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PROBLEMS IN THE REGULATION OF CARCINOGENIC CHEMICALS IN AN INTERNATIONAL PERSPECTIVE. I. INTERPRETATION OF EXPERIMENTAL DATA

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PREAMBLE

This contribution constitutes the first in a series of three articles under the same main heading; the succeeding two contributions will carry the subtitles: ***II. Determination of Carcinogenic Potency and III. A Critical Assessment of Regulatory Approaches***

ABSTRACT

The influence of non-scientific factors on risk-assessment in the regulatory context is discussed. On the purely technical level, most controversy when dealing with data interpretation has been centered around certain types of tumors caused by exposure to non-genotoxic agents in the rodent, and which many scientists consider to be of doubtful relevance to man. The reasons for decreased reliance on such toxicological findings are high spontaneous background levels and/or mechanistic considerations. Such tumors include target organs like liver, bladder, forestomach, renal tubuli, as well as certain endocrine associated tumors, like those of the thyroid, mammary glands, pituitary, and adrenal glands (phaeochromocytomas).

While discussing examples of controversial experimental carcinogens, the assessment by international organizations as well as regulatory decisions made by different national agencies in Europe and North America are presented.

RESUMEN

Se discute la influencia que tienen factores no científicos en la regulación de la estimación del riesgo. La mayor controversia que se presenta al tratar de interpretar datos a nivel puramente técnico se ha centrado sobre ciertos tumores causados por la exposición de roedores a agentes no genotóxicos, lo cual para muchos investigadores es de dudosa relevancia para el hombre. Las razones para apreciar así tales hallazgos toxicológicos están basadas en los elevados niveles espontáneos y/o en consideraciones mecanísticas. En esto se encuentran incluidos tumores en órganos blanco como hígado, vesícula, duodeno, túbulos renales, así como ciertos tumores asociados a glándulas endócrinas como tiroides, mamarias, pituitaria y adrenales (feocromocitomas).

En este trabajo se discuten ejemplos de controversia sobre carcinogénesis experimental y se presentan las evaluaciones realizadas por organizaciones internacionales así como las decisiones que sobre regulación han sido aplicadas por diferentes agencias nacionales de Europa y de Norteamérica.

GENERAL BACKGROUND

Most scientists feel, that proper risk management should be based on actual levels of risk —preferably seen in an overall perspective. Further, that the characterization and quantification of risk by the process of risk assessment should be a purely scientific undertaking. Nevertheless, a number of other factors, such as pressures from industrial interests, political considerations, media reactions, etc. will, in reality, to a lesser or greater extent influence the outcome of any regulatory

process. Even with respect to the goals set for the basic risk assessment process itself, there exists a fundamental difference between, on one hand the scientific community, and on the other the regulatory bureaucracy. Whereas scientists favor the *most likely* interpretation of existing information, in the regulatory context, a number of other factors will influence the methods chosen for risk assessment. Due to non-scientific considerations, bias may, thus, be introduced already at an early stage of the evaluation process. It is not surprising, that while virtually identical information has been used, government agencies in different countries often make widely divergent regulatory decisions regarding chemicals. Sometimes this is true even for agencies within the same nation. In countries like Sweden and the U.S. this means, that to be "on the safe side" excessively conservative estimates will be made that often exaggerate real risks. Lack of an overall policy for risk reduction and an exaggerated sensitivity to pressures from news media are other features typical for these societies. Large risks are sometimes ignored, while certain small well-publicized risks are stringently regulated. It is not always remembered, that the costs for any regulation will eventually have to be paid by the public, but industry can live with any kind of regulation as long as it is competitively neutral, and as long as there are alternative products that can be marketed. In countries, where the priorities are dominated by the interests of agriculture and industry, the notions of what is politically "safe", are obviously different.

For government and industry alike, the aspiration to promote "scientific truth" is mostly secondary to other goals. In highly developed nations, the elevated degree of technical sophistication in evaluating chemical hazards is not necessarily matched by a corresponding "political wisdom" when selecting regulatory goals and priorities (Zeckhauser and Viscusi 1990). For developing nations, where administrative structures, legislation and regulations are often modeled on the situation found in highly industrialized nations, the background for, and the context in which regulatory decisions are taken, should be carefully analyzed considering the limited total resources available to less developed societies for risk reduction of chemical hazards.

This article reviews some purely scientific problems associated with the first step of the risk assessment process for potentially carcinogenic chemicals, which is data interpretation; problems connected with high-to-low-dose extrapolation as well as determination of equivalent human dose on basis of animal experiments will be treated in the next article dealing with quantitative risk assessment. The controversy around the role of metabolic overloading at the maximum tolerated dose *(MTD)* will also be dealt with in this context; in most cited examples such overloading may influence the quantitative aspects of carcinogenesis, rather than to transform a non-carcinogen to a carcinogen. Only for a very limited number of chemicals are adequate human data available, and the emphasis will mainly be on evidence of carcinogenicity based on data obtained in experimental animals.

Mutagenicity data are per se apparently not considered as sufficient evidence for carcinogenicity in any of the OECD countries (OECD 1984, IARC 1987, IPCS 1990, U.S.EPA 1986). Life-time exposure to a chemical agent in a small rodent (usually the mouse and the rat; occasionally the hamster) still remain the only practical model for investigation *in vivo* of potential carcinogenic properties. For a positive study in such animals there has, as a general, been consensus on the main design of such investigations as well as the kind of evidence which provide additional support for carcinogenicity; existence of dose-related trends, positive findings with different routes of exposure, positive results in more than one animal strain, tumors in multiple organs, etc. Further, additional types of information that are generally taken into account include positive data from genotoxicity testing, metabolic transformation to carcinogenic products, and structural similarity to other known carcinogens (OSTP 1985, Dybing 1986). Most controversy has been centered around circumstances that would indicate that a positive finding in an experimental animal is ***not*** relevant to man, and these circumstances constitute the main topic for the analysis provided here. In this context the emphasis will be on the interpretation of the significance with respect to humans, based on mechanistic aspects of tumor induction, of certain types of tumors found in experimental animals.

Some experts have stated, that until we know the mechanism by which chemicals induce cancer, it would be premature to distinguish between various carcinogens on basis of postulated mechanism of action (Perera 1990, Cogliano *et al.* 1991, Rall 1991). Others ardently support a change of present regulatory systems like those in the U.S. and the Scandinavian countries in favor of mechanistically based assessment systems (Weisburger

and Williams 1983, Butterworth 1987b, Butterworth *et al.* 1991, Clayson 1989). The latter group of scientists point out, that present administrative approaches fail to distinguish between trivial and serious risks resulting in a misappropriation of limited resources, over-regulation, and public disinformation (Abelson 1990, Ames *et al.* 1987, Ames and Gold 1990a, b, Ames *et al.* 1990, Cohen and Ellwein 1990, Zeckhauser and Viscusi 1990, Nilsson *et al.* 1993).

