ed. In addition to all the ecological problems with polychlorinated organic compounds one should not forget the use of chlorine as a chemical weapon in early 20th century wars, and even chlorination of municipal water, important as it is in terms of public health, is creating problems according to recent reports. Concerning the situation with polychlorinated compounds, TCDD will not necessarily have been the last surprise. The past surprises have been caused by lack of knowledge. In order to avoid them in the future, information is urgently needed with respect of aspects such as the toxicity of individual compounds, extrapolation of toxicological data to men, long-term effects, risk assessment, and interactions with biological systems. However, to sit and wait for more information is not enough. Action must be taken, and this must be done on an international scale since contamination does not respect national boundaries. A concrete, if radical proposal would be to stop production of polychlorinated compounds altogether. This may not be unrealistic in light of the successful restriction in the use and production of DDT in the 1960s and PCBs in the 1970s and their substitution by second-generation products.

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## Genetic hazards

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Among toxic effects on humans the non-reversible ones are of particularly great concern. Important effects of this kind are damage to the eye lens and to the nervous system, and the teratogenic, carcinogenic and mutagenic effects. The last group is characterized by a long latency period between initiation of the damage and the phenotypic manifestation of the induced change. In particular, genetic damage, when present in cells of the germ line (mitotically dividing pre-meiotic cells and germ cells), may be expressed many generations after the primary induction of the genetic change, the mutation. Genetic injuries to somatic cells, even though they may exhibit very strong effects, e.g. play a so far not yet well understood role in the initiation of malignant transformation and tumor formation, will not add to the genetic load of future generations. They disppear from the population with the death of the carrier.

Has the chemical induction of mutations been proved for human cells? Are chemical mutagens known which can alter the human genetic material? For somatic cells this is proved daily by the therapeutic effects of genotoxic carcinostatic drugs. In this case human cancer cells are killed as a result of the chromosomal damage induced by the drugs. Mutagenic effects from a number of causes are also known for other human cells, e.g. peripheral lymphocytes (Natarajan and Obe, 1980). In contrast to this, chemically induced mutations in germ cells have not yet been proved. This lack of scientific proof of induced human germ line mutations results primarily from the tremendous technical difficulties which, at the present time, do not allow one to distinguish between rare spontaneous and rare induced mutations. On the other hand, even if direct proof is lacking, there is no scientific basis for rejecting the

argument that, in humans as in other mammals, chemicals can reach the germ cells and induce the whole spectrum of mutations possible. If one accepts this hypothesis, the next question to consider is: How long can a freshly induced mutation survive in the human gene pool? This strongly depends on the type of mutation induced.

Polyploidy is, with respect to the amount of genetic material involved, the largest genetic change. Occasionally triploid fetuses in humans (e.g. resulting from double insemination of an egg) can develop quite extensively, but the result is the premature birth of a non-viable baby. Thus polyploidy is immediately eliminated and does not add to the variation of the genetic material transmissable to future generation.

Aneuploidy is a numerical change in the chromosome set and may result from nondisjunction or chromosome loss. Since the viability of humans and mammals strongly depends on a blanced genome, most of the aneuploidies are lethal. Exceptions are numerical aberrations of the sex-chromosomes and some small autosomes. Trisomies for chromosomes 13 and 18 may survive until birth and trisomy 21 (Down syndrome) and various sex-chromosome aneuploidies can survive until the reproductive age. The transmission of the aneuploidy by the affected patient to the next generation is very rare. In experimental eukaryotic systems (fungi, plants, insects, mammals) polyploidy and aneuploidy can be induced by spindle poisons which do not directly interact with the genetic material. Aneuploidy can, in addition, result from the action of chromosome-breaking agents.

Other mutations, the chromosome aberrations, affect only parts of chromosomes. Smaller or larger sections of chromosomes may be deleted, duplicated, inverted or translocated. These mutations can affect the health of the carriers via disturbance of the gene balance, and in the case of heritable reciprocal translocations, meiotic chromosome segregation can lead to unbalanced gametes. With the exception of very small changes and the balanced types, all aberrations can be expected to be eliminated from the human gene pool shortly after appearance, usually within the first generation. Experimentally, aberrations are induced by chromosome-breaking chemicals, e.g. alkylating agents.

