RISK ASSESSMENT

Vinyl acetate

CAS-No.: 108-05-4

EINECS-No.: 203-545-4

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FINAL APPROVED VERSION

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The first draft of the Comprehensive Risk Assessment Report of **Vinyl acetate**, a substance chosen from the EU 1st Priority List in 1994, was discussed preliminary (written procedure) at the TM II / 2001 (June 2001).

The Environmental part was discussed in depth at the TM III/2002 (August 2002) and further in depth at the TC NES IV 04 (December 2004). It was distributed for the last visit written procedure in May 2005 and for the final written approval in May 2006.

The Human Health part was discussed first in depth at the TM I/2002 (March 2002), second in depth at the TM IV/2002 (December 2002), last visit at the TM II/2003 (June 2003), 2nd last visit (effects assessment only) at TC NES I/2007 and 2nd last visit (Risk characterisation) at TC NES II/2007. It was distributed for the final written procedure in December 2007 and for a further written approval in May 2008.

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Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/931 on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/942, which is supported by a technical guidance document3. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

[1] O.J. No L 084, 05/04/199 p.0001 – 0075
[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011
[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

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0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 108-05-4

EINECS No. 203-545-4

IUPAC Name Vinyl acetate

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(x) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(x) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

As a follow up to conclusion (i), which was drawn in the first draft of the Environment Section of the Comprehensive Risk Assessment Report of Vinyl acetate (August 2002), further acute and long-term toxicity tests were performed for the aquatic compartment and more data on a number of production and processing sites were obtained, leading to the following revised risk characterisation.

conclusion iii):

Terrestrial compartment

A risk to the local terrestrial compartment can be identified for the life cycle stage of vinyl acetate processing. This conclusion applies to the generic processing scenario for the local main source. The unknown processing sites account for a missing tonnage of 325,000 t/a (or 41 % of the total production volume of 800,000 t/a), compared to a verified processing tonnage of 475,000 t/a.

The risk characterisations for selected (worst case) production and processing sites with site specific data indicate no actual risk to the terrestrial compartment. However, the highest PEC/PNEC ratio calculated with site specific data (0.86) is not very far from the PEC/PNEC ratio calculated for the generic processing scenario (2.33), and there was also a risk at one of the production sites (which has been eliminated during the time of the preparation of this RAR). Consequently, it is not completely implausible that unknown processing sites pose a risk to the terrestrial environment.

The risk characterisation for the terrestrial compartment could still be revised by requesting further testing (i.e. OECD 207 Earthworm acute toxicity test and OECD 208 Terrestrial plants growth test). However, a further data improvement by means of testing is not considered a reasonable next step by the rapporteur for the following reasons.

Further information on the toxicity of vinyl acetate to terrestrial organisms allows for a re-evaluation of the potential risk that emanates from the unknown processing sites, but delays risk reduction measures in case that a risk still remains with the new data. The rapporteur therefore proposes to enter directly into the risk reduction phase, which would be more effective than further data improvement at this stage of the risk assessment.

Risk reduction measures should be considered for all facilities with a vinyl acetate processing capacity exceeding 20,000 t/a. This threshold is derived from the generic processing scenario, assuming that vinyl acetate is released to soils via sludge application and aerial deposition. Sites already applying advanced techniques would not require further consideration of risk reduction measures. It is known from some processing/production sites that techniques such as waste water distillation, sludge incineration or deposition in a landfill, waste gas incineration or vapour recovery systems can avoid significant releases of vinyl acetate to soils.

In principle, the rapporteur agrees with the proposal of SCHER that considering the volatility of the substance, terrestrial plants might be exposed via air. However, no guidance is available how to derive a PNEC for the air compartment. In addition, the available guidelines for terrestrial plants (OECD 208 / 227) are designed to include exposure through soil or by wet residues (e.g. spray drift of plant production products). To determine effects of volatile compounds, testing needs to be conducted in closed greenhouses or chambers under continuous exposure conditions. We feel this kind of testing is not appropriate for vinyl acetate, because no indications for high toxicity for plants exist. For other soil organisms, a continuous exposure to volatile compounds like vinyl acetate is negligible since the substance is expected to evaporate from the soil.

conclusion ii):

Aquatic compartment

A risk to the local or regional aquatic compartment (surface water and sediments) was not identified for production and processing of vinyl acetate. This conclusion applies to all sites.

Atmospheric compartment

Based on a qualitative risk characterisation, no unacceptable risk for the atmosphere is expected from vinyl acetate. The substance is rapidly removed from air by chemical

breakdown, adsorption to airborne particles or aerosols, and wet deposition. Furthermore, air concentrations indicate a negligible risk with regard to ecotoxicity.

Non-compartment specific effects

Non compartment specific effects (secondary poisoning) of vinyl acetate are not expected as there is no indication that the substance has potentially bioaccumulative properties.

Marine Assessment including PBT assessment

VAM does not meet the PBT/vPvB criteria. A risk for the regional or local marine environment is not expected.

Human Health

Human Health (toxicity)

Workers

conclusion iii)

Conclusion iii is expressed for the endpoint of carcinogenicity and repeated dose toxicity after inhalation and carcinogenicity after dermal contact. Skin contact of vinyl acetate should be reduced at scenario 2a (manufacturing of formulations and products, vinyl acetate monomer), even if evaporation of the substance reduces the contribution of dermal exposure.

On the background of cancer risks and repeated dose toxicity, air concentrations of vinyl acetate at the workplace should be controlled to a level in the range of 17.6 mg/m^3 (critical exposure level). Conclusion iii is derived for repeated dose toxicity after inhalation for scenario 2 (manufacturing of formulations and products). This scenario with an exposure value of 14.6 mg/m^3 is a borderline case. Since there is the uncertainty concerning the reduction of the intraspecies factor and the mode of action for carcinogenesis for this borderline scenario conclusion iii is drawn.

Consumers

conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Man exposed indirectly via the environment

conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Human Health (risks from physico-chemical properties)

Conclusion (ii)

There is at present no need for further information and/or testing and no need for riskreduction measures beyond those which are being applied already.

1 GENERAL SUBSTANCE INFORMATION

Identification of the substance

CAS No.: 108-05-4

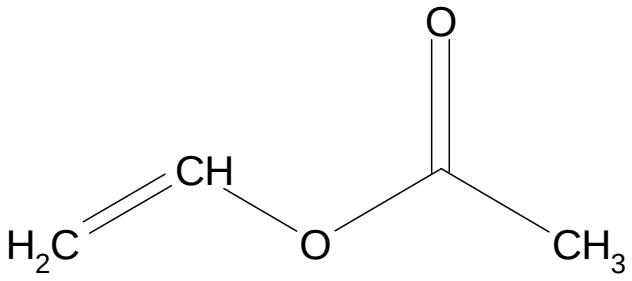
EINECS No.: 203-545-4

IUPAC Name: Vinyl acetate

Synonyma: Acetic acid vinyl ester

Empirical formula: $C_4H_6O_2$

Structural formula:



Molecular weight: 86 g/mol

Purity/impurities, additives

Purity: 99.8 % w/w

Impurities: ≥ 0.03 - 0.1 % w/w water

≥ 0.005 - 0.01 % w/w acetic acid

≥ 0.005 - 0.02 % w/w acetaldehyde

Additives: hydrochinone or hydrochinone mono methyl ether as stabilisator

0.00015 - 0.002 % w/w

Table 1-1: Physico-chemical properties

Physical state	liquid at 20 °C	
Melting point	- 93.2 °C	Handbook of Chemistry and Physics, 1980 - 1981
Boiling point	+ 72.7 °C	Merck Index, 1996
Relative density	0.932 at 20 °C	Merck Index, 1996
Vapour pressure	120 hPa at 20 °C	Hoechst AG, 1994c
Surface tension	24 mN/m at 20 °C (pure substance)	Handbook of Chemistry and Physics, 1980 - 1981
Water solubility	20 g/l at 20 °C	Merck Index, 1996
Partition coefficient	logPow 0.7	Hoechst AG, 1992
Flash point	- 8 °C (DIN 51755)	CHEMSAFE, 1994
Auto-flammability, ignition temperature	385 °C (DIN 51794)	CHEMSAFE, 1994
Explosive properties	not explosive	due to structural reasons
Oxidizing properties	not oxidizing	due to structural properties

Vapour pressure:

Vapour pressure values were available from safety data sheets, product information and literature without any details about the used methods. All values correlate well with the boiling point of 72.7 °C (Clausius-Clapeyron).

Water solubility:

The water solubility is 20 g/l at 20 °C according to safety data sheets and product information. No further information about the determination method is available.

Partition coefficient:

According to literature data the partition coefficient was determined experimentally (HPLC method: logPow 0.21, shaking method: logPow 0.73). The calculation with CLOGP3-Software resulted in a logPow of 0.7. Due to the agreement of experimentally and calculated values a logPow of 0.7 was used for the risk assessment.

Classification

- (Classification according to Annex I, 19th ATP)

F	R 11	Highly flammable

- (Proposal of the rapporteur)

The current classification included in Annex I to Directive 67/548/EEC is only due to human health effects and does not yet consider environmental effects of vinyl acetate. Based on the data presented in the environmental section of this risk assessment report and the criteria of Directive 93/21/EEC, the rapporteur proposes the substance not to be classified and labelled for environmental risks.

The classification included in Annex I to Directive 67/548/EEC does not yet include toxic effects. According to the data presented below and the criteria of Directive 93/21/EEC, vinyl acetate should be classified as follows and assigned the risk phrases:

Harmful	R 20	Harmful by inhalation
Irritant	R 37	Irritating to respiratory system
Carcinogenic, Cat. 3	R 40	Limited evidence of a carcinogenic effect

Human data on irritation/corrosion caused by vinyl acetate are not available. In the only valid test (RCC, 2003) mild irritative effects on the skin of rabbits was observed that do not need classification. The eye irritation potential of the compound seems to be mild as judged on the basis of poorly documented eye irritation tests with rabbits. Hence, no classification for eye irritative effects is needed.

Acute inhalation tests with rats demonstrated severe irritation in the respiratory tract of the animals. Thus, vinyl acetate should be labelled “R 37 Irritating to respiratory system”.

Results from an animal skin sensitization study (Buehler Test) showed a moderate skin sensitising potential of vinyl acetate (commercial grade). With the use of the Local Lymph Nodes Assay (LLNA) no significantly positive stimulation responses were detected at concentrations of 5% - 100%. Overall, the outcome of both studies may indicate that vinyl acetate is not devoid of a skin sensitising potential. The results of the LLNA do confirm the weak-moderate effects seen in the Buehler test. However, since the positive threshold level was not exceeded in the LLNA, classification and labelling with R 43 is not warranted.

There is clear evidence for the carcinogenicity of vinyl acetate in two animal species and in both sexes. The carcinogenic potential was demonstrated for the inhalation and oral route of administration. Vinyl acetate exposure produced tumors at the site of first contact along the exposure routes. A thresholded mode of carcinogenic action is thought to be active. The observed tumor responses are reflecting the target site-specific enzyme activities:

Following inhalation and oral exposure vinyl acetate is rapidly hydrolysed by carboxylesterases leading to the formation of acetic acid and acetaldehyde which is further converted into acetic acid in the presence of aldehyde dehydrogenases. Intracellular aldehyde dehydrogenase activity is limited, at higher concentrations of vinyl acetate it will not be sufficient for the oxidation of generated acetadehyde. Thus, at high vinyl acetate concentrations non-physiological concentrations of acetaldehyde are produced. Acetaldehyde is a physiological intermediate with low background concentrations. Its adverse effects (genotoxicity and mutagenicity) are limited to non-physiologically high concentrations. Therefore, a threshold mode of action is assumed for vinyl acetate.

Above threshold concentrations, cytotoxicity (only at the olfactory mucosa), mitogenic actions and genotoxic actions occurred.

Data on vinyl acetate are in line with the idea that vinyl acetate genotoxicity is mediated by acetaldehyde. Increasing concentrations of acetaldehyde produce genotoxic actions at the site of contact. It has to be taken into consideration that acetaldehyde occurs naturally in mammalians cells and is part of the physiological cellular metabolism.

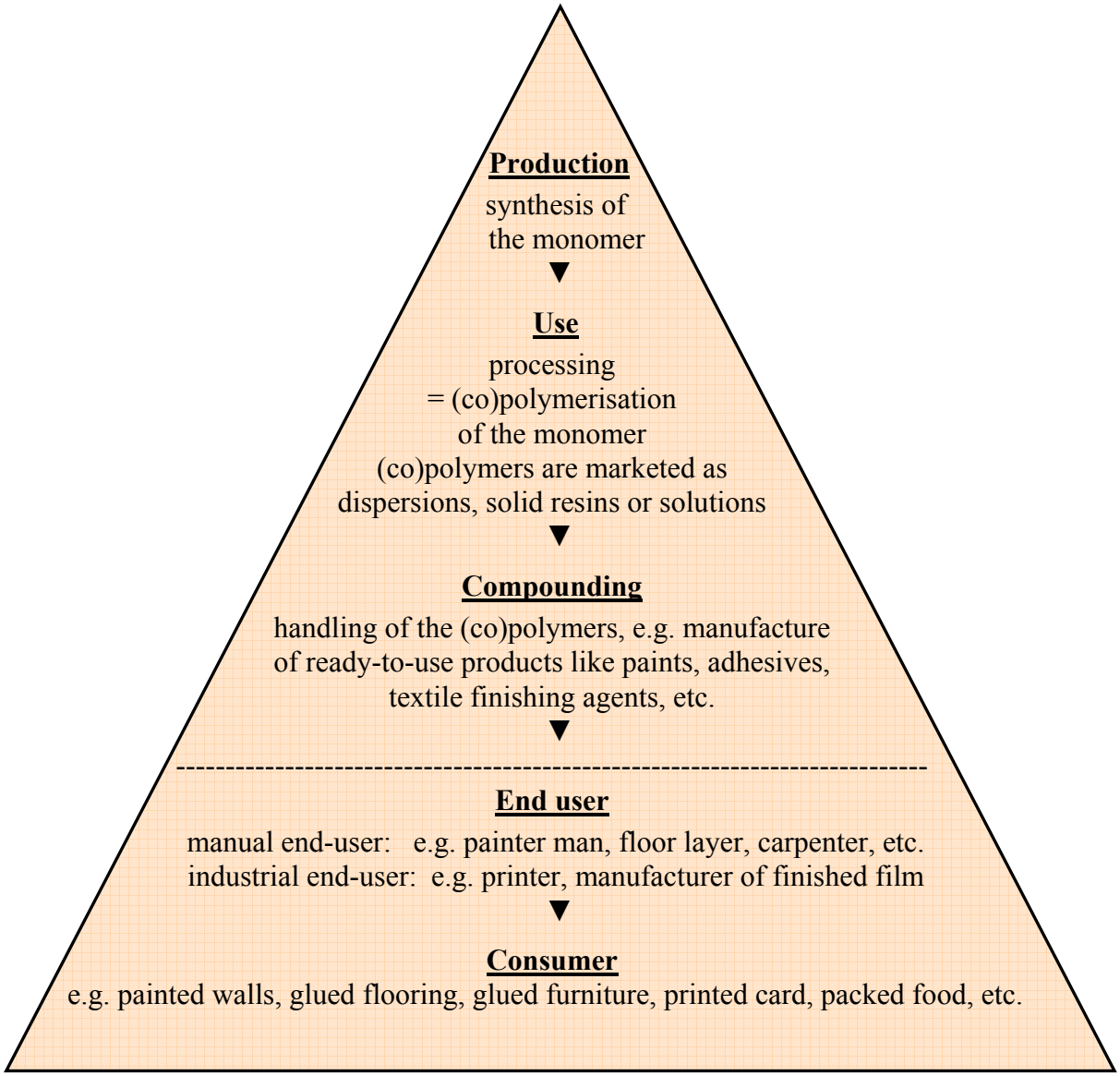
From animal data it is concluded that vinyl acetate might pose a cancer risk for humans exposed to the substance via the inhalation or oral route. Carcinogenicity is thought to act via a secondary mechanism and the concern may only be relevant above threshold concentrations. The observed effects are thought to be relevant for the human. For the respiratory tract humans may be less sensitive than the rat due to a lower carboxylesterase activity in the nasal mucosa.

In Germany, vinyl acetate is assigned to the MAK-pregnancy category "D" denoting that the current data base is not sufficient for final evaluation of developmental toxicity. However it is outlined that vinyl acetate was evaluated for reproductive toxicity in one species (rat) already with negative results. If this latter outcome could be verified in additional species vinyl acetate could be assigned to category "C" denoting that no risk for adverse developmental effects have to be expected for female workers in compliance with the respective MAK value of 10 ppm.

2 GENERAL INFORMATION ON EXPOSURE

2.1 EXPOSURE SOURCES

Exposure of man and environment to vinyl acetate monomer (VAM) may result from releases during each of the consecutive steps downstream the supply chain. These are production of the substance, polymerisation of the monomer, compounding operations involving the polymers, and eventually use of the end products.



The first three stages in the chain, i.e. manufacture of the monomer, polymerisation of (co)polymers, and compounding of (co)polymers are in most cases conducted by different companies and at separate locations. Notwithstanding, there is a small number of sites where both production of the monomer and further polymerizing is carried out, while there are other cases where VAM is (co)polymerized and subsequently also compounded.

Only a few manufacturers of VAM exist in the EU, whereas the number of companies polymerising the monomer and in particular the number of downstream users of the polymers is considerably higher. For example, there are no more than two VAM manufacturers in Germany that use parts of the produced VAM volume to manufacture vinyl acetate (co)polymers. Similarly, just six out of fifty-three European manufacturers of coating/printing inks manufacture the required polymer constituents from the monomer on-site.

Production sites of VAM and facilities producing the (co)polymers, but also compounding operations are typical point sources of VAM emissions. Any VAM entering the environment from the latter type of operation results from releases of monomer residuals remaining with the processed (co)polymers.

Finally, diffusive releases of residuals may occur during end-use of the finished products that contain vinyl acetate (co)polymer constituents.

2.1.1 Production

Vinyl acetate is produced by a catalytic vapour-phase reaction of ethylene and acetic acid in gas phase, liquid phase or a fluidised-bed process.

2.1.1.1 Global production figures

The worldwide demand for VAM reportedly amounted to 4.3 million t/a in 2003, representing an overall operating rate of about 86 % of a maximum global production capacity of 5 million t/a. The main production capacities are located in Asia (43 %) and North America (34 %), followed by Western Europe on third rank (18 %) (Chemical Week, 2003).

2.1.1.2 Production in Western Europe

A questionnaire was launched by the European Chemical Industry Council (CEFIC) among member companies in 2000 and updated in 2002 to collect data on produced and processed volumes of vinyl acetate monomer in Europe. Five producers of VAM were identified with a nominal production capacity of 800,000 t/a (cf. Table 2-1). Fluctuating operating rates (additional capacities brought online, partly offset by shutting down other plants) make it difficult to provide a general figure for the annual production volume of VAM in the EU.

For comparison, the production volume of VAM reported by the CEFIC Acetyls Sector Group (ASG) for Western Europe was about 715,000 tonnes in 2002. Additionally, total purchases[4] in 2002 accounted for almost 250,000 tonnes. Along with an opening stock[5] of approximately 80,000 tonnes, the “total supply” for 2002 amounted to 1,045,000 tonnes.

[4] Purchase from outside Western Europe but also from co-producers in Western Europe

[5] Includes all product produced or purchased but not yet invoiced or transferred to internal captive use, e.g. product in storage or in transit (should be equal to the closing stock of the previous period).

From the total supply of about 1,045,000 tonnes, some unquantified amounts were exported outside Western Europe again (figures were not given by industry for confidentiality reasons). With an end stock[6] of less than 50,000 tonnes in 2002, approximately 750,000 tonnes of VAM were marketed in Western Europe that year (all figures provided by CEFIC ASG).

The outcome of the CEFIC survey was in poor consistency with data retrieved from the IUCLID database (as of May 1999). A number of the companies included in the CEFIC survey were not covered by the IUCLID data - and vice versa. Several of the enterprises had discontinued to exist as a business entity, owing to an intensive restructuring process of the market (mergers, acquisitions and divestitures of business segments). In some cases it turned out that some IUCLID-mentioned capacities had been only sales bureaus, whereas other facilities had meanwhile been shut down. IUCLID data were therefore left aside.

2.1.2 Processing

Unlike for production, no exact figure of the processed volume of vinyl acetate can be extracted from the statistics provided by the CEFIC Acetyls Sector Group (ASG). The provided data is difficult to balance for import/export and purchases/sales as ASG statistics comprise all Western European countries, which does not coincide with EU countries, and some parts of the statistics are confidential, thus were not disclosed to the rapporteur.

According to the CEFIC Acetyls Sector Group, the total supply of VAM in Western Europe was approximately 1,045,000 tonnes in 2002, based on a production volume of about 715,000 t, total purchases of about 250,000 t and an opening stock of about 80,000 t. When exports, sales, captive use and closing stock are deducted, approximately 750,000 t entered the open market in Western Europe in 2002. From this tonnage, an unknown amount is sold to non-EU countries in Western Europe (Switzerland, Turkey, Iceland, Norway), with the remaining tonnage of VAM being sold and processed in the EU. Some amounts of the total VAM supply of 1,045,000 tonnes is directed to captive use[7] and also processed in the EU, but figures on captive use are not provided. As sales to non-EU countries in Western Europe and captive use were not quantified, the exact processing volume in the EU cannot be determined. It is therefore assumed to be in the range of 750,000 – 800,000 tonnes per year.

Responses to the CEFIC questionnaire came either from individual processors or industry associations of processing companies. One industry association (CEPE) did not provide information broken down to individual members or processing sites, but for reasons of confidentiality submitted aggregated, in part statistically evaluated data (max./min. values or average means) which were compiled from a questionnaire among member companies. In addition to the CEFIC data, other processing sites could be identified and consulted. Based on this data collection, the actual total processing volume of VAM may amount to 474,900 tonnes per year in the EU (cf. Table 2-1).

[6] Closing or end stock of the previous period. Includes all product produced or purchased but not yet invoiced or transferred to internal captive use, e.g. product in storage points or products in transit.
[7] All product transferred internally in Western Europe whether from own production or purchased.

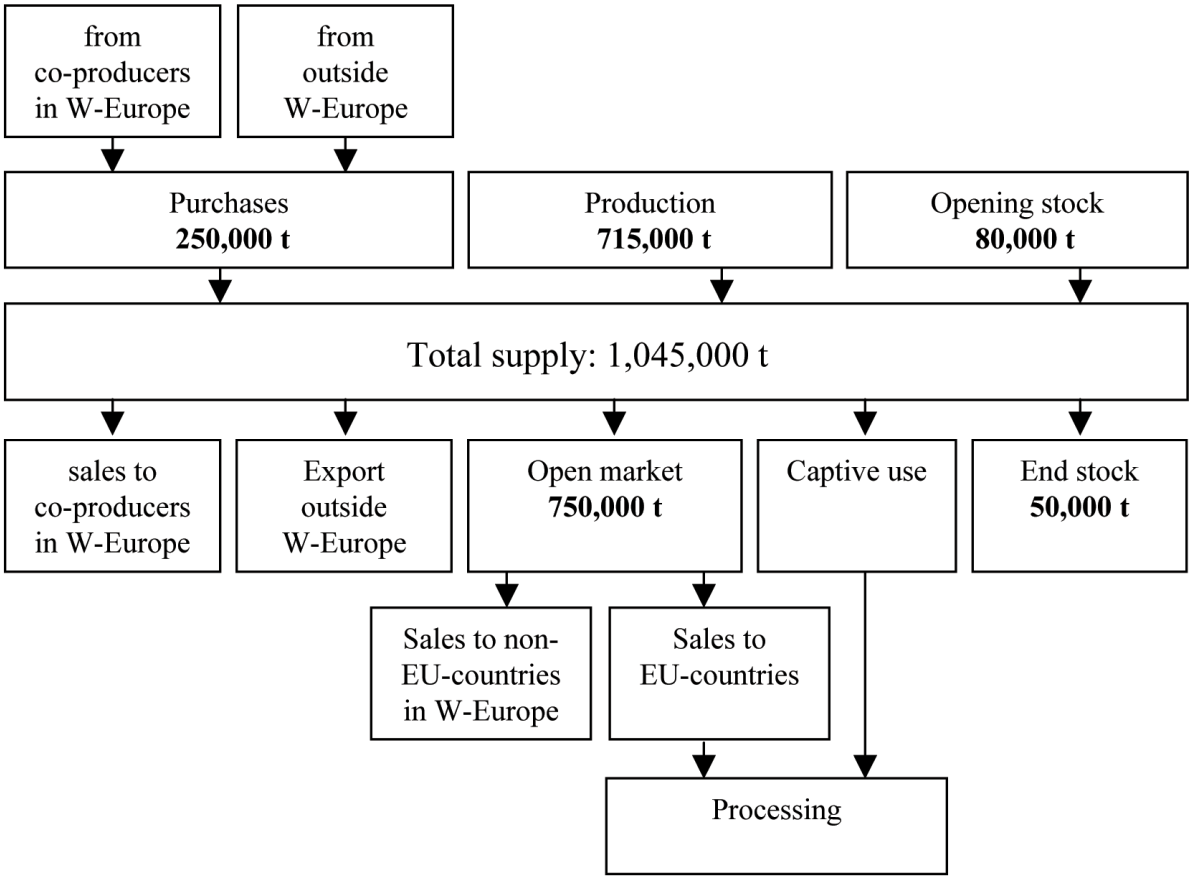


Figure 2-1 Market statistics of VAM for 2002, based on CEFIC Acetyls Sector Group.

It has to be concluded that the data provided on production and processed volumes of VAM do not match very well:

	Based on single sites:	**Based on market statistics :**
Production	800,000 tonnes	715,000 tonnes
Processing	474,900 tonnes	750,000 - 800,000 tonnes (estimate)

This imbalance may be due to a number of reasons:

- Figures on volumes were reported for different business years and reflect the dynamics of a market which is characterized by continuous restructuring activities
- Some annual production data are based on nominal capacities, thus do not reflect actual figures which may change from year to year
- Production figures are rounded to nearest 10,000 t/a
- Downstream users did not reply to the questionnaire
- Data are not corrected for import/export or sales/purchases

The maximal difference between reported production volume and specific processing data for individual sites is 325,000 t/a (= 800,000 t/a - 475,000 t/a). It is therefore assumed that a number of processing sites exist that were not covered by the CEFIC questionnaire. As no information on these sites is available, a generic risk characterization will be conducted. A fraction of main source of 0.15 and 300 emission days are assumed (Tab. B3.2 of the TDG) to estimate the fraction of the total emissions that is expected to be released through the main local point source (worst case scenario for the local environment). The estimated total emissions that are released during processing of the total volume of 325,000 t/a are included in the calculation of the regional and continental background concentrations.

Specific local risks were calculated for the production and processing sites that could be identified (cf. Table 2-1). In several cases it could not be clarified whether the term “use” in the questionnaire was interpreted by the responders as (co)polymerisation, or compounding of already polymerised material, or both. If not specified otherwise it was assumed that the data referred to (co)polymerisation, although this may overestimate emissions in some cases. For confidentiality reasons, sites were coded. Five producers of VAM were identified being located in four EU member states (France, Germany, Spain and UK). Identified processing plants are located in Belgium, Denmark, Finland, France, Italy, Germany, the Netherlands, Spain and UK. No information on geographical locations was disclosed by industry associations that provided only aggregated data.

Table 2-1: Production and/or processed volumes of VAM identified within the EU

Site code	Produced volumes	Processed volumes	Data source
1		30,000	CEFIC
2		10,000	CEFIC
3a		6,000	CEFIC/CIRFS
3b		10,000	CEFIC/CIRFS
3c		8,000	CEFIC/CIRFS
3d		4,000	CEFIC/CIRFS
4	110,000		CEFIC
5		90,000	CEFIC
6		25,000	CEFIC
7*		10,810	CEPE
8		15,000	CEFIC
9		9,100	CEFIC
10		15,000	CEFIC
11		110,000	CEFIC
12		36,000	CEFIC
13		280	CEFIC
14		2,000	CEFIC/FEICA

Site code	Produced volumes	Processed volumes	Data source
15		2,200	CEFIC/FEICA
16		400	CEFIC/FEICA
17		300	CEFIC/FEICA
18		14,000	CEFIC/FEICA
19		25,000	CEFIC/FEICA
20**	0	0	CEFIC/FEICA
21	200,000		CEFIC
22	100,000		CEFIC
23		17,000	CEFIC
24		5,000	pers. communicated
25		6,910	pers. communicated
26	140,000		pers. communicated
27	250,000		pers. communicated
28		8,000	pers. communicated
29		6,500	pers. communicated
30		8,400	pers. communicated
∑	**800,000**	**474,900**	

CEFIC: European Chemical Industry Council
CEPE: Conseil Européen des Industries des Peintures, Encres d'Imprimerie et Couleurs d'Art
CIRFS: Comité International de la Rayonne et des Fibres Synthetiques
FEICA: Fédération Européenne des Industries de Colles et Adhésifs

* As communicated by industry association CEPE, most manufacturers buy polymer emulsions (PVA) ready for use and do not polymerise VAM themselves: 52 companies reported in a questionnaire to use PVA at 116 different sites for making printing inks, paintings and coatings. The total volume of VAM polymers was 43,524 t in 2000. The processing volume in the table refers to the two companies still polymerising VAM.

** confirmation of conducting merely compounding operations, i.e. handling of the (co)polymers.

2.2 USES

Vinyl acetate monomer is solely used as an intermediate in chemical industry for manufacturing (polymerisation) of vinyl acetate (co)polymers (IC 3 / UC 33). Hence it is concluded that the entire production volume of VAM is used up for the manufacture of various (co)polymers, mainly polyvinyl acetate.

Apart from manufacture of homopolymers the monomer is combined with other monomers like ethylene, vinyl chloride and acrylic acid esters to form various types of co-polymers. According to the Directory of Chemical Producers Western Europe (SRI International, 1997/98) two types of co-polymers involving ethylene are produced:

(i) ethylene-vinyl acetate co-polymer resins (EVA) with a production capacity of approximately 1 million t/a, and

(ii) vinyl acetate-ethylene co-polymer resins (VAE) with a production capacity of approximately 300,000 t/a.

Polymers manufactured from VAM are used in a broad spectrum of products, including water-based paints, printing inks, lacquer, ceramic, adhesives for packaging and construction, paper finishing, and protective colloids for various materials. Other important products include textile fibres, paper coating and inks (Table 2-2). The content of residual VAM in homo- and co-polymers depends on the product, spanning a wide range of reported values of less than 5 ppm up to 6,000 ppm (Celanese, 2000). According to the CEFIC compilation of data from industry, the majority of polymers have an average content of around 1,000 - 2,000 ppm or less. Ullmann (1998) provides a quantitatively weighted median value for the monomer content of roughly 3,000 ppm. The latter value will be used for estimating diffuse releases of VAM from (co)polymers and end-use products in this document.

In addition, vinyl acetate (co)polymers are used as feedstock for manufacture of vinyl alcohol (co)polymers (e.g. polyvinyl alcohol). Polyvinyl alcohol is obtained by hydrolysing polyvinyl acetate. The production capacity within the EU is reported at > 100,000 t/a (SRI International, 1997/98). Commercial grades of polyvinyl alcohol differ in the degree of polymerisation (molecular mass) and degree of hydrolysis (residual polyvinyl acetate content). Along with saponification (alkaline hydrolysis of fatty acid esters) of the acetyl moieties of polyvinyl acetate, monomer residuals are getting eliminated as well. A minor fraction of polyvinyl alcohol ends up in the manufacture of polyvinyl acetales, e.g. of polyvinyl butyral (PVB).

A broad quantitative breakdown of the use pattern of vinyl acetate is available for Germany for 1990. In this year, approximately 68 % of the produced vinyl acetate was used mainly for the manufacturing of polyvinyl acetate and, to a less extent, for the manufacturing of copolymers. 32 % of the produced vinyl acetate was manufactured to polyvinyl alcohol (BUA, 1993). Vinyl acetate occurs as residual monomer in homo- and copolymers based on vinyl acetate and in products or formulations based on these polymers. As polyvinyl alcohol is produced by trans esterfication (saponification) of vinyl acetate (co)polymers, residual vinyl acetate monomer does not occur in polyvinyl alcohol and in polymers derived from polyvinyl alcohol (Celanese, 2000). Hence the manufacturing of polyvinyl alcohol is not relevant in the context of residual monomer content.

Use pattern of vinyl acetate:

Main category	**Industry category**	**Use category**
Non dispersive use	Chemical industry: used in synthesis (IC 3)	Intermediate: monomer in the production of polyvinyl acetate (UC 33)

Table 2-2: Vinyl acetate in the value chain: (co)polymers are used in a wide spectrum of products (Wacker Chemie, 2002)

					Building material	**Printing ink**	**Paint**	**Film**	**Lacquer**	**Adhesive**	**Cosmetic, hairspray**	**Plastic**	**Paper finishing**	**Textile finishing**	**Non woven**
VAM	+	VAM	→	polyvinyl acetate	x	x	x		x	x		x	x	x	
VAM	+	acrylate	→	vinyl acrylic co-polymer	x		x						x	x	x
VAM	+	ethylene	→	EVA				x		x		x			
VAM	+	ethylene	→	VAE	x		x			x		x	x	x	x
VAM	+	vinyl chloride	→	VC copolymer		x			x	x		x	x		x
VAM	+	carbonic acid	→	VA carbonic acid copolymer							x				
VAM	+	vinyl ester	→	VA vinyl ester copolymer	x		x				x				

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.1.1 Releases into the environment

Vinyl acetate is used as an intermediate in the production of a range of polymeric materials. Releases of the monomer into the environment may be expected during various life cycle stages and from the final products and articles generated from VAM (cf. chapter 2.1). VAM is a comparatively volatile substance that is likely to evaporate into the atmospheric compartment, primarily via off-gas. Likewise, owing to the high water solubility of VAM, emissions may occur to the aqueous phase if the substance is getting into contact with water. Waste water may in turn be a relevant source for emissions to the atmosphere during treatment (e.g. stripping).

3.1.1.1.1 Production of the monomer

Vinyl acetate is produced by a catalytic reaction of ethylene and acetic acid. All commercial producers of vinyl acetate communicated closed system facilities in a largely continuous process. Nevertheless, start-up, shut-down and purification operations may result in some releases of VAM to waste products. Incineration of waste was reported by all but one producer, flue gas is commonly flared, and waste water typically undergoes a distillation procedure in order to regain the dissolved monomer. Measured values of VAM in waste water discharges from closed production systems show that losses can be significantly reduced by an adequate process design including pre-treatment of the waste water.

The aggregated capacity of the five vinyl acetate production facilities identified across the EU amounts to approximately 800,000 tonnes per annum (chapter 2.1.1.2). Specific emission data for releases of VAM to water and air have been provided by all producers. The figures are set out in Table 3-1 below.

From combined measured and estimated data it is concluded that emissions to surface water from production totalled approximately 0.486 t/a while emissions to air accounted for about 2.867 t/a.

Table 3-1: VAM releases from production to the aquatic and atmospheric compartment

Site	Production (t/year)	Release to surface water (kg/year)	Comment	Direct release to atmosphere (kg/year)	Comment
4	110,000	< 331	water treated within process (distillation) and then discharged to biological WWTP; concentration below d.l. of 50 µg/l	367	below d.l., figure communicated by company
21	200,000	< 155	emission to biological WWTP provided	-	incineration
22	100,000	< 0.36	water treated within purifying unit and external WWTP; calculated from d.l. of 0.1 mg/l and site-specific data	-	incineration
26	140,000	-	water is treated within process (distillation, no STP); no VAM detected in waste water*	2,500	emission reduction measures currently implemented***
27	250,000	-	plant designed to reduce emissions (no STP)**	-	waste gas from routine venting is flared
Σ	**800,000 t/year**	**486.36 kg/year**		**2,867 kg/year**	

* The waste water is treated within the process by distillation (no STP) and sent to a collection basin where organic compounds are measured before release to a waste water discharge canal and then to a river. Organic compounds are mostly acetates and acetaldehyde. No VAM is detected in the discharge (detection limit of < 1 ppm by ionic chromatography). Any residual VAM molecules are assumed to hydrolyse completely at a pH of 12 in the waste stream.

** The company operating the plant reported that new technology had been implemented in the recently commissioned facility in order to contain and recover VAM in the process (closed system process, distillation of wastewater for re-introduction of material in the process, incineration of remaining waste).

*** State of the art emission reduction measures (vapour recovery techniques) are currently being implemented in order to meet the technical standards of other production facilities in Europe and to obtain a permit in 2007 that is consistent with IPPC requirements. Emission reduction measures will be fully operative in 2009. The remaining emissions are expected to be approximately 2.5 tonnes per year.

3.1.1.1.2 Processing of vinyl acetate monomer

Table 2-2 shows the most common (co)polymers generated from VAM. It could not be elucidated for all processing sites, which notified being "users" of VAM in the CEFIC questionnaire, what kind of operations they actually perform. If not indicated otherwise, the term “use” or “processing” of VAM was interpreted as polymerisation of the monomer. Roughly, three types of processors can be distinguished:

- a few large-scale facilities which manufacture (co)polymers from VAM for resale
- some small- to medium-scale (SMC) facilities polymerising for captive use, e.g. manufacturers of paints, coatings or fibre
- the majority of SMC facilities which do not perform polymerisation but purchase VAM (co)polymers for compounding, i.e. mixing of vinyl acetate (co)polymers with e.g. fillers, binders and other polymeric dispersions

A default emission factor for waste water of 0.7 % of the annually processed volume is provided in the TGD for intermediates in chemical synthesis. The emission factor foreseen in the TGD is not considered representative for vinyl acetate and replaced by a more specific value of 0.02 %, which was communicated by industry representatives at a technical meeting[8] in 2002. The factor of 0.02 % is backed by the available site-specific data. The majority of facilities emitted less than 0.01 %, many even less than 0.001 % of their tonnage to waste water. Three sites emitted between 0.01 and 0.02 % of their tonnage, while only two exceeded the latter value. The highest emission factors were 0.04 and 0.15 %. The value of 0.02 % provided by industry is therefore assumed to be a realistic factor for estimating emissions.

Unlike for some of the SMC facilities, waste gas from routine venting is typically collected and flared in large operational sites. If no specific data were provided by the companies, it was assumed that 0.1 % of the polymerised material is being released to the atmosphere as VAM, which is the default emission factor for air provided in the TGD. The rapporteur considered to extrapolate information from the available site specific data in order to obtain a more representative fraction of release to air. The site specific emission factors ranged from less than 0.01 % to 0.5 %. Most facilities emitted between 0.01 and 0.1 % of their tonnage to air. The fraction of 0.1 % foreseen in the TGD is therefore considered a realistic and conservative value for estimating releases to air.

[8] As agreed on TM III'02, a technical meeting with industry took place in Frankfurt, Germany, in October 2002 to further the discussion on information and data to be included in the exposure scenario.

Table 3-2: VAM releases from processing sites to the aquatic and atmospheric compartment

Site	Processing (t/year)	Release to surface water (kg/year)	Comment	Direct release to atmosphere (kg/year)	Comment
1	30,000	15.8	calculated from d.l. of 50 µg/l, specific STP flow	430	figure provided by company in CEFIC questionnaire
2	10,000	1550	d.l. of 10 µg/l specific STP flow	2,630	from CEFIC questionnaire
3a 3b 3c 3d	6,000 10,000 8,000 4,000	100.2	calculated from given effluent concentrations and STP default values	28,000	from CEFIC questionnaire
5	90,000	331	see production site 4 (Table 3-1)	367	from CEFIC questionnaire
6	25,000	22.2	calculated from annual emission to waste water and STP flow	11,100	from CEFIC questionnaire
7*	10,810	3	generic calculation	7,110	aggregated data provided by CEPE max. value given mean: 1126 kg/a
8	15,000	230	annual emission to waste water, specific STP flow	31,000	from CEFIC questionnaire
9	9,100	5	annual emission to waste water, specific STP flow	14,000	from CEFIC questionnaire
10	15,000	1.13	annual emission to waste water, specific STP flow	14,000	from CEFIC questionnaire
11	110,000	84.5	d.l. of 5.7 µg/l and STP flow	-	incineration
12	36,000	720	no specific data on emissions but on STP flow	7,500	worst case scenario for diffuse releases, below d.l. at waste gas incinerator
13	280	-	no water involved in process	280	estimate based on release of 1 kg/t

Site	Processing (t/year)	Release to surface water (kg/year)	Comment	Direct release to atmosphere (kg/year)	Comment
14	2,000	40	generic calculation	2,000	from CEFIC questionnaire
15	2,200	6.5	annual emission to waste water, specific STP flow	1,500	from CEFIC questionnaire
16	400	-	no water involved in process	70	from CEFIC questionnaire
17	300	6	generic calculation	400	from CEFIC questionnaire
18	14,000	10	annual emission to waste water, specific STP flow	8,000	from CEFIC questionnaire
19	25,000	249	annual emission to surface water, specific STP flow	7,000	from CEFIC questionnaire
20**	0	0.1	annual emission to waste water	1	pers. commun.
23	17,000	-	pers. commun.	< 1.05E-05	pers. commun.
24	5,000	225	calculated from d.l. of 5 mg/l and effluent flow from process	24,925	pers. commun.
25	6,910	138	generic calculation	3,500	pers. commun.
28	8,000	160	generic calculation	8,000	estimate based on release of 1 kg/t
29	6,500	7.5	annual emission to waste water, specific STP flow	200	pers. commun.
30	8,400	1.39	calculated from given effluent concentrations and STP flow	-	pers. commun.
Σ	**474,900 t/year**	**3,906 kg/year**		**172,013 kg/year**	

* Aggregated data. The processing volume of 10,810 t/a in the table refers to the two companies still conducting polymerisation of VAM themselves. The total volume of ready to use *VAM polymers* in manufacture, however, added up to 43,524 tonnes in 2000. The magnitude of emissions in relation to *total VAM use* was specified as a maximum of 30 kg/year to waste water treatment plants, and a maximum of 7,110 kg/year to air by industry association CEPE.

** confirmation of conducting merely compounding operations, i.e. handling of the (co)polymers

As the difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a) is rather high, a generic worst case

scenario is included in the risk assessment to estimate the fraction of release at the local main source. The estimate is based on:

- a fraction of main source of 0.15 (Tab. B3.2 of TDG)
- a duration of emission of 300 d (Tab. B3.2 of TDG)
- a waste water flow of 10,000 m^3/s and a dilution of D = 40
- an emission factor to waste water of 0.02 % for processing
  (personal communication, technical meeting with industry representatives in 2002)
- a release fraction of 0.1 % of the polymerised material to air (as VAM)

For the local main source, a release of 9.75 t/a to waste water, of 975 kg/a to surface water, and of 48.75 t/a to the atmosphere is calculated.

For the whole processing volume of 325,000 t/a, a release of 65 t/a to waste water, of 6,5 t/a to surface water and of 325 t/a to the atmosphere is derived.

3.1.1.1.3 Compounding and use of materials containing vinyl acetate (co)polymers

Finished (co)polymers contain various concentrations of VAM residuals. Owing to the high volatility of VAM, it is anticipated that these residuals are exhaustively released during further life-cycle stages down the supply chain such as compounding into ready-to-use products.

Further diffusive releases from end-use products during service lifetime (e.g. adhesives, paints, fillers, coating materials, plasters and primers for porous building materials in the construction industry and in finishing agents; Ullmann, 1998) are expected to be low. Nevertheless, a contribution of these products to regional background concentrations was considered. Assuming that (co)polymers have on average a VAM content of 3,000 ppm (cf. section 2.2), the diffusive emissions may amount to 3,000 t/a based on a VAM market supply of roughly 1,000,000 t/a (cf. section 2.1.1.2). To estimate the fractional release to air and water, the fugacity of VAM was calculated (cf. Table 3-6): accordingly, 2,700 t/a (90 %) were assumed to be released to air while 300 t/a (10 %) would enter the aquatic compartment.

Polyvinyl alcohol is produced by trans-esterfication (saponification) of vinyl acetate (co)polymers. After this additional processing step, residual VAM will not be present in the finished product. Therefore, releases of VAM from polyvinyl alcohol were not estimated.

3.1.1.1.4 Storage and Transport

Gaseous releases of VAM could occur during storage and transport of the substance due to its high vapour pressure. However, a contribution to air emissions of these operations was not assumed because the use of supposedly closed-system containers and storage systems was reported. Furthermore, no tool is currently available to estimate the fractions which might be released during storage and transport despite the taken precautionary measures.

3.1.1.2 Degradation in the environment

3.1.1.2.1 Degradation in the atmosphere

Direct photolysis

Vinyl acetate molecules do not absorb light at wavelengths longer than 250 nm (Daniels, 1983). As solar irradiation wavelengths in the troposphere exceed 290 nm, no direct photo-transformation of VAM is expected to occur in this layer of the atmosphere.

Indirect photolysis

VAM undergoes rapid photooxidative transformation in air. It predominantly reacts with hydroxyl radicals and to a minor extent also with ozone by addition to the olefinic moiety.

Calculations were performed with the AOPWIN ™ (v1.91) computer program based on experimentally determined rate constants both for OH-radicals and ozone (cf. Appendix).

Based on a mean atmospheric concentration of [OH] = 5 x 10^5 cm^{-3} and an overall OH rate constant of 26.3 x 10^{-12} cm^3 $molec^{-1}$ s^{-1} (Atkinson et al., 1982), an atmospheric half-life of 14.6 hours is estimated for VAM. Assuming a rate constant of 25 x 10^{-12} as included in the AOPWIN ™ Experimental Database (Saunders et al., 1993), an atmospheric half-life of 15.4 hours results. The OH rate constant of 26.3 x 10^{-12} cm^3 $molec^{-1}$ s^{-1} and estimated atmospheric half-life of 14.6 hours were used in the risk assessment.

From a mean ozone concentration of 7 x 10^{11} cm^{-3}, a half-life of 6.55 days is derived.

Table 3-3: Reactivities and half-lives for phototransformation of VAM in the troposphere

Oxidative species	Species concentration	Rate constant (cm^3 $molec^{-1}$ s^{-1})	Half-life (days)	Source
OH radicals	5.0E+05 cm^{-3}	26.34E-12	0.608 (e)	Atkinson et al. (1982)
OH radicals	5.0E+05 cm^{-3}	25.00E-12	0.642 (e)	Saunders et al. (1993)
Ozone	7.0E+11 cm^{-3}	17.50E-19	6.55 (e)	Atkinson et al. (1982)
Ozone	4 - 8 ppm	3.2 ± 0.47E-18	2 (m)	Grosjean and Grosjean (1998)
NO_x	0.2 - 2.0 ppm		0.2 - 0.3 (m)	Joshi et al. (1982)

(e) = estimated (m) = measured value

In smog chamber experiments with NO_x concentrations simulating rural and urban atmospheres, photooxidative half-lives of VAM were determined to 4.1 ± 0.1 and 6.5 ± 3.0 hours, respectively (Joshi et al., 1982). The initial VAM concentration was 4 ppm (v/v) while NO_x levels were either 0.2 ppm (v/v) or 2 ppm.

3.1.1.2.2 Degradation in the aquatic compartment

Abiotic degradation

VAM undergoes hydrolysis in surface and groundwater. A hydrolytic half-life of 7.3 days at pH 7 and 25 °C was estimated for the compound (Mabey and Mill, 1978). From this value, a half-life of 17 days can be derived for a generic environmental temperature of 12 °C, as foreseen in the TGD. As pH decreases, the hydrolysis rate decreases and half-life increases. Main hydrolysis products of VAM are acetic acid and acetic aldehyde (Daniels, 1983; Stuckey et al., 1980).

Half-lifes of VAM in basic and acidic aquatic environments could not be obtained. However, hydrolysis rate constants were determined for VAM residuals in water-based dispersion paints, having typically an alkaline pH of 8 – 9. One paint was acidified to pH of 5.5 in order to determine the rate constant in an acidic solution. At pH 8.6, the half-life of VAM was found to be less than 4 days at room temperature, and a rate constant of 1.68 l mol^{-1} s^{-1} was experimentally determined. At a pH of 5.5, hydrolysis was found to be distinctly slower with a rate constant of 0.002 l mol^{-1} s^{-1} (kinetic data provided by industry, 2002). The observed hydrolysis rates in water-based and dispersion paints give further indication that hydrolysis of VAM decreases with pH, but cannot be transferred to aquatic environments.

The estimated half-life of 17 days at standard environmental parameters (pH 7 and 12 °C) is used in the risk assessment of VAM.

Biodegradation

In an initial degradation step, VAM is enzymatically hydrolysed to acetate and acetaldehyde, followed by either mineralization or biotranformation into biomass.

Based on a Ready Biodegradability Modified MITI Test I (OECD test guideline 301 C), measuring the mineralization of a test substance on the basis of oxygen consumption, vinyl acetate is classified as readily biodegradable (Chemicals Inspection and Testing Institute (Ed.), Japan, 1992). In the test, a biodegradation rate of 82-98 % was observed by BOD after a test period of 14 days and using non-adapted, activated sludge under aerobic conditions (pH 7, temperature 25 ± 1 °C, concentration of test substance at test begin: 100 mg/l).

The results of several other laboratory studies also demonstrate that VAM is readily biodegraded by domestic sewage effluent microorganisms both under aerobic (e.g. Pahren and Bloodgood, 1961; Price et al., 1974) and anaerobic conditions of sludge digesters (e.g. Chou et al., 1979; Stuckey et al., 1980). Although the tests were not conducted according to standardized test guidelines, they provide further evidence for the above classification as readily biodegradable.

In a more recent study (Nieder et al., 1990) it was demonstrated that VAM is biotransformed in samples of soil sludge and sewage under aerobic and anaerobic conditions. Four yeasts and 13 bacteria feeding aerobically on VAM were isolated. Rates of transformation were higher under aerobic conditions, but anaerobic and aerobic degradation pathways both yielded the same products, i.e. acetaldehyde as an intermediate and acetic acid as a final product. An aerobic transformation half-life of 12 hours was obtained with one of the bacterial isolates, whereas a half-life of 60 hours was found for the non-enzymatic hydrolysis in a sterile medium.

No tests are available that simulate the biodegradation of VAM in surface waters. Therefore, the rate constant and half-life for biodegradation in surface waters has to be derived based on the results from the available screening tests. For readily biodegradable substances a rate constant of 0.047 d^{-1} that is equivalent to a half-life of 15 d is proposed in the TGD (Table 3-4).

3.1.1.2.3 Degradation in soil and sediment

Vinyl acetate is also transformed by biotic and abiotic hydrolysis in soils and sediments. As described in the preceding section, microbial isolates from sludge and soil were found to be capable of utilizing VAM as a sole carbon source (Nieder et al., 1990).

There is evidence that some soil organisms are also capable of decomposing polymers of vinyl acetate. In an aqueous test medium containing 4.5 g/l of polyvinyl acetate as the sole carbon source, the polymers were biotransformed by oil fungi during an incubation period of 15 days at room temperature (Garcia Trejo, 1988). It should be noted, however, that de-polymerisation is assumed to be the limiting step. Further biodegradation is rapid so that relevant VAM concentration in environmental compartments are not to be expected.

Biodegradation rate constants were derived for the different environmental compartments according to section 2.3.6.5 of the TGD, based on the ready biodegradability of VAM as shown in a number of studies and a $Kp_{soil} < 100$. The rate constants are compiled in Table 3-4 below.

Table 3-4: Biodegradation rate constants of VAM in different environmental compartments

Compartment	Biodegradation rate
activated sludge (STP)	$k_{STP} = 1\ h^{-1}$
surface water	$k_{SW} = 0.047\ d^{-1}$
sediment	$k_{sed} = 0.00231\ d^{-1}$
soil	$k_{soil} = 0.023\ d^{-1}$

3.1.1.3 Distribution

No information on transport and partitioning of VAM between different environmental media was found in the available literature or was provided by industry. The distribution of the compound between air, water, sediment, and soil compartments following release to the environment was therefore assessed based on the physical and chemical properties of VAM.

3.1.1.3.1 Adsorption

Measured data on adsorption and desorption of VAM to soil or sediment could not be obtained. However, given an octanol - water partition coefficient of log K_{ow} = 0.73, it is concluded that VAM has only a low potential to adsorb to soils or sediments.

Using the QSAR regression equation given in the TGD for non-hydrophobic substances, an organic carbon - water partition coefficient of K_{oc} = 24.2 l/kg was calculated from the log K_{ow}.

Further solid - water partition coefficients (K_p) were derived from this K_{oc} for the various environmental compartments by using the generic values given in the TGD for organic carbon contents of soils and sediments (cf. Table 5, section 2.3.4 in the TGD).

Table 3-5: Partition coefficients of VAM for various compartments, derived according to the standard environmental characteristics of the TGD

Compartments	**Partition coefficients (l/kg)**
soil – water	Kp_{soil} = 0.484
sediment – water	Kp_{sed} = 1.211
suspended matter – water	Kp_{susp} = 2.421
activated sludge – water	Kp_{sludge} = 8.958
raw sewage – water	Kp_{sewage} = 7.263

3.1.1.3.2 Volatility

Given the high vapour pressure of VAM and a Henry's law constant of H = 51.6 Pa m^3 mol^{-1} (at 20 °C), a relevant transfer from the aqueous to the atmospheric compartment is expected. This transfer is mainly caused by volatilisation and air stripping during treatment of waste water, and to a lower degree by volatilisation from surface waters. The transfer to the atmosphere is to some extent counterbalanced by the high water solubility of the compound. Based on the Henry's law constant, VAM can be regarded as a moderately volatile compound.

3.1.1.3.3 Modelling of distribution

The (steady state) distribution of VAM between various environmental compartments at 12 °C was estimated by means of the Mackay level 1 six compartment fugacity model, employing the EQC Model v.1.0 and the physico-chemical data compiled in Table 1-1. The output, as shown in Table 3-6, indicates that the major environmental compartment for VAM is air, with a smaller proportion entering the aqueous compartment and just negligible quantities being predicted for other compartments.

Table 3-6: Mackay level 1 environmental distribution of vinyl acetate calculated from the OECD EQC model v.1.0 at 12 °C

Compartments	Distribution (%)
air	91.5
water	8.4
soil	0.037
sediment	< 0.001
suspended sediment	< 0.001
fish	< 0.001

3.1.1.3.4 Elimination in Sewage Treatment Plants (STP)

The fraction of VAM degraded in a municipal standard STP as well as the distribution of the residual VAM leaving the STP was estimated from SIMPLETREAT 3.0. Calculations were performed using the partition coefficients Kp_{sludge} = 8.958 l/kg, Kp_{sewage} = 7.263 l/kg and a first order biodegradation rate constant for VAM in a STP of 1 h^{-1}.

Table 3-7: Modelled fate of vinyl acetate in a standard municipal STP, according to the provisions of section 2.3.7 of the TGD

Fraction	Percent
directed to air	11.6
directed to sludge	0.2
degraded	78.2
total removal from waste water	90.0
directed to water	10.0

3.1.1.3.5 Accumulation

Bioaccumulation test results of VAM are not available. However, as a result of the high vapour pressure, ready degradability and low lipophilicity of the substance, it is neither expected to bioconcentrate in terrestrial or aquatic organisms, nor to biomagnify in food chains. This appraisal is further backed by results from QSAR calculations (BCFWIN v.2.14) which give a BCF of 3.16 (log BCF = 0.50) based on a log K_{ow} = 0.73 of VAM.

Geoaccumulation of VAM is not expected, based on a K_{oc} value of 24.2 l/kg along with effective biotic and abiotic breakdown processes in soil, hydrolysis and ready biodegradability in water, and rapid photooxidative breakdown of VAM in the atmosphere.

3.1.2 Exposure Assessment for the Aquatic Compartment

Exposure of the aquatic compartment to VAM may result from the production of VAM, from the polymerisation of the monomer, from compounding of polymer-containing formulations and eventually during subsequent use of the products. Water is generally involved in the stages of VAM production and consecutively VAM polymerisation as well as cleaning of the systems. In polymeric material, the residual monomer concentration decreases rapidly due to "outgassing". As the average VAM content in (co)polymers is assumed to be 3,000 ppm, it can be concluded that diffusive releases from end-use products will be a major exposure pathway. However, as the major amount of the residual monomer material will be released in the early stages of product lifetime, releases to the aquatic compartment after disposal to landfills is assumed to be relatively low compared to production and processing point sources.

Local point emission sources of VAM for the aqueous compartment can be roughly grouped into three categories:

- large-scale production and/or polymerisation of the monomer
- medium-scale sites which comprise polymerisation of VAM and compounding
- compounding operations

While there exist few large scale producers and/or polymerisers, the number of medium-scale polymerisers and notably compounders is considerably higher. An increasing lack of knowledge about VAM emissions down the supply chain has to be noticed. To this end the final destination of hundred thousands of tonnes of VAM produced or imported into Western Europe could not be elucidated within the scope of this report.

3.1.2.1 Estimation of local environmental concentrations ($Clocal_{water}$)

In general the local concentrations in water were calculated using the approach outlined in the TGD (section 2.3.8.3) and the corresponding annexes (A- and B-tables). The calculations involved the following steps:

(1) Emission per day: Derivation of the local daily emission rates ($Elocal_{water}$), based on emission data provided by industry or default values from the TGD.

(2) Influent concentration: Conversion of the local emission rate to a concentration in untreated waste water ($Clocal_{infl}$) by the effluent volume (default of 2,000 m^3/day or 10,000 m^3/day depending on tonnage, or site-specific data).

(3) Effluent concentration: Calculation of the concentration in the STP effluent ($Clocal_{effl}$) assuming a remaining fraction of 0.1 (90 % removal rate according to SIMPLETREAT calculation) of the influent going to the STP effluent.

(4) Concentration in surface water: Derivation of the local concentration in the aqueous compartment from the equation $Clocal_{water} = Clocal_{effl} / D$, with D = 10 or D = 40 depending on tonnage, or specific dilution factors supplied for the individual site.

(5) Average annual concentration in surface water: The average annual concentration was derived by multiplication of $Clocal_{water}$ by X/365, yielding $Clocal_{water\_annual}$, with X being the specific number of operation days per annum or a generic value of $T_{emission}$ = 300 days.

(6) PEClocal: The $PEClocal_{water}$ was derived by adding the $PECregional_{water}$ to the calculated value of $Clocal_{water}$, with $PECregional_{water}$ being taken from Simplebox calculations (see Appendix 4).

(7) Average annual $PEClocal_{water\_ann}$: This parameter was derived by addition of $PECregional_{water}$ to the calculated value of $Clocal_{water\_annual}$.

3.1.2.1.1 Production of the monomer

VAM is manufactured in closed systems at elevated temperature and pressure. Fractional distillation is applied to separate different constituents from the waste water and recuperate VAM to enhance reaction yields. Hence VAM concentration in waste water of production facilities is generally low. Site-specific data were made available from all VAM producers by providing figures either on annual loads to waste water or surface water or measured concentrations in waste water (cf. Table 3-8).

All producers of VAM are either located at large rivers, estuaries, or at the sea. Hence dilution of the waste water is high. Leaving aside shut-downs for reasons of maintenance work, continuous production was reported by all VAM manufacturers.

Table 3-8: Emissions to water from production sites of VAM in the European Union

Site	Production (t/year)	Release to STP (kg/year)	Release to surface water (kg/year)	$Clocal_{water}$ (µg/l)	Specific information provided/used
4	110,000	3.31E+03	< 3.31E+02	< 2.09E-01	effluent concentration below d.l. of 50 µg/l; STP effluent flow and dilution data
21	200,000	1.55E+03	<1.55E+02	6.80E-02	emission load, STP effluent flow and dilution data
22	100,000	3.60E+00	3.60E-01	4.00E-03	effluent concentration below d.l. of 0.1mg/l; STP effluent flow data
26	140,000	no STP*	-	-	effluent concentration below d.l. of 1mg/l; at pH 2 VAM hydrolyses completely in the waste stream
27	250,000	no STP**	-	-	TOC of waste water, dilution data
Σ	**800,000 t/year**	**4.86E+03 kg/year**	**4.86E+02 kg/year**		

* The waste water is treated within the process by distillation (no STP) and sent to a collection basin where organic compounds are measured before release to a waste water discharge canal and then to a river. No VAM is detected in the discharge (detection limit of < 1 ppm by ionic chromatography). Any residual VAM molecules are assumed to hydrolyse completely at a pH of 12 in the waste stream. ** The company operating the plant reported that new technology had been implemented in the recently commissioned facility in order to contain and recover VAM in the process (closed system process, distillation of wastewater for re-introduction of material in the process, incineration of remaining waste).

3.1.2.1.2 Processing of the monomer

Release from point-sources: verified processing sites

An emission factor for wastewater of 0.02 % was used in the calculations for the aquatic compartment if no site-specific emission data were provided (personal communication, technical meeting with industry representatives in 2002, cf. section 3.1.1.1.2).

Where no site-specific information on the flow rate of the STP or the receiving water course was given, the generic scenario of the TGD was applied: For low production volume facilities (≤ 1000 t/a), a domestic treatment plant scenario with a flow rate of 2,000 m^3/d and a dilution factor of 10 in surface water was assumed, whereas a flow rate of 10,000 m^3/d and a dilution

factor of 40 was assumed for high production volume facilities[9]. Most operators, however, provided specific data which were used in the calculations.

Pretreatment of raw waste water is common practice, e.g. by adding special zeolites or by electroflotation. The solid phase is subsequently removed (e.g. with a filter press), while the pre-purified waste water is sent to a municipal STP for further treatment. It is expected that at least 50 % of the residual VAM in waste water is retained with the filter cake. A preliminary purification step can therefore substantially reduce the content of total organic carbon in the waste water. Regarding the reported site-specific emissions or concentrations in waste water that were used in the exposure calculations, it is assumed that any internal pre-treatment is already reflected in the figures provided, if not indicated otherwise.

In few cases, VAM production and polymerisation were conducted at the same location, although not necessarily by one single company. If so, the individual emissions of both operations were added up to calculate one combined local concentration in the receiving surface water.

Table 3-9: Emissions of VAM to surface water from processing (polymerisation) sites of VAM in the European Union (fibre industry and paint/coating industry excluded)

Site	Processing (t/year)	Release to STP (kg/year)	Release to surface water (kg/year)	$Clocal_{water}$ (µg/l)	Specific information provided/used
1	30,000	1.58E+02	1.58E+01	4.81E-04	calculated from d.l. of 50 µg/l, STP effluent flow, dilution
2	10,000	1.55E+04	1.55E+03	6.60E-02	calculated from d.l. of 10 µg/l, STP effluent flow, dilution
3a	6,000	6.00E+02	6.00E+01	1.00E+01	calculated from d.l. of 100 µg/l
3b	10,000	3.00E+02	3.00E+01	5.00E-01	effluent concentr. of 50 µg/l
3c	8,000	2.40E+01	2.40E+00	4.00E-01	effluent concentr. of 4 µg/l
3d	4,000	7.80E+01	7.8E+00	1.3E+00	effluent concentr. of 13 µg/l
5	90,000	3.31E+03	3.31E+02	2.09E-01	calculated from d.l. of 50 µg/l, STP effluent flow, dilution
6	25,000	2.22E+02	2.22E+01	3.04E+00	calculated from emission to waste water, dilution
7*	10,810	3.00E+01	3.00E+00	5.00E-01	generic calculation
8	15,000	2.30E+03	2.30E+02	2.30E+00	emission to waste water, STP effluent flow
9	9,100	5.00E+01	5.00E+00	9.81E-01	emission to waste water, STP effluent flow

[9] default values according to emission scenario document for IC-3 (chemicals used in synthesis, TGD part IV)

Site	Processing (t/year)	Release to STP (kg/year)	Release to surface water (kg/year)	$Clocal_{water}$ (µg/l)	Specific information provided/used
10	15,000	1.13E+01	1.13E+00	3.10E-03	emission to waste water, STP effluent flow
11	110,000	8.45E+02	8.45E+01	1.32E-01	calculated from d.l. of 5.7 µg/l, STP effluent flow, dilution
12	36,000	7.20E+03	7.20E+02	2.10E+00	STP effluent flow, dilution
13	280	0.00E+00	0.00E+00	0.00E+00	no water used in process
14	2,000	4.00E+02	4.00E+01	7.69E+00	generic calculation
15	2,200	6.50E+01	6.50E+00	1.03E+01	emission to waste water, STP effluent flow, dilution
16	400	0.00E+00	0.00E+00	0.00E+00	no water used in process
17	300	6.00E+01	6.00E+00	1.16E-05	generic calculation
18	14,000	1.00E+02	1.00E+01	1.27E+00	emission to waste water, STP effluent flow
19	25,000	2.49E+03	2.49E+02	9.61E+00	emission to surface water, STP effluent flow
20**	0	1.00E+00	1.00E-01	1.91E-07	emission to waste water
23	17,000	0.00E+00	0.00E+00	0.00E+00	no emission to waste water
24	5,000	2.25E+03	2.25E+02	7.23E-03	calculated from d.l. of 5 mg/l, STP effluent flow, dilution
25	6,910	1.38E+03	1.38E+02	2.67E-05	generic calculation
28	8,000	1.60E+03	1.60E+02	1.33E+00	generic calculation
29	6,500	7.50E+01	7.50E+00	2.03E-03	emission to waste water, STP effluent flow, dilution
30	8,400	1.39E+01	1.39E+00	1.78E-03	emission to waste water, STP effluent flow, dilution
Σ	**474,900 t/year**	**39,063 kg/year**	**3906 kg/year**		

* The processing volume of 10,810 t/a in the table refers to the two companies still conducting polymerisation of VAM themselves. The total volume of ready to use *VAM polymers* in manufacture, however, added up to 43,524 tonnes in 2000. The magnitude of emissions in relation to *total VAM use* was specified as a maximum of 30 kg/year to waste water treatment plants by industry association CEPE.

