Recommendation from the Scientific Committee on Occupational Exposure Limits: Risk Assessment for Vinyl Chloride

8 hour TWA	:	-
STEL (15 mins)	:	-
Additional classification	:	-

Assessed risk of hepatic angiosarcoma, upon exposure for working lifetime:

Vinyl chloride exposure (ppm)	Angiosarcoma risk
1	3×10^{-4}
2	6×10^{-4}
3	9×10^{-4}

Substance identity and properties:

Vinyl chloride	C_2H_3Cl
Classification:	Carc. Cat. 1; R45
Synonyms:	Chloroethylene
Chemical name:	Chloroethene
Structural formula:	$CH_2 = CH - Cl$
CAS number:	75 - 01 - 4
EINECS number:	602 - 023 – 00 - 7
Molecular weight:	62.5
Melting point:	-159.7°C
Boiling point:	-13.8°C
Vapour pressure:	3 333 mbar at 20°C
Conversion factors:	1 ppm = 2.59 mg/m^3
(20°C, 101.3kPa)	1 mg/m^3 = 0.385 ppm

Occurrence and use:

Vinyl chloride (VC) is a high-volume industrial chemical that is used as a monomer in the production of polyvinyl chloride (PVC). It is of very low acute toxicity, characterised by prenarcotic and narcotic effects at airborne concentrations of 1% (10 000 ppm) and higher. Following recognition of its carcinogenicity in humans, strict regulations have been applied in all industrialised countries.

Low levels of vinyl chloride, arising from degradation of chloroethylene solvents (trichloroethylene, perchloroethylene), are of environmental concern at polluted sites (Kielhorn *et al.* 2000). Close to landfills and areas contaminated with chlorinated hydrocarbons, this dechlorination process may lead to significant levels of VC in ambient air, leachate and groundwater. Low levels of VC have also been identified in tobacco smoke (cigarettes and small cigars). For an extended discussion of these aspects, see IPCS-WHO (1999).

General background of carcinogenicity of vinyl chloride

The toxicology and carcinogenicity of VC have been widely studied during the last 25 years, and a number of assessments of carcinogenic risk have been carried out, based on both occupational epidemiology and animal experimental data. Vinyl chloride is classified as a human carcinogen by the European Union (EU) (category l) and the International Agency for Research on Cancer (IARC) (group l). The established carcinogenicity is the preponderant toxicological effect of VC.

The SCOEL has therefore been asked to provide a human carcinogenicity risk assessment as background for possible further regulatory action by the EU.

As in the case of other chemical carcinogens, the assessments of risks concerning the environmental exposure and exposures in the occupational environments follow lines that are often considerably different. A number of available carcinogenic risk evaluations for the general (not occupationally exposed) population are based on epidemiological studies on workers (as, for instance, in the case of WHO Air Quality Guidelines, 1987, 1999 and 2000). Recent risk evaluations also include the consideration of toxicokinetics in experimental animals and humans, by using experimental data and Physiologically-Based Pharmacokinetic Models (PBPK models), in order to account for interspecies differences in physiology and VC metabolism.

Toxicokinetics and toxicodynamics of VC

The main metabolic route of VC, after inhalation or ingestion, is by oxidation via cytochrome P-450 (CYP2E1), which leads to the formation of chloroethylene oxide. This is a highly reactive epoxide which rapidly rearranges to chloroacetaldehyde (Bolt 1978, Bartsch *et al.* 1979, Bolt *et al.* 1981). The detoxification of these two reactive metabolites is through conjugation with glutathione, catalysed by glutathione S-transferase(s).

