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HOW RELEVANT ARE HIGH DOSES IN MUTAGENICITY AND CARCINOGENICITY STUDIES IN ANIMALS?

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Summary

Toxicity, including genotoxicity of chemicals, is frequently affected by metabolic conversion of the chemicals by the host, leading to inactive derivatives. Inactivation mechanisms can be overcome by high doses of a chemical resulting in a greatly altered dose—effect relationship and drastically increased toxicity. This is exemplified by the dose-dependent effect of chloroform and 1,1-dichloroethylene in animals, by investigations on the glutathione-dependent mutagenicity of chlorodinitrobenzene in the Ames test, and by the effects resulting from inhibited metabolic inactivation of naphthalene in isolated hepatocytes.

At low doses genotoxic effects of chemicals may be prevented by metabolic inactivation, e.g. chloroprene is mutagenic in vitro but not carcinogenic, possibly due to glutathione conjugation. Moreover, disputable data on mutagenicity or carcinogenicity of styrene must be judged in that its mutagenic metabolite styrene oxide is inactivated by efficient cellular epoxide hydrolase activity in vivo.

Hepatic monoxygenase activity creates mutagenic hydrogen peroxide which is trapped in vivo by catalase and glutathione peroxidase. Thus, information on the mechanism and extent of inactivation is essential for extrapolation from high doses of chemicals used in toxicity studies in animals to low doses that are relevant to human exposure.

Introduction

For risk estimation of man's exposure to carcinogenic chemicals it is customary to extrapolate from high doses at which tumor induction is observed in animals to low doses in the human environment. However, there is increasing evidence that several carcinogens induce other biological responses at low doses than those at high doses. Furthermore, species differences in the metabolic activation and inactivation processes may also question a linear dose—effect relationship.

An evaluation of these facts is essential because one has to be aware that man is continuously exposed to small amounts of mutagens and carcinogens. Commoner et al. (1978) reported the presence of mutagens in broiled hamburgers; the presence of mutagenic activity in charred fish and beef was previously reported by Nagao et al. (1977) and Sugimura et al. (1977). Such mutagens apparently stem from pyrolisation of the amino acids tryptophan and glutamic acid (Sugimura, 1978). Nitrosamines present in food (Spiegelhalder et al., 1979; Scanlan et al., 1980) are constantly formed in the human stomach when secondary and tertiary amines are ingested together with nitrite (Liljinsky, 1977; Tannenbaum, 1980). Chloroform has been used for many years in tooth pastes and in cough syrup formulations and it is a drinking-water contaminant whenever chlorine is added for hygienic reasons (Fishbein, 1979; Stockinger, 1977). Sugimura recently evaluated that by the age of 50 a person has eaten approx. 10 tons dry weight of food (Sugimura, 1978). Even though concentrations of environmental contaminants in food are very low, their continuous intake could be sufficient to cause genetic damage possibly resulting in the appearance of cancer.

Models to extrapolate from high to low doses and between species

Animal studies to investigate the potential hazards of such chemicals are usually performed at relatively high levels of exposure (Crump et al., 1976; Cornfield, 1977; Scientific Committee, 1978; Maugh, 1978; Tomatis, 1979; Carter, 1979; Interagency Regulatory Liaison Group, 1979). In general, human risk is determined by extrapolating from the high doses used in the animal experiments to the much lower levels to which man is actually exposed assuming that no threshold exists. This is based on the concept that cancer is induced after the reaction of a single or several molecules with DNA or other macromolecules in a cell. Once such a reaction has occurred the cell is damaged irreversibly and initiates a new disorganized cell line which finally produces the tumor. This mechanism implies that there will be a certain tumor incidence in a population no matter how long the dose of the carcinogen has been. In this case, cancer incidence at low doses will be a linear function of the high doses.

The formula generally applied in extrapolation from high to low doses is (Reitz et al., 1978): Linear $P = \beta \times d$ where P is the fractional incidence of excess tumors, d the average daily dose in mg/kg and β is a dimensionless parameter to be estimated from the experimental data. Additionally, a species correction factor can be applied to β which has been chosen as the cube root of the ratio of the body weights to calculate the dose per surface area. This always implies that man is considered to be more sensitive to a carcinogen than mice or rats. Rall (1960) established this correlation with 18 anti-neoplastic agents. They produced, however, direct toxicity, and Rall stated that these compounds are not involved in the various drug-metabolizing systems. However, there is ample evidence that most of the environmental chemicals undergo metabolism by the host, and it becomes evident from this that the rate of metabolism must be included into the process of risk estimation.

Reitz et al. (1978) suggested therefore that the relative rates of production of toxic metabolites be introduced into risk estimation. And, indeed, relative

carcinogenicity of chloroform, vinylidene chloride, 2-acetylaminofluorene and trichloroethylene in rats and mice correlated well with the relative rate of metabolic activation to a reactive intermediate of each of these chemicals.

The resolution of these approaches by animal experiments seems to be impossible. To decide which of the correction factors is correct would mean that data on the response to low doses of the chemical concerned would have to be collected. But this would mean measuring low incidences of cancer in animal populations, say 0—15%, and these low incidences can only be distinguished with any degree of statistical certainty from the natural rate of cancer in animals by trials involving impossibly large numbers of both control and treated animals. Thus, in practice, only relatively high doses can yield statistically significant data. But in many cases such high doses may produce cancer simply because their very quantity overwhelms the biochemical pathways that would detoxify smaller, more realistic doses.

Since no conclusive solution of the problem can be obtained from the standard carcinogenicity or mutagenicity test procedures the only approach to solve the problem should be the evaluation of mechanisms which may affect the tumor incidence at low and high doses of carcinogens. This may provide a more solid basis for the presently highly speculative and uncertain quantitative risk assessment.

In general several mechanisms are considered to be involved in the inactivation of chemical carcinogens. Important contributors to these mechanisms are membranes that may not permit potential carcinogens to come into contact with DNA and enzyme systems for repair of damage to DNA. Some evidence also suggests that the immune system can identify and destroy many kinds of operant cells produced by chemicals that slip through the other defenses.

Moreover, enzyme systems for metabolic toxification and detoxification modify the biological impact of chemicals and have to be considered in the course of risk estimation.

This is explained in more detail by discussing the following points:

(1) Is there any indication of threshold doses of carcinogenic chemicals in man due to metabolism?

(2) Are the high doses of carcinogens used in animal studies generally representative for the low doses to which man is exposed?

(3) Does metabolism result in qualitative and quantitative differences in the susceptibility of different species to carcinogens?