** confirmation of conducting merely compounding operations. i.e. handling of the (co)polymers

Release from point-sources: non-verified generic site

Based on a difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a), a generic scenario was calculated for the local main source (fraction of main source = 0.15, cf. section 3.1.1.1.2).

A release of 9.75 t/a to waste water, of 975 kg/a to surface water and a $Clocal_{water}$ of 8.12 µg/l are derived.

Release from diffuse sources

A quantitative breakdown of the use pattern of VAM is a difficult task to perform since the notified annual production volume in Western Europe (800,000 t/a) considerably exceeds that of the verified industrial uses (475,000 t/a). For a conservative estimate of the diffusive releases from end-use products during service lifetime, a VAM total market supply of about 1,000,000 t/a and an average VAM content of 3,000 ppm in (co)polymers is assumed, leading to an estimated total release of 3,000 t/a, with a fractional release of 300 t/a (10 %) directed to the aquatic compartment (cf. section 3.1.1.1.3).

3.1.2.1.3 Manufacture of synthetic fibres

According to information provided by the industry association of European Fibre Producers CIRFS (**C**omité **I**nternational de la **R**ayonne et des **F**ibres **S**ynthetiques) in the CEFIC questionnaire of 2000, seven sites in five different countries (including Turkey) were using VAM in the manufacture of acrylic fibres at approximately 60,000 t/a. With a share of around 25 % accounting for Turkey, 45,000 t/a were processed at Western Europe sites.

More recent data provided by CIRFS in 2004 reveals that acrylic fibre production in the EU takes place at four sites with a combined capacity of up to 28,000 t/a (sites 3a-3d, Table 3-9).

VAM is used in the production of acrylic fibre strands in proportions of less than 10 % by weight in the finished product. The filaments are coagulated and subsequently stretched in wet processes, thereby leaving some VAM in the process water. Reportedly, the process water is circulated in closed loop systems and finally discharged. All following calculations are based on site-specific effluent concentrations and default values for STP effluent flow and dilution.

Emissions from downstream manufacturing are "none or very low" according to the information provided by CIRFS in 2000.

3.1.2.1.4 Manufacture of coatings and printing inks

As communicated by industry association CEPE (**C**onseil **E**uropéen des Industries des **P**eintures, **E**ncres d'Imprimerie et Couleurs d'Art), most of the manufacturers buy polymer emulsions (PVA) ready for use in paints and do not polymerise VAM by themselves.

According to a questionnaire launched by CEPE in the year 2000, 52 companies used vinyl acetate polymers (PVA) at 116 different sites in the EU for making printing inks, paintings and coatings. The total quantity of processed polymers added up to 43,524 tonnes, of which 40,419 tonnes were used in water-born products. The largest volume of PVA used on one site was 8,900 tonnes (figure for 2002).

According to information provided by CEPE in 2003, only two companies still conducted polymerisation of VAM in the EU in 2002, at one single site each. Reported annual volumes were about 7,120 tonnes and 3,690 tonnes, respectively (combined capacity of 10,810 tonnes).

For reasons of confidentiality only ranges of processed PVA volumes and arithmetic means of emission data have been provided by CEPE rather than specific values of individual sites. Absolute quantities of VAM emissions to water were reported by CEPE to be in a range of 0 - 30 kg/a (mean of 2 kg/a). Related to the site-specific processed VAM volumes, the emissions to water were between 0 - 2 ppm, at a mean of 0.7 ppm. Employing the generic values tabled below, a local concentration of $Clocal_{water}$ = 0.5 µg/l is predicted from a reported max. annual emission to waste water of 30 kg (site 7 in Table 3-9).

Table 3-10: Parameters used in calculation of $Clocal_{water}$ for a coatings and printing inks site

Parameter	**Figure**	**Source**
Maximum annual emission to waste water	30 kg/a	provided by CEPE in 2002
Operation	300 days *	TGD default, B 3.9
STP effluent flow	2,000 m³/d	TGD default
Dilution factor	10	TGD default

* Albeit the number of days where batches containing VAM polymers being manufactured were reported to cover a span of 4 to 365 days per year (mean 127 d/a) it could not be elucidated if continuous operation (= 365 d/a) can be attributed to the highest volume.

According to CEPE, waste water from the manufacturing plants handling the vinyl acetate monomer is typically treated on-site to remove solids (oligomers) prior to discharge to a municipal waste water treatment plant. Measured VAM concentrations after this pretreatment were reported in a range of 1 - 10 µg/l, at a mean of 6 µg/l. If the highest measured concentration of 10 µg/l is chosen for calculation (instead of a maximum annual emission of 30 kg) and assuming a 90 % removal rate and a dilution factor of 10 in the receiving river, a local concentration of $Clocal_{water}$ = 0.1 µg/l was calculated for surface waters. However, the above given $Clocal_{water}$ of 0.5 µg/l was used as a worst case value for the risk characterization for coatings and printing inks manufacturers (no. 7).

Typical contents of VA polymers in paints and inks span a broad range of 0.6 % up to 60 %, averaging 18 %. As notified by 53 manufacturers of paints and inks, the VAM residuals in their products range from 0.0003 % to 0.2 %, averaging 0.07 % (aggregated data, CEPE 2000). In the process of incorporating the polymer emulsions into the paint product, however, it can be expected that the residual VAM content is decreased due to evaporation or

hydrolysis into acetic acid and acetaldehyde. At an alkaline pH of 8 – 9 which is typical for finished water-based paints, VAM residuals within the formulated paints are rapidly hydrolysed at a rate constant of k_{Hydro} = 1.6 l mol^{-1} s^{-1} (corresponding to a half-life of less than 4 days). Therefore, residual VAM levels in water-based paints will be negligibly low once the product enters the market and is used. In contrast, hydrolysis is distinctively slower in acidified dispersion paints, with a rate constant of k = 0.002 l mol^{-1} s^{-1} (kinetic data provided by industry in 2002).

3.1.2.1.5 Manufacture of adhesives

According to the European Adhesives Manufactures Association FEICA (**F**édération **E**uropéenne des **I**ndustries de **C**olles et **A**dhésifs) the vast majority of manufacturers of adhesives and tackifiers undertake only compounding, thereby utilizing ready-made polymer formulations. Nonetheless, some sites were found out to conduct also VAM polymerisation for captive use. Unlike for other industry associations which submitted data in aggregated form, manufacturers of adhesives provided data on individual sites for the CEFIC questionnaire (sites 14-20, Table 3-9)

3.1.2.1.6 Paper recycling and use of printing inks and paintings

From the high vapour pressure of the monomer, it is concluded that paper and cardboard will have lost all VAM residuals during service lifetime. When finally arriving at the recycling plant no residuals are anticipated to be included in the material any more. Hence, recycling of paper and cardboard is not deemed to be a relevant source of VAM for the hydrosphere. The same applies to printing inks, coatings and paintings. No PEClocal for paper recycling was therefore derived.

3.1.2.2 Measured data in aqueous environment

Measured data of VAM in the aqueous compartment are either not available from the open literature or relate to emission levels several decades ago. The available data may therefore be representative for the historical but not for the present situation.

In the CEFIC questionnaire, producers and users of VAM were asked to provide monitoring data on VAM concentrations in the surface water bodies receiving their discharges. The companies either stated that no data is available, or commented that VAM cannot be measured in surface water bodies as the concentration in the WWTP effluent is already below the analytical detection limit. The notified detection limits at the outlets ranged from 5 - 100 µg/l.

3.1.3 Exposure Assessment for the Atmosphere

3.1.3.1 Estimation of local concentrations ($Clocal_{air}$)

Within the CEFIC questionnaire some responding companies (majority of the VAM producers and several sites undertaking polymerisation) provided data on the VAM quantities released to air on an annual basis, or provided figures on measured aerial concentrations. Some other companies indicated data on request.

3.1.3.1.1 Production of the Monomer

Almost all of the producers of VAM confirmed that routine venting of the plant is carried out and that the exhaust gas is flared. However, there is no evidence that this can be assumed for all plants. Moreover, diffuse releases from flanges, pumps etc. cannot be totally excluded.

Measured site-specific data was provided by all but one facility, and was used to override defaults in calculations. The producer communicating no data argued that no particular control data is available because of running a "closed circuit system" without emissions. Due to the high volatility of vinyl acetate, the amount which is released via indirect emissions from STP cannot be ignored. In the tables below the amounts from production/processing and from STP are summed up under "total releases to air".

Table 3-11: Estimation of local VAM concentrations in air and soil for VAM production sites

Site	$Clocal_{air}$ ($\mu g/m^3$)	DEP_{total_ann} ($mg/m^2 \cdot d^1$)	Comments	Direct releases to air (t/year)	Total releases to air (t/year)
4	0.3	8.20E-04	maximum value calculated from limit of detection	< 0.37	7.51E-01
21	1.35E-01	1.95E-04	incineration and indirect release from STP	0	1.78E-01
22	3.87E-04	5.57E-07	incineration and indirect release from STP	0	4.18E-04
26	2.88E+02	2.38E-01	specific data	2.5	2.5
27	0	0	air emissions do not occur due to closed system, distillation of waste water and waste incineration (no STP)	0	0
Σ				**2.87**	**3.43**

3.1.3.1.2 Processing of the Monomer

Release from point-sources: verified processing sites

The term "use" was interpreted by respondents to the CEFIC questionnaire in different ways: as synonym for any kind of activity downstream of production, i.e. polymerisation of VAM and/or handling and packing of the polymer solutions and resins (see steps 2 and 3 in section 2.1). Consequently, it could not be clarified which kind of activities each "user" actually performed.

Some of the respondents provided site-specific emission loads of VAM to the atmosphere. Releases were estimated for those sites that provided no data, with estimates ranging from as low as 0.4 x 10^{-3} % up to 0.2 % of the processed volume. The facilities which manufacture high volumes of the polymer(s) all reported low emissions of approximately 5 x 10^{-3} kg/t, due to incineration of off-gas. Small and medium-scale facilities typically emit around 1 kg/t to air, although some employ some sort of purification equipment.

Table 3-12: Estimated local concentrations of VAM in air and deposition to soil

Site	$Clocal_{air}$ ($\mu g/m^3$)	DEP_{total_ann} ($mg/m^2 \cdot d^1$)	Comments	Direct releases to air (t/year)	Total releases to air (t/year)
1	3.28E-01	4.91E-04	site-specific data	4.30E-01	4.48E-01
2	2.00E+00	4.85E-03	site-specific data	2.63E+00	4.43E+00
3a	5.56E+00	8.09E-03	based on estimates	6.0E+00	6.07E+00
3b	9.27E+00	1.30E-02	based on estimates	1.0E+01	1.003E+01
3c	7.41E+00	1.10E-02	based on estimates	8.0E+00	8.003E+00
3d	3.71E+00	5.35E-03	based on estimates	4.00E+00	4.009E+00
5	2.93E-01	8.23E-04	site-specific data	3.67E-01	7.51E-01
6	8.45E+00	1.20E-02	site-specific data	1.11E+01	1.11E+01
7	5.4	7.8E-03	based on estimates	7.11E+00	7.113E+00
8	2.40E+01	3.40E-02	site-specific data	3.10E+01	3.13E+01
9	1.10E+01	1.50E-02	site-specific data	1.40E+01	1.40E+01
10	1.10E+01	1.50E-02	site-specific data	1.40E+01	1.40E+01
11	1.58E-03	2.27E-06	site-specific data	0	2.08E-03
12	5.71E+00	9.14E-03	site-specific data	7.50E+00	8.34E+00
13	2.99E-01	4.31E-04	calculation based on default of 1 kg/t	2.80E-01	2.80E-01
14	2.14E+00	5.58E-03	calculation based on default of 1 kg/t	2.00E+00	3.62E+00
15	1.60E+00	2.32E-03	site-specific data	1.50E+00	1.51E+00

Site	$Clocal_{air}$ ($\mu g/m^3$)	DEP_{total_ann} ($mg/m^2 \cdot d^1$)	Comments	Direct releases to air (t/year)	Total releases to air (t/year)
16	9.7E+01	1.40E-01	site-specific data	7.00E-02	7.00E-02
17	3.71E-01	5.43E-04	site-specific data	4.00E-01	4.07E-01
18	7.41E-03	1.10E-02	site-specific data	8.00E+00	8.01E+00
19	6.49E-03	9.72E-03	site-specific data	7.00E+00	7.29E+00
20	9.27E-04	1.49E-06	site-specific data	1.00E-03	1.12E-03
23	8.01E-06	1.15E-08	site-specific data	1.05E-05	1.10E-05
24	1.90E+01	2.80E-02	site-specific data	2.49E+01	2.52E+01
25	3.24E+00	4.88E-03	site-specific data	3.50E+00	3.66E+00
28	7.41E+00	1.10E-02	site-specific data	8.00E+00	8.19E+00
29	2.22E-01	3.34E-04	site-specific data	2.00E-01	2.09E-01
30	1.48E-03	2.13E-06	site-specific data	0	1.60E-03
Σ				**1.72E+02**	**1.78E+02**

Release from point-sources: non-verified generic site

Based on a difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a), a generic scenario was calculated for the local main source (fraction of main source = 0.15, cf. section 3.1.1.1.2).

A $Clocal_{air}$ of 45 $\mu g/m^3$, a DEP_{total_ann} of 5.5E-02 $mg/m^2 \cdot d^1$, a direct release of 48.75 t/a and a total release of 3.33 E+02 t/a to air are derived.

Release from diffuse sources

For a conservative estimate of the diffusive releases from end-use products during service lifetime, a VAM total market supply of about 1,000,000 t/a and an average VAM content of 3,000 ppm in (co)polymers is assumed, leading to an estimated total release of 3,000 t/a, with a fractional release of 2,700 t/a (90 %) directed to the atmosphere (cf. section 3.1.1.1.3).

3.1.3.1.3 Manufacture of synthetic fibres

As communicated by CIRFS (2002), approximately 1 per mill (1 kg/t) of VAM is released to air. For fibre strands production (sites 3a-3d, Table 3-12), local concentrations in air and corresponding deposition rates are therefore based on a fraction of emission of 0.001 (0.1 %).

3.1.3.1.4 Manufacture of coatings and printing inks

According to aggregated data provided by industry association CEPE (Conseil Européen des Industries des Peintures, Encres d'Imprimerie et Couleurs d'Art), the air emissions of facilities manufacturing paints and printing inks range from zero to > 7 t/a, with a mean of 1.1 t/a (based on 53 companies responding to a CEPE questionnaire in 2000).

The maximum reported value of 7.11 t/a was used to calculate local air concentrations ($Clocal_{air}$) and deposition rates (DEPtotal) for a printing ink / coating manufacturing site as a worst case scenario, following the provisions of the TGD (chapter 2.3.8.2). The results are provided in Table 3-12 (site 7).

Annual release to air by this industry sector is approximately 0.1 % of the reported annual use volume of 43,524 t/a. An estimated release of 43.5 t/a by all facilities manufacturing paints and printing inks is used in the regional assessment.

3.1.3.1.5 Manufacturing of adhesives

The calculated local concentrations of VAM in air and soil adjacent to adhesive manufacturing sites are included in Table 3-12 (sites 14-20).

3.1.3.2 Measured data in the atmosphere

Only very few measured vinyl acetate concentrations in ambient air are available. Vinyl acetate was detected in the atmosphere of an industrial area in Texas (USA) in 1974 in concentrations up to 2 mg m^{-3}. In the ambient air of a landfill in New Jersey (USA), concentrations of 0.5 µg m^{-3} were measured in 1976. In a more recent study of 1990/91, cited in the SIDS Dossier, vinyl acetate was not detected in ambient air in Texas (representing the background concentration) at a detection limit of 2 ppb.

A monitoring campaign on non-methane volatile organic compounds (VOCs) was carried out in Italy, Spain and Germany, encompassing urban areas, suburban areas, forest-rural and remote areas. VAM was not detected in all samples but occurred in "traces" in 6 % of the samples from remote areas, with "traces" meaning "less than 0.5 % w/w of a total VOC concentration of 20-200 µg/m^3" (Ciccioli et al., 1994). The results were interpreted by the authors in a way that biogenic sources contribute substantial parts to the overall emissions of VOCs. In addition, VAM was formed under conditions simulating incineration of pine wood and wheat straw. Forest fires and agricultural practises were therefore deemed to be a further anthropogenic source of VAM, particularly in Mediterranean countries (Ciccioli et al., 2001).

3.1.4 Exposure assessment for the terrestrial compartment

VAM may enter the terrestrial compartment by aerial deposition or by spreading of contaminated sewage sludge on soils. Based on the water solubility and octanol-water partition coefficient of VAM, it is anticipated that VAM is relatively mobile in soil. This is backed by QSAR calculations on the K_{oc}, suggesting only little affinity to organic material.

Information provided by industry indicates that only small amounts of industrial sludge from VAM production and processing facilities is spread on soils. The majority of companies indicated that contaminated sludge is either incinerated or landfilled.

Sludge from production sites is either incinerated (2 sites), landfilled (1 site) or does not emerge at all since no WWTP is involved (2 sites). The same applies also for major polymerisation facilities. Several facilities indicated pre-treatment (e.g. electroflotation) of waste water on-site. Such treatment upstream of discharge to municipal sewer systems is to reduce TOC contents. The cakes from filter presses are usually incinerated or transported to a landfill. However, no data was provided for a few small-size facilities.

Estimates on the fate of VAM in waste water treatment plants revealed that only 0.2 % of the VAM load entering the WWTP will remain within the sludge. Further reduction in sludge concentrations is anticipated from subsequent digestion. From these considerations it is concluded that the main source of release of VAM to soil will be deposition from the atmospheric compartment after local releases to air from point sources. Small quantities of VAM entering the soil will be swiftly degraded (section 3.1.1.2.3).

As a worst case approach, soil concentrations were estimated for the two sites having the highest deposition rates of $DEPtotal_{ann}$ = 238 µg m^{-2} d^{-1} (site 26) and $DEPtotal_{ann}$ = 140 µg m^{-2} d^{-1} (16) according to the provisions of the TGD. Both facilities do not operate a STP so that sludge application is not included in the estimate. Soil concentrations were also calculated for several sites that had relatively high deposition rates and reported that sludge is being used on agricultural soils (Table 3-13).

Table 3-13: Local concentrations of VAM in soil and in soil porewater

	DEP_{total_ann} ($mg/m^2 \cdot d^1$)	C_{sludge} (mg/kg)	Clocal for					
			soil (mg/kg)	agric. soil (mg/kg)	grass-land (mg/kg)	soil porewater (mg/l)	agric. soil porewater (mg/l)	grassland porewater (mg/l)
26	2.74E-03	No STP	9.95E-05	9.95E-05	1.16E-04	1.84E-04	1.84E-04	2.154E-04
16	1.40E-01	No STP	5.16E-03	5.16E-03	6.03E-03	9.42E-03	9.42E-03	1.10E-02
3a	8.09E-03	0.56	6.13E-04	3.56E-04	3.63E-04	1.12E-03	6.50E-04	6.62E-04
3b	1.30E-02	0.28	6.36E-04	5.08E-04	5.67E-04	1.17E-03	9.27E-04	1.04E-03
3c	1.10E-02	0.02	4.18E-04	4.08E-04	4.74E-04	7.63E-04	7.44E-04	8.66E-04
3d	5.35E-03	0.07	2.40E-04	2.05E-04	2.34E-04	4.38E-04	3.77E-04	4.28E-04
6	1.20E-02	1.71	1.40E-03	6.17E-04	5.58E-04	2.55E-03	1.13E-03	1.02E-03

Furthermore, a generic scenario was calculated for the local main source (fraction of main source = 0.15, cf. section 3.1.1.1.2), based on a difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a). From a DEP_{total_ann} of 5.5E-02 $mg/m^2 \cdot d^1$ and a C_{sludge} of 20.9 mg/kg, a $Clocal_{soil}$ of 0.014 mg/kg is calculated.

3.1.5 Non compartment specific exposure relevant to the food chain

As the physical-chemical properties of VAM do not indicate any relevant bio-accumulation potential, the potential for secondary poisoning is considered to be low.

3.1.6 Regional concentrations

For the estimation of the regional background concentrations, the following releases from diffuse as well as point sources are taken into account.

Point source releases to surface water

For the production and processing sites included in the CEFIC survey (cf. Table 3-1 and Table 3-2), annual releases of vinyl acetate to waste water were calculated, either based on default estimations or site-specific data provided. The identified production and processing sites have a combined annual load of 40 tonnes VAM, which is expected to end up in waste waters.

Not included in this estimated total release are the sites located at the sea (3b, 22, 27). The marine sites were exempted as they are not assumed to contribute to the PECregional for surface (fresh) water, that is used for risk characterisation of inland sites in section 3.3.1. A marine risk assessment is included in Section 3.3.5.2.

Accounting for the difference between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a), a generic scenario was calculated for the remaining processing volume of 325,000 t/a. A release fraction of 0.02 % for waste water as communicated by industry was assumed to calculate a total release of 65 tonnes/year.

Point source releases to air

For the production and processing sites included in the CEFIC survey (cf. Table 3-1 and Table 3-2), annual releases of vinyl acetate to atmosphere were calculated, either based on default estimations or site-specific data provided. The identified production and processing sites have a combined annual release of 175 tonnes VAM, which is expected to be released to the atmosphere. SimpleBox calculations of the PECregional for air take these direct releases as well as indirect releases of VAM from STPs into account.

Similar to surface water, a generic scenario was calculated. Applying a release fraction of 0.1 % to air as communicated by industry to the remaining processing volume of 325,000 t/a, results in a total release of 325 t/a.

Point source releases to soil

No point sources were identified for the soil compartment.

Diffuse releases

Residual monomers of vinyl acetate are diffusively released from products containing vinyl acetate polymers to the environment. Due to the high vapour pressure of the substance, it is assumed that around 90 % of the residual vinyl acetate contained in polymeric products enter

the atmosphere, whilst 10 % are released into water during processing and formulation of the polymeric products.

Based on a residual VAM content of 3,000 ppm and a processing volume of roughly 1,000,000 tonnes, an annual release of 3,000 t/a can be estimated. It is assumed that 2,700 t/a (90 %) of the total annual release enter the atmosphere, whilst 300 t/a (10 %) are released into waste water. Contrary to TGD specifications it is assumed that 100 % of the waste water is treated in WWTP because all waste water is produced by industrial facilities.

The following table summarizes the estimated emissions of vinyl acetate from different point and diffuse sources to water and atmosphere:

	Emission to waste water (tonnes/year)	**Direct release to air (tonnes/year)**
Release from point-sources: verified processing sites	40.31	174.52
Release from point-sources: non-verified (based on 325,000 t/a)	65.00	325.00
Diffuse release from end-use products containing VAM	300.00	2,700.00
Total	405.31	3,199.52
Regional (10 %)	40.53	319.95
Continental (90 %)	364.78	2,879.56

Continental and regional background concentrations of vinyl acetate were calculated. A share of 90 % of the total releases was allotted to the continental scale, while 10 % was allocated to the regional sector. The software tool SimpleBox 2.0a was used to predict the following continental and regional environmental concentrations (see Appendix 4):

Table 3-14: Regional and continental concentrations (PECs) according to SimpleBox 2.0a

Continental PECs		**Regional PECs**	
$PECcont_{surfacewater}$	9.22E-07 mg l^{-1}	$PECreg_{surfacewater}$	8.33E-06 mg l^{-1}
$PECcont_{air}$	1.87E-06 mg m^{-3}	$PECreg_{air}$	9.67E-06 mg m^{-3}
$PECcont_{agr.soil}$	1.10E-07 mg kg_{wwt}^{-1}	$PECreg_{agr.soil}$	9.05E-07 mg kg_{wwt}^{-1}
$PECcont_{agr.soil,porew}$	2.02E-07 mg l^{-1}	$PECreg_{agr.soil,porew}$	1.66E-06 mg l^{-1}
$PECcont_{nat.soil}$	4.64E-08 mg kg_{wwt}^{-1}	$PECreg_{nat.soil}$	2.40E-07 mg kg_{wwt}^{-1}

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Effects assessment for the aquatic compartment (including sediment)

3.2.1.1 Test results

3.2.1.1.1 Criteria for validation

Despite being a HPVC with a market supply of more than 1 million tonnes per year, only a comparatively small data set is available on the ecotoxic effects of VAM. Toxicity studies can only be classified as valid if suitable precautions have been taken to prevent decreasing exposure concentrations of the test substance due to volatilisation during the test (e.g. flow-through or sealed vessels). Furthermore, exposure concentrations should be measured in the course of the study. Test results which were obtained under static conditions without analytical determination of test substance concentrations can only give a rough estimate of the ecotoxic effects, since the possible decrease in test concentrations due to volatilisation is not considered. In these cases it has to be assumed that the effective concentrations are lower as relevant parts of the test substance might have evaporated during the course of the test.

The most relevant test results for aquatic vertebrates, invertebrates and algae are summarized in Table 3-15 and are described in more detail below.

Table 3-15: Summary of the most relevant results of toxicity tests with aquatic vertebrates, invertebrates and plants (algae)

Species	Duration	Test design / remarks	Endpoint	Concentration (mg/l)	Reference	Validity
Vertebrates						
Lepomis macrochirus	96 h	static (open system) endpoint mortality	96 h-LC_{50}	adult: 18 (n)	Pickering & Henderson (1966)	not valid
Pimephales promelas	96 h	static (open system) endpoint mortality	96 h-LC_{50}	adult: 19.7 (n) 1 d old juveniles: 14-15 (n) 2 d old juveniles: 15 (n)	Pickering & Henderson (1966)	not valid
Pimephales promelas	34 d	OECD 210 (Fish Early Life Stage) flow-through, analytical data	34 d-NOEC 34 d-LOEC	0.55 (m) 0.93 (m)	ASG/CEFIC (2003b)	valid
Invertebrates						
Daphnia magna	48 h	OECD 202, semi-static, sealed test vessels, analytical data, endpoint immobilisation	24 h EC_{50} 48 h-EC_{50}	24.0 (m) 12.6 (m)	ASG/CEFIC (2003a)	valid
Daphnia magna	24 h	static (open system) endpoint immobilisation	24 h-LC_{50}	52 (n)	Bringmann & Kühn (1982)	not valid
Artemia salina	24 h	static (open system) endpoint mortality	24 h-LC_{50}	45 (n)	Price et al. (1974)	not valid

Species	Duration	Test design / remarks	Endpoint	Concentration (mg/l)	Reference	Validity
Ophryotrocha diadema (sediment dwelling, marine)	48 h	determined one week after exposure, endpoint mortality	48 h-LC_{50}	33 (n)	Parker (1983)	not valid
Plants (algae)						
Pseudokirchneriella subcapitata *(Selenastrum capricornutum)*	96 h	OECD 201, static, sealed test vessels with zero headspace, analytical data	72 h-E_RC50 72 h-NOEC	12.7 (m) 5.96 (m)	ASG/CEFIC (2003c)	valid
Scenedesmus quadricauda	8 d	cell multiplication inhibition test	8 d-EC_3	370 (n)	Bringmann & Kühn (1977a)	not valid
Microcystis aeruginosa	8 d	cell multiplication inhibition test	8 d-EC_3	35 (n)	Bringmann & Kühn (1976, 1978a, 1978b)	not valid

(n) nominal concentration (m) measured concentration

3.2.1.1.2 Fish

Acute toxicity

Pickering and Henderson (1966) examined the effects of short term exposure of VAM in different species of fish. Tests were performed according to the methods recommended by the American Public Health Association (Standard Methods for the Examination of Water and Waste Water, 11th Ed., 1960). Fish were exposed in open, static systems for 96 hours. Different concentrations of test solution were prepared in a logarithmic series ranging nominally from 10 to 100 mg/l (temperature 25 °C, dissolved oxygen 7.8 mg/l, pH 7.5 - 8.2, water hardness as EDTA 20 - 172 mg/l, no aeration throughout the test period). The test results obtained for bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*) are summarized in Table 3-15. As no chemical measurements were available the derived effect concentrations were related to nominal concentrations. The test results are therefore only of limited use for the effects assessment of VAM.

Long-term toxicity

The chronic toxicity of VAM to the early life-stages of fathead minnow (*Pimephales promelas*) was examined under flow-through conditions by ASG/CEFIC (2003b) according to OECD-Guideline 210. One hundred embryos (< 30-hours post fertilization, 4 replicates of 25 embryos) per dose level and control were exposed to nominal concentrations of 0.389, 0.648, 1.08, 1.80, 3.00 and 5.00 mg vinyl acetate per litre. All test aquaria were sampled weekly throughout the duration of the study (days 0, 7, 14, 21, 28 and 34) and the collected samples were analysed for vinyl acetate using GC/EI-MS. The measured test solution concentrations were variable and did not remain within +/- 20 % of the mean measured concentrations over the course of the study. Study average percent of target values for vinyl acetate ranged from 41.8 to 60.8 % and resulted in mean measured exposure concentrations of 0.163, 0.353, 0.551, 0.930, 1.71 and 3.04 mg/l.

The test system was maintained for 31 days post hatch of the control embryos (34 days total). Based on mean measured concentrations, the no-observed-effect concentration (NOEC) and lowest observed-effect concentration (LOEC) were statistically determined for the following endpoints:

- percent of embryos that hatched (embryo survival)
- time to hatch (day-to-mean hatch)
- percent of normal larvae (larvae exhibiting no lethal or sublethal effects) at test termination
- percent larvae survival
- percent overall survival and growth (length and weight)

If the raw data (or alternatively the transformed variables after logarithmic inverse or square root transformation) were found to be both normal (Shapiro-Wilk's test) and homogeneous (Bartlett's test), a parametric analysis was conducted with a Dunnett's test. Data that was not

normally and/or homogeneous were analysed parametrically with a Steels' Many-one rank test or Kruskal-Wallis test.

The test results can be summarised as follows:

- No statistically significant ($\alpha = 0.05$) effects were observed for the percent of embryos hatched and the time to hatch endpoints up through the highest exposure level of 3.04 mg/l tested for these endpoints.

- No statistically significant ($\alpha = 0.05$) effects were observed for the percent normal larvae at test termination, the percent larvae survival, and the percent overall survival up to the exposure concentration of 0.551 mg/l. Statistically significant effects for these endpoints were observed at exposure levels of 0.93 mg/l and above. These effects showed a clear concentration-dependency: While 93.9 ± 13.8 % of the larvae survived and showed normal development at a test concentration of 0.551 mg/l, this fraction was reduced to 78.0 ± 5.2 % at the next higher exposure level of 0.93 mg/l, to 32.5 ± 11.0 % at 1.71 mg/l, and to zero at the highest exposure level of 3.04 mg/l.

- No statistically significant (alpha = 0.05) differences in growth (lengths, weights) of surviving organisms at study termination were observed up through the highest exposure level tested for this endpoint (1.71 mg/l).

Therefore, NOEC and LOEC were determined with 0.551 and 0.930 mg/l.

Table 3-16: Chronic toxicity of VAM for early life stages of *Pimephales promelas*

Parameter	**NOEC (m) (mg/l)**	**LOEC (m) (mg/l)**
Weight (mg)	1.71	> 1.71
Length (mm)	1.71	> 1.71
Percent embryos hatched	3.04	> 3.04
Time to hatch	3.04	> 3.04
Percent normal larvae	0.551	0.930
Percent larvae survival	0.551	0.930
Percent overall survival	0.551	0.930

(m) measured concentration

3.2.1.1.3 Aquatic invertebrates

Acute toxicity

An acute 48-hour *Daphnia magna* toxicity test with VAM was conducted under semi-static conditions according to the OECD-Guideline 202 by ASG/CEFIC (2003a). The study was performed with 20 daphnia (ten individuals per replicate with two replicates per dose level) exposed to nominal test concentrations of 4.28, 7.13, 11.9, 19.8, 33.0 and 55 mg vinyl acetate per litre. Due to the volatility of the test material serum bottles were filled to capacity and

sealed. In order to maintain the exposure concentrations and dissolved oxygen levels test solutions were renewed after 24 hours. The concentration of vinyl acetate in the test solutions were confirmed in new and old test media via GC/EI-MS. The time-weighted mean (TWM) measured values ranged from 66.9 to 110 % of target concentration resulting in TWM measured concentrations for the study of 2.91, 4.77, 9.77, 14.9, 31.0 and 60.5 mg vinyl acetate / l. A 24 h-EC50 of 24.0 mg/l (95 % confidence limits 20.2-29.0 mg/l) and a 48 h-EC50 of 12.6 mg/l (95 % confidence limits 11.2-14.4 mg/l) were obtained from this study, indicating that toxicity is increasing with the duration of exposure.

Additional static tests are available using *Daphnia magna* (Bringmann and Kühn 1982), the brine shrimp *Artemia salina* (Price et al., 1974) and the marine polychaete *Ophryotrocha diadema* (Parker, 1983). As only nominal concentrations are reported, these studies are regarded as invalid and not suitable for effects assessment purposes, but are nevertheless described in more detail below for completeness.

In an acute immobilization study with *Daphnia magna* (Bringmann and Kühn 1982) a 24 h-EC_{50} of 52 mg/l related to nominal concentrations was derived. The corresponding LC_0 and LC_{100} value was 17 mg/l and 128 mg/l. The test was performed in an open, static system (local tap water free from chlorine, saturated with oxygen / content of at least 2 mg/l, temperature: 20 °C, pH at beginning of test: 7.8 - 8.2, water hardness: 16° dH.).

Price et al. (1974) examined the toxicity of vinyl acetate to the brine shrimp *Artemia salina*. In a static test conducted with artificial sea water a 24 h-LC_{50} of 45 mg/l based on nominal concentrations was found. During the study bottles were loosely capped and incubated at ambient temperature (24.5 °C). After incubation the number of live and dead shrimp were noted by viewing the shrimp in the bottle aided by a colony counter.

Another static toxicity test in filtered sea water (temperature: 21 °C) was carried out by Parker (1983) using the marine polychaete *Ophryotrocha diadema* as test organism. For the study a half-logarithmic series of concentrations was applied. After an 48 hours exposure period, surviving animals were transferred into pure sea water, where they were allowed to recover for one week. Any animals which died during this period were recorded along with the earlier mortalities in the test. A 48 h-LC_{50} of about 33 mg/l (nominal concentration) was derived from this study for vinyl acetate.

Long-term toxicity

Tests on long-term effects of vinyl acetate on aquatic invertebrates are not available.

3.2.1.1.4 Aquatic algae

The only reliable test result for aquatic algae was obtained from a growth inhibition test with the freshwater green alga *Pseudokirchneriella subcapitata* (ASG/CEFIC, 2003c). Due to the volatility of vinyl acetate several deviations of the standard algal bioassay were made in order to maximize the retention time of the substance in the test medium over the exposure period:

- The study was performed under zero headspace conditions in sealed test vessels.
- In order to avoid CO_2-limitation the content of sodium carbonate in the growth medium was increased by a factor of five.

- Test vessels were not shaken during the incubation period.
- For the daily cell density determinations test vessels were vigorously shaken prior to unsealing to suspend as much biomass as possible.
- After sampling, additional test medium from a surrogate test vessel was added in order to maintain zero headspace conditions.

The algae were exposed to six nominal test concentrations of 1.56, 3.13, 6.25, 12.5, 25.0, 50 and 100 mg vinyl acetate / l over a 96-hour period. Since the concentration of vinyl acetate declined over time (after 72 hours between 75 and 30 % of the target concentrations were still present in the test solutions), time weighted means (TWM) were calculated leading to 72-hour TWMs of 0.331, 0.733, 1.58, 5.96, 6.57, 14.9 and 34.7 mg/l. Based on these TWMs the following test results were obtained:

Table 3-17: Toxicity of vinyl acetate in algae *(Pseudokirchneriella subcapitata)*

Parameter	**Endpoint**	**Concentration (mg/l)** (m)	
Growth rate	72 h-ERC50	12.70	(95 %-CI: 9.50-17.1)
	NOEC	5.96	
Biomass	72 h-EBC50	7.48	(95 %-CI: 1.60-34.9)
	NOEC	1.58	

(m) measured concentration

For comparison purposes a second test using standard algal bioassay techniques was conducted by the same authors. Since the concentration of vinyl acetate declined very rapidly (after 24 hours only 0.4 - 0.8 % of the target concentration was found in the highest tested concentrations of 50 - 200 mg/l) and the vinyl acetate concentrations tested were not set high enough to determine a concentration-response curve, the test results of the zero-headspace bioassay are regarded as more reliable.

The green algae *Scenedesmus quadricauda* was investigated in a cell multiplication study by Bringmann and Kühn (1977a). Under this method, the onset of cell multiplication inhibition by different substances was determined. From this study an 8d-TGK value of 370 mg/l for vinyl acetate was obtained. Another cell multiplication inhibition test with the blue-green algae *Microcystis aeruginosa* as test organism found an 8d-TGK value of 35 mg/l (Bringmann and Kühn, 1976, 1978a, 1978b).

In both tests, the substances were dissolved in distilled water (temperature 27 °C, pH 7 at beginning of test). As the effect concentrations were related to nominal concentrations and presumably the algae were not in the exponential growth phase during the whole test duration, both studies are classified as not valid.

3.2.1.1.5 Microorganisms

The toxicity of vinyl acetate to different microorganisms was examined by Bringmann and coworkers using cell multiplication inhibition tests. The following test results based on nominal concentrations were obtained:

Table 3-18: Toxicity of VAM to microorganisms

Species	Effect	Endpoint	Concentration (mg/l)	pH / temp.	Reference
Pseudomonas putida	inhibtion of cell multiplication	16 h-EC_3	6 (n)	7.0 / 25 °C	Bringmann & Kühn (1976, 1977a)
Chilomonas paramaecium (Protozoa)	inhibtion of cell multiplication	48 h-EC_5	9.5 (n)	6.9 / 20 °C	Bringmann et al. (1980)

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC)

3.2.1.2.1 Surface water ($PNEC_{aqua}$)

Due to the volatility of vinyl acetate, only effect values based on analytically measured concentrations are suitable for derivation of the PNEC. Reliable acute test results are available for aquatic invertebrates and algae. For aquatic vertebrates a valid long-term test result is reported. As long-term NOECs from species representing two trophic levels are available (algae and fish), an assessment factor of 50 is proposed. Applying this factor to the long-term NOEC as determined in an ELS test using *Pimephales promelas*, the PNEC for aquatic ecosystems is calculated as follows:

$$\mathbf{PNEC_{aqua} = 0.55\ mg/l\ /\ 50 = 0.011\ mg/l}$$

3.2.1.2.2 Sewage treatment plants ($PNEC_{STP}$)

According to the procedure described in the TGD for assessing the toxicity of a substance to microorganisms in order to identify adverse effects in WWTPs, an assessment factor in the range of 10 to 100 is applied for tests on microorganisms with different sensitivities and different endpoints.

For vinyl acetate, an EC_3-value of 6 mg/l is reported for *Pseudomonas putida*, which can be considered as a NOEC-value for microorganisms. For the determination of a $PNEC_{STP}$, an assessment factor of 10 is proposed for this kind of test result, which leads to:

$$\mathbf{PNEC_{STP} = 6\ mg\ l\ /\ 10 = 0.6\ mg/l}$$

3.2.1.2.3 Sediment ($PNEC_{sed}$)

Information on toxic effects of vinyl acetate on benthic organisms is not available. A provisional $PNEC_{sed}$ can be calculated using the equilibrium partitioning method of the TGD:

$$\mathbf{PNEC_{sed}} = \frac{K_{susp\_water}}{RHO_{susp}} \times PNEC_{water} = \frac{1.51\ m^3 \cdot m^{-3}}{1300\ kgwwt\ m^{-3}} \times 0.011\ mg \cdot l^{-1} \times 1000 = \mathbf{1.27\ E\text{-}02\ mg/kg}$$

As measured data on concentrations of vinyl acetate in sediment is not available either, the PEC has to be derived with the same method. If both PEC and PNEC are calculated using the equilibrium partitioning method, the same risk ratios as for surface water are obtained. It is therefore referred to the risk characterisation for surfaces waters in section 3.3.1.

The above equation only considers uptake via the water phase, but uptake may also occur via other exposure pathways like ingestion of sediment. However, these other exposure pathways are considered negligible due to the low adsorptive properties of vinyl acetate and the low affinity to organic material. In addition, VAM is degraded hydrolytically and biologically in sediments. A significant transfer of VAM into the sediment-phase is therefore not expected.

As the equilibrium partitioning method will yield the same risk ratios as for surface water and as a significant transfer of VAM into the sediment-phase is not expected, a quantitative risk assessment for sediments is not considered necessary.

3.2.2 Effects assessment for the terrestrial compartment

3.2.2.1 Terrestrial effect data

No terrestrial effect data based on standard soil test organisms (e.g. earthworms, collembolan, microorganisms) is available.

Burditt et al. (1963) investigated the potential of using vinyl acetate as a fumigant to eggs and larvae of fruit flies *Ceratitis capitata* and *Dacus dorsalis* (both Diptera). Effect values were based on 2 h exposure followed by 48 h of observation.

A further fumigation test with terrestrial organisms was carried out at 25 °C for 3 h by Ittah and Zisman (1992) using *Theba pisana* (Mollusca: Helicidae) as test organism. In this study the fumigation was also followed by 48 h of observation. The derived effect values from both studies are summarized in the table below.

Table 3-19: Fumigation tests with fruit flies (*Ceratitis capitata* and *Dacus dorsalis*) and mollusc (*Theba pisana*)

Species	Age	Effect	Duration of fumigation	Effect	Concentr. (g/m³)	Reference
Ceratitis capitata	24 h old eggs	mortality	2 h	2 h-LC_{50} 2 h-LC_{95}	53 90	Burditt et al. (1963)
Ceratitis capitata	mature larvae	mortality	2 h	2 h-LC_{50} 2 h-LC_{95}	43 60	Burditt et al. (1963)
Dacus dorsalis	24 h old eggs	mortality	2 h	2 h-LC_{50}	> 96	Burditt et al. (1963)
Dacus dorsalis	mature larvae	mortality	2 h	2 h-LC_{50} 2 h-LC_{95}	46 70	Burditt et al. (1963)
Theba pisana	differing ages	mortality	3 h	3 h-LC_{31}	2.3	Ittah & Zisman (1992)

3.2.2.2 Calculation of $PNEC_{soil}$

The available test results on terrestrial organisms (i.e. diptera and mollusca) are originated from fumigation studies. The $PNEC_{soil}$ cannot be deduced from these data. According to the TGD, the risk assessment is performed on the basis of the equilibrium partition method (EPM) if no standard test data with soil dwelling organisms is available (equation 72):

$$\mathbf{PNEC_{soil}} = \frac{K_{soil\_water}}{RHO_{soil}} \times PNEC_{water} = \frac{0.931\,m^3 \cdot m^{-3}}{1700\,kgwwt \cdot m^{-3}} \times 0.011\,mg \cdot l^{-1} \times 1000 = \mathbf{6.02E\text{-}03\ mg/kg}$$

3.2.3 Effects assessment for the atmosphere

3.2.3.1 Biotic effects

Data on toxicity of airborne VAM are very scarce.

For the mollusc *Theba pisana*, a 3 h-LC_{31} of 2.3 g/m^3 is reported. This effect concentration is in the same order of magnitude as the results from the acute inhalation studies with mammals.

No experimental data on toxicity of VAM to terrestrial plants is available. Therefore no $PNEC_{air}$ can be calculated for terrestrial vegetation.

3.2.3.2 Abiotic effects

3.2.3.2.1 Global warming

Vinyl acetate absorbs solar irradiation wavelengths in the infrared region of 1,000 - 1,200 nm. Compared to the global warming effects caused by other substances (e.g. carbon dioxide) emitted from anthropogenic sources, the contribution of vinyl acetate to the overall man-made global warming potential (GWP) plays only an insignificant role, taking into account the released amounts and the short half-life of VAM in air.

3.2.3.2.2 Troposheric ozone

Ground-level ozone is formed by the reaction of VOCs and NO_x in the presence of heat and sunlight. To this end vinyl acetate enhances formation of ground level ozone. The reactions that form ozone do not take place instantaneously, but can take hours or days. Nevertheless it cannot be excluded that for specific meteorologic conditions and intensive solar irradiation the contribution to ozone peaks may be substantial in the immediate vicinity of emission sources. On the regional scale, however, there are sources of (biogenic) volatile substances being of much more significance. Notwithstanding any feasible measure should be undertaken to abate releases of vinyl acetate to air in order to curb formation of ground level ozone. As compared to other volatile organics ozone-forming ability of vinyl acetate was found to be moderate (Joshi et al., 1982).

3.2.3.2.3 Stratospheric ozone (ODP)

The atmospheric lifetime of VAM is too short to allow for transport of relevant shares up to the stratospheric layer. In addition, the substance does no include any halogen atoms which could be split off and thereby form any free radicals that are capable of decomposing stratospheric ozone.

3.2.3.2.4 Acidification

Vinyl acetate is easily decomposed in the atmosphere, forming acetaldehyde and subsequently acetic acid. Following wet and dry deposition the substance may enter surface water bodies. However, acetic acid is not a strong acid (pKa = 4.76). Compared to atmospheric loads of other substances that form stronger acids such as NO_x and SO_2, the acidification potential of vinyl acetate is relatively low and the overall impact on surface water bodies negligible.

3.2.4 Non compartment specific effects relevant to the food chain

As vinyl acetate does not present indications of a bioaccumulation potential, an effect assessment for secondary poisoning is not required.

3.3 RISK CHARACTERISATION

As a follow up to conclusion (i), which was drawn in the first draft of the Environment Section of the Comprehensive Risk Assessment Report of vinyl acetate (August 2002), further acute and long-term toxicity tests with aquatic organisms were performed and more data on a number of production and processing sites could be obtained, leading to the following refined and revised risk characterisation.

3.3.1 Aquatic compartment (incl. sediment)

Sewage treatment plants

Based on a $PNEC_{STP}$ of 600 µg/l (cf. section 3.2.1.2.2) and PEC_{STP} values derived for the different production, processing and manufacturing sites (= $Clocal_{eff}$ values, equation 38 of the TGD), the following PEC : PNEC ratios for waste water treatment plants were calculated.

Table 3-20: Local risk characterisations for STPs

Site	Exposure source	PEC_{STP} (µg/l)	PEC_{STP} : $PNEC_{STP}$
1	processing	5.00E+01	0.08
2	processing	1.00E+01	0.02
3a	processing	1.00E+02	0.17
3b	processing	5.00E+01	0.08
3c	processing	4.00E+00	0.01
3d	processing	1.30E+01	0.02
4/5	production/processing	5.00E+01	0.08
6	processing	3.04E+01	0.05
7	processing	5.00E+00	0.01
8	processing	2.30+01	0.04
9	processing	9.81E+01	0.16
10	processing	3.10E-02	0.00
11	processing	5.70E+00	0.01
12	processing	2.57E+01	0.04
13	processing	0.00E+00	-
14	processing	7.69E+01	0.13
15	processing	1.04E+01	0.02
16	processing	0.00E+00	-
17	processing	1.16E-04	0.00

Site	Exposure source	PEC_{STP} (µg/l)	PEC_{STP} : $PNEC_{STP}$
18	processing	1.27E+01	0.02
19	processing	9.61E+01	0.16
20	processing	1.91E-06	0.00
21	production	1.03E+01	0.02
22	production	4.00E-01	0.00
23	processing	0.00E+00	-
24	processing	1.21E+02	0.20
25	processing	2.67E-04	0.00
28	processing	5.33E+01	0.09
29	processing	8.11E-01	0.00
30	processing	7.70E-01	0.00

An evaluation of the inhibition of microbial activity in STPs seems reasonable only for those sites where vinyl acetate-containing waste water is released to biological treatment plants. The production sites which are not connected to a biological WWTP, as their waste water is treated within the process (site 26 and 27), were thus excluded from the risk characterisation. As VAM is typically produced in closed systems and dissolved vinyl acetate monomers are often recovered from waste water streams, i.e. by fractional distillation to increase reaction yields, residual levels in the waste water are generally low (cf. section 3.1.2.1.1). For the remaining production sites (4, 21 and 22) discharging to waste water treatment plant, no risk was therefore identified.

Based on a difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a), a generic scenario was calculated for the local main source (fraction of main source = 0.15). A PEC_{STP} of 325 µg/l was derived, resulting in a PEC : PNEC ratio of 0.54.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion applies to all production, processing and manufacturing sites.

Surface water

A $PNEC_{aqua}$ of 11 µg/l for surface water has been determined (cf. section 3.2.1.2.1).

Using the $PNEC_{aqua}$ and a $PECreg_{surfacewater}$ of 8.35E-03 µg/l (cf. section 3.1.6), the risk ratio for the regional environment is calculated as follows:

$$PECreg_{surfacewater} : PNEC_{aqua} = 8.35E\text{-}03\ \mu g/l : 11\ \mu g/l = 7.59E\text{-}04$$

From the $PNEC_{aqua}$ and the estimated $PEClocal_{water}$ (= $Clocal_{water}$ + $PECreg_{surfacewater}$) for the different production and processing sites, the risk ratios for the local environments are calculated as follows:

Table 3-21: Local risk characterisations for surface water (freshwater)

Site	Exposure source	$PEClocal_{water}$ (µg/l)	$PEClocal_{water}$: $PNEC_{aqua}$
1	processing	8.83E-03	0.00
2	processing	7.44E-02	0.01
3a	processing	1.00E+01	0.91
3b	processing	see marine risk assessment	
3c	processing	4.08E-01	0.04
3d	processing	1.31E+00	0.12
4/5	production/ processing	2.17E-01	0.02
6	processing	3.05E+00	0.28
7	processing	5.08E-01	0.05
8	processing	2.31E+00	0.21
9	processing	9.89E-01	0.09
10	processing	1.14E-02	0.00
11	processing	1.40E-01	0.01
12	processing	2.11E+00	0.19
13	processing	8.35E-03	0.00
14	processing	7.70E+00	0.70
15	processing	1.03E+01	0.94
16	processing	8.35E-03	0.00
17	processing	8.36E-03	0.00
18	processing	1.28E+00	0.12
19	processing	9.62E+00	0.87

Site	Exposure source	$PEClocal_{water}$ (µg/l)	$PEClocal_{water}$: $PNEC_{aqua}$
20	processing	8.35E-03	0.00
21	production	7.64E-02	0.01
22	production	see marine risk assessment	
23	processing	8.35E-03	0.00
24	processing	1.56E-02	0.00
25	processing	8.38E-03	0.00
26	production	8.35E-03	0.00
27	production	see marine risk assessment	
28	processing	1.34E+00	0.12
29	processing	1.04E-02	0.00
30	processing	1.01E-02	0.00

For the difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a), a generic scenario was calculated based on a fraction of main source of 0.15. A $PEClocal_{water}$ of 8.13 µg/l was derived, resulting in a PEC : PNEC ratio of 0.74.

A risk to the local or regional aquatic compartment was not identified for production and processing of vinyl acetate. This conclusion applies to all sites and the generic scenario.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Sediment

Information on toxic effects of vinyl acetate on benthic organisms is not available, neither are measured data on concentrations of vinyl acetate in sediment. Both PEC and PNEC can be calculated using the equilibrium partitioning method, which will result in the same risk ratios as were obtained for surface water.

This approach only considers uptake of vinyl acetate via the water phase, but uptake may also occur via other exposure pathways like ingestion of sediment. These other exposure pathways are considered negligible due to the low adsorptive properties of vinyl acetate and the low affinity to organic material. In addition, VAM is degraded hydrolytically and biologically in sediments. A significant transfer of VAM into the sediment-phase is therefore not expected.

On the basis of the available information, it is concluded that an unacceptable risk for sediments and sediment dwelling organisms is generally not to be expected from vinyl acetate.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

3.3.2 Atmosphere

Due to the high volatility of VAM it is expected that – once released into the environment – the main compartment for environmental exposure is air. This finding is supported by the results of environmental distribution models based on the physico-chemical data of VAM.

VAM beaks down rapidly in the atmosphere with a globally and seasonally averaged half-life of approximately 0.6 days (cf. section 3.1.1.2.1.) Hence transport from points of release over long distances can be excluded.

Although no breakdown products have been identified it seems likely that even more polar fragments are generated through addition to the olefinic bonds. Among the expected reaction products is formaldehyde (Atkinson, 1995). Besides photooxidation, wet deposition of VAM and its breakdown products is expected to be an important physical removal process from the atmosphere. Concurrently, cloud droplets further contribute to hydrolytic degradation of VAM in moist air. Since acetaldehyde and its oxidation product acetic acid show some acidification potential, their wet deposition adds to acidification of weakly buffered surface waters. From the results of this risk assessment it is however concluded that the specific contribution of VAM degradation products will not be of any relevance as compared with other substances.

From its high vapour pressure and low K_{ow} value it is concluded that either adsorption to airborne particles and aerosols is of relevance. In addition there is no indication that VAM significantly adds to adverse effects on other parameters of atmospheric quality like global warming or ozone depletion.

At air concentrations of approximately 2 g/m^3 significant biotic effects were observed. However, the data basis is far too scarce to derive a PNEC from this test result. As the highest

calculated local concentration of approx. 0.3 µg/m^3 is several orders of magnitude lower, further tests on this endpoint seem not to be of high priority.

It is concluded that an unacceptable risk for the environment via the atmosphere is not expected from vinyl acetate.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

3.3.3 Terrestrial compartment

No terrestrial effect data based on standard soil test organisms (e.g. earthworms, collembolan, microorganisms) is available. A $PNEC_{soil}$ = 6.02E-03 mg/kg was therefore derived using the equilibrium partition method of the TGD.

It is expected that the main pathway of VAM immission into soil is aerial deposition after local releases to air from point sources, rather than sludge application: Only 0.2 % of the VAM load entering a STP is expected to adsorb to sludge, and most companies producing or processing VAM reported either incineration or landfill of STP sludge.

As a worst case approach, soil concentrations were estimated for the two facilities with highest aerial deposition rates. Both facilities do not operate a STP so that sludge application is not included in the estimate (site 26 and 16). Furthermore, soil concentrations were calculated for several sites that had relatively high aerial deposition rates and reported sludge application on agricultural soils.

From the $PNEC_{soil}$ and the $PEClocal_{soil}$ (= $Clocal_{soil}$ + $PECreg_{nat.soil}$) for the selected production and processing sites, the risk ratios for the local environment are calculated as follows:

Table 3-22 Local risk characterisations for terrestrial soil

Site	Exposure source	$PEClocal_{soil}$	$PEClocal_{soil}$: $PNEC_{soil}$
26	production (no STP)	9.97E-05	0.02
16	processing (no STP)	5.16E-03	0.86
3a	processing	6.13E-04	0.10
3b	processing	6.36E-04	0.11
3c	processing	4.18E-04	0.07
3d	processing	2.40E-04	0.04
6	processing	1.40E-03	0.23

A risk to the terrestrial compartment at the *known* production and processing sites of vinyl acetate is not expected. However, a potential risk was identified for the generic scenario calculated for the local main source (fraction of main source = 0.15), based on a difference of 325,000 t/a between nominal production volume (800,000 t/a) and verified processing capacities (total of 475,000 t/a). A $PEClocal_{soil}$ of 0.014 mg/kg was derived, resulting in a PEC : PNEC ratio of 2.33.

Conclusion (iii): There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

3.3.4 Non compartment specific effects relevant to the food chain

As there is no indication for vinyl acetate to be a potentially bioaccumulative substance, it was abstained from assessing effects for secondary poisoning.

3.3.5 Marine Assessment including PBT Assessment

3.3.5.1 PBT Assessment

The following table shows the PBT/vPvB criteria as defined in the TGD and the respective values of vinyl acetate as derived in chapter 3 of this risk assessment.

Table 3-23 PBT/vPvB criteria according to TGD and data for vinyl acetate

	PBT-criteria	vPvB-criteria	VAM
P	half-life > 60 d in marine water or > 40 d in freshwater or half-life > 180 d in marine sediment or > 120 d in freshwater sediment	half-life > 60 d in marine water or freshwater or half-life > 180 d in marine or freshwater sediment	readily biodegradable in surface water, half-life: 15d (section 3.1.1.2.2)
B	BCF > 2,000	BCF > 5,000	BCF of 3.16 (section 3.1.1.3.5)
T	chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	not applicable	34 d NOEC (fish): 0.551 mg/l (section 3.2.1.1.2)

Vinyl acetate was found to be readily biodegradable in sewage treatment plants and is also considered as readily biodegradable in surface water. No standard tests on degradation of vinyl acetate in soil or sediment are available, but different studies indicate that the substance is biotransformed and abiotically degraded in soil and sediment.

A BCF of 3.16 was derived from log Kow of 0.73. Measured BCF are not available.

A 34 d NOEC of 0.551 mg/l was found for fathead minnow (*Pimephales promelas*).

It can be concluded that vinyl acetate does not meet any of the PBT/vPvB criteria.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

VAM does not meet the PBT/vPvB criteria.

3.3.5.2 Marine Assessment

There is no indication that VAM persists or accumulates in the environment. However, it is known that two production sites (site 22 and 27) and one processing site (site 3b) discharge to the marine or estuarine environment. PEClocal for these sites are based on site-specific data in combination with default values from the TGD. Two facilities operate a WWTP (site 22 and 3b). The company operating the other production plant (site 27) reported that new technology had been implemented in the recently commissioned facility in order to contain and recover VAM in the process (i.e. closed system process, distillation of wastewater for re-introduction of material in the process, incineration of remaining waste). Thus VAM in the waste water is either recycled or remaining material is incinerated.

As a first approach, the marine regional background concentration was derived from the fresh water regional background concentration by division by 10:

$$PEC_{regional\_seawater} = 8.35E\text{-}03\ \mu g/l / 10 = 8.35E\text{-}04\ \mu g/l$$

The $PEC_{regional\_seawater}$ was added to the $Clocal_{water}$ to obtain the respective $PEClocal_{water}$ for the marine sites. The $PNEC_{marine}$ was derived by dividing the $PNEC_{aqua}$ by 10:

$$PNEC_{marine} = PNEC_{aqua} / 10 = 11\ \mu g/l / 10 = 1.1\ \mu g/l$$

Based on these values, the following PEC : PNEC ratios < 1 for all sites are derived.

Table 3.24 PEC : PNECseawater ratios for marine sites

Site		$Clocals_{seawater}$ (µg/l)	$PEClocal_{seawater}$ (µg/l)	$PEClocal_{seawater}$: $PNEC_{marine}$
3b	processing	5.00E-01	5.01E-01	0.46
22	production	4.00E-03	4.84E-03	0.00
27	production	0.00E+00	8.35E-04	0.00

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all identified marine sites.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Vinyl acetate is a monomer, solely used to manufacture vinyl acetate (co)polymers. For production volumes see section 2.

A quantitative breakdown of the use pattern is available for Germany, where in 1990 app. 68 % of the produced vinyl acetate was mainly used for the production of polyvinyl acetate and, to a less extent, for the production of copolymers. 32 % of the produced vinyl acetate was manufactured to polyvinyl alcohol (BUA, 1993).

Vinyl acetate occurs as residual monomer in homo- and copolymers based on vinyl acetate and in products / formulations based on these polymers. As polyvinyl alcohol is produced by trans-esterfication (saponification) of vinyl acetate (co)polymers, residual vinyl acetate monomer does not occur in polyvinyl alcohol and in polymers derived from polyvinyl alcohol (Celanese, 2000).

The content of residual vinyl acetate monomer in homo- and copolymers depends on the product and the field of application (< 2 - 3000 ppm, Celanese, 2008). The quantitatively weighted median value of this content amounts to 3000 ppm (Ullmann, 1998).

Polymers based on vinyl acetate are ingredients of a variety of products, which are applied in e.g. adhesives, paints, fillers, coating materials, plasters and primers for porous building materials in the construction industry and in finishing agents (Ullmann, 1998).

For workers exposure to vinyl acetate occurs primarily via inhalation of the vapour and by immediate dermal contact with the substance.

There is no direct consumer use of vinyl acetate monomer. However, consumer exposure may result from the use of polymerized vinyl acetate products containing very small amounts of residual monomer such as binders and additives for paints and plasters, glues (e.g. for carpets or structural members), and coatings.

Among 42 products listed in the Swedish product register which contain vinyl acetate, 6 products were found to be used by consumers. In the Norwegian product register, 15 products which contain vinyl acetate have been listed for use in the consumer field.

In the Federal Republic of Germany, products containing polyvinyl acetate in which vinyl acetate may be present as a residual monomer are used in the consumer field. Thus, the consumer may be exposed to vinyl acetate; the exposure may take place via the inhalation, dermal and oral routes.

Vinyl acetate may be released to the environment, principally to the atmosphere, as a result of emissions from manufacturing, processing, and storage facilities. The short atmospheric half-life, the rapid hydrolysis in water, the low n-octanol/water partition coefficient, and the ability of biological fluids of mammals and other organisms to degrade vinyl acetate will lead to rapid removal. Little or no vinyl acetate is detected in environmental samples, including those from hazardous waste sites (VATG, 1994).

4.1.1.2 Occupational exposure

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario. For scenarios with the reasonable worst case estimates leading to a risk, in addition, typical values are assessed, if the necessary information is available. The typical exposure is an estimate of the approximate location of the median levels of exposure over the whole spectrum of likely circumstance. The combination of both levels gives an impression on the distribution of the exposure levels for a specific scenario and might serve as an extended basis for a comprehensive risk characterisation and for the discussion of risk reduction measures.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90^{th} percentiles are derived as representing reasonable worst case situations. For the purpose of exposure assessment only data measured later than 1990, if available, are taken. Scenarios are clustered as far as possible to make the description transparent.
Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:
Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.
Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.
There is an agreement between the EU-memberstates, within the framework of existing substance, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided, that for a certain scenario gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criteria. For most down stream uses it is commonly known, that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since often quantitative information on dermal exposure is available, the EASE model is used for assessing dermal exposure, at the most.

Industrial activities involving monomeric vinyl acetate present opportunities for exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

The following occupational exposure limits (OEL) and short term limits (STEL) apply in the EU and the USA:

Table 4.1.1.2.: Occupational exposure levels (EU, USA)

Country	OEL	STEL	Source
Finland	36 mg/m^3 (10 ml/m^3)	71 mg/m^3 (20 ml/m^3)	FIN, 2007
Austria, Greece	36 mg/m^3 (10 ml/m^3)	70 mg/m^3 (20 ml/m^3)	A, 2007, GR 2001
Belgium, Spain	36 mg/m^3 (10 ml/m^3)	54 mg/m^3 (15 ml/m^3)	B 2007, SP 2008
USA: ACGIH, Italy, Portugal	35 mg/m^3 (10 ml/m^3)	53 mg/m^3 (10 ml/m^3)	USA, 2008, I, 2008, POR, 2004
Ireland	30 mg/m^3 (10 ml/m^3)	60 mg/m^3 (20 ml/m^3)	IRL, 2007
Denmark, France, Norway	30 mg/m^3 (10 ml/m^3)	-	DK, 2007, F, 2008, NO, 2007
Sweden	18 mg/m^3 (5 ml/m^3)	35 mg/m^3 (10 ml/m^3)	S, 2007
Germany, The Netherlands	18 mg/m^3 (5 ml/m^3)	-	GER, 2007, NL, 2007

Occupational exposure to vinyl acetate may occur in the following areas:

- production of vinyl acetate and polymerisation in the chemical industry (scenario 1),
- manufacturing of formulations and products (scenario 2),
- use of formulations and products containing residual vinyl acetate (scenario 3).

For the manifold uses of vinyl acetate (co)polymers with residual vinyl acetate monomer content < 2 – 3000 ppm, low levels of exposure are to be expected. In spite of this, on account of the carcinogenic effect of vinyl acetate, these scenarios are included in the occupational exposure assessment. Due to hydrolysis of vinyl acetate monomer, a further decrease of the residual monomer content occurs.

Exposure during the skilled trade use of hair care products (3 w/w vinyl acetate copolymer) and car polishing products (1.5 w/w vinyl acetate copolymer) are regarded to be of minor relevance since the monomer concentration is approx. 100 times below the polymer concentration. Therefore these scenarios are not included in this occupational exposure assessment.

