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PROBLEMS IN THE REGULATION OF CARCINOGENIC CHEMICALS IN AN INTERNATIONAL PERSPECTIVE II. DETERMINATION OF CARCINOGENIC POTENCY

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ABSTRACT

A characterization of the dose-response relationship for induction of tumors by chemicals at levels to which humans are exposed is a prerequisite for quantitative cancer risk assessment. If such data are derived from animal experiments, a valid extrapolation from the studied animal species to man must be made. The present article reviews the most important procedures for high-to-low-dose extrapolation, highlighting some of the limitations of currently used mathematical models. The problems associated with the use of the maximum tolerated dose (MTD) in cancer bioassays are also discussed. Based on mechanistic as well as pharmacokinetic considerations, pitfalls in the determination of carcinogenic potency is illustrated by discussing some well-known human and experimental carcinogens, like inhibitors of DNA-repair and some substances with promoter-like action. Determination of protein and DNA adducts from ethylene oxide and propylene oxide demonstrate the usefulness of the target dose concept for interspecies comparison.

RESUMEN

La caracterización del comportamiento dosis-respuesta en tumores inducidos por agentes químicos a niveles en que están expuestas las personas es un prerrequisito para la evaluación cuantitativa del riesgo al cáncer. Si tales datos se derivan de experimentos con animales, es válida la extrapolación de las especies animales estudiadas al hombre. Este artículo revisa los procedimientos más importantes para la extrapolación de dosis altas a bajas, destacando algunas de las limitaciones de los modelos matemáticos comunmente empleados. Se analizan también los problemas asociados con el uso de la dosis máxima tolerada (DMT) en los bioensayos de cáncer. Los defectos al obtener la potencia carcinogénica basada en consideraciones mecanísticas así como farmacocinéticas surgen de la discusión sobre carcinógenos experimentales y humanos bien conocidos, como inhibidores de la reparación del ADN y sustancias con actividad similar a los promotores. La determinación de aductos de proteinas y ADN de los óxidos de etileno y propileno demuestran la utilidad del concepto de dosis blanco para realizar la comparación entre especies.

GENERAL BACKGROUND

In a situation where exposure of the human population to a multitude of carcinogenic agents present in air, food, and drinking water is unavoidable, it is, obviously, necessary to estimate their carcinogenic potencies in order to make meaningful predictions of carcinogenic risk. Carcinogenic potency may vary by several orders of magnitude among chemicals, as exemplified by the two established human carcinogens *benzene and bis(chloromethyl)ether,* with an estimated potency difference of about 10,000 (IRIS 1993). However, adequate quantitative data is available from humans only for very few chemicals. When using information obtained in experimental animals for risk assessment purposes, a qualitative assessment of the relevance for man of the induced experimental tumors must always be carried out before extrapolating any experimental dose-response data. Some common problems associated with this first phase of cancer risk assessment was dealt with in a preceding article (Nilsson 1993).

TABLE I. INCIDENCE OF TUMORS IN TREATED GROUPS OF EXPERIMENTAL ANIMALS REQUIRED FOR SIGNIFICANCE (p=0.05) DEPENDING ON GROUP SIZE AND INCIDENCE OF SPONTANEOUS TUMORS IN CONTROLS

Incidence of tumors in controls	Number of animals per group				
(%)	10	25	50	75	100
0	50	20	12	8	6
10	70	40	28	24	21
20	80	52	40	36	34
30	90	64	52	47	45
40	100	72	62	58	55

HAZARD ASSESSMENT: HIGH-TO-LOW DOSE EXTRAPOLATION MODELS

Statistical Power of Resolution for Animal Bioassays - Use of the MTD. Provided that the tumors induced in an animal study are thought to be relevant to the human exposure situation, the crucial issue is to define the shape of the dose-response curve from the animal data. However, in cancer risk estimation the dose range of interest usually lies far below the dose range accessible for animal experimentation. In **Table I** the incidence of tumors in treated groups of experimental animals required for statistical significance at the 5% level is given, depending on group size and incidence of spontaneous tumors in controls. As seen from this table, when utilizing the usual size of dose groups consisting of 50 animals, a 28% increase in incidence of tumor bearing animals is required when the incidence for the same type of tumor is 10% in the control group, and a 40% increase is necessary in order to detect a statistically significant increase when the background incidence is 20%. For large human populations incidences at 1% and below have considerable toxicological significance, a degree of resolution that cannot be achieved in regular cancer testing. To compensate for this lack of sensitivity, the test animals are given extremely high doses, including the so called *maximum tolerated dose (MTD)* as the highest tested dose.

Although correctly motivated from the statistical point of view, the use of the MTD in cancer testing in experimental animals has become a hotly contested issue for other reasons. Many substances with an inherently low toxicity are required to be administered in unrealistically large amounts in order to achieve the MTD, a dosing schedule that may result in so called *metabolic overloading,* implying possible changes of metabolic pathways and/or depletion of natural protective agents. As to the latter effect, there has been considerable discussion of the implications of glutathione depletion at high exposure levels with respect to the fate of reactive electrophilic intermediates. Such mechanisms have, e.g., been cited to account for the bone marrow toxicity and carcinogenicity of *benzene,* where reactive quinoid intermediates species are formed during metabolism (Irons 1985, Parke 1989). However, although metabolic overloading undoubtedly may occur under MTD dosing conditions, in many cited examples such overloading seems primarily to affect the shape of the dose-response curve, rather than to transform a non-carcinogen to a carcinogen.

Dihalomethanes, like methylene chloride, are detoxified by the liver microsomal mixed-function oxygenase (MFO) system as well as by a metabolic pathway involving initial conjugation with glutathione. The oxidative pathway in mice and rats —which generates CO as one end product— is saturated at concentrations of a few hundred ppm, while the glutathione-S-transferase pathway shows no appreciable saturation up to about 10,000 ppm. Since glutathione is regenerated during the operation of both pathways, the pool of this reductant is not appreciably diminished. Further, following high exposures in the mouse, there is a loss of P-450 from Clara cells of the lungs, resulting in a 50% loss of metabolic capacity by this pathway. No such decrease was found for the glutathione-S-transferase dependent reactions (Andersen *et al.* 1987, ECETOC 1989).

Whereas tumor incidence in mice showed a good correlation with the amount of methylene chloride metabolized by the glutathione dependent pathway, it could not be adequately correlated with production of intermediates via the MFO pathway. Using pharmacokinetically based modeling, a tissue target dose (see below) is obtained that is lower in comparison with conventional extrapolation methods by at least an order of magnitude (Andersen *et al.* 1987). Below, inorganic arsenic will cited to illustrate the conse-quences of another type of metabolic overloading which may occur in humans.

Although it is generally agreed upon that the MTD should induce no life-shortening toxicity, or no more than a 10% decrease in body weight gain (Haseman 1985), it is not realized, that certain other effects, including cell proliferation, might also be regarded as a sign of toxicity that should possibly be included as one consideration in setting the MTD (Swenberg and Maronpot 1991). As discussed in the previous article (Nilsson 1993), induced cell proliferation will per se cause an increase in the incidence of tumors in organs like liver, bladder, thyroid, and forestomach in rodents. Since several non-genotoxic carcinogens induce

such cell proliferation at high doses, e.g. in tissues like the rodent liver, the inclusion of cell proliferation as an index of toxicity for setting of MTDs would certainly challenge a number of current regulatory decisions.

High-to-low-dose extrapolation. From the mechanistic point of view there are two basically different types of dose-response relationships: (a) for endpoints that imply the existence of a definite dose-threshold (most toxicological effects, teratogenic action, and at least some types of cancer promotion) and (b) stochastic phenomena which have no real threshold (mutations and cancer initiation). To estimate the carcinogenic action at low doses various approaches have been used. For events following relationships of type (a), safety factors or so called tolerance distribution models are used, whereas linear (mechanistic) models have often been employed to describe stochastic phenomena. Only a few of the more commonly used models will be mentioned here, and the reader is advised to consult an appropriate review for a comprehensive treatment of the subject (Hanes and Wedel 1985, Zeise *et al.* 1987, Freedman and Navidi 1989, Johannsen 1990).

