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Genetic risks caused by occupational chemicals

Use of experimental methods and occupational risk group monitoring in the detection of environmental chemicals causing mutations, cancer and malformations

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Key words: occupational chemicals, cancer, mutation, malformation.

It is estimated that chemicals play a predominant role in the etiology of a majority of human cancers (14, 56). The best documented examples of the effects of chemicals are provided by malignancies associated with certain industrial processes (126). The possible genetic health hazards associated with chemicals, including carcinogenesis, are more difficult to evaluate in the human environment in general since exposure commonly involves very low concentrations, extends through a long life-span, and affects a large part of the population.

Chemical safety evaluation is of great concern in occupational hygiene. The preventive aspects are far-reaching, and in this review we merely note some specific points. More industrial epidemiology is

needed, and we see no reason why detailed records of occupational exposure to chemicals should not be compiled and incorporated into record linkage systems. More collaborative contacts are required between epidemiologists and laboratory scientists, and more experimental work is needed to help them evaluate the effects of chemicals with putative genetic risk, particularly in circumstances where workers may be exposed to mixtures of chemicals. Existing animal tests for carcinogenicity need to be refined, and new short-term tests must be devised which can provide accurate screening for potential carcinogens, mutagens, and teratogens. While the risk for carcinogens is of the most immediate concern, it should be born in mind that increased mutational risk may also have serious cumulative implications for human genetic health.

There are tens of thousands of untested chemicals in the human environment, and some attempt must be made to identify the ones that are potentially hazardous to man. While carcinogenesis in man has

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a definite end-point in the manifestation of a malignant tumor and is an emotive issue, the phenomenon of genetic damage in man is more difficult to demonstrate. Nevertheless, there is every reason to expect that environmental factors, both physical and chemical mutagens, constitute an important etiologic factor in genetic diseases and congenital malformations.

The emphasis in the treatment of human health problems should be changed from the high efficiency in medical care to more effective prevention of disease and ill health. The aim of the present review is not to stress the problems of occupational cancer and chemical hazards, but rather

to elucidate the possibilities for their prevention. Such prevention can be achieved through the use of short-term screening methods to predict long-term health hazards, including mutations, cancer and malformations, and to monitor workers at risk.

THE HUMAN CHEMICAL ENVIRONMENT

The increase in the number of different chemical substances in man's environment is due to the rapid strides of scientific and technical progress. The computer register of the American Chemical Society's Chemical Abstracts Service contained over 4 million distinct entities in 1977, with an average growth rate of about 6,000 per week (94).

It has been estimated that there are about 63,000 chemicals in common use, and other compounds are coming onto the market at a rate of perhaps 1,000 a year. It is problematic that the means for establishing the long-term effects of these chemicals are far from perfect. To date, the primary concern has been acute toxicity, and only recently have carcinogenic, mutagenic, and teratogenic properties been emphasized. Control of such chemicals is extraordinarily complex due to the difficulty of extrapolating experimental data to humans, the long latency period between exposure and adverse recognizable damage, the lack of public awareness of the threats to health, and the huge number of interfering chemicals.

There are about 25,000 occupational chemicals with a potential mutagenic hazard (92). By now about 7,000 chemicals have been tested for carcinogenicity in animals, and a little more than 1,500 have been reported to be suspected carcinogens. From critical evaluations the International Agency for Research on Cancer (IARC) has compiled a list of some 30 chemicals for which there is convincing evidence for carcinogenicity in humans. At present, the world capacity for the adequate animal testing of chemicals for carcinogenicity is about 500 a year (95).

Fig. 1. Structures of directly acting carcinogens and/or mutagens. Asterisks indicate the electrophilic atoms attacked by nucleophiles; broken lines indicate the bonds broken in nucleophilic substitution reactions.

MOLECULAR MECHANISMS IN CARCINOGENESIS, MUTAGENESIS AND TERATOGENESIS

Chemical carcinogens and mutagens have been observed to bind covalently to cellular macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins (128). However, the consequences of the binding of carcinogens to proteins or RNA are not yet understood in relation to malignant transformation. No simple hypotheses have been evolved to explain the long latency periods of such epigenetic changes or their transmission to daughter cells.

Some carcinogens and mutagens can react *directly* (fig. 1) with nucleic acids and other macromolecules in the cells without metabolic activation (152). However most chemical carcinogens and mutagens require a bioactivation process to be effective, i.e., they act *indirectly* (fig. 2). The biotransformation of xenobiotics (foreign compounds) is usually a two-step process.

First the compound is oxidized, reduced, or split in a hydrolysis reaction. Apolar compounds are usually oxidized by the monooxygenase system, a multienzyme complex attached to the endoplasmic reticulum. This enzyme machinery is the most active in the liver, but activity is also detected in extrahepatic tissues (145). The enzyme complex contains a family of cytochrome P-450s, which activate oxygen and bind the substrate. The immediate product of monooxygenation can be labile and reactive compounds such as epoxides, free radicals, and N-hydroxy compounds (fig. 2). These electrophilic products readily react with the nucleophilic sites of proteins and nucleic acids (74, 101, 152).

The second stage of biotransformation, conjugation, involves the addition of an intermediary metabolite or other chemical structures to the functional group formed in the first stage of biotransformation. In mammals the metabolite is usually a glucuronic acid or a sulfuric acid residue. Other important metabolites include amino acids such as glycine (hippuric acid synthesis) or amino acid derivatives such as glutathione. Epoxides can also conjugate with water to form a dihydrodiol in a reaction catalyzed by epoxide hydra-

tase. The conjugation stage increases both the molecular weight and the polarity of the compound. Consequently, the conjugated compound is excreted in bile or urine. The conjugation reaction usually completely inactivates the compound, and thus the reaction is a true detoxication (1). There are, however, a few cases in which the conjugate is more active than the parent compound (1, 121).

Chemical carcinogens and mutagens can bind covalently or noncovalently to DNA. Covalent bonds may be stable for extended periods of time and therefore are possibly related to carcinogenic and mutagenic mechanisms. However, even noncovalent binding such as intercalation may induce mutations. Carcinogens are reported to bind covalently to nucleic acid bases or to phosphodiester bonds (128). All four types of bases and particularly their N and O atoms exhibit binding. Generally, binding to purine bases (adenine and particularly guanine) is more active than binding to pyrimidine bases (fig. 3).

Chemical carcinogens are thought to act by causing errors (mutations) in DNA replication (41, 91, 178). Mutations can be divided into two categories, microlesions and macrolesions (17, 41). In eucaryotes (higher organisms), both types of muta-

Fig. 2. Examples of indirectly acting carcinogens or mutagens of industrial importance [world production over 500,000 tons per year (40)].

tions can be observed: (a) microlesions at the molecular level (point mutations) or (b) macrolesions at the chromosomal level, consisting of structural changes in the chromosomes (deletions, rearrangements, breaks) or of numerical changes in the genome (aneuploidy, polyploidy) (fig. 4). In prokaryotes (bacteria), possessing a simple genetic machinery, mutations may only occur at the molecular level as alterations in the nucleotide sequence of DNA, i.e., base-pair substitutions or frameshift changes.

Fig. 3. Some sites of attack by chemical carcinogens in DNA (PAH = polycyclic aromatic hydrocarbons, A = aromatic amines; B = bifunctional alkylating agents; N = nitroso compounds) [Modified from Sarma et al. (128)].

Point mutations can be caused by a misreading of a base. This type of point mutation is called a base substitution mutation. Such mutations are frequently caused by small DNA adducts, e.g., methylating agents. If the DNA base is changed from one purine to another (or from one pyrimidine to another), it is called a transition. If the change leads to mutation from purine to pyrimidine (or vice versa), it is called a transversion.

Bulky carcinogens, e.g., aromatic amines, may cause errors in DNA replication due to the insertion or deletion of nucleotides. This type of point mutation is called a frameshift mutation. Conceivably, the cross-linking of DNA strands, caused by bifunctional alkylating agents, may also lead to point mutations.

The generation of microscopic mutations (macrolesions) is poorly understood. It is known that covalently bound carcinogens may cause breaks in the sugar-phosphate backbone of DNA by at least two mechanisms. Carcinogens may bind to phosphoric acid residues to form labile phosphotriesters; depurination, resulting from certain types of carcinogen-purine adducts, also labilizes phosphodiester bonds. After DNA replication a single-strand break would lead to a double-strand break, probably observed also microscopically (128).

An increased body of evidence indicates that the same chemicals may exhibit mutagenicity, teratogenicity, and carcinogenicity (table 1). It is very likely that, at least in these cases, the chemicals exert their teratogenic and carcinogenic effects by inducing mutations. Teratogens, as well as mutagens, may act directly or after metabolic activation, and form covalent DNA adducts. Many electrophilic reactants and bifunctional alkylating agents have been shown to act by such mechanisms (54). The action of the teratogens requiring metabolic activation may be enhanced by inhibitors of fetal epoxide hydratase (11). Glutathione appears to have a protective function against the action of some teratogens, as its depletion with diethyl maleate increases teratogenic potential (54). Therefore, it is reasonable to suspect that the teratogenic effects of some chemicals may result from metabolic activation, particularly if it occurs in the

Fig. 4. Classification of various mutational types (see text).

embryonic tissue during organogenesis. The human fetus may be especially vulnerable to transplacental chemicals because of the early development of the enzymatic biotransformation system (116).