4.1.1.2.1 Production of vinyl acetate and polymerisation in the chemical industry (scenario 1)

Vinyl acetate is produced by a vapour phase reaction between ethylene and acetic acid in the presence of a palladium catalyst or between acetylene and acetic acid in the presence of a zinc acetate catalyst (Zenz and Cordaso, 1994). The substance is manufactured continuously in closed systems at increased temperature and high pressure. It is to be assumed that the substance is processed daily. Consequently the duration and frequency of exposure are assumed to be daily and for the entire length of the shift. According to information provided

by one producer, filling devices are equipped with local exhaust systems and, for the purpose of dispatch, vinyl acetate is filled into tank cars by means of a gas displacement device.

Vinyl acetate is used primarily to produce polymers like polyvinyl acetate, polyvinyl alcohol (via transesterification (saponification) of polyvinyl acetate), and a variety of copolymers. According to information provided by the producers, the polymerisation process takes place discontinuously or continuously in a closed system (Celanese, 2000). These polymers, usually formed as emulsions, suspensions, solutions, or resins are used to prepare different products e. g. adhesives, paints, paper coatings and textile finishing agents (Zenz and Cordaso, 1994). In part, the further processing is performed immediately after production, so that transfer or filling activities are avoided to a large extent.

The other main use of vinyl acetate is the production of polyvinyl alcohol being also performed in closed systems. Residual vinyl acetate monomer does not occur in polyvinyl alcohol and in polymers derived from polyvinyl alcohol (Celanese, 2000).

Since vinyl acetate is highly flammable it is to be expected, that high exposure levels at the workplace are avoided. For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached. According the information provided by the companies, exposure is possible during sampling, maintenance, transfer, charging of catalysts, filter changing, drumming and cleaning. Inhalation exposure in other areas is normally minimised by technical equipment (e.g. special designed filling stations, local exhaust ventilation).

Workers normally use PPE (gloves, eye glasses) and, during cleaning activities, respiratory protection in addition (Celanese, 2000).

Inhalation Exposure

Workplace measurements

Table 4.1.1.2.1: Vinyl acetate exposures at workplaces in the chemical industry during production and polymerisation

Job category / activities	Year of measure-ment	Number of samples	Range of measurement data [mg/m³]	Geometric mean [mg/m³]	95th-percentile [mg/m³]	Duration and frequency
8-h time weighted average						
Production						
maintenance	1992	2	< 1.8 – 3.5	-	-	-
sampling, loading	-	6/year (p)	0.2 – 1.4	-	-	-
production	since 1978	3/year	< 17.8	-	-	-
Polymerisation	since 1994	427	0 – 22.9	0.2	2.6	-
	1997 –1999	20 (s)	0.007 – 4.5	0.1	2.9	-
	1994 – 1999	265 (p)	<0.006 – 11	<0.03	0.4	-
	1994 – 1999	38 (p)	<0.004 – 2.4	<0.03	2	-
			2.6			
	1995 – 2000	5		-	-	-
Polyvinyl alcohol production	-	2/year (p)	0.2 – 1.4		-	-
Short term values						
Production						
Maintenance	1997	2	< 2 – 3.9	-	-	-

p: personal sampling; s: stationary sampling

For the purpose of measuring vinyl acetate in the air at the workplace, the substance is adsorbed to activated charcoal and then desorbed using carbon disulphide and determinated gas-chromatographically. The detection limit of the method amounts to 0.09 mg/m³ (sampling volume: 25 l) (BASF AG, 1999).

Due to the measurement method and the sampling strategy which were applied, the currently available measurement results (Table 4.1.1.2.1.) are regarded as valid.

Three producers provide information on the collective of exposed workers: 35 – 40 workers. From the descriptions it is concluded that the exposure scenarios relating to the production and the polymerisation of vinyl acetate can be clustered. The collectives of measurement results comprise exposure levels from 0 – 22.9 mg/m³ with four 95th percentiles up to 2.9 mg/m³. From the data given in table 4.1.1.2.1 regarding production and polymerisation,

3.0 mg/m³ (based on 95th percentile of 2.9, round off) is derived as the reasonable worst case of inhalation exposure in the chemical industry.

Conclusions

For the assessment of risks of inhalation exposure to vinyl acetate during the production and polymerisation in areas with high levels of protection belonging to the chemical industry an 8-h time weighed average concentration (8-h TWA) of 3.0 mg/m³ (95th percentile, round off) should be taken to represent a reasonable worst case situation.

Dermal exposure

When producing and polymerising vinyl acetate dermal exposure could occur during activities like drumming, sampling, cleaning, maintenance and repair work. For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows:

Input parameters: Non dispersive use, direct handling, intermittent
Level of exposure: 0.1 – 1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (palms of hands) the model yields an exposure level of 42 - 420 mg/person/day.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) during exposure relevant activities is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance.

According to information provided by the manufacturers, in case of vinyl acetate, suitable gloves (tested according EN 374) are worn. Further information shows that the permeation time of the recommended gloves is longer than normal operation activities length (vinyl acetate handling is less then 30 minutes per shift). Apart from this the gloves ought to be changed immediately by extensive contamination with vinyl acetate.

The exposure assessment is made based on the assumption, that a vast majority of workers belonging to scenario 1 are protected by suitable gloves. However, in spite of this, dermal exposure may occur due to e. g.

- unintended contamination during the handling of used gloves,
- limited protection of suitable gloves at real working conditions (e. g. mechanical stress).

As a rule, for the use of suitable gloves, low levels of daily dermal exposure are to be expected. Since no measurement results are available, a protection efficiency of 90 % is taken as a default value leading to an exposure level of 42 mg/person/day.

Besides in the case of vinyl acetate, the predominant effect reducing potential dermal exposure is the very high volatility of the substance (vapour pressure 12 kPa) which leads to considerable low retention times of the substance on the skin or on the protective gloves. This exposure reducing effect cannot be considered if workers have continuous direct contact with the substance, e.g. dipping hands into the substance. For using vinyl acetate as a chemical intermediate, this situation (occlusive exposure) is regarded to be rather non-probable. Furthermore, it is assumed, that non-occlusive exposure is the predominant exposure situation. In annex 1, the calculation of the evaporation time of the pure substance is described. For vinyl acetate with the EASE estimate of 1 mg/cm², an evaporation time of 10 second (T = 30°C) is calculated. For vinyl acetate on the gloves, an assumed temperature of 20°C leads to a similar evaporation time. This values should be regarded to represent the order of magnitude, since it is not known in how far the interaction of the skin with the substance influences the evaporation time.

In conclusion, the short retention time of vinyl acetate on the skin leads to considerably lower dermal exposures than predicted by the EASE model which considers dermal exposure during the whole shift (0 – 42 mg/person/day).

Conclusions

For assessing the health risks from daily dermal exposure in the area of production and polymerisation (scenario 1), an exposure level of 0 – 42 mg/person/day should be taken. The higher level is regarded to represent the reasonable worst case situation. This exposure assessment is based on the information provided by the producers, that suitable gloves are worn and takes into account the possible dermal exposure under actual workplace conditions.

On account of the high vapour pressure of vinyl acetate, the resulting retention time of the substance on the gloves or the skin is shortened and lower levels of dermal exposure than the estimated one are to be expected.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Manufacturing of formulations and products (scenario 2)

According to information provided by industry there is normally no direct use of vinyl acetate monomer in formulating facilities and exposure is limited to the use of vinyl acetate (co)polymers containing small amounts of residual monomer. Vinyl acetate monomer itself is not used in formulations. However, some adhesive producing facilities use vinyl acetate monomer for (co)polymerisation and perform the adhesive formulation subsequently. Therefore, this exposure scenario comprises the polymerisation step (use of vinyl acetate monomer) and the formulation step (residual vinyl acetate in (co)polymers).

The content of residual vinyl acetate monomer is < 2 - 3000 ppm in the different (co)polymers. It depends on the further application of the (co)polymer. During the further processing of the (co)polymers, residual monomer may be released. The content of residual vinyl acetate monomer in water-based systems decreases by hydrolysis (BUA, 1993).

Formulations and products based on vinyl acetate (co)polymers like adhesives and paints contain < 2 - 3000 ppm residual vinyl acetate monomer, which normally evaporates when applied by the end user.

The processing to various products (formulation e.g. adhesives, paints) may not be limited to the large-scale chemical industry but occurs in small and medium-sized chemical companies, too. For this case, in principle, it cannot be excluded that, in addition to the level of technical protection described for the large scale industry, open systems without local exhaust ventilation are used (Voullaire and Kliemt, 1995). According to this scenario, higher inhalative and, as a result of the non regular use of gloves, higher dermal exposures are expected if vinyl acetate (co)polymers and compounds are handled, e.g. during filling, sampling, charging, cleaning, maintenance and repair works. Vinyl acetate monomer is normally handled in closed systems, but some tasks, e.g. pumping and filling, are performed using the monomer.

Inhalation exposure

Workplace measurements

Measurement data is only available from the adhesive production.

Table 4.1.1.2.2: Vinyl acetate exposures at workplaces for adhesives production

Job category / activities	Years of measure-ment	Number of samples	Range of measurement data [mg/m^3]	50th percentile [mg/m^3]	90th percentile [mg/m^3]
Adhesives production					
Formulation (FEICA, 2001) (no information on sampling and duration of sampling)	1997 – 2000	60	0.07 – 32	3.9	-
	1995 – 2000	100	10.3 – 14.6	12.5	-
	1996, 2000	2	1.8 - 3.9	-	-
	1998, 1999	-	3.2 – 46	-	-
	1995	1	3.1		
Pumping of monomer vinyl acetate into tank	1998, 1999	-	39 – 46.8 (30 min)	-	-
Preparation of pre-emulsion	1998, 1999	-	< 3.6 (90 min)	-	-
Pumping of pre-emulsion into reactor	1998, 1999	-	18 (60 min)	-	-
8 h time weighed averages					
Filling, cleaning, opening of reaction vessels (BGAA, 1996 and BG Chemie 2002)	1990 – 1995	10 (p)	< 64	6	56
	1996 – 2002	23	-	3.1	14.6

p: personal sampling

According to information provided by the adhesive producers 2 – 70 workers are exposed.

Most of the data given in table 4.1.1.2.2 cannot be regarded as shift averages but - with some restrictions as exposure during activities, which do not last the whole shift.

The data regarding adhesives production (table 4.1.1.2.2) shows one measurement collective with results from 10.3 - 14.6 mg/m³ and a 50th percentile of 12.5 mg/m³. But there are also measurement collectives with wide ranges from 3.2 mg/m³ to 47 mg/m^3. The sampling durations are in part given as 30 min – 60 min. Calculated shift averages thus amount to 2 – 3 mg/m³. The highest measured result was obtained during pumping vinyl acetate monomer into a monomer tank.

Measurement results from workplaces in the adhesives industry with partly lower level of protection obtained during filling, cleaning and opening of reaction vessels were provided by the German Employer's Accident Liability Insurance Association (BGAA, 1996 and BG Chemie 2002,, c.f. table 4.1.1.2.2). It can be seen, that measurement data obtained 1996 – 2002 are considerably lower than those obtained 1990 – 1995. The 90th percentile of 56 mg/m³ is reduced to 14.6 mg/m³. It is described that the two highest results (56 mg/m³ and 64 mg/m³, 1990-1995) were obtained during polymerisation of monomer vinyl acetate, in part with not sufficient control measures in place. If necessary, the situation has been changed by the companies. BGAA states, that the old data were in part worst case measurements. Taking into accpunt, that the new data were obtained in 10 companies, they can be regarded as representative for the exposure situation.

Workplace measurements obtained during the manufacture of polymer dispersions in adhesive production facilities have been submitted by the Federal Monitoring Authorities in Germany for the period 1990 – 1993. Air measurements revealed concentrations between < 0.1 – 40mg/m^3 (n = 10, personal sampling) with a mean value of 22 mg/m^3. A higher value of 70 mg/m^3 was obtained with stationary sampling. The second highest value amounts to 40 mg/m^3. The workplaces were only partially equipped with local exhaust ventilation. Since only limited information is available on the results, the measurement results are not included in table 4.1.1.2.2.

Taking into account the short term exposures of up to 46.8 mg/m³, the shift averaged given by BGAA seems to be quite plausible. Based on the available measurement results, 14.6 mg/m3 (90th percentile of a collective of measured results) is regarded to represent a reasonable worst case situation in the adhesive production industry. In addition, a short term exposure level of 47 mg/m³ should be taken for assessing the risks of daily inhalation exposure.

Conclusions

At present, the information on exposure levels in the formulation industry is limited. Measurement results are available from the adhesive production only. This fact might indicate that exposure levels are at highest in this industrial sector and possibly lower in the formulation of paints. However, the available information cannot be seen as a proof for this assumption.

The available exposure levels indicate that exposure up to 46 mg/m³ (see table 4.1.1.2.2) (maximum value 70 mg/m³, see text on data from the Federal Monitoring Authorities) occurs. The high exposure level indicates that monomeric vinyl acetate is handled in the adhesive producing companies (polymerisation step).

For assessment of the health risks from daily inhalation exposure to vinyl acetate during the formulation of products (here: adhesives), an 8-h time weighed average concentration (8-h TWA) of 14.6 mg/m³ (90th percentile of a measurement collective) should be taken to represent a reasonable worst case situation. It should be noted that considerably lower exposure levels are to be expected if only the formulating step is performed (no handling of monomer vinyl acetate).

Short term exposures up to 47 mg/m³ (duration 30 min) obtained during the handling of vinyl acetate monomer, e.g. pumping into storage tanks, should be taken into account.

Dermal exposure

According to the description given above, the use of vinyl acetate monomer (polymerisation step) is regarded to represent the reasonable worst case situation. The exposure level estimated for handling (co)polymers with residual monomer (formulation step) is appropriate for the typical exposure.

For the handling of vinyl acetate monomer as well as (co)polymer (residual monomer: < 3000 ppm) during the compounding of formulations and products (e.g. adhesives) in small and medium-sized chemical enterprises it cannot be excluded that gloves and eye protection are not regularly worn and that both, immediate dermal contact and exposure to eyes caused by hand-eye-contacts occur. Dermal exposure is assessed for the unprotected worker using the EASE model:

Input parameters:	non-dispersive use, direct handling, intermittent
Exposure levels:	0.1 – 1 mg/cm^2/day

If vinyl acetate monomer is used (polymerisation step), dermal exposure amounts to 42 – 420 mg/person/day. The upper value is regarded to represent the reasonable worst case situation. For vinyl acetate with the EASE estimate of 1 mg/cm², an evaporation time of 10 second (T = 30°C) is calculated. For vinyl acetate on the gloves, an assumed temperature of 20°C leads to a similar evaporation time.

Taking into account the content of residual vinyl acetate monomer of < 3000 ppm in the vinyl acetate (co)polymer, the estimated level of dermal exposure reduces to < 0.0003 - < 0.003 mg/cm^2/day (formulation step). Considering an exposed area of 420 cm^2 (palms of two hands), dermal exposure amounts to 0.13 – 1.3 mg/person/day. For the assessment of the health risks, the upper value should be taken. On account of the high vapour pressure of vinyl acetate (12 kPa) and the resulting short retention time on the skin, lower levels of dermal exposure than the estimated ones are to be excepted.

4.1.1.2.3 Use of formulations and products containing residual monomeric vinyl acetate (scenario 3)

There is no direct use of vinyl acetate monomer. However, exposure may result from the use of formulations and products like paints and plasters, glues (e.g. for carpets or structural members), and coatings, which are based on vinyl acetate (co)polymers containing small amounts of residual monomer. Formulations and products based on vinyl acetate (co)polymers like adhesives and paints contain < 2 - 3000 ppm residual vinyl acetate, which normally evaporates when applied by the end user. Due to hydrolysis of the residual monomer, its content might be reduced.

The main uses of polyvinyl acetate and vinyl acetate copolymers are applications in polymer dispersions. Homopolymer and copolymer dispersions are important binders for indoor and outdoor paints, special coatings, and textile finishing agents. Dispersions are used for plasters and water-thinable adhesives. Special types are applied in wood paints as well as in coatings for paper and cardboard, in adhesives and textile finishing agents (Ullmann, 1998).

Quantitatively less important than polyvinyl acetate dispersions are solid resins which are used as binders in paints for paper, cardboard, wood, leather, certain plastics and in impregnation coatings. Vinyl acetate (co)polymers are an extremely important raw material in the adhesives industry. Copolymerisation of vinyl acetate with other monomers allows specific improvement of certain properties. These copolymers are used in heat-sealable finishes on paper, cardboard and aluminium foil, as a raw material for wash-off adhesives, textile finishing agents and marking inks (Ullmann, 1998).

Inhalation and dermal exposure to traces of residual monomer is possible during a variety of activities depending on the product in use, e. g. charging, mixing and coating work as well as cleaning work. As a worst case it is assumed that these works are performed daily during the whole shift.

Inhalation exposure

Workplace measurements

Workplace measurements obtained during the use of vinyl acetate products have been submitted by German Employer's Accident Liability Insurance Association for the period 1990 - 1995 (BGAA 1996).

Table 4.1.1.2.3: Vinyl acetate exposures at different workplaces (BGAA 1996)

Job category / activities	Years of measurement	Number of samples	Range of measurement data [mg/m^3]	Geometric mean [mg/m^3]	95th percentile [mg/m^3]	Duration and frequency
8-h time weighted average						
Wood working industry (hot melting glues)	1990 - 1995	162	-	1	2	-
Coating works (painting)	1990 - 1995	28	-	1	1	-

Air emission of residual vinyl acetate monomer during gluing of a carpet in a closed room with a glue containing 20-30 w/w polymer dispersion has been detected as 2.6 mg/m^3 (0.7 ml/m^3) (Hoechst AG, 1993a). Based on the conditions of exposure (closed room, limited ventilation) this value is regarded to represent a worst case situation.

FIN provided data from silk screen printing (0.18 mg/m^3) and paper cardboard coating, paste mixing and coating (6 ± 7.7 mg/m^3). Since no further information is available, this data cannot be considered in exposure assessment.

Conclusions

Because of the low residual monomer content in formulations, on the average 3000 ppm, low inhalation exposure levels are to be expected. This is confirmed by the available measurement results. For the area of the application of products (e.g. adhesives, paints) on the basis of polymeric vinyl acetate 2.6 mg/m^3 (determined during air emission monitoring) should be taken as a worst case for assessing the risk of inhalation exposure.

Dermal exposure

For the handling of various polyvinyl acetate products in industrial and skilled trade areas it cannot be excluded that gloves are not regularly worn and that immediate dermal contact occurs (Voullaire and Kliemt 1995). Since detailed information on the possible activities are missing, for a rough estimation, dermal exposure is assessed with the EASE model using the category "wide dispersive use":

Input parameters:	wide dispersive use, direct handling, intermittent
Exposure level:	1 - 5 mg/cm^2/day.

Taking into account the content of residual vinyl acetate monomer of < 2 – 3000 ppm in the different products, the estimated level of dermal exposure reduces to < 0.003 - < 0.015 mg/cm^2/day. Considering an exposed area of 840 cm^2 (both hands, worst case), dermal exposure amounts to 2.5 – 12.6 mg/person/day. The higher value is regarded to represent the reasonable worst case. It is to be assumed, that the content of residual monomer in the dispersions is decreased by chemical reactions of vinyl acetate, e.g. further polymerisation or hydrolysis. In addition, dermal exposure may be lowered by the evaporation of the substances (vapour pressure: 12 kPa).

4.1.1.2.4 Summary

Based on the available information, the exposure assessment reveals that handling the monomeric substance in the areas of production and polymerisation, the formulation of adhesives as well as the use of formulations, especially of adhesives, are the main source for occupational exposure. Direct uses of the substance other than polymerisation are not known. Applications of the manifold products containing vinyl acetate in traces as residual monomer, e.g. adhesives, coating materials and paints, are regarded to be of minor relevance.

The relevant inhalation and dermal exposure levels are given in tables 4.1.1.2.4 A and 4.1.1.2.4 B, respectively.

For the large-scale chemical industry, it is assumed that the production and polymerisation of vinyl acetate is mainly performed in closed systems with high levels of protection. Exposure occurs if the closed systems are breached for certain activities e. g. filling, cleaning and sampling in the area of manufacturing and polymerising of vinyl acetate monomer (scenario 1). Dermal exposure is assessed using the EASE model (scenario 1). In addition, the high vapour pressure of vinyl acetate is taken into account as an exposure reducing effect.

According to information provided by industry, there is normally no use of vinyl acetate monomer in formulating facilities and exposure is limited to the use of vinyl acetate (co)polymers containing < 2 – 3000 ppm residual monomer. However, some adhesive producing facilities use vinyl acetate monomer for (co)polymerisation and perform the formulation subsequently. Therefore, this exposure scenario comprises the polymerisation step (vinyl acetate monomer) and the formulation step (use of residual vinyl acetate in (co)polymers). Generally, in formulating facilities different levels of protection are realised. In part, processes like filling and mixing are performed in open systems.

Measurement results are available from formulating adhesives only (scenario 2). This fact might indicate, that exposure to vinyl acetate at other formulating processes. e.g. of paints, is lower. The available exposure levels reveal that exposure of 47 mg/m³ vinyl acetate occurs. On the background of the information that normally only vinyl acetate (co)polymer is used, these high levels are non-plausible. At present it is assumed, that the high levels relate to the use of vinyl acetate monomer (polymerisation step). If no monomer is handled and a company uses only vinyl acetate (co)polymers (formulation step), exposure is expected to be considerably lower.

The widespread industrial and skilled-trade applications of polymeric dispersions and solid resins containing residual vinyl acetate monomer (< 2 – 3000 ppm) comprise uses in paints, lacquers, adhesives, plasters and coating materials (see chapter 4.1.1.2.3). In scenario 3 a worst case situation is described assuming 3000 ppm residual monomer and daily exposure during the whole shift.

Table 4.1.1.2.4 A: Summary of inhalation exposure data of vinyl acetate which are relevant for occupational risk assessment

Inhalation exposure								
Area of production and use	**Form of exposure**	**Activity**	**Duration [hs/day]**	**Frequency [days/year]**	**Shift average [mg/m^3]**	**Method**	**Short-term exposure[mg/m^3]**	**Method**
1. Production and polymerisation in the chemical industry	vapour (liquid)	filling, sampling, cleaning, repair, maintenance	8 h / day	daily	**3.0** (reasonable worst case)	95th percentile	-	-
2. Manufacturing of formulations and products (e.g. adhesive production)	vapour (liquid)	filling, sampling, cleaning, repair, maintenance	8 h / day (assumed)	daily	**14.6** (reasonable worst case)	90th percentile	47 (30 min, use of vinyl acetate monomer)	measure-ment value
3. Use of formulations and products containing residual vinyl acetate monomer (< 2 – 3000 ppm)	vapour (liquid)	different activities, e.g. coating, painting	8 h / daily (assumed)	daily	**2.6** (worst case)	laboratory experiment, worst case conditions	-	-

Table 4.1.1.2.4 B: Summary of dermal exposure data of vinyl acetate which are relevant for occupational risk assessment (reasonable worst case)

Dermal exposure								
Area of production and use	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Contact level [(1)]**	**Level of exposure [mg/cm²/day]**	**Exposed area [cm²]**	**Shift average [mg/p/day]**	**Method (use of PPE)**
1. Production and polymerisation in the chemical industry	liquid	filling, sampling, cleaning, repair, maintenance	daily	intermittent	0.1 [(2)]	420 (palms of hands)	**42** [(2)]	EASE, exp. judgement (suitable gloves)
2. Manufacturing of formulations and products (e.g. adhesive production) a) polymerisation step (vinyl acetate monomer) b) formulation step (vinyl acetate (co)polymer)	liquid	filling, sampling, cleaning, repair, maintenance	daily	intermittent	1 [(2)] 1 [(3)]	420 (palms of hands) 420 (palms of hands)	**420 [(2)] (a)** **1.3 [(3)] (b)**	EASE, (without gloves) EASE, (without gloves)
3. Use of formulations and products containing residual vinyl acetate monomer (< 2 – 3000 ppm)	liquid	different activities, e.g. coating, painting	daily	intermittent	5 [(3)]	840 (hands)	**12.6** [(3)]	EASE (without gloves)

(1) Contact level according to the EASE model

(2) The EASE estimate is largely reduced because of the short duration time of dermal exposure. The retention time of pure vinyl acetate is calculated to 1 second for 0.1 mg/cm² and to 10 seconds for 1 mg/cm² (order of magnitude) independent on the use of gloves (non-occlusive exposure).

(3) Worst case estimation assuming 3000 ppm residual monomer. Due to the high vapour pressure of the substance shortened retention times on the skin are to be expected leading to considerable lower dermal exposure levels than estimated with the EASE model. For vinyl acetate in mixture the retention time cannot be calculated because of the complex composition of the mixtures and their specific drying behaviour.

4.1.1.3 Consumer exposure

According to the Swedish product register, products containing vinyl acetate are used by consumers in Sweden. Such products are e.g. moisture barrier paints and other paints and lacquers/varnishes on a solvent basis, adhesives, glues, dispersion adhesives, hotmelt adhesives and sealing compounds. The consumer products are offered in wholesale and retail trade, e.g. in repair shops for motor vehicles and motorcycles as well as for personal and household goods (as per February 1995).

In the Norwegian product register, 15 products for use in the consumer field have been listed in which vinyl acetate is used as a component of paints for the exterior and interior application to houses (as per 16 December 1994). No information is available for the amount of vinyl acetate in the formulations.

In the Federal Republic of Germany, polyvinyl acetate is known to be used e.g. as a component of adhesives (vinyl acetate polymer content up to 36%), car polishing products (primers, polyvinyl acetate copolymer content up to 1.5%), hair care products (hair setting lotions, vinyl acetate copolymer content up to 3%, hair care products) and in plastics for toys and those coming into contact with foods. These products contain traces of vinyl acetate as a residual monomer; in adhesives (e.g. film and surface adhesives), the residual monomer content is 0.1 - 0.2% (voluntary reporting by the manufacturers/distributors etc. to the BgVV (as per 01.04.1996); Umbach, 1995; Ellenhorn and Barceloux, 1988). This content would be similar to 1000 - 2000 ppm.

In case reports from the Swiss Toxicological Information Centre, polyvinyl acetate is stated as a component of paints and lacquers/varnishes (binding materials) for households (Velvart, 1993). According to the data provided by the manufacturers, the concentration of polyvinyl acetate is up to 13%, and that of vinyl acetate as a residual monomer up to a maximum of 0.3% in the (co)polymer. In water-based paints residual vinyl acetate monomer hydrolyses rapidly.

According to information provided by the industry, poly vinyl acetate is used in printing inks. The maximum polymer concentration is 60%, the maximum concentration of the residual monomer is 0.2%; this results in a maximum content of vinyl acetate monomer of 0.12%.

Inhalation exposure

Measured exposure data from paints

At present, „solvent- and emission-free“ paints and plasters are available based on binders and additives with a content of volatile organic components (VOC; sum of vinyl acetate, acetaldehyde, and formaldehyde) below 100 ppm (Zeh et al., 1994).

In an exposure study monitoring volatile components of polyvinyl acetate (PVA) based paints all fixed-station area air samples collected during application and drying of the painting process and analyzed for vinyl acetate showed concentrations below the analytical limit of detection (Kominsky and Freyberg 1992). The average environmental limit for detection with regard to fixed-station area air samples (measurement during application and drying up to 6

hours after application) was 10 ppb (= 36 µg/m³). The average environmental limit of detection for personal breathing zone concentrations during paint application was 220 ppb.

The content of vinyl acetate monomers (VAM) in the samples of paint used during the study were <1 to 8.2 ppm (Kominsky and Freyberg, 1992). Shortly after production of paints relatively high amounts of residual monomers of ≈ 3200 ppm can be found. In the process of incorporating the resin into the paint product, the residual VAM content of the resin is lost or converted to hydrolysis products (acetic acid and acetaldehyde). This conclusion is supported by the results of the air monitoring for vinyl acetate during and after paint application.

Surfaces coated with products containing polymer dispersions of vinyl acetate are generally free of vinyl acetate before drying since due to the difference in vapour pressure vinyl acetate evaporates more quickly than water (Wacker-Chemie, 1993). In addition, vinyl acetate hydrolyses at pH 8 to 9 (usual pH in paints) with a half-life of less than 4 days. With respect to potential vinyl acetate evaporation from glues of building material, due to the high vapour pressure of vinyl acetate building material might be free of vinyl acetate after a short time and not lead to a long-term vinyl acetate burden in the rooms (Wacker-Chemie, 1993).

From this point of view it can be expected that vinyl acetate emission is very low from paints. In an experimental study, where paints have been spiked with vinal acetate, Zeh et al. (1994, 2000) have evaluated VOC emissions from paints and, as a constituent of VOC's, vinyl acetate. The experiments showed excellent correlation between measured and modelled data. With a paint spiked with 1200 ppm, room air levels were found of 10 mg/m³. Extrapolating these findings to paints containig concentrations in the range < 10 ppm, the room concentrations of vinyl acetate after use of paints shound not exceed levels in the range of 0.1 mg/m³.

Adhesives for carpets and other film and surface adhesives

Polyvinyl acetate is used as a component of film and surface adhesives, e.g. for carpets and floor sealings; 0.1 - 0.3% of vinyl acetate are contained in the products as residual monomer.

The monitored air emission of vinyl acetate during gluing of a carpet with a VAM containing glue in a closed room has been detected at 2.6 mg/m^3 (glue formulation: 20-35% dispersion; the maximum VAM content of the dispersion is typically 0.1-0.3%) (Hoechst, 1993).

This information can be used to estimate an acute exposure to the person who is gluing the carpet (normally workplace exposure). From other studies (Zeh, 1994, 2000) it is known that vinyl acetate concentrations will decline quickly and remaining long-term exposure may be low. There is no further information on room air levels although gluing carpets may serve as a considerable source of exposure to vinyl acetate.

Emission of vinyl acetate from carpets

Vinyl acetate had been found after 16 hours of equilibration in a concentration of less than 2 µg/m^3 (limit of detection) in air over three different carpets (two with and one without a finish containing up to 2000 ppm vinyl acetate; carpet size 0.2 m^2, test chamber size 0.5 m^3, air exchange rate 0.5 h^{-1} (Deutsches Teppichforschungsinstitut e.V., 1992)).

In an US study the air emissions of four new carpets (typically installed in residences, schools, and offices; the carpet samples were collected at the manufacturers' mills

immediately following production) were measured in room air under simulated indoor conditions in a 20 m^3 environmental test chamber over a period of one week following installation. After 3 hours the initial concentration amounted about 290 ppb (1.04 mg/m^3) and after 168 hours about 10 ppb (0.036 mg/m^3). The emission rate was estimated to be 853 $\mu g/m^2$ per hour after 24 hours and 103 $\mu g/m^2$ per hour after 168 hours. Altogether the carpets emitted 85 mg/m^2 of vinyl acetate over the period of 168 hours (Hodgson et al., 1992a, 1992b). The room air ventilation rate was 1 h^{-1}.

The results of these studies obviously show, that the emission of vinyl acetate varies greatly.

For risk assessment therefore the highest amounts that have been measured should be taken into consideration. As a realistic worst case estimate, the concentrations of vinyl acetate from carpet emissions would then amount to about 1000 µg/m³ (~ 300 ppb). For chronic exposure, the concentration measured by Hodgson et al. (1992a, 1992b) after 168 h equilibrium of 36 µg/m³ (≈ 10 ppb) should be taken.

Chronic exposure results in ≈ 0.2 µg/kg/h for women (activity level: resting) and ≈ 0.4 µg/kg/h for men (activity level: resting) accounting for 4.6 and 9.2 µg/kg bw per day, respectively. For 3 year-old children, the result of estimation of chronic exposure by inhalation is 0.78 µg/kg/h (18 µg/kg bw per day).

For calculating exposures the following data have been used (AIHC, 1994; AUH, 1995):

	3-year-old children	Adult (women)	Adult (men)
Body weight (TGD defaults)	11 kg	60 kg	70 kg
Breathing volume (resting)	0.24 m³/h[10]	0.3 m³/h	0.7 m³/h
Time of residential stay	23 h	23 h	23 h

The inhalation exposure (Exp_{inh}) has been calculated by the equation

$Exp_{inh} = \frac{c_{air} * r_{inh}}{BW}$, where c_{air} is the room air concentration obtained by measurements and r_{inh} the breathing volume (see table).

Exposure to car polishing products (primers)

In car polishing products (primers), polyvinyl acetate copolymer is used in a concentration up to 1.5%. The residual monomer content of vinyl acetate is very low so that the consumer exposure to vinyl acetate in this field of use may be neglected.

Exposure from printing inks

[10] conservative unfavorable assumption for breathing rates considering chronic exposure over whole day (Finley, et al. 1994)

The use of printing inks containing vinyl acetate may lead to an exposure of consumers. Printing inks are used for example in computer ink-printers. For this purpose, the ink-solvents must volatilize very quickly. The content of a cartridge is 50 ml, the vinyl acetate residual monomer content is then 0.06 g. If the cartridge is used for 1 month, the daily release of ink will amount to 0.002 g. The liberation of this amount in a room of 40 m³ results in a concentration of 0.05 mg/m³.

Dermal exposure

Exposure to hair setting lotions

The Scientific Committee (Scientific Committee, 1994; Colipa 1989) has stated a consumer exposure to hair setting lotion up to 1 g/day. Hair setting lotions contain up to 3% vinyl acetate copolymer which corresponds to a daily consumer exposure of 30 mg vinyl acetate copolymer. Assuming a residual monomer content of 0.2% vinyl acetate, the consumer exposure to vinyl acetate will be 60 µg vinyl acetate daily or 1 µg/kg bw and day.

Oral exposure

Exposure to plastics coming into contact with food etc.

Substances that will come in contact with food as monomers for the manufacture of plastics are regulated under the "Regulations to amend the Regulations on Specific Commodities under the Foods Act, 1994". In this regulation, a maximum value of 12 mg/kg migrating from articles is allowed. This means, that an amount of 12 mg of the vinyl acetate residual monomer may migrate from one kg of food. Taking into consideration, that the real migration rate of vinyl acetate from plastic material is not known, and according to the proposed classification of vinyl acetate migration from articles must be held as low as possible.

A tolerable daily intake (TDI) value of 0.2 mg/kg bw has been established[11].

Conclusion

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[11] A tolerable daily intake (TDI) value of 0.2 mg/kg bw has been established. This rather high value was derived by taking the NOAEL and application of safety factors. The amount of the daily intake from migration can be roughly estimated assuming the following parameters: A can with a weight of 500 g containing 0.1% of vinyl acetate residual monomer contains a volume of one liter liquid. Assuming that 0.1% of the residual monomer will migrate every day, then an amount of 0.5 mg will migrate per day, which would result in an oral exposure of about 0.01 mg/kg bw if one person will drink the total content of the can. Therefore, it seems unlikely that the regulatory limits will be reached.

Following the exposure assessment there is no direct exposure of the consumer to vinyl acetate besides of the release of monomers from polymers. Overall exposure of consumers to vinyl acetate results mainly from inhalation due to emissions from carpets, paints and adhesives. However, there is a lack of sufficient data on other uses. For comparison with acute effects, the estimate for short-term exposure from carpets will be used (concentrations of 1 mg/m³). With regard to chronic effects, the concentration of 0.036 mg/m³ for long-term exposure will be used resulting in an exposure of 9.2 µg/kg bw/d for men, 4.6 µg/kg bw/d for women, and 18 µg/kg bw/d for children. For the risk characterisation a value of about 10 µg/kg bw/d for adults will be used.

The dermal exposure to vinyl acetate is estimated to be about 1 µg/kg bw/d.

The sum of all types of exposure resulting from residual monomeric vinyl acetate (reasonable worst case) is estimated to be in the range of 5 to about 20 µg/kg bw/d.

4.1.1.4 Indirect exposure via the environment

According to Appendix VII of chapter 2 of the TGD, the indirect exposure to humans via the environment, i.e. through food, drinking water and air is estimated.

As a worst case scenario, the maximum intake due to exposure in the vicinity of a vinyl acetate production facility is calculated (site 26). This is compared to an average intake due to exposure via the regional background concentration.

The resulting total daily dose is: $DOSE_{tot\_local}$ = 36 µg / kg $_{bw}$ d

$DOSE_{tot\_regional}$ = 2.47 E-03 µg / kg $_{bw}$ d

The calculated total doses comprise the following routes:

Route	**Percent of total dose**	
	local	**regional**
Drinking water	1.26	9.64
Fish	2.97E-05	0.44
Stem	0.36	0.33
Root	0.24	0.37
Meat	2.04E-04	2.41E-04
Milk	3.80E-03	4.50E-03
Air	98.13	89.22

The main route of indirect exposure of both scenarios is the intake via inhalation of air.

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

Vinyl acetate is hydrolysed by carboxylesterases to acetic acid and acetaldehyde which is subsequently oxidized to acetic acid by aldehyde dehydrogenases. Acetate enters the citric cycle in an activated form as acetyl coenzyme A. Vinyl acetate metabolism not only takes place in the liver but also in several tissues which might be of relevance for the toxicological effects elicited by vinyl acetate.

The main pathway for the metabolism of vinyl acetate is shown in Fig. 1.

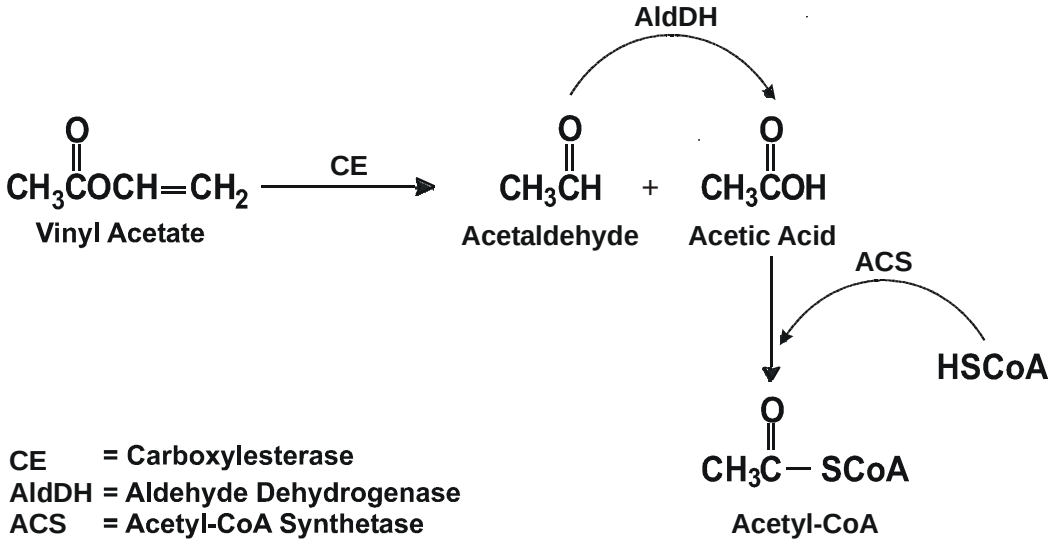

Fig. 1:

Biotransformation Pathways for Vinyl Acetate

(Modified from Bogdanffy et al., 1998)

Animal data – in vivo

Inhalative exposure

Four CD rats (Sprague-Dawley-derived), 2 males and 2 females, were exposed nose only to an atmosphere of nominal 750 ppm of ^{14}C-vinyl acetate (labelled at the vinyl moiety) for 6 h (2.68 mg/l x 0.8 l/min/kg x 360 min ≈ 772 mg/kg bw). The authors presented the results (radioactivity in urine, faeces, expired air, cage washing and carcasses) as percentage of the total radioactivity recovered because the precise amount of (^{14}C)-vinyl acetate given to the animals was not known. The tables and appendices were not available to the rapporteur, therefore data are given as described by the authors. During a 96 h period the mean proportions of the recovered (not the administered!) radioactivity found in urine, faeces and expired air were 4.8, 3.6, and 74.6%, respectively. The major portion of radioactivity was eliminated in the first 24 h. A mean of 16.4% of the total recovered radioactivity was present in the carcass after 96 h. In case of nose-only inhalation of 750 ppm (772 mg/kg bw) about 83% of the recovered radioactivity were found in the expired air (as CO_2), urine and faeces. Based on the fact, that the percentages of radioactivity presented were based on the recovered amount of radioactivity and taking into consideration that presystemic metabolism in the nasal cavity occurs, no conclusions about the amounts of systemically available vinyl acetate can be drawn from this investigation. For investigation of tissue distribution (number of animals not available) radioactivity was investigated immediately, 1, 6 and 72 h after a 6 h exposure to a nominal dose of 750 ppm ^{14}C-vinyl acetate. Immediately after exposure, the highest mean concentrations of radioactivity were found in harderian gland, ileum, submaxillary salivary gland and the contents of the gastrointestinal tract. Radioactivity was widely distributed with relatively high levels present in liver, kidneys, lung, brain, stomach, colon and ovaries. 1, 6 and 72 h after exposure, the pattern of distribution was approximately the same although concentrations had declined. 72 h after exposure, the harderian gland, adrenal gland and ovaries contained the highest mean levels of radioactivity (Strong et al., 1980; the appendices and tables of the study were not available to the rapporteur).

Two male Wistar Rats exposed to vinyl acetate (stabilised with 0.01% hydroquinone) concentrations varying between 200 and 2000 ppm in closed chambers with an exposure time of 1.4 h or less demonstrated dose dependent elimination kinetics. The authors concluded that the metabolic pathways became saturated when vinyl acetate exposure levels exceeded 650 ppm (2320 mg/m^3) (Simon et al., 1985a).

Vinyl acetate deposition was measured in the isolated upper respiratory tract (URT) of anaesthetised adult male CrlCD:BR rats at exposure concentrations ranging from 73 to 2190 ppm during 1h inhalation under unidirectional flow conditions (flow rate 100 ml/min) (Plowchalk et al., 1997). The URT was isolated by inserting a polyethylene tube into the trachea to the level of the nasopharynx of sodium pentobarbital anaesthetised (70 mg/kg bw, ip) rats (250 to 350 g). A separate cannula was inserted into the trachea, toward the lungs, to maintain a patent airway. A valve system allowed alternative switching between two impingers to enable the continuous collection of expired air. Each animal was then placed into a holder in a supine position and the nose was inserted with Batelle-style nose-only restrainers into an exposure chamber. Chamber atmosphere (C_{in}, 73 to 2190 ppm vinyl acetate in nitrogen, 34 °C, humidity 88%) was drawn through the URT under constant velocity (nominal 100 ml/min) and unidirectional flow conditions. The air exiting the nasal cavity (C_{exp}) was trapped in an impinger. Preliminary experiments showed that approximately 8 min of

exposure was required for vinyl acetate to achieve a steady state in nasal tissues. After 8 min of equilibration, impinger samples were collected, approximately every 4 min, for up to 40 min and analysed for vinyl acetate and acetaldehyde by gas chromatography. Fractional deposition (equivalent to extraction) of vinyl acetate in the URT was calculated as (C_{in} - C_{exp})/C_{in}. Fractional deposition of vinyl acetate from the nasal cavity was non-linear with reference to exposure concentration and ranged from 36 to 94%, with greatest uptake at lowest vinyl acetate concentrations. While the exposure concentration was increased from 76 to 550 ppm, uptake decreased progressively to about 40% and remained at this level up to approximately 2000 ppm. When simulations were performed without carboxylesterase activity in nasal mucosa, it could be calculated, that vinyl acetate deposition due to blood-flow extraction is minimal and accounts for less that 15 % of total deposition (Plowchalk et al., 1997; Bogdanffy et al, Bericht 1998). Acetaldehyde was found in expired air at all vinyl acetate exposure concentrations. With increasing the vinyl acetate exposure, concentration of acetaldehyde in expired air increased. At vinyl acetate exposure of approximately 1000 ppm the concentration of acetaldehyde in the expired air was 277 ppm (499 mg/m^3). To study the effect of carboxylesterase inhibition on vinyl acetate deposition, rats (n = 10) were pre-treated for 3 days with one ip injection per day (0.2 ml/100g bw) of a 5% BNPP (bis(p-nitrophenyl)phosphate, an inhibitor of carboxylesterase) suspension in saline. Pre-treatment of rats with BNPP significantly decreased fractional deposition of vinyl acetate. At exposure concentrations ranging from 76 to 663 ppm, BNPP pre-treatment reduced fractional deposition by at least 55% compared to that seen in naive rats. The appearance of acetaldehyde in the expired air and the inhibition of its formation by the inhibitor BNPP are indicative for a carboxylesterase-mediated metabolism of vinyl acetate.

Oral exposure

Rats were administered oral doses of ^{14}C-vinyl acetate (labelled at the vinyl moiety, 1 ml of a 10000 ppm (v/v) aqueous solution, overall dose level 297 mg/kg bw) by gastric intubation. The dosing regimen was 6 times 1 hour apart. During the dosing regime and subsequent 96 h collection period, a mean of 64.4% of the administered radioactivity was excreted (1.4% in faeces, 1.8% in urine and 61.2% in exhaled air). In addition a mean of 5.4% was found in the carcass at 96 h. The major portion of the urinary radioactivity was excreted within the first 24 h. Most of the radioactivity eliminated by the expired air was recovered during the 6 h dosing regime and the first 6 h after dosing. This portion of radioactivity was recovered from the traps designed for collecting carbon dioxide. The authors of the study suppose, that the unaccounted 30.1% of the dose were most likely lost in the expired air, which escaped from the metabolism cages when the animals were removed for dosing. There was a wide tissue distribution of radioactivity following administration of ^{14}C-vinyl acetate by the oral route. One hour after the sixth dose the highest mean concentrations of radioactivity were found in the harderian gland and the submaxillary salivary gland. High levels were also found in the liver, kidney, stomach, ileum, colon and gastrointestinal tract contents. Low concentrations of radioactivity were found in fat. Attempts have been undertaken to determine vinyl acetate metabolites in urine and faeces. No radiolabelled carbonates or bicarbonates were found in urine or feces. Thin layer chromatography of urine indicated that there was one major radioactive fraction and several minor fractions (Strong et al., 1980). Exhaled radioactivity was entirely present as ^{14}C carbon dioxide. Therefore it can be concluded, that 63 % of orally applied ^{14}C vinyl acetate is excreted as metabolites.

A single oral dose of nominal 1.17 mg of ^{14}C-vinyl acetate (labelled at the vinyl moiety) was orally administered to albino mice of the CDI strain (3 male, 3 female). Animals were taken

for analysis 1, 6 and 72 h after administration. Whole-body autoradiograms indicated a wide distribution of radioactivity. High concentrations were observed in harderian and salivary glands, gastrointestinal mucosa, liver and brown fat (Strong et al., 1980).

Solutions of 0.9 % saline containing various concentrations of vinyl acetate were placed in the mouth of anesthetized male F344 rats for 10 min and then analyzed for acetaldehyde by HPLC (Morris et al., 2002). Acetaldehyde was detected in these solutions providing evidence that metabolism of vinyl acetate occurs in vivo in oral tissues.

Dermal exposure

There are no studies on absorption, metabolism, distribution and excretion of vinyl acetate after dermal exposure. A study has been undertaken to determine acute dermal toxicity (Mellon Institute, 1969), see also section "Acute toxicity". In brief, all animals died after occlusive application of 16 ml vinyl acetate per kg bw on to the skin of rabbits. After occlusive application of 4 ml/kg, gross pathology revealed congestion of the lungs and liver, mottled spleen and kidney and prominent liver acini. The results indicated that vinyl acetate might be systemically bioavailable after dermal application.

Other routes

Injection of vinyl acetate (i.p.) to female Chester-Beatty rats (0.8 ml/kg bw) resulted in a decrease of hepatic glutathione level to 77% of the normal value 30 min after application (Boyland and Chasseaud, 1970). Similar decreases of hepatic non-protein sulfhydryls were reported after i.p. vinyl acetate injection to mice (300 mg/kg bw), Wistar rats (300 or 450 mg/kg bw) and guinea pigs (500 mg/kg bw) (Holub and Tarkowski, 1982). It is assumed that these findings may indicate conjugation with glutathione. However due to the limited data the relevance of this pathway cannot be assessed.

Human data – in vivo

Inhalative exposure

To provide validation data for the application of the PBPK model developed by Bogdanffy et al. (1999b; see also section PBPK modelling) in humans, controlled human exposures to inhaled vinyl acetate were conducted (Hinderliter et al., 2005). Air was sampled by a probe inserted into the nasopharyngeal cavity of five volunteers (two women, three men). Volunteers were instructed to inhale and exhale through the nose. Sampling was carried out during exposure to labelled $^{13}C1$, $^{13}C2$-vinyl acetate during resting and light exercise at three exposure levels (1, 5 and 10 ppm nominally). Both, labelled vinyl acetate and the major metabolite acetaldehyde from the nasopharyngeal region were sampled at a calibrated flow rate of 12 l/h and analysed in real time utilising ion trap mass spectrometry (MS/MS). Measurements were taken every 0.8 sec in an exposure period of 2 to 5 min resulting in data during all phases of the breathing. The rate of sampling was rapid enough to capture much of the behaviour of vinyl acetate in the human nasal cavity including inhalation and exhalation. However, the sampling was not frequent enough to accurately capture the peak concentration

in every breath. The method is free of interference from endogenous compounds and its detection limit is in the single digit ppb level. An acoustic rhinometry system was used to measure the cross-sectional area and volume of the nasal cavity. The complete set of human exposures resulted in a data set with over 22000 data points of vinyl acetate and acetaldehyde.

Oral exposure

There are no toxicokinetic data on human oral vinyl acetate exposure.

Dermal exposure

There are no toxicokinetic data on human dermal vinyl acetate exposure.

In vitro data (both animals and humans)

Carboxylesterase activity

Carboxyl esterase activity in the nose

Vinyl acetate hydrolysis has been studied in vitro in the nasal tissues of F344 rats and B6C3F1 mice. Homogenates of nasal respiratory and olfactory mucosa, adjusted to pH 7.4, were incubated with vinyl acetate at 37°C for 10 min (respiratory mucosa) or 2 min (olfactory mucosa). It was shown that nasal esterases catalyse formation of acetaldehyde and acetic acid with high efficiency. V_{max} values for rat and mouse respiratory tissue ranged from 22 to 46 µmoles/min/mg protein (22 to 24 for rat; 30 to 46 for mouse) which is in the same range as the data obtained in rat liver microsomes (23 µmoles/min/mg protein, Simon et al., 1985a). V_{max} for rat and mouse olfactory tissue ranged from 89 to 165 µmoles/min/mg protein (88 to 92 for rat, 95 to 165 for mouse). K_m values obtained for rat and mouse nasal tissues ranged from 0.30 to 1.07 mM and were similar among olfactory and respiratory epithelium (Bogdanffy and Taylor, 1993). The enzyme kinetic data for carboxylesterase mediated hydrolysis of vinyl acetate do not account for the species differences in susceptibility to the carcinogenic effects of vinyl acetate. Values are listed in Table 1.

Carboxylesterase activities have also been investigated in nasal (respiratory, olfactory) tissue from different species using p-nitrophenolbutyrate (Bogdanffy et al., 1987) or methylmethacrylate (Mainwaring et al., 2001) as substrates for carboxylesterase (see Table 2). Higher carboxylesterase activities could be determined in olfactory tissue of rats and mice compared to respiratory tissue. However, it is difficult to draw firm conclusions concerning carboxylesterase activities in nasal tissues of humans and rodents, because vmax values in human samples were obtained when analyzing just one substrate concentration. In the case of human olfactory tissue, only S9, but not microsomes have been analyzed (Mainwaring et al., 2001) which makes species comparison difficult.

Since the nasal cavity epithelium is composed of a heterogeneous cell population, measurement of xenobiotic metabolizing enzymes using homogenates could yield artificial results. Therefore, an in vitro gas uptake technique has been established in order to enable more accurate measurement of kinetic constants (Bogdanffy et al., 1998). By applying this method, whole rat turbinates from CrlCD:BR rats covered with either respiratory or olfactory epithelium or sections of human nasal tissue (middle turbinate or dorsal meatus) were placed in a sealed scintillation vial. Vinyl acetate vapour was injected into the headspace over the tissue and its loss from the vial headspace and the appearance of acetaldehyde were determined and modeled using a distributed parameter model that reflects tissue histochemistry. In this model the individual compartments are said to represent epithelial cells, basal cells, or submucosal tissue in the respiratory and olfactory mucosa. Rat turbinate surface areas were estimated using a three-dimensional, anatomically "accurate" computer model of the nasal passages from one F344 rat. Human turbinate surface areas were estimated from the tissue samples. Vapour is transported to the air: tissue interface by diffusion. The diffusivity of the vapour in the tissue is approximated by its diffusivity in water. The vapour diffusivity in all the tissue subcompartments is assumed to be equal. Metabolic activity is assumed to be located in the tissue compartments according the observations from enzyme histochemical and immunohistochemical studies. The kinetic parameter estimates were compared to those derived from homogenate experiments using two normalization procedures. When the normalization took into account tissue architecture (epithelial cell volume vs. tissue wet weight), a good agreement between homogenization technique and gas uptake technique could be observed in animal tissues. No clear conclusions on species differences could be drawn from these investigations, because dependent on the scaling technique, different results were obtained for respiratory and olfactory carboxylesterase activities in rats and humans. An overview about carboxyl esterase activities in different rat and human tissues is given in Table 1. Major drawbacks of this technique were that mucosal and blood flow and cofactor loss were not taken into account by the in vitro gas uptake methodology and that only one rat has been used to create a computer model.

Carboxyl esterase activity in the oral cavity

Vinyl acetate hydrolysis has been investigated in homogenates from oral cavity mucosa which has been obtained from different regions of the oral cavity of male F344 rats and male BDF mice. Homogenates were incubated for 30 min with various concentrations (0.05 – 10 mM) of vinyl acetate and the production of acetaldehyde was quantified by HPLC. All tissue regions possessed vinyl acetate hydrolysis activity. In both species thy hydrolysis activity was greatest in the the dorsal interior regions. Activity in the other oral regions was 2-15-fold lower (Morris et al., 2002). Values are given in table 1.

Carboxyl esterase activity in the skin

Carboxyl esterase activities have been determined using different ester substrates (but not vinyl acetate itself) in rat and human skin microsomes but also in viable skin using flow-through diffusion cells. Clark et al. (1993) investigated enzyme mediated hydrolysis of fluazifop butyl in human and rat skin post-mitochondrial fraction (with and without the esterase inhibitor BNPP). McCracken et al. (1993) investigated hydrolysis of four different ester substrates in rat skin microsomes and cytosol. Lobmeier et al. (1996) investigated the

hydrolysis of different 4-hydroxybenzoic acid esters in homogenates from different human skin compartments. Frederick et al. (1992) investigated the enzyme-mediated hydrolysis of ethylacetate in rat skin homogenates in comparison to other tissues. It could be demonstrated that rat and human skin both possess carboxyl esterase activities. Results are presented in Table 2. From the investigations by Frederick et al. (1992) it can be demonstrated, that carboxyl esterase activity in skin homogenate was lower compared to lung or liver, but higher compared to blood.

Carboxyl esterase activity in the blood, liver and lung

Vinyl acetate was rapidly degraded in the presence of human plasma and whole blood. Half lives for the breakdown of vinyl acetate, using initial concentrations of 30-130 ppm (0.33-1.41 mM) were approximately 150 sec (2.5 min) with plasma and 210 sec (3.5 min) with whole blood (0.1 M phosphate buffer, pH 7.4, at 37º C). The reaction was shown to be an enzyme-mediated hydrolysis, which was characterised by a stoichiometric formation of acetaldehyde (Strong et al., 1980).

The enzyme-mediated hydrolysis of vinyl acetate has been studied in vitro with microsomes from rat liver and lung, rat and human plasma and three different purified esterases (acetylcholine esterase, butyrylcholine esterase and carboxyl esterase). The incubation was carried out at 37°C and pH 8.0. Concentrations of vinyl acetate varied between 1.2 mM and 30 mM in the experiments designed for determination of K_m and V_{max}. Kinetic data show that microsomes (rat liver: V_{max} = 23 µmol/min/mg protein, rat lung: V_{max} = 6 µmol/min/mg protein) as well as purified carboxyl esterase (from porcine liver) are capable to form acetic acid from vinyl acetate (V_{max} = 238 µmol/min/mg protein), enzyme activity in rat plasma (V_{max} = 0.6 µmol/min/mg protein) and human plasma (V_{max}= 0.7 µmol/min/mg protein) is lower (Simon et al., 1985a).

Degradation of 200 µM vinyl acetate from in vitro systems containing 1 ml blood or 0.5 ml plasma, or 0.5 ml red blood cells from rats or volunteers, diluted in 0.1 M sodium phosphate buffer pH 7.4, was measured at 37°C. Vinyl acetate disappeared with a half-life of 4.1 min in human blood, and below 1 min in rat blood. In rat plasma vinyl acetate was hydrolysed with a half life of 1.2 min. Preincubation of rat plasma with BNPP lowered the rate of disappearance, suggesting that the reaction was catalysed by carboxylesterases (Fedtke and Wiegand, 1990).

Frederick et al. (1992) compared ethyl acrylate hydrolysis in tissue homogenates from different rat tissues. Results are presented in Table 2: Carboxylesterase activities (based on substrates other than vinylacetate).

Table 1: summary of vinyl acetate hydroxylase activities (carboxyl esterase activities) in human and animal tissues

Tissue	K_m	V_{max}	Literature
rat respiratory tissue (determined in homogenates from respiratory mucosa)	0.43 mM in males 0.30 (0.34)* mM in females	22.03 [µmol/mg prot/min] in males 23.86 (25.14)* [µmol/mg prot/min] in females	Bogdanffy and Taylor, 1993
mouse respiratory (determined in homogenates from respiratory mucosa)	0.42 mM in males 0.76 (0.93)* mM in females	29.6 [µmol/mg prot/min] in males 46.3 (51.78)* [µmol/mg prot/min] in females	Bogdanffy and Taylor, 1993
rat respiratory tissue (determined in homogenates from respiratory mucosa, scaled to whole tissue based on wet weight)	0.037 mg/ml	35.7 mg/h	Bogdanffy et al., 1998
rat respiratory tissue (determined in homogenates from respiratory mucosa, scaled to whole tissue based on epithelial volume)	0.037 mg/ml	64.9 mg/h	Bogdanffy et al., 1998
rat respiratory tissue (determined by the gas uptake technique, scaled to whole tissue based on wet weight)	0.04 mg/ml	10.8 mg/h	Bogdanffy et al., 1998
rat respiratory tissue (determined by the gas uptake technique, scaled to whole tissue based on epithelial volume)	0.04 mg/ml	63.0 mg/h	Bogdanffy et al., 1998
Human respiratory tissue (determined by the gas uptake technique, scaled to whole tissue based on epithelial volume)	0.05 mg/l	441 mg/h	Bogdanffy et al., 1998
Human respiratory tissue (determined by the gas uptake technique, activity per specimen (middle turbinate)	0.05	1.5 mg/h	Bogdanffy et al., 1998
Human respiratory tissue (determined by the gas uptake technique, based on epithelial cell volume)	0.05	0.57 mg/h/mm^3	Bogdanffy et al., 1998

Tissue	K_m	V_{max}	Literature
rat olfactory (determined in homogenates from respiratory mucosa)	0.33 (0.55)* mM in males 0.2 (0.51)* mM in females	88.8 (113.5)* ([µmol/mg prot/min] in males 91.52 (135.52)* ([µmol/mg prot/min] in females	Bogdanffy and Taylor, 1993
mouse olfactory (determined in homogenates from respiratory mucosa)	0.52 (1.07)* mM in males 0.31 (0.41)* mM in females	164.6 (253.8)* [µmol/mg prot/min] in males 95.5 (107.56)* [µmol/mg prot/min] in females	Bogdanffy and Taylor, 1993
rat olfactory tissue (determined in homogenates from olfactory mucosa scaled to whole tissue based on wet weight)	0.028 mg/ml	133.1 mg/h	Bogdanffy et al., 1998
rat olfactory tissue (determined in homogenates from olfactory mucosa scaled to whole tissue based on epithelial volume)	0.028 mg/ml	39.9 mg/h	Bogdanffy et al., 1998
rat olfactory tissue (determined by the gas uptake technique, scaled to whole tissue based on wet weight)	0.05 mg/ml	5.8 mg/h	Bogdanffy et al., 1998
rat olfactory tissue (determined by the gas uptake technique, scaled to whole tissue based on epithelial volume)	0.05 mg/ml	45.4 mg/h	Bogdanffy et al., 1998
Human olfactory tissue (determined by the gas uptake technique, scaled to whole tissue based on epithelial volume)	0.05 mg/ml	45.0 mg/h	Bogdanffy et al., 1998
Human olfactory tissue (determined by the gas uptake technique, activity per specimen (dorsal meatus))	0.05 mg/ml	0.9 mg/h	Bogdanffy et al., 1998
Human olfactory tissue (determined by the gas uptake technique, scaled to epithelial cell volume)	0.05 mg/ml	1.94mg/h/mm^3	Bogdanffy et al., 1998
Rat oral cavity mucosa	0.49 mM (dorsal interior mucosa) 0.41 mM (dorsal exterior mucosa)	90 nmol/min (dorsal interior mucosa) 50 nmol/min (dorsal exterior mucosa)	Morris et al., 2002

Tissue	K_m	V_{max}	Literature
Mouse oral cavity mucosa	0.97 mM (dorsal interior mucosa) 0.91 mM (dorsal exterior mucosa)	24 nmol/min (dorsal interior mucosa) 2.6 nmol/min (dorsal exterior mucosa)	Morris et al., 2002
rat liver microsomes	0.73 mM	23 µmol/mg prot/min	Simon et al., 1985
rat lung microsomes	6.1 mM	6.2 µmol/mg prot/min	Simon et al., 1985
rat plasma	4 mM	0.56 µmol/mg prot/min	Simon et al., 1985
human plasma	7.1 mM	0.69 µmol/mg prot/min	Simon et al., 1985
Rat liver homogenates	Not given in the report	133.3 µmol/min/g liver (homogenate)	Strong et al., 1980
Mouse liver homogenates	Not given in the report	140.0 µmol/min/g liver (homogenate)	Strong et al., 1980

*values in brackets: different equations have been used for calculation; see Bogdanffy and Taylor, 1993.

Table 2: carboxylesterase activities (based on substrates other than vinylacetate)

Tissue	Substrate	K_m	V_{max}	Literature
rat respiratory mucosa	p-nitrophenylbutyrate	20.8 mM (males) 20.9 mM (females)	0.099 µmol/mg prot/min (males) 0.106 µmol/mg prot/min (females)	Bogdanffy et al., 1987
rat olfactory mucosa	p-nitrophenylbutyrate	34.1 mM (males) 31.2 mM (females)	0.605 µmol/mg prot/min (males) 0.593 µmol/mg protein/min (females)	Bogdanffy et al., 1987
mouse respiratory mucosa	p-nitrophenylbutyrate	40.4 mM (males) 25.6 mM (females)	0.174 µmol/mg prot/min (males) 0.124 µmol/mg protein/min (females)	Bogdanffy et al., 1987
mouse olfactory mucosa	p-nitrophenylbutyrate	23.1 mM (females) 21.8 mM (females)	0.467 µmol/mg prot/min (males) 0.444 µmol/mg pro/min (females)	Bogdanffy et al., 1987
Rat respiratory tissue	Methylmetacrylate	0.15 mM (microsomes)	14.3 nmol/mg protein/min (microsomes)	Mainwaring et al., 2001

Tissue	Substrate	K_m	V_{max}	Literature
			3.5 nmol/mg protein/min) (S9)*	
Human respiratory tissue	Methylmethacrylate	Not determined	2.7 nmol/mg protein/min (microsomes)* 0.15 nmol/mg protein/min (S9)*	Mainwaring et al., 2001
Rat olfactory tissue	Methylmethacrylate	0.14 mM (microsomes)	38.6 nmol/mg protein/min (microsomes) 12 nmol/mg protein/min (S9)*	Mainwaring et al., 2001
Human olfactory tissue	Methylmethacrylate	Not determined	0.46 nmol/mg protein/min (S9)*	Mainwaring et al., 2001
Rat liver microsomes	Methylmethacrylate	0.1 mM	46.5 nmol/mg protein/min	Mainwaring et al., 2001
Human liver microsomes	Methylmethacrylate	Not determined	494 nmol/mg protein/min*	Mainwaring et al., 2001
Human skin post-mitochondrial fraction	Fluazifop butyl	420 µM (median from skin samples from different areas; n = 7)	61 nmol/min/g (median from skin samples from different areas; n = 7)	Clark et al., 1993
Rat skin post-mitochondrial fraction	Fluazifop butyl	248 µM ± 30.6 (mean, n=7)	740 nmol/min/g ± 90 (mean, n=7)	Clark et al., 1993
Rat skin microsomes	Fluazifop-butyl Carbaryl Paraoxon Phenylacetate	14 ± 2.8 µM 32 ± 4.2 µM 950 ± 100 µM	0.02 µmol/min/g 0.2 nmol/min/g ND 1.13 µmol/min/g	McCracken et al., 1993
Rat skin cytosol	Fluazifop-butyl Carbaryl Paraoxon Phenylacetate	42 ± 2.8 µM 36 ± 3.8 µM 340 ± 50 µM	0.4 µmol/min/g 0.5 nmol/min/g ND 3.44 µmol/min/g	McCracken et al., 1993
Rat liver homogenate	Ethyl acrylate	1.9 µmol/ml tissue	31.7 µmol/min/ml tissue	Frederick et al., 1992

Tissue	Substrate	K_m	V_{max}	Literature
Rat lung homogenate	Ethyl acrylate	1.88 µmol/ml tissue	5.3 µmol/min/ml tissue	Frederick et al., 1992
Rat skin homogenate	Ethyl acrylate	5.45 µmol/ml tissue	0.86 µmol/min/ml tissue	Frederick et al., 1992
Rat Arterial blood	Ethyl acrylate	4.6 µmol/ml tissue	0.18 µmol/min/ml tissue	Frederick et al., 1992

* the values for rat S9 fractions and for all human tissues are for a single rate measured at a single substrate concentration of 1.0 mM.