The TD_x Model and the Benchmark Dose Approach --Carcinogens are graded by some Scandinavian regulatory agencies in potency categories according to their estimated TDx value, that is, the lowest dose that produces a statistically significant tumorigenic response (Nordic Council of Ministers 1985). This crude approach, which does not take the shape of the dose-response curve into account, can not be compared with the models described below. However, it should be realized, that its primary purpose has not been to calculate absolute risks, but to compare potencies of various carcinogens on a relative scale. However, even for this objective its application may lead to misleading results for compounds with radically different shape of the dose-response curves in the more relevant lower part of the curve. This is illustrated in **Fig. 1.**

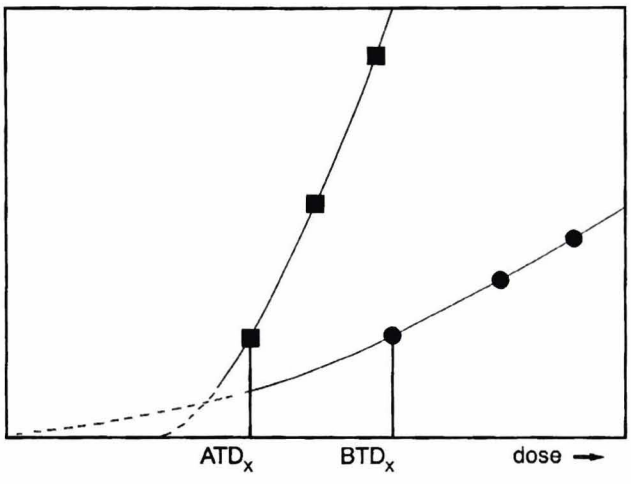

Fig. 1. Comparison of the dose-response curves for two carcinogens characterized by different slopes in the experimentally accessible dose range

The three measured data points for cancer incidence as a function of dose describe a steep inclination for **Compound A** suggesting a dose threshold, whereas for **Compound B** it is much more shallow, but indicative of a linear component in the low-dose region. Within the dose-range accessible to animal experimentation, Compound A, has a lower TD_x, and, therefore, appears to be the more potent carcinogen of the two. This is obviously not so in the usually much more relevant low-dose region, and the TD_x approach may, therefore, result in a gross overestimation of risk for compound A. Further, an unnecessary element of imprecision is introduced by allowing the lowest dose chosen in a particular bioassay that happens to induce a statistically significant increase in tumor incidence decide a cancer potency estimate. Using another dose selection scheme for the same type of study this value may change considerably. In the Nordic Council of Ministers' report the influence of the shape of the dose-response curve was discussed, and it was e.g. noted, that supralinearity could result in underestimation of the cancer incidence in the low-dose region (p. 45). However, no method for correcting TD_x values for such deviations was proposed.

When comparing carcinogenic potencies of different compounds in the dose range accessible to experimentation, improved precision may be obtained by using a "benchmark dose" which is based on data from the entire dose-response curve rather than on a single arbitrary lowest-observed-effect-level (LOEL), like TD_x. Since low-dose extrapolation is not involved, several descriptive models may be used for curve fitting. In most cases it is difficult to estimate the an excess cancer risk that is less than 10% above the background spontaneous incidence level, and the effective dose corresponding to this excess risk, ED10, has therefore often been chosen (Kimmel and Gaylor 1988). In principle, the procedure involves the following steps: the dose-response curve is fitted to the data and the upper confidence limit on the curve is obtained. For many sets of data the linearized multistage model may modified for this purpose (Crump 1984b). From this curve the ED10 is then found, and the corresponding lower confidence limit on the dose which gives a 10% excess risk, LED10, estimated (see **Fig. 2**). Although a "benchmark dose" derived in this fashion may be employed as a more adequate substitute for the TD_x values in comparing carcinogen potencies, its greatest utility lies in providing better approximations of ADI (acceptable daily intake) values for non-stochastic effects.

Tolerance distribution models assume that each member of a given population has a threshold, or tolerance level, below which the individual will not respond to exposure, and that the variability among individuals can be expressed as a probability distribution (probit, logit, Mantel-Bryan, Weibull, etc.). Out of these models, the **Weibull model,** which was introduced by a Swedish scientist in 1951 (Weibull 1951), has found a certain use for regulatory purposes. This model appears to be very flexible and easily fitted to most data sets (Carlborg 1981), especially to data that

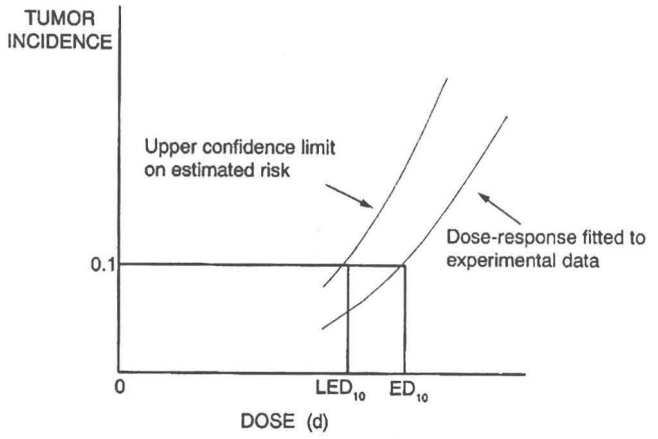

Fig. 2. Graphical illustration of the estimation of benchmark dose (adapted from Kimmel and Gaylor 1988)

exhibit threshold-like appearance (e.g. the ED01 bladder tumor data for 2-acetylaminofluorene, 2-AAF; Littlefield *et al.* 1979). The main reservation has been made as to the validity of its biological basis (Zeise *et al.* 1987).

Linear (Mechanistic) Models assume that a certain number of reactions, events, or "hits" (a concept derived from radiation biology) or transition stages, related to a critical target in the cell (DNA) are necessary to transform a normal cell to a cancer cell. In the earlier models, response was mostly considered as a function of time, and although early appearance of tumors in a particular study would call for a separate analysis incorporating time-to tumor data, most of these models are now used in their dichotomous dose-response forms.

There seems to be some consensus, that unless available data are sufficiently accurate to exclude models involving a linear component in the low-dose region, or when a genotoxic mechanism might be involved, tolerance distribution models like Mantel-Bryan's probit model and multi-hit models should not be used (Swedish Cancer Committee 1984, U.S.EPA 1986a). The latter type of models may, on the other hand, be appropriate for promotive agents as well as for several types of "epigenetic" (nongenotoxic) carcinogens".

The **one-hit model** for carcinogenesis, proposed by Iversen and Arley (1950), assumes that a single "hit" at the critical target within a cell is sufficient for cell initiation and transformation. The model can be traced back to the one-hit model for effects of X-rays in cellular systems proposed by Crowther in 1924 (Crowther 1924) and may be derived as follows:

At very low radiation doses the number of hits induced by ionizing radiation will be proportional to the number of targets available. With increasing dose some events will occur in targets that have already been hit, so the number of effective hits will decrease with increasing exposure. If the number of original targets is N_O, N the number of remaining unaffected targets at dose D, then

(4) $-dN/dD = kN;\ dN/N = -kdD$

This integrates to

(5) $\ln(N/N_O) = -kD$ or

(6) $N/N_0 = e^{-kd} = S$ = fraction unaffected targets

When translated into cancer initiating events, the probability of a target, like DNA in a cell, being affected at dose D is one (total probability of being affected) minus the probability of not being affected:

(7) $P(D) = 1 - e^{-kd}$

At very low doses this one-hit model gives a straight line for the dose-response curve through the origin. It is now seldom used, because it has only one parameter and does not give a good fit with respect to the basically sigmoid-shaped dose-response curves characteristic of more complete data sets. It can be regarded as a special case of the multi-hit or multistage models.