Indication of threshold doses due to metabolic inactivation

(a) Carcinogenicity of estrone

The hormone estrone, for example, has been shown by several investigators to be carcinogenic when given to laboratory animals in large doses (IACR, 1974). However estrone is present in very small concentrations in all humans, yet without demonstrable evidence of harm. It is present in much higher concentrations in women than in men which might be at least part of the cause of the much greater incidence of breast cancer in women.

Moreover, glucuronidation of estrogens seems to be a significant inactivating mechanism. A recent report by Fishman et al. (1979) indicates a correlation

between low glucuronidation capacity and the occurrence of breast cancer in women. These authors studied steroid excretion in the urine of 30 young women who by genetic analysis were at risk for familial breast cancer as compared to a carefully matched control group. Highly significant differences were observed in the excretion of glucuronides of estrone and estradiol with the high-risk women exhibiting lower values than the controls. Besides the finding of a potential discriminant for identifying women at high risk for breast cancer, this observation strongly indicates that the status of inactivation mechanisms affects susceptibility to potential carcinogens.

(b) Increased DNA-repair by reactive oxygen species

Recently, we demonstrated that reactive oxygen species, which are formed during monooxygenase-mediated metabolism of chemicals, induce DNA-repair activity in the human lymphoblastoid cell line NC37 BaEV (Andrae and Greim, 1979). This was observed in the course of measuring the induction of DNA-repair replication by mutagens requiring metabolic activation (Fig. 1). Muta-

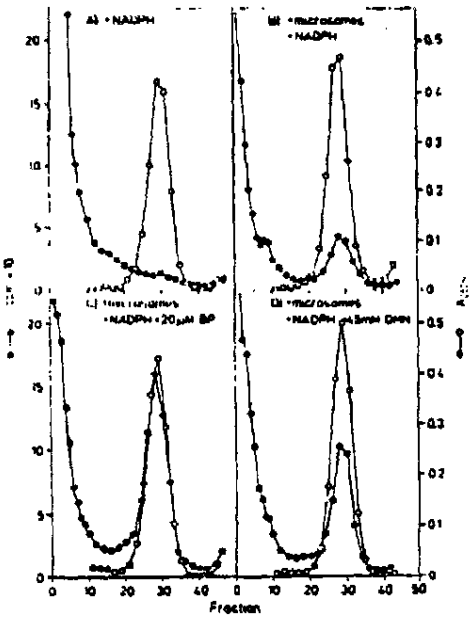

Fig. 1. Repair replication in NC37 BaEV cells. Cultures were incubated in the presence of 1 µM 5-fluorodeoxyuridine and 10 µM 5-bromodeoxyuridine for 1 h. After addition of 2.5 mM hydroxyurea cells were incubated for 3 h with *A*: NADPH; *B*: 0.5 mg protein/ml liver microsomes of clophen A 50 pretreated rats + NADPH; *C*: liver microsomes + NADPH + 20 µM benzo(a)pyrene; *D*: liver microsomes + NADPH + 45 min mM dimethylnitrosamine, and labeled for 4.5 h with 10 µCi [^{3}H]thymidine (40Ci/mmole). Cells were lysed in 0.5% sodiumdodecylhydrogensulfate, digested with proteinase K (50 µg/ml) and sedimented in alkaline CsCl-gradients (Andrae and Greim, 1979). The location of [^{3}H]-label at normal density (coincident with the absorbance peak) is indicative for repair replication.

gens such as benzo[*a*]pyrene and dimethylnitrosamine induce repair activity. However, in control experiments without the mutagen a marked incorporation of repair label was observed. The incubate was comprised of washed microsomes of phenobarbital-treated mice, NADPH and hydroxyurea which is used to suppress semiconservative DNA synthesis. When this inhibitor was omitted, no repair replication occurred. Hydroxyurea-induced repair was dependent on the presence of microsomes and NADPH, and was reduced to 50% by the metabolic inhibitor SKF 525-A. This highly indicates that hydroxyurea-induced repair is a consequence of microsomal monooxygenase-mediated metabolism. Since addition of glutathione (GSH) and superoxide dismutase reduced and catalase prevented hydroxyurea-induced DNA repair, hydrogen peroxide (H_2O_2) or reactive oxygen species deriving from H_2O_2 are likely to be the genotoxic agents. As H_2O_2 production generally occurs during monooxygenase function (Hildebrandt and Roots, 1975; Jones et al., 1978) we investigated the effect of ethylmorphine. Accordingly, ethylmorphine induced DNA repair in the test system but the effect was prevented in the presence of catalase (Fig. 2). Other in vitro cell systems, e.g. human A549 lung tumor cells, apparently have a sufficient H_2O_2-inactivation capability as these systems did not show enhanced repair activity.

The precise mechanisms by which reactive oxygen species are trapped in vivo remain unknown. GSH peroxidase is likely to be involved. It is evident that efficient H_2O_2-inactivation must be present in vivo to prevent reactive oxygen species from exceeding a no-effect concentration and thus preventing genotoxic effects.

(c) Chloroprene: Mutagenicity but no carcinogenicity

Further evidence for no-effect levels due to metabolic inactivation is given by toxicity studies on chloroprene (2-chloro-1,3-butadiene).

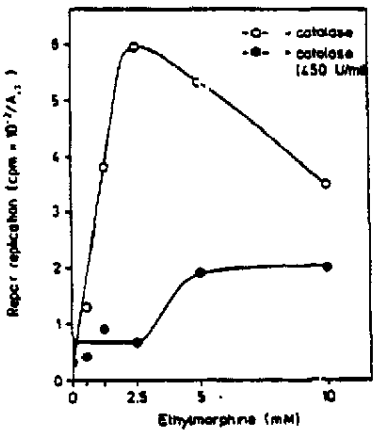

Fig. 2. Repair replication in NCI-H EsEV cells in the presence of liver microsomes isolated from phenobarbital-treated mice, NADPH and ethylmorphine. For details see legend to Fig. 1.

Chloroprene is a reactive chemical which is widely used in the manufacturing of the synthetic rubber neoprene. Chloroprene has been suggested to be responsible for the increased incidence of skin and lung cancer in workers exposed to the chemical. However, the data are questionable and no carcinogenic effects of chloroprene have been noted in animal studies to date (Fishbein, 1979; Ponomarkov and Tomatis, 1980). Bartsch et al. (1975, 1979) have demonstrated a slight mutagenicity of chloroprene in *Salmonella typhimurium* strains without metabolic activation. Mutagenicity was increased about 3-fold in the presence of S9 fractions. These observations were considered to reflect the probable formation of an epoxide intermediate of chloroprene.