Chloroethylene oxide and chloroacetaldehyde react with nucleic acid bases, forming DNA adducts. The *in vivo* formation of four etheno-DNA adducts has been experimentally demonstrated. These appear highly persistent and can cause defective transcription (miscoding). Targets for alkylation in nucleic acids are adenine, guanine and cytosine moieties. Biologically relevant "etheno" adducts, e.g. 1,N^6-etheno-deoxyadenosine and 3,N^4-etheno-deoxycytidine, are easily formed *in vitro* on incubation of DNA with vinyl chloride and rat liver microsomes (Laib and Bolt 1980). The metabolism of VC is a dose-dependent, saturable process (Bolt and Filser 1983). Inhalation toxicokinetics of vinyl chloride and vinyl bromide show linearity within a lower dose range, but saturation of metabolism occurs at inhalation concentrations of about 500 ppm (Gehring *et al.* 1978, Bolt and Filser 1983). This metabolic saturation leads to non-linearity of tumour response with inhalation concentration at high doses of vinyl chloride in inhalation bioassays (Gehring *et al.* 1978, 1979).

Studies of VC metabolism and the interpretation of carcinogenic dose-response in experimental animals represent one of the first integrations of metabolic data and associated criteria into carcinogenic risk assessment (Gehring *et al.* 1978, Bolt *et al.* 1980). A first attempt at extrapolating human cancer risks due to vinyl chloride from experimental animals to humans has been published by Gehring *et al.* (1979). However, at that time the authors did not yet incorporate the metabolic differences between the species. In fact, humans metabolise vinyl chloride more slowly than do rats, when expressed relative to body weight (Buchter *et al.* 1978). The use of the "physiologically-based pharmacokinetic" (PBPK) models to consider metabolic data is now the main tool for this purpose. VC is a typical example of an industrial chemical for which the risk assessment requires such an approach (Clewell *et al.*, 1995; Reitz *et al.*, 1996, IPCS-WHO, 1999).

Selection of the type of low-dose extrapolation

Although vinyl chloride is an established cause of human liver angiosarcoma, the evidence in humans is inconclusive as to whether it also causes other neoplastic and non-neoplastic chronic liver diseases as well as neoplasms in other (extrahepatic) organs. Furthermore, the shape of the dose-response curve for angiosarcoma is uncertain. For instance, in a very recent study Ward *et al.* (2001) extended for approximately 8 years the mortality and cancer incidence follow-up data of 12 700 male workers in the vinyl chloride industry in four European countries. A total of 53 deaths from primary liver cancer (standardised mortality ratio 2.40, 95% confidence interval = 1.80-3.14) and 18 incident cases of liver cancer were identified, including 37 angiosarcomas, 10 hepatocellular carcinomas and 24 liver cancers of other and unknown histology. In Poisson regression analyses a marked exposure response for all liver cancers, angiosarcoma and hepatocellular carcinoma was observed. The exposure response for liver cancer in analyses restricted to cohort members with cumulative exposures of <1 500 parts per million/year was close to that estimated for the full cohort (relative risk of 2.0 per logarithmic unit of cumulative dose). No strong relation was observed between cumulative vinyl chloride exposure and other cancers (Ward *et al.* 2001). From this and other epidemiological studies, no clear statement as to the shape of the dose-tumour response curve for humans can be inferred.

From animal experimental data (long-term bioassays in rats) effective doses of the reactive metabolite(s) were calculated using the PBPK model of Clewell *et al.* (1995).

The initial VC metabolism was hypothesised to occur via two saturable pathways, one representing low-capacity, high-affinity oxidation by cytochrome P450 CYP2E1 and the other representing higher-capacity, lower-affinity oxidation by other isozymes of cytochrome P450. This calculation showed that the model was linear up to nearly 100 mg/m^3, and the calculated equivalence factor was later used to convert the risk from the inhalation experiments in animals (in dosimetric units) to human risk values.

Another line of research investigated the biological response of rat liver to low vinyl chloride doses. Investigations of cancer prestages, as biomarkers, may provide a tool to study dose-response relationships, down to ranges where the carcinogenic risk caused by the substance can no longer be distinguished from non-exposed controls in long-term cancer bioassays.