There are also differences between mutagens, teratogens, and carcinogens. While carcinogenesis may be caused mainly by chemicals (mutagens) binding covalently to DNA, mutagenesis and teratogenesis may additionally be brought about by noncovalent interactions such as intercalation. While mutations and malformations are caused by chemicals interfering with DNA replication, such as base analogues and spindle (microtubule)

poisons, malformations may additionally be brought about by a large number of chemicals (e.g., antimetabolites, amino acid antagonists, enzyme inhibitors and nonspecific cytotoxins) exerting their effects on functions other than DNA replication (156).

DNA lesions can be assayed by a number of techniques. Mutations, for example, can be scored in microbial and eucaryotic tests (discussed in the section Short-Term Screening Tests). Special tester strains of bacteria have been developed to differentiate frameshift and base-substitution mutations. DNA breaks may be assayed for in alkaline sucrose gradients, which

Table 1. Comparison of experimental mutagenic, carcinogenic and teratogenic effects of some chemicals. [Modified from Brusick (17) and Harbison (54)]

Substance	Mutagen ^a	Carcinogen ^a	Teratogen ^a
Polycyclic aromatic hydrocarbons			
Benzo(a)pyrene	+	+	+
Benzanthrane	+	+	+
Methylcholantrene	+	+	+
Nitroso compounds			
Dimethylnitrosamine	+	+	+
Methylnitrosourea	+	+	+
Bifunctional alkylating agents			
Busulfan, cyclophosphamide, myleran	+	+	+
Nitrogen mustard	+	+	+
Other compounds metabolized to electrophilic reactants			
Benzene	(+)	±	+
Urethane	+	+	+
Acetylaminofluorene	+	+	+
Cycasin	+	+	+
Aflatoxin B ₁	+	+	+
Carbon tetrachloride	—	+	+
Metals			
Mercury, organic	+	..	+
Lead, salts	(+)	+	+
Arsenic, Na ₃ AsO ₄	(+)	+	+
Cadmium, CdCl ₂ , SdSO ₄	+	+	+
Anesthetics			
Halothane	(+)	..	+
Pesticides			
Captan	+	+	+
Folpet	+	+	+
Tobacco smoke	+	+	+

^a + = positive; (+) = insufficiently positive; ± = contradictory; — = negative.

give an indication of the molecular size of DNA (23, 158). DNA replication errors can be measured in vitro with purified DNA polymerase and synthetic templates. Errors in DNA replication (infidelity) have been shown to increase, when the template has been treated with known carcinogens (135).

Cells have mechanisms that tend to remove carcinogens bound to DNA and to repair the lesions introduced. The repair mechanisms consist of several functions such as error recognition and degradative (endonuclease and exonuclease) and synthetic (polymerase and ligase) functions (22). Three different repair processes have been identified in mammalian cells: (a) photoreactivation repair repairing ultraviolet damage, (b) excision repair occurring throughout the cell cycle, and (c) postreplication repair confined to the period of DNA replication.

The first evidence of the importance of DNA repair mechanisms as derived from

fibroblast cultures was obtained from patients suffering from *Xeroderma pigmentosum*, an inherited skin disease. The patients are known to be sensitive to ultraviolet light, which induces skin tumors. DNA repair activity in fibroblasts obtained from the patients is very low, and thus their DNA is vulnerable to damage. Direct evidence has later accumulated revealing that the DNA repair mechanisms are able to remove chemical carcinogens from DNA and repair the strand (22, 133). Consequently, the activity of DNA repair mechanisms possibly influences the dose-effect relationships of carcinogen action. Even so, there is not necessarily a threshold for the action of chemical carcinogens and mutagens.

BIOLOGICAL SIGNIFICANCE AND MANIFESTATION OF MUTATIONS IN MAN

Fig. 5. Schematic representation of mutations induced in germ cells (germlinal) and in somatic cells during embryonic (early) or postnatal (late) development. Only germlinal mutations can be passed on to the next generations, while somatic mutations are only transmitted to the cell line derived from the mutant cell.

Mutations are stable heritable changes in genetic material. They are the primary source of genetic variation and thus a necessary prerequisite for all organic evolution. According to experimental evidence a large portion of the mutations induced in higher organisms are deleterious or lethal to the individual. Thus it is only logical to suppose that an abrupt significant increase in the mutation frequency of any species, including man, is a serious threat to the future of the species. Due to the recessive nature of most mutations the consequences of the genetic load, i.e., hereditary diseases and disorders, would be manifested only generations later — even if the load would result from mutations induced by the mistakes of the present generation.

Mutations that cause sterility, embryonic loss, or early lethality are detrimental in their biological fitness value, but have little impact on society. Thus mutations that are more fit biologically may actually be a heavy burden on society, if the affected persons require lifetime medical or institutional care.

Another extension of the mutational event in multicellular organisms relates to

the position of the target cell in the individual. Mutations that occur in somatic cells are transmissible only horizontally to daughter cells, whereas those that occur in the germ-line cells may be transmitted vertically to subsequent generations. This situation implies that their manifestations may be completely different (fig. 5).

Germinal point mutations from generations back form the human genetic load which occasionally manifests itself, e.g., as rare recessive genetic diseases. About 2,800 of such monogenous disorders are already known, and it has been estimated that about 2 % of the human population is affected by monogenous defects (98).

Constitutional chromosomal changes usually become manifest as severe malformations or syndromes (34). Among newborns the prevalence of chromosomal changes is about 0.6 %, and more than 90 % of such changes derive from new mutations in the parental germ cell. The most frequent chromosomal aberration found among newborns is the Down syndrome (mongolism or trisomy-21), which occurs at a ratio of 1/650 newborns. Sex chromosome aneuploids, such as the Klinefelter syndrome in males (with the chromosome constitution 47,XXY) or the Turner syndrome in females (with the chromosome constitution 45,X) are found in the population at a rate of 2.5/1,000 newborns. However, inherited germinal chromosome aberrations are subject to severe selection during gestation, so that

the frequency of chromosomal aberrations among spontaneously aborted fetuses or stillbirths is in the range of 50 %. These aberrations mainly consist of aneuploids, i.e., numerical changes in the chromosomes such as trisomies (~ 40 %) , X-monosomy (~ 20 %) and polyploidy (~ 20 %) (20, 138). This phenomenon reveals that certain abnormal chromosome constitutions, e.g., polyploidy and most monosomies, almost invariably lead to death of the fetus.

Knowledge of the biological manifestations of somatic mutations is far less documented. There is increasing evidence in favor of the role of somatic lesions in the etiology of cancer (19, 52). While tumor induction and malignant transformation may both be multistage processes, genetic mechanisms are likely to be involved in various ways, and the genomic information may not only be affected at the molecular level, but also at the chromosomal one. In fact, recent staining techniques producing microscopically identifiable chromosome banding have provided interesting evidence on specific chromosomal alterations in certain human cancers (90, 125).

Furthermore, somatic mutations may be involved in cellular — and finally individual — aging processes (110), and even in the development of some constitutional and degenerative diseases (9, 28) (fig. 6). Cardiovascular diseases have also been suggested to be due to irregularities of

Fig. 6. Possible manifestations of somatic and germinal mutational events in man.

gene expression (76). The proportion of congenital malformations caused by mutations — either germinal or somatic during embryonal development — is still a matter of controversy. Some estimates vary between 20—30 % (153). Theoretically, as well as from experimental evidence with known mutagens, it can be expected that probably even a higher percentage of congenital malformations is due to chemicals acting transplacentally at certain sensitive stages of organogenesis.

SHORT-TERM SCREENING TESTS

Mutagenicity bioassays

The basis for mutagenicity tests with experimental objects derives from the fact that the genetic material is similar in all living organisms. Furthermore, the transmission of genetic information, as well as its transcription, basically follows the same mechanisms in all eucaryotes. Therefore, experimental demonstrations of mutagenic activity always indicate a potential risk to human genetic health. According to experimental evidence with short-term mutagenicity tests there is a high correlation also for a carcinogenic risk of the chemical (96, 150). Thus the detection of compounds capable of inducing mutagenic alterations in the experimental genome also

points to the carcinogenic and possible teratogenic risk of this compound.

Around 100 different mutagenicity assays have been more or less widely used to detect mutagenic compounds in the environment. The various tests can be grouped according to the test object and the biological mechanism screened (table 2). Several expert groups (25, 35) have expressed the opinion that, due to the complexity of mutational events, a battery of different short-term tests should be used for reliable results. For this reason the test system has to be reproducible, reliable, rapid, and economical and be able to answer the question of the ability of the chemical or its metabolites to cause either point mutations in the DNA or chromosomal and/or genome mutations. Although no single assay can accomplish this task, practical demands have led to the "tier approach" ideology (16) in many proposed guidelines for the evaluation of genetic toxicologic safety. However, administrative actions may be taken at any level of experimental evidence.

Bacterial screening tests. At present the most widely employed test for the routine screening of mutagenic chemicals or metabolites is the *Salmonella/microsome test* developed by Ames and his co-workers (2) and known as the *Ames test*. This assay evaluates a test chemical in a rapid sensitive screen utilizing the $9000 \times g$

Table 2. Screening procedures for the identification of mutagens (short-term tests) and their potential to detect different types of mutations (35).