Why was chloroprene shown to be mutagenic but not carcinogenic? In analogy to vinylchloride and 1,1-dichloroethylene, a subsequent detoxification of the chloroprene by conjugation with glutathione has been suggested (Haley, 1978; Plugge and Jaeger, 1979). Since in vitro mutagenicity test procedures do not include sufficient glutathione (Summer et al., 1980), the apparent discrepancy of mutagenicity in vitro without producing carcinogenicity in the animal may be explainable. We tested the possible involvement of glutathione in inactivation in isolated rat hepatocytes and in the whole animal (Summer and Greim, 1980).

Similar to the rat liver, cellular glutathione decreased in isolated rat hepatocytes to about 50% of the control values within 15 min in the presence of 3 mM chloroprene (Fig. 3). This depletion was concentration-dependent and increased with time. In hepatocytes of animals pretreated with phenobarbital or Clophen A50, 3 mM of chloroprene almost completely depleted glutathione within 30 min.

This strongly indicates that a Phase I reaction, presumably epoxide for-

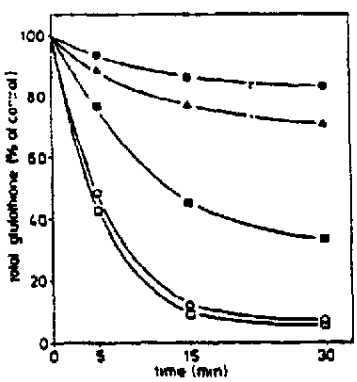

Fig. 3. Glutathione depletion in isolated rat hepatocytes by chloroprene. Data are from 1 representative experiment out of 3 with different cell preparations. Control level of cellular GSH amounted to 19.9 ± 2.1 nmoles/mg cell protein. Closed symbols: Hepatocytes (5 mg cell protein/ml) of untreated animals incubated with 0.5 mM (●), 1.0 mM (▲) and 3.0 mM (■) chloroprene. Open symbols: Hepatocytes from either phenobarbital- (○) or Clophen A 50- (□) pretreated animals incubated with 3 mM chloroprene.

mation which is enhanced after Clophen A50- or phenobarbital-pretreatment, precedes glutathione conjugation.

Glutathione-dependent detoxification in the animal was further verified by determining urinary thioether excretion comprised of GSH conjugates and mercapturic acids. Chloroprene administration to rats resulted in a dose-dependent increase in the excretion of urinary thioethers (Fig. 4). This increase was reversible and completed within 24 h at all dose levels administered. At dose levels of 50 and 100 mg chloroprene/kg, the additional excretion of urinary thioethers was almost 200 and 450 μmoles/kg daily, respectively.

It is to be noted that no linear dose—response relationship was observed in thioether excretion, indicating that at higher doses of chloroprene the availability of cellular glutathione becomes rate-limiting.

Thus, a dose level at which high concentrations of chloroprene overcome GSH-inactivation is suggested. Only sufficiently high doses are expected to induce carcinogenicity. They have not been used so far in carcinogenicity studies.

Effects of high doses on metabolism

(a) Overwhelmed metabolic inactivation

The latter observation suggests that high doses of a chemical may have other biological effects than lower doses by overcoming metabolic inactivation mechanisms.

Vinylidene chloride (1,1-dichloroethylene) is also conjugated to glutathione. In the rat, glutathione is depleted by exposure to high levels of 1,1-dichloroethylene (Jaeger et al., 1974). Fasting of rats prior to 1,1-dichloroethylene exposure further depleted the glutathione and, at the same time, dramatically increased the hepatotoxicity of the chemical. Furthermore, McKenna et al. (1977) reported that a sudden disproportionate increase in macromolecular binding of 1,1-dichloroethylene metabolites occurred when glutathione was depleted by more than 30%. These observations are strongly in favour of the protective function of such inactivating systems.

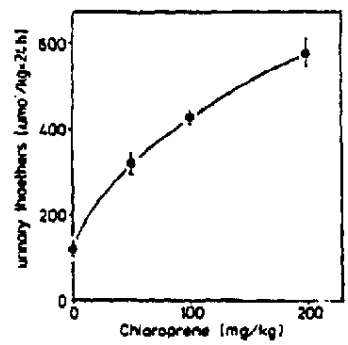

Fig. 4. Chloroprene-dependent excretion of thioethers in the urine of rats. Chloroprene was administered in olive oil by stomach tube. Data represent means ± S.D. from 4 animals.

(2) Naphthalene: Protein binding and conjugation

Isolated hepatocytes metabolized naphthalene to water-soluble compounds (Schwarz et al., 1980). Metabolism of the hydrocarbon was linear for 1 h reaching a plateau after 2 h. During biotransformation of naphthalene reactive intermediates were formed which became irreversibly bound to cellular protein. Binding almost paralleled the increase in the formation of metabolites. Formation of water-soluble compounds and binding was due to metabolism since frozen-thawed cells in the presence of 1 mM SKF 525-A showed neither formation of water-soluble compounds nor binding to cellular macromolecules.

Conjugation with UDP-glucuronic acid and sulfate is one of the main pathways of naphthalene metabolism in hepatocytes (Bock et al., 1976). We inhibited these conjugation mechanisms by interfering with the synthesis of their respective co-factors. Addition of D-galactosamine reduces levels of uridine-diphosphoglucuronic acid by trapping uridine-triphosphate and by inhibiting UDPG dehydrogenase activity (Bauer and Reutter, 1973). Sulfation is inhibited by incubation of the cells in sulfate-free medium which decreases synthesis of 3'-phosphoadenosine-5-phosphosulfate (PAPS) and, thereby, sulfation (Schwarz, 1980). Incubation of hepatocytes in a sulfate-free medium in the presence of 3 mM D-galactosamine did not affect formation of water-soluble metabolites from naphthalene. However, a drastic increase in covalently bound metabolites was demonstrated (Fig. 6).

It is evident from this that cellular formation of reactive and toxic species of chemicals is frequently counteracted by metabolic detoxification. A disproportionate increase in the toxic effects can occur when high concentrations of a chemical are present which overcome inactivation processes.