It seems obvious, that the mechanism of action of cancer induction involves a chain of events with a degree of complexity that may keep generations of scientists busy until they are all resolved. However, as Butterworth and Eldridge (1992) aptly puts it; we may not understand all details of such a series of events, but the general idea, e.g. of DNA reactivity resulting in mutations as a key mode of action still proves to be a useful concept. For such a generalized approach based on identifying crucial common denominators in the mechanism of action, a defined ***mode of action*** has been proposed by these authors as the bases for assessing chemical carcinogens.

Due to the introduction of certain elements based on mode of action, the cancer hazard assessments carried out by the **EC nations** and **Canada** are often different form those of the **U.S.** and some of the **Scandinavian countries.** In order to get a better understanding of the underlying problems involved, illustrative examples have been chosen below which cover some of the most relevant controversial issues.

INTERPRETATION OF CERTAIN TYPES OF TUMORS FOUND IN EXPERIMENTAL ANIMALS

For non-genotoxic compounds, in particular, different opinions as to the relevance for man have been expressed for the following types of tumors induced in experimental animals: *Rodent liver tumors, follicular cell tumors of the thyroid, tumors of the renal proximal tubuli in rats, testicular tumors in male rats of some strains, tumors of the rodent forestomach and bladder, adrenal phaeochromocytomas in the rat, lymphomas and lung adenomas in the mouse, certain endocrine associated tumors like pituitary, testicular and mammary tumors* (IPCS 1990), as well as certain splenic fibrosarcomas (Bus and Popp, 1987). To gain credibility, the question of diminished relevance of certain types of tumors has mostly been linked with some hypothesis or other concerning the underlying mechanism for their induction.

Liver Tumors. Primary liver cancer is relatively rare in man. However, when testing for carcinogenicity, mouse strains characterized by an extremely high background incidence of such tumors —like the hybrid B6C3F1 mouse— have commonly been used e.g. in the U.S. National Toxicology Program. For the latter strain, Doull *et al.* (1983) gives a background incidence range of 18 to 47% in males and 3 to 8% in females, whereas Tarone *et al.* (1981) cite a maximum of 58% in untreated males. The wisdom of investigating potential carcinogenic effects with respect to liver in such an animal model has been widely questioned and the issue extensively reviewed (Nutrition Foundation 1983, Clayson 1989, IPCS 1990; Butterworth *et al.* 1991). For this reason, many national as well as international expert bodies have expressed the opinion, that an increase in incidence of rodent liver tumors may be of little relevance in predicting human cancer risk, and that it is inadvisable to classify a substance as likely to be carcinogen to humans solely on the basis of an increased incidence of mouse liver tumors. For many years this has been the position of **Joint FAO/WHO Meeting of Experts on Pesticide Residues (JMPR)** as well as the **International Program on Chemical Safety** (IPCS 1990). In the case of pesticides like *DDT* and *lindane,* the authorities in the **Netherlands** as and in the **U.K.** have relied on the jmpr interpretation of this issue (Nilsson *et al.* 1993). These, and other chlorinated compounds, are well-known promoters of liver neoplasia (Nutrition Foundation 1983). The same basic interpretation has also been codified in the most recent EU (1992) directive for classification and labeling.

For genotoxic as well as non-genotoxic carcinogens the mouse liver tumors are, in general, considered valid by the **U.S.EPA** (1986) as well as by the **National Swedish Chemicals Inspectorate** when extrapolating cancer risk to man, also when this is the only carcinogenic response observed. However, in the U.S. classification could be changed from "sufficient" evidence in animal studies to "limited":

> "[...] when factors such as the following, are observed: an increased incidence of tumors only in the highest dose group and/or only at the end of the study; no substantial dose-related increase in the proportion of tumors that are predominantly malignant; the occurrence of tumors that are predominantly benign; no dose related shortening of the time to the appearance

of tumors; negative or inconclusive results from a spectrum of short-term tests for mutagenic activity; the occurrence of excess tumors only in a single sex."

As demonstrated by the outcome of numerous regulatory decisions, these special conditions have rarely been found to be applicable by this agency. Within the **National Swedish Chemicals Inspectorate** a discussion paper addressing these issues was presented by Hammar (1986), but the Inspectorate has continued to follow the approach chosen by **International Agency for Research on Cancer (IARC)** for the purpose of classification with virtually no distinctions being been made on basis of type of tumor.

Those who have voiced skepticism concerning the validity of certain types of liver neoplasia in the rodent have been toxicologists at industry toxicology laboratories (ECETOC 1982), but also many prominent independent scientists engaged in basic cancer research. The main defenders have been Tomatis at the IARC (Tomatis *et al.* 1973) as well as pathologists engaged within the U.S. National Toxicology Program (Maronpot *et al.* 1987) and administrators in the regulatory agencies in the U.S. and Scandinavia. It is to be noted, that within the concerned UN organizations there has been no consensus on this issue between on one hand IARC (1987), and on the other hand, IPCS (1990).

Representatives from the National Toxicology Program have pointed out, that the data-base for the $B6C3F_1$ mouse is unique with respect to our knowledge of the spontaneous occurrence of various pathological lesions, and that the high sensitivity may represent an advantage in the detection of weak carcinogens (Maronpot *et al.* 1987). The detractors of the mouse liver lesions have, on the other hand, stressed that it is a well known fact that hormonal factors, modifications of the diet, such as methionine and choline deficiency (Ghoshal and Farber 1983, Yokoyama *et al.* 1985) can cause a significant increase in liver tumor incidence in sensitive rodent strains. Further, any substance that would affect the normal levels of the sex hormones will have the potential to indirectly influence the liver tumor incidence (Lucier 1992). Finally, compounds that have a mitogenic action in liver will also promote the appearance of liver tumors (Butterworth and Eldrich 1992, Swenberg and Maronpot 1991). The appearance of spontaneous liver tumors in the $B6C3F_1$ mouse is evidently coupled to the activation of a specific proto-oncogene (Fox *et al.* 1990). What is actually studied with non-genotoxic substances in such animal models is —according to many scientists— cancer promotion rather than the action of complete carcinogens, an effect that should not result in cancer classification.

Against this view the argument has been forwarded, that in reality promoters may be as important —if not more important— in the etiology of human cancer. Further, that the division of carcinogens into the two groups of genotoxic and non-genotoxic agents is not feasible and represents an oversimplification (Hammar 1986). This is often parried by pointing out, that the doses used in the animal tests to elicit such "promotion" are mostly unrealistically high and orders of magnitude above those normally encountered. Further, it is the opinion of many experts, that at least for certain types of well investigated groups of substances, like the peroxisome proliferators and certain thyreostatic compounds, a distinction between genotoxic and non-genotoxic carcinogens is both feasible and serves a useful purpose in risk assessment (Butterworth and Eldridge 1992).