Organisms/test systems	Point mutations	Repair	Chromosome mutations	
			Structural	Numerical
Prokaryotes				
<i>S. typhimurium</i> ^a	+	+		
<i>E. coli</i> ^a	+	+		
<i>B. subtilis</i> ^a	+	+		
Eucaryotes				
Fungi ^a	+	+		+
<i>Drosophila</i>	+		+	+
Micronucleus test (rodents)			+	+
Sister chromatid exchanges (rodents) ^a		(+)?	(+)?	
Spot test (mouse)	+			
Cell cultures ^a	+	+	+	

^a Metabolic activation system can be added to the test.

supernatant fraction from rat livers as the metabolic activation system. The mutagenic potential of a chemical is determined from measurements of the number of reverse mutations in special strains of *Salmonella typhimurium* auxotrophs, i.e., strains requiring nutrients for their growth. The results are compared with positive and negative controls.

The tester strains have been selected for their sensitivity and specificity to being reverted to prototrophy by different types of mutational events. All of the *Salmonella* strains used in the test are histidine auxotrophs, and biotine is needed. The strains TA 98, TA 1537, and TA 1538 can be used to detect mutagens causing frame-shift mutations, while TA 100 and TA 1535 are used to detect mutagens causing base-pair substitutions in bacterial DNA.

The greatest advantage of the Ames test is that it gives a rapid method for detecting chemicals which are able to affect the bacterial chromosome — basically naked DNA. The demonstration of about a 90 % correlation between known carcinogens and mutagenicity in the Ames system (96, 118, 119, 140) implies that a positive test result indicates with high probability that the chemical is a potential carcinogen.

The same test design can be used in the *fluctuation test*, which is more sensitive than the traditional Ames test on plates (49). However, this test is more laborious and has higher demands for aseptic conditions. It appears to be very promising for monitoring employees exposed to hazardous chemicals in the work environment.

Use of Drosophila for mutagenicity testing. Mutagenicity tests with the classical genetic test organism, the fruit fly, *Drosophila melanogaster*, have proved to be sensitive and versatile in detecting chemical mutagens.

With the use of different mating schemes and different marker strains it is possible to screen for different types of mutational events, from point mutations to chromosomal changes. An advantage of *Drosophila* is that its main metabolic pathways for xenobiotics are similar to those of mammals (136). With successive matings, different spermatogonial stages can be studied easily. In addition both

somatic mutations (chromosome loss, specific locus reversions) and somatic cross-over can be studied in the *Drosophila* system (155).

In the correlative studies by Vogel (150) the most sensitive test system in *Drosophila* proved to be the *recessive lethal mutation test*, which is the easiest to carry out with the marked X chromosome as the background (thus over 20 % of the total DNA of the genome or over 1,000 loci are simultaneously screened for mutations). The test can be carried out in two generations, and therefore the experiment lasts only six weeks. In this test about a 94 % correlation has been obtained with known chemical carcinogens (150). Another advantage of *Drosophila* derives from the inducibility of the biotransformation enzymes analogous to those of mammals (31, 93).

Genetic tests with mammals. The need for in vivo mammalian tests in the evaluation of human risk is obvious. The test most often used for detecting germinal point mutations in mammals is the *specific locus test*, which is based on the use of genetically marked strains of mice. The test involves crossing a treated mouse carrying dominant homozygous markers with a recessive homozygous mouse. Thus forward mutations induced in the marker loci are revealed by the appearance of a recessive trait in the offspring. Since the number of marker genes is low (about 10), and even if the induced mutation frequency is as high as 1×10^{-4} , the number of mice needed for the study of even a single compound is several hundreds with tens of thousands of progeny (131).

The use of the *dominant lethal test* in mammals is generally considered to be insensitive. The test involves consecutive matings of the treated male animal to females that are then killed in midterm pregnancy and examined for fetal death. The assay reveals germinal mutations in the male, mainly chromosome breakage or small deletions rather than point mutations. Probably also a high incidence of nondisjunction can be detected (6). Even though the test has the advantage of using intact mammals, it cannot be practically applied to the routine screening of chemicals because of the large number of animals needed (several hundreds).

Cytogenetic methods. Cytogenetic tests are based on the visual observations of chromosomal damage either in in vitro cell cultures or in samples taken from test organisms exposed in vivo. Species with a low chromosome number and large chromosomes are the most popular for cytogenetic assays.

The advantage of the in vitro tests is that the exposure can be accurately determined. Even the metabolic activation system can be incorporated into cell cultures for additional activation capacity.

In the traditional cytogenetic assay the chromosomal aberrations are examined in metaphase cells. Thus chromosomal breaks or rearrangements or changes in the number of chromosomes can be observed (37).

A few years ago a method was developed which enables observation of another chromosomal parameter, namely, the frequency of exchanges between sister chromatids [sister chromatid exchange (SCE)]. This method (87), applicable also for exposed humans or in vitro techniques, has proved to be sensitive to mutagenic and/or carcinogenic exposure (36). It should be noted that the mechanisms involved in the induction of SCE and chromosome aberrations are different, and the results are not always parallel (53, 99).

Tests for DNA repair and cell transformation

Tests for repair synthesis. DNA alteration is an essential stage in the mechanism of chemical carcinogenesis and mutagenesis. One method of measuring DNA damage is to assay DNA repair in the cultured cells challenged with the test chemical. There are a number of recent reviews on the methodology of DNA repair tests (22). The techniques for detecting DNA repair or the unscheduled synthesis of DNA can therefore be of great use when an alteration in cellular DNA is to be demonstrated.

Cell transformation assays. If the addition of a chemical causes normal tissue cultures of mammalian cells to be transformed to cultures characteristic of malignant cells, the chemical is considered to be a potential carcinogen. Transformed cells are characterized by various growth parameters, such as loss of contact inhibition of growth and growth in semisolid

agar.

Cell transformation assay has been shown to be as equally predictive of carcinogenicity as the Ames test, and by inference, therefore, it should be equally predictive of mutagenicity (118). Baby hamster kidney cells (BHK 21/C12), human diploid lung fibroblasts (WI 38), or human liver cells (Chang) have been used by the Imperial Chemical Industries study group with the activating rat liver homogenate fraction (S-9 mix). Survival and transformation frequency have been calculated. The test is not as rapid as the Ames test, but it seems to be a very promising system for a preliminary transformation screen (119).

While human cells or normal diploid cells are obviously desirable for mutation assays of cultures, they are more difficult to handle from a screening viewpoint than malignant cell cultures due to their low plating efficiencies and lack of perpetual proliferation. Lymphoma cells, which grow in suspension, are even easier to handle than cells which grow as a monolayer. Lymphoma cells in cultures can readily detect directly acting mutagens and carcinogens (3) and may be used also in combination with S-9 mix to detect indirectly acting mutagens and carcinogens.

The cell transformation test is based on the actual transformation of in vitro cultures of normal mammalian cells into tumor cells by brief exposure of these cell cultures to small amounts of carcinogenic agents. On a flat surface in the culture medium, normal cells divide and spread by forming a one- or few-cell thick layer that shows an ordered pattern under the microscope. "Transformed" cells spread in a characteristically disordered fashion and pile on top of each other. Moreover, transformed cells, when reinjected into athymic (nude) mice or other animals of the strain from which the original normal cells were harvested, develop into full-fledged malignant tumors.

Screening for teratogens and embryotoxic agents

Current knowledge on the common characteristics of mutagenicity and teratogenicity should implicate a potential teratogenic risk for any chemical that is found to be mutagenic. Thus any of the afore-

mentioned test systems may give circumstantial evidence of teratogenicity. However, a number of the animal systems used in experimental teratology are analogous to the application of long-term animal tests in the testing for carcinogenicity. Each animal species has special characteristics in relation to teratological testing (130, 153). Rodents have been used extensively, mainly because of their easy maintenance, large litter size, and frequent pregnancies. Since rats and mice have litters of 5–10, the number of pregnant animals needed for each determination is not very high. Hamsters, although having a smaller litter size, are particularly suitable for teratological testing involving chromosome analysis because hamster chromosomes can easily be identified. Chick embryos have some advantages over mammals in that they are cheap and easy to maintain and the embryos may be affected directly without a placental barrier. For these reasons the chick embryo has been used for teratogenic screening (46, 144). Of course, the phylogenetic difference between man and bird poses uncertainties for the extrapolation of experimental findings from the chick embryo to man.

The teratogenic potential of chemicals is dependent on several variables (153). The species, as well as the strain, may be important, as was shown in the case of thalidomide, to which man is much more sensitive than rodents. It is likely that metabolic differences between the species underly such differential sensitivity. Moreover, the vulnerability of the embryo is very dependent on the developmental stage during testing, the time of organogenesis usually being sensitive. The teratogen may have a selective effect on the organ developing at the time of administration, as was the case with thalidomide. Finally, the dose is an important variable. The teratogenic potential may be expressed within a small concentration range; at a lower concentration no effect is noted; at a higher concentration, embryotoxicity or maternal lethality may be observed. Thus, a comprehensive scheme for teratological testing involves two or more animal species, two or more developmental periods, and several concentrations of the chemical.

POSSIBILITIES FOR MONITORING POPULATIONS AT RISK

Mutagenicity in the urine of exposed workers

The possibilities to detect a causal response for occupational mutagens/carcinogens in the human population are still limited. Urine of anesthesiologists has been found to be mutagenic in the conventional Ames assay (97), and mutagenicity was detected in the urine of workers who were accidentally exposed to epichlorohydrin (79). Furthermore, mutagenic metabolites have been detected in the urine of patients treated with antineoplastic drugs (134).

