecten in het water, van chemicaliën en afvalwater: Afbreekbaarheid, Toxiciteit, Bioaccumulatie', Central Laboratory TNO (1977). This manual of methods was produced at the request of The Netherlands Government Department of Transport and Public Works, North Sea Directorate.

A limited number of copies of the Netherlands edition is available on request to this department (An English translation is in press).

Location: Schoemakerstraat 97, 2628 VK Delft, The Netherlands

Postal address: P. O. Box 217, 2600 AE Delft, The Netherlands

Contact person: Dr H. J. Hueck

Institute for Organic Chemistry (OCI)

The department of biochemistry and microbiology of the OCI has a long tradition of activities in the field of plant protecting agents, especially fungicides.

In the field of toxicology, facilities are at present available for the study of degradation of chemicals in plants or soil, both in the laboratory and in the field. In addition, mobility of chemicals in soils can be studied. Microbiological conversions of compounds by pure cultures of bacteria or fungi are also subjects of study. All investigations are supported by the extensive analytical facilities of the institute. The study of the toxicity of compounds for micro-organisms comprises investigations on the biochemical mode of action of fungicides and bactericides. Toxicity of chemicals for the microbial population in soil can also be determined.

Location: Croesestraat 79, 3522 AD Utrecht, The Netherlands

Postal address: P. O. Box 5009, 3502 JA Utrecht, The Netherlands

Contact person: Dr A. Kaars Sijpesteijn

Medical Biological Laboratory (MBL)

In the MBL a number of research projects concerned with the influence of toxic compounds and ionizing and non-ionizing radiation (micro-waves, radar) on the organism are in progress.

In general this research is performed with small mammals (mice, rats, guinea pigs); occasionally the bacterial model is used, and rarely humans. Research is especially directed towards the influence of the above mentioned factors on the CNS, muscle, the cardio-vascular system, the immune system and the lungs, as well as on mutagenesis and DNA-repair mechanisms. The direct consequences of exposure to the compounds and radiations mentioned and also their mode of action and the possibilities for prevention and treatment of their consequences, are being investigated.

An important part of the research program is directed towards problems of industrial toxicology, 'biological monitoring' and early detection of physical damage. As far as the latter is concerned the program attempts to include both background research and short- and long term routine testing for toxicological damage.

Location: Lange Kleiweg 139, 2288 GJ Rijswijk, The Netherlands

Postal address: P. O. Box 45, 2280 AA Rijswijk, The Netherlands

Contact person: Dr W. F. Stevens

Prins Maurits Laboratory (PML)

Institute for Chemical and Technological Research

The objective of the chemical research in this institute is protection against toxic compounds in the environment, primarily protection of the armed forces against chemical warfare agents. The institute has 30 years of experience in this field.

The increased demand, especially during the last decade, for research on environmental protection and occupational health in industrialized and densely populated areas, has widened the scope of the work of the institute and its cooperation with other disciplines in TNO.

The institute has modern facilities and highly skilled personnel to investigate compounds with extreme acute toxicity using analytical, organic, physico chemical, biochemical and physical techniques. In the field of mammalian-, and ecotoxicology the institute has special experience in:

synthesis of (labeled) toxic compounds

percutaneous absorption studies

sampling and identification in the atmosphere, water, soil and biological material

photodecomposition studies

Location: Lange Kleiweg 137, 2288 GJ Rijswijk, The Netherlands

Postal address: P. O. Box 45, 2280 AA Rijswijk, The Netherlands

Contact person: Dr H. Kienhuis

REP-institutes

REP is the designation for three collaborating institutes of the TNO Organization for Health Research TNO: the Radiobiological Institute, the Institute for Experimental Gerontology and the Primate Centre.

Their contribution to toxicology studies is related to the special facilities which are available in this institutes. The facilities for study of late effects of radiation have made it possible to study the toxicity, carcinogenicity and other late effects of radioactive compounds in rodents. The availability of different species of monkeys made it possible to study agents which potentially enhance arteriosclerosis while the availability of germ-free animals provide possibilities for the analysis of the effect of the intestinal micro flora on the activation or inactivation of toxic compounds.

Location: Lange Kleiweg 151, 2288 GJ Rijswijk, The Netherlands

Postal address: P.O. Box 5815, 2280 HV Rijswijk, The Netherlands

Contact person: Prof. Dr L. M. van Putten

TNO

TNO is the Netherlands abbreviation for the Organization for Applied Scientific Research in the Netherlands.

Established in 1930 by an act of Parliament, TNO's aims are described as 'to ensure that applied scientific research is put at the service of the community in the most efficient manner possible'.

In the more than 45 years since its foundation, TNO has become one of the largest Research and Development (R&D) organizations in the entire Western world with almost 5000 employees, 1000 of which have an academic degree. R&D is carried out in 35 well equipped laboratories, called the TNO 'institutes'. The TNO-act specifies that the Government will grant TNO an annual subsidy. Another important source of revenue is payment for R&D carried out for government, provincial and local authorities, consumer organizations, and national and international industries and sponsors throughout the world. In 1979 the total budget of TNO amounted to 500 million Dutch guilders.

The research and development activities of TNO can be divided in different main sectors, such as: industrial research, health research, nutrition and food research and national defense research.

For general information concerning TNO and its work consult:

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Design

BSRS, Amsterdam

Typesetting and printing

Drukkerij Vijlbrief, Haarlem

Co-ordination

Information Department TNO, The Hague

and Ms Flora de Vrijer, Zeist

Photography

Aerocarto KLM, p. 12 and 15