Peroxisome proliferators (PPs) constitute a distinct class of rodent hepatocarcinogens that cover a wide range of structurally disparate chemicals (Reddy and Lalwani 1983, Stott 1988, ECETOC 1992). The most striking finding upon exposure to these compounds is the proliferation in hepatocytes of the cytoplasmic organelles, peroxisomes, which is accompanied by the induction of ß-oxidation of fatty acids and liver hyperplasia. Apart from various drugs and high volume industrial chemicals like *di-(2-ethylhexyl)phthalate* (DEHP) and *trichloroacetic acid,* several pesticides, like *2,4-D, 2,4,5-T,* and *fomesafen* belong to this class. However, peroxisome proliferation was first discovered (Paget 1963) for *clofibrate* (ethyl-p-chlorophenoxy-2-isobutyrate) and subsequently found to be associated with a number of other hypolipidemic drugs.

The mechanism of carcinogenic action of PPs is enigmatic. Thorough investigations of representative members of this group of compounds, like clofibrate and DEHP, have failed to reveal any significant genotoxic action, and an indirect mechanism for carcinogenesis has, therefore, been proposed involving a dose threshold (for a review, see Butterworth 1987a, Stott 1988, Nilsson *et al.* 1991, ECETOC 1992). A serious drawback when assessing the absence of genotoxic action of PPs was, that most systems used for assessing the genotoxic potential of these agents were not relevant to the liver —the main target tissue for toxicological effects in mammals. However, when evaluating

such end points as UDS and DNA single strand breaks in hepatocytes exposed *in vivo*, Nilsson *et al.* (1991) failed to detect evidence of genotoxic action in the rodent liver, even under conditions of extreme oxidative stress.

One favored hypothesis for cancer induction involves damage to DNA, mediated by reactive molecular species which are derived from the hydrogen peroxide that is generated by the peroxisomes (Reddy and Lalwani 1983). Leakage of peroxide from these subcellular particles during ß-oxidation of fatty acids has been detected in perfused livers (Conway *et al.* 1987). Oxidative damage —in the form of increased double bond conjugation and peroxidation of membrane lipids as well as lipofuschin accumulation— has been demonstrated after prolonged treatment with potent PPs (Goel *et al.* 1986, Lake *et al.* 1987). A modest increase in the formation of 8-hydroxydeoxyguanosine —a known reaction product from attack by hydroxyl radicals (Kasai *et al.* 1986)— has also been detected in liver DNA under such conditions in rats (Kasai *et al.* 1989). Finally, by means of postlabeling techniques, unidentified DNA-adducts have been identified in rat liver DNA after long-term oral administration of ciprofibrate (Randerath *et al.* 1991).

However, it is quite possible that the formation of the low levels of 8-hydroxydeoxyguanosine found, the DNA-adducts detected by postlabeling, and similar markers of oxidative damage represent artifacts. This seems quite certain in the case (Fahl *et al.* 1984) of the single strand breaks ("nicks") in supercoiled SV40 virus induced by isolated metabolizing peroxisomes derived from rats treated with such an inducing agent (Nilsson *et al.* 1991). Actually, the capacity to induce peroxisomal proliferation is not matched by the capacity of these agents to induce liver tumors. A better correlation has been found to exist between the carcinogenic potency and the ability of these compounds to induce a persistent increase in replicative DNA synthesis (Marsman *et al.* 1988, Cattley and Popp 1989). The stimulation of cell division will increase the chances of fixation of DNA-damage, as well as enhance the conversion rate of initiated cells to tumor cells. The latter observation is consistent with the finding, that many PPs have been found to act as promoters of liver tumors in rodents (Reddy and Rao 1978, Glauert *et al.* 1986, Rao *et al.* 1986, Ward *et al.* 1986, Preat *et al.* 1986a, b, Abdellatif *et al.* 1990, Nilsson *et al.* 1991).

Since the biological effects induced by PPs may conceivably be the result of some metabolic product(s) derived from the breakdown of these substances, the metabolically inert PP, perfluorooctanoic acid, was studied by us and found to be active as a promoter of liver tumors in the rat induced by diethylnitrosamine in an initiation-selection-promotion protocol (Abdellatif *et al.* 1990, Nilsson *et al.* 1991a).

Apart from the PP per se, a likely candidate representing the "ultimate" peroxisome proliferating agent is its acyl-CoA derivative, which may interact with a recently identified, specific PP-activated receptor (Isseman and Green 1990). Thus, it is possible that this group of agents act by a mechanism similar to TCDD and hormones (See below under *Receptor-Mediated Carcinogenesis).*

An overwhelming volume of data support the notion that PPs are true "epigenetic" carcinogens. Conflicting evidence as to the contrary may be given other interpretations. This brings their promoting action into focus. In absence of a plausible mechanism for initiation, the promoting activity by itself would seem insufficient to explain the carcinogenic response induced by some of the more potent PPs. However, these agents seem to exhibit different characteristics as compared to classical promoters of liver neoplasia, like phenobarbital. Thus, whereas phenobarbital causes an increase in the number of preneoplastic foci in a liver initiated by treatment with diethylnitrosamine, a potent PP, like nafenopin or WY-14,643, does not increase the number of such foci appreciably, but causes a great increase in seize of these, predominantly basophilic foci (Preat *et al.* 1986a, Cattley and Popp 1989, Marsman and Popp 1989). Further, the foci thus induced seem to have a much higher likelihood to progress to hepatocellular carcinomas. Recent findings to the effect that PPs effectively boost the selection of transformed cells give support to the notion, that these compounds act mainly by selecting already initiated hepatocytes that are always present in the rodent liver in such a population of cells (Schulte-Herrmann *et al.* 1983). This hypothesis has been strengthened by the fact that an increased yield of tumors is obtained by treating old rats for the same length of time with a potent proliferator than when exposing young individuals (Cattley *et al.* 1991). For the above reasons, the significance of peroxisome associated liver tumors for man under realistic exposure conditions appear doubtful in most cases.

The high-volume chemical DEHP has been classified by **IARC** (1982) as a 2B carcinogen on basis of liver tumors in rodents, and the **U.S.EPA** has made a similar classification (IRIS 1993). However,

since a relatively low carcinogenic potency has been assigned (IRIS 1991) to this plasticizer [q* = 0.02 (mg/kg and day)$^{-1}$], its use has not been curtailed to any significant extent by regulatory action in the U.S., European regulatory agencies have, in general, not classified DEHP as a carcinogen. The main reasons given for downgrading the evidence of carcinogenicity obtained in rodents (hepatocellular carcinoma) for this class of compounds are the following (ECETOC 1992):

- Peroxisome proliferators are non-genotoxic.
- Rodents seem to represent a less appropriate experimental model for potential toxicity (including carcinogenicity) of this class of compounds in humans; most induce peroxisome proliferation in rats and mice but not in guinea pigs, marmosets or human hepatocytes *in vitro*.