We have successfully used the sensitive fluctuation test to detect mutagenic metabolites in the urine concentrates of exposed workers (Falck et al., unpublished results). Mutagenicity has been detected in urine concentrates from heavy smokers, a finding similar to the results recently obtained with the conventional Ames assay (157). The fluctuation test has been applied to some occupational risk groups, e.g., workers in the rubber industry, and it revealed mutagenicity in concentrated urine (Falck et al., unpublished results).

Many chemicals are excreted into the urine as active mutagenic metabolites, while some mutagenic chemicals are excreted as nonmutagenic conjugates. With the use of an extraction and concentration procedure to separate the urinary components, it is possible to characterize the mutagenic components in urine. In the analysis of urine, the sample is incubated with rat liver homogenate (containing hydrolytic enzymes, e.g., β -glucuronidase) to hydrolyze the conjugated metabolites.

The urine test has a great potential for the screening of worker populations for possible carcinogenic agents. It is valuable for quantitating the degree of exposure of a population to potentially toxic chemicals and for identifying individuals who are at risk.

Screening for chromosomal changes in exposed groups

Unfortunately, very little is known about additional genetic ill health in man that

would possibly be related to an increased germinal mutation rate caused by exposure to environmental mutagens (142). Due to the very low mutation rate (1×10^{-5} — 10^{-6} per locus), diagnostic problems and register handicaps, it is practically impossible to obtain statistically significant information about a possible increase of the monogenous genetic diseases induced by environmental mutagens. Naturally only the very few dominant mutations with unambiguously identifiable phenotypes could be used for monitoring. Suggested marker phenotypes include such human dominant disorders as the Marfan syndrome (frequency 0.015/1,000), aniridia (frequency 0.01/1,000), retinoblastoma (frequency 0.05/1,000), or achondroplasia (frequency 0.1/1,000). Screening for chromosomal changes is statistically more rewarding (13). Recent evidence suggests a rise in the frequency of chromosomal translocations causing such anomalies as the Down syndrome (trisomy 21) and the Patau syndrome (trisomy 13) (62).

At present the best available method to assess possible genetic damage in human risk groups occupationally or environmentally exposed to exogenous agents is the metaphase analysis for the detection of chromosome aberrations. The simplest procedure is to use peripheral blood samples from the persons under study. The cells are cultured for 2—3 d in the presence of a mitogen, which induces the lymphocytes to undergo mitosis. The most tedious step of the procedure is the microscopical analysis of the metaphases since at least 100 metaphases should be scored for each individual.

This system can be used to evaluate genetic damage that has occurred in the exposed individual *before* the blood sample was taken. However, no accurate timing of the mutational event can be achieved because the population of lymphocytes stimulated during culturing may be long-lived. Thus the only real *in vivo* sample to be used for the detection of chromosomal damage in exposed humans can be obtained from bone marrow, where cells proliferate spontaneously.

The method for detecting SCE (see the Cytogenetic Methods section) is sensitive in detecting mutagens and known carcinogens both *in vitro* and *in vivo* even

though the results do not always parallel those obtained with the use of chromosome-breaking agents [e.g., in exposure to vinyl chloride (53) and to styrene (99)]. In studies of exposed populations the SCE method has proved to be sensitive in detecting significantly higher frequencies of SCEs in heavy smokers (86) and laboratory personnel (44).

A clear causal relationship exists between high lymphocyte aberration frequency and increased cancer incidence at the population level (table 4). The most clear-cut correlation is revealed in populations exposed to ionizing radiation, either occupationally or in other situations (12). A causal correlation between chromosomal aberrations in peripheral blood and a later increase in cancer incidence has been shown in several groups exposed to radiation, such as in patients receiving thorium dioxide for angiography (39) or patients in radiotherapy due to ankylosing spondylitis (18). Evidence of chemical exposures causing a high chromosomal aberration rate and malignant diseases in the same risk group is still quite weak, the best documented case being occupational exposure to vinyl chloride (table 4).

Epidemiologic monitoring

Long-term harmful effects of chemicals may appear, e.g., as spontaneous abortion, malformation, and cancer. Similarities between teratogenesis and carcinogenesis have been reviewed elsewhere (54, 77, 103). Mutations induced by chemical and physical factors appear to be the common underlying mechanism for the two conditions. Mutations have also been suggested to be an important cause of spontaneous abortions, as a large number of the aborted embryos contain chromosome anomalies (21). Moreover, many aborted fetuses appear to be malformed (21, 80, 132), and therefore it is possible that the etiologies of abortions and malformations are partly similar.

Occupational exposures have been assumed to account for anywhere between 3 and 40 % of all cancers today and in the near future (15, 24, 57, 156). A number of industrial agents and processes have been identified as carcinogenic in epidemiologic studies. However, because of

mixed exposures to carcinogens and the long latency periods in the development of cancer, it is difficult to apply epidemiologic surveys as means of occupational health policy. By the time a cancer hazard is identified, the industrial processes may have changed, and the preventive measures would have become less effective. The monitoring of childhood cancers may be a better instrument for environmental surveillance, as the cancers evolve in a relatively short time and the etiologic factors are therefore more obvious (102, 154). It has been shown that the X-raying of and viral infections in pregnant women predispose the embryo to the risk of childhood cancer (102). Work with experimental animals has helped to identify tens of chemicals which can pass through the placenta and cause cancer transplacentally (122). However, evidence linking chemical exposures to childhood cancer in humans exists for only a few substances. Workers exposed regularly to anesthetic gases in operating rooms are reported to have children with an increased risk of cancer (66, 69). The daughters of women treated with diethylstilbestrol during pregnancy carry an increased risk of vaginal and cervical cancer at puberty (55). The intake of barbiturates by pregnant women may predispose the child transplacentally to brain tumors. Moreover the administration of barbiturates to children may also cause a risk (47). Some other risk factors for transplacental carcinogenesis may be exposure of pregnant women to certain chlorinated pesticides (65) and smoking during pregnancy (109). Attempts to correlate the incidence of childhood cancer to the parents' occupation have been conflicting so far (51). Studies with specific data on chemical exposures are likely to be more informative in this respect.

The incidence of congenital malformations also display an extensive geographic variation. In worldwide surveys the incidence has been shown to vary 5- to 100-fold (88), although it is likely that such a large variation is partially due to differences in reporting and statistical practices. The prevalence of neural tube defects varies about 10-fold between European regions (88).

In Finland the incidence of cleft lip and

palate, as well as defects of the central nervous system, varies more than two-fold between different regions (48, 129). It is thus possible that environmental factors play an important, but yet undefined, role in the etiology of congenital malformations. Studies relating the socioeconomic status of the family and the prevalence of malformations have reported a gradient in Great Britain and the United States, while no such difference has been detected in Hungary or among Jews in Israel or the United States (88). A number of reports have suggested that operating room staff have more malformed offspring than the population at large (26). Preliminary studies with nurses using hexachlorophene (52) and a case study on workers exposed to styrene in the reinforced plastics industry (60) suggest an increased liability to malformations in the offspring. As registers of malformations are now kept in several countries, it is likely that the information on the etiologic factors of human malformations is rapidly expanding and clarifying the strength of the environmental factors.

Abortion is defined as a loss of nonviable products of conception from the uterus. The period when abortions are observed spans from the time of conception to about 20 weeks of gestation; thereafter the miscarriage is classified as stillbirth (21).

According to prospective studies the frequency of spontaneous abortions in humans ranges between 14 and 30 % of all pregnancies. Retrospective studies have estimated the frequency to be about 15 % or less (21). The differences in these figures are mainly due to the difficulty of observing pregnancies and fetal losses during the first few weeks after conception. For this reason the overall rate is believed to be as high as 30 % (21). Fetal losses are more likely to occur in the early weeks of pregnancy, and the frequency decreases considerably after the first trimester.

Morphological inspection of the abortuses may be a valuable source for teratological monitoring. The specimen that is intact enough for inspection (usually over 60 % of the material) may reveal fetal malformations in at least 5 to 10 % of the cases (130, 132). The percentage is markedly higher than the frequency of con-

genital malformations (1 to 3 %) (50). The reason for the concentration of malformations in the abortion material is the decreased survival of the malformed fetus. It has been estimated that fetuses with neural tube defects miscarry at a frequency of 60 to 90 % (88). Chromosome studies of aborted fetuses have revealed that anomalies are about 60 to 100 times more frequent in spontaneous abortions than in livebirths, amounting to about 50 % of all aborted fetuses (13, 79, 138).

Up to now very few chemicals have

been shown to cause spontaneous abortions, and most textbooks do not list exogenous chemicals as an etiologic factor. However, cigarette smoking (58) and some occupational exposures have recently been reported to carry an increased risk for spontaneous abortions (table 3). Female anesthetists and operating room nurses (4, 66, 69, 81, 124), laboratory technicians (83, 139), female employees in a copper smelter (113, 114), and the wives of vinyl chloride polymerization workers (66) are suggested to have an increased risk of abortion.

Table 3. Occupational exposures with reported risk of spontaneous abortion in retrospective studies.