Such a parameter is the development of pre-neoplastic enzyme-altered foci in the liver. Adenosine triphosphatase (ATPase)-deficient foci were quantitated by Laib *et al.* (1985) as an endpoint of VC effect in the 'rat liver foci bioassay'. The quantity of foci area follows dose-time response relationships identical to those observed for the induction of liver tumours (Kunz *et al.* 1983). In order to study dose-dependence, hepatocellular ATPase-deficient foci were evaluated after subchronic exposure of newborn rats to VC. Wistar rats were exposed from day 1 after birth over 10 weeks to 10, 40, 70, 150, 500 and 2 000 ppm VC (8 h/day; 5 days/week). One week after cessation of exposure hepatic ATPase-deficient foci were quantitated. In a subsequent investigation at a lower dose range, groups of female and male Wistar and Sprague-Dawley rats were exposed (8 h/day; 5 days/week) to 2.5, 5, 10, 20, 40 and 80 ppm VC. Exposure started at day 3 of life and lasted for 3 weeks. After cessation of exposure the animals were maintained for 10 weeks without further treatment until ATPase-deficient foci were quantitated. Below the range of saturation of metabolism of VC (i.e. below 500 ppm exposure), both sets of experiments revealed a straight linear relationship between the dose of VC and the % foci area (related to liver area) which was induced. Within the dose range investigated, i.e. down to 2.5 ppm airborne exposure, the dose-dependence was linear, and no obvious threshold for the induction of pre-neoplastic foci by VC was apparent (Laib *et al.* 1985). This shows that, down to low doses of VC, no 'threshold' dose exists. Investigations on covalent binding of (^{14}C)-VC to cellular macromolecules support this view, and the linearity of the dose-response curves disproves the hypothesis of a 'threshold' for the carcinogenic action of VC in rats (Schumann *et al.* 1982).

It has been stated that vinyl chloride cancer risk assessments based on animal studies tend to overestimate the human risk of angiosarcoma of the liver (Kielhorn *et al.* 2000). However, in the absence of clear data on the quantitative nature of carcinogenic dose-response of VC in humans, and considering the above-mentioned experimental data, it appears prudent to the SCOEL to consider a linear (non-threshold) dose-response for the assessment of human cancer risks associated with vinyl chloride exposures.

Available cancerogenic risk evaluations of VC

Epidemiologically-based risk estimations (low-dose linear extrapolation)

(a) WHO (1987,1999, 2000): The World Health Organisation has reconfirmed an earlier assessment of 1987. Angiosarcomas of the liver were considered, together with other tumours. The estimate was based on epidemiological data and on relative risks related to working environments. A "Unit Risk" for lifetime exposure to 1 µg/m^3 VC was assessed as 1 x 10^{-6} per l µg/m^3, compatible with a risk of 2.59 x 10^{-3} for 1 ppm lifetime exposure.

Assuming an overall working time corresponding to 14% of lifetime (see *Appendix*), the risk related to working time exposure would be 0.36 x 10^{-3} for 1 ppm VC.

(b) Clewell *et al.* (1995): These authors used epidemiologically derived relative risks, together with a PBPK model for taking into account aspects of metabolism of VC. The risk assessment was limited to liver angiosarcomas. The authors produced three independent evaluations, based on three different epidemiological studies, with the following results.

(l) Based on Fox and Colliers (1977): 0.71-4.22 x 10^{-3}, for a lifetime exposure to 1 ppm.

(2) Based on Jones *et al.* (1988): 0.97-3.60 x 10^{-3}, for a lifetime exposure to 1 ppm.

(3) Based on Simonato *et al.* (1991). 0.40-0.79 x 10^{-3}, for a lifetime exposure to 1 ppm.

All three estimates are very close to one another. The average of the mean values of the confidence intervals of the three risk estimates is 1.8 x 10^{-3}, for lifetime exposure to 1 ppm. This is again consistent with the WHO estimate, and leads to a risk related to overall working time (14% of lifetime) of 0.25 x 10^{-3} for 1 ppm VC.