Currently, PPs as a group are not assessed differently from other carcinogens by the U.S.EPA and the National Swedish Chemicals Inspectorate. The latter agency has refused registration for several pesticide products that induce peroxisome proliferation associated liver tumors. However, the U.S.EPA has indicated a willingness to revise its policy if it can be shown, that liver tumors induced by this group of compounds is confined to rodents, and/or if the mechanism of cancer induction can be shown to be indirect and have a threshold (Nilsson *et al.* 1993).

Bladder Tumors. 2-naphthylamine constitutes the classical example of a human bladder carcinogen, and a number of genotoxic aromatic amines have been suspected of possessing similar properties. The thorny issue of bladder tumors has mostly been centered around *non-genotoxic compounds,* as amply illustrated by the food additives *saccharin* and *cyclamate,* the carbamate insecticide *propoxur,* the fungicide *chlorothalonil,* the detergent *nitriloacetic acid,* as well as the industrial chemical *melamine* (2,4,6-triamino-s-triazine).

Since saccharin has been classified by **IARC** (1980) as a 2B carcinogen, the results from the animal studies with the artificial sweeteners deserve some additional comments. In several adequately conducted studies with only *cyclamate,* e.g. that conducted by Kroes *et al.* (1977), no treatment related increase of tumors were found, and the issue of carcinogenicity of cyclamate and saccharin was first precipitated by the finding of bladder tumors (transitional cell carcinomas) in the study conducted by Oser *et al.* (1975) using Wistar rats fed a 10:1 *mixture of sodium cyclamate: saccharin* in the diet at doses of 0, 500, 1120 and 2500 mg/kg/day for 2 years. From week 79 the original dose groups were split, and half of the survivors in each dose group received in addition *cyclohexylamine* hydrochloride in the diet (25, 76, or 125 mg/kg/day for the 500, 1120, and 2500 mg groups respectively). In a subsequent study with only sodium saccharin a dose related increase was also found for the incidence of transitional-cell papillomas and/or carcinomas when Charles River CD rats were given 4-7.5% of saccharin in the diet in a two-generation study (Schoenig *et al.* 1985).

Studies using pretreatment with a known initiator of neoplasms have demonstrated saccharin to be able to promote the local action of a known carcinogen, like N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) and N-butyl-N-(4-hydroxybutyl) nitrosamine, in the bladder (Cohen 1985). In this system epithelial ulceration, e.g. by freezing, has been found to act as an initiator (for a review, see Ellwein and Cohen 1990). Using N-methyl-N-nitrosourea Hicks *et al.* (1978) could demonstrate a similar effect using cyclamate as a promoter.

The preponderance of available evidence suggest that neither cyclamate nor saccharin are genotoxic. According to Ashby (1985) "a review of the toxicology of sodium chloride reveals a profile of genotoxic activities almost identical to that of sodium saccharin". In spite of numerous and extensive analysis of the risk of developing bladder cancer from artificial sweeteners in various populations, there has also been no convincing evidence that suggests a carcinogenic effect in humans (Ellwein and Cohen 1990).

In two adequately conducted long term feeding studies *propoxur* has been found to induce tumors of the bladder epithelium in two strains of rats after ingestion of 3000 ppm and above in the diet. Studies in mice have been negative. Based on a large number of studies, propoxur has been considered to lack significant genotoxic effects (JMPR 1989). The effects of propoxur in the rat have been considered to be very similar to those found for the artificial sweeteners and have raised doubts as to the relevance of these carcinogenic effects under normal exposure conditions:

- Extremely steep dose-response curves for carcinogenic action with tumors only apparent at the highest dose level.

- Failure to induce hyperplasia and tumors in other animal species than rats.
- Resembles the well established tumorigenic response of the rat bladder to various promoting agents.
- Absence of significant genotoxic action.

For propoxur the following additional observations have been cited in this context (JMPR 1989):

- Failure to induce such effects when rats of the same strain were given a semisynthetic diet. This could not be attributed to disparities in metabolite pattern or differences in absorption from the gut.
- Inhibition of hyperplasia induced by propoxur by lowering of urinary pH.

In other words, propoxur as well as the artificial sweeteners discussed above, adequately fits the definition of an "epigenetic carcinogen" given by Weisburger (Weisburger and Williams 1983). Although it is true, that the spontaneous incidence of bladder tumors in rats is low, chronic exposure to a number of agents that cause irritation or trauma to the bladder epithelium may stimulate mitosis and promote hyperplasia with the subsequent formation of tumors (De Groot *et al.* 1988, DeSesso 1989). Examples of such promoting agents are foreign *solid bodies, alteration of urinary pH* as well as *infectious agents,* or even *freeze ulceration* of the bladder epithelium (Cohen 1985). Other well-known chemical promoters of bladder carcinogenesis are *o-phenylphenol, butylated hydroxyanisole (BHA),* as well as the *artificial sweetening agents* discussed above, all of which induce a response in rat bladder similar to that seen for propoxur.

According to Clayson (1989), any agent or process that disrupts the urothelium, or the tight junctions between adjacent luminal cells, leads to a massive proliferation of this normally quiescent tissue. Continuation of proliferative stimulus for the major part of the life span in rodents may result in tumor formation. In mice and rats, but not in humans, the presence of bladder stone, e.g. induced by 4-ethylsulphonylnaphthalene-1-sulphonamide (Clayson *et al.* 1967), is tumorigenic. A cell proliferative response of the bladder epithelium caused by the administration of these agents at high doses is sufficient to cause tumor induction. For saccharin this does not seem to occur below an intake of 500 mg/kg/day. In summary, available evidence indicate that exposure to these compounds is associated with an insignificant cancer risk when the exposure is kept below levels inducing hyperplasia of the bladder epithelium.

Bladder tumors induced by chemicals in animal models are under all circumstances assumed to be of significance by the **National Swedish Chemicals Inspectorate** as well as by the **U.S.EPA.** The risk models used for such compounds assume the absence of any dose threshold. The U.S.EPA can make an exception only for compounds for which the neoplastic effect is linked to the formation of bladder stones.

According to the "Delaney amendment" to the U.S. Federal Food, Drug and Cosmetic Act (Sec. 409 [c][1][A]) from 1957, no additives may be present in food that causes cancer in man or animals. On basis of this clause, the **U.S. FDA** had to prohibit the use of cyclamate in 1970 on basis of tumor induction in the rodent bladder (U.S FDA 1969, 1970). In 1977 the U.S.FDA for similar reasons notified its intent to ban saccharin as well (U.S. FDA 1977), but because of the adverse reactions among consumers and many scientists, U.S. Congress was forced to pass "the Saccharin Study and Labeling Act" in 1977 that overturned the Delaney amendment for this additive (but not for cyclamate) without resolving the basic issue (U.S. FDA 1978).