Occupation	Exposed	Abortions in study group (%)	Abortions in control group (%)	Ratio (study/control)	Reference
Copper smelters	Female	28	13	2.2	Nordström et al (114)
Laboratory personnel	Female	18—33	6.7—19	1.7—2.7	Kolmodin-Hedman & Hedström (83) and Strandberg et al. (139)
Operating room personnel	Female	18—20	10—14	1.4—1.6	International Agency for Research on Cancer (70), Knill-Jones et al. (81) and Rosenberg & Kirves (124)
Vinyl chloride polymerization workers	Male	15.8	8.8	1.8	Infante et al. (66)

Table 4. Delayed manifestations in exposed groups and available data on the experimental mutagenesis, carcinogenesis, and teratogenesis of some occupationally important chemicals. (Reference source in parentheses)

Chemical compound	Increase in exposed persons ^a				Positive experimental data ^a		
	Cancer	Abortions	Malformations	Chromosomal aberrations in lymphocytes	Mutagenesis	Carcinogenesis	Teratogenesis
Acrylonitrile	(+) (7, 72)	..	..	— (143)	± (104, 147)	± (72)	± (105)
Anesthetic gases	± (27, 70)	± (70, 73)	± (4, 26)	..	± (108, 151)	± (108)	± (108)
Arsenic	± (68)	..	..	± (111)	± (8, 107)	± (8, 68, 84)	± (8, 61)
Asbestos	± (71)	..	..	..	± (63, 71)	± (71)	..
Benzene	± (69)	..	..	± (43)	± (30)	± (33, 69)	± (33)
Chloroprene	(+) (67, 72)	± (127)	..	± (78)	± (5)	± (72)	± (72)
Epichlorohydrin	.. (70)	..	..	± (85, 117)	± (137)	± (70)	..
Chromium	± (68)	..	..	± (10)	± (106)	± (68)	± (45)
Ethylene oxide	± (59)	..	..	± (32)	± (70)	.. (70)	..
Lead	± (141)	(+) (113, 114, 123)	(+) (115, 123)	± (42, 112)	± (89)	± (141)	± (38, 123)
Styrene	..	..	(+) (60)	± (99, 100)	± (146)	± (72)	(+) (144)
Vinyl chloride	± (29, 72)	± (66)	± (64)	± (53, 120)	± (68, 148)	± (72, 149)	± (75)

^a + = positive data; (+) = limited data; ± = contradictory data; — = negative data; .. = no data.

There are many reasons why the surveillance of spontaneous abortion may prove to be a valuable instrument in environmental monitoring (80, 154). First the monitoring of spontaneous abortions should furnish information on teratogens since many of the aborted embryos are malformed and most malformed ones can be acquired for inspection. Second spontaneous abortions are frequent enough to allow collection of a large sample size. In a retrospective study the incidence of spontaneous abortions is about 5—15 % of the number of births, the incidence of malformations is 1 % or less if only certain types are recorded, and the incidence of childhood cancer is about 0.2 % of the number of births. Third the time between the exposure and the outcome is shorter in spontaneous abortions than in malformations and childhood cancer. With such perspectives, it is likely that our knowledge on the interactions of chemical exposures in human abortions will greatly increase in a few years time.

TOXICOLOGY AS PREVENTIVE SCIENCE

In this article we have discussed the underlying principles of genetic toxicology and presented its major applications in the field of occupational health. The combination of laboratory testing with the experimental and epidemiologic monitoring of worker populations should make genetic toxicology a powerful instrument in the prevention of occupational and environmental genetic hazards. However, most of the methods applied in genetic toxicology are new and await their final practical validation.

In table 4 we have collected data from different epidemiologic and experimental sources on the harmful effects of some chemicals in order to review the extent of current knowledge about them and to examine the outcome of the different approaches. In reviewing the literature, not all contradictory data have been presented. Instead, we have attempted to evaluate the data or to refer to an evaluation by, e.g., the International Agency for Research on Cancer or the National

Institute for Occupational Safety and Health, U.S.A.

The data are the most complete and unanimous for vinyl chloride. For asbestos, epichlorohydrin, and chromium all data systematically point to risk, but data on malformations and abortions are lacking. For some other compounds the data are contradictory (e.g., for the animal carcinogenicity of benzene and arsenic), or important information is also lacking. In general, there is a good correlation between data on experimental mutagens and human carcinogens. It is rather astonishing that so little information is available on human abortions and malformations, even though epidemiologic studies on these conditions should be much easier than those on cancer. If one would take other chemicals as examples, the lack of data would be even more obvious.

The genetic hazards of the ever expanding introduction of chemical products into the human environment are imminent. The effects of such expansion are likely to express themselves as chronic diseases such as cancers and reproductive problems. Moreover, it is possible that environmentally induced genetic lesions contribute to the prevalence of other chronic conditions and diseases including heart and vascular diseases and aging. The possible hazards are not limited to the present generation but are likely to be transmitted to future ones. Such deleterious trends develop slowly, and they may be masked by the many health restoring activities practiced by modern societies.

Wherever environmental chemicals and pollutants end up, they usually originate from industrial facilities, where they are produced. Thus the occupational environment, where the hazardous chemicals are manufactured, is the first to be polluted, and the workers carry the brunt of the unknown. Any ill effects are likely to be experienced first by the workers involved. Thus the surveillance of occupational health is not only important for the protection of the worker, but for the protection of the public at large.

The control of chronic diseases with long latency periods requires special strategies. Particularly with respect to ever changing chemical exposures, it is not enough to wait until their ill effects ex-

press themselves as fatal conditions in large populations.

The reasonable course of action for coping with increasing rates of chronic diseases and the social costs involved is to place a stronger emphasis on prevention in terms of political, legislative, and social actions and the allocation of research funds. Preventive medicine is a good investment in every society.

REFERENCES

1. AITIO, A. (ed.). *Conjugation reactions in drug biotransformation*. Elsevier/North Holland, Amsterdam 1978, 529 p.
2. AMES, B. N., DURSTON, W. E., YAMASAKI, E. and LEE, F. D. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proc. natl. acad. sci. u.s.a.* 70 (1973) 2281—2285.
3. ANDERSON, D. Attempts to produce systems for isolating spontaneous and induced variants in various mouse lymphoma cells using a variety of selective agents. *Mutat. res.* 33 (1975) 407—416.
4. ASKROG, V. and HARVALD, B. Teratogen effekt af inhalationsanæstetika, *Nord. med.* 83 (1970) 498—500.
5. BARTSCH, H., MALAVEILLE, C., MONTESANO, R. and TOMATIS, L. Tissue mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *S. typhimurium*. *Nature* 255 (1975) 641—643.
6. BATEMAN, W. J. The dominant lethal assay in the male mouse. In: B. J. KILBEY, M. LEGATOR, W. NICHOLS and C. RAMEL (eds.), *Handbook of mutagenicity test procedures*. Elsevier Scient. Publ. Co., Amsterdam 1977, pp. 325—334.
7. BEHR, P. Controlling chemical hazards. *Environment* 20 (1978) 25—29.
8. BENCKO, V. Carcinogenic, teratogenic and mutagenic effects of arsenic. *Environ. health perspect.* 19 (1977) 179—182.
9. BENDITT, E. P. The origin of atherosclerosis. *Sci. am.* 236 (1977) 74—85.
10. BIGALIEV, A. B., TUREBAEV, M. N., BIGALIEVA, R. K. and ELEMESOVA, M. Cytogenetic examination of workers engaged in chrome production. *Genetika* 13/3 (1977) 545—547.
11. BLAKE, D. A., MARTZ, F., FAILINGER C. III and GERY-MARTZ, A. M. Arene oxide-mediated chemoteratogenesis. In: P. W. JONES and R. I. FREUDENTHAL (eds.), *Carcinogenesis* (vol. 3). Raven Press, New York, NY 1978, pp. 401—411.
12. BLOOM, A. D. Induced chromosomal aberrations in man. *Adv. hum. genet.* 3 (1972) 99—172.
13. BOCHKOV, N. P. Monitoring of human populations in connection with environmental pollution by the evaluation of chromosome anomalies. In: H. BÖHME and J. SCHÖNEICH (eds.), *Environmental mutagens*. Akademie — Verlag, Berlin 1977, pp. 167—179.
14. BOYLAND, E. A chemist's view of cancer prevention. *Proc. r. soc. med.* 60 (1967) 93—99.
15. BRIDBORD, K., DECOUFLE, P., FRAUMENI, J. F. JR., HOEL, D. G., HOOVER, R. N., RALL, D. P., SAFFIOTTI, U., SCHNEIDERMAN, M. A. and UPTON, A. C. Estimates of the fraction of cancer in the United States related to occupational factors. Presented at the AFL-CIO national conference on occupational safety and health in Washington DC, September 1978.
16. BRIDGES, B. A. The three-tier approach to mutagenicity screening and the concept of radiation equivalent dose. *Mutat. res.* 26 (1974) 335—340.
17. BRUSICK, D. J. Alterations of germ cells leading to mutagenesis and their detection. *Environ. health. perspect.* 24 (1978) 105—112.
18. BUCKTON, K. E., JACOBS, P. A., COURT BROWN, W. M. and DOLL, R. A study of the chromosome damage persisting after X-ray therapy for ankylosing spondylitis. *Lancet* 2 (1962) 676—682.
19. CAIRNS, J. The cancer problem. *Sci. am.* 233 (1975) 64—79.
20. CARR, D. H. Chromosomes and abortion. *Adv. hum. genet.* 2 (1971) 201—257.
21. CARR, D. H. Detection and evaluation of pregnancy wastage. In: J. G. WILSON and E. C. FRASER (eds.), *Handbook of teratology* (vol. 3). Plenum Press, New York, NY 1977, pp. 189—225.
22. CLEAVER, J. E. Methods for studying repair of DNA damaged by physical and chemical carcinogens. In: H. BUSCH (ed.), *Methods in cancer research* (vol. XI). Academic Press, New York, NY 1975, pp. 123—162.
23. CLEAVER, J. E., GOTH, R. and FRIEDBERG, E. C. Value of measurements of DNA repair levels in predicting carcinogenic potential of chemicals. In: R. MONTESANO, H. BARTSCH and L. TOMATIS (eds.), *Screening tests in chemical carcinogenesis*. International Agency for Research on Cancer, Lyon 1976, pp. 639—661.
24. COLE, P. Cancer and occupation: Status and needs of epidemiologic research. *Cancer* 39 (1977) 1788—1791.
25. COMMITTEE 17. Environmental mutagenic hazards. *Science* 187 (1975) 503—514.
26. CORBETT, T. H., CORNELL, R. G., ENDRES, J. L. and LIEDING, K. Birth defects among children of nurse-anesthetists. *Anesthesiology* 41 (1974) 341—344.
27. CORBETT, T. H., CORNELL, R. G., LIEDING, K. and ENDRES, J. L. Incidence of cancer among Michigan nurse-anesthetists. *Anesthesiology* 38 (1973) 260—263.