Aldehyde dehydrogenase

Aldehyde dehydrogenase in the nose

Aldehyde dehydrogenase (ALDH) activities have been investigated in nasal homogenates (prepared from respiratory and olfactory mucosa) from male F344 rats (Casanova-Schmitz et al., 1984). Activities were quantified by measuring acetaldehyde-dependent formation of NADH at 340 nm during the first 1-5 min after addition of acetaldehyde. It could be demonstrated that in both respiratory and olfactory mucosa two isozymes were present. The higher Km isozeyme of ALDH was five to eight times greater in respiratory than in olfactory tissue, whereas the low Km ALDH was comparable in respiratory and olfactory homogenates. Exposure to acetaldehyde vapor does not significantly alter the enzyme activities in the nasal homogenates. Results are presented in table 3.

Aldehyde dehydrogenase activities in whole nasal tissue homogenates (epithelia type not specified) from male B6C3F1 mice, Sprague-Dawley rats, Syrian golden hamsters and Hartley guinea pigs were determined according to the method described by Casanova-Schmitz et al. (1984) (Morris, 1997). The estimated V_{max} values for each species were normalised to the predicted minute ventilation of that species. In mouse, rat, and hamster the data were compatible with the assumption of two isozymes with an apparent high affinity and an apparent low affinity. In contrast, only a single form (high affinity) was detected in guinea pig nose homogenates. Total metabolic capacity (when normalised to predicted minute ventilation of the respective species defined as the sum of V_{max} for both high- and low-affinity isozymes, averaged 0.04, 0.16, 0.56, and 0.14 µmol/min/whole nose for nasal tissue of mouse, hamster, rat and guinea pig, respectively. The ratio V_{max}/K_m was significantly different between the species, the lowest activity observed in mouse (0.035 µmol/min/l/min) whereas hamster (0.13 µmol/min/l/min) and rat (0.22 µmol/min/l/min) showed findings in the same range (Morris, 1997). For comparison, the data are also presented in table 3. The data may indicate the existence of quantitative species differences.

Since the nasal cavity epithelium is composed of a heterogeneous cell population, measurement of xenobiotic metabolizing enzymes using homogenates could yield artificial results. Therefore, a similar approach as has been used to comparatively evaluate kinetic constants of carboxyl esterase , the gas uptake technique (see carboxylesterase activity in the nose), has been applied for acetaldehyde dehydrogenase (Bogdanffy et al., 1998). Data from Casanova-Schmitz et al. (1984) were used for homogenate-derived values. K_m and v_{max} values for aldehyde dehydrogenase activity in rats obtained by the gas uptake technique differed

from the values obtained using tissue homogenates. However, when data obtained from homogenates were scaled to whole nose based on epithelial volumes, comparable K_m and v_{max} values were obtained by the two different methodologies, which is illustrated in table 3.

Table 3: summary of acetaldehyde hydroxylase activities in human and animal tissues

Tissue	K_m	V_{max}	Literatur
rat respiratory tissue (determined in homogenates from respiratory mucosa)	20 mM (high Km) 3 x 10 exp-4 mM (low Km)	128 nmol/mg prot/min (high Km) 0.8 nmol/mg prot/min (low Km)	Casanova-Schmitz et al., 1984
rat olfactory tissue (determined in homogenates from olfactory mucosa)	22 mM (high Km) 1 x 10 exp-1 mM (low Km)	28 nmol/mg prot/min (high Km) 2.2 nmol/mg prot/min (low Km)	Casanova-Schmitz et al., 1984
rat whole nose homogenates	17 µmol/ml (high Km) 0.03 µmol/ml (low Km)	3.5 µmol/min/l/min (high Km) 0.27 µmol/min/l/min (low Km)	Morris, 1997
mouse respiratory tissue	41 µmol/ml (high Km) 0.07 µmol/ml (low Km)	1.35 µmol/min/l/min (high Km) 0.27 µmol/min/l/min (low Km)	Morris, 1997
hamster respiratory tissue	12 µmol/ml (high Km) 0.02 µmol/ml (low Km)	1.47 µmol/min/l/min (high Km) 0.31 µmol/min/l/min (low Km)	Morris, 1997
guinea pig respiratory tissue	ND (high Km) 0.1 µmol/ml (low Km)	ND (high Km) 0.64 µmol/min/l/min (low Km)	Morris, 1997
rat respiratory tissue (homogenate data from Casanova-Schmitz et al., 1984)	0.8 mg/ml	3.38 mg/h	Bogdanffy et al., 1998
rat olfactory tissue (homogenate data from Casanova-Schmitz et al., 1984)	0.8 mg/ml	0.94 mg/h	Bogdanffy et al., 1998
rat respiratory tissue (determined by the gas uptake technique, normalized to tissue	0.8 mg/ml	4.58 mg/h	Bogdanffy et al., 1998

volume)			
rat olfactory tissue (determined by the gas uptake technique, normalized to tissue volume)	0.8 mg/l	1.48 mg/h	Bogdanffy et al., 1998
human respiratory tissue (determined by the gas uptake technique, normalized to tissue volume)	1.1 mg/ml	88.2 mg/h	Bogdanffy et al., 1998
human olfactory tissue (determined by the gas uptake technique, normalized to tissue volume)	1.1 mg/ml	2.31 mg/h	Bogdanffy et al., 1998
Human oral cavity tissue (Acetaldehyde as substrate)	75 – 83 mM (high Km) 0.2 – 33 µM (low Km)		Dong et al., 1996; Yin et al., 1997
Human oral cavity		170 nmol/min/ml tissue 53 µmol/hr/ml tissue	Dong et al., 1996
Human esophagus and stomach	75 – 85 mM		Yin et al., 1997
Human saliva (benzaldehyde as substrate)	85 mM	9 nmol/min/ml saliva 11.75 µmol/hr/ml saliva	

Aldehyde dehydrogenase in the gastrointestinal tract

ALDH activity has been demonstrated in oral cavity tissue in humans (Dong et al., 1996; Yin et al., 1997). Human ALDH can be divided into two groups based on their affinity for acetaldehyde metabolism, those having a low affinity (i.e. high Km of 75-83 mM) and those with a high affinity (i.e. low Km of 0.2 – 33 µM). Dong et al. (1996) report a low affinity ALDH in the human oral cavity with a specific activity of 170 nmol/min/ml tissue in the gingival at a substrate concentration of 20 mM acetaldehyde, which is approximately equal to a human oral cavity ALDH activity of 53 µmol/hr/ml tissue. The human esophagus and stomach also contains low affinity (Km: 75 – 85 mM) ALDH activity (Yin, 1997). ALDH activity has also been demonstrated in human saliva. Sreerama and coworkers identified a low affinity ALDH (Km = 85 mM) in the human saliva with a specific activity of 9 nmol/min/ml saliva at a substrate concentration of 4 mM benzaldehyde, which is equal to a ALDH activity of 11.57 µmol/hr/ml in human saliva (Sreerama et al., 1995).

Aldehyde dehydrogenase in tissues different from nose and oral cavity

In mammalians and humans, multiple forms of ALDH exist. Important to mention are the cytosolic aldehyde dehydrogenase of class 1 (ALDH1) and the mitochondrial aldehyde dehydrogenase of class 2 (ALDH2). ALDH1 and ALDH2 have been determined in a variety of human and animal tissues (Uotila and Koivusalo, 1997). However, Acetaldehyde was not degraded in the presence of diluted human (and also rat and mouse) plasma or whole blood, even when these systems were fortified with NAD (Strong et al., 1980).

In an assay which detects both formaldehyde dehydrogenase (FAD) and ALDH activity simultaneously, formation of formate as a marker of enzyme activities could be determined in normal human bronchial epithelial cells and in normal human bronchial explants (Ovrebo et al., 2002). Nevertheless, expression of ALDH enzymes alone has not been determined so far in tissues of the upper respiratory tract of humans. Both ALDH1 and ALDH2 enzymes display Km values in the range of 0.5 mM for free formaldehyde (Mukerjee et al., 1992).

Cytochrome P450 2E1 (CYP2E1)

It has been investigated whether CYP2E1 is involved in vinyl acetate metabolism. Rats were pretreated with the CYP2E1 inhibitor diallyl sulphide. Nasal tissues were removed and assayed for CYP2E1 dependent p-nitrophenol hydroxylase activity. Nasal respiratory and olfactory microsomal CYP2E1 activity was inhibited. However, CYP2E1 inhibition had no significant effect on nasal vinyl acetate extraction. Therefore it was concluded that CYP2E1 does not contribute to local metabolism of vinyl acetate (Bogdanffy et al., 1999a). These investigations confirm previous results by Simon et al. (1985): after pre-treatment of rats with the CYP2E1 inhibitor diethyldithiocarbamate, the pharmacokinetics of vinyl acetate was not affected.

Glutathione transferases/glutathione conjugation

Based on the facts that i.p. injection of vinyl acetate to female Chester-Beatty rats resulted in a decrease of hepatic glutathione level to 77% of the normal value 30 min after application (Boyland and Chasseaud, 1970) and that similar decreases of hepatic non-protein sulfhydryls were reported after i.p. vinyl acetate injection to mice (300 mg/kg bw), Wistar rats (300 or 450 mg/kg bw) and guinea pigs (500 mg/kg bw) (Holub and Tarkowski, 1982), it has been assumed that these findings may indicate conjugation with glutathione. Unpublished results from DuPont Haskell Laboratories, however, did not confirm this assumption: no depletion of glutathione was noted in isolated nasal turbinates incubated in nutritive medium supplemented with vinyl acetate. Direct incubation of vinyl acetate with glutathione-containing solutions resulted in no loss of vinyl acetate from the headspace indicating that vinyl acetate does not readiliy react with glutathione. However, it could not be ruled out, that glutathione conjugation might occur at extremely high doses of vinyl acetate.

Physiologically based modelling - inhalation

A physiologically based pharmacokinetic (PBPK) model was developed to describe the deposition of vinyl acetate in the nasal cavity of the rat. The model estimates tissue exposure to vinyl acetate, acetaldehyde, and acetic acid in respiratory and olfactory mucosa with a submodel estimating potential intracellular pH_i change associated with vinyl acetate exposure

(Plowchalk et al., 1997). The model takes into account the known data on vinyl acetate metabolism (see fig.1).

In this model nasal cavity is described by three separate tissue compartments which are in contact with two airstreams flowing through the nose (Morris et al., 1993; Kimbell et al., 1993). Fractional airflows were set 12% for the dorsal medial airstream (olfactory and respiratory mucosa) and 88% for the lateral/ventral airstream (respiratory mucosa). In the model airflow is assumed to be unidirectional. Each tissue compartment was subdivided into layers representing mucus, epithelial cells, basal cells, and submucosal tissue. Mucus depth for both olfactory and respiratory mucosa was assumed to be 10 μm, based on morphometric analysis. Depth of the respiratory mucosa was estimated to be approximately 40 μm (epithelial cell layer 30 μm, basal cell layer 10 μm). The epithelial layer in the olfactory mucosa consisted of sustentacular cells (40 μm), sensory cell bodies (not included since these cells do not contain vinyl acetate metabolising enzymes), and basal cells (10 μm).

It was assumed that all enzymatic activity located in the submucosa was in the cell layers of the first 20 μm. Enzymes involved in vinyl acetate metabolism are assumed not to be uniformly distributed throughout the nasal cavity. Histochemical studies (Bogdanffy et al., 1987) indicate that carboxylesterase activity is located primarily in the cytoplasma of epithelium cells (strong), basal cells (moderate), and weak to negligible in goblet cells and the overlying mucus of the respiratory mucosa. Distribution of carboxylesterase activity in the olfactory tissue is primarily in sustentacular cells of the epithelia and Bowman's glands. Overlying mucus, basal cells and neuronal (sensory) cells were negative.

Aldehyde dehydrogenase is demonstrated by immunohistochemical staining primarily in the epithelial and basal cells of respiratory mucosa, was weak to negligible in basal cells and Bowman's glands and was absent in the sustentacular and sensory cells of the olfactory mucosa (Bogdanffy et al., 1986). Kinetic constants for carboxylesterase catalysed hydrolysis of vinyl acetate were estimated from in vitro determinations by Bogdanffy et al., 1993 (nasal tissue homogenates, converted from nmol/min/g protein basis to mg/h for the whole nose). Rates of aldehyde dehydrogenase activity in respiratory and olfactory mucosa were estimated from literature (Casanova-Schmitz et al., 1984) by scaling the reported values (nmoles/min/mg protein from homogenates) to a whole nose basis, assuming a tissue protein content of 90 mg protein/g and tissue weights of 111 mg respiratory mucosa/nose and 141 mg of olfactory mucosal tissue/nose.

Data for the kinetic constants and distribution of acetyl-CoA synthetase within the nasal tissue are not available. The K_m and Vmax for acetyl-CoA synthetases were estimated from values reported for muscle tissue in Wistar rat (Knowles et al., 1974). It was assumed that the enzyme was equally distributed in each tissue compartment.

Vapour is transported to the air: tissue interface by diffusion. The molecular diffusivities were extrapolated from the diffusivity of ethanol in water (Morris et al., 1993).

Perfusion of blood through the nasal cavity was modelled as blood flowing only through the two submucosal compartments in the respiratory and olfactory tissues at a total nasal perfusion rate of 13.2 ml/h (Morris et al., 1993). Symmetrical perfusion was assumed (6.6 ml/h).

In the pharmacodynamic submodel reduction of intracellular proton concentration was described to depend on intracellular buffering and transport to the extracellular compartment

by the pH-dependent Na^+/H^+ antiport. Na^+/H^+ antiport activities are not available for rat nasal tissues and were estimated from proton efflux rate versus pH data for human leukocytes (Goldsmith and Hilton, 1992).

The dosimetry model was applied to data from a series of experiments in vivo (see above, Plowchalk et al., 1997) designed to measure uptake and metabolism of vinyl acetate in the isolated upper respiratory tract of anaesthetised rats at exposure concentrations ranging from 73 to 2190 ppm during 1-h inhalation (unidirectional flow, 100 ml/min). The best model fit for the experimental data on vinyl acetate extraction was achieved when a second carboxylesterase 'high affinity pathway' was included into the model. The high-affinity carboxylesterase was placed in the mucus compartment of the model. Histochemical studies support this assignment (Bogdanffy et al., 1986). The kinetic constants derived for the high affinity pathway are similar to those that would be expected for many low molecular-weight substrates for cytochrome P-450 2E1. However, as the presence of P-450s in mucus is improbable the authors assume that the proposed high-affinity pathway is represented by an isoenzyme of carboxylesterase (see Bogdanffy et al. 1999a).

Model simulations (unidirectional flow, 188 ml/min, data at the end of 6 h-exposure of vinyl acetate for the first epithelial compartment in the tissue stack) predicted an exposure concentration-dependent increase in tissue concentrations of vinyl acetate, acetaldehyde and acetic acid in respiratory mucosa and also in olfactory epithelial mucosa (dosimeters given at vinyl acetate exposure concentrations of 50, 200, 600 and 1000 ppm). Steady-state concentrations of vinyl acetate in olfactory tissue are predicted to be much lower than in respiratory tissue at all exposure concentrations. Concentrations of acetaldehyde and acetic acid are expected to be approximately 1-2 orders of magnitude greater than vinyl acetate concentrations in all tissue compartments.

At vinyl acetate expositions of ≤ 600 ppm the concentrations of acetaldehyde in nasal olfactory and respiratory epithelium were predicted to be less than 5 mM. The greatest reduction in intracellular pH_i was predicted for respiratory mucosa .

A sensitivity analysis was conducted for four concentrations (5, 50, 200, and 600 ppm vinyl acetate) of vinyl acetate during 4-h exposures. The air flow and high-affinity metabolic parameters are the most important model parameters in relation to uptake of vinyl acetate by the rat nose (Plowchalk et al., 1997).

Bogdanffy et al. (1999b) have extended the model of Plowchalk et al. (1997) by

(1) constructing a 5-compartment model of the rat nasal cavity

(2) a 4-compartment model of the human nasal cavity and

(3) air phase resistance to mass transfer from the lumen to the air: mucus interface.

This model is modified to account specifically for the dorsal anterior patch of olfactory epithelium for the human and the rat nasal cavity. To better characterise the vinyl acetate flux to the apical regions of the olfactory compartments, the 5-compartment rat model divides the olfactory region into 2 compartments, a small dorsal anterior compartment (olfactory-1) and a larger posterior compartment (olfactory-2). The respiratory mucosa on the ventral side is also divided. This model incorporates air phase resistance to mass transfer. In the model of Plowchalk et al. (1997) equilibrium between the air and the mucus phase was assumed. A detailed quantitative estimate of the various cell layer thicknesses comprising the olfactory

and the respiratory mucosa of the rat and the human is provided by Bogdanffy et al. (1998). Distribution of enzymes to specific compartments was based on histochemical localisation and is described in detail by Plowchalk et al. (1997). Enzyme kinetic constants for carboxylesterase and aldehyde dehydrogenase were obtained from a whole tissue, in vitro, gas uptake technique (Bogdanffy et al., 1998). For rats a high affinity carboxylesterase pathway was optimised from vinyl acetate deposition data (rat nasal air stream 100 ml/min; exposure concentration ranged from 75 to 1500 ppm) in vivo. These values were adopted for humans and were scaled to account for differences in tissue amount between rats and humans. The K_m and Vmax for acetyl-CoA synthetase were estimated from values reported for muscle tissue in Wistar rat (Knowles et al., 1974), scaled to humans based on surface area. Mass transfer coefficients are results from hybrid computational fluid dynamics simulations on rat and human nasal cavity (Frederick et al., 1998). Blood perfusion was scaled to species based on cardiac output and nasal region based on surface area. Total nasal perfusion estimated to be 0.3% cardiac output compares favourably with measured values (Stott, 1983). The pharmacodynamic submodel for estimating intracellular acidification was described by Plowchalk et al., 1997. The PBPK model was used to examine the intracellular accumulation of acetic acid, acetaldehyde and protons produced during the metabolism of vinyl acetate at vinyl acetate expositions of 50, 200 and 600 ppm. At vinyl acetate exposure concentrations $\leq$600 ppm the concentrations of acetaldehyde in the olfactory-1-compartment of rats and the single olfactory compartment of humans were predicted to be <2 mM. Predictions of olfactory acidification for both rat and humans are presented. The change of intracellular pH is predicted to be slightly greater for human olfactory epithelium, than that of rats, per ppm inhaled vinyl acetate. Results for respiratory compartments are not shown**.**

To provide validation data for the application of the model in humans (Bogdanffy et al., 1999b), controlled human exposures to inhaled vinyl acetate were conducted (Hinderliter et al., 2005). Air was sampled by a probe inserted into the nasopharyngeal cavity of five volunteers (two women, three men). Volunteers were administered a nasal aerosol spray consisting of 2 % lidocaine and 0.25 % oxymetazoline hydrochloride and were instructed to inhale and exhale through the nose. Sampling was carried out during exposure to labelled 13C1, 13C2-vinyl acetate during resting and light exercise at three exposure levels (1, 5 and 10 ppm nominally). Both labelled vinyl acetate and the major metabolite acetaldehyde from the nasopharyngeal region were sampled at a calibrated flow rate of 12 l/h and analysed in real time utilising ion trap mass spectrometry (MS/MS). Measurements were taken every 0.8 sec in an exposure period of 2 to 5 min resulting in data during all phases of the breathing. The rate of sampling is rapid enough to capture many of the behaviours of vinyl acetate in the human nasal cavity including inhalation and exhalation. The sampling frequency is not short enough, however, to accurately capture the peak concentration in every breath. The method is free of interference from endogenous compounds and its detection limit is in the single digit ppb level. An acoustic rhinometry system was used to measure the cross-sectional area and volume of the nasal cavity. The complete set of human exposures resulted in a data set with over 22000 vinyl acetate and acetaldehyde data points. Data treatment was needed to identify the relevant experimental data, i. e. the peak concentrations for comparison of experimental data from bidirectional air flow (breathing cycle) with model data from unidirectional flow. After treatment, the experimentally determined concentrations of both vinyl acetate and acetaldehyde were then compared to the predicted concentrations calculated by applying the previously published human model (Bogdanffy et al., 1999b). The PBPK-model was exercised at the actual exposure concentrations. Other parameters were the same as presented in the human nasal model. Sensitivity analysis indicates that the air phase concentrations are most sensitive to the tissue:air partition coefficients, nasal airflow and the metabolic capacity of mucus layer . For each individual volunteer, the data from each exposure (i.e. three resting

and three light exercise periods) were characterised by a mean standard deviation, along with the model simulations. The concentrations of both compounds in airway appear to be linear as the model predicts. For vinyl acetate deposition data sets, the experimental measured concentrations are close to the model predicted peak concentrations (r = 0.9). Acetaldehyde data are fitted with somewhat lower precision (r=0.6). The authors give the explanation that increase in breathing rate resulted in difficulties of the analytical set up which resulted in less accurate measurements of peak concentrations. We are of the opinion, that the precision of the prediction is fair. Given the variations in the experimental data there is no significant inter-individual variation and no significant difference in nasopharyngeal concentrations of vinyl acetate and acetaldehyde based on exercise state. These results show that the human nasal model (Bogdanffy et al., 1999) predicts the experimental observations with regard to vinyl acetate concentrations and acetaldehyde wash-out in the airstream of human nasopharyngeal cavity in a concentration range from 1 to 10 ppm.

Physiologically based modelling - upper gastrointestinal tract

A physiologically based pharmacokinetic (PBPK) model was developed to describe the uptake and metabolism of vinyl acetate in the upper gastrointestinal (GI) tract following ingestion of vinyl acetate via drinking water in mice, rats and humans (Sarangapani, 2001). The model describes the upper GI tract using three compartments, one each for the oral cavity, esophagus and forestomach, respectively. Each compartment is divided into a three-layered substructure. A top lumen layer provides passage of the ingested vinyl acetate, a middle epithelial layer forms the target tissue for vinyl acetate toxicity, and a lower submucosal layer with blood perfusion clears the parent chemical and its metabolites. This model takes into account the known course on vinyl acetate metabolism (see Fig. 1). A pharmacodynamic submodel (PD model) was developed to describe the intracellular pH_i change associated with vinyl acetate exposure (see also "physiologically based modelling-inhalation"). The steady state pH of epithelial cells is determined by the balance between the rate of acid loading due to cellular metabolism, the cellular buffer strength, and the rate of proton transport due to special mechanisms. The PD model assumes the intracellular buffer to be a closed system containing bicarbonate and phosphate as buffers.

The upper GI tract epithelial and submucosal tissue thickness in rats and mice were measured from histological sectioning. The tissue thickness in humans was assumed to be same as in rats. Morris et al. (2002) measured carboxylesterase activity in the oral cavity tissue of rats and mice in homogenates of several tissue samples (see "in vitro data"). The composite activity values were scaled for tissue volume. Carboxylesterase activity in the esophagus and the forestomach was not measured. Human activity was obtained by allometrically scaling the mouse value to man. Aldehyde dehydrogenase (ALDH) activity has been demonstrated in oral cavity tissue in humans (Dong et al., 1996; Yin et al., 1997). Rates of ALDH activity in rats and mice oral cavity were assumed to be equal to that of humans. The acetyl-CoA synthetase activity was taken from values reported for muscle tissue in Wistar rats (Knowles et al., 1974). Other model parameters were either obtained from the available literature (diffusivity, cardiac output, surface area) or were estimated from experimental studies (water intake). The antiport parameter for the PD-submodel was estimated from values of cultured vascular muscle cells of rat or human lymphocytes.

The PBPK model was used to estimate steady state concentrations (24-h exposure) of acetic acid, acetaldehyde and intracellular proton concentration in the epithelial cell layer of the three tissue compartments for a range of vinyl acetate exposure concentrations from 400 to 10000 ppm in drinking water. The anterior tissue compartment along the GI tract (oral cavity)

is the tissue with the highest uptake of vinyl acetate both in rats and mice (rat data are not shown) when compared to oesophagus and forestomach (anterior-posterior gradient). The concentrations of vinyl acetate, acetaldehyde and acetic acid show a decline across the tissue layers in the mouse oral cavity for the three exposure concentrations used in the study. The amount of acetaldehyde, formed intermediately in the epithelial tissue of the mouse oral cavity is predicted to be about 0.16 and 0.63 mM at an exposure of 400 ppm and 2000 ppm vinyl acetate, respectively. Although this PBPK model focuses on the upper gastrointestinal tract and does not include systemic components, it could be concluded from the model, that the clearance of vinyl acetate and its metabolites from the submucosal tissue into systemic circulation will be negligible. The model-derived exposure-dose relationship for intracellular proton concentration is sigmoidal. The reduction in pH from the resting-phase proton concentration is about 0.4 and 0.7 pH units at a vinyl acetate exposure of 400 and 2000 ppm, respectively. Bogdanffy (2001) demonstrated this effect of intracellular acidification by exposing freshly isolated rat hepatocytes to vinyl acetate and observing transient alterations in intracellular pH by use of a pH sensitive fluorescent marker that was located within the cytoplasm of isolated hepatocytes.

Sensitivity analysis reveals that the model-estimated internal dose-metrics are sensitive to oral esterase activity, tissue thickness and antiport characteristics. Values for oral esterase activity and oral tissue thickness are available for the rat and mouse, but not for humans. Similarly, antiport characteristics for rats cultured vascular muscle cells and human lymphocyte are available, but antiport data specific to the oral cavity epithelial cells are not available. Due to these missing data the exact variability in the internal dose-metric in humans cannot be accurately predicted from the mouse-PBPK model.

Conclusion:

Following inhalation and oral exposure of rats vinyl acetate is rapidly and effectively hydrolysed by carboxylesterases leading to the formation of acetic acid and acetaldehyde which is further converted into acetic acid in the presence of aldehyde dehydrogenases. Further information on acetaldehyde and acetic acid can be obtained from separate reports (e.g. reports prepared by the German MAK Commission).

The in vivo uptake of vinyl acetate was measured in the isolated upper respiratory tract of rats (anaesthetised rat, unidirectional flow, and 1 h-exposure). Disappearance of vinyl acetate from the airstream was highest at the lowest exposure concentrations. Greater than 94% extraction was observed at vinyl acetate exposure concentrations of 76 ppm or below. With increasing exposure concentration (76 to 550 ppm), extraction decreased progressively to about 40% and remained at this level up to concentration of approximately 2000 ppm. The impact on blood-flow extraction on vinyl acetate deposition has been calculated by simulating vinyl acetate exposure in the absence of carboxylesterase activity. It could be demonstrated, that blood flow extraction accounts for less than 15 % of total vinyl acetate deposition. Hence, 15 % inhalative uptake can be taken as a worst case scenario for risk characterisation of systemic effects. However, it should be kept in mind, that vinyl acetate can be degraded in the blood with half lives between < 1 min and 4.1 min.

After oral administration of 297 mg/kg/bw ^{14}C vinyl acetate, 63 % of the applied radioactivity was excreted as metabolites in exhaled air, urine and faeces. Based on the fact that vinyl acetate can be metabolized in the upper GI tract epithelium it can be assumed, that a considerable extent of metabolism takes place presystemically which is supported by an oral

PBPK model (see below). This model led to the conclusion, that clearance of vinyl acetate and its metabolites into the systemic circulation would be negligible. Hence, 63 % absorption would represent an overestimation of systemically available amounts of vinyl acetate. However, the PBPK model developed for oral vinyl acetate exposure did not include systemic components and furthermore, the model was developed in the absence of valid data for carboxylesterases in animal and human tissues. Based on the fact, that carboxylesterases in the GI tract are lower compared to carboxylesterase activities in nasal tissues, 50 % absorption can be assumed as a worst case for systemically available amounts of vinyl acetate after oral uptake. However, no clear assumptions can be made for systemically available metabolites of vinyl acetate (acetaldehyde, acetic acid).

There are no valid quantitative data on the systemic bioavailability of vinyl acetate and its metabolites following dermal exposure. However, based on an acute dermal study in rabbits and based on the fact that carboxylesterase activities are lower in skin compared to nose or oral cavity, it can be assumed that systemic bioavailablility of vinyl acetate and/or vinyl acetate-derived metabolites is higher after dermal exposure when compared to oral or inhalative exposure. Therefore 90 % dermal absorption should be taken forward to the risk characterisation.

Local metabolism was studied in human and rat nasal respiratory and olfactory tissue with whole turbinates in vitro. The studies indicated species differences of nasal respiratory carboxylesterase activities between rats and humans. The differences varied depending on the mode of data presentation (activity per specimen/activity per epithelial cell volume/activity scaled to whole nose). Therefore, no clear conclusions on the magnitude of species differences of nasal respiratory carboxylesterase activities can be drawn from these investigations. Rat aldehyde dehydrogenase activity in respiratory epithelium was about twice that of humans. Activities of the rat olfactory enzymes (carboxylesterase and aldehyde dehydrogenase) were about equivalent to those of humans. The K_m values for both enzymes are not different between the two species. Aldehyde dehydrogenase activities determined in whole nasal tissue homogenates from mouse, rat, hamster and guinea pig showed significantly different ratios V_{max}/K_m for the various species indicating the existence of species differences.

Vinyl acetate hydrolysis has been studied in vitro in the oral mucosal tissues from the oral cavity of rats and mice. The hydrolysis activity of the oral tissues is at least 100-fold lower than that of the nasal tissues.

A physiologically based pharmacokinetic model was developed which describes the deposition of vinyl acetate in the nasal cavity of the rat. This model predicts steady state concentrations of the metabolite acetic acid after continuing 6 h-exposure in respiratory tissue which are approximately 13 times greater and in olfactory tissue which are approximately 2 times greater than those of acetaldehyde, the second metabolite. As the concentration of acids is indicative for the concentration of protons the model predicts the greatest reduction in intracellular pH_i for respiratory mucosa. Hence, pH effects should be more pronounced in this tissue as compared to other tissues. This physiologically based toxicokinetic/toxicodynamic model for rat was modified for the olfactory epithelium of the both human and rat nasal cavity. The change in intracellular pH is predicted to be slightly greater for human olfactory epithelium, than that of rats. To provide validation data for this model, controlled human exposures at exposure levels of 1, 5 and 10 ppm to inhaled vinyl acetate were conducted. Air was sampled by a probe inserted into the nasopharyngeal cavity of five volunteers at bidirectional breathing through the nose. Data from ion trap mass spectrometry measurements of labelled vinyl acetate and acetaldehyde were compared with data from the human nasal

model simulation. For the vinyl acetate data a good fit was demonstrated (r = 0.9). Acetaldehyde data are fitted with a somewhat lower precision. The results show that the human nasal model predicts the experimental observations with regard to vinyl acetate concentrations and the acetaldehyde washout in the airstream of human nasopharyngeal cavity in a concentration range from 1 to 10 ppm. However, uncertainties of the model consist in the enzyme kinetic data used to establish the model. Therefore, data on PK and PD outcome derived from the model should be taken with caution.

A similar PBPK model with a pharmacodynamic submodel for the upper GI tract of mouse, rat and man was developed to estimate oral vinyl acetate uptake, metabolism and the reduction in the intracellular pH. The model was used to estimate steady state concentrations (24 h exposure) of acetic acid, acetaldehyde and intracellular proton concentration in the epithelial cell layer for a range of vinyl acetate exposure from 400 to 10000 ppm in drinking water. Details of model simulations are given for mouse. The intracellular pH reduction from the resting-phase proton concentration is about 0.4 and 0.7 pH units at a vinyl acetate exposure of 400 and 2000 ppm, respectively. Due to missing human data (carboxylesterase activity and tissue thickness) the exact variability in the internal dose-metric in humans cannot be accurately predicted from the PBPK model.

4.1.2.2 Acute toxicity

Animal data:

Vinyl acetate exhibits low acute toxicity by the oral and the dermal way of exposure but significant acute inhalation toxicity:

Oral

An oral LD_{50} value of 3.73 ml/kg (= 3470 mg/kg) was determined in a range finding study in rats, using vinyl acetate (no data on purity): Doses of 8, 4 and 2 ml/kg body weight were administered by stomach intubation to 5 rats per dose. After application of 8 ml/kg all rats died within 24 hours, whereas after application of 4 ml/kg 3/5 rats died within 24 hours, and after application of 2 ml/kg no mortalities were observed. Clinical signs included sluggishness, gross pathology detected congestion throughout the lungs and the abdominal viscera, the liver was mottled (no more data given) (Mellon Institute, 1969).

The same oral LD_{50} value of 3.76 ml/kg (approximately 3500 mg/kg) for rats resulted from a study using 2% and 20% vinyl acetate emulsions with traganth. dyspnoe, tremors, apathy and diarrhoea were observed. At necropsy, no gross changes were detected (no further data given) (BASF AG, 1967).

Dermal

A range finding study with rabbits demonstrated a dermal LD_{50} value of 8.0 ml/kg (= 7440 mg/kg) for vinyl acetate (no data on purity): Four rabbits per dose (doses: 4, 8 and 16 ml/kg body weight) were immobilised during a 24-hours contact period with the compound retained under impervious sheeting on the clipped intact skin of the trunk. The animals were placed in a hood with their heads protruding into the room to prevent the inhalation of the vapour. After occluded application of 16 ml/kg all animals died (clinical signs: convulsions within 15-30 minutes, crying, pupils turned upward, deaths within 25-70 minutes). After occluded application of 8 ml/kg 2/4 animals died within 2 days (skin condition: erythema, oedema, necrosis). After occluded application of 4 ml/kg no mortalities and no clinical signs were observed. However, gross pathology revealed congestion of the lungs and liver, mottled spleen and kidney and prominent liver acini (Mellon Institute, 1969).

Inhalation

An acute inhalation range finding study with rats resulted in an LC_{50} value of 4490 ppm (= 15.8 mg/l/4 hours): Vinyl acetate vapour at metered concentration, not checked analytically, was generated by feeding the liquid at a constant rate down the inside of a spirally corrugated surface of a minimally heated tube, through which metered air was passed. This vapour was delivered in a glass exposure chamber to 6 male and 6 female rats per dose (doses: 8000, 4000 and 2000 ppm). While inhaling 8000 ppm all male and all female rats died during the exposure time (clinical signs: gasping at 10, prostration at 25, convulsions and death at 50 minutes to 1.5 hours). While inhaling 4000 ppm 2/6 male and 2/6 female rats died during the

exposure time (clinical signs: laboured breathing at 20 minutes, convulsions at 2.5 hours, death at 3 hours). After inhalation of 2000 ppm no rat died (clinical signs: extremities red and irritated). Gross pathology detected haemorrhages in lungs and tracheae of victims, but no remarkable effects after inhalation of 2000 ppm (Mellon Institute 1969). In another study 4000 ppm vinyl acetate (= 14.1 mg/l) killed 2-4/6 rats within an exposure time of 4 hours. No further data are submitted (Carpenter et al., 1949).

In a study on toxicity caused by inhalation of saturated vinyl acetate vapours (temperature used was 20°C) 10/12 rats died within 3 minutes of exposure. Saturated vapours were produced by blowing air through a 5 cm layer of vinyl acetate and inhalation resulted in death of 0/12 rats after 1 minute, 10/12 rats after 3 minutes and 6/6 rats after 10 minutes of exposure. Clinical signs prior to death included severe irritation of mucous membranes, laboured breathing and narcosis. At necropsy, no gross changes were detected (BASF AG, 1967).

Human data:

No human data available.

Conclusion:

Human data on the acute toxicity of vinyl acetate are not available. In tests with rats LD_{50} values were in two studies 3470 mg/kg and 3500 mg/kg, respectively. A dermal LD_{50} value of 7440 mg/kg was determined from a range finding study with rabbits. Thus, vinyl acetate needs no labelling according to EU criteria with respect to acute oral and acute dermal toxicity. Inhalation toxicity testing, however, resulted in LC_{50} values of 15.8 mg/l/4 hours and 14.1 mg/l/4 hours in rats; thus requiring classification as harmful and labelling with „R 20, harmful by inhalation“.

4.1.2.3 Irritation

4.1.2.4 Corrosivity

Animal data:

Vinyl acetate has proven to cause irritation or corrosion to the skin of rabbits depending on the duration of the skin contact.

The primary skin irritation potential of vinyl acetate (stab., 99.9% purity) was investigated according to international test guidelines by semi-occlusive application of 0.5 ml of the substance to the intact skins of one male and two female New Zealand White rabbits. Oedema was not observed, mean scores for erythema (observation 24, 48 and 72 hours after application) were calculated to be 0.7/0.3/0; erythema disappeared in all animals after 3 days. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no other clinical signs of substance related effects were observed (RCC 2003a).

In a Draize skin test with rabbits similar to current EU guidelines vinyl acetate caused skin irritation: Six rabbits were exposed by occlusion to 0.5 ml of undiluted liquid vinyl acetate each. Effects on intact skin included erythema (scores 1/1/1.5 for 4, 24, 72 h) and oedema (scores 2.7/2.2/0.3 for 4, 24, 72 h). Erythema observation at 4 and 24 h was difficult due to red staining of the application site. Subdermal haemorrhage was observed in one animal after 72 hours (Industrial BIO-Test Labs. Inc., 1972).

Mild irritation after 5-15 minutes of exposure and necrosis after 20 hours of exposure was demonstrated in a further skin irritation study: An unknown number of rabbits were dermally exposed to unknown amounts of undiluted vinyl acetate for 1, 5 and 15 minutes and for 20 hours (no information on the kind of exposure). No signs of irritation were observed after 24 hours following 1-minute exposure; slight erythema occurred 24 hours after the 5- and the 15-minutes exposures. Mild erythema and mild oedema were observed at 24 h after the 20-hours exposure. Necrosis was observed 8 days after 20 hour-exposure to the compound (BASF AG, 1967).

Long time occlusive dermal application of 8 ml/kg killed 2/4 rabbits within 2 days; these animals showed necrosis at the application site (Mellon Institute, 1969, see chapter on acute toxicity).

Application of 1-2 drops of the undiluted substance (stabilised with 3-5 ppm hydroquinone) to the eyes of rabbits after 1 hour mild (+) erythema, pronounced (++) oedema and after 24 hours mild (+) erythema and mild (+) opacity. No signs were observed after 8 days. The number of test animals was not given, scores were mild (+), pronounced (++), very strong (+++) (BASF AG, 1967).

Vinyl acetate caused mild irritation in the eyes of rabbits which was reversible within 48 h after application. The test was performed according to the current guidelines on acute eye irritation (OECD 405, B.5 92/69 EEC). The amount of 0.1 ml of undiluted vinyl acetate (purity of 99.9 %) was placed in the left conjuctival sacs of one male and two female New Zealand White rabbits. One hour after application mild redness and chemosis of conjunctivae were observed, which were fully reversible in all animals after 48 and 24 h, respectively. No changes of corneal opacity and iris were observed. The mean scores determined after 24, 48 and 72 h and relevant for classification were 1/0/0 for redness and 0/0/0 for chemosis, corneal opacity and iris (RCC, 2003c).

Five rabbits that received 0.1 ml undiluted vinyl acetate (clear liquid, purity not given) showed mild reddening 24 to 48 hours after application. In three animals chemosis was observed after 24-48 hours (without any scores). Overall, mild eye irritation was concluded (Hoechst, 1970).

Vinyl acetate has proven to cause severe irritation in the respiratory tract of rats (see chapters on acute toxicity, 4.1.2.2 and repeated dose toxicity, 4.1.2.6).

Human data:

Exposed workers are principally exhibiting local irritant reactions of the skin, eyes and respiratory tract (information by the vinyl acetate industry, without further details). Other, more substantiated information on irritating effects on humans is not available.

Conclusion:

Except a general notice from occupational use no substantiated human data on irritation/corrosion caused by vinyl acetate are available.

Due to the only valid tests (RCC, 2003a and c) mild irritative effects on the skin and eyes of rabbits were observed that do not warrant classification. Earlier studies of limited reliability indicated pronounced irritation or corrosion of skin after extended exposure periods.

Acute inhalation tests with rats demonstrated severe irritation in the respiratory tract of the animals. Thus, vinyl acetate should be labelled „R 37 Irritating to respiratory system according to the Annex I rules. In September 2007 the TC C&L agreed on R37.

4.1.2.5 Sensitisation

Animal data:

The potential of undiluted vinyl acetate (commercial grade) to produce delayed contact hypersensitivity in guinea pigs was evaluated with the Buehler method (adoption of method of Ritz and Buehler). The study was performed with 20 animals in the test group and 10 animals in the control group.

Animals were induced 3 days per week with undiluted commercial grade vinyl acetate (usually containing 1.5-20 ppm hydroquinone as a stabiliser) under an occluded patch (6 hours per exposure) placed in a restrainer. After a rest period of 2 weeks the animals were challenged with 25% vinyl acetate in acetone. The incidence and severity of skin responses in the test group (grade 1 responses: slight but confluent, or moderate patchy erythema; grade ± responses: slight patchy erythema) were greater than those produced by the naive control: Twenty four hours after removal of the test 6/20 test animals demonstrated effects grade 1 and 14/20 effects grade ±, 48 hours after removal 3/20 test animals showed grade 1 and 17/20 grade ± effects; in the control group no grade 1 and 7/10 grade ± effects were observed. Thus, the testing laboratory concluded that vinyl acetate was a skin sensitiser in guinea pigs.

Since the vinyl acetate stabiliser hydroquinone (commercial grade vinyl acetate usually contains 1.5 to 20 ppm hydroquinone) has been reported to cause skin sensitization in concentrated form, 5 ppm hydroquinone in acetone was also tested (Buehler test). Following primary challenge no grade 1 responses occurred. The incidence of grade ± responses (slight, patchy erythema) in the hydroquinone-treated group (7 of 10) was compared to that of the naive control group (7 of 10). Since incidence and severity of the responses were essentially comparable between these 2 groups, the testing laboratory concluded that sensitization had not been induced by hydroquinone (Hill Top Biolabs Inc., 1995).

Vinyl acetate (purity > 99.9%) was tested in a Local Lymph Node Assay (LLNA). A total of seven groups each of five female mice (CBA strain) were used. Five groups were treated with Vinyl acetate at concentrations of 5%, 10%, 25%, 50% (w/v) in acetone: olive oil, 4:1 (v/v) and 100% (undiluted) by topical application to the dorsum of each ear lobe (left and right) on three consecutive days. A negative control group was treated only with the vehicle. A positive control group was treated with Alpha-Hexylcinnamaldehyde at a 25% concentration. The application area of both ears of each mouse was measured for ear thickness prior to the first application, daily 24 hours after each dosing and prior to necropsy. Five days after the first topical application the mice were intravenously injected into the tail vein with ^{3}H-methyl thymidine (equal to 19.8 µC/mouse). Approximately five hours after the intravenous injection the mice were killed, auricular lymph nodes were excised and pooled per animal. Single cell suspension were prepared, washed and incubated with trichloroacetic acid overnight. ^{3}H-thymidine incorporation was measured in a scintillation counter. All animals had survived and systemic clinical were not noted. Local inflammatory reactions (increased ear thickness) were noted in all groups with the exception of the vehicle control group and the 5%-group. The Stimulation Index (SI) was below a 3-fold increase in all groups with the exception of the positive control group. Mean SI values were 2.0, 2.4, 1.9, 1.7, 1.3, 16.6 for the 5%, 10%, 25% 50%, 100% test solution and the positive control (25% HCA) (5%)(RCC, 2003b).

Data on respiratory sensitization are not available.

Human data:

No allergic reactions were observed in twenty-one chemical operators who worked in vinyl acetate production for a mean period of more than 15 years: These operators, evaluated for overall health, were presumably exposed to levels of vinyl acetate in the air in concentrations of approximately 5-10 ppm for the duration of their service. In workers which came into contact with vinyl acetate (average exposure to 17.6-65 mg/m^3 over 15.2 years, with intermittent exposures near 180 mg/m^3) no allergic reactions were observed. However, no patch test was performed (Deese and Joyner, 1969).

In workers with frequent and intensive dermal exposure to vinyl acetate, no allergic skin reactions could be detected. However, no patch test was performed (Wacker-Chemie, 1995a, Klaschka and Vossmann, 1994).

Vinyl acetate is not included in standard patch test kits and this may be the reason that data on patch testing with this substance are not available. Thus, the inconclusive or negative data as cited above cannot be used as evidence that vinyl acetate may not cause sensitization by skin contact.

There is no information available on the potential for vinyl acetate to produce respiratory sensitization in humans.

Conclusion:

No cases of skin sensitization from the handling of vinyl acetate in the workplace have been reported in the last years. However, the data obtained for humans at the workplace are of limited value for assessing skin sensitising potential of vinyl acetate. There are no data on

negative patch tests to substantiate the conclusion that the substance has no skin sensitising potential. The absence of positive findings and the absence of adequate data do not allow the conclusion that vinyl acetate has no skin sensitising potential.

Results from an animal skin sensitization study (Buehler Test) showed a moderate skin sensitising potential of vinyl acetate (commercial grade). With the use of the Local Lymph Nodes Assay (LLNA) no positive stimulation responses were detected at concentrations of 5% - 100%. Increased ear thickness after treatment with concentrations >5% support the skin irritative properties seen after prolonged dermal exposure. However, the results obtained with this LLNA may not fully reflect the potential of concentrations >10%, since higher concentrations of vinyl acetate show increasing volatility, due to decreased proportions of acetone/olive oil. As a result, samples applied to the skin of the ear may have been quickly evaporated. SI values support this assumption, since a constant decrease was obtained for concentrations > 10%.

Overall, the outcome of both studies may indicate that vinyl acetate is not devoid of a skin sensitising potential. The results of the LLNA do confirm the weak-moderate effects seen in the Buehler test. However, since the positive threshold level was not exceeded in the LLNA, classification and labelling with R 43 is not warranted. The LLNA was given a higher reliability since pure vinyl acetate was used for testing whereas a commercial grade test substance was applied in the Buehler test. In addition, the Buehler test was not fully compliant to the EU testing guideline due to some deviations of the test protocol.

No direct information is available from studies in humans on respiratory sensitization. In view of the widespread use, the absence of any reports suggests that vinyl acetate may not be a respiratory sensitiser.

4.1.2.6 Repeated dose toxicity

Animal data:

Inhalation exposure

- In a less well documented study (Gage, 1970, no details on parameters of laboratory investigations, no data on food consumption, no list of organs for microscopic examination or results) groups of 4 male and 4 female Alderly Park rats were exposed to vinyl acetate (no data on purity and details on exposure chamber) at 100, 250, 630 or 2000 ppm (calculated as 360, 890, 2200 or 7100 mg/m³) 6 hours/day, 15 days in about 3 weeks. Animals exposed to 2000 ppm showed signs of eye and nose irritation, respiratory difficulties, and reduced body weight gain (no quantitative data). At the concentration of 2000 ppm, increased numbers of macrophages in the lungs were observed. Females of the 630 ppm- and 250 ppm-groups also had lower body weight gain. Autopsy revealed normal organs up to 630 ppm, at 250 ppm blood and urine tests were reported to be normal. There were no data on these tests for other dose groups. Based on this short note, the NOAEC for systemic effects was 100 ppm (360 mg/m³) in female rats and at 630 ppm (2200 mg/m³) in male rats. The NOAEC for local effects on the respiratory tract was 630 ppm (2200 mg/m³).

- In a 4-week range-finding study (Owen, 1979a,b) groups of 5 male and 5 female Sprague-Dawley rats and CD-1 mice were exposed to 0, 50, 150, 500 or 1000 ppm (calculated as 0,

180, 540, 1800 and 3600 mg/m³) of vinyl acetate vapour, 6 hours/day, 5 days/week (no data on the purity of the test substance, acetaldehyde concentration in the exposure chamber 8 ppm). The dose level of the 50 ppm group was increased to 1500 ppm (5360 mg/m³) on exposure day 8 (in mice) and on exposure day 10 (in rats) as marked clinical effects had not been observed at 1000 ppm. The studies did not incorporate examinations on haematology, clinical chemistry or urinalysis. Multiple samples from tissues/organs were preserved, but were not processed for histopathology. The reports available did not contain figures and tables. Mice exposed to 150 ppm and rats exposed to 500 ppm or higher intermittently showed hunched posture and respiratory distress with dose-related frequencies and severity grades. A dose-related (non significant) decrease in body weight gain was noted in female rats of all treatment groups and in male rats of 50/1500 ppm- and 1000 ppm groups. Mean body weight gain was decreased in all treated mice, however it gained significance only in males and females of the 1000 ppm group and in females of the 50/1500 ppm group. No treatment-related macroscopic abnormalities were observed at necropsy, the absolute and relative weights of spleen were decreased in male mice exposed to 1500 ppm and in male rats exposed to ≥1000 ppm. Histopathological examinations of respiratory tissues from mice of all concentration groups were performed subsequently and revealed hyperplastic and metaplastic changes in the epithelium of the respiratory tract (no further details, Owen, 1980a). Concentrations ≥150 ppm in mice and ≥500 ppm in rats produced clinical signs of respiratory tract irritation. As growth retardation was not significant, the NOAEC for systemic effects was 1500 ppm in rats and mice (5360 mg/m³). However with respect to the limited test parameters, the confidence of statements on systemic toxic effects was low. The NOAEC for local effects on the respiratory tract was 50 ppm in mice (180 mg/m³) and 150 ppm in rats (540 mg/m³).

- The effects of vinyl acetate exposure on nasal epithelial cell proliferation were evaluated in male Sprague-Dawley-derived rats (5 rats per group) exposed for 1, 5, or 20 days (6 h/d, 5 days per week) to 0, 50, 200, 600, or 1000 ppm (Bogdanffy et al., 1997). The test material was 99.9% pure. Impurities consisted of acetaldehyde (100 ppm), acetic acid (50 ppm), hydroquinone stabiliser (3-7 ppm), ethylacetate (300 ppm), and methylacetate (200 ppm). Respiratory tract tissues were examined for gross alterations. Cell proliferation was assessed by histopathological evaluation of five cross sections of the nose and by immunocytochemistry (level of BrdU incorporation following BrdU injection 16 hours after the last exposure). Data on feed consumption were not reported. There were no changes in body weight except to a reduced mean body weight in the 1000 ppm group from day 3 (maximum reduction on day 5 -14%) through day 26 (-11%). Following a single exposure, rats of the 600 and 1000 ppm groups showed concentration-related minimal to moderate degeneration, necrosis and exfoliation of the olfactory epithelium and only at 1000 ppm minimal degeneration and necrosis in the respiratory epithelium. Following 5 or 20 exposures, additional lesions consisted of mild to severe regenerative hyperplasia with attenuation and/or disorganisation of the olfactory mucosa, occasionally squamous metaplasia and only after 20 exposures minimal to severe degeneration and atrophy of olfactory nerve bundles. Respiratory epithelium showed minimal regenerative hyperplasia and only after 20 exposures minimal squamous metaplasia. A significant concentration-related increase in cell labelling was observed in the basal cells of the respiratory and olfactory epithelium following a single 6-hour exposure to 600 and 1000 ppm vinyl acetate. No significant difference in labelling indexes of the respiratory and olfactory epithelium was observed after 5 days of exposure. Following 20 days of exposure, the response in the respiratory epithelium remained near control levels while that in the olfactory epithelium was significantly higher in the 600 and 1000 ppm groups

than control levels. Again basal cells were labelled, but additional labelling of cells were seen in the adluminal regions of regenerating epithelium. The NOAEC for nasal effects was 200 ppm in this study (710 mg/m³).

- In a 90-day inhalation study groups of 10 male and 10 female Sprague-Dawley rats and CD-1 mice were exposed to vinyl acetate vapours (purity of test substance 99.9%) at concentrations of 0, 50, 200 or 1000 ppm (calculated as 0, 180, 710 or 3600 mg/m³), 6 hours/day, 5 days/week (Owen, 1980a,b). Histopathological sections from several tissues from all animals of the high concentration and the control groups were examined, the nasal turbinates (without any information about the number of sections) were included. In mice, histopathology examinations were extended to 2 levels of nasal turbinates, 3 lobes of the lungs and 3 levels of the trachea for all animals of the low and intermediate groups. The reports did not contain histopathology summary tables; tabulated data were totally absent in the mouse study report. The findings of the nasal mucosa were not separately reported for the respiratory and olfactory epithelia. No feed consumption data are available. Exposure of rats to 1000 ppm vinyl acetate resulted in significantly decreased body weight gain (final body weight -19% in both sexes), clinical effects (intermittent incidence of respiratory distress, hunched postures and ruffled fur), increased lung-to-body weight ratio (assumed as due to lung congestion), and histomorphological changes in the respiratory tract (mild histiocytic alveolitis). The authors assumed that increased relative lung weights were related to congestion. This association seems not to be plausible because lung congestion was reported for one incidental death in the control group. The quality of the rat study was considered to be restricted, because most control and high dose rats suffer from parasitic infections indicated by eosinophilic gastritis and colon nematodiasis. Many rats from both groups had peribronchial/perivascular lymphoid hyperplasia, alveolar histiocytosis/ histiocytic pneumonia, chronic tracheitis and laryngitis, and lympadenitis. Except a higher incidence of alveolar histiocytosis in high dose rats (19/20 vs. 12/20 controls) no treatment-related toxic effect was seen in the organs examined. – The olfactory regions of nasal sections of 4 male and 3 female rats exposed to 1000 ppm were reviewed (Hardisty et al., 1999). The most severe lesions were seen in the dorsal medial meatus of the anterior nasal passages (level II), lesions extended posteriorly to the dorsal medial meatus of levels III and IV and the dorsal scroll of the third ethmoturbinates. Lesions were characterised by postdegenerative atrophy of the olfactory epithelium and focal erosion, with associated infiltration of neutrophils and eosinophils. Some losses of Bowman's glands and of nerve bundles in the lamina propria were observed in the most severely affected areas. There was only a minimal evidence of regeneration of the affected epithelium.

- In mice, exposure to 200 ppm vinyl acetate produced transient signs of respiratory distress and hunched posture through the first 9 days of exposure. High dose males and females exhibited ruffled fur, hunched posture and respiratory distress. Six high dose females, three high dose males and two male controls died during the course of the study, all except one death were associated to the routine blood sampling. There was a significant reduction of body weight gain in the groups of high dose males (-18%) and females (-17%). Microscopic changes including inflammatory, transudation and eosinophilic homogenous material in the nasal passages, hyperplasia of goblet cells hyperplastic and metaplastic changes of the nasal turbinates, (suspected) areas of epithelial metaplasia or hyperplasia of the trachea with loss of ciliated epithelium and epithelial atrophy, multifocal bronchiolitis, bronchiectasis, bronchial epithelial metaplasia and hyperplasia, and occasional bronchiolar and bronchial exudation were seen in high dose male and female mice. Mild to slight inflammation of the nasal turbinates were observed in one low dose female and

three intermediate dose females, a very mild accumulation of eosinophilic homogenous material was observed in the nasal passages of two low dose female mice, and two mid dose females exhibited mild bronchiolitis. The authors considered these mild changes to be similar to that seen in the control animals, although no findings were reported for control mice. Although similar lesions may occasionally be observed in historical controls, these findings were consistent to those of the high dose mice. Therefore this interpretation is considered to be equivocal. On the limited data of this studies, a NOAEC for systemic effects was 200 ppm (710 mg/m³) for the rat (due to growth retardation at 1000 ppm) and 50 ppm (180 mg/m³) for the mouse (due to hunched posture at 200 ppm and above) However, it is uncertain whether hunched posture could be interpreted as a non-specific toxic effect, more likely it seems to be associated with the respiratory symptoms. The NOAEC for local effects on the respiratory tract was 200 ppm (710 mg/m³) for rats due to respiratory distress and higher incidences of alveolar histiocytosis and 50 ppm (180 mg/m³) for mice due to respiratory distress at 200 ppm assumed that the morphologic lesions of the respiratory tract of mid and low dose were incidental.

- In a combined chronic toxicity and carcinogenic study (Owen, 1988; Bogdanffy et al., 1994b) male and female Sprague-Dawley rats and CD-1 mice including 60 animals per sex per dose and three satellite groups of 10 of each species and sex for interim evaluation at week 53 and week 83 and recovery studies (70 weeks of exposure followed by 15/16 weeks of recovery) were exposed by inhalation to vinyl acetate vapour in concentrations of 0, 50, 200, and 600 ppm (equivalent to 0, 178.5, 714, 2142 mg/m³) over a period of 2 years (6 hours/day, 5 days/week). The test material contained impurities of acetaldehyde (≤65 ppm), acetic acid (≤10 ppm), water (≤472 ppm) and hydroquinone (≤1 ppm). Examinations on hematology, clinical chemistry, urinalysis and gross and microscopic abnormalities were performed on satellite groups at week 51 and 81 and on the end of the main study. At the end of the study, a significant reduction in weight gain (no quantitative data available) was observed among rats and mice in the 600 ppm groups (final body weights -10% in males to -15% in females). Also, the overall body weight gain of 200 ppm mice was significantly lower than that of the control groups (no quantitative data reported). Increases in weight gain were noted in the recovery groups of high dose male rats, the high dose male mice and females of all doses. Exposed animals of both species at all concentrations showed rough haircoat and hunched posture. There were effects on blood glucose (reduced in high dose female rats) and urinary parameters (decreased urine volume and pH values and increased specific gravity in high dose rats) which were attributed to (nonmeasured) reduced food and water consumption. The survival rates of 600 ppm female rats were higher than that of controls, (64% vs. 36%), but overall there was no treatment-related increase in mortality in both species.

  In both species exposure-related effects were confined to the respiratory system. Mean lung weight was increased in high dose rats (relative weights) and mice (absolute and relative weights). Lung weight was also affected in low and intermediate dose rats and intermediate dose mice, however the effect was inconsistently found after several intervals of treatment. In both species of each sex, vinyl acetate induced morphological nonneoplastic lesions in the nasal cavity of the 200 and 600 ppm groups and in the trachea (mice only) and in the lungs of the 600 ppm groups (Tables 4.1.2.6 A + 4.1.2.6 B). In addition, some high dose female mice showed hyperplastic lesions in the larynx (no data on exact number of affected animals), a focus of squamous epithelial hyperplasia with dysplastic changes was noted in a single female. - In rats, nasal lesions were restricted to the olfactory mucosa consisting of atrophy and basal cell hyperplasia, in severe cases lesions were accompanied with replacement of respiratory epithelium, non-

keratinising squamous metaplasia, thickened and edematous submucosa, loss of nerve bundles and Bowman's glands or with hyperplasia of glandular structures. In the 200 ppm groups, lesions were restricted or most pronounced in the anterior part of the dorsal meatus, which is normally covered by the olfactory epithelium. At 600 ppm, the lesions extended to the posterior parts of the olfactory epithelium. In this area, a layer of stratified non-differentiated epithelium containing small foamy structures resembling nerve bundles and groups of epithelial cells resembling acinar cells of the Bowman's glands was evident in many rats of the 200 ppm groups and a few rats of the 600 ppm groups. They were often accompanied by keratinising squamous epithelium and epithelial nest-like infolds. The authors considered this being a complete regeneration. - Mice of the 200 and 600 ppm groups showed similar lesions, however differed in that the olfactory epithelium was replaced by respiratory-like epithelium and the sustentacular cells were more frequently hypertrophic and showed loss of sensory cells. In high dose mice only, nonkeratinizing squamous metaplasia of the respiratory epithelium, nasal inflammatory infiltrate and exudate were observed. Also, the trachea was affected showing hyperplastic epithelium, occasionally with epithelial flattening, exfoliation, metaplasia and fibroepithelial projections. In the lung of both species exposed to 600 ppm bronchial/bronchiolar epithelium was flattened or exfoliated, and showed intraluminal fibroepithelial projections and alveolar (or interstitial) accumulation of macrophages with pigments, foamy or eosinophilic appearance.

Treatment-related nasal and pulmonary lesions in rats and mice were similar in the interim and recovery groups to those seen in the main study and occurred at about the same incidence and the same or lower degree as in the main study. In the lungs bronchial exfoliation was not observed in any of the interim or recovery group rats. Cessation of treatment for 16 weeks showed no recovery from olfactory atrophy in rats and mice, although high dose mice did show a tendency to recover from the inflammatory changes and epithelial hyperplasia. No hyperplasia of the tracheal epithelium was seen in the recovery group of mice.

In summary, the chronic inhalation of vinyl acetate vapour ≥200 ppm induced cytotoxic effects on the epithelia of the upper and lower respiratory tract of rats and mice accompanied by nasal inflammation in mice. The NOAEC for local effects on the respiratory tract was 50 ppm for both species (180 mg/m³). Based on the reduction of body weight gain, the NOAEC for systemic toxicity was 200 ppm for rats (710 mg/m³) and 50 ppm for mice (180 mg/m³).