Especially in view of the widespread occurrence sometimes of high levels of potent natural carcinogens in food and animal feed (Doll and Peto 1981, The Swedish Cancer Committee 1984, Toth and Erickson 1986, Ames *et al.* 1987, 1990), there is widespread agreement among scientists that the Delaney amendment reflects outdated perceptions on the etiology of human cancer. However, for mainly political reasons all attempts to amend this part of the legislation have so far failed. **The United States National Cancer Institute Committee for the Review of the Data on Cyclamate,** (DHEW 1976), the **Food Additives and Contaminant Committee of Great Britain** (FACC 1982), and most recently the **Cancer Assessment Committee of the Center for Food Safety and Applied Nutrition at the U.S. FDA** (CAC 1984) and a **National Academy of Sciences —National Research Council Committee** (NAS-NRC 1985) have all concurred in finding that the experimental data do not demonstrate cyclamate to be carcinogenic. The **World Health Organization's Joint Expert Committee on Food Additives** has approved the use of cyclamate and saccharin since 1977 (WHO 1977). However, as already mentioned, **IARC** has classified saccharin as a B2 carcinogen, whereas the evidence has been considered limited for cyclamates (IARC, 1980).

The **National Swedish Food Board** initially decided to follow the decision of the U.S. FDA to issue a total ban on cyclamates and saccharin. However, in 1979 this decision was reconsidered with the result, that the use of cyclamates and saccharin again became permitted in table-top sweeteners for diabetics and others who wants to restrict their sugar intake (Slorach 1985). According to Stuart Slorach of the Swedish Food Administration (Slorach 1989), there is no general ban on the use of cyclamates in any of the Scandinavian countries. In contrast to the situation in Sweden, cyclamates can be used in soft drinks and foods in Denmark, Norway, and Finland. The more restrictive Swedish position is not based on perceived potential cancer risks, but rather reflects the more favorable relation between the ADI and sweetening potency for the alternative sweeteners saccharin and aspartame. The toxicity of the cyclamate metabolite cyclohexylamine is here perceived as the limiting factor.

As to the pesticide propoxur the authorities in **the Netherlands** do not consider that the bladder tumors induced by this compound in the rat are relevant to man and has not introduced any restrictions with respect to its use. Although the **U.S.EPA's** Peer Review Committee acknowledged the possibility that propoxur may induce tumors by a threshold mechanism, the Agency decided to classify propoxur as a B2 carcinogen classification (probable human carcinogen) (Rinde 1991). **The National Swedish Chemicals Inspectorate** has adopted an attitude similar to that of the U.S.EPA.

Tumors of the Rodent Forestomach. Although the incidence of gastric cancer has decreased over the past decades in the industrialized Western world, it remains one of the more common types of neoplasia in many other geographical areas. Especially in view of the poor clinical prognosis of cancers at this site, the presence in food of substances that can be linked to gastric cancer is of great concern.

Several experimental carcinogens induce malign and/or benign tumors in the forestomach of rodents upon oral administration. Examples are provided by e.g. the directly alkylating agents ethylene oxide and propylene oxide (Dunkelberg 1982), N-methyl-N'-nitro-N-nitrosoguanidine (Sugimura and Fugimura 1967) and at high doses, also some non-genotoxic compounds like the widely used food antioxidant, butylated hydroxyanisole, BHA (Ito *et al.* 1982, 1983a, b, 1984, 1986; Hirose *et al.* 1986) and the industrial chemical ethylacrylate (Smith 1986). Although less potent, the bread additive propionic acid has been found to induce similar effects (von Greim 1985).

In the first published investigations (Ito *et al.* 1982, 1983a; 1986) Fischer 344 rats were fed diets containing between 0.125 and 2.O% BHA for two years. At dose of 1 and 2% of BHA high incidences of papillomas and squamouscell carcinomas of the forestomach were found. In a subsequent investigation (Ito *et al.* 1984; Hirose *et al.* 1986) it could be demonstrated, that 3-BHA was much more active in inducing pappilomas than 2-BHA, and that the induction of tumors was preceded by massive cell proliferation and hyperplasia of the epithelium. In high doses BHA was also found to induce tumors of the forestomach in hamsters (Masui *et al.* 1986).

BHA has proven to be an efficient *promoter* of carcinogenesis in the forestomach as well as in the bladder of rodents (IARC 1986). However, it has also been found to exert a modest *protective action* against the carcinogenic effects of compounds like benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, diethylnitrosamine, urethane, aflatoxin B1 at other sites (Wattenberg, 1986). The two opposing effects probably mirrors different properties of BHA; neoplasia is enhanced in the forestomach and bladder due to local irritative action with subsequent cell proliferation, whereas the systemic antioxidant properties suppress the formation and/or reaction of electrophilic metabolites which may interact with DNA.

The BHA induced neoplasia in the forestomach of rodents features many similarities to the bladder neoplasia induced by propoxur discussed above:

- Steep dose-response curves for induction of tumors.
- Lack of genotoxic action (IARC 1986).
- Massive cell proliferation and hyperplasia of the epithelium at tumor inducing concentrations of BHA.
- Failure to induce a similar response in the esophagus and stomach lining of dogs, monkeys, and pigs (review by Nera *et al.* 1988).

When interpreting the results from the studies with BHA, ethylacrylate and similar compounds, it is important to realize, that the rodent forestomach lacks an equivalent counterpart in the human anatomy. Further, although squamous epithelium is also found in the esophagus of mammals, the rapid transit of ingested material limits

the dose (dose=time times exposure) to the mucosa in this region. In contrast, the rodent forestomach has a storage function, and for animals with free access to food it is never empty. This means, that the squamous epithelium of the forestomach in the positive cancer studies referred to above was *continuously exposed* to extremely high concentrations of BHA with a resulting massive cell proliferation. As for the bladder epithelium, the spontaneous incidence of tumors is low for the squamous epithelium of the forestomach. However, irritative or corrosive action results in a drastic proliferative response of these tissues. Thus, at a concentration of 2%, the mitotic index based on radiographic incorporation of labeled thymidine was found to increase by about a factor of 5 in the forestomach epithelium. Not unexpectedly, the bladder urothelium displayed similar increases in labeling index (Nera *et al.* 1988).

In summary, the induction of tumors by BHA in the rodent forestomach only occurs at an unrealistically high concentration in food of about 1% and above, and depends on the maintenance of a massive cellular proliferation of the squamous epithelium. At 0.5% and lower levels no such effects have been detected. Available evidence indicate that exposure to BHA is associated with an insignificant cancer risk when the exposure is kept below levels inducing hyperplasia of the forestomach epithelium. Similarly to the induction of bladder tumors by non-genotoxic agents, the carcinogenic response induced by BHA in the rodent forestomach represents an unspecific response of this tissue that hardly qualifies BHA for cancer classification.