28. CROW, J. F. Impact of various types of genetic damage and risk assessment. *Environ. health perspect.* 6 (1973) 1—5.
29. CREECH, J. L. and JOHNSON, M. N. Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J. occup. med.* 16 (1974) 150—151.
30. DEAN, B. J. Genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. res.* 47 (1978) 75—97.
31. DONNER, M., SORSA, M. and VAINIO, H. Recessive lethals induced by styrene and styrene oxide in *Drosophila melanogaster*. *Mutat. res.* 67 (1979) 373—376.
32. EHRENBERG, L. and HÄLLSTRÖM, T. Haematologic studies on persons occupationally exposed to ethylene oxide. In: INTERNATIONAL ATOMIC ENERGY AGENCY, *International Atomic Energy Agency report SM 92/26*. 1967 pp. 327—334.
33. ENVIRONMENTAL PROTECTION AGENCY. *Human health effects: Benzene*. Cincinnati, OH 1978. 39 p.
34. EPSTEIN, C. J. and GOLBUS, M. S. Prenatal diagnosis of genetic diseases. *Am. sci.* 65 (1977) 703—711.
35. EUROPEAN ENVIRONMENTAL MUTAGEN SOCIETY. Mutagenicity screening: General principles and minimal criteria (Committee report). *Mutat. res.* 53 (1978) 361—367.
36. EVANS, H. J. What are sister chromatid exchanges? In: A. DE LA CHAPELLE and M. SORSA (eds.), *Chromosomes today* (vol. 6). Elsevier/North-Holland, Amsterdam 1977, pp. 315—326.
37. EVANS, H. J. and O'RIORDAN, M. K. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutat. res.* 31 (1975) 135—148.
38. FERM, V. H. and CARPENTER, S. J. Developmental malformations resulting from the administration of lead salts. *Exp. mol. pathol.* 7 (1967) 208.
39. FISCHER, P., GOLOB, E., KUNZEMÜHL, E., BEN HAIM, A., DUDLEY, R. A., MÜLLNER, T., PARR, R. M. and VETTER, H. Chromosome aberrations in peripheral blood cells in man following chronic irradiation from internal deposit of Thorotrast. *Radiat. res.* 29 (1966) 505—515.
40. FISHBEIN, L. *Potential industrial carcinogens and mutagens*. Environmental Protection Agency, Washington DC 1977, 316 p.
41. FISHBEIN, L., FLAMM, W. G. and FALK, H. L. *Chemical mutagens*. Academic Press, New York, NY 1970, 364 p.
42. FORNI, A. M., CAMBIAGHI, G. and SECCHI, G. C. Initial occupational exposure to lead. *Arch. environ. health* 31 (1976) 73—78.
43. FORNI, A. M., CAPPELLINI, A., PACIFICICO, E. and VIGLIANI, E. C. Chromosome changes and their evolution in subjects with past exposure to benzene. *Arch. environ. health* 23 (1971) 385—391.
44. FUNES-CRAVIOTO, F., KOLMODIN-HEDMAN, B., LINDSTEN, J., NORDEN-SKJÖLD, M., ZAPATA-GAYON, C., LAMBERT, B., NORDBERG, E. and OLIN, R. Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rototyping factory and in children of women laboratory workers. *Lancet* 1 (1977) 322—325.
45. GALE, T. F. Embryotoxic effects of chromium trioxide in hamsters. *Environ. res.* 16 (1978) 101—109.
46. GEBHARDT, D. O. E. The use of the chick embryo in applied teratology. In: D. H. M. WOOLAM (ed.) *Advances in teratology* (vol. 5). Logos Press, London 1972, pp. 97—111.
47. GOLD, E., GORDIS, L., TONASCIA, J. and SZKLO, M. Increased risk of brain tumors in children exposed to barbiturates. *J. natl. cancer inst.* 60 (1978) 1031—1034.
48. GRANROTH, G., HAKAMA, M. and SÄXEN, L. Defects of the central nervous system in Finland: Variations in time and space, sex distribution and parental age. *Br. j. prev. soc. med.* 31 (1977) 164—170.
49. GREEN, M. H. L., MURIEL, W. J. and BRIDGES, B. A. Use of a simplified fluctuation test to detect low levels of mutagens. *Mutat. res.* 38 (1976) 33—42.
50. HAKASALO, J. K. Cumulative detection rates of congenital malformations in a ten-year follow-up study. *Acta pathol. microbiol. scand. sect. A* 242 (1973) 1—59.
51. HAKULINEN, T., SALONEN, T. and TEPPÖ, L. Cancer in the offspring of fathers in hydrocarbon-related occupations. *Br. j. prev. soc. med.* 30 (1976) 138—140.
52. HALLING, H. Misstänkt samband mellan hexaklorofen-exposition och missbildningsbörd. *Läkartidningen* 74 (1977) 542—546.
53. HANSTEEN, I.-L., HILLESTAD, L., THIS-EVENSEN, E. and STORETVEDT HELDAAS, S. Effects of vinyl chloride in man: A cytogenetic follow-up study. *Mutat. res.* 51 (1978) 271—278.
54. HARBISON, R. D. Chemical-biological reactions common to teratogenesis and mutagenesis. *Environ. health perspect.* 24 (1978) 87—100.
55. HERBST, A. L., ULFELDER, H. and POSKANZER, D. C. Adenocarcinoma of the vagina. *New engl. j. med.* 284 (1971) 878—881.
56. HIGGINSON, J. Present trends in cancer epidemiology. *Proc. can. cancer Congr.* 8 (1969) 40—75.
57. HIGGINSON, J. and MUIR, C. S. The role of epidemiology in elucidating the importance of environmental factors in human cancer. *Cancer detect. prev.* 1 (1976) 79—105.
58. HIMMELBERGER, D. V., BROWN, B. W. and COHEN, E. N. Cigarette smoking during pregnancy and the occurrence of spontaneous abortion and congenital ab-