The **multi-hit** model assumes that a target cell must absorb at least a certain number of "chemical hits" before a carcinogenic change is induced. It can be fitted to data exhibiting strong superlinearity or strong sublinearity very well, but is difficult to interpret in biological terms; the best fit for liver angiosarcomas induced by vinylchloride involves half a hit; the sublinear dose-response curve for NTA using the gamma multihit model yields 28 hits (Zeise *et al.* 1987).

The **multistage model,** first proposed by Armitage and Doll (1954), is an extension of the one-hit model. It is assumed that a normal cell must progress through a series of heritable changes before it becomes malignant. One generalized version of this model takes the form:

(8) $P(D) = 1 - \exp[-(\alpha + q_1D + q_2D^2 + ...q_kD^k)],\ k \geq 1$

Where P(D) is the probability of cancer at dose D, k is the number of stages (usually set arbitrarily by the U.S.EPA at the number of dose levels minus one), q_k are coefficients to be fit to the data, and D^k the applied dose raised to the kth power. The background cancer incidence is $1-\exp(-\alpha)$. At low doses the function becomes essentially linear, where q_1 is the slope and provides a measure of carcinogenic potency. The most likely estimate at very low doses becomes increasingly unstable with a small change in the response at experimental doses (Carlborg 1981). Therefore, a further development was the replacement of the linear term in the polynomial function by its upper 95% confidence limit to achieve

TABLE II. THE UPPER 95% CONFIDENCE LIMIT ON LIFE TIME CANCER RISK AT A DOSE OF 1 mg PER kg BODY WEIGHT AND DAY (POTENCY FACTOR, UNIT RISK, SLOPE FACTOR, CPF, q_1*) FOR SELECTED CARCINOGENS (IRIS 1993)

Compound Potency Factor	Potency Factor (by ingestion)	Potency Factor (by inhalation)
Acrylamide	4.5	4.5
Arsenic, inorg.	1.8	15
Azobenzene	0.1	0.1
Benzene	0.03	0.03
Benzidine	230	230
Benzo(a)pyrene	7	–
Beryllium	4	8
Bis(chloroethyl)ether	1	1
Bis(chloromethyl)ether	220	220
Bromodichloromethane	0.06	–
Butadiene	–	1.8
Carbon tetrachloride	0.1	0.05
1,2-Dibromoethane	85	0.8
3,3'-Dichlorobenzidine	0.5	–
1,2-Dichloroethane	0.09	0.09
Dichloromethane (Methylene chloride)	0.008	0.002
Dinitrotoluene mixture 2,4-/2,6-	0.7	–
1,4-Dioxane	0.01	–
1,2-Diphenylhydrazine	0.8	0.8
Epichlorohydrin	0.01	0.004
Ethylene oxide**	–	0.4
Folpet	0.004	–
Formaldehyde	–	0.05
Hydrazine/sulfate	3	17
4,4'-Methylene bis (N,N'-dimethyl)aniline	0.05	–
Nickel refinery dust	–	0.8
Nickel subsulfid	–	1.7
N-Nitroso-di-n-butylamine	5	5
N-Nitrosodiethylamine	150	150
N-Nitrosodimethylamine	50	50
N-Nitrosopyrrolidine	2	2
Propylene oxide	0.2	0.03

** U.S.EPA 1985a

more stable estimates of risk (q_1*) above background than are obtained for the most likely estimates. The multistage model is very flexible in fitting data sets because it is a polynomial function of dose. This so called *linearized multistage model* is currently used routinely by the U.S.EPA (Crump 1984a) and has, therefore, become the most widely used model in the world for estimation of cancer risk. It is commercially available as a program for PC*** (Global 82, Tox-Risk). The upper 95% confidence limit on life time cancer risk at a dose of 1 mg per kg body weight and day (potency factor, unit risk, slope factor, CPF, q_1*) is a regularly used estimate for calculation of human cancer risk in the U.S.

(9) total population risk (during 70 years) =

dose (mg/kg & day) x q_1* (mg/kg & day)$^{-1}$ x no. of exposed persons

In **Table II** potency factors obtained by the linearized multistage model (IRIS 1993) for some representative human and experimental carcinogens are presented.

*** Clement Associates, 1201 Gaines Street., Ruston, LA 71270, USA.

The multistage model is favored by U.S.EPA because it generally gives conservative risk estimates for low exposures, but the model has several weaknesses (Carlborg 1981, Sielken 1987, Zeise *et al.* 1987, Freedman and Navidi 1989, Johannsen 1990). For formaldehyde a much better fit to the experimental data was found using a five-stage multistage model than by using the U.S.EPA version with the conventional restrictions. Equation (8) predicts that the probability of tumor induction will eventually approach unity when the dose is sufficiently high. Thus, a good fit will not be obtained for data sets where the dose-response function rises steeply and then reaches a plateau (strongly concave, Michaelis-Menten kinetics), e.g. as for DDT, diethylstilbestrol, and vinyl chloride. To cope with such a situation, a regression procedure is used for computing the maximum likelihood polynomial function from a data set. Whenever the model does not fit the data sufficiently well, data at the highest dose are deleted, and the model is refitted to the rest of the data. This is continued until an acceptable fit is obtained (Anderson 1983). Scientifically, this represents a questionable approach.

Another defect of the linearized multistage model is that the low-risk extrapolation is relatively insensitive to the shape of the dose-response function in the observable range. Thus, data with very steep dose-response curves give risk estimates that are very similar to those produced by data sets characterized by very flat dose-response curves (Carlborg 1981). Thus, a main criticism has been that the use of the U.S.EPA model may result in overly conservative estimates in the former case. In fact, the upper confidence limit in the linearized multistage model can result in a non-zero estimate of risk for data sets that do not show carcinogenicity (Johannsen 1990).

The U.S.EPA is currently considering some drastic changes of its 1986 Guidelines (U.S.EPA 1986a). In addition to the introduction of mechanistic considerations based on "mode of action", basically, linear extrapolation will only be used for compounds with a known, or suspected genotoxic mode of action. Instead of applying the default linear multistage model, a simple extrapolation will be used by drawing a straight line from the origin to the ED_{10} for cancer incidence (R. Hill, personal communication). However, to an even greater extent than for the multistage model, such a method will greatly exaggerate risk for such genotoxic carcinogens which have a very steep dose-response (e.g. for propylene oxide and formaldehyde) and where strongly promotive factors operate in the high dose range used in animal studies, but not at low exposures.

A more recent development is the Moolgavkar-Knudson model that is based on cellular dynamics and transformations incorporating time-to-tumor data (Moolgavkar and Knudson 1981). Although conceptually attractive, it is a very complex model where some of the in-put parameters are difficult to obtain.

Multiplicative models - Ehrenberg and co-workers (von Bahr *et al.* 1984, Ehrenberg 1991, Ehrenberg and Scalia-Tomba 1991, Ehrenberg and Tornqvist 1992) have used a multiplicative two-stage model that incorporates an initiation step, characterized by one-hit kinetics as well as a function describing promotion with a tolerance distribution, where the probability for tumor induction, P, is the product of the probability for initiation (P_{ini}) and the probability for promotion (P_{pro}):

$$(10)\quad P = P_{ini} \times P_{pro}\ (\times P_x)$$

P_x is a correction factor. Whereas in this model initiation follows a dose-response function with a linear component at low doses, unspecific promotion is expected to follow a tolerance distribution model based on a normal, or log normal Gaussian distribution - as has been demonstrated for a compound like tetradeconylphorbolacetate (TPA; Ewing *et al.* 1988). In eq. (11) a general form of this model is given

$$(11)\quad P(D) = (1 - \exp(-(\delta + \Gamma D))\ \phi\ (\alpha + \beta D)$$

Where D is the dose, ϕ the standard normal integrated distribution function, ß, and g are parameters to be adapted to the particular set of data analyzed, whereas at low doses α and δ represent the background values for promotion and initiation, respectively. A model similar to the one proposed by Ehrenberg *et al.* is applicable to radiation induced cancer in experimental animals (Storer *et al.* 1988), and is the type of model recommended by the U.S. National Research Council (1990) for low-dose extrapolation of radiation induced cancer in man.