The relevance for man of the tumors induced by the alkylating agents mentioned initially can hardly be questioned, but **IARC** has also classified BHA as a B2 carcinogen on basis of the forestomach tumors induced in rats and hamsters (IARC 1986). However, supported by a massive expertise, the tumors associated with the commonly used antioxidant BHA, as well as the bread additive propionic acid have —to the knowledge of this author— not been considered relevant with respect to the human exposure situation by the authorities responsible for food control in any country. For obscure reasons, the Delaney amendment has not been invoked in the **U.S.** for BHA. The substance is a "generally recognized as safe" (GRAS) food antioxidant that may be used in concentrations not exceeding 0.02% of the total fat or oil content of a particular food. The **Commission of the European Communities** (1978) has established a temporary ADI of 30 mg/adult for BHA or BHA combined with butylated hydroxytoluene (BHT). Similarly, the **Joint FAO/WHO Expert Committee on Food Additives** retains the temporary ADI of 0-0.5 mg/kg bw for BHA or combined BHA, BHT and tert-butylhydroquinone (WHO 1983). In **Sweden** it is used under the code E 320 in a variety of fatty food products, like margarine.

Thyroid Follicular Cell Carcinomas. Thyroid follicular neoplasia (adenomas and carcinomas) has been associated with *diets deficient in iodine* (Bielschowsky 1953, Schaller and Stevenson 1966, Ohshima and Ward 1986), *partial thyroidectomy* (Dent *et al.* 1956), the *implantation of thyroid stimulating hormone (TSH) secreting pituitary tumors* (Haran-Ghera *et al.* 1960), *natural goitrogens* (in rape seed and cabbage), chemotherapeutic agents like *sulfamethoxazole, sulfathiazole, amitrole (pesticide), ethylene thiourea (ETU), chlorinated aromatics (PCBs, TCDD),* as well as certain complex anions like *perchlorate* or *tetrafluoroborate* (For a review see Hill *et al.* 1989). In man the only recognized specific thyroid carcinogen seems to be ionizing radiation (Hill *et al.* 1989).

Many thyreostatic compounds inhibit thyroid hormone synthesis by blocking iodine incorporation into the thyroid hormone precursors. This leads to stimulation of the anterior pituitary to secrete TSH which, in turn, results in thyroid hypertrophy, hyperplasia and eventually to development of neoplasia of the thyroid, but sometimes also of the pituitary. The most common mechanisms are the inhibition of peroxidase action essential for the hormone synthesis as well as interference with the thyroid hormone transport system. TSH increases the synthesis of RNA, protein and activates the oncogenes *c-myc* and *c-fos* (Tramontano *et al.* 1986). As to the genotoxicity of the three classes of thyroid carcinogens, *thionamides* (thioureas, etc.) and *amitrole,* complex *halogenated hydrocarbons* (chlorinated paraffins, aldrin, etc.) and *aromatic amines* (4,4'-methylenedianiline, etc.), in general, only the aromatic amines have demonstrated consistent activity (U.S.EPA 1988).

Any agent or process that inhibits the biosynthesis, secretion, or transport of thyroid hormones will lead to an increase in TSH secretion, which in turn stimulates proliferation of the follicular thyroid cells. If prolonged, such an actions will eventually result in neoplasia. The phenomenon is well known in the field of pharmaceuticals. Thus, it has been known for many years that the administration of certain sulfonamides, like *sulfmethoxazole,* to

the rat induces thyroid hyperplasia and tumors (MacKenzie and MacKenzie 1943, Swarm *et al.* 1973) and has not the least detracted from their clinical use.

The generation of this type of tumors in rodents by compounds that cause thyroid-pituitary hormonal imbalance represents one instance where the **U.S.EPA** has acknowledged the existence of an indirect mechanism of action, resulting in a threshold under which no induction of cancer is expected to occur (Paynter *et al.* 1988; U.S.EPA 1988). This type of mechanism, no doubt, accounts for the carcinogenic effects to this target organ induced by e.g. the ethylenebis-dithiocarbamate fungicides (EBDCs) and their breakdown product, ethylene thiourea (ETU) (IPCS 1988). The reason why U.S.EPA still classifies the EBDCs (and ETU) as B2 carcinogens is that ETU (like amitrole), in addition to thyroid tumors, also induces an increased incidence of liver tumors in the B6C3F1 mouse. In **Sweden** the Inspectorate has classified these compounds as carcinogens. Because of their non-genotoxic mechanism of action the EBDCs and ETU are, on the other hand, evaluated using a threshold approach in **the Netherlands, U.K.** as well as in **Germany,** where the relevance of the liver tumors in the mouse model has been rejected for these compounds (Nilsson *et al.* 1993).

Tumors of the proximal tubuli in the rat kidney are induced by several non-genotoxic compounds like *branched chain aliphatic hydrocarbons (e.g. 2,2,4-trimethylpentane), unleaded gasoline, d-limonene, isophorone, cinnamyl anthranilate,* and *many chlorinated compounds* (e.g. by 1,4-dichlorobenzene) and seems to be associated with the accumulation of a low molecular weight protein, a2u-globulin, in the kidney tubules in rats (Swenberg *et al.* 1989, Borghoff *et al.* 1991).

Particularly high amounts of α2μ-globulin is synthesized by the male rat liver, transported by the blood stream to the kidney and excreted by the primary urine. It is subsequently partially (50%) reabsorbed in the tubuli. Compounds inducing a "hydrocarbon nephropathy" ("protein droplet nephropathy") cause excessive accumulation of droplets of α2μ-microglobulin in the lysosomes of the tubular cells of the kidney tubuli that later results in cell necrosis and formation of granular casts. These pathological changes are associated with compensatory cell proliferation, and eventually in the induction of tumors. The biological role of α2μ-globulin is not known, but it appears to have the capacity to transport nephrotoxic compounds and/or their metabolites. Of interest is the observation, that the same protein seems to be involved in the binding of lead in the inclusion bodies seen in the rat after exposure to this metal (Fowler *et al.* 1993). Other mammalian species than the rat, including NIH Black rats, guinea pigs, mice, dogs, as well as monkeys do not seem to synthesize the α2μ-microglobulin and do not develop protein droplet nephropathy after exposure to the chemicals referred to above. Thus, a great deal of evidence point to the fact that tumors of the proximal convoluted tubuli in the rat kidney associated with the accumulation of α2μ-microglobulin is a species specific response, and that compounds having been classified on basis of such effects should be reassessed in light of this new evidence.