TABLE 4.1.2.6 A

Summary of statistically significant non-neoplastic changes in lungs and nose in rats: main study

	Incidence of Lesions Other than Tumors [a]							
	Males				Females			
Concentration (ppm):	Control	50	200	600	Control	50	200	600
Lungs:	(58)	(59)	(60)	(60)	(60)	(60)	(60)	(59)
Bronchial exfoliation								
very slight	0	0	0	8**	0	0	0	0
slight	0	0	0	26**	0	0	0	4
moderate	0	0	0	2	0	0	0	0
Intraluminal fibrous projections								
very slight	0	0	0	16 ***	0	0	0	3
slight	0	0	0	14 ***	0	0	0	28 ***
moderate	0	0	0	1	0	0	0	8 **
severe	0	0	0	0	0	0	0	1
Pigment macrophage								
very slight	1	0	0	0	0	0	0	1
slight	1	3	3	33 ***	6	4	1	10
moderate	0	0	1	2	0	0	0	4
Peribronchiolar/perivascular lymphoid aggregates								
very slight	5	1	0 *	0 *	0	1	2	0
slight	15	18	21	14	11	14	14	23 *
moderate	1	4	1	2	2	1	2	5
Nose:	(59)	(60)	(59)	(59)	(60)	(60)	(60)	(59)
Olfactory epithelial atrophy								
very slight	0	1	4	0	0	1	4	0
slight	0	2	47 ***	7 *	0	0	23 ***	18 ***
moderate	0	0	2	33 ***	0	0	0	30 ***
severe	0	0	0	10 **	0	0	0	3
Olfactory epithelial squamous metaplasia								
very slight	0	0	0	2	0	0	5	4
slight	0	0	0	12 **	0	0	0	26 ***
moderate	0	0	0	9 **	0	0	0	7**
severe	0	0	0	1	0	0	0	0

[a] Figures in parenthesis present the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher´s) test between each treatment and control incidence is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
Cited from Bogdanffy et al. (1994b)

TABLE 4.1.2.6 A

Summary of statistically significant non-neoplastic changes in lungs and nose in rats: main study

(Continued)

	Incidence of Lesions Other than Tumors [a]							
	Males				Females			
Concentration (ppm):	Control	50	200	600	Control	50	200	600
Nose:	(59)	(60)	(59)	(59)	(60)	(60)	(60)	(59)
Olfactory epithelial regeneration								
very slight	0	0	3	0	0	0	3	2
slight	0	0	30***	1	0	0	16***	7**
moderate	0	0	2	0	0	0	3	0
Olfactory epithelial inflammatory cell infiltrate								
very slight	0	0	0	1	0	0	0	0
slight	0	0	0	7 *	0	0	0	5*
moderate	0	0	0	1	0	0	0	1
Epithelial nest-like infolds	0	0	0	0	0	0	1	0
very slight	0	0	1	0	0	0	0	0
slight	0	0	15***	5	0	0	5	5*
moderate	0	0	1	5	0	0	0	2
Olfactory epithelial leukocytic exudate								
very slight	0	0	0	0	0	0	1	0
slight	0	0	0	11***	0	0	0	5 *
moderate	0	0	0	2	0	0	1	3
severe	0	0	0	1	0	0	0	0
Basal cell hyperplasia								
very slight	2	5	3	1	0	0	7*	0
slight	0	0	40***	21***	0	0	24***	35***
moderate	0	0	11***	22***	0	0	3	16***
severe	0	0	0	2	0	0	0	0
Turbinate leukocytic exudate								
very slight	0	2	0	0	1	1	3	1
slight	4	8	5	5	4	3	3	7
moderate	3	6	3	8	0	1	1	7 **
severe	0	0	0	1	0	0	0	0
Submucosal inflammatory cell infiltrate								
slight	2	0	1	2	0	0	0	0
moderate	1	3	1	6	0	0	0	5 *
severe	0	0	0	1	0	0	0	0
very severe	0	0	1	0	0	0	0	0

[a] Figures in parenthesis present the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher´s) test between each treatment and control incidence is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, cited from Bogdanffy et al. (1994b)

TABLE 4.1.2.6 B

Summary of statistically significant non-neoplastic changes in lungs and nose of mice: main study

	Incidence of Lesions Other than Tumors [a]							
	Males				Females			
Concentration (ppm):	Control	50	200	600	Control	50	200	600
Lungs:	(51)	(51)	(56)	(53)	(56)	(55)	(55)	(51)
Accumulation of alveolar macrophages								
very slight	5	1	4	3	5	2	6	1
slight	10	2 *	4	7	3	8	4	10
moderate	0	4	8 **	4	2	1	1	12 **
severe	1	1	4	0	1	3	1	1
Intra-alveolar eosinophilic material								
very slight	0	0	3	1	0	0	2	1
slight	3	1	1	19 ***	0	0	0	7 **
moderate	0	0	0	10 **	0	0	1	15 ***
severe	0	0	0	2	0	0	0	1
Accumulation of brown pigmented macrophages								
very slight	2	2	1	11 *	3	5	1	2
slight	0	0	5	12 ***	1	1	4	21 ***
moderate	0	0	1	1	0	0	0	2
Intraluminal fibroepithelial projections								
very slight	0	1	2	3	1	0	0	6
slight	0	0	0	17 ***	0	2	1	19 ***
moderate	0	0	0	3	0	0	0	7 **
Bronchial gland dilatation	14	16	26	17	8	17	20 *	15
Bronchial/bronchiolar epithelial flattening and/or exfoliation								
very slight	0	0	0	4	0	0	0	4 *
slight	1	0	0	25 ***	0	0	0	28 ***
moderate	0	0	0	7 *	0	0	0	4 *
severe	0	0	0	0	0	0	0	1
Bronchial/bronchiolar epithelial disorganisation								
very slight	0	0	0	0	0	0	0	5 *
slight	0	0	0	11 **	0	1	0	18 ***
moderate	0	0	0	4	0	0	0	0

[a] Figures in parenthesis present the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher´s) test between each treatment and control incidence is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, cited from Bogdanffy et al. (1994b)

TABLE 4.1.2.6 B

Summary of statistically significant non-neoplastic changes in lungs and nose of mice: main study

(Continued)

	Incidence of Lesions Other than Tumors [a]							
	Males				Females			
Concentration (ppm):	Control	50	200	600	Control	50	200	600
Nose:	(52)	(48)	(53)	(50)	(56)	(57)	(55)	(51)
Inflammatory exudate	0	0	2	15***	0	0	1	5**
Mucosal inflammatory infiltrate	1	0	0	12**	1	2	0	5
Submucosal gland hyperplasia								
slight	3	3	28***	25***	2	5	42***	35***
moderate	0	0	8**	15***	0	0	7**	13***
Olfactory epithelial atrophy (mainly dorsal meatus)								
very slight	0	0	2	0	0	0	0	0
slight	0	0	5	0	2	4	8	0
moderate	0	0	28***	2	0	0	26***	0
severe	0	0	4	3	0	0	4	1
Olfactory epithelial atrophy (widespread)								
slight	0	0	1	0	0	0	0	0
moderate	1	0	8*	5	0	0	12***	5*
severe	0	0	4	39***	0	0	2	45***
Squamous metaplasia at the naso/maxilloturbinate region								
very slight	0	0	0	0	0	0	0	1
slight	1	1	2	13**	4	2	0	13*
moderate	0	1	0	11***	0	0	0	6**
severe	0	0	0	0	0	0	0	1
Replacement olfactory by respiratory epithelium								
slight	0 0	0	5	11***	0	0	15***	10***
moderate	0	0	1	0	0	1	5*	10***
severe		0	0	0	1	0	0	0
Trachea/bronchi:	(49)	(46)	(51)	(48)	(55)	(56)	(52)	(48)
Epithelial hyperplasia	0	0	2	19***	1	1	0	11***

[a] Figures in parenthesis present the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher´s) test between each treatment and control incidence is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, cited from Bogdanffy et al. (1994b)

Oral application

- In oral dose range-finding studies, groups of 5 male and 5 female rats or mice (Sprague-Dawley, CD-1) received vinyl acetate in drinking water at concentrations of 0, 50, 200, 1000 or 5000 ppm (rats, equivalent to 5, 20, 100 or 500 mg/kg bw, calculated on an assumed water consumption of 10% of body weight) and 0, 50, 150, 1000 or 5000 ppm (mice, equivalent to 7.5, 22.5, 150, or 750 mg/kg bw, calculated on an assumed water consumption of 15% of body weight) for 4 weeks (Gale, 1979; no data on purity, frequency of dose solution preparation or analytical concentrations). Vinyl acetate was analysed by the commercial supplier containing 3 ppm acetic acid and 21 ppm acetaldehyde. The concentration of acetic acid was confirmed after delivery and after study completion. Test solutions were daily prepared containing the nominal vinyl acetate concentrations including a corrective over-formulation for losses within 24 hours. Formulations were analysed for vinyl acetate concentrations prior to the administration during the first days (rat, mouse) and at Day 29 (rat only) of the treatment period and reanalysed for stability 24 hours later. During the 4th week of treatment the dose level of 50 ppm was increased to 10000 ppm. No deaths occurred during the course of the study in any of the experimental groups. With the exception of one single female mouse at 5000 ppm female mice, which showed tremor and hypothermia, all animals remained healthy throughout the study. Reduction of body weight gain and water consumption was observed in rats, particularly in females, at ≥ 1000 ppm, in male rats at ≥ 5000 ppm and in male mice at 5000 ppm. Reduced water consumption was also apparent in male mice treated at ≥ 1000 ppm and in female mice at 5000 ppm, the latter consumed less food than control females. Lower feed consumption in rats was confined to females treated at 10000 ppm. Data on mean body weight were not reported in the summary document available, the only quantitative data given was that reduction in feed consumption was 11% during the first 3 weeks of the study. Absolute and relative liver weights in rats of both sexes and female mice were lower in all treated groups compared to the controls. There are data on liver histopathology from 4 male and 4 female rats of the control and 5000 ppm groups showing mild multifocal portal and intralobular hepatitis similar to those lesions commonly observed in rats of this age and strain. No other organs were processed for microscopy and no laboratory examinations on clinical chemistry or hematology parameters were conducted. Thymus weights were reduced in male mice exposed to 5000 ppm. There was no evidence of any treatment-related gross abnormalities in organs. The results provided the basis for selection of appropriate dose levels for 13-week studies. The NOAEL for rats was 200 ppm (20 mg/kg bw) (based on reduced body weight gain at 1000 ppm) and in mice, the NOAEL was 1000 ppm (150 mg/kg bw) (based on tremor and, hypothermia in a single female mouse and reduced thymus weight at 5000 ppm). Reductions of water consumption and in body weight may reflect bad palatability of the test solutions, the toxicological relevance reduced liver weights (in the absence of any corresponding morphological or laboratory abnormalities) is equivocal.

- In oral 13-week studies accepted as valid according to the requirements of the directive 793/93/EEC, groups of 10 male and 10 female Sprague-Dawley rats and CD-1 mice were administered vinyl acetate in drinking water at concentrations of 0, 200, 1000 and 5000 ppm (calculated mean dose levels: male rats 31, 163, 684, female rats 36, 193, 810 mg/kg bw/d; male mice 11, 60, 285, female mice 10, 72, 281 mg/kg bw/d) (Gale, 1980a, 1980b). The test substance was 99.9% pure and contained 0.01% acetaldehyde, 0.005% acetic acid, and 0.4% water. There were no deaths during the study and all animals appeared normal. A slight, non-significant degree of growth retardation became prevalent in male rats of the high dose group the mean body weight of these animals was 8% lower than that

of controls. The weekly water consumption of male and female rats at 5000 ppm was significantly lower than that of the controls. The overall water consumption was 23% and 25% lower than in control males and females. A less marked reduction in water consumption was transiently seen for mid dose male and female rats. No treatment-related effects were seen with respect to hematology, blood chemistry, organ weights, or histopathology. In conclusion, administration of vinyl acetate in the drinking water for 13 weeks at concentrations up to 5000 ppm produced no evidence of organ toxicity in rats and mice representing the NOAEL for both species.

- 90-day drinking water studies were conducted in rats and mice to determine whether repeated exposure to vinyl acetate correlates with tissue toxicity, measured primarily by cell proliferation in and histopathology of upper digestive tract tissues (DuPont, 2000, Valentine et al., 2002). Males only were used since they appeared to be slightly more susceptible to neoplasia than females in the Japanese oral cancer study (Umeda et al., 2004a). Test solutions were prepared twice weekly, samples of the low and high dose solutions were analysed for concentrations, stability and pH value.

  Five groups of 20 male CDF (F-344)/CrlBr rats were administered vinyl acetate in the drinking water for 92 days at concentrations of 0, 1000, 5000, 10000, and 24000 ppm vinyl acetate (99.98%) (DuPont, 2000). Animals were weighed and individually observed for body weight development, water consumption and clinical signs of toxicity on a weekly basis. On test days 1, 8, 29, and 92, 5 rats per group were prepared for evaluation of oral cavity cell proliferation using pulsed 5-bromodeoxyuridine(BrdU) uptake techniques and histopathology of the upper digestive tract tissues (oral cavity, esophagus, forestomach, duodenum). Evaluations of the oral cavity for cell proliferation and histopathology were limited to level III (identical to level III of the oral cancer study by Umeda et al., 2004a) and level VI from high dose rats and controls.

  Drinking water solutions were daily prepared. Vinyl acetate concentrations taken from solutions in drinking water bottles were stable within 10% of nominal concentrations for at least 4 days. The pH of test solutions decreased with increase of the vinyl acetate concentration and time, e.g., from 5.21 on day 0 to 3.74 by day 4 at 2400 ppm. At this concentration, spontaneous hydrolysis of test compound led to an increase of the acetic acid concentration from 55 ppm at day 0 to 650 ppm by day 4.

  No mortalities or clinical signs of toxicity were attributed to vinyl acetate administration. Rats in the 5000, 10000, and 24000 ppm group had significantly lower mean body weights (-8.8, -7.4, -6-1%) and mean body weight gains (-13.4, -11, -9.2%) during the study relative to controls. Rats in the 10000 and 24000 ppm group significantly lower mean daily food consumption (-6, -7.4%) compared to controls. Mean daily water consumption was consistently and significantly lower during the study in rats administered water solutions containing 5000, 10000, and 24000 ppm vinyl acetate (-28, -37, -40.3%). Based on the overall mean water consumption data, the mean daily intake of vinyl acetate in rats from the 1000, 5000, 10000, and 24000 ppm groups was 81, 350, 660, and 1400 mg/kg bw, respectively. No test substance-related gross lesions were observed in the oral mucosa, esophagus, or forestomach of rats at any exposure level. Compared to controls, no test-substance related microscopic lesions were observed in the oral mucosa of rats at 24000 ppm. In rats of this group, significant increases in mean cell proliferation occurred in the upper jaw on days 29 and 92 and in the lower jaw on test days 1 and 29. Labelling indices were increased less than 2-fold above the appropriate controls for these groups and were considered to be of equivocal biological significance.

A similar study was conducted in B6D2F1/CrlBr mice (BDF1) (20 males/group) that received same concentrations of vinyl acetate. Evaluations of the upper and lower jaws for cell proliferation and histopathology were initially limited to level III and V from high-dose and control mice. Subsequently, the lower jaw from the intermediate groups sacrificed at 92 days was additionally evaluated to determine a NOEL for cell proliferation.

All mice survived to their scheduled termination without any clinical sign of treatment-related toxicity. No vinyl acetate induced effect on body weight was observed. At various intervals during the study, mice from all test groups had significantly lower mean food consumption compared to controls. However for the period day 0-92 no significant difference was observed. The mean daily water was significantly lower in mice from all test groups at various intervals and for the period day 0-92 (-23, -30.5, -31.4, -35%). The mean daily intake of vinyl acetate in mice from the 1000, 5000, 10000, and 24000 ppm groups was 250, 1200, 2300, and 5300 mg/kg bw. No test substance-related gross lesions were observed in the oral mucosa, esophagus, or forestomach of mice at any exposure level. Compared to controls, no test-substance related microscopic lesions were observed in the oral mucosa of mice at 24000 ppm. Significant and dose-related increases in mean cell proliferation occurred in the lower jaw of mice from the 10000 and 24000 ppm groups but only at 92 days. The increases were approximately 2.4- to 3.4-fold above the control groups mean for the 10000 and 24000 groups, respectively. Based on the magnitude of the increases and the dose-related nature of the response, these increases were considered to be compound-related.

The author concluded that increases in cell proliferation in the lower jaw of mice qualitatively and quantitatively paralleled the tumor outcome data of the Japanese oral cancer study (Umeda et al., 2004a). The incidence of oral cavity neoplastic lesions was higher in the lower jaw than the upper jaw in either rats or mice (these details were not yet submitted to the rapporteur) and the incidence of these oral cavity lesions was about 3-fold higher in mice compared to rats. It was reported to be unknown whether the increased sensitivity in the lower jaw is attributable to an innate difference in tissue responsiveness or due other phenomena, such as a gravity dependent enhancement in tissue dose to vinyl acetate. The increases in cell proliferation also paralleled the administered dose of vinyl acetate in rats (3- to 4-fold less on body weight basis) and mice.

- No treatment-related histopathological findings were reported in a short communication on dose-range finding studies in rats and mice (no further data on strains, animals/sex/group, etc.) at concentrations of 0, 600, 1500, 3800, 10000 or 24000 ppm vinyl acetate in the drinking water after 13 weeks. Water consumption was affected at 24000 ppm (calculated on an assumed water consumption of 10% in rats, resp. 15% in mice to be equivalent to 2400 mg/kg bw/d for rats, resp. 3600 mg/kg for mice) (Umeda et al., 2004a, main study reported in 4.1.2.8).

Other routes

- No information on prolonged vinyl acetate exposure via other routes was available.

Human data:

- No human data are available.

Summary on repeated dose toxicity

- Inhalation exposure

No premature deaths were confined to prolonged vinyl acetate treatment up to the highest tested concentrations of 2000 ppm in the rat and of 1000 ppm in mice. Rough haircoat and hunched posture was observed on rats and mice exposed to vapour concentrations of 50 ppm and higher for 2 years (Bogdanffy et al., 1994b, Owen, 1988). Prolonged inhalation exposure of rodents to higher concentrations of vinyl acetate vapour induced clinical signs of growth retardation, eye and nose irritation, hunched posture, rough fur, respiratory distress and reduced urine volume. A reduction of body weight gain observed in mice ≥ 200 ppm and in rats ≥ 600 ppm after exposure over 2 years may be associated to non-specific toxicity of vinyl acetate. It was interpreted to be attributed to lower food and water consumption, but the 2-year study did not contain any data on the consumption of water/food and the food efficiency. Reduced urine volume observed in rats at 600 ppm of the 2-year study (Bogdanffy et al., 1994b) were not accompanied by other morphological or functional abnormalities. No clear toxic effect could be identified in parenchyma outside the respiratory tract.

Main effects of vinyl acetate were the local toxic effects on the upper and lower respiratory tract. Rats and mice exposed repeatedly demonstrated degeneration, partly accompanied by regenerative/reparative processes and occasionally inflammation, hyper- and metaplasia of the surface lining epithelia of the nose. Lesions of the nasal cavity were found in the epithelium of the olfactory and respiratory regions being more pronounced in the olfactory epithelium. Rats exposed to concentrations of 200 ppm or higher over a period of 2 years (Bodganffy et al., 1994b, Owen, 1988) developed degenerative lesions of the olfactory mucosa. In the respiratory epithelium of the nasal turbinates, lesions were evident in this species at 1000 ppm after 4 weeks of vinyl acetate inhalation (Bogdanffy et al., 1997). At the end of the study, cell proliferative rates were significantly increased in the olfactory epithelium at ≥600 ppm, but no response was seen in the respiratory epithelium up to 1000 ppm. Mice exposed at 200 ppm for a period of 2 years showed degenerative lesions of the olfactory mucosa. Squamous metaplasia were noted in the respiratory mucosa at a concentration of 600 ppm accompanied by prominent inflammatory infiltration (Bogdanffy et al., 1994b). Hyperplastic and metaplastic alterations of the larynx and trachea together with epithelial desquamation and fibrotic reactions of the tracheal epithelium were exclusively found in mice at a concentration of 600 ppm within 2 years (Bogdanffy et al., 1994b, Owen, 1988). Similar changes were found in the bronchial and bronchiolar airways of rats and of mice at this concentration and exposure duration. In addition histiocytic cell accumulation in the alveoli and interstitium of the lung was observed being possibly related to increased lung weights.

- Oral administration

No specific organ toxicity was recorded after repeated oral administration with drinking water of vinyl acetate to rats and mice. A subchronic 13-week study revealed a slight (non-significant) reduction of food consumption and growth retardation in male rats at 5000 ppm (calculated to correspond to 684 mg/kg bw/d) (Gale, 1980a, b) that was supposed to be related to the 23% reduction in water consumption. No evidence of organ toxicity was detected in rats and mice of all dose groups. Thus, the NOAEL for both species was estimated to be 5000 ppm.

Elevated rates of cell proliferation in the mucosa of the oral cavity was observed in mice exposed for 92 days to vinyl acetate concentrations of 10000 ppm (5300 mg/kg bw/d) and rats exposed to 24000 ppm (1400 mg/kg bw/d) (DuPont, 2000; Valentine et al., 2002). The NOAEC for the mitogenic response was 5000 ppm (1200 mg/kg bw/d) in the mouse study and 10000 ppm (6600 mg/kg bw/d) for the rat study.

No/Lowest-observed-effect concentrations/levels

- Inhalation exposure

The combined chronic toxicity and carcinogenicity study on Sprague-Dawley rats and CD-1 mice (Owen, 1988, Bogdanffy et al., 1994b) was selected for the derivation of effect levels.

For local effects on the respiratory tract:

2-year study/Sprague-Dawley rats and CD-1 mice/$NOAEC_{local}$ 50 ppm (Equivalent to 178.5 mg/m³)

For systemic toxic effects:

2 year study/CD 1 mice/$NOAEC_{sys}$ 50 ppm (equivalent to 178.5 mg/m³)

- Oral administration

The 90-day drinking water studies of Gale (1980a,b) were performed according to current testing guidelines and therefore were considered to represent the most reliable data with respect to oral repeated-dose toxicity.

90-day study/Sprague-Dawley rats and CD-1 mice/NOAEL 5000 ppm (Equivalent to 684 mg/kg bw/d for male rats, 810 mg/kg for female rats, 285 mg/kg bw/d in male mice, 281 mg/kg bw/d for female mice).

This value is well corresponding to the NOAEL from long-term studies (Section 4.1.2.8) which was 1000 ppm for Sprague-Dawley rats receiving drinking water for 2 years (Bogdanffy et al., 1994a, Shaw, 1988). The NOAEL of 5000 ppm observed in mice treated for 2 years were considered to be less reliable, because the study did not contain laboratory investigations (Maltoni et al., 1997).

The 5000 ppm vinyl acetate concentration in drinking water is proposed as a NOAEL for systemic effects (684 mg/kg bw/d for the rat, 281 mg/kg bw/d for the mouse) for quantitative calculations in the risk characterisation.

Classification

As lesions of the respiratory tract epithelia occurred at concentrations above the critical concentration values according to the Annex VI criteria, there is actually no need for classification and labelling with respect to repeated dose toxicity.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Studies in vitro: bacterial gene mutations

Vinyl acetate was negative in bacterial gene mutation assays with Salmonella typhimurium strains TA 98, TA 100, TA1535 and TA1537 (McCann et al., 1975) with and without rat liver S-9 activation. Vinyl acetate was also negative in bacterial gene mutation assays with Salmonella typhimurium strains TA 98, TA 100, TA 1535 and E. coli WP2 uvr/pKM 101 with and without metabolic activation (JETOC, 2004). Also a negative result was obtained with the strains TA 100 and TA1530 after metabolising vinyl acetate with mouse liver S-9 mix and gas phase exposure of the bacteria up to 2% vinyl acetate (Bartsch et al., 1979).

Jung et al. (1992) described a collaborative study to evaluate Salmonella typhimurium TA 102 with three laboratories. Vinyl acetate was negative in all laboratories; experimental details are

available only for one lab (Hoechst, 1988). Here, vinyl acetate, diluted in ethanol, gave negative results in three tests for doses up to 5000 µg/plate with and without S-9 mix.

Vinyl acetate was also negative in several investigations for which no adequate descriptions are available: bacterial gene mutation test in various Salmonella strains (Florin et al., 1980; Lijinski and Andrews, 1980; Brams et al., 1987) and SOS-chromotest with E. coli (Brams et al., 1987).

In vitro tests: bacterial genotoxicity

	Concentration range					
Test system	With S-9 mix	Without S-9 mix	Result	Toxicity	Remarks	Reference
Salm. typh. TA98, TA100, TA1535, TA1537	yes	yes	neg	no data	rat liver S-9 mix; tested doses not clearly specified	McCann et al., 1975
Salm. typh. TA100, TA1530	0.003%, gas phase		neg	total toxicity at 0.02% and higher doses	mouse liver S-9 mix with and without cofactors	Bartsch et al., 1979
Salm. typh. TA102	up to 5000 µg/plate	up to 5000 µg/plate	neg			Jung et al., 1992
Salm. typh. TA98, TA100, TA1535, TA1537	no data	no data	neg	no data	screening paper without details	Florin et al., 1980
Salm. typh. TA98, TA100, TA1535, TA1537, TAQ1538	up to 1000 ug/plate	up to 1000 ug/plate	neg	no data	screening paper without details; use of rat and hamster liver S-9 mix	Lijinski and Andrews, 1980
Salm. typh. TA97, TA87, TA100	100 to 500 ug/ml	100 to 500 ug/ml	neg	no data	screening paper without details	Brams et al., 1987
Salm. typh. TA98, TA100, TA1535, TA1537 E. coli WP2 uvr/pKM 101	1.22-5000 µg/plate	1.22-5000 µg/plate	neg	no data	only test result tables, original data in Japanese	JETOC, 2004
E. coli PQ37	130 to 8600 ug/ml	130 to 8600 ug/ml	neg	no data	screening paper without details	Brams et al., 1987

Studies in vitro: chromosomal mutations, micronuclei, mouse lymphoma assay

Norppa et al. (1985) investigated the induction of chromosomal aberrations in human lymphocytes (whole blood cultures and isolated lymphocytes) after 48-h treatment with vinyl acetate without S-9 mix. Positive effects were obtained for doses of 0.2, 0.5 and 1 mmol/l (isolated lymphocytes) or 0.5 and 1 mmol/l (whole blood cultures). Extremely steep dose-effect relationships were observed; the maximum effect was ca. 90% aberrant cells. Toxicity data were not given.

In a similar investigation by Jantunen et al. (1986), with 24-h treatment of human lymphocytes (isolated lymphocytes and whole blood cultures), dose-dependent positive effects were found for doses of 0.25 to 2.0 mmol/l. Moderate to strong cytotoxic effects were seen for doses of 0.5 mmol/l and higher (inhibition of mitotic activity). Mustonen et al. (1986) used vinyl acetate as positive control in a chromosomal aberration test with human lymphocytes; 0.5 mmol/l led to an aberration frequency of 17.1% (excluding gaps).

In an in vitro micronucleus test with human lymphocytes without S-9 mix (Mäki-Paakanen and Norppa, 1987), increased micronucleus frequencies were obtained for doses of 0.25 and 1.0 mmol/l vinyl acetate with effects of 3.2% and 3.1% micronucleated cells (negative control, 0.9%). Data on toxicity were not given.

A mouse lymphoma assay was reported to be positive with and without S-9 mix (Kirby, 1983); however, a full report with detailed data is lacking. Vinyl acetate was tested in concentrations ranging, with S-9 mix, from 1.8 to 5 µl/ml and, without S-9 mix, from 1.9 to 4 µl/ml; the highest dose of 5 µl/ml corresponds to 4650 µg/ml or 54 mmol/l. With S-9 mix, induced mutant frequencies were 2.4- to 7.4-fold that of the concurrent negative control; relative total growths ranged from 10 to 62%. Without S-9 mix, 5- to 10.3-fold increases in mutation frequencies were observed; relative total growths were between 5 and 26%. Further details on the investigation are not available. Therefore, the relationship between genotoxicity and cytotoxicity cannot be evaluated. Furthermore, it cannot be deduced whether the genetic effects were preferentially due to gene or chromosome mutations.

In vitro tests: chromosomal mutations, micronuclei, mouse lymphoma assay

	Concentration range					
Test system	With S-9 mix	Without S-9 mix	Result	Toxicity	Remarks	Reference
chrom. ab. in human lymphocytes	not done	0.1 to 1 mmol/l	pos	no data	48-h treatment; pos at 0.2, 0.5 and 1 mmol/l (isol. lymp.), or 0.5 and 1 mmol/m (whole blood); extremely steep dose-effect-relationship; max. eff. ca. 90% aberrant cells	Norppa et al., 1985
chrom. ab. in human lymphocytes	not done	0.125 to 2.0 mmol/l	pos	clear inhibition of mitotic activity at 0.5 mmol/l and higher	24-h treatment; pos at 0.25 to 2.0 mmol/l (isol. lymph. and whole blood); max. eff. >40% aberrant cells	Jantunen et al., 1986
chrom. ab. in human lymphocytes	not done	0.5 mmol/l	pos	no data	vinyl acetate was used as positive control	Mustonen et al., 1986
micronuclei in human lymphocytes	not done	0.125 to 2.0 mmol/l	pos	no data	48-h treatment; pos for doses of 0.5 and 1.0 mmol/l; max. eff. 3.2% micronucleated cells (negative control, 0.9%)	Mäki-Paakanen and Norppa, 1987
mouse lymphoma assay	1.8 to 5 µl/ml	1.9 to 4 µl/ml	pos	with S-9 mix, total growth ranged from 10 to 62%; without S-9 mix, from 5 to 26 %	full report lacking; no data on mutation frequencies; no colony-sizing	Kirby, 1983

Studies in vitro: SCE

Vinyl acetate clearly induced sister-chromatid exchanges (SCE) in human lymphocytes in the absence of S-9 mix. According to Norppa et al. (1985) after 48-h treatment all tested doses ranging from 0.1 to 1 mmol/l were positive; the highest dose led to an SCE frequency of 120 per cell. He and Lambert (1985) demonstrated that the SCE induction was dependent on the treatment time. With 1-h treatment doses in the mmol/l-range were needed for positive effects; this investigation suffers from the disadvantage of unusual high negative controls of ca. 20 SCE per cell.

SCE induction by vinyl acetate was also demonstrated in CHO cells (Norppa et al., 1985). With 24-h treatment time without S-9 mix, effects were similar to those observed in human lymphocytes. After 4-h treatment effects were stronger in presence than in absence of S-9 mix.

In vitro tests: SCE

	Concentration range					
Test system	With S-9 mix	Without S-9 mix	Result	Toxicity	Remarks	Reference
human lymphocytes	not done	0.1 to 1 mmol/l	pos	strong inhibition of cell proliferation at high doses, but SCE induction not limited to doses with strong toxicity	48-h treatment; pos at all tested doses; extremely steep dose-effect relationship; max. SCE frequency ca. 120 per cell	Norppa et al., 1985
CHO cells	0.2 to 2 mmol/l	0.1 to 5 mmol/l	pos	strong inhibition of cell proliferation at high doses, but SCE induction not limited to doses with strong toxicity	24-h treatment without S-9 mix: strong pos effect at all tested doses; 4 h treatment: stronger effect with S-9 mix than without	Norppa et al., 1985
human lymphocytes	not done	0.1 to 2.4 mmol/l	pos	no data	investigation of time- and dose-dependency: with 1-h treatment doses in the mmol/l-range needed for positive effects; extremely high negative controls of ca. 20 SCE per cell	He and Lambert, 1985

Studies in vitro: DNA strand breaks

Induction of DNA strand breaks in human lymphocytes by vinyl acetate was analysed using the alkaline elution technique by Lambert et al. (1985). 4-h treatment with concentrations of 10 and 20 mmol/l did not result in an increase of DNA strand breaks.

In vitro tests: DNA strand breaks

	Concentration range					
Test system	With S-9 mix	Without S-9 mix	Result	Toxicity	Remarks	Reference
alkaline elution, human lymphocytes	not done	10 and 20 mmol/l	neg	no data	4-h treatment	Lambert et al., 1985

Studies in vitro: DNA-protein crosslinks (DPX)

Kuykendall and Bogdanffy (1992) demonstrated the potential of vinyl acetate for induction of DNA-protein crosslinks (DPX) with purified plasmid DNA and calf thymus histone proteins. Doses ranging from 10 to 100 mmol/l were used. Induction of DPX was bound to the presence of rat liver microsomes.

Induction of DPX was also found in isolated cells from rat nasal tissues (respiratory and olfactory; Kuykendall et al., 1993). With treatment times of 1 to 2 h, very high doses ranging from 25 to 75 mmol/l were needed for induction of DPX rates over the background level.

In the alkaline elution investigation of Lambert et al. (1985; see 'DNA strand breaks' above) the rate of X-ray induced DNA strand breaks was decreased by vinyl acetate which indicates the formation of DPX; vinyl acetate doses were 10 and 20 mmol/l.

In vitro tests: DNA-protein crosslinks

	Concentration range					
Test system	With S-9 mix	Without S-9 mix	Result	Toxicity	Remarks	Reference
DPX, isolated DNA + histone proteins	10 - 100 mmol/l	10 - 100 mmol/l	pos		use of rat liver microsomes instead of S-9 mix; pos only in presence of microsomes	Kuykendall and Bogdanffy, 1992
DPX, cells from rat nasal tissues (respiratory and olfactory)		5 to 75 mmol/l	pos	totally toxic after 2-h exposure to 50 mmol/l	12- to 15-fold increase of DPX levels after 1-2 h exposure; LOEC 25 mmol/l	Kuykendall et al., 1993
alkaline elution, human lymphocytes	not done	10 and 20 mmol/l	pos	no data	the induction of DNA strand breaks by X rays was decreased by treatment with vinyl acetate	Lambert et al., 1985

4.1.2.7.2 Studies in vivo

Studies in vivo: micronuclei and chromosomal aberrations in bone marrow cells

A bone marrow micronucleus test with mice was done with single intraperitoneal administration of doses ranging from 250 to 2000 mg/kg; sampling was 30 h after treatment (Mäki-Paakanen and Norppa, 1987). Negative findings were obtained for doses of 250 and 500 mg/kg. After treatment with 1000 and 2000 mg/kg vinyl acetate weak increases in micronucleus frequencies were found: 1.33% and 1.57% micronucleated polychromatic erythrocytes as compared to 0.6% in the negative control. However, these doses were in the highly toxic range and were lethal to 6 out of 14 or 8 out of 14 treated animals. The ratio of polychromatic to normochromatic erythrocytes, which indicates local cytotoxicity in the bone marrow, was clearly depressed at these doses. The relevance of these findings is highly questionable.

Micronucleus frequencies were also determined within 3-months toxicity studies with rats and mice. Bone marrow smears were analysed at the end of treatment and it was found that micronucleus frequencies in vinyl acetate treated animals were not significantly different from

control animals. Treatments of rats and mice were done via drinking water (1000, 5000 and 10000 ppm in drinking water; Gale, 1980a,b) or by inhalation (50, 200 and 1000 ppm; Owen, 1980a; 1980b). At the higher doses toxic effects were seen in all studies. All these investigations were done according to inadequate methodologies, e.g. the analysed cell type was not specified and positive controls were lacking. Therefore, the findings are of low reliability.

In a publication without detailed description of the methodology, results of a chromosomal aberration test with male Wistar rats were reported (Nersesyan et al., 1990). Vinyl acetate was given via the i.p.-route, the only dose was 160 mg/kg which corresponds to 20% of the LD_{50}; sampling of bone marrow was done 26 h after treatment. From the 5 animals analysed, 8.2% of the cells carried chromosomal aberrations (negative control, 0.6%); gaps were included and the spectrum of induced aberrations was quite unusual. A positive control was not included and coding of the slides was not mentioned. Altogether, this finding is of low reliability.

In vivo tests: micronuclei (MN) and chromosomal aberrations (CAb) in bone marrow

Test system	Doses	Exposure regimen	Sample time	Result	Local cytotox	General toxicity	Remarks	Reference
MN in mouse bone marrow	250 to 2000 mg/kg	1 x i.p.	30h	pos	500 mg/kg and higher	ca. 50% lethality at 1000 and 2000 mg/kg		Mäki-Paakanen and Norppa, 1987
MN in rat bone marrow	1000 to 5000 ppm	over 3 months in drinking water		neg	no data	no	inappropr. methodology	Gale, 1980a
MN in mouse bone marrow	1000 to 5000 ppm	over 3 months in drinking water		neg	no data	no	inappropr. methodology	Gale, 1980b
MN in rat bone marrow	50 to 1000 ppm	inhalation over 3 months		neg	no data	no	inappropr. methodology	Owen, 1980a
MN in mouse bone marrow	50 to 1000 ppm	inhalation over 3 months		neg	no data	no	inappropr. methodology	Owen, 1980b
CAb in rat bone marrow	160 mg/kg	1 x i.p.	26 h	pos	no data	20% of LD-50	no detailed data	Nersesyan et al., 1990

Studies in vivo: SCE in bone marrow cells

Vinyl acetate induced sister-chromatid exchanges (SCE) in bone marrow cells of male BDF rats (3 per group) after single intraperitoneal application (Takeshita et al., 1986). Weak effects were found at doses from 370 mg/kg upwards in non-hepatectomised rats and from 470 mg/kg upwards in hepatectomised rats. The maximum effect was ca. a doubling of the spontaneous SCE frequency at

560 mg/kg. The treatments were without clear influence on cell proliferation behaviour. Data on general toxicity were not given; it can be estimated that 370 and 470 mg/kg correspond to half of the LD50.

In vivo tests: SCE bone marrow

Test system	Doses	Expos. regimen	Sample times	Result	Local cytotox.	General toxicity	Reference
SCE, rat bone marrow	370 to 560 mg/kg	1 x i.p.	21 h	pos	no	no data	Takeshita et al., 1986

Studies in vivo: DNA binding in rat liver

Binding to liver DNA was studied after administration of [^{14}C]-vinyl-labelled vinyl acetate to groups of 3 rats (male and female F344, male Wistar) (Simon et al., 1985b). The test substance was given orally (1mCi = 0.1 mmol per animal) or by inhalation (1200 to 1800 ppm). Four hours after start of exposure the livers were processed. Although radioactivity was measured in DNA and nucleoproteins, none of those DNA adducts were found which occur after exposure to analogue vinyl halides and vinyl carbamate. The authors discussed that these findings should be viewed along with the rapid degradation of vinyl acetate to acetaldehyde and acetic acid by blood esterases. These intermediates may enter the metabolic C_2-pool (via acetyl-CoA) giving rise to unspecific incorporation of the label into purine nucleotides.

In vivo tests: DNA binding

Test system	Doses	Expos. regimen	Result	Remarks	Reference
DNA adducts, rat liver	1200 to 1800 ppm	4 h inhalation	neg		Simon et al., 1985b
DNA adducts, rat liver	0.1 mmol / animal	1 x orally	neg		Simon et al., 1985b

Studies in vivo: germ cell effects

Micronucleus frequencies in early spermatids of mice were analysed by Lähdetie (1988) 13 days after a single i.p. administration of vinyl acetate doses ranging from 250 to 1000 mg/kg. Doses of 750 and 1000 mg/kg were in the lethal range where 8 out 9 or 1 out of 4 animals died; group sizes were quite low (1 to 4 animals). No increase in micronucleus frequencies was seen.

Sperm abnormalities in mice were investigated 3 and 5 weeks after start of 5 daily i.p.-doses of 125 to 1000 mg/kg vinyl acetate (Lähdetie, 1988). At 1000 mg/kg no treated animal survived. At 750 mg/kg 1 out of 5 treated mice survived; the only surviving mouse showed an increased frequency of abnormal sperms 5 weeks after treatment, but not 3 weeks after treatment.

All animals survived after i.p.-doses of 500, 250 and 125 mg/kg. Marked decreases in weight gain were reported for doses of 500 and 250 mg/kg.

At 500 mg/kg increased frequencies of sperm anomalies were detected in 2 of 7 animals at 3 week-sampling (variation, ca. 2-14%) and in 4 out of 8 animals at 5 week-sampling (extremely high variation, ca. 1 to 62%). At 250 and 125 mg/kg no effects on sperm abnormality were observed.

Altogether, the effects on sperm abnormality were limited to doses in the highly toxic range. Furthermore, effects on sperm abnormality are not specific for mutagens but may be due to spermatotoxicity. Therefore, these findings are of low relevance for assessment of mutagenicity.

In vivo tests: germ cell effects

Test system	Doses	Expos. regimen	Result	General toxicity	Remarks	Reference
MN in early spermatids of mice	250 to 1000 mg/kg	1 x i.p., 13 days recovery	neg	lethality at 750 and 1000 mg/kg	only 1 to 4 mice per group	Lähdetie, 1988
sperm abnormality in mice	125 to 1000 mg/kg	daily i.p. for 5 days	pos	lethality at 750 (4/5) and 1000 mg/kg (5/5)	effects limited to doses with high toxicity and not specific to mutagenesis	Lähdetie, 1988

Studies in vivo: Human studies - chromosomal aberrations

27 workers in the polyvinyl acetate production were analysed with respect to their frequencies of peripheral lymphocytes with chromosomal aberrations (Shirinian and Arutyunyan, 1980). No exposure data were given. Aberration frequencies varied from 2.2% (1976) to 2.5% (1977) and 2.4% (1978). In a negative control group of 20 workers from the non-chemical industry the aberration frequency was 1.0% in 1978 (not analysed in 1976 and 1977). The negative control group was not 'matched', confounding factors were not considered. The authors do not claim a 'positive' result and no clear conclusion can be drawn.

Human studies: chromosomal aberrations

Test system	Exposure	No. of subjects	Matched negative control	Authors concl.	Genetic effect	Reference
chromos. aberrations in peripheral lymphocytes	not specified	27	no	?	2.2 to 2.5 % (1.0% in negative control)	Shirinian and Arutyunyan (1980)

Genotoxicity of the acetaldehyde: possible contribution to vinyl acetate genotoxicity

Acetaldehyde is a metabolite of vinyl acetate through esterase-mediated metabolism. It is discussed that vinyl acetate exhibits its genotoxicity via acetaldehyde. For example Kuykendall and Bogdanffy (1992) demonstrated that vinyl acetate induces DPX via acetaldehyde, and according to Norppa et al. (1985) chromosomal damage induced by vinyl acetate in mammalian cell cultures is through formation of acetaldehyde.

A comprehensive overview on the genotoxicity of acetaldehyde is given by the SCCNFP Opinion on acetaldehyde (2004). According to this overview acetaldehyde is not mutagenic to the standard battery of Salmonella typhimurium strains with and without metabolic activation. In a number of investigations with mammalian cells in vitro various genetic effects were induced. The genotoxicity of acetaldehyde was not dependent on the presence of external metabolisation systems. The lowest concentrations with positive genetic effects were in the ranges of 4.4 µg/ml for chromosomal aberrations and micronuclei and 1.3 µg/ml for SCE. With respect to other positive genetic effects, such as mutations in the mouse lymphoma assay, DNA binding and DNA strand breaks, higher lowest-observed-effect concentrations were reported. Doses of 4.4 and 1.3 µg/ml correspond to 0.1 and 0.03 mmol/l.

In vivo, two investigations are available on the induction of micronuclei by acetaldehyde. In bone marrow cells of mice intraperitoneal administration of high acetaldehyde doses induced micronuclei (Morita et al., 1997). The lowest doses with observed effect were about 200 mg/kg in two independent investigations; this is approximately 50% of the LD_{50}. In mouse spermatids no micronuclei were induced after single intraperitoneal administration of doses up to 500 mg/kg (Lähdetie, 1988).

Acetaldehyde is a weak inducer of SCE in rodent bone marrow cells in Chinese hamsters (single intraperitoneal administration of 0.5 mg/kg; Korte et al., 1981) and mice (single intraperitoneal injections of 0.4 µg per animal [ca. 16 µg/kg]; Obe et al., 1979).

DNA-protein crosslinks (DPX) were induced in nasal respiratory mucosa cells of Fischer 344 rats after inhalation exposure of 1000 or 3000 ppm (Lam et al. 1986). Similar ranges of DPX were produced following a single 6 h exposure and after 5 days of daily 6 hour inhalation period. Lower concentrations of 100 and 300 ppm were negative. Olfactory epithelium did not response at the end of a 6-hour exposure time at concentrations of 1000 and 3000 ppm, but exposure on 5 days resulted in a significant increase of DPX rates at 1000 ppm (no results on other test concentrations). The magnitude of response at 1000 ppm in vivo (DPX production of +12-13% at the end of 6 hour inhalation on 1 day or 5 days) was in the same range in homogenates of respiratory mucosa incubated with 100 mM acetaldehyde (DPX +14%).

Acetaldehyde is a naturally occurring substance in the metabolic pathways of animals and humans (metabolism of ethanol and sugars). It occurs in small quantities in human blood. Therefore, it may well be that acetaldehyde expresses its genotoxic potential in case of metabolic overload.

Genotoxicity of acetic acid: possible contribution to vinyl acetate genotoxicity

The vinyl acetate metabolite acetic acid is negative in bacterial genotoxicity tests (McCann et al., 1975; Hude et al., 1988; Zeiger et al., 1992).

Controversial findings were reported on in-vitro clastogenicity of acetic acid in CHL cells. According to Ishidate (1988; see also: Ishidate et al., 1984) acetic acid was negative for doses up to 1000 µg/ml (= 16.7 mmol/l) without S-9 mix. Morita et al. (1990) found positive effects with and without S-9 mix for doses of 12 and 14 mmol/l (721 and 842 µg/ml), a dose of 16 mmol/l (961 µg/ml) was totally toxic. Morita et al. (1990) concluded that acetic acid itself was non-clastogenic, but the effect was due to, unspecific, decreased pH values.

In an in vitro SCE test acetic acid induced a marginal response at 5 and 10 mmol/l (Sipi et al., 1992). The maximum effect was a 1.5-fold increase and probably due to unspecific cell-cycle delay.

In a gene mutation test with transgenic mice a 2-fold increase in mutation frequency was obtained after single dermal administration of 30 µg/kg acetic acid (Myhr, 1991); a lower (12 µg/kg) as well as a higher dose (48 µg/kg) were without effect. In this early investigation on this test system no analysis of the mutation spectrum was done. Therefore, it cannot be deduced from the test data whether the effect is due to mutations induced by acetic acid or due to the clonal growth of a cell carrying a mutation (an effect which is known for promoters).

Overall, the genotoxicity data on acetic acid give no relevant evidence that the genotoxic potential of vinyl acetate may be due to acetic acid.

Conclusion:

Vinyl acetate is negative in bacterial mutagenicity tests.

In mammalian cell cultures various cytogenetic effects were induced in the absence of S-9 mix (chromosomal aberrations, micronuclei, SCE) and in the presence of S-9 mix (SCE; chromosomal aberrations and micronuclei not analysed with S-9 mix). The lowest positive concentrations ranged from 0.1 to 0.2 mmol/l. A positive mouse lymphoma assay is in line with these results, but it cannot be deduced whether the positive effect is due to chromosomal or to gene mutations (no colony sizing). Mammalian cell culture investigations on DNA strand breaks (DSB) and DNA protein crosslinks (DPX) were negative (DSB), or extremely high concentrations were needed for positive effects (DPX).

Very few reliable data are available on the in vivo mutagenicity of vinyl acetate. A weak induction of micronuclei in mouse bone marrow cells was clearly limited to intraperitoneal doses in the LD50 range (1000 and 2000 mg/kg bw). In rats no induction of micronuclei was observed in spermatids (screening assay with intraperitoneal doses up to 1000 mg/kg bw). Further tests on induction of micronuclei or chromosomal aberrations were of too low reliability.

Also in an SCE test with rats positive effects were weak and limited to high and probably highly toxic intraperitoneal doses (370 and 470 mg/kg bw). Such weak increases in SCE frequencies may well be induced by unspecific effects on the cell cycle.

No specific DNA binding was observed in rat livers after inhalation or oral administration.

Induction of sperm abnormalities in mice again was limited to doses in the toxic range. Furthermore, it is not specific for mutagens.

No clear conclusion can be drawn from a human study on the possible induction of chromosomal aberrations in workers exposed to vinyl acetate.

Genotoxicity data on vinyl acetate metabolites are in line with the hypothesis that vinyl acetate genotoxicity is mediated by acetaldehyde. The genotoxicity of acetaldehyde is possibly limited to an overloading of defence mechanisms.

Altogether, vinyl acetate has a mutagenic potential, which is preferentially expressed as clastogenesis. The data on in vivo genotoxicity are difficult to interpret, since their majority is of low reliability, or the effects are not specific to mutagenicity. The most important effect, a weak induction of micronuclei in mouse bone marrow, is limited to intraperitoneal doses of high toxicity. Therefore, it is unlikely that the genotoxic potential of vinyl acetate is expressed in germ cells in man. However, genotoxic effects locally in directly exposed tissues (site of first contact) cannot be excluded; the occurrence and strength of the effects will be dependent on the metabolic capacity of the directly exposed tissue.

No classification of vinyl acetate in terms of germ cell mutagenicity is proposed.

4.1.2.8 Carcinogenicity

Animal data:

Inhalation exposure

- In a combined chronic toxicity and carcinogenic study (Owen, 1988; Bogdanffy et al., 1994b) male and female Sprague-Dawley rats and CD-1 mice including 60 animals per sex per dose and three satellite groups of 10 of each species and sex for interim evaluation at week 53 and week 83 and recovery studies (70 weeks of exposure followed by 15/16 weeks of recovery) were exposed by inhalation to vinyl acetate vapour in concentrations of 0, 50, 200, and 600 ppm over a period of 2 years (6 hours/day, 5 days/week). Exposure-related tumor response was observed in the nasal cavity of rats only. Concomitant degenerative, hyperplastic and metaplastic changes of the respiratory tract are described in 4.1.2.6 (Tables 4.1.2.6.A + 4.1.2.6.B). A tumor response of other organs related to vinyl acetate treatment was not evident in rats and mice.

TABLE 4.1.2.8.A

Summary of Statistically Significant Neoplastic Changes in Lungs and Nose of Rats by Vinyl acetate:

Main Study

	Incidence of Tumors (Numeric)[a]							
	Males				Females			
Concentration (ppm):	Control	50	200	600	Control	0	200	600
Lungs:	(58)	(59)	(60)	(60)	(60)	(60)	(60)	(59)
Well differentiated adenoma [B]	0	0	0	0	1	0	0	0
Nose:	(59)	(60)	(59)	(59)	(60)	(60)	(60)	(59)
Inverted papilloma [B]	0	0	0	4	0	0	0	0
Squamous cell carcinoma [M]	0	0	0	2	0	0	0	4
Papilloma [B]	0	0	1[§]	0	0	0	0	0
Carcinoma in situ [M]	0	0	0	1	0	0	0	0
Total benign tumors	0	0	1	4	0	0	0	0
Total malignant tumors	0	0	0	3	0	0	0	4
Total nasal tumors	0	0	1	7**	0	0	0	4
Larynx:	(59)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Squamous cell carcinoma [M]	0	0	0	0	0	0	0	1

[a] Figures in parenthesis represent the number of animals from which this tissue was examined microscopically. [B] = benign, [M] = malignant. Significance of differences in a pairwise (Fisher's) test between each treatment and control incidence is represented by ** ($p<0.01$).
Cited from Bogdanffy et al. (1994b), [§] exophytic papilloma in the olfactory mucosa zone, confirmed by personal communication

A total of 11 nasal cavity tumors (Table 4.1.2.8.A) classified as papilloma, squamous cell carcinoma, carcinoma in situ in olfactory regions, and papilloma and squamous cell carcinoma in respiratory or anterior non-olfactory regions (distribution see Table 4.1.2.8.B) were evident in 7 males (11.9%) and 4 females (6.7%) of the high dose rat group. In the intermediate dose groups a single tumor (benign papilloma of the olfactory region) was found in a male rat. In the larynx a squamous cell carcinoma was found in a high dose female. No tracheal or treatment-related lung tumor was observed. No respiratory tract tumor was seen in the control and low dose groups except a single adenoma of the lung in a control female.

Table 4.1.2.8.B Regional distribution of vinyl acetate-induced nasal tumors in rats

Site of origin	Tumor type
Olfactory region	2 inverted papillomas
	1 exophytic papilloma[§]
	1 squamous cell carcinoma
	1 carcinoma in situ
Respiratory region	2 inverted papillomas
Cuboidal epithelium of zone B[a]	2 squamous cell carcinoma
unknown	3 squamous cell carcinoma

#Cited from Bogdanffy et al., 1994b, [§] in a male of the 200 ppm group

[a] Zone B is located within a cross section of the nose at the level of the nasolacrimal duct medial/ventral to the incisor tooth root

In mice, no treatment-related increased incidence of tumors was observed in the nose, larynx, trachea, lung, or other tissues of mice in the main study and in the satellite groups. A single squamous cell carcinoma in a major lung bronchus of a high dose male and a single adenocarcinoma in the lung of a control male was noted.

Oral application

- The offspring of F_o-animals which received vinyl acetate in drinking water from the time of gestation at target concentrations of 0, 200, 1000 and 5000 ppm were investigated in a chronic and carcinogenicity study (Bogdanffy et al., 1994a; Shaw, 1988). Vinyl acetate was administered to male and female Sprague Dawley rats (60 offspring/sex/dose of the main study and 10 offspring/sex/dose for interim sacrifices at week 52 and week 78) via drinking water in concentrations of 0, 200, 1000, and 5000 ppm (average vinyl acetate consumption: 0, 10, 47, and 202 mg/kg/day in males, 0, 16, 76, 302 mg/kg/day in females) over a period of 2 years. The test substance contained contaminants in concentrations of ≤11.5 ppm acetic acid and ≤71 ppm acetaldehyde, ≤389 ppm water, the test solution was prepared daily. They were examined for clinical symptoms, body weight, food and water consumption, parameters of hematology, clinical chemistry and urinalysis, gross and microscopical abnormalities. Numerous organs were examined histopathologically, but no tissues from the oral cavity (except the tongue) were listed as protocol organs. Rats of the 1000 and 5000 ppm groups consumed less water and rats of the 5000 ppm groups had decreased food consumption. Mean body weight gain of 5000 ppm males was significantly reduced compared to controls during the first (-11%) and second years (-17%). Mean body weight gain of 5000 ppm females was depressed (-11%) only during the second year. No treatment-related effect was seen for the mortality rates, or the test parameters of hematology, clinical chemistry or urinalysis. There was no evidence of systemic target organ toxicity. Some organ weights (brain, kidney, heart, liver, spleen, adrenal, pituitary) were altered at ≥1000 ppm, but were not associated with histopathological changes and were therefore considered to be related to lower body weight. The high concentration did not exceed the MTD since feed and water consumption can be attributed to the lower body weight gain. The authors concluded that there was no treatment-related oncogenic effect. Squamous carcinomas were seen in the oral cavity of two high dose males and additional single other oral cavity tumors

were observed in treated animals (fibrosarcoma in one low and mid dose males each, an odontoma in a mid dose female, and a malignant schwannoma in a high dose female). In addition, a single sarcoma was found in the stomach of a high dose male. All reported tumors (including those in control and treated animals, which were not reported here) were considered to be within normal rates and within the biological variation of the strain and age of rat. Due to the reduced body weight gain at 5000 ppm, the NOAEL for nonneoplastic effects was 1000 ppm (47 mg/kg bw/d in male rats, 76 mg/kg bw/d in female rats).

For reason of possible target organs not being routinely examined and the fact that dose-related and significantly increased tumor rates in this study were absent did not give sufficient proof that carcinogenic potential of vinyl acetate was negative. Even when oral cavity was not examined as a protocol organ, two squamous carcinomas were seen in high dose males. As squamous cell carcinomas were not seen in control animals and rarely occur in historical control rats of this strain, it comes into question - due the rapporteur's opinion - that this finding may be related to vinyl acetate administration. Assumption is supported by concordance with data from other oral cancer studies in rats and mice that reported squamous cell carcinomas in the oral cavity (Umeda et al., 2004a).

- The chronic toxicity study of Lijinsky and Reuber (1983) revealed equivocal results concerning higher incidences of tumors in animals after oral administration of vinyl acetate. Groups of 20 male and female F344 rats received vinyl acetate dissolved in drinking water at 1000 or 2500 ppm over a period of 100 weeks and were observed up to 130 weeks. Three types of neoplasm appeared at higher incidences in the treated groups. These were neoplastic nodules in the liver (males 0/20, 3/20, 2/20; females 0/20, 0/20, 6/20, historical controls 3-9.1%), adenocarcinomas of the uterus (0/20, 1/20, 5/20; historical controls 0.8%-10.8%), C-cell adenomas and carcinomas of the thyroid (males 2/20, 2/20, 1/20; females: 1/20, 2/20, 6/20, historical controls: 5-7.6%). Data on historical controls were reported from internal control animals and from literature. The study performance was inadequate since the vinyl acetate solutions were prepared only once a week, which resulted in hydrolytic degradation. Bogdanffy et al. (1994a) reported a 6% to 14% degradation of vinyl acetate in tap water after 1 day and 40% to 60% over a 7-day period. The group size was small comparing to test standards for carcinogenicity studies and only gross lesions were processed for histopathology.

- In contrast to this, a recent carcinogenicity study of the Japan Bioassay Research Center (Umeda et al., 2004a, Celanese, 1998) reported to be in conformity to OECD Guideline 453 indicated that higher tumor incidences were induced by oral treatment with vinyl acetate. Groups of 50 male and female F344/DuCrj rats received drinking water containing 0, 400, 2000, and 10000 ppm vinyl acetate (98%, major impurities: water, acetic acid (no data on ppm), and 5 ppm hydroquinone, test solution was prepared twice a week) for 104 weeks (equivalent analysed doses in mg/kg bw/d, at 400 ppm: 21 in males and 31 in females, 2000 ppm: 98 in males, 146 in females, 10000 ppm: 442 in males and 575 in females). Groups of 50 male and female Crj:BDF1 mice also received drinking water containing 0, 400, 2000, 10000 ppm vinyl acetate for 104 weeks (equivalent mean doses in mg/kg bw/d calculated from body weight and amount of water consumed: 400 ppm: 42 in males, 63 in females, 2000 ppm: 202 in males, 301 in females, 10000 ppm: 989 in males and 1418 in females).

- The concentrations prepared for each group varied in comparison to the set concentration within a range of 81%-120.8% for the 400 ppm group, 89-102.7% for the 2000 ppm group, and 71.4-112% for the 10000 ppm group in the rat study and for the mice, values ranged from 81-113.3% for the 400 ppm group, 88.4-118.4% for the 2000 ppm group, and 74.1-120% for the 10000 ppm group. The concentrations of vinyl acetate in the drinking water was measured with a gas

chromatograph and stability of vinyl acetate in the drinking water was also measured in the preparations around the time of administration of the animals over a four-day period. A comparison of the results was reported to confirm stability (no quantitative data available). During the study, the general condition of the animals was observed once daily, the body weight and food consumption were estimated once weekly for the first 14 weeks, thereafter body weight was checked once per two weeks and food consumption once monthly. For the first 14 weeks water consumption was estimated twice a week and once per two week thereafter. Hematology and clinical chemistry examinations were performed in animals surviving until the time of scheduled necropsy during the 104th week after a fasting period of at least 18 hours. Urinalysis was done on fresh urine collected from the animals as surviving until the last week of administration. At present, no summary tables or individual data are available on laboratory examination. All animals underwent necropsy at the end of the study (week 97 for the rats, week 94 for the mice), organ weight was measured on scheduled sacrificed animals on brain, lung, liver, spleen, heart, kidney, adrenal, testis, ovary. During the study the body weight and food consumption were estimated once weekly for the first two weeks, thereafter body weight was checked once per two weeks and food consumption once monthly. For the first two weeks water consumption was estimated twice a week, and once per two week thereafter. Hematology and clinical chemistry examinations and urinalysis were performed (no data on time). All animals underwent necropsy, organ weight was measured on scheduled sacrificed animals on brain, lung, liver, spleen, heart, kidney, adrenal, testis, ovary. Histopathology examination of all animals included 37 organs/tissues (including the nasal cavity and the oral cavity with sections on three different levels each on the maxilla and mandible).

- The vinyl acetate concentrations in the drinking water measured 4 d after the preparation were found to decrease to 72-80% of the initial concentrations for rats and 86-96% for mice. The acetic acid concentration and the pH measured 4 d after the preparation were 9.2 ppm and pH 4.0 for the 400 ppm vinyl acetate-formulated drinking water, 47 ppm and pH 4.2 for the 2000 ppm water and 263 ppm and pH 3.6 for the 10000 ppm water at the end of the 4-day administration period.

- In the rat, no significant difference between the survival of the treated groups and that of the control group was observed in the males or females. A slight decrease in mean final body weight was observed in the high dose groups (-8% in males, -10% in females) compared to the control groups. As outlined in figures on body weight development, the growth curve from week 1 by week 16 was similar for all treatment and control groups. In this group, water consumption was on average 82% for the male rats and 75% for the female rats of that of the control groups. No significant difference in food consumption was seen for male and female groups that received vinyl acetate compared to the control values. Hematology tests reveal elevated concentrations for haemoglobin and MCH in high dose female rats. Male rats of this group exhibited an elevated albumin/globulin ratio and decreased levels of total cholesterol, phospholipids and calcium. Urinalysis revealed lower urinary pH values in male rats of the 400 and 10000 ppm groups. At necropsy, mandibular nodules were noted in three high dose males and in 1 female in the 400 ppm group in the mandibular region and in the maxillary region of 1 female of the 10000 ppm groups. A decrease in the actual weights of the kidneys and livers of male rats that received 10000 ppm. There were higher incidences of tumor rates in the oral cavity, esophagus, and stomach that were considered to be treatment-related (Table 4.1.2.8.C). In the oral cavity, increased numbers of squamous cell carcinomas were found in exposed groups of females (control: 0/50, 400 ppm: 1/50, 2000 ppm: 1/50, 10000 ppm: 3/50 with significance $p<0.05$ in the Peto test, not significant in the Fischer's exact test and Cochran Armitage test), a significantly increased incidence occurred in males (control, 400 ppm and 2000 ppm: 0/50, 10000 ppm: 5/50 with significance $p<0.01$ in the Peto test and $p<0.05$ in

Fischer's exact test and Cochran Armitage test). Squamous cell carcinoma metastasis to the tongue was noted in 1 male of the 10000 ppm group. Squamous cell papilloma was observed in 2/50 males in the 10000 ppm group (total tumor rate in high dose males 14%). In this dose group, basal cell activation was also observed in 2/50 males and 1/50 females. Epithelial dysplasia was seen in 2/50 of the 10000 ppm females. Mapping of neoplastic and preneoplastic lesions in the rat oral cavity of the high dose group showed that lesions were distributed throughout the cavity, but 4 of 8 carcinomas occurred at level VI (mandibular region at the anterior edge of the lower molar teeth). Tumors or lesions presumed to be preneoplastic lesions of the esophagus and stomach were only evident at 10000 ppm. One female out of 50 had a squamous cell carcinoma of the esophagus. In 1/50 males and 1/50 females, oesophageal squamous cell hyperplasia was observed. In addition, basal cell activation was observed in 4/50 females. No stomach tumors were found, but basal cell activation occurred in 2/50 males and 5/50 females in the 10000 ppm group.

Peto test revealed an increasing trend in the occurrence of interstitial cell tumors in the testes of males (control group: 42/50; 400 ppm group: 40/50; 2000 ppm group: 44/50; 10000 ppm group: 47/50), and an increased trend in the occurrence of adenocarcinoma in the mammary glands of females (control group: 0/50; 400 ppm group: 0/50; 2000 ppm group: 0/50; 10000 ppm group: 3/50). Both tumor rates were within the range of historical control data of the testing centre (82-98% (mean 89.6%) for interstitial cell tumors and 0-6% range (mean 2%) for mammary adenocarcinomas).

Comment: The selected doses in this rat study albeit there are very high in the top dose were not considered to exceed the MTD since no indication on non-specific or specific systemic organ toxicity could be identified. The slight decrease in final body weight and increased haemoglobin values in female rats were most likely attributable to the significantly reduced water consumption. Lower kidney and liver weights might be reflective for lower body weight gain in high dose males, no lesions indicative for organ toxicity were reported. No indications for clinically observed cachexia were reported, and tumor masses in the oral cavity of 1 or 2 animals out of 50 animals/dose groups were not to assume to interfere with food uptake because of the low number of animals affected; as to be expected the mean group food consumption in the dose groups was comparable to control values.

The increased numbers of squamous cell tumors (malignant and benign) in the oral cavity of male and female rats of the high dose groups were considered as treatment-related. In the context of increased incidences of squamous cell carcinomas of the oral cavity in high dose males and females together with the occurrence of some benign tumors and preneoplastic lesions of this cell type at this dosage and under consideration of the fact that spontaneous tumors of this type and at this site are rare, the authors in agreement with the rapporteur also considered single squamous carcinomas at 400 and 2000 ppm likely to be associated to vinyl acetate treatment. The same can be assumed for all effects on the esophagus and stomach.

- In mice no significant difference between the survival of the treated groups and that of the control group was observed in the males or females. A decrease in final body weight was observed in the high dose groups after week 60 with lower final body weights by 30% in males, and by 18% in females (starting after week 80) compared to the control groups. As outlined in figures on body weight development, the growth curve from week 1 by week 12 was similar for all treatment and control groups. Decreased mean water consumption was noted in the males of the 10000 ppm group (-15%), and dose-related in all female dose groups (-24% in the 400 ppm group, -8% in the 2000 ppm group, -17% in the 10000 ppm group, compared to control group values). There was no significant difference in food consumption between treated and control

groups. Hematology examinations revealed increased counts of platelets, neutrophils and decreases in lymphocyte counts for high dose males and decreased MCHC for high dose female. In males of the 400 group and males and females of the 10000 ppm groups serum glucose was reduced. In the males of 10000 ppm group, increased albumin/globulin ratios, increased ALP activity, and decreased total cholesterol, triglyceride and calcium levels were observed. Urinalysis in this group revealed decrease urinary pH values and increased urinary protein, in females at this dose level increased urinary protein and ketone bodies were noted. Clinical observation revealed tumor masses in the oral cavity in 6 males and 6 females of the 10000 ppm groups, the first appearance was noted in periods between week 65-78 in males and week 92-104 in females. At necropsy, mandibular nodes were seen in 3 male and 5 females, maxillary nodes occurred in 3 males and 1 female in the 10000 ppm groups. Decreases in absolute weights and increases of relative weights of several organs were seen in male and female mice in the high dose group and the author thought this to be due to decreased body weight. Tumors associated with the vinyl acetate treatment were observed in the oral cavity, esophagus, forestomach and larynx. In the 10000 ppm group, squamous cell carcinomas of the oral cavity occurred in 13/50 males and 15/49 females (both significant) and squamous cell papilloma occurred in 4/50 males and 34/49 females (total incidence of squamous cell tumors in males 32% and in females 37%), but no squamous cell tumors were seen in the control and low dose groups. Only at 10000 ppm, epithelial dysplasia was observed in 24/50 males and 17/49 females. From doses at 2000 ppm and higher, squamous cell hyperplasia was also observed in 2/50 males and 1/50 females at 2000 ppm, and in 13/50 males and 6/49 females at 10000 ppm. Basal cell activation was found in 1/50 males and females each at 2000 ppm and in 18/50 males and 17/49 females at 10000 ppm. More than 60% of the male mice and 35% of the female mice had two or more preneoplastic lesions at different sites of the oral cavity; their incidence was highest at level V of the mandibular region (at the middle point between lower incisor teeth and molar teeth). In the esophagus, there were squamous cell tumors consisting of carcinomas in 7/50 males and 1/49 females of the 10000 ppm group and of one single papilloma in a 2000 ppm female. In the 10000 ppm group, lesions of preneoplastic nature were epithelial dysplasia (2/50 males, 7/49 females, squamous cell hyperplasia (2/50 males and 2/49 females), and basal cell activation (9/50 males and 15/49 females). In the forestomach, squamous cell carcinomas occurred in 7/50 males and 3/49 females of the high dose group in comparison to none in the control and other dose groups. Benign squamous cell papilloma was evident in 2/50 males and 1/49 females of this dose group. Males at 10000 ppm had epithelial dysplasia (1/50), squamous cell hyperplasia (3/50), and basal cell activation (1/50). Squamous cell hyperplasia occurred in 2/50 females at 400 ppm and 4/49 at 10000 ppm and basal cell activation was observed in 1/49 females at 10000 ppm. Furthermore, squamous cell carcinomas in the larynx were seen in 2/50 males at 10000 ppm, 1/50 females at 2000 ppm and 1/49 females at 10000 ppm. In this group, there also occurred epithelial dysplasia (2/50 males and 3/49 females), squamous cell hyperplasia (1/50 males) and basal cell activation (3/50 males and 6/49 females). The stomach tumors were reported to arise from the forestomach in treated mice, for basal cell activation of the rat stomach no certain localisation was noted. No other tumor type at any other organ/tissue site increased in both studies. Metastasis of squamous cell carcinomas in the oral cavity were found in the lungs and lymph nodes of two males and two females in the 10000 ppm group and in the salivary glands of another female of this group. Squamous cell carcinoma metastases in the lungs with origin from the esophagus or stomach were also seen in two other males, squamous cell carcinoma in another female metastasised to the kidney, pancreas and lymph nodes. In the 10000 ppm groups, a total of 9 males and 7 females died of tumors in the above mentioned target organs such as oral cavity (6 males and 4 females), in the stomach (1 male and 2 females) or larynx (1 male and 1 female) and in the esophagus (1 male). The authors concluded that squamous cell tumors (malignant and benign) occurring in the oral cavity,

larynx, esophagus, and stomach of male and female mice were caused by the administration of vinyl acetate. Atrophy of the salivary glands was noted in 6 males and 4 females in the 10000 ppm group which were afflicted with tumors on the mandible and was thought to be a secondary change to tumor growth.