Risk estimates based on experimental data (low-dose linear extrapolation)

(a) Clewell *et al.* (1995)*:* The authors used the linearised multistage model, together with a PBPK model (in order to take into account the dose-related metabolic rates in experimental animals and humans, to provide a suitable interspecies extrapolation). The experimental data included two experimental studies by Maltoni *et al.* (1981; 1984) with inhalation of VC by rats and mice, another study by Maltoni *et al.* (1981, 1984) using gavage treatment of rats, and a study by Feron *et al.* (1981) with oral exposure of rats through the diet. The risk assessment was limited to liver angiosarcomas. For the present purpose, only the risk estimates based on inhalation experiments (Maltoni *et al.*, 1981, 1984) are considered, which seems appropriate in view of human inhalation exposure. As an average of upper confidence limits calculated for humans on the basis of all inhalation studies in mice and rats, the authors arrived at a lifetime risk for 1 ppm VC inhalation of 3.0 x 10^{-3}.

Assuming an overall working time corresponding to 14% of lifetime, the associated working time (14% of lifetime) risk would be 0.42 x 10^{-3} for 1 ppm VC.

An improved physiologically-based pharmacokinetic (PBPK) model for VC was later

developed by Clewell *et al.* (2001). The initial metabolism of VC was described as occurring via two saturable pathways, one representing low capacity-high affinity oxidation by CYP2E1 and the other representing higher capacity-lower affinity oxidation by other isozymes of P450, producing in both cases chloroethylene oxide and chloroacetaldehyde as intermediate reactive products. Depletion of glutathione by reaction with both reactive metabolites was also considered. Animal-based risk estimates for human inhalation exposure to VC, using total metabolism estimates from the PBPK model, were basically found to be consistent with the previous risk estimates (v.s.).

(b) Reitz *et al.* (1996): The authors also used the linearised multistage model, together with PBPK modelling. The experimental data analysed included two experimental studies by Maltoni *et al.* (1974; 1981; 1984) in rats and mice. The upper confidence limit of lifetime risk at 1 ppm VC was calculated as 1.5×10^{-3}, corresponding to a working time (14% lifetime) exposure risk at 1 ppm VC of 0.21×10^{-3} for l ppm VC.

(c) US EPA (2000): The agency used the linear "default method" as well as the linearised multistage model (with practically the same result), together with a PBPK model (in order to take into account the dose-dependent metabolic rates in experimental animals and humans and to provide a suitable interspecies extrapolation). The experimental data analysed were again those of Maltoni *et al.* (1974; 1981; 1984) in female rats. The risk assessment considered liver angiosarcomas, together with hepatomas and neoplastic nodules. The risk estimate presented by the agency, as the upper confidence limit, was for continuous lifetime exposure during adulthood: 11.4×10^{-3} for l ppm VC. Again, assuming an overall working time to be 14% of lifetime, the risk becomes about 1.6×10^{-3} for l ppm VC.

All these risk estimates, based on different sets and categories of data (animal experiments, epidemiological studies), lead to risk estimates that are basically consistent, if the variability and uncertainty of such estimates is taken into account.

Conclusion

Vinyl chloride is an established carcinogen, both in humans and in experimental animals. The primary target of its carcinogenicity is the liver, although there is clear experimental and suggestive human evidence that it also acts at extrahepatic sites. The primary, and most typical, liver tumour is angiosarcoma (hemangioepithelioma), but experimental data also demonstrate formation of hepatocellular carcinomas.

The SCOEL was asked to perform an assessment of human risk of carcinogenicity, related to workplace conditions. As a first step, available data were reviewed, which indicated that a linear high dose–low dose extrapolation of tumour risk was the most appropriate way in this case.

On this basis, the available quantitative risk assessments were reviewed, including those based on human epidemiological data and those based on extrapolation from animal data, by means of PBPK modelling.

The different approaches resulted in final risk estimates which were basically consistent with one another. As a result, it was inferred from epidemiological studies that a

continuous exposure for working life (estimated to be 14% of the total lifetime) to *1 ppm vinyl chloride would be associated with a cancer risk for hepatic angiosarcoma of about 0.3 x* 10^{-3}.