In toxicology, such threshold levels on the basis of overwhelmed inactivation have long been established. For example, furosamide, a diuretic, is excreted predominantly unaltered in the urine when low therapeutic doses are given. Administration of high doses, however, overwhelm renal clearance and cause a disproportionate increase in toxic metabolites which react with macromolecules. Moreover, bromobenzene is metabolized in the liver to the reactive 3,4-bromobenzene oxide. This molecule is detoxified by glutathione con-

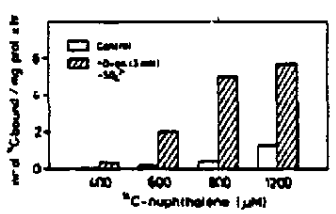

Fig. 6. Effect of 3 mM D-galactosamine (D-gs) and sulfate depletion on irreversible binding of [^{14}C] naphthalene. Hepatocytes were prepared from 200 g Sprague—Dawley rats by in situ perfusion with collagenase. Hepatocytes (1.25×10^6 cells) were incubated in Waymouth MB 752:1 medium in spinner flasks. Metabolism was stopped with ice-cold *n*-hexane. "Water soluble metabolites" of naphthalene were quantified by liquid-scintillation counting of the water phase plus neutralized NaOH extract of the organic phase containing the phenolic products. To determine irreversible binding of ^{14}C radioactivity, 0.5 ml of the cell suspension was sonified twice with 0.5 ml ethanol. The subsequent solvent extractions were performed as previously described (Hesse et al., 1978).

jugation. When high doses of bromobenzene are given, glutathione is depleted and the reactive metabolites interact with cellular macromolecules (Gillette, 1974a, b). Other chemicals for which there is evidence that high doses cause a disproportionate increase in toxicity and possibly carcinogenicity are aspirine, salicylamide, acetaminophen, styrene, ethylene glycol and aniline (Gehring et al., 1976).

(b) Impaired metabolic activation

On the other hand, potential carcinogens not only overwhelm inactivating capabilities but also inhibit metabolic activation reactions. The animal bioassay results on vinylchloride of Maltoni (1977), Lee et al. (1978) and Viola et al. (1971) suggest that inhalation beyond 150 ppm results in a smaller increase in carcinogenic response in rodents than that predicted from a linear extrapolation of the responses in the dose range of 0—150 ppm. Recent pharmacokinetic studies indicate that at high doses of vinyl chloride the mechanisms which metabolically activate this chemical are saturated. Bolt et al. (1980) observed no further increase of DNA and protein-bound vinyl chloride metabolites in rats beyond a certain level of exposure. A similar effect is reported with carbon tetrachloride by Ugazio et al. (1972). A preceding low dose of CCl_4 significantly increased the LD_{50} of rats when a high dose was given thereafter.

Modification due to species differences in metabolism

Moreover, species differences in the metabolic activation and inactivation systems further complicate extrapolation from high doses given in animal studies to the low doses to which man is exposed.

Several well-established examples indicate qualitative and quantitative species differences in the activating and inactivating metabolism interfering with the capability of the reactive intermediates to bind to the genetic material.

(a) Species differences in metabolic activation

(1) 2-Acetylaminofluorene

For example, 2-acetylaminofluorene (2-AAF), a strong hepatocarcinogen in male rats, is without effect in guinea pigs. 2-AAF is an indirect carcinogen. It requires aromatic *N*-hydroxylation forming the *N*-hydroxy-2-AAF (Miller et al., 1961). Since guinea pigs do not have this enzyme this species is insensitive to the carcinogen although they develop tumors when the *N*-hydroxy product is given (Miller et al., 1964).

Other species differences in the carcinogenicity of 2-AAF cannot be related to this activating mechanism. However, carcinogenicity of 2-AAF is also dependent on the formation of a sulfo-derivative. This reaction is catalysed by a *N*-sulfotransferase which differs quantitatively in several animal species. DeBaun et al. (1970a, b) determined the activity of this enzyme and were able to explain further the species differences of 2-AFF carcinogenicity (Table 1). Male rats, which are highly sensitive to this carcinogen, possess a high sulfotransferase activity. It is low in female rats and in mice of both sexes which are

TABLE 1

HEPATOCARCINOGENICITY OF 2-ACETYLAMINOFLUORENE AS RELATED TO SULFO-TRANSFERASE ACTIVITIES IN SEVERAL SPECIES

De Baun et al., 1970a, b.

		Hepatocarcinogenicity	Sulfo-transferase activity[a]
Rat	M	+++	23.0
	F	+	4.0
Mouse	M	+	0.5
	F	+	0.5
Guinea pig	M	—	0.8
Rabbit	M	—	2.0

[a] μmoles/0.04 ml supernatant per 20 min.

less susceptible than the male rat. The other species tested which have moderate or lower sulfotransferase activities are not known to produce hepatocarcinoma due to 2-AAF.

(2) Chloroform

Moreover, not only qualitative but also quantitative differences in the metabolizing rate exist between the different animal species and man. Several investigators have studied the metabolism of chloroform in rats and mice and have concluded that mice metabolize chloroform more rapidly than do rats (Pohl, 1979). The most complete studies are those of Brown et al. (1974) who administered a 60 mg/kg dose of chloroform orally to rats, squirrel monkeys and 3 strains of mice (Table 2). Very little unmetabolized chloroform was recovered from the mice. In the rat, 3 times as much unmetabolized chloroform was found, while in the monkey 13 times as much unaltered chloroform was exhaled. Fry et al. (1972) administered chloroform to human volunteers and found that 17—66% of the material was exhaled unchanged. However, the dose of chloroform used in these investigations was only 7 mg/kg. Thus, the relative amount of chloroform that would be metabolized at the higher doses used in the animal experiments may even have been overestimated. Based on these investigations, Reitz et al. (1978) suggested man to be the least sensitive of the species to the carcinogenic action of chloroform. This is also based on the data of Weiss et al. (1977) who observed that man normally metabolizes

TABLE 2[a]

INTERSPECIES DIFFERENCES IN CHLOROFORM METABOLISM AS DETERMINED BY URINARY EXCRETION OF THE UNCHANGED CHEMICAL

	Oral dose (mg/kg)	Unchanged $CHCl_3$ excreted (% of dose)
Mouse	60	6
Rat	60	20
Monkey	60	78
Man	7	17—66

[a] Modified from Reitz et al. (1978).

materials much more slowly than the small laboratory animals such as mice or rats. Consequently, a direct extrapolation from carcinogenicity tests on chloroform in mice or rats would overestimate the risk of man to this potential carcinogen.

(3) Halogenated ethylenes

In addition to chloroform, there are several other examples where the relative carcinogenicity of chemicals correlates well with their rate of activation to a reactive species. 1,1-Dichloroethylene is metabolized more extensively in the mouse than in the rat (McKenna et al., 1977). Correspondingly, tumors have been observed in mice exposed to 1,1-dichloroethylene (Maltoni, 1977), whereas in rats, although conflicting results appeared, no tumor formation seems to be related to this chemical (Fishbein, 1979; Maltoni, 1977; Rampey et al., 1977; Maltoni et al., 1977).