- normality. *Am. j. epidemiol.* 108 (1978) 470—479.
59. HOGSTEDT, C., ROHLEN, O., BERNDTSSON, B. S., AXELSON, O. and EHRENBORG, L. Kohortstudie av dödsorsaker hos anställda i etylenoxidframställning. *Läkartidningen* 75 (1978) 3285—3287.
 60. HOLMBERG, P. C. Central nervous defects in two children of mothers exposed to chemicals in the reinforced plastics industry. *Scand. j. work environ. & health* 3 (1977) 212—214.
 61. HOOD, R. D. and BISHOP, S. L. Teratogenic effects of sodium arsenate in mice. *Arch. environ. health* 24 (1972) 62—65.
 62. HOOK, E. B. The ratio of de novo unbalanced translocation to 47, trisomy 21 Down syndrome. *Mutat. res.* 52 (1978) 427—439.
 63. HUANG, S. L., SAGGIORO, D., MICHELMANN, H. and MALLING, H. V. Genetic effects of crocidolite asbestos in Chinese hamster lung cells. *Mutat. res.* 57 (1978) 225—232.
 64. INFANTE, P. F. Oncogenic and mutagenic risks in communities with polyvinyl chloride production facilities. In: U. SAFIOTTI and J. K. WAGONER (eds.), *Occupational carcinogenesis. Ann. n.y. acad. sci.* 271 (1976) 49—57.
 65. INFANTE, P. F., EPSTEIN, S. S. and NEWTON, W. A. Blood dyscrasia and childhood tumors in exposure to chlor-dane and heptachlor. *Scand. j. work environ. & health* 4 (1978) 137—150.
 66. INFANTE, P. F., MC MICHAEL, A. J., WAGONER, J. K., WAXWEILER, R. J. and FALK, H. Genetic risks of vinyl chloride. *Lancet* 1 (1976) 734—735.
 67. INFANTE, P. H., WAGONER, J. K. and YOUNG, R. J. Chloroprene: Observations of carcinogenesis and mutagenesis. In: H. H. HIATT, J. D. WATSON and J. A. WINSTEN (eds.), *Origins of human cancer*. Cold Spring Harbor Publications 1977, pp. 205—217.
 68. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monographs on the evaluation of carcinogenic risk of chemicals to man* (vol. 2). Lyon 1973. 184 p.
 69. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monographs on the evaluation of carcinogenic risk of chemicals to man* (vol. 7). Lyon 1974. 326 p.
 70. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monographs on the evaluation of carcinogenic risk of chemicals to man* (vol. 11). Lyon 1976. 306 p.
 71. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monographs on the evaluation of carcinogenic risk of chemicals to man* (vol. 14). Lyon 1977. 106 p.
 72. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monographs on the evaluation of carcinogenic risk of chemicals to man* (vol. 19). Lyon 1979. 513 p.
 73. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Transplacental carcinogenesis*. Lyon 1973. 181 p.
 74. JERINA, D. M. and DALY, J. W. Arene oxides: A new aspect of drug metabolism. *Science* 185 (1974) 573—582.
 75. JOHN, J. A., SMITH, F. A., LEONG, B. K. J. and SCHWETZ, B. A. The effects of maternally inhaled vinyl chloride on embryonal and fetal development in mice, rats and rabbits. *Toxicol. appl. pharmacol.* 39 (1977) 497—513.
 76. JUCHAU, M. R., BOND, J. A. and BENDITT, E. P. Aryl 4-monooxygenase and cytochrome P-450 in the aorta: Possible role in atherosclerosis. *Proc. natl. acad. sci. u.s.a.* 73 (1976) 3723—3725.
 77. KALTER, H. Some relations between teratogenesis and mutagenesis. *Mutat. res.* 23 (1975) 29—36.
 78. KATOSOVA, L. D. Cytogenetic analysis of peripheral blood of workers engaged in the production of chloroprene. *Gig. tr. prof. zabol.* 10 (1973) 30—39.
 79. KILIAN, D. J., PULLIN, T. G., CONNER, T. H., LEGATOR, M. S. and EDWARDS, H. N. Mutagenicity of epichlorohydrin in the bacterial assay system: Evaluation by direct in vitro activity and in vivo activity of urine from exposed humans and mice. *Mutat. res.* 53 (1978) 72.
 80. KLINE, J. STEIN, Z., STROBINO, B., SUNER, M. and WARBURGTON, D. Surveillance of spontaneous abortions: Power in environmental monitoring. *Am. j. epidemiol.* 106 (1977) 345—350.
 81. KNILL-JONES, R. P., MOIR, D. D., RODRIGUES, L. V. and SPENCE, A. A. Anesthetic practice and pregnancy: Controlled survey of women anesthetists in the United Kingdom. *Lancet* 1 (1972) 1326—1328.
 82. KNUDSON, A. Overview: Toward the understanding and control of cancer. In: J. R. FRAUMENI (ed.), *Persons at high risk of cancer*. Academic Press, New York, NY 1975, pp. 503—514.
 83. KOLMODIN-HEDMAN, B. and HEDSTRÖM, L. Enkät undersökning hos kemikalieexponerad laboratoriepersonel rörande spontanaborter. *Läkartidningen* 75 (1978) 3044—3045.
 84. KRAYBILL, H. Carcinogenicity of arsenic in experimental studies. In: B. W. CARNOW (ed.), *Health effects of occupational lead and arsenic exposure — A symposium*. U. S. Department of Health, Education and Welfare, Washington, DC 1975.
 85. KUČEROVÁ, M., ZHURKOV, V. S., POLIKOVA, Z. and IVANOVA, J. E. Mutagenic effect of epichlorohydrin: II. Analysis of chromosomal aberrations in lymphocytes of persons occupationally exposed to epichlorohydrin. *Mutat. res.* 48 (1977) 355—360.
 86. LAMBERT, B. LINDBLAD, A., NORDESKJÖLD, M. and WERELIUS, B. In-

- creased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas* 88 (1978) 147—149.
87. LATT, S. A., ALLEN, J. W., ROGERS, W. E. and JÜRGENS, L. A. In vitro and in vivo analysis of sister chromatid exchange formation. In: B. J. KILBEY, M. LEGATOR, W. NICHOLS and C. RAMEL (eds.), *Handbook of mutagenicity test procedures*. Elsevier Scient. Publ. Co., Amsterdam 1977, pp. 275—291.
 88. LECK, I. Correlations of malformation frequency with environmental and genetic attributes in man. In: J. G. WILSON and F. C. CLARK (eds.), *Handbook of teratology* (vol. 3). Plenum Press, New York, NY 1977, pp. 243—244.
 89. LEONARD, A., DEKNUDT, G. and GILLIAVOD, N. Genetic and cytogenetic hazards of heavy metals in mammals. *Mutat. res.* 29 (1975) 280—281.
 90. LEVAN, G. and MITELMAN, F. Chromosomes and the etiology of cancer. In: A. DE LA CHAPELLE and M. SORSA (eds.), *Chromosomes today* (vol. 6). Elsevier/North-Holland, Amsterdam 1977, pp. 363—371.
 91. LOEB, L. A., SPRINGGATE, C. F. and BATULLA, N. Errors in DNA replication as a basis of malignant changes. *Cancer res.* 34 (1974) 2311—2321.
 92. LOPRIENO, N. Occupational hazards: Mutagenic activity of industrial compounds. In: H. BÖHME and J. SCHÖNEICH (eds.), *Environmental mutagens*. Akademie-Verlag, Berlin 1977, pp. 153—160.
 93. MAGNUSSON, J. and RAMEL, C. Mutagenic effects of vinyl chloride on *Drosophila melanogaster* with and without pretreatment with sodium phenobarbiturate. *Mutat. res.* 57 (1978) 307—312.
 94. MAUGH, T. H. Chemicals: How many are there? *Science* 199 (1978) 162.
 95. MAUGH, T. H. Chemical carcinogens: The scientific basis for regulation. *Science* 201 (1978) 1200—1205.
 96. MC CANN, J. and AMES, B. Detection of carcinogens as mutagens in the *Salmonella/microsome* test: Assay of 300 chemicals. Discussion. *Proc. natl. acad. sci.* 73 (1976) 950—954.
 97. MC COY, E. C., HANKEL, R., ROBBINS, K., ROSENKRANTZ, H. S., GINFFRIDA, J. G. and BIZZARI, D. V. Presence of mutagenic substances in the urines of anesthesiologists. *Mutat. res.* 53 (1978) 71.
 98. MC KUSICK, V. A. *Mendelian inheritance in man: Catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes* (4th ed.) John Hopkins University Press, Baltimore, MD 1975, p. 837.
 99. MERETOJA, T., JÄRVENTAU, H., SORSA, M. and VAINIO, H. Chromosome aberrations in lymphocytes of workers exposed to styrene. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 259—264.
 100. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
 101. MILLER, J. A. and MILLER, E. C. Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: H. H. HIATT, J. D. WATSON and J. A. WINSTEN (eds.), *Origins of human cancer*. Cold Spring Harbor Laboratory 1977, pp. 605—627.
 102. MILLER, R. W. Aetiology of childhood cancer: Epidemiological approach. In: H. B. MARSDEN and J. K. STEWARD (eds.), *Tumors in children* (2nd ed.). Springer-Verlag, Berlin 1976, pp. 14—27.
 103. MILLER, R. W. Relationship between human teratogens and carcinogens. *J. natl. cancer inst.* 58 (1977) 471—474.
 104. MILVY, P. and WOLFF, M. Mutagenic studies with acrylonitrile. *Mutat. res.* 48 (1977) 271—278.
 105. MURRAY, F. J., SCHWETZ, B. A., NITSCHKE, K. D., JOHN, J. A., NORRIS, J. M. and GEHRING, P. J. Teratogenicity of acrylonitrile given to rats by gavage or by inhalation. *Food cosmet. toxicol.* 16 (1978) 547—551.
 106. NAKAMURO, K., YOSHIKAWA, K., SAYATO, Y. and KURATA, H. Comparative studies of chromosomal aberration and mutagenicity of trivalent and hexavalent chromium. *Mutat. res.* 58 (1978) 175—181.
 107. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH. *Occupational exposure to inorganic arsenic (new criteria)*. U. S. Dept. of Health, Education and Welfare, Washington, DC 1975. 127 p.
 108. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH. *Occupational exposure to waste anesthetic gases and vapors*. U. S. Dept. of Health Education and Welfare, Washington, DC 1977. 194 p.
 109. NEUTEL, C. I. and BUCK, C. Effect of smoking during pregnancy on the risk of cancer in children. *J. natl. cancer inst.* 47 (1971) 59—63.
 110. NICHOLS, W. W. Somatic mutation in biological research. *Hereditas* 81 (1975) 225—236.
 111. NORDENSON, I., BECKMAN, G., BECKMAN, L. and NORDSTRÖM, S. Occupational and environmental risks in and around a smelter in northern Sweden: II. Chromosomal aberrations in workers exposed to arsenic. *Hereditas* 88 (1978) 47—50.
 112. NORDENSON, I., BECKMAN, G., BECKMAN, L. and NORDENSON, I. Occupational and environmental risks in and around a smelter in northern Sweden: IV. Chromosomal aberrations in workers exposed to lead. *Hereditas* 88 (1978) 263—267.
 113. NORDSTRÖM, S., BECKMAN, L. and NORDENSON, I. Occupational and environmental risks in and around a smelter in northern Sweden: III. Frequency of spontaneous abortion. *Hereditas* 88 (1978) 51—54.