There is reason to believe, that in view of the existing natural high background level of initiations, the promotive environment may, perhaps, be the most important factor in deciding the incidence of various kinds of cancers in humans (Doll and Peto 1981, Swedish Cancer Committee 1984). Many promotive effects appear to be characterized by a dose-threshold. However, most of the modeling carried out for low-dose extrapolation of promotive effects does not consider a situation, where a population is already subjected to such a high level of promotive stress, and where an additional promotive insult may result in a dose-response relationship that lacks a dose threshold. Such additivity presupposes a common mechanism of action, but its general role in human populations is at the present time difficult to quantify. The multiplicative model of Ehrenberg *et al.* will, in fact, accomodate such a situation. Thus, when a background promotion exists ($\alpha_o > 0$) for a particular site, a linear dependence of cancer incidence on dose will be obtained at doses approaching zero. However, when there is no background promotion ($\alpha_o = 0$), the dose-response curve will exhibit a no-effect threshold, at least in materials of limited size (e.g. for an animal bioassay).

von Bahr *et al.* (1984) have analyzed various sets of experimental data with respect to choice of extrapolation models. Only in the case of the "mega-mouse study" cited above (Littlefield *et al.* 1979) with 2-AAF and in a few other examples was it possible to select an appropriate model with a reasonable degree of confidence. However, it was

clearly shown that, depending on the situation, both tolerance distribution models as well as mechanistic models may be justified. Thus, in the case of 2-AAF, a good fit to the data for liver tumors was obtained with multistage models of the Armitage-Doll type. The Ehrenberg model gave an excellent fit to the liver tumor data for 2-AAF in the ED01 study utilizing 24,000 mice (Littlefield *et al.* 1979), especially for the 24 months sacrifice, but also gave a good fit with respect to the bladder tumor data. With respect to these tumors, out of 13 options, only the probit model as well as the Ehrenberg model showed a good fit (the Weibull model was not included). This probably reflected a different type of induction mechanism at this particular site, possibly related to a promotive action. Further, of the models studied, only the multiplicative model was found to give an adequate fit to data obtained from studies for induction of skin tumor after dermal application of B(a)P. For vinylchloride this model could be applied, as well as one of the models developed by Cornfield to fit a Michaelis-Menten type of kinetics. However, the Cornfield model, although providing a good fit to high doses, will not allow for a low dose linear term.

The adherence to one single model to cover all situations without taking due consideration to all relevant biological data (epigenetic/genotoxic action, promotive mechanisms, etc.), and sometimes even ignoring available data points in the high dose part of the dose-response curve when trying to obtain a fit to the model used (e.g. the successive exclusion of high dose data points in the application of the linearized multistage model), may have given mathematical modeling an undeservedly bad reputation among biologists. Although the data sets derived from conventional carcinogenicity studies in animals are limited, the intelligent use of mathematical procedures can, at least in some cases, lead to the rejection of a certain type of model for that particular situation, and thereby improve the confidence in low-level risk extrapolation. Of all models that so far have been tested the Ehrenberg model seems to combine biological credibility with a considerable flexibility in accomodating threshold as well as nonthreshold dose-response relationships.

PITFALLS IN THE DETERMINATION OF CARCINOGENIC POTENCY- MECHANISTIC CONSIDERATIONS

The following main type of errors are common in risk assessments carried out by government regulatory bodies: (a) Failure to consider the mechanism of action of a potential carcinogen and (b) inadequacies associated with assessing dose due to the metabolism of the compound, i.e. lack of adequate pharmacokinetic considerations. Below, some illustrative examples of questionable high-to-low-dose extrapolations are discussed.

Agents Interfering with DNA-Repair - The dose-response relationship for inactivation of a specific enzyme(s), existing in several identical copies in one and the same cell, is characterized by a definite dose-threshold. Thus, it seems questionable if stochastic models (linear models) for dose-response extrapolation should be used for carcinogens acting by interfering with enzymes or proteins involved in, e.g., DNA-repair. For this reason an assessment of the effects on the genetic material is of considerable importance for quantitative risk assessment of some carcinogens. However, among industrially important chemicals there seems to exist sufficiently good evidence which makes such a mechanism plausible only for *inorganic arsenic* as well as for lead, and possibly also for *nickel*.

Inorganic arsenic is an established human carcinogen, inducing skin cancers upon ingestion, and cancer of the lung upon long-term exposure by inhalation (IARC 1980, IPCS 1981). Whether arsenic ingestion induces cancer at other sites has been much debated, but there are certain indications pointing in this direction (Bates *et al.* 1992). Inorganic arsenic is unique in that it has not been possible to consistently induce cancer in an experimental animal.

Exposure to potentially toxic concentrations of inorganic arsenic is a world wide problem that is associated with occupational exposure, primarily in smelters, but in many areas also due to the presence of appreciable levels of inorganic arsenic in drinking water. For the general population being exposed to inorganic arsenic skin and lung cancers have been the main toxicological end point that has been the driving force for risk assessment. Since exposure to inorganic arsenic to some extent is unavoidable, regulatory agencies have been compelled to apply some kind of quantitative risk assessment basically using two different approaches: until now the **U.S.EPA** has been using extrapolation models with a linear component in the low dose region for cancer risk assessment. The 33rd Meeting of the Joint **FAO/WHO Expert Committee on Food Additives** in 1989, on the other hand, recommended as provisional maximum tolerable daily intake for inorganic arsenic of 2 µg/kg/day for an adult person based on the threshold concept (WHO 1990). This recommendation has been found acceptable by most countries. The U.S.EPA is currently considering to lower its current MCL (Maximum Contamination Level) for drinking water in the U.S. of 50 ug/L, which is identical with the WHO recommended value.

Results from bacterial mutation tests —like the Ames' test— have largely been negative. In some studies clastogenic effects as well as SCEs have been observed *in vitro* (For a review, consult IARC 1980, 1987b, de la Rosa *et al.* 1994). While arsenic compounds have not been shown cause point mutations, it has a co-mutagenic effect in bacteria and in mammalian cells with different types of chemical mutagens (Rossman *et al.* 1977, Lee et. al. 1986) as well as with UV (Rossman 1981, Lee *et al.* 1985, Li and Rossman, 1991). Rossman and co-workers have provided evidence that arsenic interferes with the later repair steps, such as DNA ligation (Rossman *et al.* 1977, Li and Rossman 1989a, 1989b). It is noteworthy, that defects in DNA ligase has been found in cells from patients with Bloom's syndrome (Willis and Lindahl 1987, Chan and Becker 1988). This recessive hereditary disease is characterized by a greatly increased spontaneous incidence of cancer.

However, arsenic may interfere with other steps involved in repair. Thus, Snyder (1994) recently reported, that arsenite inhibits the removal of UV-induced thymidine dimers from cellular DNA by excision repair. Interestingly, arsenite does not exert any enhancing effect of somatic recombinations induced by radiation and alkylating agents in Drosophila (de la Rosa *et al.* 1994). In this system arsenic has a clear inhibiting effect, possibly indicating interference with one or more enzymes involved in the somatic recombination process.

In one study mice were given 10 or 100 ppm sodium arsenite in their drinking-water for 8 weeks; some animals were given a single i.p. injection of 2 mg/kg bw tris(1-aziridinyl)phosphine oxide (TEPA). Arsenic treatment alone caused a slight increase in chromosomal aberrations in bone-marrow cells; the higher dose of arsenic potentiated the chromosome-damaging effect of TEPA. Administration of 100 mg/L sodium arsenite in drinking-water for 8 weeks also enhanced the occurrence of dominant lethals induced in male mice treated with 1 mg/kg TEPA; arsenic alone did not significantly increase the frequency (Sram 1976). This was interpreted by the investigator as an indirect evidence that arsenic interferes with DNA repair. Recently we have demonstrated that arsenic greatly increases the yield of chromosomal aberrations and sister chromatid exchanges induced by X-rays and UV in human fibroblasts and human peripheral lymphocytes in vitro (Jha *et al.* 1992). Similar effects have been obtained in human lymphocytes by a California laboratory using the DNA crosslinking agent diepoxybutane (Wiencke and Yager 1992).