- Comment: The observation that the high concentration (10000 ppm) administered to the top dose group animals have caused a reduction in final mean body weight might indicate that the MTD was exceeded. But, the following reasons support the view that the MTD was not reached: There was no significant difference in survival rates between dose and control groups, although relatively high numbers of animals died because of squamous cell tumors. Lower final body weight can be attributed to reduced water consumption. Growth curves seems to follow the curves of water consumption with a delay of some weeks. Both effects developed during the late phase of the study, reduction in water consumption began after week 60 (males) or 80 (females). The depressing effect of lower water consumption may be superimposed by the development of squamous cell tumors that resulted in a number of deaths. No indication on vinyl acetate-related systemic toxicity either as non-specific or organ-specific adverse effects could be identified. Otherwise, if the MTD would be exceeded, toxic signs were also to be expected for the early phase of a study where the mg/kg/d doses were much higher than in aged animals. The rapporteur's comment is in agreement with the conclusion of the investigators that the MTD is not exceeded (Umeda et al., 2004b).

- The authors concluded from these 2-year carcinogenicity studies in rats and mice that vinyl acetate given in drinking water caused squamous cell tumors in areas of the upper digestive tract with direct contact (predominantly in caudal and exterior regions of the mandible that were highly exposed) and squamous cell hyperplasia, basal cell activation and epithelial dysplasia represented early stages of squamous cell tumors. The lowest concentration associated with squamous cell tumors was 400 ppm in female rats and 2000 ppm in female mice. This is in line with the dose-related increases of tumor incidences at 31 mg/kg/d and 61 mg/kg/d, respectively, calculated as dose/bodyweight. A BMDL10 of 477 mg/kg/d for the combined incidences of squamous cell carcinomas and papillomas in the oral cavity of rats and mice (both species and both sexes combined for calculation) was estimated (BMDS version 1.3.1, US EPA's NCEA, multistage model).

Table 4.1.2.8.C: Percentages of tumors and presumed preneoplastic lesions in a two-year carcinogenesis study on F344 rats and BDF1 mice with drinking water administration of vinyl acetate (Umeda et al., 2004a)

RAT		10000 ppm		2000 ppm		400 ppm		control	
organ	Tumor type/preneoplasia	m#	f	m	f	M	f	m	f
oral cavity	squamous cell carcinoma	**10.0*↑**	**6.0↑**	-	**2.0↑**	-	**2.0↑**	-	-
	squamous cell papilloma	4.0	-	-	-	-	-	-	-
	basal cell activation	4.0	2.0	-	-	-	-	-	-
	epithelia dysplasia	-	4.0	-	-	-	-	-	-
esophagus	squamous cell carcinoma	-	2.0	-	-	-	-	-	-
	squamous cell hyperplasia	2.0	2.0	-	-	-	-	-	-
	basal cell activation	-	8.0	-	-	-	-	-	-
stomach	basal cell activation	4.0	10.0	-	-	-	-	-	-
MOUSE		10000 ppm		2000 ppm		400 ppm		control	
organ	Tumor type/preneoplasia	m	f	m	f	M	f	m	f
oral cavity	squamous cell carcinoma	**26.0**↑↑**	**30.6**↑↑**	-	-	-	-	-	-
	squamous cell papilloma	**8.0↑↑**	**6.0↑↑**	-	-	-	-	-	-
	squamous cell hyperplasia	26.0	12.2	4.0	2.0				
	basal cell activation	36.0	34.6	2.0	2.0				
	epithelial dysplasia	48.0	34.6						
esophagus	squamous cell carcinoma	**14.0*↑↑**	2.0	-	-	-	-	-	-
	squamous cell papilloma	-	-	-	2.0	-	-	-	-
	squamous cell hyperplasia	4.0	4.0	-	-	-	-	-	-
	basal cell activation	18.0	30.6	-	-	-	-	-	-
	epithelial dysplasia	4.0	14.2	-	-	-	-	-	-
forestomach	squamous cell carcinoma	**14.0*↑↑**	**6.1↑↑**	-	-	-	-	2.0	-
	squamous cell papilloma	4.0	2.0	-	-	-	-	-	-
	squamous cell hyperplasia	6.0	8.1	-	-	-	4.0	-	-
	basal cell activation	2.0	2.0	-	-	-	-	-	-
	epithelial dysplasia	2.0	-	-	-	-	-	-	-
larynx	squamous cell carcinoma	4.0	2.0	-	2.0	-	-	-	-
	squamous cell hyperplasia	2.0	-	-	-	-	-	-	-
	basal cell activation	6.0	12.2	-	-	-	-	-	-
	epithelial dysplasia	4.0	6.0	-	-	-	-	-	-

m males, f females, - no tumor observed, #no of animals: 50 (except high-dose female mice: 49)
statistical results only on tumors: sign. difference to controls * $p<0.05$, ** $p<0.01$ in Fisher's exact test positive trend in Peto test or in Peto test and Cochran-Armitage test↑ sign. increase $p<0.05$ ↑↑ sign. increase $p<0.01$

- Another recent lifetime study on the carcinogenicity of vinyl acetate by Maltoni et al. (1997) reported increased tumor rates in Swiss mice orally treated with vinyl acetate. The study was started in the 80's and the study design did not show conformity with the current guidelines for cancer studies. Daily fresh prepared vinyl acetate solutions were administered in ad libitum supplied drinking water at 0, 1000 and 5000 ppm (calculated to be equivalent to 0, 150, or 750 mg/kg bw/d based on a water consumption of 15% of body weight) for 78 weeks. Data on vinyl acetate purity and analytical concentration in the drinking water and on water consumption were not given, but the test substance were reported to contain impurities of benzene (30-45 ppm), methyl and ethyl acetate (50 ppm), crotonaldehyde (6-16 ppm), acetaldehyde 2-11 ppm), and acetone (330-500 ppm). Male and female breeders (13-14 males and 37 females/group) and of male and female offspring (37-39 males and 44-48 females/group) were treated starting at 12th day of pregnancy. The animals were submitted daily to a clinical observation, the body weights were registered weekly within the first 13 weeks and every two weeks thereafter until the end of treatment at week 78, and than every 8 weeks until the end of the study. The evidence of gross lesions was checked weekly for the first 13 weeks and thereafter every second week. All animals were necropsied and histopathology was performed on 28 organs/tissues and any other organ with macroscopic lesions. With respect of the presumed target organs, five sections of the head were only included in animals treated longer than 100 weeks. Other target organs such as the tongue, esophagus, stomach, intestine were also examined microscopically, but not the larynx. No treatment-related effect on the body weights of male and female breeders and slight decreases in mean body weight in male and female offspring was seen. No treatment-related effect on survival rates occurred at the end of the study, the survival rates of the male and female offspring were slightly higher than control animals. It was reported that no behavioural changes or non-neoplastic lesions were observed by gross inspection and histological examination. Higher incidence of tumors and presumed neoplastic lesions occurred in treated animals in the following organs (see Table 4.1.2.8.D): Zymbal gland, oral cavity, tongue, esophagus, forestomach, glandular stomach, lung, liver, and uterus. The increase of stomach tumors was considered to be borderline increase, higher rates of hepatocarcinomas were only seen among male offspring. The tumors of the lung, liver, uterus and Zymbal gland were not consistent with the findings of the Japanese study (Umeda et al., 2004a). Regarding relatively high spontaneous occurrence of tumors at these sites in the control groups of the study, the interpretation of these results is difficult. As the test substance contained impurities of known carcinogens (e.g. benzene, hydroquinone), the authors assumed that these impurities were relevant for the tumor response. The rapporteur recognises the reduced reliability of this study due to impure test substance and the non-concordance with guideline testing procedures. However, this study showed some consistency with other studies. Compared to control animals that showed normal tissues (except some tongue tumors) there were markedly increased incidences of squamous cell tumor and dysplasia along the exposure route (oral cavity, tongue, esophagus, forestomach). For most effects on the epithelial surface of the upper digestive tract, the offspring were more sensitive than the parent animals. Tumors of this type at these sites were consistent to data from BDF1 mice in the study of Umeda et al. (2004a). The assumption of the authors is considered to be unlikely, as the concentrations of impurities were extremely low. Based on this study lacking laboratory investigations, the NOAEL for nonneoplastic lesions was 5000 ppm in mice (750 mg/kg bw/d).

Table 4.1.2.8.D: Percentages of tumors and presumed preneoplastic lesions in a lifetime carcinogenesis study in Swiss mice with drinking water administration of vinyl acetate for 78 weeks (Maltoni et al., 1997)

group	concentrations	**5000 ppm**				**1000 ppm**				**control**			
		breeders		offspring		breeders		Offspring		breeders		offspring	
organ	**tumor/preneoplastic lesion**	13 m	37 f	49 m	48 f	13 m	37 f	37 m	44 f	14 m	37 f	38 m	48 f
Zymbal glands	carcinoma	-	2.7§	4.1	8.3	-	-	-	4.5	-	-	-	-
	squamous cell dysplasia	7.7	16.2	8.2	22.9	-	8.1	-	4.5	-	2.7	5.3	6.3
oral cavity	squamous cell carcinoma	7.7	2.7	20.4	18.8	-	-	-	-	-	-	-	-
tongue	squamous cell carcinoma	7.7	8.1	14.3	25.0	-	2.7	-	-	-	-	2.6	-
	squamous cell dysplasia	-	8.1	8.2	14.6	-	-	-	2.3	-	-	-	-
esophagus	squamous cell carcinoma	-	16.2	24.5	37.5	-	-	-	-	-	-	-	-
	acanthoma	-	2.7	-	6.3	-	-	-	-	-	-	-	-
	squamous cell dysplasia	30.8	16.2	8.2	14.6	-	-	-	-	-	-	-	-
forestomach	squamous cell carcinoma	-	8.1	4.1	14.6	-	-	-	-	-	-	-	-
	acanthoma	7.7	13.5	16.3	22.9	-	-	2.7	-	-	-	-	-
	squamous cell dysplasia	-	-	2.0	-	-	-	-	-	-	-	-	-
glandular stomach	adenocarcinoma	7.7	2.7	-	2.1	-	-	-	-	-	-	2.6	-
	adenomatous polyp	-	-	-	-	-	-	2.7	-	-	-	-	-
	glandular dysplasia	-	-	6.1	-	-	-	-	2.3	-	-	-	-
lung	adenocarcinoma	15.4	2.7	4.1	6.3	15.4	2.7	-	2.3	14.3	-	2.6	-
	adenoma	23.1	16.2	22.4	22.9	15.4	16.2	16.2	6.8	14.3	8.1	15.8	12.5
	animals bearing lung tumors	30.8	18.9	26.5	22.9	30.8	16.2	16.2	9.1	28.6	8.1	18.4	12.5
liver	animals bearing hepatocarcinoma/s	15.4	-	34.7	4.2	30.7	-	21.6	-	21.4	-	26.3	2.1
uterus	adenocarcinoma		16.2		16.7		5.4		13.6		2.7		10.4
	leiomyosarcoma		5.4		8.3		-		4.5		2.7		-
	leiomyoma		5.4		12.5		-		6.8		2.7		4.2
	total malignant uterus tumors		21.6		25.0		5.4		18.2		2.7		10.4

m males f females, - no tumor observed, § percentages, no statistical evaluation available

- Vinyl acetate was administered in drinking water at doses of 0, 1000 and 5000 ppm to 17-week old Sprague-Dawley rats (breeders, 13 or 14 males/group and 37 females/group) and to 12-day embryos (offspring, 53-107 males/group and 57-99 females/group) (Minardi et al., 2002). Treatment was continued for 104 weeks, thereafter animals were kept receiving tap water until their spontaneous death. Vinyl acetate purity was > 99%, impurities were benzene 30-45 ppm, methyl and ethyl acetate 50 ppm, crotonaldehyde 6-16 ppm, acetaldehyde 2-11 ppm, and acetone 330-5000 ppm. In treatment groups, the rates of total malignancies and carcinomas and/or precursor lesions were increased in oral cavity, lips, tongue, oesophagus, and forestomach.

- It was reported that there were no differences between treated animals and controls in mean body weight, survival, behaviour, or treatment-related non-oncological pathological changes (no summary data included in the publication).

- Tumors of the oral cavity and lips occurred significantly more frequent in offspring males and females (24.5% and 15.5%, respectively, versus 0% in the controls, Table 4.1.2.8.E) exposed to 5000 ppm concentration of vinyl acetate. No significant effect was seen at the end of life in the rat groups (breeders), which were treated from the 17th week onwards. Oral cavity squamous cell carcinomas were seen in two females (5.4%) of the 5000 ppm and in one female of the 1000 ppm (breeder) group. No significant tumor response was seen at the tongue and the esophagus. Single females of the breeder group at 1000 and 5000 ppm and two females of the offspring group had squamous cell carcinomas of the tongue. However, squamous dysplasia was commonly observed in all treated groups of females, their incidences were significantly higher in the 5000 ppm (breeders and offspring) compared to the controls. In the esophagus, only one squamous cell carcinoma was observed in a male offspring exposed to a concentration of 5000 ppm. In contrast, the incidences of squamous dysplasia were significantly higher in the breeder and offspring groups at 5000 ppm compared to the control groups. Spontaneous dysplasia in the forestomach was seen in a number of control animals of the breeders and the offsprings (3.7-8.1%). Their rates increased significantly in offspring males and females at 1000 and 5000 ppm. (16.1% up to 24.6%). Squamous cell tumors at this site were significantly more frequent in the offspring males at 1000 and 5000 ppm and in the offspring females at 5000 ppm.

- In conclusion, treatment-related significantly increased rates of tumors were seen in the oral cavity and lips in the 5000 ppm offspring groups of both sexes and in the forestomach of the offspring groups of male rats at 1000 ppm and above and of female rats at 5000 ppm. Squamous dysplasia considered as precursor lesions were increased in theses groups and also in the breeder groups at 5000 ppm. Although the numbers of animals in the breeders group were limited, the animals of the offspring group at which the treatment started at day 12 of pregnancy appeared to be more sensitive towards tumor and precursor development.

- The study was not compliant to the current standard for a carcinogenicity study. The major limitations were the reduced number of animals tested in the breeders group, the variability of animal numbers among the test groups and the prolongation of the post-treatment period until to the spontaneous deaths. Instead of a study termination animals were allowed to live until their spontaneous death and the tumor incidences were not corrected for the lifetime. No data on food and water consumption were available.

Table 4.1.2.8.E: Percentages of tumors and presumed preneoplastic lesions in a life-time carcinogenesis study in Sprague-Dawley rats with drinking water administration of vinyl acetate for 104 weeks (Minardi et al., 2002)

group	concentrations	**5000 ppm**				**1000 ppm**				**control**			
		breeders		offspring		breeders		Offspring		breeders		offspring	
organ	**tumor/preneoplastic lesion**	13 m	37 f	53 m	57 f	13 m	37 f	83 m	87 f	14 m	37 f	107 m	99 f
oral cavity	squamous cell carcinoma		5.4	**24.5****	**15.8****	-	2.7	-	-	-	-	-	-
	squamous cell dysplasia			5.7					2.3			0.9	
tongue	squamous cell carcinoma	-	2.7	1.9	3.5	-	2.7	-	-	-	-		-
	squamous cell dysplasia	-	**18.9***	5.7	**15.8**** **§**	-	8.1	-	2.3 **§**	-	-	-	-
esophagus	squamous cell carcinoma	-	-	1.9	-	-	-	-	-	-	-	-	-
	squamous cell dysplasia	15.4	**21.6*** **§**	**35.8****	**40.4**** **§**	7.7	5.4§	-	4.6§	-	2.7	-	-
forestomach	squamous cell carcinoma	7.7	8.1	**13.2****	**7.0***	7.7	-	**7.2***	3.4	-	-	-	-
	squamous cell dysplasia	30.8	**29.7***	**24.5**** **§**	**24.6**** **§**	23.1	5.4	**19.3**** **§**	**16.1*** **§**	7.1	8.1	3.7	4.0

*p<0.05, ** p<0.01 using χ2test, § p<0.001 using Cochrane-Armitage test for dose-response relationship

Other information after oral administration

- Newborn Wistar (4-5 animals per sex per group) rats were exposed orally to 100 or 200 mg/kg bw/d vinyl acetate (99% pure, impurity: 100 ppm hydroquinone) in condensed milk twice a day for three weeks. A subgroup of animals was additionally treated with phenobarbital in drinking water for 8 weeks to stimulate the growth of potentially occurring preneoplastic liver foci. 14 weeks after the start of the study livers were dissected. No ATPase-free and gamma-GT-positive areas in the liver as indication for the development of liver nodules could be found (Laib and Bolt, 1986).

Summary on carcinogenicity of vinyl acetate in experimental animals.

Vinyl acetate induced an increased number of nasal tumors (mainly papillomas and squamous cell carcinomas) in various regions of the nasal mucosa of rats after long-term inhalation. The total incidence was significantly increased at a concentration of 600 ppm, but a single papilloma already developed at 200 ppm. No significant tumor response was seen in mice after long-term inhalation of vinyl acetate vapour. Occasionally single squamous cell tumors occurred at other sites of the respiratory tract in rats and mice.

Although the complete report was not available, published information from a recent oral cancer study in F344 rats and BDF1 mice (Umeda et al. 2004a) demonstrated significantly increased rates of squamous cell tumors in the oral cavity (rats and mice), esophagus and forestomach (mice) after a 2-year administration of 10000 ppm vinyl acetate with the drinking water (equivalent mean doses in rats were 442 mg/kg bw/d for males, 575 mg/kg bw/d for females, in mice 989 mg/kg bw/d for males, 1418 mg/kg bw/d for females). Maximum increase of tumor incidences was found in the oral cavity in both species. Squamous cell carcinomas were already observed at a dose of 400 ppm in

female rats (31 mg/kg bw/d). Consistently in another life-time study on a breeding and offspring generation of mice (Maltoni et al., 1997) which did not met actual standards on cancer bioassays, squamous cell tumors were also observed with increased incidences in several sites of the gastrointestinal tract (oral cavity, tongue, esophagus, forestomach) at a concentration of 5000 ppm in the drinking water (calculated dose 780 mg/kg bw/d). In addition, higher incidences of adenocarcinomas of the glandular region of the stomach were found in high-dose male breeders. Also some other organs (lung, liver, uterus) showed increased rates of benign and malignant tumors compared to that of the control groups. Tumors of the liver and the uterus have also been seen in the Lijinsky study (Lijinsky and Reuber, 1983). However, both studies hampered from methodical insufficiencies. Further, these data were inconsistent to the absence of parenchymal tumors in other more valid studies. Therefore interpretation of these tumors remains unclear. With respect to the carcinogenic potential of vinyl acetate, the results of Lijinsky and Reuber (1983) were considered not to be reliable due to several methodological deficiencies. No indication for an increased incidence of enzyme-altered liver foci was seen in another study (Laib and Bolt, 1986).

No clear positive tumor response was found in another oral rat cancer study at vinyl acetate concentrations up to 5000 ppm (Shaw, 1988, Bogdanffy et al., 1994a). Except the tongue, tissues of the oral cavity were not included as standard protocol tissues for histopathology. Although, this study showed the occurrence of two squamous cell carcinomas in the oral cavity of males of the 5000 ppm group.

Recently published data on rats exposed to drinking water containing 1000 or 5000 ppm vinyl acetate confirmed significant increases in squamous cell carcinomas of the oral cavity and the forestomach (Minardi et al., 2002). Treatment of offsprings resulted in higher tumor rates than in rats with treatment begin at week 17 of life. However, this study has a number of limitations in its design. Thus, tumor response along the gastrointestinal could be interpreted to be supportive to the results from the Umeda study.

Human data:

Information on repeated human exposure to vinyl acetate is small. Quantitative data on exposure and effects were not well investigated or documented. Workers had also been exposed to other compounds, so that effects cannot be attributed clearly to vinyl acetate. Confounding factors (i.e. smoking habits) were not ruled out. Therefore the relevance of the observed effects to evaluate risks to human health is questionable. Despite of these limitations some data from literature are cited below:

- A retrospective study on 21 chemical operators in a production plant with mean age of 45.3 years and mean exposure time of 15.2 years to vinyl acetate vapour with concentrations up to 49.3 ppm (TWA 5.2-8.2 ppm) revealed no vinyl acetate-related injury or differences in medical and biochemical parameters. Local irritant reactions were attributed to occasionally high acute exposures (Deese and Joyner, 1969).

- In a cohort study, 4806 male workers who were exposed to 19 different chemicals (vinyl chloride, polyvinylchloride dust, chlorinated solvents, acrylates, acrylonitrile and others) including vinyl acetate, between the years 1942 and 1973 had an excess risk of cancer of the respiratory system and the CNS. A subgroup (of cases with lung cancer) with undifferentiated large cell lung cancer was associated to a slightly higher cumulative exposure to vinyl acetate (Waxweiler et al., 1981).

- A nested case-control study (Ott et al., 1989) was undertaken in a cohort of 29139 men employed in two chemical manufacturing facilities and a research and development center, who had died in 1940-1978 with non-Hodgkin`s lymphoma, multiple myeloma, lymphocytic or non-lymphocytic leukemia. Exposure odds ratios (OR) were examined in relation to 111 work areas, 21 specific chemicals (OR based on an ever/never basis), and 52 chemical activity groups. Exposure to vinyl acetate was associated with non-Hodgkin's lymphoma in seven of 52 men (OR 1.2), multiple myeloma in three of 20 men (OR 1.6), non-lymphocytic leukemia in two of 39 men (OR 0.5), and with lymphocytic leukemia in two of 18 men (OR 1.8). Examination of OR related to the exposure duration was not done because of the OR <1.3 or number of cases <4.

Summary of human data

Data on human experience were not specifically associated to the exposure with vinyl acetate. In summary, the above-mentioned epidemiological data are insufficient to evaluate the carcinogenic potential of vinyl acetate in humans.

Other information from metabolites

Toxicity/carcinogenicity data from both metabolites, acetaldehyde and acetic acid, were considered with respect to their possible role in vinyl acetate toxicity:

Acetaldehyde

With respect to the effects from external exposure to acetaldehyde the endogene level of acetaldehyde has to be taken into consideration:

Endogene acetaldehyde:

Acetaldehyde is a naturally occurring substance in the metabolic pathways of animals and humans (metabolism of ethanol and sugars). It occurs in low concentrations in human blood.

A range of 0.1 - 2.1 µM for pulmonary blood acetaldehyde concentrations has been calculated in normal control subjects (Eriksson, 1987). The exact determination of the endogenous blood acetaldehyde level is impaired by the difficulty to exclude an artefactual acetaldehyde formation during the analytical procedures. Whole blood-associated acetaldehyde (WBAA) levels in teetotalers of 7.9 ± 0.7 µM (102 males) and 7.7 ± 0.6 µM (123 females) were measured by Halvorson et al. (1993). Acetaldehyde levels of 0.7 ± 0.5 µM in male human volunteers (n = 4) were obtained in the supernatants without precipitates from whole blood after headspace chromatography (Fukunaga et al., 1993).

In male Long-Evans rats treated with the aldehyde dehydrogenase inhibitors cyanamide and disulfiram endogenous blood concentrations of up to 2-5 µM were measured whereas no endogenous blood acetaldehyde could be detected in control animals (Eriksson, 1985). These results may indicate the existence of an endogenous acetaldehyde in protein bound forms.

Repeated dose effects following external exposure to acetaldehyde:

Inhalation exposure/animal data

- Although explant experiments on rat nasal turbinalia with olfactory or respiratory epithelium failed to demonstrate a cytotoxic potential of acetaldehyde at a concentration of 50 mmol on the olfactory and respiratory epithelia (Kuykendall et al., 1993), repeated dose studies demonstrated cytotoxic properties of acetaldehyde on the rat nasal epithelium.

- No histopathological abnormality was observed in the respiratory epithelium of rats (5 males/group), which were nose-only exposed to 750 ppm or 1500 ppm acetaldehyde on 3 consecutive days (6 h/d) (Cassee et al., 1996). In the olfactory region, a few necrotic cells were seen in 3/5 rats at 750 ppm, and 4/5 exposed to 1500 ppm showed cell necrosis (range few to many necrotic cells).

- In rats exposed to 0, 400, 1000, 2200 or 5000 ppm (790-9000 mg/m^3) acetaldehyde on 4 weeks (6 h/d, 5 d/w) treatment-related changes at 5000 ppm were dyspnoe, excitation during the first 30 min of each exposure, yellow-brown fur, severe growth retardation, more neutrophils and less lymphocytes in the blood, a reduced production of urine with a high density, increased lung weights, and severe degenerative, hyperplastic and metaplastic changes of the nasal, laryngeal and tracheal epithelium. The most severe lesions of the nose were found in the olfactory epithelium, but focal lesions were also observed especially in the posterior part of the nasal cavity covered with respiratory epithelium. Some minor alveolar alterations such as accumulation of pigment-laden macrophages and focally increased cellularity/thickening of alveolar septa were occasionally observed in the 5000 ppm group. Major lesions seen at 1000 and 2200 ppm (1800-3960 mg/m^3) comprised growth retardation and an increased production of urine in males, slight to moderate degeneration with or without hyper- and metaplasia of the olfactory epithelium, and only at 2200 ppm, minimal epithelial changes in the larynx and trachea. Focal degenerative lesions without hyperplasia were also observed single males and half of the females at 1000 and 2200 ppm of acetaldehyde. The only change observed at the 400 ppm level that could be attributed to acetaldehyde was slight degeneration of the nasal olfactory epithelium seen as loss of microvilli and thinning and disarrangement of the layer of epithelial cells (Appelman et al., 1982).

- A concentration without histopathological alterations on the respiratory tract was estimated to be 150 ppm (6 h/d, 5d/wk) in a 4-week inhalation study on male Wistar rats (Appelman et al., 1986). Nasal lesions observed at 500 ppm were similar as described for 400 ppm consisting of degeneration of the olfactory epithelium with a mean severity of slight to moderate.

- In a carcinogenicity study on rats exposed to 0, 750, 1500, 3000 (gradually reduced to 1000 ppm) ppm acetaldehyde vapour (6 h/d, 5 d/w) for up to 28 months treatment related-effects included increased mortality, growth retardation, nasal tumors (Table 4.1.2.8.F), and non-neoplastic nasal changes in each of the test groups. At day 468, the mortality rate in the high-dose group was 50% (28/55) for males and 42% (23/55) for females. By day 715, all high dose rats hat died and, at termination of the study at day 844, only a few animals were still alive in the mid-dose group. At the end of the study dose-related nasal changes comprised: degeneration, metaplasia and adenocarcinomas of the olfactory epithelium at all exposure levels. The respiratory epithelium showed squamous metaplasia and squamous cell carcinomas of the respiratory epithelium at the 2 highest exposure levels, and slight to severe rhinitis and sinusitis in top-concentration rats (Table 4.1.2.8.G). In the larynx, hyperplasia and keratinised squamous metaplasia of the epithelium in the vocal cord region were seen in many rats of the mid- and top-concentration groups. One female rat of the 1500 ppm group had developed a laryngeal carcinoma in situ (Woutersen et al., 1986).

TABLE 4.1.2.8.F

Tumor incidence in the nasal cavity of rats exposed to acetaldehyde vapour for at most 28 months

	Tumor incidences [a]							
Site/type of tumor	**Males**				**Females**			
	control	750	1500	3000/ 1000	control	750	1500	3000/ 1000
Nose	(49)#	(52)	(53)	(49)	(50)	(48)	(53)	(53)
Papilloma	0	0	0	0	0	**1**	0	0
Squamous cell carcinoma	**1**	**1**	**10***	**15*****	0	0	**5**	**17*****
Carcinoma in situ	0	0	0	1	0	0	3	5
Adenocarcinoma	0	**16*****	**31*****	**21*****	0	**6***	**26*****	**21*****
Larynx	(50)	(50)	(55)	(52)	(53)	(52)	(54)	(54)
Carcinoma in situ	0	0	0	0	0	**1**	0	0

[a] The number of animals examined is given in brackets.
Statistics: Fisher Exact Test *P<0.05 **P<0.012 ***P<0.001
Number of tissues examined (Data from Woutersen et al. 1986)

TABLE 4.1.2.8.G: Treatment-related non-neoplastic lesions in the nasal cavity of rats exposed to acetaldehyde vapour for at most 28 months

Site/type of lesions	Males				Females			
	control	750	1500	3000/1000	control	750	1500	3000/1000
Nose	(49)	(52)	(53)	(49)	(50)	(48)	(53)	(53)
Respiratory epithelium								
Squamous metaplasia without keratinisation	0	**1**	**11****	**1**	0	**3**	**14*****	0
Squamous metaplasia with keratinisation	0	0	**5**	**19*****	0	**1**	**16*****	**18*****
Papillomatous hyperplasia	0	0	0	**2**	0	0	0	**6***
Simple/pseudoepitheliomatous hyperplasia	0	**5**	**16*****	**8****	0	**3**	**31*****	**9****
Olfactory epithelium								
Squamous metaplasia without keratinisation	0	0	0	0	0	0	**1**	**1**
Squamous metaplasia with keratinisation	0	0	0	**3**	0	0	0	0
Basal cell hyperplasia without atypia	0	**37*****	**9****	0	0	**42*****	**19*****	0
Basal cell hyperplasia with atypia	0	**1**	**17*****	0	0	0	**5**	0
Submucosal atypical basal cells	0	0	**23*****	0	0	0	**31*****	**2**
Proliferation of glands in the thickened submucosa	0	0	**14*****	**5**	0	**4**	**18*****	**5**
Larynx	(50)	(50)	(51)	(47)	(51)	(46)	(47)	(49)
Squamous metaplasia without keratinisation	**2**	**2**	**10***	**9***	**1**	0	**6**	**9***
Squamous metaplasia with keratinisation	**1**	**4**	**13*****	**32*****	0	**3**	**17*****	**23*****
Proliferation of dysplastic epithelium	0	0	**1**	0	0	**1**	**4**	**2**
Lungs	(55)	(54)	(55)	(52)	(53)	(52)	(54)	(54)
Keratinised stratified squamous metaplasia of bronchial epithelium	0	0	0	**1**	0	0	0	0

The number of animal examined is given in brackets.
Statistics: Fisher exact Test *P<0.05 , **P<0.01 , ***P<0.001, Data from Woutersen et al. 1986

Lesions comparable to those seen after 28 months were observed in the acetaldehyde exposed rats of additional interim sacrifice groups killed at 13, 26 (subgroups of each 5 animals/sex/group) and 52 weeks (10 animals/sex/group) after the same dose regimen as reported above (Wouterson et al., 1986) clearly demonstrating severe abnormalities in the nose and larynx, minor changes in the trachea and nasal tumors (Woutersen et al. 1984). Of the animals killed at these intervals, only one had a tumor of the respiratory tract: a female in the high dose group killed in week 53, bearing a nasal squamous-cell carcinoma.

- Increased numbers of nasal tumors were also evident in rats which inhaled 750, 1500 or 3000/1000 ppm acetaldehyde vapour for 78 weeks as well as in rats exposed to acetaldehyde for 52 weeks followed by a recovery period of at most 26 weeks (Woutersen and Feron, 1987).

- Inhalative exposure of hamsters to 2500 ppm (4500 mg/m³) gradually decreased to 1650 ppm (2970 mg/m³) for a period of 52 weeks (7 h/d, 5 d/w) resulted in hyper- and metaplastic changes of the nasal epithelium, and hamsters developed nasal tumors (3/52) and laryngeal tumors (11/47) (Feron et al., 1982).

Oral administration/animal data

- A subacute oral toxicity study of acetaldehyde was carried out in Wistar-derived rats (Cpb:WU) (Til et al., 1988). Groups of ten male and female rats received drinking water containing 0, 25, 125 and 675 mg/kg bw/d acetaldehyde for a period of 4 weeks. Food and water intake was decreased in the top dose groups. There was no significant effect on parameters of hematology, clinical chemistry and urinalysis. The only adverse effect was slight to moderate hyperkeratosis of the stomach of high dose males and females.

- Soffritti and his colleagues (Soffritti et al., 2002) published the results of an oral carcinogenicity study that may correspond to the positive carcinogenicity bioassay that were announced by Maltoni et al. (1997). Acetaldehyde was administered to 50 male and 50 female Sprague-Dawley rats for 104 weeks in drinking water at concentrations of 0, 50, 250, 500, 1500, or 2500 mg/L. Increased rates of tumors at several organs were observed in treated groups. However, the effects were not dose-related and no clear conclusion could be drawn from this study.

- In a study on cell proliferative activity, 20 Wistar rats were given either water containing acetaldehyde at a concentration of 120 mM (324 mg/kg/d) or tap water to drink for 8 months (Homann et al., 1997). Tissue specimens were taken from the tongue, epiglottis, and forestomach of each animal and immunohistochemically stained for markers of cellular proliferation (Ki67 nuclear antigen) or differentiation (cytokeratins 1,4,10,11,14, and 19). The mean epithelial thickness of each sample was measured via light microscopy and differences between control and treated groups were analysed by use of unpaired Student's *t* test. Although no tumors were observed, staining for cytokeratins 4 and 14 revealed an enlarged basal layer of squamous epithelia in the rats receiving acetaldehyde. In these animals, cell proliferation was significantly greater than that observed in the control animals for samples from the tongue ($p<0.0001$), epiglottis ($p<0.001$) and

forestomach ($p<0.0001$). In addition, the epithelia from acetaldehyde-treated rats were significantly thicker than in epithelia from control animals ($p<0.05$ for all three sites).

Oral uptake/human data

- Alcoholic consumption is a well-known risk factor for squamous cell cancer of the oropharynx, larynx and esophagus. Findings of consistence in the mutational spectrum induced by acetaldehyde in human lymphocytes and in the p53 tumor suppressor gene of oesophageal tumors were interpreted to support the assumption that tumors of the upper gastrointestinal tract are attributable to acetaldehyde which is the primary oxidation product of ethanol (Noori and Hou, 2001).

Other data relevant for acetaldehyde carcinogenicity

- In the recent evaluation on acetaldehyde given by the Scientific Committee on Cosmetic Products and Non-Food, (SCCNFP, 2004) data including additional human data from case were reported:

  Acetaldehyde is a naturally occurring substance, also in human metabolic pathways. It is metabolised to acetic acid. Acetaldehyde itself is the main metabolite of ethanol and this reaction is catalysed by alcohol dehydrogenases (ADH). Five ADHs have been characterized in humans, two of which (ADH2 and ADH3), are known to be polymorphic. In particular, polymorphism for ADH3 seems to strongly influence the metabolism of ethanol to acetaldehyde, with ADH3 1 allele carriers being faster metabolisers than ADH3 2 carriers. Acetaldehyde is metabolised by phase II enzymes, including aldehyde dehydrogenases (ALDH) and glutathione S-transferases (GST). ALDH2 is polymorphic; its mutant allele, ALDH2 2, which leads to enzyme inactivity, is prevalent in Asian populations. GSTM1 is also polymorphic, with a null genotype GSTM1 0 present mainly in European populations (Coutelle et al., 1997). Therefore, carriers of ADH3 2, ALDH2 2 and GSTM1 0 alleles are likely to be exposed to higher levels of acetaldehyde than are other people, following intake of a comparable amount of alcohol. Supportive data were gained from testing in ALDH2-/- knockout mice in which a doubling of blood acetaldehyde concentrations and more severe toxicity was observed in comparison to wild-type (ALDH2+/+) mice (Isse et al., 2005).

  An increased relative frequency of bronchial and oral cavity tumours was found among nine cancer cases in one study of chemical workers exposed to various aldehydes. Three case–control studies assessed the risk of oral, pharyngeal, laryngeal and oesophageal cancer following heavy alcohol intake, according to genetic polymorphism of enzymes involved in the metabolism of ethanol to acetaldehyde (alcohol dehydrogenase 3) and in the further metabolism of acetaldehyde (aldehyde dehydrogenase 2 and glutathione S-transferase M1). Despite limitations in the study design and the small size of most of the studies, these studies consistently showed an increased risk of alcohol related cancers among subjects with the genetic polymorphisms leading to higher internal doses of acetaldehyde following heavy alcohol intake as compared to subjects with other genetic polymorphisms.

  Oesophageal tumours have been associated with genetically determined high metabolic levels of acetaldehyde after drinking alcohol. A Japanese case–control study (Yokoyama et al., 1996) of ALDH2-related risk for oesophageal squamous-cell carcinoma in alcoholics (40 cases and 55 controls) and non-alcoholic drinkers (29 cases and 28

controls) during 1991–95 showed a higher risk for oesophageal cancer in those with one ALDH2 2 allele in both alcoholics (crude odds ratio, 7.6; 95% confidence interval (CI), 2.8–20.7) and non-alcoholic drinkers (odds ratio, 12.1; 95% CI, 3.4–42.8). Mantel–Haenszel adjustment for age and daily alcohol consumption had virtually no influence on the risk estimates [adjusted odds ratios not given]. As persons who have the mutant ALDH2 2 allele have a high concentration of blood acetaldehyde after drinking alcohol, the results of this study were interpreted as strongly suggesting a carcinogenic role of acetaldehyde in humans.

The IARC has concluded that there is inadequate evidence in humans for the carcinogenicity of acetaldehyde and that there is sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde.

The overall conclusion was that acetaldehyde is possible carcinogenic to humans (Group 2B).

In the EU, acetaldehyde is a carcinogen classified in EU as a Category 3 carcinogen and a Category 3 mutagen.

Acetic acid

Inhalation exposure/no repeated dose animal study with guideline equivalence is available.

The lowest concentration inducing histopathological lesions in the nasal mucosa of mice exposed for 4, 9 or 14 days (6 h/d) was 55.4 ppm acetic acid (Zissu, 1995). Only the olfactory mucosa was affected.

A 4-hour nose-only inhalation of acetic acid at target concentrations of 600 µl/l or 1300 µl/l (0.6 or 1.3 ppm) followed by a 20 hour observation period resulted in very focal lesion in the respiratory epithelium of the dorsal meatur of level 1 of the nasal cavity in three out of five exposed rats (Kilgour et al., 2000). In vitro exposure to 53.3 mM acetic acid, a concentration calculated to correspond to the in vivo air concentration, to olfactory and respiratory turbinates for 4 or 24 hour resulted in a significant reduction in ATP and potassium concentrations.

Discussion on vinyl acetate carcinogenesis

The manufacturing industry postulated a non-genotoxic mode of action for vinyl acetate-induced carcinogenic effects being similar for the respiratory tract and the gastrointestinal tract. The acceptance of this hypothesis will be discussed in the following part of this assessment report for each organ system as suggested by the IPCS framework on cancer risk assessment subdivided into: postulated mode of action, key events associated with hypothesis, dose-response relationship, temporal relationship, strength, consistency, specificity of association, biological plausibility and coherence, inconsistencies, uncertainties and data gaps, assessment of mode of action for tumors (according to the IPCS framework on cancer risk assessment, 1999).

I. Respiratory tract tumors:

Ia. Postulated mode of action

Bogdanffy and his co-workers postulated that vinyl acetate metabolism in the respiratory and olfactory epithelium of the nasal mucosa increases local concentration of intracellular H^+ and hence produced tissue acidification. This lowered pH causes toxicity, atrophy, degeneration and subsequently reparative hyperplasia. The stimulation of hyperplastic activity for a significant time of the animal's life span increases the probability of mutation and tumor formation. This hypothesis emphasises the role of cytotoxicity with a minor contribution of the acetaldehyde as a mutagenic DNA-protein cross-linking agent. It was stated that 'with sufficient genetic damage, induced by spontaneous mutation and acetaldehyde induced DNA-protein cross-links (DPX) olfactory degeneration progresses to a state of elevated proliferation and eventually, at high vinyl acetate to neoplastic transformation' (Bogdanffy and Plowchalk, 2001). Later on, the considerations on the mode of action was slightly modified (Bogdanffy, 2002, Hinderliter et al., 2005). Intracellular acidification was still thought to be the crucial step in a sequence of responses including acidification-induced mitogenic cellular proliferation of nasal respiratory epithelium and acidification-induced cytotoxic cell proliferation in olfactory epithelium.

Ib. Key events associated with hypothesis

Step 1: From inhalation to cytotoxicity:

- *Metabolic enzyme activities* (Table 4.1.2.8.H and Table 4.1.2.8.I):

The enzymes responsible for the hydrolysis of vinyl acetate to acetic acid and acetaldehyde, and its further oxidation to acetic acid are carboxylesterase and aldehyde dehydrogenase (see 4.1.2.1). In principle, both were detected in the respiratory and olfactory epithelium of the rat nasal cavity (Bogdanffy et al., 1986, 1987, 1998, 1999a).

Carboxylesterase

Earlier data on tissue homogenates and using p-nitrophenyl butyrate to measure carboxyl esterase activity pointed to the fact that a higher sensitivity of the olfactory epithelium to toxic effects of vinyl acetate could be attributed to 6fold higher hydrolytic enzyme activity at this epithelium (Bogdanffy et al., 1987). These results were confirmed in tissue homogenates, where carboxylesterase-mediated hydrolysis of vinyl acetate was more efficient (4-7fold, related to protein content) in the olfactory epithelium than in the respiratory epithelium (Bogdanffy and Taylor, 1993). K_m was in the same low range in the olfactory and respiratory epithelium.

Data using an in vitro gas uptake method on organ explants from rat turbinates or sections of intact nasal tissue from humans demonstrated much lower esterase activity than seen in tissue homogenates. When the epithelial volume was considered into calculations, a similar esterase activity in both (olfactory and respiratory) regions was observed for the rat and a much higher activity was found for the respiratory epithelium for the human (Bogdanffy et al., 1998).

Data on carboxylesterase activity in human nasal tissues are sparse. Mainwaring et al. (2001) found that the ratio of carboxylesterase activity (methyl methacrylate as substrate) between olfactory and respiratory microsomes was similar for the rat as well as for the man Esterase activity for both species was about 3fold higher in olfactory microsomes which is in line with a 2fold higher activity in olfactory microsomes reported by Bogdanffy et al. (1999a). In respiratory microsomes Mainwaring et al. (2001) estimated a 4fold higher activity for the rat than in the human samples. The activity in rat olfactory S9 mix (24fold) was much higher than in olfactory S9 mix from human origin. Although there may be some carboxylesterases activity outside the microsomes, as demonstrated for liver cells, where carboxylesterase activity has also been identified in the cytosol (Tabata et al., 2004), the low values for V in olfactory and respiratory microsomes or S9-mix may indicate a lower hydrolytic activity in the human nasal tissues compared to those of the rat.

Table 4.1.2.8.H: Metabolic rate (V) for carboxylesterase activity (towards methyl methacrylate) in nasal tissues (adopted from Mainwaring et al., 2001)

Tissue	V (nmol/min/mg protein)	
	Human	Rat
Respiratory microsomes	2.7[a]	14.3
Respiratory S9	0.15[a]	3.5[a]
Olfactory microsomes	No data	38.6
Olfactory S9	0.48[a]	12[a]
Liver microsomes	494.0[a]	46.6

[a]The values for rat S9 fractions and for all human tissues are for single rate measured at substrate concentration of 1.0 mM methyl methacrylate.

A more than 35fold higher activity of carboxylesterase was also reported for rat nasal tissue homogenate (pooled from all regions, no region identified) compared to human respiratory epithelium from polyp tissue (which may not be representative for enzyme activity in normal tissue, expression is reduced in hyperplastic epithelium, see also Lewis et al., 1994) at similar enzyme affinity, when α-naphthyl butyrate was used as a substrate (Mattes and Mattes, 1992). While p-nitrophenyl butyrate was shown to be a competitive substrate of rat nasal α-naphthyl butyrate esterase, it did not affect human α-naphthyl butyrate esterase indicating that there are differences in substrate specificity of rat and human carboxyl esterases. Due to the limitations by tissue selection, this preliminary study could only be interpreted to support the rats' significantly higher enzyme activity.

Liver microsomal carboxylesterases are inducible by exogenous compounds (Satoh and Hosokawa, 1995). Yet unknown, this could not be excluded for the nasal epithelial cells.

Aldehyde dehydrogenase

Specific aldehyde dehydrogenase activity is more than 2-fold higher in tissue homogenates from the respiratory mucosa than in homogenates from the olfactory mucosa (Stanek and Morris, 1999). After adjustment to the cell volume the aldehyde dehydrogenase activity of the respiratory epithelial cells was threefold higher in rats than that of humans (Bogdanffy et al., 1998).

Two aldehyde dehydrogenases were identified in the rat nasal tissues: a high K_m isoenzyme, which was 5-8fold more efficient in the respiratory mucosa than in the olfactory mucosa (not normalised to cell volume), and a low K_m isoenzyme of aldehyde dehydrogenase (with comparable low activity in both areas) (Casanova-Schmitz et al., 1984).

- *Localisation of enzyme activity in various cell types of the upper respiratory tract:*

Carboxylesterase

In the rats' olfactory epithelium, carboxylesterase was histochemically (Bogdanffy et al., 1987) and immunohistochemically (Lewis et al., 1994) most prominently localised in the Bowman's glands, the ducts of the Bowman's gland and in sustentacular cells of the olfactory epithelium, and it was absent in the basal cells, the neuronal sensory cells and the overlying mucus. The apical (supranuclear) and basal portions of the sustentacular cell cytoplasm were intensely immunoreactive for carboxylesterase (Olson et al., 1993). The exact subcellular location of carboxylesterase has not yet been identified for nasal mucosa cells. In many other tissues, carboxylesterases from the mammalian multigene family were mainly localised in the endoplasmatic reticulum (Potter et al., 1998, Satoh and Hosokawa, 1995). A striking abundance of endoplasmatic reticulum in the supranuclear area of the rat sustentacular cells where P450 enzymes are localised (Farbman, 1992) suggests that carboxylesterases may also be associated to the endoplasmatic reticulum in this type of cell (as in other tissues, Satoh and Hosokawa (1995)). Together with the absence of carboxylesterase enzyme activity in the neuronal cells this indicates that sustentacular cells are involved in the vinyl acetate metabolism.

At the respiratory epithelium various cell types of the epithelium (ciliated epithelial cells and cuboidal cells > nonciliated cells) demonstrated strong to moderate carboxylesterase activity by immunohistochemical staining. The goblet cells and the submucosal seromucous glands showed a negative or weak staining. Consistent with the low immunoreactivity, the overlying mucous was negative. Carboxylesterase activity of the basal cells of the respiratory epithelium was reported to be weak-moderate by Bogdanffy et al. (1987), and no or weak immunoreactivity was found by Lewis et al. (1994) and Olson et al. (1993). No presence of carboxylesterase could be attributed to the mucous by in situ immunostaining or by determination of activity in nasal lavage fluid: The Vmax value of nasal lavage carboxylesterase activity was 0.134 µmol/min/mg and 160times lower than that observed in nasal respiratory mucosal homogenates und far more lower than in the olfactory region (Bogdanffy et al., 1999a).

Similar to the rat, carboxylesterase immunoreactivity was diffuse in ciliated and secretory cells of the normal respiratory mucosa of the human, some subepithelial glands were also

positive (Lewis et al., 1994). Sections were from eight patients (7 females, 7 non-smokers) showing a large variation across samples from normal respiratory epithelium, local inflammation to hyperplasia and squamous hyperplasia where carboxylesterase immunostaining was completely absent. Human data on carboxylesterase (immuno-) localisation in samples of the olfactory region were not available.

The observations on enzyme activity, distribution and cytosolic localisation in nasal mucosa cells does not support Bogdanffy's assumption that carboxylesterase is bound to or embedded in the plasma membrane of the nasal epithelium, and may act as a protective mechanism by removal of toxicants *prior* to the entry into the cell cytoplasm. With respect to the sustentacular cells, the main site of carboxylesterase in the olfactory epithelium, there is no evidence of a secretory function for mammalian species (Farbman, 1992). As indicated by the absence of carboxylesterase in mucous, no hydrolytic cleavage occurs preliminary to the vinyl acetate uptake.

Aldehyde dehydrogenase

Immunohistochemically, aldehyde dehydrogenase was detected in the respiratory epithelium (ciliated epithelial cells>nonciliated epithelial cells>goblet cells>cuboidal cells), while the olfactory epithelium showed negligible immune-reactivity (weak in the basal cell layer, weak to negligible reaction in Bowman's glands, no immune-reactivity in sustentacular and sensory cells) (Bogdanffy et al., 1986). In addition, epithelial cells of the trachea and ciliated cells of the lower bronchioles demonstrated little activity, which increased distally, and Clara cells of the lower bronchioles showed high activity of aldehyde dehydrogenase.

At the subcellular level aldehyde dehydrogenase isoenzymes are located in the cytosol and in the mitochondria. Intracellular distribution for aldehyde dehydrogenase for human nasal tissue is not known. There are at least two isoenzymes of aldehyde dehydrogenase, a high affinity mitochondrial aldehyde dehydrogenase-2 and an aldehyde dehydrogenase-1 in the cytosol. Polymorphism of isoenzymes which impair enzyme activity and acetaldehyde metabolism are well known in subpopulations of Asian origin and may result in higher tissue concentration of a acetaldehyde after alcohol consumption (Ginsberg et al. 2002) .

The following Table 4.1.2.8.I summarises the available in situ immunohistochemistry data, in vitro data on tissue homogenates, and data on enzyme activity for the respiratory and olfactory epithelium in the rat and the man.

Overall, data from in-situ investigations using (immuno-)histochemistry methods and semiquantitative determination of staining intensity were consistent with the results from investigations on the enzyme activity of carboxylesterase and aldehyde dehydrogenase. The differences in epithelial thickness and the dilution effects by a contribution of non-epithelial cells in the cell homogenates may account for uncertainties in the estimated absolute values for enzyme activity, especially for human tissues. No (or few) data are available on the isoenzymes of carboxylesterases and aldehyde dehydrogenases in nasal tissues from the rat compared to those of the human.

Table 4.1.2.8.I Data on enzyme localisation and activity in the nasal mucosa of rat and man

	Respiratory epithelium (RE)		Olfactory epithelium (OE)		Remark	Reference
	Rat	Man	Rat	Man		
Carboxylesterase						
Enzyme activity in cell homogenates	V_{max} 35.7 mg/h if normalised to epithelial volume: V_{max} 64 mg/h Prediction from gas uptake model: V_{max} 63 mg/h	undetectable	V_{max} 133.1 mg/h or normalised to epithelial volume: V_{max} 39mg/h Prediction from gas uptake model: V_{max} 45.4 mg/h	Undetectable, except for 1 sample: 478 nmol/min/mg protein	Rat: Esterase activity in RE less than 2fold higher than in OE (Consider: related to tissue wet weight, no correction to protein content!) Uncertainty in estimation of enzyme activity in human OE sample by 5time thickness of human mucosa, dilution effect of submucosal tissue and only one sample analysed. Activity in human sample was 200times less than in rat OE (see also Bogdanffy andTaylor, 1993)	Bogdanffy et al. 1998
Enzyme activity in microsomes or S9 mix	V_{max} 14.3 nmol/min/mg protein 3.5 nmol/min/mg protein	V_{max} 2.7 nmol/min/mg protein 0.15 nmol/min/mg protein	V_{max} 38.6 nmol/min/mg protein 12 nmol/min/mg protein	V_{max} No data* 0.48 nmol/min/mg protein (only one individual)	Methyl methacrylate as substrate, at concentration of 1.0 mM Carboxylesterase activity 3fold higher in OE than in RE *OE tissue gained not sufficient to prepare microsomal fractions	Mainwaring et al., 2001

Enzyme activity in microsomes	23.8 µmol/min/mg/protein		41.4 µmol/min/mg/protein		Activity higher in OE than in RE	Bogdanffy et al., 1999a
Enzyme activity in cell homogenates	+ V_{max} 22-46 µmol acetaldehyde/min/mg protein K_m 0.3-0.43 mM		+++ V_{max} 89-165 µmol acetaldehyde/min/mg protein K_m 0.2-0.55 mM		Vinyl acetate as substrate Enzyme activity in OE was 4-7fold higher than in the RE	Bogdanffy and Taylor, 1993
Enzyme activity in cell homogenates	V_{max} 0.1 µmol/min/mg protein K_m 20.8 ±1.7 mM±SE		V_{max} 0.6 µmol/min/mg protein 34.4 K_m ±3.9 mM±SE		p-Nitrophenyl butyrate as substrate Enzyme activity in OE was 6fold higher than in RE	Bogdanffy et al., 1987
Immunohistochemistry	Ciliated cells: +, Goblet cells: ± Basal cells: - submucosal glands: ±	All ciliated and secretory cells: + submucosal glandular epithelium: some +	Bowman‘s glands/-ducts: + Sustentacular cells: + Basal cells: - Neuronal cells: –	No data		Lewis et al., 1984
Immunohistochemistry	Overall: + At cellular level: Mucous: – Ciliated cells, cuboidal cells: ++, nonciliated cells, basal cells + Goblet cells, submucosal seromucous glands: ±		Overall: +++ At cellular level: Mucous: – Bowman‘s glands/-ducts: +++ Sustentacular cells: ++ Basal cells: ± Neuronal cells: –			Bogdanffy et al., 1987;

Aldehyde dehydrogenase						
Enzyme activity in tissue homogenates	64 nmol/min/mg protein		28 nmol/min/mg protein		60 mM acetaldehyde as a substrate Enzyme activity 2fold higher in RE than in OE	Stanek and Morris, 1999
Enzyme activity in cell homogenates	V_{max} 3.38 mg/h	No data	V_{max} 0.94 mg/h	No data	Rat: Enzyme activity 3fold lower in OE than in RE	Bogdanffy et al., 1998
Enzyme activity	High K_m isoenzyme: 128 nmol/min/mg protein low K_m isoenzyme: 0.8 nmol/min/mg protein		High K_m isoenzyme: 28 nmol/min/mg protein low K_m isoenzyme: 2.2 nmol/min/mg protein		High K_m isoenzyme 5-8times higher activity in RE than in OE, low K_m isoenzyme activity comparably low in both epithelia	Casanova-Schmitz et al., 1984)
Immunohistochemistry	Ciliated cells +++ nonciliated cells ++ goblet cells + cuboidal cells: ±		Sustentacular cells: – Sensory cells: – Bowman's glands: ± Basal cells: +		Overall negligible enzyme activity in the olfactory mucosa	Bogdanffy et al., 1986

- *Considerations on the role of enzymes:*

Regarding that the olfactory region was more sensible towards the cytotoxic effect and the tumor response (as indicated by the lower concentrations at which preneoplastic lesions occurred, 200 ppm, see Table 4.1.2.6.A), and the regional characteristics of the enzymes involved, the local concentration of acetaldehyde might be of critical value for the regional susceptibility for tumorigenicity.

Bogdanffy et al. (1998) considered the metabolite acetic acid to be the initiator of cellular degeneration. Indeed, in vitro testing demonstrated cytotoxic potential of acetic acid. 1 h-exposure of rat nasal turbinate explants of respiratory and olfactory mucosa induced cytotoxic effects at 50 mM acetic acid (without a clear difference in susceptibility among olfactory and respiratory mucosa) (Kuykendall et al., 1993). Cytotoxicity was examined after 1 hr exposure to concentrations of 50 mM of test substances by enzyme leakage of acid phosphatase as into the media. No cytotoxic effect was observed on olfactory and respiratory mucosa by acetaldehyde, while a 3-fold enzyme release versus control levels by vinyl acetate (no difference among olfactory and respiratory epithelium), and a 4-fold release by acetic acid were seen. At equimolar concentrations, acetaldehyde demonstrated no potential for cytotoxicity and vinyl acetate was less cytotoxic than acetic acid. No significant difference in cytotoxicity was found between the olfactory and the respiratory epithelium. The absence of cytotoxicity in the acetaldehyde testing appears not plausible (at least for the respiratory epithelium where the activity of aldehyde dehydrogenase is high) assuming that acetic acid should rapidly be produced by aldehyde dehydrogenase activity. The nil response may indicate that either acetaldehyde did not reach the cytosol, no acetic acid was produced (e.g. by enzyme inactivity in the in vitro model) or that the produced acetic acid (which dissociates into acetate anions and protons) which were buffered. No cytotoxic effect was observed at 25 mM vinyl acetate for incubation times up to 2 hours (no data for acetic acid). Using the transformation into the inhalation concentration as proposed by Bogdanffy (2002) 25 mM corresponds to about 100 ppm vinyl acetate (50 mM ≈ 200 ppm). Results of in-vitro tests could be interpreted to fit well for the lowest observed effect level for cytotoxicity in the area of the olfactory region, which was 200 ppm. However, in-vivo observations on the respiratory epithelium revealed cytotoxicity at 1000 ppm.

Assuming that acetic acid will be the main cytotoxic agent, the lack of cytotoxicity up to 600 ppm in the respiratory epithelium may indicate that the local concentration of acetic acid in this area does not result in cellular damage. Thus the occurrence of tumors in this region (at 600 ppm) could not be attributed to acetic acid-related cytotoxicity.

For mechanistic considerations, the in situ distribution and activity of involved enzymes carboxylesterase and aldehyde dehydrogenase are of special interest. Both nasal regions, the respiratory and olfactory epithelium are capable to metabolise vinyl acetate because the presence of both enzymes has been demonstrated by immunostaining and activity measurements.

Regarding the different mucosal regions, the enzyme activity of carboxylesterase is higher in the olfactory region (4-7fold, related to protein content) compared to those of the respiratory region (Bogdanffy et al., 1999a, Bogdanffy and Taylor, 1993). This difference is consistent with the intensity of immunostaining in these regions (Olson et al., 1993).

Comparing the absolute capacities among the enzymes, data indicated that the capacity of aldehyde dehydrogenase to aldehyde dehydrogenase is much lower than that for carboxylesterase to vinyl acetate in rat nasal tissues. In the olfactory epithelium, Vmax of aldehyde dehydrogenase was lower than the activity of carboxylesterase (30-40fold lower towards their respective substances). The difference is less marked for the respiratory epithelium, aldehyde dehydrogenase activity is 13-19fold lower than the carboxylesterase activity (Bogdanffy et al., 1998).

It is concluded that at the same local concentration of vinyl acetate above K_m of carboxylesterase, the intraepithelial concentration of acetaldehyde in the olfactory epithelium is expected to exceed that of cells in the respiratory epithelium. Because of the differences in V_{max} of aldehyde dehydrogenase as compared with carboxylesterase at concentrations above K_m the oxidation (and thereby the detoxification) of acetaldehyde to acetic acid is limited and may result in intracellular accumulation of acetaldehyde. From the absence of aldehyde dehydrogenase activity in the sustentacular cells and sensory cells it may be assumed that acetaldehyde concentrations will be higher at the same external vinyl acetate concentration.

Compared to the olfactory epithelium, the lower carboxylesterase activity in the respiratory epithelium yielded a lower in situ production of acetic acid and acetaldehyde. This might be reflected by the higher LOAEC for cytotoxic effects (1000 ppm) and hyperplastic lesions (1000 ppm) in the respiratory epithelium than that for the olfactory epithelium (LOAEC 200 ppm for both effects, see Table 4.2.1.8.E).

Quantitative information on aldehyde dehydrogenase activities in the anterior (respiratory mucosa) and posterior (olfactory mucosa) nasal tissues of F344 rats is given in a paper by Stanek and Morris (1999). Activities averaged 210 and 160 nmol/min, respectively, corresponding to 64 and 28 nmol/min/mg protein. Nasal uptake was measured at three inspired concentrations of 10, 300, and 1500 ppm acetaldehyde and resulted in an uptake efficiency of 54, 37, and 34% demonstrating a concentration dependence of uptake. Stanek and Morris (1999) elaborated convincing evidence that the underlying saturable process is the metabolism of acetaldehyde by aldehyde dehydrogenase. Capacity limitation for nasal ALDH is suggested to occur at inspired concentrations of 300 ppm or more.

Carboxylesterase localisation indicates intracellular metabolism of vinyl acetate in the above cited cell types, but did not explain why selective toxicity was evident in carboxylesterase-negative olfactory neuronal cells. Bogdanffy et al. (1998) concluded that the toxic effect was more likely a result from cell-specific sensitivity to cytotoxicity and to a lesser extent the function of metabolic capacity. The discrepancies between cells with metabolic capacities and affected neuronal cells could suggest that additional mechanisms are involved (e.g. selective sensitivity of cells, local distribution of metabolic products). The latter has been discussed by Bogdanffy who assumed intercellular communication via tight junctions to play a role in transportation of acid metabolites from the sustentacular cells to the sensory cells, the most sensitive target cells of the olfactory mucosa (Bogdanffy, 1990). Indications on a potential of acetaldehyde to disrupt tight junctions are available from a human colonic mucosa model (Basuroy et al., 2005). Indirect effects following damage to the sustentacular cells could explain high vulnerability of neuronal (sensory) cells to vinyl acetate (as for many esters). These cells are adjacent to the sensory cells and have supporting functions. The toxic insult (here: by increase of tissue concentration of acetaldehyde and/or other metabolites) primarily damages the sustentacular cells, their dysfunction may initiate the neuronal cell death. Since no aldehyde dehydrogenase activity was localised in the sustentacular cell, the detoxification

capacity through oxidation of acetaldehyde is lacking. This observation is in line with experience from other substances (e.g. methyl bromide, Schwob et al., 1995) where the inhalation of a gas that needs metabolic transformation for toxification results in selective vulnerability of sustentacular cells and olfactory sensory neuronal cells whereas the basal cells are spared from toxic insult. Also, from others it was suspected in earlier studies that toxic metabolites might diffuse from cells of the Bowman's glands (Olson et al. (1993).

For interspecies comparison, data on the absolute enzyme capacities in rats and humans indicated that the capacity of aldehyde dehydrogenase is much lower than that for carboxylesterase, in rats and in human nasal tissues. In the olfactory epithelium, Vmax of aldehyde dehydrogenase was 30-40fold lower for the rat and 20fold for humans than the activity of carboxylesterase. The difference is less marked for the respiratory epithelium; aldehyde dehydrogenase activity is 13-19fold lower in rats and 5fold lower in humans than the carboxylesterase activity (Bogdanffy et al., 1998). Taking these data into the account, there is no indication on a higher sensitivity of humans exposed to vinyl acetate compared to that observed in rats.

- *Contribution of cellular acidification:*

Due to the low activity of aldehyde dehydrogenases in the olfactory region the metabolic oxidation of acetaldehyde and thereby additional generation of acetic acid is considered of minor importance for the olfactory region. Compared to the olfactory mucosa, the lower carboxylesterase activity and the relatively high activity of aldehyde dehydrogenase in the respiratory region is supposed to prevent the accumulation of acetaldehyde in this area by the oxidative pathway generating acetic acid. However, at higher vinyl acetate concentrations aldehyde dehydrogenase capacity may be exceeded and intracellular accumulation of acetaldehyde will occur.

It has been assumed that the intracellular production of acetic acid exceeds cellular buffering capacity and results in elevated H^+ concentrations which is thought to be associated with cytotoxicity (Plowchalk et al., 1996, 1997). The existence of intracellular acidification was supported by findings on cultured carboxylesterase-containing hepatocytes exposed to various concentrations of a vinyl acetate-PBS solution (10-1000 µM). Dose-dependent reductions of intracellular pH values were observed at concentrations of 10-200 µM (expected mucous concentration resulting from inhalation exposure to 50-670 ppm vinyl acetate), a marked decrease in intracellular pH appeared at 500 µM, no further drop occurred at 800 and 1000 µM. The maximal drop was 0.5-0.6 pH units. The reduction of intracellular pH was rapidly reversible within hepatocytes after withdrawal of vinyl acetate (Bogdanffy, 2002).