Independent data, derived from animal experiments and using PBPK modelling, point to a similar order of magnitude (between 0.2 and 1.6 x 10^{-3}), and thus confirm this approach.

References

ATSDR - US Dept. of Health and Human Services (l997) Toxicological Profile for Vinyl Chloride, ATSDR, Atlanta, Georgia

Bartsch H, Malaveille C, Barbin A, Planche G (1979). Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes, and dichlorobutenes produced by rodent or human liver tissues. Arch Toxicol 41: 249-277

Bolt HM (1978) Metabolic activation of halogenated ethylenes. In: Remmer H, Bolt HM, Bannasch P, Popper H, eds. Primary Liver Tumours. Lancaster, UK, MTP Press, pp. 285-294

Bolt HM, Filser JG (1983) Quantitative Aspekte der Kanzerogenität von Vinylbromid. Verh Dtsch Ges Arbeitsmedizin (Gentner, Stuttgart) 23: 433-437

Bolt HM, Filser J, Laib RJ, Ottenwälder H (1980) Binding kinetics of vinyl chloride and vinyl bromide at very low doses. Quantitative aspects of risk assessment in chemical carcinogenesis. Arch Toxicol Suppl 3: 129-142

Bolt HM, Laib R, Filser JG, Ottenwälder H, Buchter A (1981) Vinyl chloride and related compounds: mechanisms of action on the liver. In: Berk PD, Chalmers TC, Frontiers in Liver Disease. New York: Thieme-Stratton, pp. 80-92

Buchter A, Bolt HM, Filser J (1978) Pharmakokinetik und Karzinogenese von Vinylchlorid. Arbeitsmedizinische Risikobeurteilung. Verh Dtsch Ges Arbeitsmed (Gentner, Stuttgart) 18:111-124

Clewell HJ *et al.* (l995) Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: Examples with vinyl chloride and trichloroethylene Chemosphere 31: 2561-2578

Clewell HJ, Gentry PR, Gearhart JM, Allen BC, Andersen ME (2001) Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. Sci Total Environ 274: 37-66

Feron VJ, Hendriksen CFM, Speek AJ *et al.* (1981) Lifespan oral toxicity study of vinyl chloride in rats. Food Cosmetics Toxicol 19: 317-333

Fox AJ, Collier PF (l977) Mortality experience of workers' exposure to vinyl chloride monomer in the manufacture of polyvinyl chloride in Great Britain. Br J Ind Med 34: 1-10

Gehring PJ, Watanabe PG, Park CN (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation: Example: vinyl chloride Toxicol Appl Phramacol 44: 581-591

Gehring PJ, Watanabe PG, Park CN (1979) Risk of angiosarcoma in workers exposed to vinyl chloride as predicted from studies in rats. Toxicol Appl Pharmacol 49:15-21

IARC (1987) Vinyl Chloride, IARC Monographs, Suppl. 7, International Agency for Research on Cancer, Lyon

IPCS-WHO (l999): Vinyl Chloride, Environmental health Criteria 215, International Programme on Chemical Safety, World Health Organisation, Geneva

Jones RW, Smith DM, Thomas PG (1988) A mortality study of vinyl chloride monomer workers employed in the United Kingdom in 1940-1974 Scand J Work Environ Health 14: 153-160

Kielhorn J, Melber C, Wahnschaffe U, Aitio A, Mangelsdorf I (2000) Vinyl chloride: still a cause for concern. Environ Hlth Perspect 108: 579-588

Kunz HW, Tennekes HA, Port RE, Schwarz M, Lorke D, Schaude G (1983) Quantitative aspects of chemical carcinogenesis and tumor promotion in liver. Environ Hlth Perspect 50: 113-122

Laib RJ, Bolt HM (1980) Trans-membrane-alkylation. A new method for studying irreversible binding of reactive metabolites to nucleic acids. Biochem Pharmacol 29: 449-452