Reports on the cytogenetic effects of arsenic in humans have been conflicting. Some studies claim that arsenic induces sister chromatid exchanges (SCEs) in humans in vivo (Burgdorf *et al.* 1977, Wen *et al.* 1982), whereas other have failed to confirm these observations (Nordenson *et al.* 1978, 1979). Increased frequencies of chromosomal aberrations have been found in arsenic exposed humans in Sweden, Hungary, and Bulgaria (Nordenson *et al.* 1978, 1979, Paldy *et al.* 1991, Nilsson *et al.* 1993), whereas Ostrosky-Wegman *et al.* (1991) failed to detect such an effect in exposed individuals from the Lagunera area in northern Mexico.

In summary, there is evidence that cancer induction by arsenic may be linked to disturbance of DNA repair. If the mechanism of cancer induction by arsenic is linked to interference with this type of processes, it might be possible to define a "safe" exposure level with respect to the carcinogenic effects of arsenic, providing support for WHO's current use of safety factors when deriving an acceptable daily intake (ADI) for inorganic arsenic. In view of these considerations the carcinogen potency factor of 1.75 per mg and kg body weight and day derived by the U.S.EPA should probably be adjusted upwards considerably.

Interaction between arsenic and other agents - Studies of smelter workers in U.S.A., Sweden, and Japan have convincingly demonstrated an association between occupational arsenic exposure and lung cancer mortality. Based on several studies U.S.EPA estimated (IRIS 1993) a potency factor via inhalation for arsenic of 15 (mg/kg and day)$^{-1}$, or 0.0013 (ug/m^3)$^{-1}$, assuming that 20 m^3 air is inhaled/day and 30% absorption of inhaled arsenic occurs in the lungs (IRIS 1993). In addition to the mechanistic considerations referred to above, this value may represent an overestimate for the following reasons. In the studies used by U.S.EPA for risk assessment differences in smoking habits between miners and the main population do not seem to have been adequately taken into account. This is of great importance in view of the strong synergistic effect that has been found between arsenic exposure and smoking, and which is illustrated in **Table III** (Pershagen *et al.* 1981). Another possible confounding factor is the interactive effects with promoters and other carcinogenic agents present in the occupational environment per se, like SO_2, radon, heavy metals, etc.

TABLE III. RELATIVE RISKS OF LUNG CANCER IN ARSENIC EXPOSED SMOKING AND NON-SMOKING SMELTER WORKERS (FROM PERSHAGEN *et al.* 1981)

	Relative risk	
exposed cohort	smokers	non-smokers
no exposure to arsenic	4.9	1.0
exposed to arsenic	14.6	3.0

Inorganic lead compounds - Several lead compounds have been found to induce benign and malignant tumors in the kidney of rodents following oral or parenteral administration. Lead is bound by a low molecular protein in the kidney tubules forming typical inclusion bodies representing a main sink for lead in this organ. The formation of such inclusion bodies is associated with an increased rate of mitoses that may eventually result in the production of neoplastic growth (Fowler *et al.* 1994).

Lead(2) compounds do not induce point mutations in bacterial systems or SCEs in mammalian cells; they do not induce direct DNA damage like strand breaks or DNA protein crosslinks. Like arsenic, lead(2) enhances the mutagenic effect of UV and increases the number of SCEs in V79 Chinese hamster cells at concentrations as low as 0.5 μM. Lead(2) also causes an accumulation of DNA strand breaks after UV irradiation, indicating an interference with the polymerization or ligation step in excision repair (Hartwig *et al.* 1994). Although less well investigated than arsenic in this respect, available evidence seem to indicate that lead(2) induces genotoxic effects by an indirect mechanism, and the induction of renal cancer in rodents is likely to have a dose threshold.

Compounds With Mainly Promoter-Like Action - In the previous article (Nilsson 1993), qualitative aspects of

several types of agents with a promoter-like action in target tissues like liver, bladder, thyroid, forestomach, etc. were discussed. Below, some quantitative aspects of dose extrapolations involving two other promoters of tumors in rodents are examined; trichloroethylene and chlorinated dibenzo-p-dioxins.

Trichloroethylene was selected by the **National Swedish Chemicals Inspectorate** as one candidate for its risk reduction program based *inter alia* on tumors induced in rodents. Using the linearized multistage model, U.S.EPA has determined a unit risk (q_1*) for this solvent by inhalation exposure of 0.011 per mg/kg and day on basis of the induction of mouse liver tumors (U.S.EPA 1985c). This is to be compared with a potency estimate for benzene of 0.029 per mg/kg and day based on extensive human data (IRIS 1993). Considering the large uncertainties involved, the carcinogenic potencies for the two solvents are according to these estimates similar. Although having a low potency, the carcinogenic action of benzene has been detected in limited cohorts of workers chronically exposed to levels in the range of 10-200 ppm (Ott *et al.* 1978, Rinsky *et al.* 1981). If the carcinogenic potency of trichloroethylene, a widely used dry cleaning solvent, were indeed similar to that of benzene, an increased cancer incidence would definitely have been detected in several well studied cohorts, in general involving much higher exposures (Axelsson *et al.* 1978, 1984, Tola *et al.* 1980). Although in some of the cohorts exposed to trichloroethylene increased incidences of certain types of tumors were found (IRIS 1993), there is a lack of consistency between the various epidemiological studies with respect to tumor site. A recent follow-up study by Axelsson's group has provided additional support for the lack of appreciable carcinogenicity of trichloroethylene in humans (Axelsson *et al.* 1994).

As for most other substances it is, of course, difficult to exclude that trichloroethylene possesses a very low carcinogenic potential. However, it can safely be stated, that the cancer potency factor derived by the U.S.EPA from the rodent studies represents a considerable overestimation of human risk. The application of the linearized multistage model for the mouse liver tumors is also questionable; trichloroethylene has a negligible genotoxic activity and the liver tumors in mice are evidently induced by the metabolic production of the peroxisome proliferator, trichloroacetic acid (Elcombe *et al.* 1985). Further, trichloroethylene has been shown to act as a tumor promoter in the mouse (Randall and Sipes 1984). Another intermediate in the metabolism of trichloroethylene is chloral hydrate which has been widely used as a hypnotic and a sedative. The slight increase in kidney tumors, that were reported in male rats after high exposures, was accompanied by overt signs of nephrotoxicity and only apparent around the MTD (NTP 1988, 1990), and the relevance of the animal data for man has been questioned (Steinberg and DeSesso, 1993).

2,3,7,8-tetrachlorodibenzo-p-dioxin - Few chemical compounds have been investigated in such detail as have the polychlorinated dibenzo-p-dioxins (PCDDs), in particular 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and related compounds, and the subject has been extensively reviewed (Nordic Council of Ministers 1988, U.S.EPA 1988b, 1988c, IPCS 1989). The carcinogenic properties of these agents have been the focus of much controversy, and opinions on how to assess the potency of these substances for the purpose of quantitative risk assessment have differed widely —not only between countries— but also between regulatory bodies within the same country. This lack of consensus to a large extent reflects basic differences in opinion as to the mechanism of action of PCDDs. However, the **U.S.EPA** seems to be the only regulatory agency in the world which uses a non-threshold extrapolation model in this context for risk assessment purposes.