Uncertainty existed about the carboxylesterase activity of hepatocytes, which may differ significantly from those of the respiratory and olfactory mucosa cells. Earlier studies showed a hydrolysis rate about an order of magnitude higher in the rat liver than in the lung (no examination of nasal tissues) (Simon et al., 1985a). For the rat model, the carboxylesterase activity appears to be comparable in the liver and the olfactory microsomes and was nearly 3fold higher than in the respiratory epithelium microsomes (Mainwaring et al., 2001). In contrast, human liver microsomal activity towards methyl methacrylate is much higher (>100fold) than those of the olfactory and respiratory epithelium.

Interestingly this model showed that no (acute) cytotoxic effect was associated with the lowered pH-value up to the maximum reduction of 0.55 units at 500 µM and above (corresponding to inhaled concentrations above 1000 ppm). As cells returned to pre-treatment pH-values within 50 sec after treatment with 200 µM vinyl acetate, a high rate of proton efflux was thought to prevent from intracellular proton accumulation. The assumption of the authors that the proton export is in the same range as its intracellular production appears to conflict to the proposal that acidification through acetic acid is - via cytotoxicity - the initial step in tumorigenesis. The lack of cytotoxicity is in line with resistance of hepatocytes reported by Farkas and Tannenbaum (2005). A high concentration of 1000 µM vinyl acetate induced no secretion of protein and urea and some release of lactate dehydrogenase from hepatocytes that indicated 20 percent of cell death occurred at 24 hours after treatment.

Corresponding in-vivo data on the effect of vinyl acetate on intracellular pH of nasal mucosa cells were not available. In vitro exposure of isolated respiratory and olfactory epithelium cells to concentrations of 100-1000 µM vinyl acetate resulted in concentration-related decrease in intracellular pH in the respiratory epithelial cells (Lantz et al., 2003). A slight reduction in intracellular pH was seen at 100 µM, a plateau was reached at 500 µM and 1000 µM did not further increase the acidification. The maximal decrease was –0.3 pH units (pH_i 7.16 vs. resting pH_i 7.49). In the olfactory epithelium, a bimodal distribution of pH values was observed. In contrast to the respiratory epithelium cells, olfactory epithelial cells consisted of two populations with different behaviour. Only one population responded dose-dependently to 100, 250, 500 and 1000 µM vinyl acetate treatment by reduction in intracellular pH. The maximum decrease in the responders was –0.3 pH units (peak pH_i changed from 7.32 in control cells to 7.0 for the highest concentration). Changes in pH values were reversible in all cells after washing.100 µM vinyl acetate did not decrease pH when a carboxylesterase inhibitor (BNPP) inhibited carboxylesterase. Interestingly, a plateau for maximal acidification was also reached for respiratory cells at 500 µM as in the liver cell model of Bogdanffy (2002). The lowest concentration tested (100 µM) already disturbed the intracellular proton homeostasis, but the overall extent of acidification was rather low (-0.06 in the respiratory epithelium and – 0.03 in the olfactory epithelium). - Additional studies by Lantz et al. (2003) on explants from ethmoturbinates (olfactory) and maxilloturbinates (respiratory) using pH-sensitive fluorescence imaging responses to vinyl acetate observed only minimal acidification (-0.05 pH units) in the presence of 1000 µM vinyl acetate in the respiratory epithelial cells (peak pH_i 7.4 in cells of control explant). Accordingly to the isolated cells, cells of the olfactory epithelium explant (peak pH_i 7.4 in cells of control explant) responded by a bimodal distribution of cells with reductions of -0.2 and -0.8 pH units indicating the presence of two different cell populations. In conclusion, the overall lower acidification of the respiratory epithelium appears to correspond to the lower carboxylesterase activity compared to the olfactory epithelium. However, the difference in responses produces uncertainty in the data reliability (e.g. -0.3 pH units in isolated respiratory cells and –0.05 pH units in the respiratory epithelial cells of the explant model). Responsive and non-responsive cell populations may reflect the differences in carboxylesterase activity among cell types of the olfactory epithelium (lack of enzyme in neuronal cells, negligible in basal cells, and marked activity in sustentacular cells and Bowman's glands).

As acetic acid is responsible for intracellular acidification, the lack of further decrease in pH values above 500 µM in the above mentioned in vitro models indicates that the production of acetic acid is limited. The relevance of the minimal drop in pH (-0.2 pH units in hepatocytes, -0.2 pH units in respiratory epithelial cells, -0.14 pH units in olfactory epithelial (Bogdanffy, 2002, Lantz et al., 2003)) at tumor dose-equivalent concentrations of 200 µM for cell

proliferative activity or tumor growth is questionable. The lacking reactivity of hepatocytes at minor pH drops is in line with the observed tolerance of tracheal mucous epithelium to acidity. In tracheal tissue explants a reduction in ciliomotility, cell membrane destruction and intra- and intercellular oedema started in acidic environment at pH 6.70 and complete ciliostasis occurred below pH 4.9 (Holma et al., 1977).

Whether the predictions on carboxylesterase activity and intracellular acidification from the modelling in hepatocytes, nasal mucosa cells and explants are assignable in a quantitative manner to the in vivo situation of the human nasal targets remains uncertain. Considering the resistance to cytotoxicity and minor reductions in intracellular pH in hepatocytes exposed to up to 1000 µM vinyl acetate, which were consistent to the low extent of acidification and its reversibility observed in in-vitro single cell or turbinate explants of the respiratory and olfactory epithelium, the overall low aldehyde dehydrogenase activity in rat and human olfactory mucosa (which limits the additional production of acetic acid), and the lack of cytotoxicity up to 1000 ppm for the respiratory mucosa, where tumors were already seen, lead to the conclusion that the contribution of intracellular acidification as the presumed mode for tumor development is – if at all- only minor.

A validation study for the application of a human PBPK model with controlled human inhalation exposures to vinyl acetate showed that the human nasal model predicts the experimental observations with regard to vinyl acetate and acetaldehyde in the airstream of human nasopharyngeal cavity in a concentration range from 1 to 10 ppm (Hinderliter et al., 2005).

Step 2: From cytotoxicity to cancer:

The degeneration and inflammation of the olfactory epithelium observed in the toxicity and carcinogenicity studies after repeated/prolonged inhalation exposure gave indication that cytotoxicity of target cells contributed to the tumor development in this nasal region. Concomitant lesions representing attempts to repair/regenerate the damaged olfactory epithelium were observed (Bogdanffy et al., 1994b). In the view that finally tumors occurred at this site, metaplastic and hyperplastic changes could be interpreted to represent the sequential steps in carcinogenesis. The olfactory epithelium was more sensitive to develop degenerative lesions than the respiratory epithelium (Bogdanffy et al., 1997).

Early degenerative changes were evident in the sustentacular cells, but these cells primarily targeted by cytotoxic effects of vinyl acetate were not identical to the cell types of tumor origin. As no tumor of the neuro(esthesio-)blastoma type were found in carcinogenicity studies on vinyl acetate, cells of tumor origin are more likely less differentiated cells with origin of the basal cell, intermediary cells of squamous type or of the respiratory-cell like type rather than of the olfactory sensory cell type. The majority of tumors seen in the nose were of the squamous cell type indicating that cells of squamous metaplasia or surviving basal cells that undergo squamous cell differentiation were the tumor cell origin. Increased regenerative cell proliferative activity could trigger the accumulation of cells with spontaneous DNA damage and thereby cause an increase in cancer. In addition, cells replacing the olfactory epithelium may have an altered susceptibility to noxious insults.

No data on carboxylesterase activity and aldehyde dehydrogenase activity are available for cells of hyperplasia and respiratory metaplasia in the olfactory epithelium. In human samples of hyperplastic tissue of the respiratory mucosa, a dramatic reduction in enzyme

immunostaining was found and enzyme was lacking in squamous metaplasia. The absence of activity fits well to the lack of carboxylesterase activity in the squamous epithelium in the cranial portion of the nose (Olson et al., 1993). It might be assumed that the lack of carboxylesterase in hyperplastic/metaplastic respiratory tissue protects against the vinyl acetate toxicity at this sub-site. However, chronic damage of the olfactory epithelium characteristically consists of a mixture of different epithelial/cell types (respiratory-like hyperplastic area, squamous metaplasia and dysplasia, atrophic/hypertrophic olfactory areas).

There remain uncertainties on the exact mechanisms of the olfactory epithelium cell death and the disturbances in control of regenerating cell proliferation leading to carcinogenesis.

Ic. Dose-response relationship

Degenerative (atrophic) lesions of the olfactory mucosa were seen in rats at concentrations of 200 ppm or higher when exposed over 2 years (Bogdanffy et al., 1994b, Owen, 1988). In this species, the respiratory epithelium was less sensitive to damage showing no lesions up to 600 ppm (Bogdanffy et al., 1994b), the lowest concentration with respiratory epithelial damage was 1000 ppm vinyl acetate after 4 weeks of inhalation (Bogdanffy et al., 1997). Lesions of the lower parts of the respiratory tract (larynx, trachea, bronchi, bronchioli) were seen at 600 ppm vinyl acetate in rats.

A clear increase of nasal tumors at the olfactory, respiratory and other/unknown localisation occurred in rats at concentrations of 600 ppm. The total incidence of (all) nasal tumors was 11.9% in male rats and 6.8 % in female rats of the group (see Table 4.1.2.8.A).

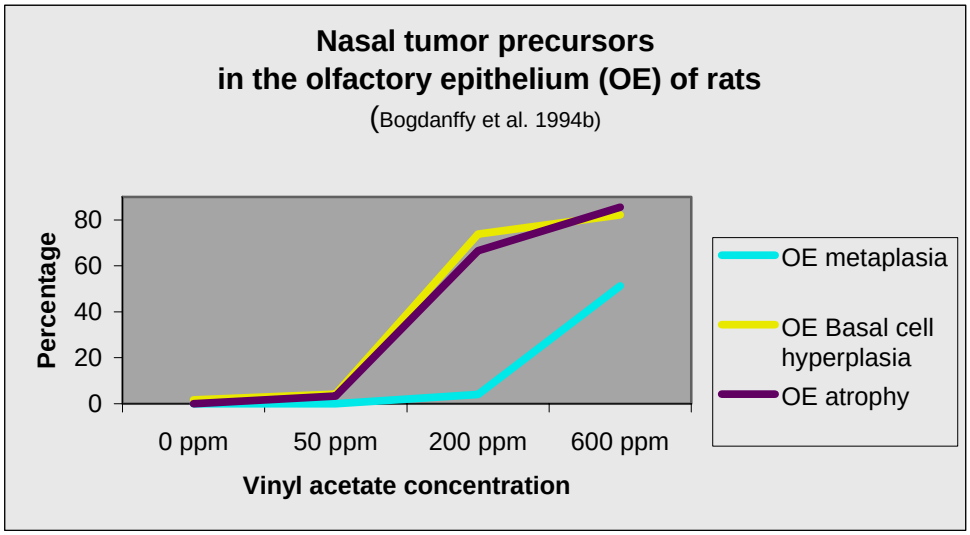

Figure 4.1.2.8.A

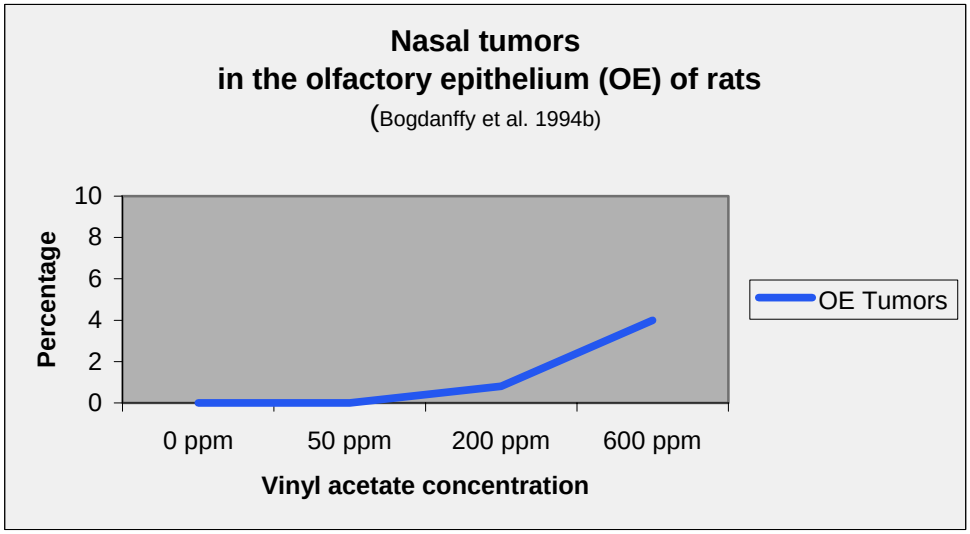

Figure 4.1.2.8.B

Regarding males and females at each dose group together (see above Figure 4.1.2.8.B), the incidence of tumors with site of origin in the olfactory region was 4% at 600 ppm (in 118 males and females). With respect to the olfactory epithelium, toxic lesions occurred at lower concentrations (200 ppm) (see above Figure 4.1.2.8.A) than those inducing an increase of tumor incidences (600 ppm). Dose-dependently the restorative basal cell proliferation increased in parallel to the degenerated (atrophic) olfactory epithelium. The dose-response curves for all effects assumed to be precursors of tumor development were non-linear, the NOAEC was 50 ppm. Olfactory epithelium metaplasia was occasionally seen at 200 ppm (in 5/60 female rats, none in males). A single benign tumor was seen in the area with olfactory epithelium in a male rat at 200 ppm. As spontaneous tumors at this site are very rare, an association to vinyl acetate exposure could not be excluded. A significant increase in metaplastic lesions was evident at the clearly tumorigenic concentration of 600 ppm.

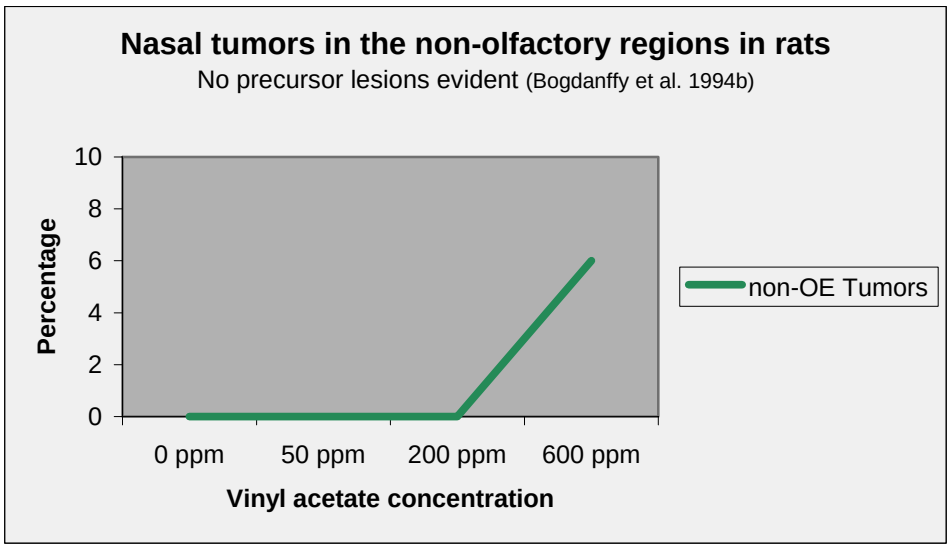

Figure 4.1.2.8.C

Tumors at other non-olfactory regions (tumors of unknown origin were included here) occurred in 6% of male and female rats (7 out of a total of 118) (Figure 4.1.2.8.C). At the respiratory epithelium of the rat nose no cytotoxicity and no other precursor lesion was reported at 600 ppm, the concentration which induced tumors at this site after 2-year inhalation (Bogdanffy et al., 1994b). Toxic effects at this site occurred only at dose levels above those inducing tumors.

A dose-response relationship of degenerative (atrophic) and other precursor effects (basal cell hyperplasia and metaplasia) and tumor development was identified for the olfactory region. No dose- response relationship was evident for cytotoxicity or other metaplastic changes could not be identified for the respiratory region.

Id. Temporal relationship

A temporal relationship of cytotoxicity-related tumor growth can be assumed for the tumors of the rat olfactory epithelium, because early degenerative findings were seen in studies with 4-week duration (Bogdanffy et al., 1997). As no underlying toxic event could be identified in the respiratory epithelium of the rat nose at or below the tumor-inducing dose, a statement on the time progression is not possible.

No complete recovery of the olfactory atrophy was observed in the recovery groups of the combined chronic and carcinogenicity studies on rats and mice (Owen 1988, Bogdanffy et al., 1994b). This is comparable to the recovery status known from acetaldehyde studies. A replacement of the damaged olfactory epithelium by respiratory-like epithelium (without sensory cells) as well as regeneration with a less differentiated olfactory-like epithelium was reported (Woutersen and Feron, 1987).

Ie. Biological plausibility and coherence

From historical data it is known that nasal tumors have a very low spontaneous incidence in aging rats (St. Clair and Morgan, 1992, Brown et al., 1991). Malignant nasal neoplasm were also rare in humans (overall rate <1/100 000; Lund, 1994).

The sequence of events (cytotoxicity, hyperplasia, benign and malignant tumors) observed at the olfactory epithelium is consistent with the general knowledge on the tumor pathogenesis of substances for which genotoxicity could be excluded as mode of action. In case of vinyl acetate it has to be discussed whether acetaldehyde genotoxicity contributes to the sequence of events.

The evidence of tumors of squamous origin indicated that DNA damage occurred in cells of squamous metaplasia or in surviving basal cells that are precursor cells of squamous cell metaplasia or squamous cell tumors.

Vinyl acetate effects seem to coincide with acetaldehyde effects. Nasal tumors were observed after long term inhalation of vinyl acetate or acetaldehyde. In comparison with the vinyl acetate data mentioned above, the primary tumor site after chronic acetaldehyde exposure of rats also was the nose, and tumors of the larynx were occasionally observed in this species after chronic inhalation to each of the test substances. Except those nasal adenocarcinomas were not found in vinyl acetate cancer studies, tumor response from both substances was comparable at least for the rat. Whereas vinyl acetate was not tested in the hamster, the prevalent tumor site was the larynx, and only few tumors were seen in the nose of

acetaldehyde exposed hamsters (Feron et al., 1982). This indicates that these localisation do have the enzymatic capabilities; the difference in distribution was suspected to be due to species-specific differences in susceptibility of the epithelium to acetaldehyde, a difference in impact of acetaldehyde on the epithelium, a difference in breathing pattern, and/or a combination of all factors (Wouterson et al., 1984).

The nature of the effect - cytotoxicity – evoked by inhalation of acetaldehyde on nasal tissues seems to be comparable to that of vinyl acetate. The rat respiratory epithelium was shown to be less sensitive towards toxic lesions than the olfactory epithelium. (Wouterson et al., 1984, 1986, Appelman et al., 1982).

If. Inconsistency, uncertainties and data gaps

The presence of degenerative lesions at the olfactory epithelium demonstrates its specific sensitivity to cytotoxicity. The evidence of dose and temporal relationships and the evidence of sequential alterations (cytotoxicity, hyper- and metaplasia, benign and malignant tumors) support that cytotoxicity is associated to the tumor development. This was seen in a long-term study on the rat, but was not consistent to the negative results from the mouse cancer study.

- Interspecies comparison to the mouse:

Degenerative lesions of the olfactory mucosa were seen in mice at concentrations of 200 ppm or higher when exposed over 2 years. Treated mice showed lesions at the respiratory epithelium at 200 ppm after 2-year inhalation of vinyl acetate (Bogdanffy et al., 1994b, Owen, 1988). Lesions of the lower parts of the respiratory tract (larynx, trachea, bronchi, bronchioli) were seen at ≥200 ppm vinyl acetate in mice.

In mice, it is noteworthy that toxic lesions beginning at 200 ppm at both localisations of the nasal epithelia, but no nasal tumor occurred at concentrations tested up to 600 ppm. Reparative replacement of the olfactory epithelium by respiratory-like epithelium and squamous metaplasia as well as hyperplasia of the submucosal glands were similar to the effects seen in the rat, but no progression to tumors occurred in this target region. It can not be excluded that the single squamous cell carcinoma of the lung bronchus was also associated to the vinyl acetate treatment occurring at the same concentration which induced epithelial exfoliation/disorganisation and fibrohyperplastic projections of the bronchus/bronchioles with high incidences (Bogdanffy et al., 1994b, Owen, 1988).

In mice vinyl acetate is also efficiently hydrolysed in nasal mucosa. Since the values for carboxylesterase activities in the respiratory and olfactory regions were in the same range (olfactory: 0.4-0.5 µmol/min/mg protein in the mouse compared to 0.6 µmol/min/mg protein in the rat, respiratory: 0.1-0.2 µmol/min/mg protein in the mouse compared to 0.1 µmol/min/mg protein in the rat, Bogdanffy et al. 1987) or were found at even higher ranges as in the rat (Bogdanffy and Taylor, 1993), a significant tumor response in the nasal mucosa should be expected for the mouse. This raises questions on species-specific differences in the susceptibility of the nasal mucosa. Is the mouse nose less sensitive to tumor generation (consider the tumors of the gastrointestinal tract of the mouse)? Or does the mouse have a high activity of aldehyde dehydrogenase (in contrast to the low ALDH activity in rat olfactory mucosa) that effectively contributes to acetealdehyde elimination and thereby prevents in situ accumulation of acetaldehyde? Determination of aldehyde dehydrogenases activity revealed low Vmax in whole nasal tissue homogenates of mouse and hamster compared to rats

(Morris, 1997). However, origin of epithelial material was not specified and whole tissue homogenates might be less predictive.

DNA adduct formation was observed in the rat nose, but no data were available for the nasal mucosa of the mouse. To explain the lack of nasal tumors in mice, further data are needed.

The mouse was negative for the inhalation route, but sensitive towards tumor development for the oral route (see 4.1.2.8).

- Uncertainties related to the role of intracellular acidification:

The role of pH changes remained unsolved for some critical points. In olfactory epithelium, target cells undergoing cell death (primarily sustentacular cells) were not identical with cells that were involved in regenerative proliferation. There are two types of basal cells, the horizontal basal cells and the globosal basal cells. In healthy tissue, the latter represents the majority of basal cells and is mainly involved in normal regeneration of olfactory cells (Ohta and Ichimura, 2001). Even if there is no significant reduction in intracellular pH values that plays a critical role in the process of cell damage, is there any pH-dependent effect on proliferation stimulus on basal cells? Comparing to other toxicants that also induce selective cell death in sustentacular/sensory cells, reparative cell replication appears to be a unique mechanism that is not necessarily linked to cellular acidification in degenerating cells. The evidence of tumors at non-olfactory sites demonstrated that proliferative activity was not necessarily associated with cell death and therefore might not be related to the cell acidification.

Bogdanffy (2002) claimed that intracellular acidification as demonstrated in the hepatocyte model precedes the morphological signs of cytotoxicity. However, 1000 µM vinyl acetate was not cytotoxic in hepatocytes at the end of 6 days of culturing as evidenced by protein and LDH leakage (Farkas and Tannenbaum, 2005). Further proof is needed that intracellular acidification is the initial lesion in cytotoxicity, at least for the olfactory region.

A reduction of intracellular pH as demonstrated in the rat hepatocytes (Bogdanffy, 2002) was – even at a lower level - assignable to cells of the olfactory and respiratory mucosa. For the rat, carboxylesterase activity appears to be in the same range in the rat liver as in the rat olfactory mucosa as demonstrated in microsomes using methyl methacrylate as a test substrate (Mainwaring et al., 2001) (Table 4.1.2.8.H). In contrast, carboxylesterase activity is much higher in the human liver than in the human olfactory mucosa.

The changes in intracellular pH were relative small in the test systems mentioned above. However, no prediction could be made on pH homeostasis after prolonged exposure. The experimental studies available examined vinyl acetate or acetaldehyde effects following acute (seconds to minutes) exposure. No data exist on the consequences of prolonged acidification.

The small reduction of intracellular pH may be explained by the low activity and nearly absence of aldehyde dehydrogenases in the olfactory mucosa, which limits the production of acetic acid. Therefore the intracellular acidification may be a process of minor relevance for (cyto-)toxicity and tumor induction at this site. Besides the uncertain relationship between small changes in pH and cytotoxicity other pH-related effects on cell transformation could be considered. On irradiated cells with DNA double-strand breaks it was demonstrated that lowering of the extracellular pH from 6.8 to 6.0 causes inhibition of DNA repair (Jayanth et al., 1994). Whether this is relevant for the carcinogenicity of vinyl acetate is unknown.

- Uncertainties related to the modes of action for the respiratory mucosa:

Beside the nasal tumors originating from the olfactory epithelium, there were also some nasal tumors, which were located in areas of the nasal cavity consisting of non-olfactory epithelial types (Table 4.1.2.8.B). No toxic event was identified as a critical step in the development of these tumors, e.g. at the respiratory epithelium of the nose. Whereas rhinitis and metaplasia occurred in the respiratory epithelium of the mouse nose, cytotoxic and/or inflammatory responses to vinyl acetate exposure were not reported in rat studies at concentrations which induce tumors (Owen, 1988, Bogdanffy et al., 1994b). Thus, clear data demonstrating that the toxic lesions in this area were associated to disregulated cellular regeneration resulting in tumor formation are missing. The absence of cytotoxic events at the respiratory epithelium at tumor-inducing concentrations demonstrated that cytotoxicity did not seem to be the necessary precondition for carcinogenicity of vinyl acetate.

Increased rates of cell turnover were observed in both respiratory and olfactory mucosa after a single 6-hour exposure. When vinyl acetate vapour was inhaled on 20 days (5 d/wk, 6 h/d) (Bogdanffy et al., 1997), increased cell proliferation was only evident in the olfactory mucosa. These findings were inconsistent with the fact that tumors were observed at both regions.

Also, it was suggested that acidification causes mitogenic response as a critical step in tumor growth of the respiratory mucosa. In-vitro exposure of cells of a Barrett's adenocarcinoma cell line to acid media increases cell proliferation and decreases apoptosis via an activation of the mitogen-activated protein kinase pathway (MAPK) (Souza et al., 2002). However, the mode of the development of Barrett's carcinoma of the oesophageal squamous mucosa appears of limited predicted value, since in this in-vitro model cells were exposed to markedly lower pH values (pH 4.0) than those observed in the nasal mucosa following vinyl acetate exposure. Up to now no substantial evidence of decreased apoptosis has been shown for vinyl acetate-related acidification and no studies investigating mitogenic effects for the inhalation route were available. Instead, some data from oral administration of acetaldehyde and vinyl acetate may be informative: Long-term cell proliferation experiments demonstrated mitogenic effects of acetaldehyde which induced diffusely enlarged basal cell layer and increased cell proliferation rates (Homann et al., 1997). Compared to vinyl acetate metabolism to acetic acid where 3 protons were delivered per molecule vinyl acetate, acetaldehyde oxidation delivers only 1 proton per molecule. This indicates that the mitogenic response is more likely an effect of acetaldehyde than an effect of acidification. The mechanism by which acetaldehyde exerts its mitogenic effect is yet unknown. Whereas only one acetaldehyde concentration (120 mM = 324 mg/kg bw/d) was applied with drinking water in the Homann study, the study of Valentine et al. (2002) observed concentration-dependent increases in cell proliferation rates in the oral mucosa of rats receiving 10000 ppm vinyl acetate or more with the drinking water. No effect was observed at 1000 and 5000 ppm, cytotoxicity was absent in all concentrations tested. In conclusion, acetaldehyde and vinyl acetate have mitogenic properties, that of vinyl acetate is likely to be mediated through acetaldehyde and has a clear no-effect level.

- Uncertainties related to genotoxic effects:

Since no tumors occurred below the LOAEC for cytotoxic effects in the olfactory region it is unlikely that genotoxic effects were active below the threshold concentration for cytotoxicity.

In contrast no dose-response could be identified for preceding events in the non-olfactory regions. Dose-related mitogenic effects were demonstrated for the oral route where it is assumed that vinyl acetate exerts its acetaldehyde-caused clastogenic effects most efficiently in proliferating cells. This might also be assumed for the sites of contact following inhalation. However, at the moment there are no studies confirming the hypothesis and identifying a LOAEC for this effect in the respiratory tract.

Ig. Actual considerations on the assessment of the mode of action for tumors of the respiratory tract (see Table 4.2.1.8.K)

Acetaldehyde is the critical metabolite with a carcinogenic potential.

The distribution and capacity of enzymes responsible for vinyl acetate toxicification and detoxification are of major importance to create a preliminary understanding of the modes of carcinogenic action. The present understanding is that acetaldehyde plays a critical role in the tumorigenicity of vinyl acetate.

The principal similarities of toxic and carcinogenic effects of vinyl acetate to those of acetaldeyhde suggest that the hydrolysis product acetaldehyde is the active carcinogenic metabolite of vinyl acetate.

Acetaldehyde is a biological constituent of many cells and is – beside the release from the man-made sources - ubiquitarily present in many foods and natural sources. Exposure to vinyl acetate induces concentration-related increases in intracellular concentrations of the hydrolytic products acetaldehyde and acetic acid. Efficiency of the absorption is optimal at lowest concentrations and decreases with increasing concentration (see 4.1.2.1). Increasing concentrations of the vinyl acetate may result in an accumulation of acetaldehyde when the cellular detoxification capacity of aldehyde dehydrogenase is overwhelmed. The toxicity and/or carcinogenicity reflect the function of metabolic capacity of the target epithelium. The regional and cellular susceptibility to cytotoxicity corresponds to the distribution and activity of the enzymes involved.

Acetaldehyde is thought to be the responsible metabolite for the genotoxic effects seen in mammalian cell cultures on vinyl acetate.

A hypothesis of a cytotoxicity-related carcinogenesis is likely to induce tumors in the olfactory region.

- The rat olfactory tissue efficiently catalyses the formation of acetaldehyde and acetic acid.

- Absorption and metabolic transformation of vinyl acetate by carboxylesterase is a concentration dependent process. Due to the absent or low aldehyde dehydrogenase activity (as compared to the carboxylesterase activity) only low concentrations of acetaldehyde can be oxidised. Above a yet non-identified exposure concentration, the production of acetaldehyde exceeds further oxidation, which results in an intracellular accumulation of acetaldehyde.

- Acetic acid-related cytotoxicity following vinyl acetate hydrolysis appears to be the initial event in tumorigenicity for tumors with origin in the olfactory epithelium.

- A direct genotoxic action of the metabolite acetaldehyde on the restorative epithelium could contribute to tumor initiation.

- Cell death induces increased cell turn over of surviving cells or basal cells. DNA synthesis is elevated and the incidence of genotoxic effects is expected to increase.

In conclusion, it appears plausible to explain the sequential steps from cytotoxicity to carcinogenesis for the olfactory epithelium. The sites of epithelial damage correlated with increased cell proliferation as evidenced by basal cell hyperplasia and transformation to squamous metaplasia. The latter was considered to be a precursor lesion of benign and malignant tumors that were squamous cell papillomas and carcinomas.

The contribution of intracellular acidification as the initial step leading to cytotoxicity and thereby to carcinogenicity remains uncertain. Short term testing indicated that the degree of intracellular acidification was only minor and reversible. Also, the site-specific changes in intracellular acidification as predicted in the PBPK-model (Plowchalk et al., 1997) were not consistent with the in-vivo preference of cytotoxicity. Genotoxicity data indicate a possible genotoxic effect of vinyl acetate mediated by its metabolite acetaldehyde, which may contribute to the tumor development at concentrations, which were cytotoxic.

At present, a narrative for a threshold concentration for tumor development could be identified for the olfactory region by its cytotoxicity.

A threshold mode of action is thought to be active for tumors with origin in the other nasal regions.

For the other (non-olfactory) tumor sites of the upper respiratory tract (mainly the nasal respiratory epithelium), cytotoxicity was clearly not a critical event in tumor formation, because tumors occurred at lower exposure concentrations than cytotoxicity. The predictions from the PBPK model, with respect to the degree of acidification and thereby of the cytotoxic response did not reflect the observed study data. During the last years, scientific working groups recognised the lack of evidence for the early proposals on acidification as the relevant mode and introduced considerations about the role of intracellular acidification in stimulation of cell growth and transformation (Lantz et al., 2003). These secondary responses occur only at higher doses.

The following aspects support the hypothesis of a threshold mode of action:

- Absorption and metabolic transformation of vinyl acetate is a concentration dependent process.

- Carboxylesterase activity in the respiratory epithelium is at least half than that of the olfactory epithelium in man and rats. The hydrolysis rates of vinyl acetate and thereby the intracellular concentrations of acetic acid and acetaldehyde are expected to be markedly lower than in the olfactory epithelium.

- Aldehyde dehydrogenase (ALDH) is the key enzyme for the elimination of acetaldehyde. Its activity is more than 2fold higher in the respiratory epithelium than in the olfactory

epithelium. The enzyme activity is sufficient to cope with low concentrations of vinyl acetate and its produced metabolite acetaldehyde. At high concentrations of intracellular acetaldehyde ALDH activity will not be sufficient to oxidise all acetaldehyde to acetic acid and acetaldehyde may accumulate. Saturation of metabolism of acetaldehyde by ALDH indicating limited enzyme capacity is suggested to occur at acetaldehyde concentrations of 300 ppm (Stanek and Morris, 1999).

- No toxicity was observed in respiratory epithelium up to 600 ppm in 2-year studies. Regarding acetic acid as the cytotoxic metabolite, acetic acid generation at 600 ppm is below the threshold for cytotoxicity.

- No increase in proliferative activity either measured as proliferation index or as hyperplastic/metaplastic changes up to 600 ppm vinyl acetate in (LOAEC 1000 ppm). It is assumed that increased cell proliferative activity is acetaldehyde-related. As no cytotoxicity or other relevant effect was seen up to 600 ppm, no significant intracellular concentration of acetaldehyde is expected to occur.

- In accordance to data from oral studies on mitogenic action of vinyl acetate a mitogenic action could be assumed above a threshold concentration. Due to the lack of adequate studies on the respiratory tract, evidence from studies on gastrointestinal tract was considered: Basal cell hyperplasia and epithelial hyperplasia in the gastrointestinal tract without preceding cytotoxicity were evident at ≥10000 ppm (NOAEL 5000 ppm ≈1200 mg/kg/d, 92-day mouse study, Valentine et al., 2002).

- Tumor response was seen at ≥600 ppm vinyl acetate, no tumors were observed at concentrations below.

- It is assumed that acetaldehyde-caused genotoxicity is active at vinyl acetate concentrations that cause acetaldehyde accumulation.

- The toxicological relevance of minimal reduction in pH remains questionable.

- Actually, an exact threshold concentration for acetaldehyde accumulation could not be estimated for the non-olfactory region.

- Since no biological response in the respiratory mucosa was observed in long-term inhalation studies up to 200 ppm vinyl acetate exposure, it is concluded that the cellular integrity is not disrupted.

Table 4.1.2.8.K: Critical steps in vinyl acetate tumor formation in the respiratory tract:

<table>
<tr><td colspan="3">VA Absorption
⇩</td></tr>
<tr><td colspan="3">Metabolism of vinyl acetate to acetaldehyde and acetic acid in:</td></tr>
<tr><td>Olfactory epithelium
Sustentacular cells + neuronal (sensory) cells:
Carboxylesterase activity +++
ALDH activity -
⇩</td><td>Olfactory epithelium
Basal cells + Bowman’s glands:
Carboxylesterase activity +++
ALDH activity (+)
⇩</td><td>Respiratory epithelium
Respiratory epithelium cells:
Carboxylesterase activity +
ALDH activity +++
⇩</td></tr>
<tr><td>High acetaldehyde production
No detoxification of acetaldehyde
Intracellular accumulation of acetaldehyde
Cytotoxicity at 200 ppm
⇩</td><td>High acetaldehyde production
Very limited detoxification of acetaldehyde
Intracellular accumulation of acetaldehyde
⇩</td><td>Limited acetaldehyde production
A. Up to a threshold concentration: Efficient detoxification though high ALDH capacity
No toxic effect up to 200 ppm
B. Above threshold concentration: Imbalance of acetaldehyde production an detoxification
Intracellular accumulation of acetaldehyde
⇩</td></tr>
<tr><td colspan="2">Secondarily to cytotoxicity of the primary target cells:
Restorative cell proliferation of surviving basal cells
Replacement by respiratory-like epithelium
and squamous cell metaplasia
⇩</td><td>Cell proliferation due to direct mitogenic effec
⇩</td></tr>
<tr><td colspan="2">Genotoxic effects of acetaldehyde on proliferating cells (inclusive tumor relevant genes)
Tumor initiation and development
Tumor response: LOAEC 600 ppm
Cytotoxicity: LOAEC 200 ppm/NOAEC 50 ppm</td><td>Genotoxic effect of acetaldehyde on proliferating cells (inclusive tumor relevant genes)
Tumor initiation and development
Tumor response:
LOAEC 600 ppm/NOAEC 200 ppm</td></tr>
</table>

II. Gastrointestinal tract tumors

IIa. Postulated mode of action

From the recently reported carcinogenicity studies in rats and mice (Umeda et al., 2004a, Maltoni et al., 1997, Minardi et al., 2002) which at the first time gave indication that vinyl acetate is also carcinogenic after oral administration, it is assumed that carcinogenicity of vinyl acetate is a direct effect on the surface epithelium of the oral cavity, larynx, esophagus, and forestomach exposed. Temporarily, it was assumed that vinyl acetate metabolism in these tissues leads to cytotoxicity accompanied by reparative hyperplasia. The continuous reparative stimulus in the presence of high concentrations of acetic acid and acetaldehyde then leads to mutation, cellular transformation, and tumor development.

Meanwhile increased cell proliferation due to mitogenic response to the long-term uptake of vinyl acetate was discussed to lead to tumor formation instead of apparently absent cytotoxicity-related mode of action (Valentine et al., 2002). Similar to the nasal regions, it was thought that in the presence of excess, non-physiological levels of acetaldehyde and low pH inducing mutations in genetic material will result in tumor formation.

IIb. Key events associated with the hypothesis

Hydrolytic esterase activities, mainly carboxylesterases, were also found in the mucosal epithelium of the oral cavity (Yamahara and Lee, 1993). Different mucosal regions of the oral cavity possess vinyl acetate hydrolysis activity in the concentration range of 0.05-10 mM vinyl acetate. The enzyme activity V_{max} was 90 and 6 nmol/min in rat and mouse oral mucosal tissue, respectively. The K_m for vinyl acetate hydrolysis were 0.5 and 0.9 mM, respectively, and were at about 100fold below the concentrations of vinyl acetate that were administered orally suggesting that saturating conditions would exist**.** The total activity was similar in pooled homogenates from F344 rats and BDF mice. About 2% of total activity were found in the oral rinse fluid; the saliva and/or oral bacteria are possible sources of esterase activity. In both species the dorsal interior region contained more activity than other regions (Morris et al., 2002). No indication on cytotoxicity was observed in the oral rinse fluid assessed for leakage of acid phosphatases and Evans blue dye after intravenous administration. The concentration of acid phosphatase was not significantly altered. Evans blue dye was significantly increased at 10 mM (intended to correspond to the dosage of 24000 ppm in the 90-day study, DuPont, 2000). However, Evans blue extravasation is not specific, since sensory nerve activation and/or increased salivation could also interfere with plasma extravasation.

The distribution and immune-reactivity of carboxylesterases in the oral mucosa have been characterised by Robinson et al. (2002). In general, the staining was most intensive in the stratum corneum, less prominent in the stratum spinosum and (nearly) absent in the basal cell layer. The staining was markedly variable among different sites within the oral cavity (range from slight to intensive). High staining intensity was found in the buccal mucosa and the upper palate, which is in line with the high activity reported for the dorsal interior (hard palate) region by Morrris et al. (2002). Overall the carboxylesterase activity is significantly lower in the oral mucosa than in the olfactory epithelium (about 100fold).

Furthermore, aldehyde dehydrogenase activity is present in the upper gastrointestinal tract. Enzyme activities were detected in human mucosa of the gingiva, tongue, oral cavity and stomach (Dong et al., 1996, Hedberg et al., 2000, Yin et al., 1997). The aldehyde

dehydrogenase activities at 20 mM acetaldehyde were determined to be 169 ±19 and 50.3 ±8.1 nmol/min/g tissue for the gingiva and tongue, respectively (Dong et al., 1996) It is also assumed that rats and mice possess aldehyde dehydrogenase activity in the oral cavity. For rats, enzyme activity was demonstrated for the stomach, liver, small intestine, large intestine, and rectum (Koivisto and Salaspuro (2003), Pronko et al., 2002, Green et al., 2002), but so far mouse data on aldehyde dehydrogenase were only available for the forestomach and stomach (Green et al., 2002). Determination of enzyme activity using 5 mM acetaldehyde revealed lower activity than in the respiratory tract, at least for the rat (Pronko et al., 2002).

Data on in situ distribution among the different regions and cell layers and data on the intracellular localisation of aldehyde dehydrogenase in the upper digestive tract are yet not available. No information about the enzymatic activity is available for the oral cavity and esophagus.

The potential of vinyl acetate for reducing the intracellular pH was confirmed in a slice preparation of buccal mucosal cells from the mouse (Nakamoto et al., 2005). The pH dropped 0.35 pH units during a 2.5 min exposure time to a concentration of 1 mM vinyl acetate. While no response was seen at 0.1 mM vinyl acetate, dose-dependent reduction in intracellular pH occurred at concentrations from 0.3-1 mM (ΔpH ca. 0.1 at 0.3 mM). Interestingly, the extent of acidification was greater for isolated single cells of buccal mucosa than for intact epithelium.

In line with the foregoing examinations, none of the oral cancer studies reported that cytotoxicity of the surface epithelia of the gastrointestinal tract nor any other organ lesion occurred in rats and mice treated with vinyl acetate via drinking water. Measurement of cell proliferation in 92-day drinking water studies demonstrated significantly increased proliferation rates of oral mucosa cells in rats and mice without any histopathological lesions of the surface epithelia (DuPont, 2000, Valentine et al., 2002).

IIc. Dose-response relationship

There are no data on dose response of effects after oral administration suggestive to be a critical *cytotoxic* step in the tumor development.

Lesions which accompanied the vinyl acetate-induced tumors in mice receiving vinyl acetate by the drinking water for 2 years were basal cell activation, epithelial dysplasia and squamous cell hyperplasia of the gastrointestinal tract epithelia (Umeda et al., 2004a). Because of their nature it can be supposed that they represent preneoplastic alterations in the sequential development to squamous cell papillomas and carcinomas. At some target localisations, these lesions were also evident at concentration below tumor-inducing concentrations of 10000 ppm (see Table 4.1.2.8.C). The lowest dose at which squamous cell hyperplasia of the stomach occurred was 400 ppm in the mouse study of Umeda et al. (2004a). The earliest changes in the oral cavity were basal cell activation and squamous cell hyperplasia at 2000 ppm. Mice, which received vinyl acetate via drinking, water for 78 weeks (Maltoni et al., 1997) showed tumors and dysplastic lesions of the gastrointestinal tract at 5000 ppm (Table 4.1.2.8.D). Relatively low, but in comparison to the controls elevated incidences of tumors or dysplasias were found in some test groups at 1000 ppm but data were inconsistent between groups of breeders and offspring. For these groups, it can be argued that the treatment duration of 78 weeks was too short and that the treatment-free period until the end of the study allowed at least in part recovery from dysplasia and hyperplasia. A clear increase in

dysplastic changes was seen at the end of life of rats treated for 104 weeks with drinking water containing ≥1000 ppm vinyl acetate (Minardi et al., 2002).

There seems to be some evidence that a dose-relationship of hyperplastic and dysplastic lesions with the tumor growth which started at 2000 ppm in the oral mouse cancer bioassay. In the rat, preneoplastic lesions were seen at the same dose which induced a marked increase of tumors in the oral cavity (10000 ppm, Umeda et al., 2004a; Table 4.1.2.8.A), but no preneoplasias were found at lower dosages. Single squamous cell carcinomas of the oral cavity occurred in female rats at the lower doses of 400 ppm and 2000 ppm without being accompanied by any other change. Although the incidences of this single tumor are not significantly different from control group, the tumors were of the same nature as those seen at 10000 ppm and could therefore not excluded to be treatment-related. With a conservative view, the no-effect level for tumor response was 2000 ppm in mice and no such effect-level was derived for the rat, where the lowest concentration tested was the lowest-observed effect level of 400 ppm.

Cell proliferative hyperactivity was a clearly dose-related effect occurring only at high concentrations. 2- to 3-fold increases in cell proliferation rates were found in mice after 92 days of exposure to 10000 or 24000 ppm vinyl acetate compared to control mice (Valentine et al., 2002). In rats, cell proliferation was also significantly increased at 24000 ppm. Increase at this concentration was lower than in mice (less than 2-fold higher than in control rats) which may be attributed to lower doses per g bodyweight in rats (1400 mg/kg bw/d) than in mice (2300 or 5300 mg/kg bw/d).

Increases in proliferation rates were higher in mandibular than in maxillar regions and were interpreted to parallel the tumor outcome (Umeda et al., 2004a). Also, the distribution pattern might be reflective for the normal proliferative activity which was shown to be highest in the floor of mouth and ventral tongue of samples from healthy humans (Thomson et al., 1999). The incidence of oral cavity neoplastic lesions was reported to be higher in the lower jaw than the upper jaw either in rats and mice and the incidence of these oral cavity lesions was about 3-fold in mice compared to rats. Although hydrolytic activity was 2-15-fold higher in homogenated mucosa from the dorsal interior region than in other regions (Morris et al., 2002), the prevalence of tumors in the lower jaw can be explained by gravity-related exposure of the lower jaw regions during feed consumption. In addition, the metabolic activity of homogenated tissues may not correctly reflect the in situ activity.

The effective tumor doses of vinyl acetate doses appeared high for the oral route compared to the tumor effective concentration of the inhalation route. This may be discussed as related to relatively low vinyl acetate hydrolytic activity in the oral cavity, which was 100-fold less than those in nasal tissue homogenates.

The highest concentrations/dosages chosen in the studies available were considered not to exceed the maximal tolerated dose (MTD) (cf. Appendix Vinyl Acetate_MTD_0209). Unspecific signs of toxicity such as reduction of body weight gain were attributable to lower water consumption (where measured) and/or feed consumption (secondarily to the low water uptake?), as no indications on target organ toxicity or other signs of systemic toxicity were identified.

IId. Temporal relationship

As no underlying toxic event was evident in oral studies, and none of the cancer studies contained interim sacrifice groups, no data are available to give information on the temporality of sequential steps for tumor development based on cytotoxicity as the initial step. The appearance of mitogenic effects after 92-days of drinking water is assumed to be a precursor event in tumor development (DuPont, 2000). The relatively small increase of proliferative index was interpreted as well corresponding to the long latency period of tumor development.

IIe. Strength, consistency, specificity of association

In contrast to the mode of action postulated, the evidence of basal cell activation observed at several sites of the gastrointestinal tract and increase in cell proliferation in oral cavity mucosa gave arguments for that the development of tumors was preceded by increased proliferative activity of basal epithelial cells without any cell death preceding. The normal differentiation and maturing of cells was replaced by dysplastic and metaplastic changes representing intermediate stages of tumor development. This observation and the absence of preceding cytotoxicity does not support the postulated a cytotoxicity-related mode of action.

IIf. Biological plausibility and coherence

Hyperproliferation may be associated to inflammation and cell death of superficial epithelial cells in spontaneous human diseases or in rodents exposed to xenobiotica. This general assumption is insufficient to proof that vinyl acetate carcinogenesis needs such toxic precursor stages.

At 10000 ppm vinyl acetate, higher tumor incidences in the oral cavity in mice than in rats parallels the higher cell proliferative activity in mice (Valentine et al., 2002).

IIg. Inconsistency, uncertainties and data gaps

Overall, the database for the oral route is less than for the inhalation route.

The oral carcinogenicity studies did not give any hint for cytotoxicity as a first step of tumor development. Additionally, none of the short and medium term oral studies in rats neither the 90-day drinking water studies in rats and mice demonstrated irritative/toxic effects on the gastrointestinal tract epithelium.

The test substance administered in positive oral cancer studies contained impurities, which were suspected to contribute to carcinogenesis. The test substance of Japanese oral cancer study contains 5 ppm hydroquinone, a metabolite of benzene, which was shown to increase tumor rates in the kidney of male F344 rats, the incidences of mononuclear cell leukemia in female F344 rats, and of hepatocellular tumors in female B6C3F1 mice (Kari et al., 1992). The test substance of the Maltoni study (Maltoni et al., 1997) contained benzene (30-45 ppm) methyl and ethyl acetate (50 ppm) crotonaldehyde (6-16 ppm), acetaldehyde (2-11 ppm). acetone (330-500 ppm). At least the tumors of the Zymbal glands observed in the Maltoni study may be discussed to be associated to the benzene impurity (Maltoni et al., 1989, NTP, 1986, Huff et al., 1989). However, the concentrations of impurities were extremely low to support this assumption.

The higher tumor incidences in mice than in rats, both exposed to a concentration of 10000 ppm, were neglected if the intake of vinyl acetate per kg bodyweight was calculated (Valentine et al., 2002). Although tumor rates should be expected to mirror the vinyl acetate concentration, an explanation for the species differences could not yet be given (e.g., due to lack of data on aldehyde dehydrogenase activity).

Nakamoto et al. (2005) stressed the importance of intracellular acidification for the induction of the mitogenic response conferring the vinyl acetate-related cell proliferation to those observed in the gastrointestinal reflux disease (Barrett's oesophagus). Whether the excess drop of pH seen in Barrett's disease could be transferred to vinyl acetate exposure remains speculative due to the little reduction in intracellular pH.

IIh. Actual considerations on the assessment of mode of action for tumors of the gastrointestinal tract

Based on the actual information, the assumption of cytotoxicity-mediated carcinogenicity of vinyl acetate was considered not to be plausible for the gastrointestinal tract. Up to now, the link between observed cytotoxicity and tumors in the gastrointestinal tract of animal studies supporting the postulated mode of action is missing. Therefore, the contribution of other mode of actions has to be considered. Although the overall database is much less than for the inhalation route there are similarities among the tumor sites.

Acetaldehyde is the critical metabolite with a carcinogenic potential.

Although carboxylesterase activity is low in the epithelium lining of the gastrointestinal tract, acetaldehyde is thought to be significant for tumor development. The high test concentrations needed to induce tumor response are thought to be reflective of the low hydrolytic activity.

A thresholded mode of action is assumed to be active in the tumor development in the gastrointestinal tract.

- Vinyl acetate is hydrolysed by the mucosal carboxylesterase to acetic acid and acetaldehyde.

- Due to the fact that cytotoxicity could be ruled out as a precedent lesion and considering that the metabolism is in principle comparable to the nasal regions the pathways of tumor development in the upper gastrointestinal tract is expected to be comparable to the non-olfactory (respiratory) regions.

- Acetaldehyde is oxidised by aldehyde dehydrogenase. The enzyme activity will be high enough for the oxidation of the physiological concentrations of acetaldehyde. As significant increases in tumor response were seen at rather high concentrations of vinyl acetate (10000 ppm for rat and mouse (Umeda et al., 2004a), ≥5000 ppm for mice (Maltoni et al., 1997, 1000 ppm for the rat (Minardi et al., 2002)) enzyme activity might cope for the low concentrations of produced acetaldehyde without any harmful effect on cell viability and integrity. However, it is unlikely that there is enough activity for

effective acetaldehyde oxidation at higher concentrations of vinyl acetate; and acetaldehyde may accumulate.

- Accumulated acetaldehyde produces genotoxic effects.

- High concentrations of vinyl acetate are mitogenic. A dose-related increase of cell proliferative activity increased dose-related above 10000 ppm in mice (≥2300 mg/kg bw/d), no effect was seen at 5000 ppm. In rats, a less than 2-fold increase in proliferative activity was observed at 24000 ppm in rats (1400 mg/kg bw/d).

- Cytotoxicity was not obvious up to tumor-inducing concentrations and therefore could be ruled out as a precedent change in the tumor development. The cleavage product acetic acid is also discussed to induce intracellular acidification. A reduction of intracellular pH was also seen for buccal mucosa. As for the nasal mucosa the extent of pH-reduction is low, which raises the question on the significance of this low range shift in intracellular pH.

- The exact concentration of vinyl acetate where production of acetaldehyde and its removal starts to be imbalanced could not be estimated by data available. It is therefore proposed to select the most sensitive biological effect with putative toxicological relevance as a starting point to estimate a threshold. Since no clear NOAEL could be determined from the carcinogenicity studies, the vinyl acetate concentration (400 ppm) inducing the single tumors in the rat oral cavity were chosen as the LOAEL.

Conclusion:

In animals, long-term inhalation and oral administration of vinyl acetate produce tumors at the primary site of exposure, the surface epithelium of the respiratory tract and of the upper gastrointestinal tract. There was no indication on systemic toxicity on any other organ or tissue outside the administration routes. Tumors observed in other organs following oral uptake could not clearly be attributed to vinyl acetate administration.

Experience from human exposure did not give indication that vinyl acetate exposure was associated to increased tumor rates.

Based on animal data a clear tumor response to vinyl acetate exposure was seen at 600 ppm (rat, Bogdanffy et al., 1994b) for the inhalation route; for the oral route a marked increase of tumor rates has been observed at 1000 ppm (rat (offspring); 70 mg/kg bw/d, Minardi et al., 2002), at 5000 ppm (mouse; 750 mg/kg/day, Maltoni et al., 1997) and at 10000 ppm (male rat 442 mg/kg bw/d, female rat 575 mg/kg bw/d, Umeda et al., 2004a). Due to limitations in the study design in the studies of Maltoni et al. and Minardi et al., the study of Umeda is considered of higher predictivity. For lower doses, there is concern that occasional findings of tumors of the same types that have been observed at high doses and which might also be related to vinyl acetate exposure. Thus, the lowest concentration suspected to be carcinogenic was 200 ppm for the inhalation route (LOAEC) and 400 ppm for the oral route (LOAEL, male rat 21 mg/kg bw/d, female rat 31 mg/kg bw/d, Umeda et al., 2004a). Clearly tumor free dosages were not established in carcinogenicity studies for the oral route, since 400 ppm were the lowest concentrations tested. Subchronic inhalation studies did not reveal any indication on proliferative response at concentrations of 50 ppm, and this concentration was verified in

carcinogenicity studies. Therefore the concentration of 50 ppm is considered as NOAEC for risk characterisation on carcinogenicity via inhalation. For the oral route, no NOAEL was estimated, 400 ppm (21 mg/kg bw/d male rat, 31 mg/kg bw/d female rat) is proposed as LOAEL for the risk characterisation.

Table 4.1.2.8.L: Effective concentrations for toxic and proliferative responses to vinyl acetate at tumor sites

Target tissues	**Upper respiratory tract**		**Upper digestive tract**
Effect	Olfactory region	Other non-olfactory regions	
Cytotoxicity			
Epithelial degeneration/necrosis /atrophy	Rat ≥200 ppm* Mouse ≥200 ppm*	Rat 1000 ppm **	No indication of cytotoxicity up to 24000 ppm***
No-adverse-effect concentration for toxic effects	Rat ≥50 ppm* Mouse ≥50 ppm*	Rat 600 ppm* Mouse 600 ppm*	Rat 24000 ppm# Mouse 24000 ppm#
Preneoplasias and Neoplasias			
Hyperplasias Metaplasias	Rat ≥200 ppm* Mouse ≥200 ppm*	Rat ≥1000 ppm** Mouse ≥600 ppm*	Mouse ≥2000 ppm##
Weak or questionable tumor response	Rat ≥200 ppm*		Rat ≥400 ppm## Mouse **2000 ppm**##
Clear tumor response	Rat ≥**600 ppm***	Rat ≥**600 ppm***	Rat **10000 ppm**## Mouse ≥**5000 ppm**###
Concentration without tumor response	Rat **50 ppm*** Mouse 600 ppm*	Rat 200 ppm* Mouse 600 ppm*	Rat LOAEC 400 ppm Mouse 2000 ppm###

*Bogdanffy et al., 1994b **Bogdanffy et al., 1997 #DuPont, 2000 ## Umeda et al., 2004a###Maltoni et al., 1997

In conclusion, vinyl acetate exposure produced tumors at the site of first contact along the exposure routes. A thresholded mode of carcinogenic action is thought to be active. The observed tumor responses are reflecting the target site-specific enzyme activities:

Following inhalation and oral exposure vinyl acetate is rapidly hydrolysed by carboxylesterases leading to the formation of acetic acid and acetaldehyde which is further converted into acetic acid in the presence of aldehyde dehydrogenases. Intracellular aldehyde

dehydrogenase activity is limited, at higher concentrations of vinyl acetate it will not be sufficient for the oxidation of generated acetaldehyde. Thus, at high vinyl acetate concentrations non-physiologically high concentrations of acetaldehyde are produced. Acetaldehyde is a physiological intermediate with low background concentrations. Its adverse effects (genotoxicity and mutagenicity) are limited to non-physiologically high concentrations. Therefore, a threshold mode of action is assumed for vinyl acetate.

Above threshold concentrations, cytotoxicity (only at the olfactory mucosa), mitogenic actions and genotoxic actions occurred.

Cytotoxicity mainly contributed by acetic acid is the earliest lesion in the olfactory mucosa. Next stages in the continuum to tumor development include the responsive restorative cell proliferation and simultaneously occurring genotoxic effects of acetaldehyde.

Increased cell proliferative activity was observed at high concentrations of acetaldehyde or vinyl acetate. Its occurrence was not linked to cell toxicity as a precondition.

Data on vinyl acetate are in line with the idea that vinyl acetate genotoxicity is mediated by acetaldehyde. Increasing concentrations of acetaldehyde produce genotoxic actions at the site of contact. It has to be taken into consideration that acetaldehyde occurs naturally in mammalians cells and is part of the physiological cellular metabolism.

The systemic bioavailability of vinyl acetate or its metabolite is low (cf. 4.1.2.1). In vivo genotoxicity tests showed that systemic genotoxicity appears to be limited to toxic doses. This is in line with the absence of systemic carcinogenic effects.

The threshold concentration leading to acetaldehyde accumulation could not yet be estimated. Using in vitro test systems as a surrogate for a site of contact model, in vitro data for several genotoxic endpoints are suggesting for a threshold concentration above which acetaldehyde exerts its genotoxic action. A NOAEC of 0.1 mmol/l for chromosomal aberrations and 0.03 mmol/l for SCE was determined. Since for the in vivo situation no biomarker for the limitation of acetaldehyde oxidation is available, it is proposed to use the identified NOAEC, respectively the LOAEL from the most sensitive biological effects as a surrogate to derive a threshold concentration for risk characterisation purposes.

Overall, it is considered that the critical events in vinyl acetate carcinogenesis do fit to the criteria for the exceptional cases where genotoxic action is thought to be thresholded[12].

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[12] Technical Guidance Document on Risk Assessment, Human Health Risk Characterisation, revised chapter, 4.13.3

There are two general cases where mutagenicity may be shown to have a threshold: (1)… does not apply here.(2) where the toxico-kinetic considerations clearly demonstrate that mutagenic metabolites will only be produced in vivo at very high exposures to the parent substance which are unlikely to be achieved in realistic human exposure scenarios. For example, where the active metabolite is only produced by a metabolic pathway that occurs when other preferential pathways are saturated, or where there is very rapid removal of the active metabolite by conjugation or detoxification, such that no biological significant amounts reach the DNA in vivo, except when these pathways are overwhelmed.

From animal data it is concluded that vinyl acetate might pose a cancer risk for humans exposed to the substance via the inhalation or oral route. Carcinogenicity is thought to act via a secondary mechanism and the concern may be relevant above threshold concentrations.

The observed effects are thought to be relevant for the human. For the respiratory tract humans may be less sensitive than the rat due to a lower carboxylesterase activity in the nasal mucosa.

Classification

According to the EU criteria for classification vinyl acetate is proposed to be classified as a Carcinogen, Category 3 and labelled as Harmful, Xn, R 40.

Justification:

- There is no adequate database on humans.
- Relevant cancer studies for the classification proposal were Owen (1988), Bogdanffy et al. (1994b), Umeda et al., (2004a), Minardi et al. (2002) and Maltoni et al. (1997). Test concentrations in these studies did no exceed the MTD (cf. Appendix Vinyl Acetate_MTD_0209).
- Vinyl acetate was carcinogenic in two animal species each at both sexes.
- Carcinogenic potential was demonstrated for two administration routes: inhalation and oral.
- Vinyl acetate was carcinogenic at the site of first contact, the surface epithelium along the exposure routes.
- Spontaneous rates of nasal tumors and epithelial tumors from the upper and lower airways and from the upper gastrointestinal tract in the test species used are known to be very low.
- All target organs of tumor development were considered to be relevant for humans.
- No species-specific mode of action for vinyl acetate carcinogenesis was identified.
- The carcinogenic effect of vinyl acetate is thought to be related to genotoxic activity of the metabolite acetaldehyde. Comparable tumor findings and genotoxicity data from acetaldehyde support this assumption.
- A threshold mechanism is thought to be active. Tumor development is reflecting the target site-specific enzyme activities involved in the hydrolysis of vinyl acetate and the metabolism of acetaldehyde. At higher concentrations the enzyme activities will not be high enough for the oxidation of generated acetaldehyde. Then acetaldehyde accumulates intracellularly and is causing increased cell proliferative activity, increased DNA adduct formation and DNA damage (clastogenicity). Cell proliferative activity of acetaldehyde and DNA adduct formation are only active above certain (threshold) concentrations.

- As for acetaldehyde, mitogenic action was seen at high local concentrations of vinyl acetate. 10000 ppm or higher concentrations of vinyl acetate produced proliferative hyperactivity in mice whereas no such effect was observed at concentrations up to 5000 ppm. The exact mechanisms how the mitogenic response is initiated are unknown.

- Cytotoxicity was assumed as one contributing mode of carcinogenesis in the olfactory mucosa diminishing the resistance of the olfactory mucosa due to its site-specific high carboxylesterase activity and the low aldehyde dehydrogenase activity. The assumption was thought to be supported by consistent dose-response and time-response relationships between cytotoxic effect and tumor growth.

- In the olfactory epithelium a multistage hypothesis of carcinogenesis is likely to be based to initial cytotoxicity, responsive cell proliferation and associated with genotoxic action of its metabolite acetaldehyde.

- For the other tumor sites, the non-olfactory (respiratory) epithelium as well for the mucosa of the upper gastrointestinal tract, concentration-dependent increased cell proliferation coupled with the genotoxic action of acetaldehyde at high concentrations of acetaldehyde were assumed to result in tumor development.

- For the inhalation route, cytotoxicity in the olfactory mucosa is thought to be the most sensitive effect related to the tumor response and is therefore taken for quantitative risk assessment. Cytotoxicity and related reparative cell proliferation are assumed to be early events in the tumor development in this region. A NOAEC (50 ppm) established for the cytotoxic effects in the olfactory mucosa is proposed to be used as a threshold concentration. For the oral route, the lowest tumor dose (400 ppm corresponding to 21 mg/kg bw/d) is proposed as basis to calculate a threshold concentration.

In September 2007 the TC C&L agreed on Carc. Cat. 3; R40.

4.1.2.9 Toxicity for reproduction

Animal data:

Fertility impairment

Groups of male and female Crl:CD(SD) rats were administered vinyl acetate in drinking water at nominal concentrations of 0 (control), 200, 1000 or 5000 ppm v/v (equivalent to about 20, 100, or 500 mg/kg bw/d, calculated on an assumed water consumption of 10% of body weight) over two generations. The test solutions were freshly prepared daily and over-formulated by 5% to correct for vinyl acetate losses over 24 hours. F_0 rats (18 males and 36 females per dose group) received vinyl acetate for 70 days prior to mating and the treatment continued throughout gestation and lactation. From the first litters groups of 25 male and 25 female pups (F_1) were selected. They continued to receive vinyl acetate for 70 days after weaning and were then allowed to mate within their groups to produce the second generation (F2). The study was terminated following weaning of the F2 litters.

The reproductive performance of the F0 and F1 animals was evaluated from indices which included litter number, viability and size. Weights of the F1 and F2 pups were recorded from birth to weaning (on lactation days 1, 7, 21) as were developmental and functional parameters (on lactation day 21). Post-mortem examinations were carried out on the F0 and F1 parental animals and on 10 male respectively female pups from each group of the F1 and F2 litters. Neither clinical abnormalities nor treatment-related mortality was observed in any of the groups. Water consumption was significantly reduced in the 5000 ppm groups in the F0 and F1 generations and in the 1000 ppm females. This was associated with significantly reduced body weight gain during lactation in the F0 females at 5000 ppm and in the F1 females at 5000 and 1000 ppm. Pup weights in the F1 generation, but not in the F2 generation were statistically significantly lower at 5000 ppm than those of controls when evaluated on lactation day 21. No effects were observed in the reproductive performance of the F0 animals, but the mating of the F1 animals resulted in a lower number of pregnancies in the 5000 ppm group (19/24) compared with controls (24/25) leading to a reduced number of litters. The difference was slight and not statistically significant, however the effect was attributed to lower fertility. Therefore an additional cross-mating trial of control and 5000 ppm male and female rats of the F1 generation was performed. Fewer pregnancies occurred when the control females were mated with the 5000 ppm males resulting in a mating index of 76% and a fertility index of 100% indicating that the lower numbers in pregnancies were rather attributable to poor mating than to impaired fertility. No decrease was apparent when the 5000 ppm females were mated with the control males. All other observations, including those made at necropsy and subsequent histopathological examinations showed no adverse effects due to vinyl acetate. There were no compound-related testicular changes evident in this study.