No guidelines have been issued by regulatory agencies on how to deal with these type of tumors. Whereas there has been a reluctance to classify such products as unleaded gasoline and branched chain hydrocarbons as carcinogens, the non-genotoxic *p-dichlorobenzene* (moth repellent) has been considered as a carcinogen by the **U.S.EPA** (B2) as well as by the Pesticide Registration Division of the **National Swedish Chemicals Inspectorate.** It should be mentioned, however, that in the case of the U.S. and Sweden these decisions were also based on the induction of liver tumors in the $B6C3F_1$ mouse by this compound. **U.S.EPA's** Environmental Health Committee to the Science Advisory Board has recently reassessed the significance of these tumors (U.S.EPA 1991). It was concluded, that compounds producing renal tumors in male rats attributable solely to chemically induced α2μ-globulin accumulation should **not** be used for human cancer hazard identification or for dose-response extrapolations.

Splenic Fibrosarcomas. The majority of compounds that induce this kind of tumors are structurally related to *aniline.* The non-genotoxic compounds aniline and *sulphonyldianiline* are potent inducers of methemoglobinaemia, a toxic effect that is accompanied by deposition of massive erythrocyte debris, particularly iron derivatives, in the spleen. Bus and Popp (1987) have suggested, that neoplasia may occur as an indirect effect of this deposition followed by induction of splenic hyperplasia. Although the hypothesis seems plausible, it needs further confirmation.

Rodent Leukemias and Lymphomas. The over-all age corrected incidence of this group of cancers is typically rather stable in human populations in industrialized countries, showing much less geographic variations than e.g. cancer of the skin, esophagus, prostate, rectum, etc. (Doll and Peto 1981). It has usually been reported as less than 10% of the total incidence. In the F344 rats and $B6C3F_1$ mice used in the NTP bioassays, leukemias and lymphomas demonstrate a high and variable incidence. In the F344 rat, these neoplasms have been reported to occur in controls at a rate of 30±11% in males, and 19%±7% in females. The corresponding incidences for the B6C3F1 mice was 13±7% in males, and 27%±10% in females (Haseman *et al.* 1984).

One complicating factor in the interpretation of findings of such tumors in rodents is the occurrence of viral factors (Wogan 1984). This is particularly so in the mouse which often are infected with retroviruses, such as murine leukemia viruses (MuLV). The majority of strains of the laboratory mouse, including the $B6C3F_1$ hybrid, contain endogenous retroviruses, i.e. genes encoding for retroviruses. In some mouse strains (e.g. AKR), retrovirus activation has been shown to precede spontaneous leukemia, and the presence of an endogenous retrovirus has been cited to explain the marked difference between the incidence of lymphoma in the B6C3F1 (50%) and NIH Swiss mice (10%) exposed to butadiene (Irons 1988). Insufficient data exist on how stimuli of different types affect the induction of retrovirally associated leukemias and lymphomas, but the finding of several non-genotoxic chemicals (diallyl phthalate, 2,7-dichlorobenzo-p-dioxin, isophorone, chlorinated paraffins, allylisourea, phenestrin) that have induced these types of tumors in the mouse seem to motivate caution in the interpretation of this group of tumors.

Testicular Tumors. In rats testicular neoplasms of interstitial cell origin (Leidig cells) occur commonly in old individuals from some strains. Haseman *et al.* (1984) gives an average of 88+9% for the Fischer 344 rat at 110-116 weeks of age, an incidence which increases to about 96% for life-span data (Solleveld *et al.* 1984). Needless to say, few conclusions may be drawn from induction of tumors at this site when using such an animal model.

Pancreatic Tumors. Spontaneous tumors of the exocrine pancreas is usually uncommon in the rat. However, corn oil gavage has been shown to significantly increase the incidence of this rare tumors by a hitherto unknown mechanism (Haseman *et al.* 1984). Further, for the Fischer F344 male rat (Solleveld *et al.* 1984) there is a drastic increase in the incidence of pancreatic acinar cell adenomas from that seen at 110-116 weeks (0.3%) to the incidence observed at termination of a life-span study (7%).

RECEPTOR-MEDIATED CARCINOGENESIS

Clonal expansion of initiated cells represents the key feature of tumor promotion. There is a growing body of evidence that such a selection and expansion is frequently mediated by a receptor-mediated mechanism, mostly based on a hormonal type of action. Among such types of action, steroid and peptide hormone pathways can be distinguished. Steroids mainly interact with receptors in the cytosol and/or nucleus with the formation of a receptor/ligand complex that binds to specific DNA sites. This binding may then lead to transcriptional activation of specific genes. Peptide hormones, on the other hand, seem to interact with cell membrane receptors resulting in the synthesis of secondary messengers that may have a central role in a range of regulatory processes. One typical feature of endocrine organ neoplasias and mammary tumors is that the incidence often increases strikingly towards the end of the life span of the animal (Solleveld *et al.* 1984). The main problems with interpretation of the effects of receptor mediated carcinogenesis in experimental animals are mostly, but not exclusively, concerned with the quantitation of human risk; the mechanism of action would implicate the existence of a definite dose-threshold. Carcinogens for which this type of mechanism has been invoked are *estrogens, polychlorinated dibenzo-p-dioxins (PCDD), phorbol esters, and recently also the peroxisome proliferators.* PCDDs will be discussed in the context of quantitative risk assessment in a following article.

Estrogen promoted neoplasia. With an annual incidence of almost 2 000 cases per million, breast cancer ranks as one of the most prevalent cancers in women. However, there is a marked difference in the incidence of breast cancer in different geographic areas; thus it is about six times higher in Caucasians than in Japanese (Doll and Peto 1981). Long before diet was recognized as an contribut-

ing factor in the induction of this group of cancers, the important role of estrogens had clearly been established. Thus, already at the beginning of this century, it was known that bilateral ovariectomy could slow down the progression of breast cancer, and subsequently it was demonstrated that estrogens will stimulate the proliferation of epithelial cells in the mammary gland, uterus and vagina. In the case of ovarian failure, when estrogen stimulation ceases, the incidence of breast cancer is reduced to about 1% of that seen in normal women (For a review, consult Lyons *et al.* 1958).

Tumors of the mammary gland occur frequently in the rat. Non-metastazing fibroadenomas are most common, and for this tumor a range of 16-44% has been reported for different laboratories for the Fisher 344 rat. The corresponding average incidence of mammary adenocarcinoma is, on the other hand, only about 2% for this strain (Haseman *et al.* 1984). In Sprague Dawley and Charles River CD rats considerably higher incidences of adenomas as well as of adenocarcinomas have been reported (McCormick and Moon 1985). The incidence of mammary gland neoplasms increases drastically towards the end of the life-span (Solleveld *et al.* 1984). The incidence of mammary tumors is low in the $B6C3F_1$ mouse, but not so for some other strains (Sher 1974). The dog is another species used in toxicological research where mammary gland adenomas occur commonly, and where, in particular, interpretation of the effect of oral contraceptive hormones has been the subject of much controversy.