Several studies indicate, that chronic administration of low levels of 2,3,7,8-TCDD to rodents is associated with an increased tumor incidence. Thus, in the chronic study by Kociba *et al.* (1978) feeding with 0.01 mg/kg and day induced an increased incidence of hepatic hyperplastic nodules in female rats, and at 0.1 mg/kg and day a sta-tistically significant increased incidence in females of hepatic carcinomas, nodules, and lung carcinomas were seen; squamous-cell carcinomas of the nasalturbinates and of the tongue were found in both sexes, as well as of the hard palate in males. Interestingly, a dose dependent, highly significant decrease in the incidence of tumors of the mammary glands, pituitary, and uterus was also noted. As to the tumors of the lung, hard palate, and nasal tur-binates observed in the Kociba study, it has been pointed out (Nordic Council of Ministers 1988) that these tumors probably were the result of a localized carcinogenic response to inhaled particles containing 2,3,7,8-TCDD that has not been observed in investigations using other routes of exposure. In the study conducted by the National Toxicology Program (NTP 1982) in rats, where dioxin was administered by gavage, no tumors were found in the respiratory tract, and the only treatment-related neoplasias were hepatic nodules and carcinomas of the females, and possibly follicular cell adenomas or carcinomas of the thyroid.

In the NTP (1982) study involving mice, males were exposed to 0.0014, 0.007, or 0.07 μg/kg and day while females were given 0.006, 0.03, or 0.3 μg/kg and day. At the highest doses an increased incidence of liver tumors was found in animals of both sexes as well as of follicular-cell adenomas of the thyroid in females. The onset of tumors occurred late in the study and generally at similar times as for control groups. Application of 2,3,7,8-TCDD to the skin of mice was found to be associated with fibrosarcomas in females. The tumors were preceded by inflammation and necrosis of the underlying subcutaneous tissue (NTP 1982).

The preponderance of evidence so far accumulated suggests that PCDDs lack significant genotoxic properties (IPCS 1989). However, 2,3,7,8-TCDD as well as other PCDDs are extremely potent tumor promoters (Pitot *et al.* 1980). An important clue when identifying a "pure" promoter is the reversibility of clonal expansion after removal of the promoting agent. In the case of PCDDs such an experiment would, unfortunately, not be meaningful in view of

the long half-life of these substances in the tissues. The fact that the incidence of liver carcinoma is increased in female but not in male rats (Kociba *et al.* 1978, NTP 1982) as well as the observation, that ovariectomy protected against the tumor promoting action of 2,3,7,8-TCDD (Lucier 1992), clearly, reflect the importance of TCDD-estrogen interaction in the carcinogenic action of this compound.

Above all, it is the proven powerful promotive action of 2,3,7,8-TCDD (plus the interaction with estrogens mentioned above), seen together with the inability to cause reproducible genotoxic effects in mammalian systems, that have convinced many scientists that dioxins should be regarded as tumor promoters rather than complete carcinogens. The extreme variations in sensitivity, not only between species, but also between strains of the same species has repeatedly been pointed out as another anomality (IPCS 1989). Thus, whereas the LD_{50} is about 1 mg/kg for the guinea pig, the corresponding value for hamsters is 5,000 mg/kg. Further, a variation in sensitivity *between various strains* of rats of more than 200 has been found (IPCS 1989).

Poland and co-workers (1976) have shown that TCDD binds to a specific receptor with a very high affinity, the Ah receptor, located in the cytosol and/or nucleus of most tissues. Many chemicals that bind to the Ah receptor —like other PCDDs the polychlorinated dibenzofurans (PCDFs) and some PCB congeners— produce the same spectrum of effects as TCDD which is mostly in proportion to their affinity to this receptor (for a review, consult Lucier 1992). In order to exert its effect, the TCDD-Ah complex requires activation in order to bind to dioxin-responsive regions of the DNA. One of the effects of binding to these regions is the transcriptional activation of the genes (CYP1A1 and CYP1A2) for production of cytochromes P450c (P_1-450) and P450d which are involved in the oxidative metabolism of various types of compounds.

In addition to induction of mixed function oxidation, UDP glucuronyltransferase and other enzymes, the binding to the Ah receptor is linked to the expression and functioning of a number of endocrine factors that influence cell differentiation and proliferation like the epidermal growth factor (EGF) receptor, estrogen receptor, tumor necrosis factor a, and gastrin. It is interesting to note, that whereas the dose-response relationship for induction of P450c does not correlate well with the induction of preneoplastic foci in the rodent liver, a good correlation has been reported with effects on the EGF and estrogen receptors.

In addition, a number of toxic responses like immuno-toxicity, teratogenicity, neurotoxicity, and gastrointestinal lesions seem to be related to the binding to the Ah receptor. Thus, mouse strains with a defective receptor are much less responsive to the toxic effects of TCDD. However, the binding affinity to the Ah cannot alone explain the various aspects of the toxicity of TCDD. Thus, although the guinea pig is about 5,000 times more sensitive to the acute toxic effects of TCDD, the amount and binding affinity of the Ah receptor is about the same in the two species (Gasiewicz and Rucci 1984). Further, in the promotion of skin cancer in the mouse by TCDD, besides the Ah locus, a second distinct locus, the hr locus seems to be involved (Poland *et al.* 1982).

Many epidemiological studies - case control studies as well as cohort studies - have assessed the possible association between cancer and exposure to various chemical products (chlorophenoxy herbicides, chlorophenols) containing PCDDs as impurities have been equivocal. There is a likelihood that confounding factors have played a significant role in the studies showing an association between increased incidence of neoplasia and exposure. Several episodes of significant exposure of humans to 2,3,7,8-TCDD and to other PCDFs have been recorded. With respect to these findings IPCS (1989) notes, that "In spite of many clinical and follow-up studies, no clear cut persistent systemic effects have been delineated, except for chloracne."

On basis of available evidence **U.S.EPA, IARC,** as well as most government regulatory agencies have concluded that there is sufficient evidence of carcinogenicity for 2,3,7,8-TCDD in experimental animals. On the other hand, neither U.S.EPA nor IARC consider that the evidence linking 2,3,7,8-TCDD to neoplastic disease in humans is adequate (IARC 1987a, U.S.EPA 1988b, 1988c). Using the linearized multistage model, U.S.EPA determined on basis of available rodent bioassays a 0.006 pg/kg and day dose to represent an upper-bound confidence limit for a risk of one in a million (U.S.EPA 1985b). This estimate was widely criticized and has subsequently been reconsidered and revised by the Agency (U.S.EPA 1988b, 1988c). In **Table IV** risk specific doses (doses corresponding to a life-time risk of one in a million) and reference doses calculated by different regulatory organizations, scientific organizations as well as by individual scientists are given.

U.S.EPA has acknowledged, that the use of the linearized multistage model would not be appropriate if a PCDD, like 2,3,7,8-TCDD, acts as a promoter. Further, this Agency recognizes that promotion, or indirect effects, can be regarded as plausible mechanisms of action. On the other hand, U.S.EPA has pointed out that it is difficult on basis of available evidence to rule out the possibility that 2,3,7,8-TCDD possesses some degree of initiating capacity at low doses. The same can, of course, be said for any agent that induces tumors in an experimental animal. While IPCS (1989) and regulatory agencies in most nations regard 2,3,7,8-TCDD and other PCDDs as epigenetic carcinogens and/or tumor promoters with a definite action threshold, U.S.EPA has chosen to retain the linearized multistage model, while adjusting the estimated dose required to give a life-time excess cancer incidence of one in a million (risk specific dose) upwards to a value for 2,3,7,8-TCDD of 0.1 pg/kg and day.