Gene mutations can be very small changes in the DNA, e.g. the replacement of only one base pair by a 'wrong' one (base pair substitution). Such a mutation usually results in the substitution of 1 amino acid in the polypeptide for which the particular gene is coding. It may eventually lead to a non-functional gene product. A very different type of gene mutation results from additions or deletions of base pairs from the DNA sequence. In this case the reading frame used in the translation of the base sequence into an amino acid sequence is shifted following the mutant site. Addition/deletion mutations are therefore also called frameshift mutations. In general a shortened peptide with a tail of wrong amino acids will result.

In humans nearly 3000 genetic traits are known which show Mendelian inheritance, and the majority of which result from various kinds of gene mutations (McKusick, 1978). Among newly-induced gene mutations, those which are neutral or beneficial in the heterozygous condition but deleterious in the homozygous condition demand a high level of concern for subsequent generations. This group of mutations, if induced in cells of the germ line, has the highest probability of becoming part of the genetic load of human populations and may become expressed many generations after the initial carriers have died.

How large is the genetic load of human populations today? A number of studies are available which deal with this difficult problem. Although details of the results vary, the overall picture is always the same. In general one finds that about 1.5% of the live-born children suffer from clinically well-defined genetic diseases. The table gives an example how the different types of mutations are represented. An estimate of the total genetic burden is extremely difficult to obtain because, in addition to the well defined traits, a large number of polygenic traits which are characterized by a complex inheritance also contribute to the load. It is estimated that maybe 2-3% of the population suffer from mutations in such traits, which can lead to cleft lip, spina bifida, clubfoot, congenital heart defects, etc. (see Brusick, 1980). If one realizes that on a worldwide scale these abstract percentages are represented by thousands and thousands of affected people, it is obvious that all possible measures should be taken to avoid any additional increase of this load.

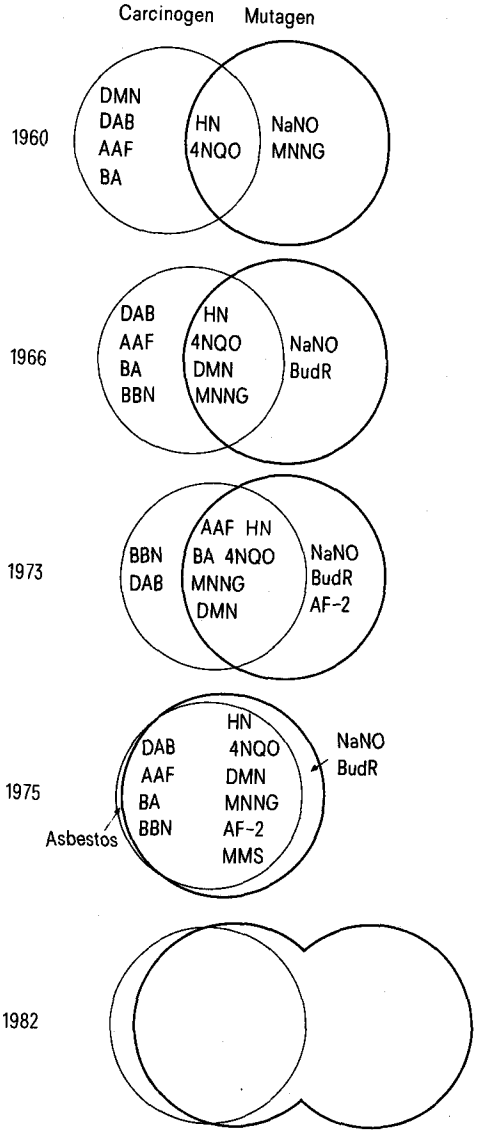
This goal can only be reached if it is possible to detect mutagenic hazards and if measures are taken to eliminate avoidable exposures. To define mutagenic hazards 2 steps are needed: the 1st step is the experimental detection of chemicals which have a potential for inducing genetic damage; the 2nd step is the risk evaluation for exposed humans. The latter is particularly important if for one reason or another a certain exposure of people in the reproductive age turns out to be unavoidable (e.g. special medications).

Potential genotoxic activity of chemicals can be detected if suitable screening systems are available. The development of such systems has to proceed from a basic idea to the definition of a practical protocol which has then to be used with a large number of reference compounds and tested in different laboratories. Only after such a validation phase, which as far as possible should also include collaborative studies, can a test be used for routine screening.