It is important to note, that for rats, a decreased calorie intake is clearly associated with decreased prevalence of mammary gland neoplasms. Likewise, increased calorie intakes may increase the incidence. For chemical treatments that affect the food intake, this becomes an important issue (Dao and Chan 1983, Haseman *et al.* 1989).

It is generally accepted, that in order to exert its biological effects estrogens must bind to a specific receptor system. Evidently, the estrogen receptors function in a manner analogous to that has been described for TCDD (Lucier 1992). In the first stages of breast cancer when the tumor cells are relatively undifferentiated, steroid hormones are necessary for progression. However, as the cancer changes to an undifferentiated state, the estrogen receptors disappear, and tumor growth becomes independent of estrogen stimulation (Henderson *et al.* 1988). It should be pointed out, that the hepatic estrogen receptor is different from the *Ah* receptor; compounds that bind to the *Ah* receptor do not bind to the estrogen receptor. Pituitary prolactin also seem to promote mammary tumorigenesis (Dao and Chan 1983).

Apart from breast cancer, estrogen receptor mediated carcinogenesis also seems to be important in rodent liver carcinogenesis. Hepatocytes contain estrogen receptors, and in two-stage models for hepatocarcinogenesis several natural estrogens, as well as xenobiotics with estrogenic activity —like certain *PCB congeners* and *α-zearanol*— have shown to exert a significant promoting action. The pesticides *kepone, DDT,* and *methoxychlor* all have weak estrogenic activities, a fact that may have significance in relation to their tumorigenic action on the rodent liver. Some, but not all liver tumors have higher than normal concentrations of the hepatic estrogen receptor, pointing towards the possibility of a differentiated response of clones of initiated cells under the influence of estrogen stimulation (Lucier 1992).

The synthetic estrogen, *diethylstilbestrol (DES),* has been shown to cause vaginal adenocarcinoma in the offspring of women treated with this hormone during pregnancy. For the various metabolites formed from DES *in vivo,* a good correlation has been found between affinity to the estrogen receptor and carcinogenic potential (Lucier 1992).

Compound Causing Neoplasms of the Pituitary. Whereas neoplasms of the pituitary are very rare in humans, adenomas of this gland is one of the most common tumors in the rat with e.g. a reported range of 18-50% for female and 5-29% for male Fischer 344 rats (Haseman *et al.* 1984).

The secretion of thyroid-stimulating hormone (TSH) from the pituitary gland is exerted by the hypothalamus by the release of the thyrotropin-releasing hormone (TRH). Imbalance of the feedback mechanisms involved will affect the thyroid as well as the pituitary. It is therefore not surprising, that some agents causing thyroid neoplasia by interfering with the synthesis and/or excretion of thyroid hormones may also cause neoplasia of the pituitary (Bielschowsky 1955, Hill *et al.* 1989). Like for mammary gland neoplasms, a low food intake may reduce the incidence of pituitary tumors in rats (Haseman *et al.* 1989).

Proliferative Lesions of the Adrenal Medulla. In IPCS Environmental Health Criteria No. 104 (IPCS 1990) it is noted with respect to the use of rats in carcinogenicity studies that

> "An overview of the literature indicates that untreated rats of various strains may exhibit widely differing incidences of lesions described as 'phaeochromocytomas'. ...There are no clear criteria for distinguishing between prominent foci of hyperplasia and benign neoplasms, and pathologists differ in the criteria that they use for distinguishing between benign and malign adrenal medullary tumors."

Wistar, Sprague-Dawley, Long Evans as well as Fischer F344 rats (but not of the Osborn-Mendel and Charles River SD strains), all have very high and variable spontaneous incidences of this type of lesions (Tischler and DeLellis 1988). Haseman *et al.* (1984) gives an incidence of 17± 9% for males and 3.5±3% for female Fischer F344 rats at 110-116 weeks, while these incidences increased to 30% in males ant to 15% in females in animals that were maintained until 10% of all individuals remained alive (Solleveld *et al.* 1984).

Among agents, that frequently induce neoplasia in the rat adrenal medulla include *estrogens, growth hormone, antithyroidal compounds, neuroleptics, alloxan, nicotine, reserpine, retinol acetate,* and *diphenylamine,* but also dietary factors like *excessive food intake* (IPCS 1990). Some authors have suggested, that the pituitary hormone prolactin plays a central role in the development of medullary neoplasia. Thus, there is evidence that hypophysectomy may reduce the incidence of phaeochromocytoma in susceptible rat strains. Growth hormone and prolactin are related peptide hormones, and at high concentrations growth hormone affect prolactin receptors. Estrogens or antithyroid compounds increase the levels of TSH which also would be expected to stimulate prolactin release. Depletion of hypothalamic dopamine by the action of reserpine, or by certain neuroleptics, could hypothetically also result in an increased prolactin production (Tischler and DeLellis 1988).

In summary, an increased incidence of adrenal medullary neoplasms in a strain of rat with a high spontaneous incidence can rarely be used as supporting evidence of carcinogenicity; for all strains and species of experimental animals particular care must be exercised in evaluating adrenal medullary tumors with respect to factors that directly or indirectly may affect endocrine functions.

CONCLUSIONS

In a modern society risk management should, ideally, be based on actual levels of risk —preferably seen in an overall perspective. In reality, a number of non-scientific factors will to a lesser or greater extent influence the outcome of any regulatory process. Due to non-scientific considerations, bias may be introduced already at an early stage of the evaluation process. For this reason, realistic risk estimates should be obtained purely on basis of scientific considerations, that must be separated from risk management.

Mammalian cancer is a group of diseases with multifactorial etiology. Although their mechanism(s) of induction is largely unknown, current knowledge may permit a rough classification of certain chemical carcinogens on basis of mode of action. Based on this concept, tumors induced experimentally in target organs of rodents like liver, bladder, forestomach, renal tubuli, as well as certain endocrine associated tumors should be interpreted with great caution.

On the level of data interpretation, there has been two basically different approaches among international agencies and national regulatory bodies. One is the *generic* approach, where —with few exceptions— all types of tumors induced in an animal model are basically considered as valid for humans, and no consideration of is taken of e.g. high spontaneous background levels and/or mode of action. Adherents to this doctrine are notably the **IARC,** the **U.S.EPA,** as well as the agencies responsible for regulation of pesticides and industrial chemicals in the **Scandinavian countries.** Proponents of an evaluation on a case-by-case basis, taking into account factors such as background incidence levels and/or mode of action, are the **IPCS (JMPR),** the authorities in countries like **Canada, Germany, the Netherlands, United Kingdom,** and recently also expressed by the **Commission of the European Communities.** Recent scientific developments appear to support the latter more flexible approach in the interpretation of experimentally induced tumors.

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