Trichloroethylene is carcinogenic in B6C3F1 mice but not in Osborn–Mendel rats (Natl. Cancer Inst., 1976). This again correlated with the greater capacity of mouse microsomes to catalyze binding of trichloroethylene metabolites to DNA (Bannerjee and van Duuren, 1978).

These data indicate that the status of metabolic activation systems in the different species significantly affects susceptibility to carcinogens in the animal and explain species differences.

(b) Species differences in metabolic inactivation

(1) Aflatoxin B_1

In addition, there is also evidence that species differences of inactivating mechanisms such as binding of the reactive intermediates with glutathione modify adverse effects of chemicals. The hepatotoxic and carcinogenic effects of aflatoxin B_1 are attributed to the reactions of metabolically formed AF B_1-2,3-oxide (Croy et al., 1978). The marked species differences in susceptibility to carcinogenicity could not be related so far to the different ability to generate the epoxide. Recently, Degen and Neumann (1978) found that AF B_1-epoxide is inactivated by conjugation with glutathione. Degen (1979) has since reported that mouse-liver preparations most effectively catalysed the formation of the glutathione conjugate. These results support the view that the lower susceptibility of mice is not a result of less effective activation but rather of more effective inactivation of the reactive AF B_1 intermediate due to the formation of glutathione conjugates.

(2) Styrene epoxide hydrolase

Oesch (1980), Oesch et al. (1973, 1974) and Jerina et al. (1977), studying the effects of microsomal epoxide hydrolase activity, further stressed the importance of considering species differences in metabolic inactivation. The hydrolase generally converts reactive epoxides into more stable dihydrodiols (Jerina et al., 1977) which are frequently further conjugated with glucuronic acid (Oesch, 1980). For example hydrolase activity is involved in the biotransformation of styrene (Leibman, 1975). It is highly suggested that hydrolase activity being present in the S9 fraction prevents styrene from being muta-

genic in the plate-incorporation assay (Greim et al., 1977) although styrene oxide, the metabolite which is formed during metabolic activation of styrene, is a potent mutagen in this test (Mily and Garro, 1976).

Epoxide hydrolase generally plays an important role in the inactivation of many aromatic and olefinic compounds of industrial interest. Since man has a much higher hydrolase activity than most of the investigated laboratory animals (Oesch et al., 1974) it is to be expected that man is the least sensitive to such compounds.

We can conclude the following from the experimental data presented: Metabolism plays an important role in the carcinogenic or mutagenic activity of chemicals. It is species-dependent, can be influenced by the dose of the chemical administered, and thus has to be considered in extrapolation from the high doses given to the experimental animal to the low doses to which man is exposed.

Regarding metabolism, roughly 4 groups of chemicals can be differentiated:

(1) Compounds which undergo neither metabolic activation nor significant inactivation, e.g. methyl methanesulfonate.

(2) Compounds which only undergo metabolic activation, e.g. dimethyl- or diethyl-nitrosamine.

(3) Compounds which only undergo metabolic inactivation, e.g. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

(4) Compounds which undergo both metabolic activation and inactivation, e.g. vinyl chloride or chloroform.

For risk estimation, it is apparent that linear extrapolation from high to low doses and from one animal species to another is only possible for compounds of Group One which require neither metabolic activation nor inactivation.

Apparent species differences in the metabolic activation and inactivation system have to be considered concerning compounds of Groups Two to Four. Extrapolation can be further complicated when high doses of the chemical inhibit or overcome metabolic activation or inactivation.

Carcinogens acting by secondary mechanisms

Another group of compounds induces tumors by secondary mechanisms, for example, by suppressing production of hormones or by inducing necrosis at high doses which finally results in tumor formation.

Several chemicals inhibit function of the thyroid gland. These goitrogens induce tumors by a secondary mechanism. Thiourea blocks thyroxin synthesis by specific inhibition of iodine peroxydation (Davidson et al., 1979). As a consequence, insufficient amounts of thyroxin are produced by the thyroid gland leading to an increased hormonal stimulation of the thyroid by the hypophysis. This results in a hypertrophy of the thyroid, and after continuous exposure, thyroid adenoma appears (IARC, 1974). Finally, carcinomas of the thyroid are observed. At the lower doses of thiourea when no hypertrophy of the thyroid is observable, carcinomas of this organ have not been detected.

Carcinomas of the thyroid have been detected in rats and mice receiving a diet of 0.25% thiourea (Purves and Griesbach, 1947). This dose is equivalent to approx. 100 mg/kg daily. When administered as a drug, repeated daily intake

of approx. 10 mg/kg was goitrogenic. Presently, thiourea is used as an industrial chemical only and sufficient measures to protect man at his working place have to be provided (Fishbein, 1979). However, workers would not be endangered by thiourea exposure unless exposed to concentrations affecting thyroid functions which can easily be detected.

Nitrilotriacetic acid and chlorothalonil, both excreted almost quantitatively via the kidney, induce urinary-tract tumors which have been preceded by tubular necrosis (Natl. Cancer Inst., 1977; Anderson and Kanerva, 1978; WHO, 1975; FAO, 1978; Hicks et al., 1975). Due to the almost 200-fold concentration of the glomerular filtrate in the tubular system, high intratubular concentrations of both compounds occur. When high doses are given to an animal, intratubular supersaturation of the chemicals with the consequences of precipitation and cristalluria appear. This frequently is associated with hematuria. Both symptoms, cristalluria and hematuria, have been observed when high concentrations of such chemicals are given. During long-term feeding of high doses to animals, tubular necrosis preceded renal-tumor formation (Anderson and Kanerva, 1978). At lower doses which do not induce tubular necrosis, neither cristalluria nor hematuria, renal necrosis or kidney tumors have been observed.

One might also refer to the tumor-inducing capability of saccharin and cyclamate. Both compounds have been shown to induce renal tumors at high doses (Hicks et al., 1975; Oser et al., 1975). Both substances, when given in large quantities to animals, increase the urinary pH substantially. This gives rise, at least in the case of saccharin as well as nitrilotriacetic acid, to subepithelial microcalculi (Anderson and Kanerva, 1978; Armstrong, 1977).

It has been demonstrated with other carcinogens that alkalization of the urine acts in a cocarcinogenic fashion, thus increasing tumor incidence. Therefore, the tumor inducing effect of such compounds may be a physicochemical one which is related to their alkalizing properties. Now if this is true, then this is a dose-related phenomenon. The doses humans would take or would be exposed to in the case of other similarly acting chemicals, would be virtually insignificant in that the buffer systems in the organism and in the urine would maintain the physiological pH of the urine. Again, extremely high doses given to animals are irrelevant to human exposure in such cases.