Laib RJ, Pellio T, Wünschel UM, Zimmermann N, Bolt HM (1985) The rat liver foci bioassay: II. Investigations on the dose-dependent induction of ATPase-deficient foci by vinyl chloride at very low doses. Carcinogenesis 6: 69-72

Maltoni C (1977) Recent findings on the carcinogenicity of chlorinated olefins, Environ Hlth Perspect 21: 1-5

Maltoni C, Lefemine C, Chieco P, Carretta D (1974) Vinyl chloride carcinogenesis. Current results and perspectives Med. Lav 65: 421

Maltoni C, Lefemine G, Ciliberti A *et al.* (198l) Carcinogenicity bioassay of vinyl chloride monomer: a model of risk assessment on an experimental basis. Environ Health Perspect 41: 2-29

Maltoni C, Lefemine G, Ciliberti A *et al.* (1984) Experimental research on vinyl chloride carcinogenesis, in Maltoni and Mehlman (eds) Archives of Research on Industrial Carcinogenesis. Vol. 2, Princeton Scientific Publishers Inc., Princeton, New Jersey

Moolgavkar SH, Luebeck EG (1990) Two event model for carcinogenesis: biological, mathematical and statistical considerations Risk Analysis 10: 323-341

Moolgavkar SH, Luebeck EG, Krewski D, Zieliski JM (1993) Radon, cigarette smoke,

and lung cancer: A reanalysis of the Colorado uranium miners' data. Am J Epidemiol 4: 207-217

Reitz RH *et al.* (1996) Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. Toxicol Appl Pharmacol 137: 253-267

Schumann AM, Watanabe PG, Reitz RH, Gehring PJ (1982) The importance of pharmacokinetic and macromolecular events as they relate to mechanisms of tumorigenicity and risk assessment, in Plaa G and Hewitt WR (eds.), Toxicology of the Liver, Target Organ Toxicology Series, Raven Press, New York, pp. 311-331

Simonato *et al.* (199l) A collaborative study of cancer incidence and mortality among vinyl chloride workers. Scand J Work Environ Health 17: 159-169

Storm JE, Rozman KK (1997) Evaluation of alternative methods for establishing safe levels of occupational exposure to vinyl halides. Regulatory Toxicol Pharmacol 25: 240-255

US EPA (1996) Proposed Guidelines for Carcinogen Risk Assessment, US Fed. Register 61, No 79, April 23, 1996. http://www.epa.gov./ORD/WebPubs/carcinogen/

US EPA (2000) Vinyl Chloride, IRIS File online, US EPA, Washington DC, available on http://www.epa.gov/iris/subst/l001.htm/

Ward E, Boffetta P, Andersen A, Colin D, Comba P, Deddens JA, De Santis M, Engholm G, Hagmar L, Langard S, Lundberg I, McElvenny D, Pirastu R, Sali D, Simonato L (2001) Update of the follow-up of mortality and cancer incidence among European workers employed in the vinyl chloride industry. Epidemiology 12: 710-718

WHO (l987) Air Quality Guidelines for Europe WHO, Copenhagen

WHO (l999) Guidelines for Air Quality, WHO, Geneva

WHO (2000) Air Quality Guidelines for Europe, WHO Regional Office, Copenhagen

This document has been edited using the assessment:

Vinyl Chloride, a Review of Recent Carcinogenic Risk Evaluations,
compiled for the SCOEL by *Giovanni Alfredo Zapponi, Istituto Superiore di Sanità, Roma, Italy*

Criteria documents used:

- US EPA (2000)
- WHO (2000)

Appendix:

General remarks on risk assessment procedures

When appropriate epidemiological data are available, the most simple procedure for low-dose risk estimations is the "Unit Risk" (U.R.) linear model, used by the WHO (1987; 2000):

$$UR=P_o(RR-l)/X$$

where: Po is the background lifetime risk, derived from age/cause-specific death or incidence rates found in national statistics or from a matched control population, RR is the relative risk (ratio of observed and expected number of cases) and X is the lifetime average exposure (standardised lifetime exposure for the study population on a lifetime continuous exposure basis).