PITFALLS IN THE DETERMINATION OF CARCINOGENIC POTENCY- PHARMACOKINETIC CONSIDERATIONS

Inorganic Arsenic Compounds - U.S.EPA's initial evaluation of cancer risk for inorganic arsenic was based on a study

TABLE IV. RISK SPECIFIC DOSES OBTAINED BY VARIOUS REGULATORY APPROACHES

Origin	Dose (pg/kg/day)	Approach
U.S.EPA 1985	0.006	no threshold
U.S.EPA 1988	0.1	no threshold
California,Toxic Air Program	0.007	no threshold
California	0.08	no threshold
U.S.FDA	13	threshold (UF = 77)
New York State	2.0	threshold (UF = 200)
West Germany	10.0	threshold (UF = 1000)
Canada*	10.0	threshold (UF = 100)
Netherlands	4.0	threshold (UF = 250)
Sweden, Norway, Denmark, Finland	5.0	threshold (UF = 200)

UF = uncertainty factor (From U.S.EPA 1988b, 1988c and Nordic Council of Ministers 1988, Zeise *et al.* 1990)

* Feeley and Grant (1993)

by Tseng *et al.* (1977), who reported increased prevalence of skin cancers in humans as a consequence of arsenic exposure from drinking water in Taiwan. Using standard U.S.EPA procedures, a carcinogenic potency factor of 15 (mg/kg and day)-1 was determined (U.S.EPA 1984), corresponding to a risk of 0.004 for a lifetime exposure to 10 µg arsenic/L in drinking water. Levels, of a similar order of magnitude are not uncommonly found in potable water in many areas of the world. Based on the compliance monitoring data available through the Federal Reporting Data System (FRDS), it has been estimated, that more than 100,000 people in the U.S. are receiving drinking water from public water supplies with arsenic levels above 50 µg/L, the current Maximum Contamination Goal Level (MCGL) in the U.S. (U.S.EPA 1986b). Although the background incidence of skin cancer of different types is difficult to establish (except for melanoma) due to under-reporting, the expected increase in skin cancer in the U.S. predicted by this risk estimate as caused by arsenic alone would hardly seem credible.

The U.S.EPA Risk Assessment Forum (U.S.EPA 1986b, 1987) has, subsequently, completed a reassessment of the carcinogenic risk associated with ingestion of inorganic arsenic and a unit risk of 1.75 per mg and kg body weight was proposed, which is a magnitude lower than the previous estimate.

This last mentioned potency estimate is probably also inflated (U.S.EPA 1988a, Boyce *et al.* 1992), in part because the amount of arsenic ingested by the Taiwanese population in question has been underestimated (solely based on drinking water consumption), in part because of pharmacokinetic and mechanistic considerations indicating a possible dose threshold. The high levels of arsenic in the artesian water on Taiwan reflect high arsenic levels in the bedrock, and with all probability also indicate the presence of elevated levels in the soils of the area. In view of the efficient uptake of arsenic in plants, the agricultural population living in the affected areas certainly has had a significant additional intake of arsenic from locally grown food. The U.S.EPA (1987) further noted that "arsenic-contaminated water was used for vegetable growing and fish farming". As mentioned before, on basis of mechanism of action it is also questionable whether linear extrapolation should at all be used for inorganic arsenic (de la Rosa *et al.* 1994).

To some extent mammals have the ability to adapt to the acute toxicity of inorganic arsenic. Among the "arsenic eaters" in Styria (Steiermark), Austria, the daily dose of arsenic could reach a level of 300-400 mg, which is 3-4 times the lethal dose under normal conditions (Lewin 1929). The mechanism of this adaptation is not well understood, but probably reflects an induction of the enzymes capable of methylating inorganic arsenic.

In mammals, most of the ingested inorganic arsenic is eliminated at a high rate mainly via the kidneys. No data are available which indicate long-term accumulation of arsenic in soft tissues. However, high levels of arsenic are maintained for longer periods of time in bone, hair and nails of exposed individuals. In rodents retention of arsenic has also been demonstrated in the squamous epithelium of the upper gastrointestinal tract, the epididymis, thyroid and lens (Lindgren *et al.* 1982). The long-term retention of arsenic in the erythrocytes of the rat seems to be peculiar for this species (Marafante *et al.* 1982). This pronounced accumulation as well as a considerable biliary excretion constitute features that makes this species a very poor model for humans in this context.

The *in vivo* methylation of inorganic arsenic, mainly occurring in the liver (Buchet *et al.* 1982), has been demonstrated in both animals and man (For a review, consult Vahter and Marafante 1988). Before methylation can take place, pentavalent arsenic must be reduced to trivalent arsenic (Vahter and Envall 1983). S-adenosyl methionine appears to be the methyl donor in these reactions catalyzed by methyltransferases (Vahter 1983, Marafante and Vahter 1984). In rabbits choline, methionine, or protein deficient diets will result in decreased methylation (Marafante and Vahter 1986, Vahter and Marafante 1987) and, presumably, result in an increase in the body burden of inorganic arsenic. Buchet and Lauwerys (1987) have also obtained evidence in rats that reduced hepatic glutathione levels is also associated with a depression of arsenic methylation. Following ingestion, or inhalation of inorganic arsenic, the major

forms of arsenic excreted in human urine are the methylated products dimethylarsinic acid (DMA) (60-80%) and monomethylarsonic acid (MMA) (10-20%) accounting for 80-90% of the excreted arsenic, the remaining fraction (10-20%) being in the form of inorganic arsenic (Smith *et al.* 1977, Tam *et al.* 1979, Buchet *et al.* 1982, Vahter 1983, 1986, Vahter and Lind 1986). Rodents differ from man in that very low levels of monomethylarsonic acid is excreted (Vahter 1983, Vahter and Marafante 1988). Consumers of marine fish, shellfish, and crustaceans, in addition, excrete significant quantities of arsenobetaine and related compounds (Vahter and Lind 1986). A fraction of the ingested inorganic arsenic is also excreted with the bile but is reabsorbed in the gut (Vahter 1983).

Under low-level exposures to arsenic, there seems to exist a balance between the amount entering the body and the amount being excreted. However, of considerable toxicological significance is the fact, that methylation efficiency decreases with increasing dose levels. In rodents (Vahter 1983) this decrease is apparent only at very high oral intakes (> 1 mg/kg bw), whereas the capacity of the human body to handle arsenic may be reduced at considerably lower exposures (Valentine *et al.* 1979, Buchet *et al.* 1981a,b). However, the dose at which this "metabolic overloading" occurs has not been determined with any precision. Also, inter-individual differences, as well as the possible role of adaptive responses in populations that have been exposed to arsenic for long time periods, are insufficiently documented. Surprisingly, no studies seem to have been conducted with respect to the metabolism of arsenic in children. In view of the long latency periods for induction of skin cancer this may be extremely important from the risk assessment point of view.

The fraction non-metabolized arsenic in the urine may not be the only relevant pharmacokinetic parameter for risk assessment of skin cancer. The most appropriate descriptor of "effective" dose would seem to be the concentration of arsenic in the target tissue, the skin. There is experimental evidence in mice that the proportion of inorganic arsenic that is retained in the organism increases with increasing dose (Vahter and Norin 1980). This could conceivably result in a non-linear relation between ingested dose and target dose.

No satisfactory animal model for the assessment of the pharmacokinetics of arsenic in man has been found. Thus, in comparison to man, rodents and dogs methylate inorganic arsenic much more effectively, and negligible quantities of monomethylarsonic acid appear in the urine of mice, rats, rabbits, or dogs (Vahter and Marafante 1988). Protein and morphological similarities corroborate the phylogenetic proximity of man and non-human primates. In a great number of aspects primates constitute the most appropriate animal models in physiological and toxicological research. Although the primitive New World marmoset monkey (Callithrix jacchus) is unique in so far as it does not methylate inorganic arsenic (Vahter *et al.* 1982). This seems also to be true for the chimpanzee (Vahter *et al.* 1994).

Tumors Induced by Chromium Compounds - According to **IARC** (1980) there is sufficient evidence for carcinogenicity of *calcium chromate* and some relatively insoluble chromium(6) compounds *(sintered calcium chromate, lead chromate, strontium chromate, sintered chromium trioxide, and zinc chromate)* in rats and limited evidence for the carcinogenicity of *lead chromate* (6)oxide and *cobaltchromium alloy* in rats. The data were found to be inadequate to evaluate the carcinogenicity of other chromium(6) compounds and chromium(3) compounds. As to carcinogenicity in man, IARC found sufficient evidence for an association between respiratory cancer and exposure during chromate production; "The epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic chromium, chromium(3) and chromium(6) or of soluble versus insoluble chromium compounds".