This sort of consideration requires more basic research than is presently available and, if true, we would be dealing with at least 2 classes of carcinogens. One group includes the classical type of the electrophilic reactants which react with target nucleophiles resulting in mutagenic or carcinogenic effects. For these mechanisms so far there is no clear evidence for a threshold. Another group of compounds which indirectly induce tumors by other mechanisms associated with extremely high doses are likely to have a threshold dose. These considerations have been taken into account only marginally so far. If this hypothesis can be clearly established, then it would be possible to deal with different classes of chemicals that induce tumors and one can start to deal with questions of acceptable dose levels. This would also imply that high doses of such compounds used in animal studies would be irrelevant to human exposure.

Effect of contaminants at high doses

Finally, the problem of the presence of impurities in chemicals being subjected to mutagenicity or carcinogenicity testing is pointed out as exemplified by trichloroethylene. This chemical has been reported to produce high incidence of hepatocellular carcinoma in both male and female mice but not in rats after high daily oral doses. (Memorandum, HEW, 1975). Pure trichloroethylene has been found to be slightly mutagenic in a modified bacterial liquid incubation system (Greim et al., 1975) and noncarcinogenic in rats, mice and hamsters (Henschler et al., 1980). Technical-grade trichloroethylene, however, contains several impurities. Using gas-chromatographic mass-spectroscopic analysis, Henschler et al. (1977) recently found 10 different compounds in trichloroethylene which amounted to 0.65% of the sample. Two of these, namely epichlorohydrin and epoxybutane, constitute strong alkylating agents which have been shown to be mutagenic (Henschler et al., 1977; Fishbein, 1976) and carcinogenic (IARC, 1976; van Duuren and Banerjee, 1976). It has been concluded that the carcinogenic effect of technical trichloroethylene is most probably due to these epoxides which are added to several brands for stabilization.

This is supported by the observations that hepatocellular carcinomas were produced in mice but not in rats (IARC, 1976). Mice show a relatively lower activity of epoxide hydrolase, the enzyme which detoxifies epoxides such as epichlorohydrin and epoxybutane (Oesch, 1973). In the carcinogenicity studies, the animals were exposed to high doses of trichloroethylene with concomitant high dose of the contaminants. Thus, when only high doses of a chemical become mutagenic or carcinogenic, possible contamination by impurities has to be considered as well.

Conclusions

First

Interspecies extrapolation and high to low doses extrapolation of chemicals undergoing metabolic activation and inactivation is most complicated. One has to consider: (1) Species differences in metabolic activation; (2) Species differences in metabolic inactivation; (3) Sufficient inactivating capacity may almost completely prevent interaction of reactive intermediates with the genetic material; (4) High amounts of a chemical may overwhelm inactivating processes with the consequence of disproportionately increased interaction with genetic material.

Second

Several chemicals act by secondary mechanisms which only become effective at doses much higher than those to which man is exposed by the chemical.

Third

When only high doses result in toxic effects including mutagenicity or carcinogenicity, contribution of impurities have to be considered as well.

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References

Anderson, R.L., and R.L. Kanerva (1978) Hypercalciuria and cristalluria during ingestion of dietary nitrilotriacetate, Fd. Cosmet. Toxicol., 16, 669—674.

Andrae, U., and H. Orvim (1978) Induction of DNA repair replication by hydroxyurea in human lymphoblastoid cells mediated by liver microsomes and NADPH, Biochem. Biophys. Res. Commun., 87, 50—58.

Armstrong (1977) Question and answer session, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Lab., pp. 1701—1708.

Banerjee, S., and E.L. van Duuren (1978) Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA *in vitro*, Cancer Res., 38, 778—780.

Bartsch, H., C. Malaveille, R. Montesano and L. Tomatis (1975) Tissue-mediated mutagenicity of vinylidine chloride and 2-chlorobutadiene in *Salmonella typhimurium*, Nature (London), 255, 641—643.

Bartsch, H., C. Malaveille, A. Barbin and G. Planche (1979) Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues, Evidence for oxirane formation by P-450 linked microsomal monooxygenase, Arch Toxicol., 41, 249—277.

Bauer, C., and W. Reutter (1973) Inhibition of diphosphoglucose dehydrogenase by galactosamine-1-phosphate and UDP-galactosamine, Biochim. Biophys. Acta, 293, 11—14.

Bock, K.W., G. van Ackeren, F. Lorch and F.W. Birke (1976) Metabolism of naphthalene to naphthalene dihydrodiol glucuronide in isolated hepatocytes and in liver microsomes, Biochem. Pharmacol. 25, 2351—2356.

Bolt, H.M., J.G. Filser, R.J. Laib and H. Ottenwälder (1980) Binding kinetics of vinylchlorides and vinyl bromide at very low doses, Arch. Toxicol., Suppl. 3, 129—142.

Brown, D.M., F.F. Langley, D. Smith and D.C. Taylor (1974) Metabolism of chloroform — The metabolism of [^{14}C] chloroform by different species, Xenobiotica, 4, 151—163.

Carter, L.J. (1979) How to assess cancer risks, Science, 204, 811—816.

Commoner, B., A.J. Vithayathil, P. Dolara, S. Nair, P. Madyastha and G.C. Cuca (1978) Formation of mutagens in beef and beef extract during cooking, Science, 201, 913—916.

Cornfield, J. (1977) Carcinogenic risk assessment, Science, 198, 693—699.

Croy, R.G., J.M. Essigmann, V.N. Reinhold and G.N. Wogan (1978) Identification of the principal aflatoxin B_1—DNA adduct formed in vivo in rat liver, Proc. Natl. Acad. Sci. (U.S.A.), 75, 1745—1749.

Crump, K.S., D.G. Hoel, C.H. Langley and R. Peto (1976) Fundamental carcinogenic processes and their implications for low dose risk assessment, Cancer Res., 36, 2973—2979.

Davidson, B., M. Soodak, H.V. Strout, J.T. Neary, C. Nakamura and F. Maloof (1979) Thiourea and cyanamide as inhibitors of thyroid peroxidase: The role of iodide, Endocrinology, 104, 919—924.

Dellarn, J.L., E.C. Miller and J.A. Miller (1970a) *N*-Hydroxy-2-acetylaminofluorene sulfotransferase: Its probable role in carcinogenesis and in protein (methionyl) binding in rat liver, Cancer Res., 30, 577—595.

DeBaun, J.R., E.Y.R. Smith, E.C. Miller and J.A. Miller (1970b) Reactivity in vivo of the carcinogen *N*-hydroxy-2-acetaminofluorene: Increase by sulfate ion, Science, 167, 184—186.