The classical model for carcinogenic risk assessment, largely used in the past for animal experiment-based risk assessment, is the Armitage-Doll multistage model. This model is based on the assumption that a single normal cell may become fully malignant only after it has undergone a sequence of irreversible heritable changes (being higher or equal to l). If the data to which the model has to be fitted refer to lifetime exposure (as generally happens for carcinogenicity experiments), the model results in the form:

$$P(d) = 1 -exp(-(q_o + q_1d + q_2d^2 + ...+ q_kd^k)$$

The form of this model most commonly used is the "Linearised Multistage Model", which, instead of the "maximum likelihood estimate" of the q_1 parameter, uses its upper confidence limit. This assumption is justified by the observation that a sufficiently small value of q_1 may result in a null estimate; conversely, the general form of the Armitage-Doll model theoretically results in a non-null value of this parameter. In practice, the model is used as descriptive interpolation of data, rather than as a theoretical model.

A more recent carcinogenic risk assessment model has been presented within the last decade, termed "Biologically-Based Models" (B-B Models), of which the "Two Stage-Clonal Expansion" models are the main representatives. These models aim at taking into account "initiation" (understood as a critical mutation event transforming a normal

stem cell into an intermediate preneoplastic cell), "promotion" (understood as the clonal expansion of intermediate cells, because of external stimuli and/or for endogenous causes) and "conversion" (understood as a second critical mutation leading to the transition of an intermediate cell to a neoplastic cell) (Moolgavkar and Luebeck 1990). The mutation steps, whenever suggested by the available experimental data, may be more than two. The use of these models implies the availability of appropriate data, more extended than required by the multistage model, and requires much more complex data processing. However, this makes it possible to consider the clonal expansion process of intermediate cells, not considered by the classical multistage model, thus representing more appropriately the basic biological processes involved in carcinogenesis. Moreover, the time dimension of exposure is adequately considered, enabling different exposure patterns to be distinguished. A model of this type has been used also for analysing epidemiological occupational data, as in the case of the Colorado Plateau Uranium Miners cohort (Moolgavkar *et al.* 1993).

Lastly, the US Environmental Protection Agency has presented new guidelines for carcinogenic risk assessment (US EPA 1996), including the use of B-B models, whenever allowed by the available data. When this is not possible, "default approaches" are proposed.

According to the US EPA, a default assumption of carcinogenicity based on linearity is appropriate when the scientific evidence supports a mode of action anticipated to be linear (e.g. DNA reactivity). Other elements of empirical support may also support an inference of linearity, e.g. the background of human exposure is on the linear part of a dose-response curve that is sublinear overall. The default assumption of linearity, according to the EPA, is also appropriate as the ultimate science policy default when evidence shows no DNA reactivity or other support for linearity, but neither does it show sufficient evidence of a non-linear mode of action to support a non-linear procedure.

Available carcinogenic risk estimates for the general population are carried out for lifetime exposure. In the case of assessments using occupational epidemiological data, the most common procedure is based on the conversion of the average inhalation daily exposure in the working environment into the average inhalation daily exposure for a lifetime (WHO 1987; 1999; 2000). This implies considering that, typically, people work for 8 hours per day, for about 240 days per year (about 2/3 of the days in a year), and for the number of years specific of the particular cases under study. The same criterion may be used for converting an average lifetime daily exposure into an average exposure in the working environment. As an example, assuming 8 working hours per day, 240 working days per year, and, lastly, a working life of 45 years and a lifetime of 70 years or more, generally a number of working years approximately 65% of the whole life, it appears that the whole time spent at work is about 14% (or about 1/7) of the whole lifetime (i.e. 8/24 x 240/365 x 45/70 = 0.14 or 1/7) for averaging exposure over time. This means that, under the above conditions, the lifetime average daily exposure to a concentration C is equivalent to an average concentration sevenfold higher in the working environment (7 x C). The same ratio holds for the cumulative exposure under the two conditions (WHO 1987; 1999; 2000; Storm and Rozman 1997).