Chromium(6) compounds are weak experimental carcinogens, and induction of tumors has only been consistently achieved with sparingly soluble chromates by employing special techniques, like intramuscular implantation, subcutaneous injection, and bronchial and intrapleural implantation (IARC 1980). In the relevant IARC (1980) monograph, where little attention was given to the important questions related to chromium speciation and pharmacokinetics, about 2% of administered chromate is said to be taken up from the gastrointestinal tract. However, this claim is based on older studies where the oxidation state of the absorbed chromium has not been verified. Recent investigations have demonstrated that, in contrast to chromium(6), chromium(3) is not taken up by mammalian cells. Further, that hexavalent chromium, which is a strong oxidizing agent, is quickly reduced to the trivalent ion in biological systems (Cohen *et al.* 1993) to a large part already in the intestinal tract (De Flora 1994). A similar reduction is mediated by alveolar macrophages and in the mucous layer of the epithelial cells of the lung. This finding is in accordance with the lack of evidence of carcinogenic activity of hexavalent chromium by oral administration. U.S.EPA has derived a carcinogenic potency factor for chromium(6) by inhalation of 41 per mg/kg and day using linear extrapolation (IRIS 1993). In view of the efficient reduction of hexavalent chromium in vivo, the validity of this risk estimate for low exposures is highly questionable.

Although Norseth (1986) seems to believe that most chromium compounds are carcinogenic, this view is not shared by many other scientists in the area (Petrilli and De Flora 1986, Levy *et al.* 1987). A key factor is decidedly the intracellular biological availability of chromium(6). Unless under conditions of overloading, soluble chromium(6) compounds are readily reduced extracellularly, whereas less soluble particulates —similarly to crystal-line nickel sulfide (Costa 1989)— may enter the cell by phagocytosis and reach the nucleus causing DNA damage.

The classification of chromium compounds by various national regulatory agencies have been inconsistent. Thus, whereas the **EEC** to a large extent has followed the IARC recommendations, **Sweden** has classified all chromates and dichromates as carcinogens.

Interspecies Extrapolations - Determination of Human Equivalent Dose - When deriving carcinogenic potency estimates the U.S.EPA utilizes a surface area correction factor for animal-to-man extrapolation to compute human equivalent dose, instead of conducting extrapolation based on dose per unit weight. The rationale underlying the use of the body surface based extrapolation is the well-known empirical observation, that metabolic rate shows a better correlation with surface area than with body weight (Adolf 1949), and the assumption that a similar relationship also holds for various toxic effects (Freireich *et al.* 1966, Davidson *et al.* 1986). The use of surface area correction increases the risk estimates for man based on experiments with mice by a factor of about 13 and based on data from rats by a factor of around 6-7. However, it is well known, that the surface based extrapolation model does not hold for some chemical substances (Krasovskii 1976). Unfortunately, adequate data are rarely available for the majority of potential carcinogens for more reliable estimates.

Conventional representations of exposure, e.g. mg/kg and day, ppm, etc., are inherently imprecise when defining dose-response relations for carcinogenic effects. The formation of reaction products (adducts) to DNA by direct acting electrophilic agents, like epoxides, is thought to be closely linked to the process of cancer initiation. The dose to DNA in target tissues, therefore, constitutes the most relevant measure of dose to be used in estimation of carcinogenic potency, and will to a greater extent reflect inter- and intra-species differences than is the case for conventional representations of exposure. Largely due to the pioneering research by the Swedish scientist Lars Ehrenberg and his co-workers, the target dose concept has been successfully applied to the risk assessment of some genotoxic chemicals, and analytical methods have been developed for this purpose (Ehrenberg *et al.* 1974, 1983, Törnqvist *et al.* 1986, Wright *et al.* 1988). The target dose, D_{target}, is defined as the time integral of the concentration, C_{target}, of an electrophilic (e.g., alkylating) agent in the environment of the DNA of target cells:

$$(13)\quad D = \int C(t)\,dt$$

The degree of alkylation, a_y, of nucleophilic sites, Y, in cellular macromolecules is directly proportional to dose according to the relationship:

$$(14)\quad a_y = k_y \times D$$

Where k_y is the second order rate constant for reaction of the electrophile with Y. Equations (13) and (14) are valid if the concentration of reaction products formed are low, and if the monitored nucleophile (Y) and the adducts are stable during the time of study. Thus, doses of electrophilic compounds/intermediates can be determined in single dose experiments in experimental animals by quantification of adducts to DNA or to proteins in various tissues.

For the simple epoxides ethylene oxide and propylene oxide the species differences in tissue dose in different species —as estimated by alkylation of nucleophilic centers in target organs per unit exposure— are much smaller than for a compound like benzo(a)pyrene which has to be metabolized to the proximate carcinogen. The rate of enzyme catalyzed elimination of the epoxides from tissues would here appear to be the key parameter in animal-to-man extrapolation. For ethylene oxide it has also been demonstrated that the target dose in man and experimental animals are very similar (Ehrenberg *et al.* 1974, OstermanGolkar *et al.* 1976, Calleman *et al.* 1978, Osterman-Golkar *et al.* 1983, Osterman-Golkar and Bergmark 1988, Duus *et al.* 1989), and we have recently shown that this is also true for propylene oxide (Nilsson *et al.* 1991, Segerbäck *et al.* 1992, 1994). For ethylene oxide as well as for propylene oxide the unit risk value derived by the U.S.EPA (U.S.EPA 1985a, IRIS 1993) should, thus, be adjusted upwards by approximately an order of magnitude.

POSSIBLE FUTURE DEVELOPMENTS

Projected life-time risks are conventionally obtained by multiplying the carcinogen potency factor at low doses with the dose, times the number of people exposed for a certain number of years. The question should be raised, however, whether this approach will by itself be sufficient to provide an adequate measure of risk without taking competing risk factors associated with aging into account. In practice, it certainly makes a lot of difference if the majority of tumors is expected to occur at a relatively early age, compared with a situation where competing causes of death will, in practice, drastically reduce the likelihood of that a certain chemically induced tumor will ever be observed. Further, experience from radiation biology has clearly demonstrated that the potency factor varies with the age of the exposed individual. In other words, the projected incidence **together with** the expected life-time shortening in case a tumor arises gives a more meaningful parameter in human risk assessment than solely providing a potency factor. More research should go into finding appropriate ways to incorporate such time-to-tumor data in the traditional derivation of estimates of unit risk. This would require a better insight in the difficult problem of comparing age-related effects and life-time dose versus daily dose in experimental animals and in man.

CONCLUSIONS

It is a fact, supported by epidemiological as well as experimental data, that the efficiency of various carcinogens to induce tumors vary enormously. For this reason the determination of carcinogenic potency of different carcinogens by high-to-low dose, as well as by interspecies extrapolation is a basic prerequisite for cancer risk assessment. Although

the data sets derived from conventional carcinogenicity studies in animals are limited, the intelligent use of mathematical procedures can, at least in some cases, lead to the rejection of a certain type of model for that particular situation, and thereby improve the confidence in low-level risk extrapolation.

When selecting adequate models for such extrapolations, due consideration should be given to mechanism of action as well as the pharmacokinetics of the agent in question. For this reason, determination of carcinogen potency must be carried out on a case-by-case basis. The multiplicative model used for radiation induced cancer, and which has been adapted to chemical carcinogens by Ehrenberg et al., seems to combine biological credibility with great flexibility to accomodate threshold as well as non-threshold dose-response relationships. By measuring target dose, e.g. in terms of protein or DNA adducts, a more relevant measure of dose may be obtained for the estimation of carcinogenic potency than conventional representations of exposure. Further efforts should be made to investigate quantitative aspects of tumor promotion, as well as obtain adequate tools to compare age related effects and life-time dose versus daily dose in experimental animals and in man.

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