The most famous test, which was developed according to this theoretical scheme is the *Salmonella*/mammalian microsome assay (Ames et al., 1977). The test is based on reversion of base-pair substitution and frameshift mutations leading from histidine auxotrophy to histidine prototrophy. Several measures have been taken to make the test as sensitive as possible for the detection of a large variety of mutagens: increased penetration of chemicals into the cells, due to the rfa mutation, leading to a defective coating of the bacterial surface (Ames et al., 1973a); sensitization to mutagenic effects by eliminating error-free DNA excision repair (Ames, 1971); increased mutagenesis from induced lesions due to the presence of the plasmid pKM101 (McCann et al., 1975); and inclusion of essential parts of the mammalian xenobiotic metabolism by addition of mammalian liver microsomes (Ames et al., 1973b). With the aid of the microbial screening tests using *Salmonella* as well as *E. coli,* a large number of chemicals were identified as mutagens. At the same time an increasing number of chemicals was found to be carcinogenic in mammalian assays. The interesting development taking place from 1960 to 1975 was the discovery of the increasing overlap of mutagenic and carcinogenic potential of chemicals. This can be shown elegantly using the Sugimura-Matsushima-Circles (fig.). They are also useful to demonstrate the most recent development. As we see in the last graph, after 1975 an ever increasing new 'bubble' grows out of the circle representing the mutagens. It represents those chemicals which turn out to be mutagenic in microbial systems but for which no information concerning possible carcinogenic effects is available. The question immediately arises: Are all these chemicals carcinogens?

One might suggest the use of animal testing to identify the carcinogens among those bacterial mutagens. Although animal testing, mainly in rats and mice, is still a key method for detection of carcinogens, these studies are long-term, very expensive and require much manpower. In addition we have to realize how tremendous the job is that has to be done. Already by the end of 1977, the Chemical Abstract Services registered well over 4 million compounds, of which about 53,000 are estimated to be in general use (Maugh, 1978). Even if only 1% of all compounds turn out to be mutagens, that would mean a sample of the order of 40,000–50,000 chemicals that have to be tested for carcinogenicity. And the number of compounds synthesized as well as the number of natural compounds isolated grows steadily. If we look at the situation realistically, we have to conclude that neither the time needed, nor the costs involved, nor the whole world's testing capacity (about 300–500 compounds per year) allow of a direct approach.

An alternative indirect approach could be the verification and extension of bacterial test data by addi-




The Sugimura-Matsushima-Circles showing the chronology of overlapping of carcinogens and mutagens (supplemented after Sugimura et al., 1976).

Abbreviations: AAF, 2-acetylaminofluorene; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; BA, benz(a)anthracene; BBN, butyl-N-(4-hydroxybutyl)nitrosamine; BudR, 5-bromodeoxyuridine; DAB, N,N-dimethyl-4-aminoazobenzene; DMN, N,N-dimethylnitrosamine; HN2, nitrogen mustard; MMS, methyl methanesulfonate; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; 4NQO, 4-nitroquinoline 1-oxide.

Frequencies of genetic disorders at birth based on studies involving over 55,000 births from different parts of the world (Carter, 1977)

| Type | | Incidence (%) |
|---|---|---|
| Unbalanced structural chromosomal anomalies | | 0.45 |
| Gene mutations | autosomal dominant | 0.90 |
| | autosomal recessive | 0.25 |
| | X-linked | 0.05 |
| Total | | 1.65 |

tional eukaryotic short-term tests. Overall, more than 130 test systems have been suggested to be useful screening assays (Hollenstein et al., 1979). Two strategies may be followed to reach the goal of identifying mutagens and genotoxic carcinogens (for detailed discussion see Brusick, 1980):

a) The hierarchical, or tier approach goes through a number of tests in a hierarchical order. The tier approach appears to be the most cost-effective approach to testing but often extends the testing time to unacceptable limits since each subsequent tier must wait until testing from the previous tier is completed.

b) The battery approach uses a number of different supplementary tests. Each short-term test has its own inherent strengths and limitations which, when used in concert, will overlap and compensate for intrinsic limitations.

Today, the tendency is toward the use of a minimal battery. Some guidelines which recommend a battery approach have identified different types of tests to be included in the battery, primarily according to the different endpoints which should be scored. What is still lacking at present is a set of scientifically sound criteria by which the members of a test battery can be selected from among the large variety of available tests.