Degen, G.H. (1979) Metabolic inactivation of aflatoxin B_1-epoxide as a cause for the difference in aflatoxin-susceptibility of rat and mouse, An in vitro investigation, Naunyn-Schmiedeberg's Arch. Pharmacol., 307, Suppl. R15.

Degen, G.H., and H.G. Neumann (1978) The major metabolites of aflatoxin B_1 in the rat is a glutathione conjugate, Chem.-Biol. Interact., 23, 239—255.

Ducks, E.L., and G. Krishna (1976) The role of glutathione in chloroform-induced hepatotoxicity, Exptl. Mol. Pathol., 24, 13—2 .

FAO Plant Production Paper, 10 Suppl. (1978) Pesticides residues in food: 1977 evaluations, pp. 87—99.

Fishbein, L. (1976) Industrial mutagens and potential mutagens, I. Halogenated aliphatic derivatives, Mutation Res., 32, 267—307.

Fishbein, L. (1979) Potential Industrial Carcinogens and Mutagens, Elsevier, Amsterdam, pp. 211—217.

Fishman, J., D.K. Fukushima, J.O. O'Connor and H.T. Lynch (1979) Low urinary estrogen glucuronides in women at risk for familial breast cancer, Science, 204, 1083—1091.

Fry, B.J., T. Taylor and D.E. Hathway (1972) Pulmonary elimination of chloroform and its metabolite in man, Arch. Int. Pharmacodyn., 196, 98—111.

Gehring, P.J., G.E. Blau and P.G. Watanabe (1976) Pharmacokinetic studies in evaluation of the toxicological and environmental hazard of chemicals, in: Advances in Modern Toxicology — New Concepts in Safety Evaluation, Hemisphere, Washington.

Gillette, J.R. (1974a) A perspective on the role of chemically reactive metabolites of foreign compounds

in toxicity. I. Correlation of changes in covalent binding of reactive metabolites with changes in the incidence and severity of toxicity, Biochem. Pharmacol., 23, 2785—2794.

Gillette, J.R. (1974b) A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity. II. Alterations in the kinetics of covalent binding, Biochem. Pharmacol., 23, 2927—2938.

Greim, H., G. Bonse, Y. Radwan, D. Reichert and D. Henschler (1975) Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation, Biochem. Pharmacol., 24, 2013—2017.

Greim, H., D. Bimboes, G. Egert, W. Göggelmann and M. Krämer (1977) Mutagenicity and chromosomal aberrations as an analytical tool for in vitro detection of mammalian enzyme-mediated formation of reactive intermediates, Arch. Toxicol., 39, 159—169.

Imley, T.J. (1978) Chloroprene (2-chloro-1,3-butadiene) — What is the evidence for its carcinogenicity? Clin. Toxicol., 13, 153—170.

Happle, R., and K. Zehtermacht (1977) Induction of hair growth in alopecia areata with dinitrochlorobenzene, Lancet, II, 1002—1003.

Henschler, D., E. Eder, T. Neudecker and M. Metzler (1977) Carcinogenicity of trichloroethylene: Fact or artifact? Arch. Toxicol., 37, 233—236.

Henschler, D., W. Reuss, H.M. Lindner, D. Reichert, E. Eder and Y. Radwan (1980) Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species, Arch. Toxicol., 43, 237—248.

Heme, S., M. Metzler and T. Wolff (1978) Activation of [^{14}C]-chlorobiphenyls to protein-binding metabolites by rat liver microsomes, Chem.-Biol. Interact., 20, 255—265.

Hicks, R.M., J.St.J. Wakefield and J. Chowaniec (1975) Evaluation of a new model to detect bladder carcinogens or cocarcinogens: Results obtained with saccharin, cyclamate and cyclophosphamide, Chem.-Biol. Interact., 11, 225—238.

Hildebrandt, A.G., and I. Roots (1975) Reduced nicotinamide dinucleotide phosphate (NADPH)-dependent formation and breakdown of hydrogen peroxide during mixed function oxidation reactions in liver microsomes, Arch. Biochem. Biophys., 171, 385—397.

IACR (1974) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 6, Sex hormones, Lyon.

IARC (1974) Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 7, Some antithyroid and related substances, nitrofurans and industrial chemicals, Lyon, pp. 96—105.

IARC (1975) Monograph series on evaluation of carcinogenic risks of chemicals to man, Vol. 11, Lyon, pp. 131—138.

Interagency Regulatory Liaison Group (1979) Scientific bases for identification of potential carcinogens and estimations of risks, J. Natl. Cancer Inst., 63, 241—268.

Jaeger, R.J., R.B. Conolly and S.D. Murphy (1974) Effect of 18 h fast and glutathione depletion on 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats, Exptl. Mol. Pathol., 20, 187—198.

Jerina, D.M., H. Lehr, M. Schaefer-Ridder, M. Yagi, J.M. Karle, D.R. Thakker, A.W. Wood, A.Y.H. Lu, D. Ryan, S. West, W. Levin and A.H. Conney (1977) Bay region epoxides of dihydrodiols: A concept explaining the mutagenic and carcinogenic activity of benzo[a]pyrene and benzo[c]anthracene, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Lab., pp. 639—658.

Jones, D.P., H. Thor, S. Andersson and S. Orrenius (1978) Detoxification reactions in isolated hepatocytes, J. Biol. Chem., 253, 6031—6037.

Lee, C.C., J.C. Bandhari, M. Winston, W.B. House, R.L. Dixon and J.S. Woods (1978) Carcinogenicity of vinyl chloride and vinylidene chloride, J. Toxicol. Environ. Health, 4, 15—30.

Leibman, K.C. (1975) Metabolism and toxicity of styrene, Environ. Health Perspect., 11, 113—119.

Lijinsky, W. (1977) Standard-setting for nitrites and nitrosamines, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Lab., pp. 1807—1811.

Maltoni, C. (1977) Vinyl chloride carcinogenicity: An experimental model for carcinogenesis studies, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.) Origins of Human Cancer, Cold Spring Harbor Lab., Cold Spring Harbor, pp. 113—146.

Maltoni, C., G. Cotti, L. Morisi and P. Chieco (1977) Carcinogenicity bioassays of vinylidene chloride, research plans and early results, Med. Lavoro, 68, 241—262.

Maugh, T.H. (1978) Chemical carcinogenesis: How dangerous are low doses? Science, 202, 37—41.

McKenna, M.S., P.G. Watanabe and P.J. Gehring (1977) Pharmacokinetics of vinylidene chloride in the rat, Environ. Health Perspect., 21, 99—105.