114. NORDSTRÖM, S., BECKMAN, L. and NORDENSON, I. Occupational and environmental risks in and around a smelter in northern Sweden: V. Spontaneous abortions among female employees and decreased birth weight in their offspring. *Hereditas* 90 (1979) 291—296.
115. NORDSTRÖM, S., BECKMAN, L. and NORDENSON, I. Occupational and environmental risks in and around a smelter in northern Sweden. VI. Congenital malformations. *Hereditas* 90 (1979) 297—300.
116. PELKONEN, O. Transplacental transfer of foreign compounds and their metabolism by the fetus. In: J. W. BRIDGES and L. F. CHASSEAD (eds.), *Progress in drug metabolism* (vol. 2). John Wiley and Sons Inc., New York, NY 1977, pp. 119—161.
117. PICCIANO, D. Cytogenetic investigation of occupational exposure to epichlorohydrin. *Mutat. res.* 66 (1979) 169—173.
118. PURCHASE, I. F. H., LONGSTAFF, E., ASHBY, J., STYLES, J. A., ANDERSON, D., LEFEVRE, P. A. and WESTWOOD, F. R. Evaluation of six short-term tests for detecting organic chemical carcinogens and recommendations for their use. *Nature* 264 (1976) 624—627.
119. PURCHASE, I. F. H., LONGSTAFF, E., ASHBY, J., STYLES, J. A., ANDERSON, D., LEFEVRE, P. A. and WESTWOOD, F. R. An evaluation of short-term tests for detecting organic chemical carcinogens. *Br. j. cancer* 37 (1978) 873—959.
120. PURCHASE, I. F. H., RICHARDSON, C. R., ANDERSON, D., PADDLE, G. M. and ADAMS, W. G. F. Chromosomal analysis in vinyl chloride-exposed workers. *Mutat. res.* 57 (1978) 325—334.
121. RANNUG, U., SUNDVALL, A. and RAMEL, C. The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium*: I. Activation through conjugation with glutathione in vitro. *Chem. biol. interact.* 20 (1978) 1—16.
122. RICE, J. M. The biological behaviour of transplacentally induced tumours in mice. In: *Transplacental carcinogenesis*. International Agency for Research on Cancer, Lyon 1973, pp. 71—83.
123. ROM, W. N. Effects of lead on the female and reproduction: A review. *Mt. sinai j. med.* 43 (1976) 542—552.
124. ROSENBERG, P. and KIRVES, A. Miscarriage among operating theatre staff. *Acta anaesthesiol. scand.* 53 (1973) 37—42.
125. ROWLEY, J. A possible role for non-random chromosomal changes in human hematologic malignancies. In: A. DE LA CHAPELLE and M. SORSA (eds.) *Chromosomes today* (vol. 6). Elsevier/North-Holland, Amsterdam 1977, pp. 345—355.
126. SAFFIOTTI, U. and WAGONER, J. K. (eds.). Occupational carcinogenesis. *Ann. n.y. acad. sci.* 271 (1976) 1—516.
127. SANOTSKY, I. V. Aspects of the toxicology of chloroprene: Immediate and long-term effects. *Environ. health perspect.* 17 (1976) 85—93.
128. SARMA, D. S. R., RAJALAKSHMI, S. and FARBER, E. Chemical carcinogenesis: Interactions of carcinogens with nucleic acids. In: F. F. BECKER (ed.) *Cancer, a comprehensive treatise* (vol. 1). Plenum Press, New York, NY 1975, pp. 235—287.
129. SAXEN, I. and LAHTI, A. Cleft lip and palate in Finland: Incidence, seasonal and geographical variations. *Teratology* 9 (1974) 217—223.
130. SAXEN, I. and RAPOLA, J. *Congenital defects*. Holt, Rinehart and Winston Inc., New York, NY 1969. 247 p.
131. SEARLE, A. G. The specific locus test in the mouse. *Mutat. res.* 31 (1975) 277—290.
132. SENTRAKUL, P. and POTTER, E. L. Pathological diagnoses on 2681 abortions at the Chicago lying-in hospital, 1957—1965. *Am. j. public health* 56 (1966) 2083—2092.
133. SETLOW, R. B. Repair deficient human disorders and cancer. *Nature* 271 (1978) 713—717.
134. SIEBERT, D. Induction of mitotic conversion in *Saccharomyces cerevisiae* by lymph fluid and urine of cyclophosphamide treated rats and human patients. *Mutat. res.* 21 (1973) 202.
135. SIROVER, M. and LOEB, L. A. On the fidelity of DNA replication. *J. biol. chem.* 252 (1977) 3605—3610.
136. SOBELS, F. H. and VOGEL, E. Assaying potential carcinogens with *Drosophila*. *Environ. health perspect.* 15 (1976) 141—146.
137. SRAM, R. J., ČERNA, M. and KUČEROVA, M. The genetic risk of epichlorohydrin as related to the occupational exposure. *Biol. Zentralbl.* 95 (1976) 451—462.
138. STEIN, Z., SUSSE, M., WARBURGTON, D., WITTE, J. and KLINE, J. Spontaneous abortion as a screening device: The effect of fetal survival on the incidence of birth defects. *Am. j. epidemiol.* 102 (1975) 275—290.
139. STRANDBERG, M., SANDBÄCK, K., AXELSON, O. and SUNDELL, L. Spontaneous abortions among women in hospital laboratory. *Lancet* 1 (1978) 384—385.
140. SUGIMURA, T., YAHAGI, T., NAGAO, M., TAKEUCHI, M., KAWACHI, T., HARA, K., YAMASAKI, E., MATSUSHIMA, T., HASHIMOTO, Y. and OKADA, M. Validity of mutagenicity tests using microbes as a rapid screening method for environmental carcinogens. In: R. MONTESSANO, H. BARTSCH and L. TOMATIS (eds.) *Screening tests in chemical carcinogenesis*. International Agency for Research on Cancer, Lyon 1976, pp. 81—104.
141. SUNDERMAN, F. W. Carcinogenic effects of metals. *Fed. proc.* 37 (1978) 40—45.
142. SUTTON, H. E. The impact of induced mutations on human populations. *Mutat. res.* 33 (1975) 17—24.
143. THIESS, A. M. and FLEIG, I. Analysis of chromosomes of workers exposed to acrylonitrile. *Arch. toxicol.* 41 (1978) 149—152.
144. VAINIO, H., HEMMINKI, K. and ELO-

- VAARA, E. Toxicity of styrene and styrene oxide on chick embryos. *Toxicology* 8 (1977) 319—325.
145. VAINIO, H. and HIETANEN, E. The role of extrahepatic metabolism in drug disposition and toxicity. In: P. JENNER and B. TESTA (eds.), *Concepts in drug metabolism*. Marcel Dekker Inc., New York, NY. (in press)
146. VAINIO, H., PÄÄKKÖNEN, R., RÖNNHOLM, K., RAUNIO, V. and PELKONEN, O. A study on the mutagenic activity of styrene and styrene oxide. *Scand. j. work environ. & health* 3 (1976) 147—151.
147. VENITT, S., BUSHELL, C. T. and OSBORNE, M. Mutagenicity of acrylonitrile (cyanoethylene) in *Escherichia coli*. *Mutat. res.* 45 (1977) 283—290.
148. VERBURGT, F. G. and VOGEL, E. Vinyl chloride mutagenesis in *Drosophila melanogaster*. *Mutat. res.* 48 (1977) 327—336.
149. VIOLA, P. L., BIGOTTI, A. and CAPUTO, A. Oncogenic response of rat skin, lungs and bones to vinyl chloride. *Cancer res.* 31 (1971) 516—519.
150. VOGEL, E. and SOBELS, F. H. The function of *Drosophila* in genetic toxicology testing. In: A. HOLLAENDER (ed.), *Chemical mutagens* (vol. 4). Plenum Press, New York, NY 1976, pp. 93—142.
151. WASKELL, L. A study of the mutagenicity of anesthetics and their metabolites. *Mutat. res.* 57 (1978) 141—153.
152. WEISBURGER, J. H. and WILLIAMS, G. M. Metabolism of chemical carcinogens. In: F. F. BECKER (ed.) *Cancer, a comprehensive treatise* (vol. 1). Plenum Press, New York, NY 1975, pp. 185—234.
153. WILSON, J. G. *Environment and birth defects*. Academic Press, New York, NY 1973. 305 p.
154. WORLD HEALTH ORGANIZATION, Regional Office for Europe. Working Group on Epidemiological Surveillance Systems. Gabrovo Bulgaria 1978.
155. WÜRGLER, F. E., SOBELS, F. H. and VOGEL, E. *Drosophila* as assay system for detecting genetic changes. In: B. J. KILBEY, M. LEGATOR, W. NICHOLS and C. RAMEL (eds.), *Handbook of mutagenicity test procedures*. Elsevier Scient. Publ. Co., Amsterdam 1977, pp. 335—373.
156. WYNDER, E. L. and GORI, G. C. Contribution of the environment to cancer incidence: An epidemiologic exercise. *J. natl. cancer inst.* 58 (1977) 825—832.
157. YAMASAKI, E. and AMES, B. A. Concentration of mutagens from urine by adsorption with the nonpolar resins XAD-2: Cigarette smokers have mutagenic urine. *Proc. natl. acad. sci. u.s.a.* 74 (1977) 3555—3559.
158. ZUBROFF, J. and SARMA, D. S. R. A nonradioactive method for measuring DNA damage and its repair in nonproliferating tissues. *Anal. biochem.* 70 (1976) 387—396.

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