A number of efforts have been initiated in order to arrive at these criteria. Systematic comparisons of test systems have been reported by Purchase et al. (1978) and most recently by Ashby et al. (1982). In a large-scale international study, 65 investigators studied with united efforts 42 coded compounds in a wide spectrum of different tests. The outcome of the study (de Serres and Ashby, 1981) confirmed the usefulness of short term tests for the detection of mutagens and genotoxic carcinogens. On the other hand, the results stressed the problems related to the routine use of the tests. Not one single test was able to give only correct answers, and quantitative reproducibility is at present limited. Therefore, for routine use a battery approach is inevitable, but a number of problems remain to be solved. Some of the particular problems pin-pointed by the International Study will be solved in a continuation study. This collaborative study on Short-Term Tests for Genotoxicity and Carcinogenicity is organized within the International Program on Chemical Safety of the World Health Organizations and will be started during the second half of 1982.

A different approach to coordinating international efforts aimed at defining problem areas in genetic toxicology, such as updating knowledge in particularly important areas, and supporting international cooperation etc. was taken by the foundation of the International Commission on Protection from Environmental Mutagens and Carcinogens (ICPEMC). The goals of this organization were described in the ICPEMC News No. 3 and a number of reports were published in Mutation Research, and others are in press.

During the last decade more and more people realized that a validated data base existed only for a very few tests. The literature is continuously collected and processed by the Environmental Mutagen Information Center (EMIC) at Oak Ridge National Laboratory (Wassom, 1973). Although the very successful work of EMIC is of indispensable value for investigators all over the world, a complementary data base containing validated actual test data turned out to be urgently needed. The creation of such a computerized retrieval system was therefore incorporated into the diverse efforts of the GENE-TOX program. The concept of the program is described by Green and Auletta (1980) and reports are continuously published in the Review Section of Mutation Research (see vol. 76, 86, 87, 98, 99). As more and more expert groups have finished their reports on particular test systems, the work of GENE-TOX phase 2 can proceed. Among the goals of this work is the definition of adequate test batteries for particular groups of chemicals, and hopefully it will also be possible to define criteria for the selection of a test battery for general screening purposes.

Although screening tests are highly developed and widely used, the area of risk estimation urgently needs further development. There exist known and suspected exposures to chemical mutagens from medical treatment, from contaminants in the food and in beverages and from other sources such as smoking. Only in a few instances are risk estimates possible: some drugs and medical treatments, hycanthone, procarbacine, Mitomycin C, isoniazid (Ehling, 1980), sulfur mustard (Fox and Scott, 1980), psoriasis treatment (Bridges et al., 1981), the pesticide dichlorvos (Ramel et al., 1980), cigarette smoking (Bridges et al., 1979) and alcohol (Obe and Ristrow, 1979). All these references show how difficult it is to arrive at conclusive estimates based on epidemiological data. Even if relevant experimental data from mouse germ cells are available, many parameters contribute to the uncertainty of the numerical values derived to express human risk. In particular, any extrapolation from experimental systems to humans has to take into account possible differences in metabolism. Within the near future we urgently need innovative research which will help to bridge the gap between the steadily increasing data base from short term tests and the estimate of the risk for the human gene pool.

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## The chemical industry between regulation and self-responsibility

by J. Rutschmann

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The chemical industry seems to have the doubtful honor of being regarded by a considerable number of people, as *a,* if not *the,* major menace to human wellbeing. It threatens its surroundings and society at large by the catastrophe-proneness of its installations and processes and the unsafeness of its products, and it poisons the environment with its gaseous, liquid and solid wastes. Regrettably, there are real and very unfortunate cases that can be cited for exemplifying all three allegations. This bad image is the result - at least in part - of the psychological rules by which individuals and collectives intuitively view and assess the risks and benefits of a given situation or activity. From this psychological point of view chemistry or the chemical industry is in a particularly unfavorable position; its *hazards* - as illustrated and widely publicized by the incidents of past years - are perceived as uncanny, insidious, unavoidable, irreversible and unnatural - and to a certain extent not all of this can be contested. On the other hand its *benefits* under the trend towards romantic 'back to nature' ideas tend to be belittled, are argued away or not recognized at all. No wonder that an intuitive and emotional risk benefit assessment can easily come out against our industry! It is also evident that such a situation is a rewarding playground for a certain part of the media and of the political spectrum.

In my short talk I shall try to systematize the potential hazards posed by the chemical industry and its products, to put them into perspective and thus to point to what I consider to be the real areas of concern and problems to be solved or solved better.

I have to base my remarks on 2 simple axioms, one concerning the benefit, the other the risk side of the problem:

1. *The chemical industry is necessary*: it is indispensable for the maintenance of a standard of living in the industrial countries that evidently corresponds to the expectations of the vast majority of our societies; and it is necessary for assuring eventual coverage of the