Memorandum, Dept. of Health, Education and Welfare, Washington, (20.3.1976).

Miller, J.A., C.S. Wyatt, E.C. Miller and H.A. Hartmann (1961) The *N*-hydroxylation of 4-acetaminobiphenyl by the rat and dog and the strong carcinogenicity of *N*-hydroxy-4-acetaminobiphenyl in the rat, Cancer Res., 21, 1465—1473.

Miller, E.C., J.A. Miller and M. Enomoto (1964) The comparative carcinogenicities of 2-acetylaminofluorene and its *N*-hydroxy metabolite in mice, hamsters, and guinea pigs, Cancer Res., 24, 2018—2026.

Mily, P., and A.J. Gatto (1976) Mutagenic activity of styrene oxide (1,2-epoxy ethyl benzene), a presumed styrene metabolite, Mutation Res., 40, 15—18.

Nagao, M., M. Honda, Y. Seino, T. Yahagi, T. Kawachi and T. Sugimura (1977) Mutagenicities of protein pyrolysates, Mutation Res., 53, 351—353.

National Cancer Institute (1976) Report on the Carcinogenesis Bioassay of Trichloroethylene, Bethesda, MD.

National Cancer Institute (1977) Bioassays of nitrilotriacetic acid (NTA) and nitrilotriacetic acid-trisodium salt-monohydrate (Na_3NTAH_2O) for possible carcinogenicity, NCI Tech. Rep. Ser. No. 6, January, DHEW Publication No. (NIH) 77—806.

Oesch, F. (1972) Mammalian epoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds, Xenobiotica, 3, 305—340.

Oesch, F. (1980) Species differences in activating and inactivating enzymes related to in vitro mutagenicity mediated by tissue preparations from these species, Arch. Toxicol., Suppl. 3, 179—194.

Oesch, F., N. Morris, J.W. Daly, J.E. Gielen and D.W. Nebert (1978) Genetic expression of the induction of epoxide hydrase and aryl hydrocarbon hydroxylase activities in the mouse by phenobarbital or 3-methylcholanthrene, Mol. Pharmacol., 8, 692—696.

Oesch, F., H. Teenen and H. Fahrländer (1974) Epoxide hydratase in human liver biopsy specimens: Assay and properties, Biochem. Pharmacol., 23, 1307—1317.

Oser, B.L., S. Carson, G.E. Cox, E.E. Vogin and S.S. Sternberg (1975) Chronic toxicity study of cyclamate: saccharine (10:1) in rats, Toxicology, 4, 315—330.

Plugge, H., and R.J. Jaeger (1979) Acute inhalation toxicity of 2-chloro-1,3-butadiene (Chloroprene): Effects on liver and lung, Toxicol. Appl. Pharmacol., 50, 565—572.

Pohl, L. (1979) Biochemical Toxicology of Chloroform, in: Hodgson, Bend and Philpot (Eds.), Reviews in Biochemical Toxicology, Elsevier/North-Holland, New York, pp. 79—107.

Ponomarkov, V., and L. Tomatis (1980) Long-term testing of vinylidene chloride and chloroprene for carcinogenicity in rats, Oncology, 37, 136—141.

Purves, H.D., and W.E. Griesbach (1947) Studies on experimental goitre, VIII. Thyroid tumors in rats tested with thiourea, Br. J. Pathol., 28, 46—53.

Rall, D.P. (1960) Difficulties in extrapolating the results of toxicity studies in laboratory animals to man, Environ. Res., 2, 360—367.

Rampey, L.W., J.F. Quast, C.G. Humiston, M.F. Balmer and E.A. Schwetz (1977) Interim results of two-year toxicological studies in rats of vinylidene chloride incorporated in the drinking water or administered by repeated inhalation, Environ. Hlth. Perspect., 21, 33—43.

Reitz, R.H., P.J. Gehring and C.N. Park (1975) Carcinogenic risk estimation for chloroform: An alternative to EPS's procedures, Fd. Cosmet. Toxicol., 13, 511—514.

Scanlan, R.A., J.F. Barbour, J.M. Hotchkiss and L.M. Libbey (1980) *N*-Nitrosodimethylamine in beer, Fd. Cosmet. Toxicol., 18, 27—29.

Schwarz, L. (1980) Modulation in sulfation and glucuronidation of 1-naphthol in isolated rat liver cells, Arch. Toxicol., 44, 137—146.

Schwarz, L., M. Merger and S. Hesse (1980) Effect of decreased glucuronidation and sulfation on covalent binding of naphthalene in isolated rat hepatocytes, Toxicology, 90, in press.

Scientific Committee, Food Safety Council (1978) Quantitative risk assessment, Fd. Cosmet. Toxicol., 16, Suppl. 2, 109—136.

Spiegelhalder, B., G. Eisenbrand and R. Preussmann (1979) Contamination of beer with trace quantities of *N*-nitroso-dimethylamine, Fd. Cosmet. Toxicol., 17, 39—42.

Stoekinger, H.E. (1977) Toxicology and drinking water contaminants, J. Am. Water Works Assoc., 69, 399—402.

Sugimura, T. (1975) Let's be scientific about the problem of mutagens in human cooked food, Mutation Res., 35, 149—152.

Sugimura, T., M. Nagao, T. Kawachi, M. Honda, T. Yahagi, Y. Seino, S. Sato, N. Matsukura, T. Matsushima, A. Shirai, M. Sawamura and H. Matsumoto (1977) Mutagens—carcinogens in food, with special reference to high mutagenic pyrolytic products in broiled foods, in: H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Cold Spring Harbor Lab., pp. 1561—1577.

Summer, K.H., and W. Göggelmann (1980) 1-Chloro-2,4-dinitrobenzene depletes glutathione in rat skin and is mutagenic in *Salmonella typhimurium*, Mutation Res., 77, 91—93.

Summer, K.H., W. Göggelmann and H. Greim (1980) Glutathione and glutathione *S*-transferases in the Salmonella mammalian-microsome mutagenicity test, Mutation Res., 70, 269—278.

Summer, K.H., and H. Greim (1980) Detoxification of chloroprene (2-chloro-1,3-butadiene) with glutathione in the rat, Biochem. Biophys. Res. Commun., 96, 566—573.

Tannenbaum, S.R. (1980) Ins and outs of nitrites, The Sciences, 20, 7—9.

Tomatis, L. (1978) The predictive value of rodent carcinogenicity tests in the evaluation of human risks, Annu. Rev. Pharmacol. Toxicol., 19, 511—530.