In summary, no effects were seen on reproductive performance, or on the resulting litters of male and female rats exposed to 200 or 1000 ppm vinyl acetate in drinking water. At 5000 ppm a lower number of females became pregnant, although cross-mating studies suggested that the effect, which was marginal and not statistically significant, was associated with male reproductive performance. Effects on litters of animals exposed to vinyl acetate were confined to the 5000 ppm group of the F_1 pups only, were a significant reduction in weight gain was observed on lactation day 21. No such effect was observed for the F_2 pups. The lower weight gain at weaning of the F_1 pups was associated with significantly reduced water consumption and body weight gain of their mothers during the lactation period. Under the conditions of the study, the NOAEL was considered to be 1000 ppm (Mebus et al., 1995).

In hybrid (C57Bl/6JxC3H/He) F_1 male mice, intraperitoneal injections of 125, 250, 500, 750, or 1000 mg/kg bw (vinyl acetate dissolved in olive oil) on 5 consecutive days resulted in 80 respectively 100% mortality at the 750 and 1000 mg/kg dose level and in a dose-related loss of body weight and a dose-dependently reduced regain in body weight during the injection period at the lower dose levels. From these generally toxic dose levels reduced mean relative testicular weight was observed at the 125 and 500 mg/kg dose levels, however not at the 250 mg/kg dose level, as well as sperm abnormalities at the dose level of 500 mg/kg bw. Epididymal sperm counts at 5 weeks after treatment amounted to 5.60 ± 0.51, 5.58 ± 1.38, 6.17 ± 1.03, and 4.10 ± 1.80 millions/ml for the control, 125, 250, and 500 mg/kg dose groups (Lähdetie, 1988).

Developmental toxicity

Groups of 23 mated female Crl:CD(SD)BR rats were given vinyl acetate in drinking water (Hurtt et al., 1995, Irvine, 1980) from day 6 to day 15 of gestation at nominal concentrations of 0 (control), 200, 1000, or 5000 ppm (about 0, 25, 100, or 500 mg/kg bw/d). The test solutions were freshly prepared daily and over-formulated by between 7 and 10% to correct for vinyl acetate losses over 24 hours. On day 20 of gestation terminal examination of the dams and their uterine contents was conducted.

There were no statistically significant differences between treated and control groups of animals in appearance, behaviour or pregnancy index. Initially, there was a slightly reduced intake in food and water associated with slight retardation of body weight gain in the 5000 ppm group; the statistically significant lowered water intake was assumed to reflect the bad palatability of the vinyl acetate water solution. No changes attributed to treatment were observed in the post mortem examinations of the dams or in the examination of fetal parameters including the total incidence of abnormalities. It was concluded that the administration of vinyl acetate in drinking water at doses up to 5000 ppm v/v, a dose which resulted in slight maternal toxicity, was not embryotoxic or teratogenic in the rat.

Groups of 24 mated female Crl:CD(SD)BR rats were exposed to vinyl acetate vapours (Hurtt et al., 1995) from day 6 to day 15 of gestation at concentrations of 0 (control), 50, 200, or 1000 ppm (equivalent to about 100, 205, and 1025 mg/kg bw/day, based on a respiratory rate of 0.8 l/min/kg for rats) for 6 h/d. At 1000 ppm, severe maternal toxicity (marked reduction in body weight gain, congestion of the lungs) and some signs of fetotoxicity occurred. Fetal toxicity was evident by a statistically significant decrease in mean fetal weight and mean crown-rump length in the fetuses of the 1000 ppm group. In addition, there was a statistically significant increase in the incidence of minor skeletal alterations (mainly delayed ossification) in fetuses from dams exposed to 1000 ppm. In summary, vinyl acetate did adversely affect both the dam and the conceptus at an inhaled concentration of 1000 ppm, but not at lower exposure levels. The NOAEC for the inhalation study was considered to be 200 ppm.

Human data:

No data available.

Summary for toxicity of reproduction

Vinyl acetate has been investigated in rats for adverse effects on reproductive performance and fertility via the oral (drinking water) route of exposure and in mice for adverse effects on male reproductive organs via the i.p. exposure route. Vinyl acetate was shown to reduce testicular weight and to induce sperm abnormalities in mice, however at toxic dose levels inducing mortality and body weight loss. Vinyl acetate was further shown to have marginal influences on reproduction at oral exposure levels that lead to significantly reduced water intake (probably due to palatability) associated with significantly reduced body weight gain. The observed effects comprised reduced pup weights and slightly lower numbers of pregnancies. Distinct reproduction related adverse effects of vinyl acetate were not evidenced from a two generation study with rats for drinking water concentrations of up to and including 1000 ppm.

The developmental toxicity of vinyl acetate has been investigated in rats via oral (drinking water) and inhalation exposure routes during organogenesis. No embryo/fetotoxic or

teratogenic effects were observed for the oral route of administration at drinking water concentrations of up to and including 5000 ppm. Fetotoxic effects, revealed during inhalation exposure, were confined to high dose levels only, where severe maternal toxicity was observed. However, vinyl acetate did not adversely affect both the dam and the conceptus at an inhaled concentration of up to and including 200 ppm.

A NOAEL/fertility of 1000 ppm (equivalent to dosages of about 100 mg/kg bw/day) was derived from a study with oral exposure, and a NOAEC/developmental toxicity of 200 ppm (equivalent to dosages of about 205 mg/kg bw/day) was derived from a study with inhalation exposure.

Comment:

In Germany, vinyl acetate is assigned to the MAK-pregnancy category "D" denoting that the current database is not sufficient for final evaluation of developmental toxicity. However it is outlined that vinyl acetate was evaluated for reproductive toxicity in one species (rat) already with negative results. If this latter outcome could be verified in additional species vinyl acetate could be assigned to category "C" denoting that no risk for adverse developmental effects have to be expected for female workers in compliance with the respective MAK value of 10 ppm.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Following oral and inhalation exposure of rats vinyl acetate is rapidly hydrolysed by carboxylesterases leading to the formation of acetic acid and acetaldehyde the latter of which is further converted into acetic acid by aldehyde dehydrogenases.

The in vivo uptake of vinyl acetate was measured in the isolated upper respiratory tract of rats (anaesthetised rat, unidirectional flow, 1 h-exposure). Disappearance of vinyl acetate from the airstream was highest at the lowest exposure concentrations. Greater than 94% extraction was observed at vinyl acetate exposure concentrations of 76 ppm or below. With increasing exposure concentration (76 to 550 ppm), extraction decreased progressively to about 40% and remained at this level up to concentration of approximately 2000 ppm. The impact on blood-flow extraction on vinyl acetate deposition has been calculated by simulating vinyl acetate exposure in the absence of carboxylesterase activity. It could be demonstrated, that blood flow extraction accounts for less than 15 % of total vinyl acetate deposition. Hence, 15 % inhalative uptake can be taken forward to risk characterisation of systemic effects by inhaltional exposure as a worst case scenario.

After oral administration of 297 mg/kg/bw ^{14}C vinyl acetate, 63 % of the applied radioactivity was excreted as metabolites in exhaled air, urine and faeces. Based on the fact that vinyl acetate can be metabolized in the upper GI tract epithelium it can be assumed, that a considerable extent of metabolism takes place presystemically which is supported by an oral PBPK model. This model led to the conclusion, that clearance of vinyl acetate and its metabolites into the systemic circulation would be negligible. Hence, 63 % absorption would represent an overestimation of systemically available amounts of vinyl acetate. However, the PBPK model developed for oral vinyl acetate exposure did not include systemic components and furthermore, the model was developed in the absence of valid data for carboxylesterases in animal and human tissues. Based on the fact, that carboxylesterase activity in the GI tract is lower compared to that in nasal tissues, 50 % absorption can be assumed as a worst case for systemically available amounts of vinyl acetate after oral uptake. However, no clear assumptions can be made for systemically available metabolites of vinyl acetate (acetaldehyde, acetic acid).

There are no valid quantitative data on the systemic bioavailability of vinyl acetate and its metabolites following dermal exposure. However, based on an acute dermal study in rabbits and based on the fact that carboxylesterase activities are lower in skin compared to nose or oral cavity, it can be assumed that systemic bioavailablility of vinyl acetate and/or vinyl acetate-derived metabolites is higher after dermal exposure when compared to oral or inhalative exposure. Based on the currently available data 90 % dermal absorption should be taken forward to the risk characterisation.

Research on human and rat nasal respiratory and olfactory tissue with whole turbinates in vitro has shown that rat nasal respiratory carboxylesterase activity was about 3-fold higher than those of humans. Rat respiratory aldehyde dehydrogenase activity was about twice that of humans. Activities of the rat olfactory enzymes (carboxylesterase and aldehyde

dehydrogenase) were about equivalent to those of humans. The K_m values for both enzymes are not different between the two species. Aldehyde dehydrogenase activities determined in whole nasal tissue homogenates from mouse, rat, hamster and guinea pig showed significantly different ratios V_{max}/K_m for the various species indicating the existence of species differences.

To provide validation data for the application of a physiologically based pharmakokinetic (PBPK) model in humans, controlled human exposures to vinyl acetate concentrations of 1, 5 and 10 ppm were conducted (5 volunteers). Data from ion trap mass spectrometry measurements of labelled vinyl acetate and labelled acetaldehyde were compared with the predictions of the human nasal model. For the vinyl acetate data a good fit was demonstrated ($r = 0.9$). The quality of the acetaldehyde data fit is less ($r = 0.6$) than that of vinyl acetate. The results show that the human nasal model predicts the experimental observations with regard to vinyl acetate concentrations and the acetaldehyde washout in the airstream of human nasopharyngeal cavity in a concentration range from 1 to 10 ppm. Uncertainties of the model consist in the enzyme kinetic data used to parameterise the model. Nevertheless, according to the sensitivity analysis the modelled data can be taken for risk characterisation.

The results of PBPK modeling are taken forward for consideration on the appropriate adjustment factors. As vinyl acetate is acting locally, a systemic metabolism has not to be considered which reduces the interspecies factor from 10 (default) to 2.5. Remaining uncertanties in toxicodynamic differences are recognized, i. e. humans could be more sensitive than rats towards local toxicity of vinyl acetate metabolites which could not be excluded due to the lack of (primarily human) data. Thus, for risk charaterization an interspecies adjustment factor of 2.5 is established. Concerning intraspecies variability the same consideration as for interspecies extrapolation applies. No systemic metabolism has to be taken into consideration, reducing the intraspecies adjustment factor to 2.5.

Vinyl acetate exhibits low acute toxicity by the oral and dermal routes, but acute inhalation toxicity is to be classified as harmful according to EU criteria. The oral LD50 for rats is found to about 3500 mg/kg body weight. Acute dermal toxicity is reported for rabbits to exceed 7400 mg/kg. However, acute inhalation toxicity is characterised by a LC50 value of 15.8 mg/l/4 hours for rats. In a scarcely documented study an LC50 value of 14.1 mg/l/4 hours is mentioned.

Except a general notice from occupational use no substantiated human data on irritation/corrosion caused by vinyl acetate are available. The pure substance shows mild irritation to skin and eyes. Acute inhalation tests with rats demonstrated severe irritation to the respiratory system (R 37). Vinyl acetate does not show corrosive properties after a single skin contact of not more than 4 hours.

There is no information available on skin sensitization in humans. No cases of sensitization from the handling of vinyl acetate in the workplace have been reported in the last years. There are no reports on allergic contact dermatitis by vinyl acetate from routine patch testing or experimental studies in man. Results from an animal skin sensitization study (Buehler Test) showed a moderate skin sensitizing potential of vinyl acetate (commercial grade). In a Local Lymph Node Assay an increased stimulation index was determined with 5 and 10 % vinyl acetate solutions but not at higher concentrations. Thus, vinyl acetate is not devoid of a skin sensitising potential. However, the phrase R 43 is not warranted.

Direct information on respiratory sensitization from studies in humans is lacking.

The major toxic effects after prolonged inhalation of vinyl acetate in experimental animals were lesions of the surface epithelium of the upper and lower respiratory tract. Degeneration, regenerative/reparative processes, inflammation, hyperplasia and metaplasia were noted in the nasal mucosa. They were most pronounced in the olfactory epithelium occurring at 200 ppm in rats and mice during and at the end of a 2-year exposure period. Lesions of the respiratory epithelium were seen in mice exposed to 600 ppm during and at the end of 2 years, whereas rats demonstrated lesions at this site only at a high concentration of 1000 ppm (4 week study). Characteristic alterations of the larynx and trachea of mice in the 600 ppm groups were hyperplasia and metaplasia along with desquamation and fibrosis in the trachea. Similar changes of the bronchial and bronchiolar airways were reported for rats and mice at this concentration at the end of the 2-year exposure period. In addition, clinical signs of non-specific toxicity and irritation were evident, but no relevant toxic effect on any organ could be identified. From the 2-year studies, the NOAEC for local toxic effect on the respiratory tract was 50 ppm (178.5 mg/m³) in rats and mice. Based on growth retardation in rats of the 600 ppm groups and mice of the 200 ppm groups, the NOAEC for systemic toxicity was considered to be at 50 ppm (mice 178.5 mg/m^3).

No specific organ toxicity was recorded after repeated oral administration of vinyl acetate with drinking water to rats and mice. A subchronic 13-week study revealed a slight (non-significant) reduction of food consumption and growth retardation in male rats at 5000 ppm (684 mg/kg bw/d). As NOAEL for systemic effects the value of 684 mg/kg bw/d (male rat) will be used in the risk characterisation.

Vinyl acetate is negative in bacterial mutagenicity tests.

In mammalian cell cultures various cytogenetic effects were induced in the absence of S-9 mix (chromosomal aberrations, micronuclei, SCE) and in the presence of S-9 mix (SCE; chromosomal aberrations and micronuclei not analysed with S-9 mix). The lowest positive concentrations ranged from 0.1 to 0.2 mmol/l. A positive mouse lymphoma assay is in line with these results, but it cannot be deduced whether the positive effect is due to chromosomal or to gene mutations (no colony sizing). Mammalian cell culture investigations on DNA strand breaks (DSB) and DNA protein corsslinks (DPX) were negative (DSB), or extremely high concentrations were needed for positive effects (DPX).

Very few reliable data are available on the in vivo mutagenicity of vinyl acetate. A weak induction of micronuclei in mouse bone marrow cells was clearly limited to intraperitoneal doses in the LD50 range (1000 and 2000 mg/kg). In rats no induction of micronuclei was observed in spermatids (screening assay with intraperitoneal doses up to 1000 mg/kg). Further tests on induction of micronuclei or chromosomal aberrations were of too low reliability.

Also in an SCE test with rats positive effects were weak and limited to high and probably highly toxic intraperitoneal doses (370 and 470 mg/kg). Such weak increases in SCE frequencies may well be induced by unspecific effects on the cell cycle.

No specific DNA binding was observed in rat livers after inhalation or oral administration.

Induction of sperm abnormalities in mice again was limited to doses in the toxic range. Furthermore, it is not specific for mutagens.

No clear conclusion can be drawn from a human study on the possible induction of chromosomal aberrations in workers exposed to vinyl acetate.

Genotoxicity data on vinyl acetate metabolites are in line with the hypothesis that vinyl acetate genotoxicity is mediated by acetaldehyde. The genotoxicity of acetaldehyde is possibly limited to an overloading of defence mechanisms.

Altogether, vinyl acetate has a mutagenic potential, which is preferentially expressed as clastogenesis. The data on in vivo genotoxicity are difficult to interpret, since their majority is of low reliability, or the effects are not specific to mutagenicity. The most important effect, a weak induction of micronuclei in mouse bone marrow, is limited to intraperitoneal doses of high toxicity. Therefore, it is unlikely that the genotoxic potential of vinyl acetate is expressed in germ cells in man. However, genotoxic effects locally in directly exposed tissues (site of first contact) cannot be excluded; the occurrence and strength of the effects will be dependent on the metabolic capacity of the directly exposed tissue.

No classification of vinyl acetate in terms of germ cell mutagenicity is proposed.

In cancer studies, vinyl acetate inhalation induced an increased number of nasal tumors (mainly papillomas and squamous cell carcinomas) in various regions of the nasal mucosa of rats. The total incidence was significantly increased at a concentration of 600 ppm (2142 mg/m^3) but a single papilloma already developed at 200 ppm. No significant tumor response was seen in a mice cancer bioassay. Occasionally single squamous cell tumors occurred at other sites of the respiratory tract in rats and mice.

Oral cancer studies were also positive. Significantly increased incidences of benign and malignant squamous cell tumors in the oral cavity in rats and mice, in the esophagus and forestomach in mice were observed in 2-year drinking water studies at vinyl acetate concentration of 10000 ppm (dose ranges in rats 364-1062 mg/kg bw/d, in mice 800-2185 mg/kg bw/d). Similar results were found in another non-guideline conform cancer study on two generations of mice treated orally with 5000 ppm vinyl acetate in the drinking water (780 mg/kg bw/d). Higher rates of tumors were noted in the oral cavity, tongue, esophagus, forestomach (squamous cell carcinoma at all sites) as well as in the glandular stomach.

Vinyl acetate is considered as a threshold carcinogen because it is thought that carcinogenic action affects the site of first contact only a high concentrations. Cytotoxicity was assumed as one underlying mode of carcinogenesis in the olfactory mucosa. The assumption was thought to be supported by consistent dose-response and time-response relationships between cytotoxic effect and tumor growth. No cytotoxicity as a prerequisite of tumor development was identified for the other tumor sites, the non-olfactory regions and the upper gastrointestinal tract. However, increases in cell proliferation combined with increased formation of DNA adducts were only seen a high concentrations of vinyl acetate. Both effects are considered to be mediated by acetaldehyde that accumulates intracellularly when the physiological balance of intracellular formation and detoxification is disrupted above a certain, albeit unknown concentration of acetaldehyde. Instead, the most sensitive effects, the cytotoxicity of the olfactory region for the inhalation route (NOAEC 50 ppm), and the tumors of the upper gastrointestinal tract for the oral route (LOAEL 400 ppm), are recommended for risk characterisation.

No adequate data from human experience are available. Based on the carcinogenic potential of vinyl acetate in two animal species and at the inhalative and oral route and the absence of reliable human data the substance might pose a cancer risk for humans. Carcinogenicity is thought to act via a secondary mechanism and the concern may only be relevant above threshold concentrations.

Vinyl acetate was shown to have marginal influences on reproduction in animal studies only at oral exposure levels that produced certain signs of general toxicity. Distinct reproduction related adverse effects of vinyl acetate were not evidenced from a two generation study on rats with drinking water concentrations of up to and including 1000 ppm (NOAEL, equivalent to a dosage of 100 mg/kg bw/d). No embryo-/fetotoxic or teratogenic effects were observed in an oral study on rats for dose levels up to and including 5000 ppm vinyl acetate. A NOAEL/fertility of 1000 ppm (equivalent to about 100 mg/kg bw/d) was derived. Fetotoxic effects, revealed during inhalation exposure to rats, were confined to high dose levels (1000 ppm) only, where severe maternal toxicity was observed. A NOAEC for developmental toxicity of 200 ppm (corresponding to 205 mg/kg bw/d) was established from this study.

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

For occupational risk assessment of vinyl acetate the MOS approach as outlined in the revised TGD is applied. This occupational risk assessment is based upon the toxicological profile of vinyl acetate (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

Systemic availability for different routes of exposure

Measurement at isolated upper respiratory tract of rats of in vivo uptake of vinyl acetate demonstrated, that blood flow extraction accounts for less than 15 % of total vinyl acetate deposition (see chapter 4.1.2.1). Hence, 15 % inhalative uptake is taken as a worst case scenario for risk characterisation of systemic effects.

After oral administration of 297 mg/kg ^{14}C vinyl acetate, 63 % of the applied radioactivity was excreted as metabolites in exhaled air, urine and faeces. However, 63 % absorption would represent an overestimation of systemically available amounts of vinyl acetate (see chapter 4.1.2.1). 50 % absorption can be assumed as a worst case for systemically available amounts of vinyl acetate after oral uptake.

Based on an acute dermal study in rabbits and based on the fact that carboxylesterase activities are lower in skin compared to nose or oral cavity, it can be assumed that systemic bioavailability of vinyl acetate and/or vinyl acetate-derived metabolites is higher after dermal exposure when compared to oral or inhalative exposure. Therefore 90 % dermal absorption is taken forward to the risk characterisation.

Altogether the following absorption percentages are used in the following risk assessment: 15% inhalative uptake for the assessment of systemic effects, 50% absorption after oral uptake of vinyl acetate and 90% absorption after dermal contact.

Occupational exposure and internal body burden

In table 4.1.3.2.A the exposure levels of tables 4.1.1.2.4.A and 4.1.1.2.4.B are summarised and the route-specific and total internal body burdens are identified. Risk assessment for combined exposure requires the calculation of a total internal body burden; to this end the derived route-specific percentages for absorption are used (15% for inhalation and 90% for dermal exposure).

Table 4.1.3.2.A: Occupational exposure levels and internal body burden of vinyl acetate

Exposure scenario		Inhalation shift average	Dermal contact shift average		Internal body burden of workers after repeated exposure		
					Inhalation[(1)]	Dermal[(2)]	Combined
		mg/m^3	mg/p/d	mg/kg/d	mg/kg/d		
1. Production and polymerisation in the chemical industry		3[(3)]	42[(5)]	0.6	0.064	0.54	0.60
2. Manufacturing of formulations and products	a) polymerisation step (vinyl acetate monomer)	14.6[(3)]	420[(6)]	6	0.31	5.4	5.71
	b) formulation step (vinyl acetate (co)polymer)		1.3[6)]	0.02		0.018	0.33
3. Use of formulations and products containing residual vinyl acetate monomer		2.6[(4)]	12.6[(6)]	0.18	0.056	0.16	0.22

[(1)] based on the assumption of 15% inhalative absorption; breathing volume of 10 m^3 per shift
[(2)] based on the assumption of 90% systemic availability of vinyl acetate after dermal contact
[(3)] reasonable worst case
[(4)] worst case
[(5)] EASE (90 % protection by suitable gloves)
[(6)] EASE (without gloves)

MOS approach

The MOS approach for human risk characterisation is described in detail in the TGD (Human Health Risk Characterisation, Final Draft). The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires to convert the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and humans should be accounted for the calculation of the corrected NOAEL. If route-specific information on oral and inhalation absorption is not available, the TGD recommends to assume a 50% oral absorption and a 100% inhalation absorption. For vinyl acetate 15% absorption after inhalation, 50% after oral exposure, and 90% absorption after dermal contact is assumed (see chapter 4.1.2.1).

For occupational risk assessment, the corrected NOAEC for inhalation accounts for the difference of the standard respiratory volume (6.7 m^3) and the respiratory volume for light activity (10 m^3).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are exposure by inhalation and dermal contact.

In addition, for risk assessment of combined exposure (exposure by inhalation and dermal contact) an adequate NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation on the one hand is based on allometric scaling (factor 4 for rats, factor 7 for mice, and factor 2.4 for rabbits). For remaining interspecies differences the TGD proposes an additional factor of 2.5. Interspecies extrapolation conceptually might be considered to consist of a toxicokinetic and toxicodynamic phase. As to vinyl acetate PBPK models essentially describe the relationship between airborne concentration of vinyl acetate and intracellular acetaldehyde concentration. However, uncertainties of the model consist in the enzyme kinetic data used to establish the model. Therefore, data on toxicokinetic and toxicodynamic outcome derived from the model should be taken with caution. For occupational risk characterisation an interspecies factor of 2.5 is proposed (abutted at the default value for the remaining uncertainties of interspecies differences). For workers, an

adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%). In the case of vinyl acetate PBPK results are available (see chapter 4.1.2.1 and 4.1.3.2), which suggest that the toxicodynamic portion of the default intraspecies factor (10 for the whole population, 5 for workers) remains as overall intraspecies factor since the toxicokinetic part (4) is not applied (no systemic metabolism as it is virtually impossible that vinyl acetate will reach the systemic circulation). The remaining uncertainties are covered by application of a reduced adjustment factor for workers in the range of 1.25 and 2.5.

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure. Since chronic studies are available for vinyl acetate, a specific factor for duration adjustment of repeated dose toxicity is not necessary.

The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For vinyl acetate no further adjustment factors are used in the risk assessment.

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called “critical exposure level”. Concern will be expressed for scenarios with occupational exposure levels higher than the relevant “critical exposure level”.

4.1.3.2.2 Occupational risk assessment

Acute toxicity

- Inhalation

LC_{50}–values of 14.1 and 15.8 mg/l (4 h) were determined in rats. No animals died at the lowest tested dose of 7 mg/l (4 h). Gross pathology revealed no remarkable effects, but red and irritated extremities were observed as clinical signs.

Comparing the LC_{50}-value of ca. 15,000 mg/m^3 and the concentration of 7,000 mg/m^3 without lethality with the highest exposure concentration of 15 mg/m^3 (scenario 2, reasonable worst case) and the highest short-term exposure of 47 mg/m^3 (scenario 2) a relevant risk concerning acute toxicity is not expected under normal workplace conditions.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

- Dermal contact

The LD_{50} in rabbits was 7,440 mg/kg (24 h, occlusive). At the lowest tested dose of 3,720 mg/kg no mortalities and no clinical signs were observed, but gross pathology revealed effects in liver, spleen, kidney and the lungs.

Comparing the LD_{50} of 7,440 mg/kg and the dose of 3,720 mg/kg (no mortalities, but effects in different organs) with the highest dermal exposure of 6 mg/kg (scenario 2a, use of vinyl acetate monomer) a relevant risk concerning acute toxicity is not expected under normal workplace conditions.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

- Irritation/Corrosivity

- Dermal/Eyes

A general notice from occupational use describes principally local irritant reactions of the skin, eyes and respiratory tract (information by the vinyl acetate industry, without further details, possibly only historical relevance).

Due to the only valid tests (RCC, 2003a and c) mild irritation on the skin and eyes of rabbits were observed that do not warrant classification. Earlier studies of limited reliability indicated pronounced irritation or corrosion of skin after extended exposure periods.

All data were not substantive enough for a classification with the corresponding R-phrases. No concern for dermal or eye irritation at the workplace is expressed.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Inhalation

According to chapter 4.1.2.3/4.1.2.4 vinyl acetate has proven to cause severe irritation in the respiratory tract of rats. Additional information on acute irritation can be obtained from chapter 4.1.2.6. Sprague-Dawley-rats were exposed for 1, 5 and 20 days in an inhalation study (6 h/day, 5 days/week) to 0, 50, 200, 600, or 1,000 ppm (Bogdanffy et al., 1997). Following the single exposure of one day, rats of the 600 and 1,000 ppm groups showed concentration-related minimal to moderate degeneration, necrosis and exfoliation of the olfactory epithelium. Additionally a minimal degeneration and necrosis in the respiratory epithelium occurred at 1,000 ppm. So the value of 200 ppm (corresponding to 710 mg/m^3) will be used as NOAEC, concerning local effects after short term inhalative exposure.

A MOS calculation would result in a value of about 79 mg/m^3 (inhalation starting point of 476 mg/m³ with the NOAEC of 710 mg/m^3 multiplied by 6.7/10 (activity-driven differences of respiratory volumes in workers) divided by a reference MOS of about 3 – 6 (intraspecies differences factor 1.25 – 2.5 and interspecies factor of 2.5).

Comparing the critical exposure level of 79 mg/m^3 with the highest exposure of 14.6 mg/m^3 (scenario 2) a relevant risk concerning irritation after inhalation is not expected under normal workplace conditions.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.2.B: Local effects after inhalative exposure to vinyl acetate

	Acute exposure,			Repeated exposure,		
Starting point for MOS calculation	476 mg/m^3			90 mg/m^3		
Reference MOS	6			5.1		
Critical exposure level	79 mg/m^3			17.6 mg/m^3		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/p/d)	MOS	Conclusions
1. Production and polymerization in the chemical industry	3	159	ii	3	30	ii
2. Manufacturing of formulations and products	14.6	33	ii	14.6	6	Borderline iii
Short-term exposure	47	10	ii			
3. Use of formulations and products containing residual vinyl acetate monomer	2.6	183	ii	2.6	35	ii

Sensitization

Dermal contact

No cases of skin sensitization from the handling of vinyl acetate in the workplace have been reported in the last years.

Results from an animal skin sensitization study (Buehler Test) showed a moderate skin sensitising potential of vinyl acetate. In a Local Lymph Node Assay (LLNA) no positive stimulation responses were seen at concentrations of 5% - 100%. However, the results obtained with this LLNA may not fully reflect the potential of concentrations >10%, since higher concentrations of vinyl acetate show increasing volatility, due to decreased proportions of acetone/olive oil. Since the positive threshold level was not exceeded in the LLNA, classification and labelling with R 43 is not warranted. No concern is expressed.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Inhalation

No information on respiratory sensitisation is available. However, in view of the fact that during all the years of use specific case reports have not been reported, vinyl acetate seems at least not to be a strong respiratory sensitizer in humans. For the time being no animal model is available which would be able to verify the question of respiratory sensitisation. In summary concern is not expressed.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Repeated dose toxicity

Local effects

Inhalation

In a combined chronic toxicity and carcinogenic study (Owen, 1988; Bogdanffy et al., 1994b) male and female Sprague Dawley rats and CD-1 mice were exposed by inhalation to vinyl acetate vapour in concentrations of 0, 50, 200, and 600 ppm (equivalent to 0, 178.5, 714, 2,142 mg/m^3) over a period of 2 years (6 hours/day, 5 days/week). Examinations on haematology, clinical chemistry, urinalysis and gross and microscopic abnormalities were performed on satellite groups at week 51 and 81 and on the end of the main study. In both species of each sex, vinyl acetate induced morphological nonneoplastic lesions in the nasal cavity of the 200 and 600 ppm groups and in the trachea (mice only) and in the lungs of the 600 ppm groups. Based on these findings the NOAEC for local effects on the respiratory tract was 50 ppm (180 mg/m^3). This NOAEC is taken as starting point for the risk assessment of local effects after repeated exposure.

The experimental NOAEC of 180 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 90 mg/m^3 (180 • 6/8 • 6.7/10)

The reference MOS accounts with the intraspecies differences with a factor of 1.25 – 2.5 (see chapter 4.1.3.2.1 reference MOS). The interspecies differences deal with a factor of 2.5 (the factor for allometric scaling is already implicitly applied). Duration adjustment is not necessary, because a long term study is available.

This would result in a reference MOS of about 3 – 6 (5 • 1.25 – 2.5) and a critical exposure level of 15 to 30 mg/m^3 depending on how big the factor for intraspecies differences is dealt with. Comparing this range for a critical exposure level of vinyl acetate with the OEL of 17.6 mg/m^3 (5 ppm) which was set by the Scientific Committee on Occupational Exposure Limits, the value is very close at the lower value of 15 mg/m^3. For pragmatic reasons the occupational exposure value of 17.6 mg/m^3 (5 ppm), which was set by SCOEL (2005) is taken forward to this risk assessment. The SCOEL value corresponds to a reference MOS value of 5.1.

The highest shift average value for inhalation is reported as 14.6 mg/m^3 for manufacturing of formulations and products of vinyl acetate in scenario 2. Compared with the OEL of 17.6 mg/m^3 the scenario reaches borderline. Conclusion iii is drawn for scenario 2 since there is the uncertainty concerning the reduction of the intraspecies factor. The other scenarios do not reach concern (for corresponding MOS values see table 4.1.3.2.B).

Conclusion iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Dermal contact

Dermal studies with repeated application are not available. Besides the tests described in chapter 4.1.2.4 only a general notice from occupational use describes principally local irritant reactions of the skin, eyes and respiratory tract.

The data demand no classification and are not substantiated enough for raising a concern for repeated dermal contact.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Systemic effects

Distinct systemic effects after inhalation or oral administration of vinyl acetate are not reported. The only indicator for systemic effects after repeated inhalation of vinyl acetate could be a decreased body weight gain observed in rats at 2,142 mg/m^3 (600 ppm) and in mice at 714 mg/m^3 (200 ppm) and above, observed in a combined chronic toxicity and carcinogenicity study (Owen, 1988; Bogdanffy et al., 1994b).

Inhalation

The combined chronic toxicity and carcinogenicity study (Owen, 1988; Bogdanffy et al., 1994b, see above), which was used for the risk assessment of local effects after repeated inhalation serves also for the risk assessment of systemic effects. A decreased body weight gain, but no target organ toxicity, was observed in rats at 2,142 mg/m^3 (600 ppm) and in mice at 714 mg/m^3 (200 ppm) and above. This decrease may be associated to non-specific toxicity of vinyl acetate. It was interpreted to be attributed to lower food and water consumption, but the 2-year study did not contain any data on the consumption of water/food and the food efficiency. Based on the reduction of body weight gain the NOAEC of 50 ppm (180 mg/m^3) of the mice is taken for the MOS calculation.

The derivation of the starting point and the reference MOS are identical with the values which are derived for carcinogenicity (values see under chapter carcinogenicity). The critical

exposure level regarding systemic toxicity after repeated inhalation is identified as 17.6 mg/m^3 (90 / 5.1).

The highest shift average value for inhalation is reported as 14.6 mg/m^3 for manufacturing of formulations and products of vinyl acetate in scenario 2. Compared with the OEL of 17.6 mg/m^3 the scenario is a borderline case. Conclusion iii is drawn for scenario 2 since there is the uncertainty concerning the reduction of the intraspecies factor. The other scenarios do not reach concern (for corresponding MOS values see table 4.1.3.2.C).

Conclusion iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Dermal contact

Dermal studies with repeated application are not available. Thus the studies with other routes of application are taken into account. A target organ of systemic toxicity was not observed in the oral or inhalation studies. The systemic LOAEC of inhalation toxicity was defined by a decreased body weight gain, which was interpreted to be based on lower food and water consumption (see above). The central drinking water study in rats and mice over 13 weeks revealed no significant systemic effects up to the highest tested dose of 5000 ppm (684 - 810 mg/kg/day for rats; 281 - 285 mg/kg/day for mice).

Since a quantitative risk assessment regarding repeated dermal contact is connected with high uncertainties regarding systemic availability, route-to-route extrapolation and the missing of distinct systemic effects, only a rough estimation is done. For this the following considerations regarding the assessment of repeated dermal exposure are taken into account: Neat vinyl acetate (1 mg/m^3) would evaporate within about 10 seconds from skin under usual working conditions of non occlusive exposure. From the experimental data there is no distinct systemic effect described (highest tested dose of 810 mg/kg/day from the drinking water study).

Compared with the highest dermal exposure of 6 mg/kg (scenario 2a, use of vinyl acetate monomer) there seems to result no concern for repeated dermal contact.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Combined exposure

Besides scenario 2 no further scenario reaches concern.

Conclusion iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Table 4.1.3.2.C: Repeated dose toxicity, systemic effects of vinyl acetate

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		90 mg/m³			no quantitative calculation, see text			no quantitative calculation, see text		
Reference MOS		5.1			-			-		
Critical exposure level		17.6 mg/m³			--			-		
		Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and polymerisation in the chemical industry		3	30	ii	0.6		ii	0.6		ii
2. Manufac-turing of formulations and products	a) polymerisation step (vinyl acetate monomer)	14.6	6.2	Border line iii	6		ii	5.71		iii[1)]
	b) formulation step (vinyl acetate (co)polymer)				0.02		ii	0.33		iii[1)]
3. Use of formulations and products containing residual vinyl acetate monomer		2.6	34.8	ii	0.18		ii	0.22		ii

[(1)]conclusion iii already results from inhalation exposure

Mutagenicity

Vinyl acetate is negative in bacterial mutagenicity tests.

The substance has a mutagenic potential, which is preferentially expressed as clastogenesis. The data on in vivo genotoxicity are difficult to interpret, since their majority is of low reliability, or the effects are not specific to mutagenicity. The most important effect, a weak induction of micronuclei in mouse bone marrow, is limited to intraperitoneal doses of high toxicity.

It is unlikely, that the genotoxic potential of vinyl acetate is expressed in germ cells in man, because vinyl acetate genotoxicity is limited to toxic doses. For vinyl acetate a threshold mechanism of action is assumed.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

Regarding the carcinogenicity of vinyl acetate, no adequate human data base is available. Long-term inhalation and oral administration of vinyl acetate in experimental animals produced tumors at the primary site of exposure, the surface epithelium of the respiratory tract and the upper gastrointestinal tract. After inhalation a tumor response to vinyl acetate exposure was seen at 600 ppm in rats (Bogdanffy et al., 1994b), for the oral route an increase of tumor rates has been observed at 10,000 ppm (male rat 442 mg/kg/day, female rat 575 mg/kg/day, Umeda et al., 2004a).

Vinyl acetate carcinogenicity is assumed to be mediated by the metabolic product acetaldehyde. Based on the nonlinear kinetics of intracellular aldehyd dehydrogenase activity there is a marked increase of intracellular acetaldehyde only at high concentrations of vinyl acetate. With reference to this non-linear dose response relationship for the critical metabolite acetaldehyde risk assessment is performed with the MOS approach.

Inhalation

The study, which serves as key study for the risk assessment of carcinogenicity of vinyl acetate is the combined chronic toxicity and carcinogenic study from Owen (1988) and Bogdanffy et al (1994b), which was already taken for the risk assessment of repeated dose toxicity. Exposure-related tumor response was observed in the nasal cavity of rats at the highest concentration of 600 ppm. A total of 11 nasal cavity tumors were evident in 7 males and 4 females, classified as papilloma and/or carcinoma cell types (see table 4.1.2.8.A). In the intermediate group (200 ppm) one single tumor (benign papilloma) was found in a male rat. No respiratory tract tumor was seen in the control and low dose groups (50 ppm) except for a single adenoma of the lung in a control female. From this study a NOAEC of 50 ppm (180 mg/m^3) is derived and used as a threshold concentration.

The experimental NOAEC of 180 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average

working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 90 mg/m^3 (180 • 6/8 • 6.7/10).

The reference MOS would account with the intraspecies differences with a factor of 1.25 – 2.5 (see chapter 4.1.3.2.1 reference MOS). The interspecies differences would deal with a factor of 2.5 This would altogether result in a reference MOS of about 3 to 6. The critical exposure level regarding carcinogenicity would then lie between 15 and 30 mg/m^3 (90 / 6 or 3), depending on how big the intraspecies differences are dealt with. Looking at the recommendation of the Scientific Committee on Occupational Exposure Limits, the committee decided in 2005 to set an OEL for vinyl acetate of 17.6 mg/m^3 (5 ppm). This value is in the same order than the lower value of 15 mg/m^3. For pragmatic reasons the occupational exposure value of 17.6 mg/m^3 (5 ppm), which was set by SCOEL (2005) is taken forward in this risk assessment. The SCOEL value corresponds to a reference MOS value of 5.1.

For scenario 2 (manufacture of formulations and products), the exposure is 14.6 mg/m^3. Since there is the uncertainty concerning the reduction of the intraspecies factor and the mode of action for carcinogenesis for this borderline scenario conclusion iii is drawn. The other scenarios are out of concern. For corresponding MOS values see table 4.1.3.2.D.

Conclusion: iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Dermal contact

Dermal carcinogenicity studies are not available. An oral drinking water study with rats (Umeda et al., 2004a) showed higher incidences of tumor rates at the local site of contact: squamous cell carcinoma in the the oral cavity, esophagus, and stomach, which were considered to be treatment related.

Vinyl acetate should be able to reach vivid skin cells and a potential to induce skin tumours cannot be excluded. A quantitative approach is not possible, since a quantitative route to route extrapolation of local effects is generally not performed. For risk characterisation purposes it should be further considered, that the high vapour pressure of vinyl acetate leads to reduced retention time on skin absorption.

The highest dermal exposure value (6 mg/kg/day) was estimated for the use of pure vinyl acetate during manufacture of formulations and products (scenario 2a). The magnitude of this exposure value is also based on the knowledge, that gloves are not regularly worn in small and medium-sized enterprises. The other scenarios have exposure values at least one order of magnitude below (<0.6 mg/kg/day). This is due to a high acceptance of gloves in the large-scale chemical industry (scenario 1) and the fact, that vinyl acetate is only available as a residual monomer (< 2 – 3000 ppm) in scenario 2b and 3.

Based on this information and the fact that especially the high doses led to a significant carcinogenic response in experimental studies, scenario 2a with the highest exposure due to non-effective skin protection is considered to be associated with a significant risk, which

should require further specific measures. The other scenarios (scenario 1, 2b, 3) are considered to be of lower concern and a low risk level is assumed.

Concern for scenario 2a (manufacturing of formulations and products with the vinyl acetate monomer, see table 4.1.3.2.D).

Conclusion: iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Combined exposure

Concern was already expressed for scenario 2a after dermal contact. Thus Scenario 2a reaches also concern for combined exposure.

Because of the lower exposure values for scenarios 1, 2b and 3 no concern is expressed.

Conclusion: iii)

There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Table 4.1.3.2.D: Carcinogenicity of vinyl acetate

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		90 mg/m³			no quantitative calculation, see text			no quantitative calculation, see text		
Reference MOS		5.1								
Critical exposure level		17.6mg/m³								
		Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and polymerisation in the chemical industry		3	30	ii	0.6		ii	0.6		ii
2. Manufac-turing of formulations and products	a) polymerisation step (vinyl acetate monomer)	14.6	6.2	Border line iii	6		iii	5.71		iii[(1)]
	b) formulation step (vinyl acetate (co)polymer)				0.02		ii	0.33		iii[1)]
3. Use of formulations and products containing residual vinyl acetate monomer		2.6	34.8	ii	0.18		ii	0.22		ii

[(1)]conclusion iii already results from inhalation and/or dermal exposure

Reproductive toxicity

Fertility impairment and developmental toxicity

With respect to toxicity for reproduction no human data are available.

Distinct reproduction related adverse effects of vinyl acetate are not reported from a two generation drinking water study with rats for concentrations including 1000 ppm.

The developmental toxicity of vinyl acetate has been investigated in rats via oral (drinking water) and inhalation exposure routes during organogenesis. No embryo/fetotoxic or teratogenic effects were observed for the oral route of administration at drinking water

concentrations including 5000 ppm. Fetotoxic effects, revealed during inhalation exposure, were confined to high dose levels only, where severe maternal toxicity was observed.

In addition, any specific teratogenic potential and/or impairment of embryo/fetal development are not indicated from the same data. Therefore no MOS calculation is performed for this endpoint.

Conclusion ii

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.2.3 Summary of conclusions for the occupational risk assessment

Table 4.1.3.2.E indicates the toxicological endpoints of concern for vinyl acetate. Concern results for carcinogenicity and repeated dose toxicity. For the other endpoints no concern is expressed.

Table 4.1.3.2.E: Endpoint-specific overall conclusions

Toxicological endpoints		General conclusion	Exposure Scenarios
Acute toxicity	inhalation	ii	
	dermal	ii	
	combined	ii	
Irritation/ Corrosivity	dermal	ii	
	eye	ii	
	acute respiratory tract	ii	
Sensitisation	skin	ii	
	respiratory	ii	
Repeated dose toxicity	inhalation, local	iii	2a
	inhalation, systemic	iii	2a
	dermal, local	ii	
	dermal, systemic	ii	
	combined, systemic	iii	2a
Mutagenicity		ii	
Carcinogenicity	inhalation	iii	2a
	dermal	iii	2a
	combined	iii	2a(1)

Reproductive toxicity	inhalation	ii	
	dermal	ii	
	combined	ii	

(1)conclusion iii already results from inhalative and/or dermal exposure, therefore no specific concern for the combined exposure scenario is indicated

Risk estimation is mainly based on animal inhalation studies and oral studies. The following absorption percentages are assumed for the different exposure ways: 15% inhalative uptake for the assessment of systemic effects, 50% absorption after oral uptake of vinyl acetate and 90% absorption after dermal contact.

The most pronounced effects of vinyl acetate are local effects after repeated inhalation contact and carcinogenicity. The value of 17.6 mg/m^3 designates the critical exposure level as well for carcinogenicity as repeated dose toxicity after inhalation. Following inhalation, the substance is rapidly hydrolysed by carboxylesterases leading to the formation of acetic acid and acetaldehyde which is further converted into acetic acid. At higher concentrations of vinyl acetate the capacity of the intracellular enzymes is not big enough to oxidize all of the generated acetaldehyde. Thus, at high vinyl acetate concentrations, non-physiologically high concentrations of acetaldehyde are produced. This intermediate, develops adverse effects at non-physiologically high concentrations, which result in the formation of tumors at the exposed tissue. Because of the carcinogenic effects only occur at the site of first contact at high concentrations of vinyl acetate, the substance is considered as a threshold carcinogen.

On the background of cancer risks and repeated dose toxicity (local and systemic effects), air concentrations of vinyl acetate at the workplace should be controlled to a level in the range of 17.6 mg/m^3 (critical exposure level). However, scenario 1 and 3 of the inhalation exposure scenarios, specified in this report describe lower exposure values and are thus out of concern. Scenario 2 (manufacture of formulations and products), with an exposure value of 14.6 mg/m^3 is a borderline case. Since there is the uncertainty concerning the reduction of the intraspecies factor and the mode of action for carcinogenesis for this borderline scenario conclusion iii is drawn for repeated dose toxicity and carcinogenesis. Skin contact of vinyl acetate should be reduced in scenario 2a (manufacturing of formulations and products, vinyl acetate monomer), even if evaporation of the substance reduces the contribution of dermal exposure.

4.1.3.3 Consumers

Following the exposure assessment there is no direct exposure of the consumer to vinyl acetate besides of the release of monomers from polymers. Overall exposure to vinyl acetate occurs mainly via inhalation due to emissions from carpets, paints and adhesives. For comparison with acute effects, the estimate for short-term exposure to concentrations of a about 1 mg/m³ from carpets will be used. With regard to chronic effects, the concentration of 0.036 mg/m³ for long-term exposure will be used resulting in an exposure of 9.2 µg/kg bw/d for men, of 4.6 µg/kg bw/d for women, and of 18 µg/kg bw/d for children. The inhalation exposures numbers representing residual monomeric vinyl acetate still overestimate realistic

chronic exposure because according to the biochemical data remarkable amounts of vinyl acetate will be metabolized rapidly to acetaldehyde in the olfactory and respiratory epithelium (see chapter 4.1.2.1) thus only very small amounts of vinyl acetate will reach the systemic circulation. Furthermore, the consumer may be exposed to vinyl acetate by using hair setting lotions (1 µg/kg bw/d) by the dermal route and by migration of the substance from plastics coming into contact with foods via oral route. Since this exposure scenario is regulated (see chapter 4.1.1.3) there is no need for MOS calculation.

Acute Toxicity

Following the exposure assessment, consumers are not expected to be exposed to vinyl acetate in the range of doses which can be derived from acute oral or dermal toxicity figures based on animal LD 50 values (oral and dermal: > 3500 mg/kg body weight). Therefore the substance is of no concern in relation to acute oral or dermal toxicity.

The inhalation route of exposure should be of no concern, because in rats vinyl acetate has demonstrated LC50 values of > 14 mg/l/4h. For short-term exposure from carpets concentrations of 0.001 mg/l have been calculated.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Irritation/Corrosivity

Vinyl acetate causes only mild irritation to the skin and eyes of rabbits, but causes severe irritation in the respiratory tract of rats. Human data on irritation/corrosivity caused by vinyl acetate are not available.

Following the exposure assessment it can be assumed that consumers are exposed only to such concentrations which are far below the effective concentrations.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Sensitization

Data obtained from a Buehler Test demonstrate that vinyl acetate (commercial grade) is a moderate skin sensitizer. The results of a Local Lymph Node Assay confirm the weak to moderate effects in the Buehler Test, but do not warrant classification and labelling.

No reports of allergic contact dermatitis caused by vinyl acetate from routine patch testing or experimental studies in man are available. Furthermore, no direct information is available from studies in humans on respiratory sensitization. In view of the widespread occupational use, the absence of any reports suggests that vinyl acetate may not be a respiratory sensitizer.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Repeated dose toxicity/Non-neoplastic lesions

Following the exposure assessment there may be chronic exposure to vinyl acetate resulting from release of residual monomers out of polymers.

The major toxic effects after prolonged inhalation of vinyl acetate in experimental animals were lesions of the surface epithelium of the upper and lower respiratory tract. Degeneration, regenerative/reparative processes, inflammation, hyperplasia and metaplasia were noted in the nasal mucosa as non-neoplastic effects. These effects were most pronounced in the olfactory epithelium occurring at 200 ppm in rats and mice during and at the end of a 2-year exposure period. Lesions of the respiratory epithelium were seen in mice exposed to 600 ppm during and at the end of 2 years, whereas rats demonstrated lesions at this site only at a high concentration of 1000 ppm (4 week study). Characteristic alterations of the larynx and trachea of mice in the 600 ppm groups were hyperplasia and metaplasia along with desquamation and fibrosis in the trachea. Similar changes of the bronchial and bronchiolar airways were reported for rats and mice at this concentration at the end of the 2-year exposure period. In addition, clinical signs of non-specific toxicity and irritation were evident, but no relevant toxic effect on any organ could be identified. The NOAEC for local toxic effects on the respiratory tract of 50 ppm (178.5 mg/m³) was derived from the 2-year studies on rats and mice. Based on growth retardation in rats of the 600 ppm groups and due to hunched posture in mice of the 200 ppm groups, the NOAEC for systemic toxicity was considered at 50 ppm for mice (178.5 mg/m^3) and 200 ppm for rats (714 mg/m³).

No specific organ toxicity was recorded after repeated oral administration of vinyl acetate with drinking water to rats and mice. A subchronic 13-week study revealed a slight (non-significant) reduction of food consumption and growth retardation in male rats at 5000 ppm (684 mg/kg bw/d).

For the decision on the appropriateness of MOS, the following aspects regarding the critical effect as well as exposure have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were published in peer reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognized guidelines and to GLP.

The findings of all studies are not contradictory so that the judgement can be based on the database (cf. 4.1.2.6 and 4.1.2.8).

There are no reasons to assume limited confidence.

- uncertainty arising from the variability in the experimental data

The studies cited above consistently indicated degeneration of the nasal epithelium in several studies on rats and mice. From a well performed 2-year inhalation study on rats and mice a NOAEC for local effects of 178 mg/m^3 (50 ppm) was derived and the results were in conformity with the findings of the other studies.

- intra- and interspecies variation

The results of PBPK modeling are taken forward for consideration on the appropriate adjustment factors. As vinyl acetate is acting locally, a systemic metabolism has not to be considered, which reduces the interspecies factor from 10 (default) to 2.5. Remaining uncertanties in toxicodynamic differences are recognized, i. e. humans could be more sensitive than rats towards local toxicity of vinyl acetate metabolites which could not be excluded due to the lack of (primarily human) data. Thus, for risk charaterization an interspecies adjustment factor of 2.5 is established. Concerning intraspecies variability the same consideration as for interspecies extrapolation applies. No systemic metabolism has to be taken into consideration, reducing the intraspecies adjustment factor to 2.5.

- the nature and severity of the effect

The main effects considered as „critical effects“ are the degenerative lesions of the olfactory mucosa (irreversible, serious health effect).

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Because of the seriousness of the effect there is concern, which has to be expressed in the magnitude of the MOS.

- dose-response-relationship

In rats as well as mice no steep dose-response-relationship is observed for the irritation effects at the olfactorium. At the LOAEC (714 mg/m^3, 200 ppm) only minimal to slight changes in the olfactory epithelium were observed.

There is no reason to assume concern which has to be expressed in an increased MOS taking into account the exposure level.

- differences in exposure (route, duration, frequency and pattern)

Following the exposure assessment, the consumer may be exposed to vinyl acetate via inhalation, whereas oral and dermal exposures are assumed of minor importance.

a) Inhalation route

The NOAEC used for the discussion of the MOS regarding exposure from carpets is derived from a 2-year inhalation study on rats. Because vinyl acetate acts primarily at the nasal cavity, systemic effects have not been considered. Moreover, the NOAEC for systemic effects was considered to be 714 mg/m^3 for rats and 178 mg/m^3 for mice in the same study.

b) Oral route

The oral route is not relevant for risk characterization (see introductory remarks).

c) Dermal route

Following the exposure assessment, the consumer may be exposed dermally to vinyl acetate via usage of hair setting lotions. The estimated dermal body burden (1 µg/kg bw/d) with an assumed absorption of 100% is compared with a NOAEL from an oral 90-day study due to the lack of a dermal study.

There are no reasons to assume that special concern can be derived from this procedure nor from the available toxicokinetic information (concerning absorption via different routes was set with 100%).

- the human population to which the quantitative and/or qualitative information on exposure applies

Following the inhalation exposure there is no reason to assume a special risk for elderly, children or other people suffering from special diseases.

- other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for

Inhalation exposure scenario

Long-term exposure to 0.036 mg/m³ vinyl acetate is assumed to result via emission from carpets. The margin of safety for non-neoplastic effects between the

measured exposure level of 0.036 mg/m^3

and the

NOAEC for local irritation effects of 178 mg/m^3

is judged to be sufficient taking into account the nature of the observed effects (degenerative lesions of the olfactory mucosa) and the fact that no steep dose-response relationship is observed for the these effects. Considering the short-term exposure, see section Acute toxicity.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

MOS for

Dermal exposure scenario

The calculation of the dermal exposure due to hair setting lotions leads to an external exposure of 1 µg/kg bw/d. The margin of safety between the

external dermal exposure 1 µg/kg bw/d

and the

oral NOAEL of 684 mg/kg bw/d

is judged to be sufficient.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Mutagenicity

Vinyl acetate is not mutagenic to bacteria, but it induces chromosomal aberrations, gene mutations and SCE in several tests with mammalian cells from different sources in culture. Furthermore, at high concentrations the formation of DNA-protein-crosslinks and DNA-DNA-crosslinks is shown with mammalian cells.

The in vivo genotoxicity of vinyl acetate appears to be limited to toxic doses. Therefore, the substance may express its genotoxic potential only when defence mechanisms are overloaded and thus it may be reasonable to assume a threshold mechanism of action for vinyl acetate genotoxicity.

Taking into account the low consumer exposure (in the range of up to 20 µg/kg bw/d) based on the present knowledge it may be concluded that there is presently no concern regarding in vivo mutagenicity.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

In cancer studies, vinyl acetate inhalation induced an increased number of nasal tumors (mainly papillomas and squamous cell carcinomas) in various regions of the nasal mucosa of rats. The total incidence was significantly increased at a concentration of 600 ppm (2142 mg/m^3) but a single papilloma already developed at 200 ppm. Thus, the NOAEC of 50 ppm (178 mg/m^3) will be used for the risk characterisation. No significant tumor response was seen in a mice cancer bioassay. Occasionally single squamous cell tumors occurred at other sites of the respiratory tract in rats and mice.

For the oral route a marked increase of tumor rates has been observed at 1000 ppm (rat (offspring); 70 mg/kg bw/d), at 5000 ppm (mouse; 750 mg/kg/d) and at 10000 ppm (male rat 442 mg/kg bw/d, female rat 575 mg/kg bw/d). Due to limitations in the study design in the studies of Maltoni et al. (1997) and Minardi et al. (2002), the study of Umeda et al. (2004a) is considered of higher predictivity (cf. 4.1.2.8). For lower doses, there is concern that occasional findings of tumors of the same types that have been observed at high doses and which might also be related to vinyl acetate exposure. Thus, the lowest concentration suspected to be carcinogenic was 400 ppm for the oral route (LOAEL, male rat 21 mg/kg bw/d, female rat 31 mg/kg bw/d, Umeda et al., 2004a). Clearly tumor free dosages were not established in carcinogenicity studies for the oral route. Thus, 400 ppm (21 mg/kg bw/d male rat) is proposed as LOAEL for oral risk characterisation. Since carcinogenicity is a critical toxicological endpoint a risk characterisation for the oral route was calculated.

Vinyl acetate exposure produced tumors at the site of first contact along the exposure routes by inhalative and oral uptake. A thresholded mode of carcinogenic action is thought to be active. Uncertainties exist in the proposed mode of action and should taken into account.

MOS for

Inhalation exposure scenario

Long-term exposure to 0.036 mg/m³ vinyl acetate is assumed to result via emission from carpets. The margin of safety for non-neoplastic effects between the

measured exposure level of 0.036 mg/m^3

and the

NOAEC 178 mg/m^3

is judged to be sufficient.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

MOS for

Oral exposure scenario

The oral route is not relevant for risk characterization (see introductory remarks).

Reproductive toxicity

Since no hazards have been identified regarding fertility and developmental toxicity a risk characterisation should not be performed. This decision is further supported by the fact that the systemic availability of vinyl acetate is very low.

4.1.3.4 Man exposed indirectly via the environment

Indirect exposure to humans via the environment occurs mainly by air. Following the local scenario data (at a point source) a maximum intake of a total daily dose of 0.036 mg/kg bw/d is calculated. For the regional scenario, the respective figure is 0.00247 µg/kg bw/d.

The main route of indirect exposure of both scenarios (local and regional) is the intake via inhalation of air (cf. 4.1.1.4). Therefore the local concentration of 0.288 mg/m^3 for site 26 was selected (Table 3-11; highest value, in chapter 3.1.3.11) and used for risk characterisation.

Repeated dose toxicity

Inhalation

Local effects on the respiratory tract are not to be expected because of the low concentrations in the air of 0.288 mg/m^3 (local exposure). The NOAEC for local toxic effects on the respiratory tract of 50 ppm (178.5 mg/m³) was derived from the 2-year studies on rats and mice.

MOS for

Inhalation exposure scenario

The margin of safety for non-neoplastic effects between the

estimated exposure level of 0.288 mg/m^3

and the

NOAEC 178 mg/m^3

is judged to be sufficient.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Mutagenicity

Vinyl acetate is not mutagenic to bacteria, but it induces chromosomal aberrations, gene mutations and SCE in several tests with mammalian cells from different sources in culture. Furthermore, at high concentrations the formation of DNA-protein-crosslinks and DNA-DNA-crosslinks is shown with mammalian cells. The in vivo genotoxicity of vinyl acetate appears to be limited to toxic doses thus it may be reasonable to assume a threshold mechanism of action for germ cell mutagenicity.

Taking into account the exposure estimates resulting for a point source from a worst case calculation and from regional background concentrations it may be concluded that there is presently no concern regarding in vivo mutagenicity.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

Vinyl acetate inhalation induced an increased number of nasal tumors (mainly papillomas and squamous cell carcinomas) in various regions of the nasal mucosa of rats (cf. 4.1.2.8, 4.1.3.3). The total incidence was significantly increased at a concentration of 600 ppm (2142 mg/m^3) but a single papilloma already developed at 200 ppm. Thus, the NOAEC of 50 ppm (178 mg/m^3) will be used for risk characterisation purposes.

MOS for

Inhalation exposure scenario

The margin of safety for neoplastic effects between the

estimated exposure level of 0.288 mg/m^3

and the

NOAEC 178 mg/m^3

is judged to be sufficient.

Conclusion ii)

There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Reproductive toxicity

Since no hazards have been identified regarding fertility and developmental toxicity a risk characterisation should not be performed. This decision is further supported by the fact that the systemic availability of vinyl acetate is very low.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

Refer to chapter 4.1.1.1

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

Vinyl acetate is not explosive.

4.2.2.2 Flammability

Vinyl acetate is highly flammable.

4.2.2.3 Oxidizing potential

Due to its chemical structure, vinyl acetate is not expected to possess any oxidizing properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Vinyl acetate is highly flammable. Adequate worker protection measures must be observed. Risk reduction measures beyond those which are being applied already are not considered necessary.

Conclusion: ii

4.2.3.2 Consumers

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(x) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(x) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

As a follow up to conclusion (i), which was drawn in the first draft of the Environment Section of the Comprehensive Risk Assessment Report of Vinyl acetate (August 2002), further acute and long-term toxicity tests were performed for the aquatic compartment and more data on a number of production and processing sites were obtained, leading to the following revised risk characterisation.

conclusion iii):

Terrestrial compartment

A risk to the local terrestrial compartment can be identified for the life cycle stage of vinyl acetate processing. This conclusion applies to the generic processing scenario for the local main source. The unknown processing sites account for a missing tonnage of 325,000 t/a (or 41 % of the total production volume of 800,000 t/a), compared to a verified processing tonnage of 475,000 t/a.

The risk characterisations for selected (worst case) production and processing sites with site specific data indicate no actual risk to the terrestrial compartment. However, the highest PEC/PNEC ratio calculated with site specific data (0.86) is not very far from the PEC/PNEC ratio calculated for the generic processing scenario (2.33), and there was also a risk at one of the production sites (which has been eliminated during the time of the preparation of this RAR). Consequently, it is not completely implausible that unknown processing sites pose a risk to the terrestrial environment.

The risk characterisation for the terrestrial compartment could still be revised by requesting further testing (i.e. OECD 207 Earthworm acute toxicity test and OECD 208 Terrestrial plants growth test). However, a further data improvement by means of testing is not considered a reasonable next step by the rapporteur for the following reasons.

Further information on the toxicity of vinyl acetate to terrestrial organisms allows for a re-evaluation of the potential risk that emanates from the unknown processing sites, but delays risk reduction measures in case that a risk still remains with the new data. The rapporteur therefore proposes to enter directly into the risk reduction phase, which would be more effective than further data improvement at this stage of the risk assessment.

Risk reduction measures should be considered for all facilities with a vinyl acetate processing capacity exceeding 20,000 t/a. This threshold is derived from the generic processing scenario, assuming that vinyl acetate is released to soils via sludge application and aerial deposition. Sites already applying advanced techniques would not require further consideration of risk reduction measures. It is known from some processing/production sites that techniques such as waste water distillation, sludge incineration or deposition in a landfill, waste gas incineration or vapour recovery systems can avoid significant releases of vinyl acetate to soils.

In principle, the rapporteur agrees with the proposal of SCHER that considering the volatility of the substance, terrestrial plants might be exposed via air. However, no guidance is available how to derive a PNEC for the air compartment. In addition, the available guidelines for terrestrial plants (OECD 208 / 227) are designed to include exposure through soil or by wet residues (e.g. spray drift of plant production products). To determine effects of volatile compounds, testing needs to be conducted in closed greenhouses or chambers under continuous exposure conditions. We feel this kind of testing is not appropriate for vinyl acetate, because no indications for high toxicity for plants exist. For other soil organisms, a continuous exposure to volatile compounds like vinyl acetate is negligible since the substance is expected to evaporate from the soil.

conclusion ii):

Aquatic compartment

A risk to the local or regional aquatic compartment (surface water and sediments) was not identified for production and processing of vinyl acetate. This conclusion applies to all sites.

Atmospheric compartment

Based on a qualitative risk characterisation, no unacceptable risk for the atmosphere is expected from vinyl acetate. The substance is rapidly removed from air by chemical breakdown, adsorption to airborne particles or aerosols, and wet deposition. Furthermore, air concentrations indicate a negligible risk with regard to ecotoxicity.

Non-compartment specific effects

Non compartment specific effects (secondary poisoning) of vinyl acetate are not expected as there is no indication that the substance has potentially bioaccumulative properties.

Marine Assessment including PBT assessment

VAM does not meet the PBT/vPvB criteria. A risk for the regional or local marine environment is not expected.

Human Health

Human Health (toxicity)

Workers

conclusion iii)

Conclusion iii is expressed for the endpoint of carcinogenicity and repeated dose toxicity after inhalation and carcinogenicity after dermal contact. Skin contact of vinyl acetate should be reduced at scenario 2a (manufacturing of formulations and products, vinyl acetate monomer), even if evaporation of the substance reduces the contribution of dermal exposure.

On the background of cancer risks and repeated dose toxicity, air concentrations of vinyl acetate at the workplace should be controlled to a level in the range of 17.6 mg/m^3 (critical exposure level). Conclusion iii is derived for repeated dose toxicity after inhalation for scenario 2 (manufacturing of formulations and products). This scenario with an exposure value of 14.6 mg/m^3 is a borderline case. Since there is the uncertainty concerning the reduction of the intraspecies factor and the mode of action for carcinogenesis for this borderline scenario conclusion iii is drawn.

Consumers

conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Man exposed indirectly via the environment

conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Human Health (risks from physico-chemical properties)

Conclusion (ii)

There is at present no need for further information and/or testing and no need for riskreduction measures beyond those which are being applied already.

6 REFERENCES

AIHC (1994): American industrial health council. Exposure factors handbook Update co-ordinator, Suite 760, 2001 Pennsylvania Ave. NW, Washington DC 20006-1807 (AIHC-Report)

Appelman, L.M., Woutersen, R.A., Feron, V.J. (1982): Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23: 293-307

Appelman, L.M., Woutersen, R.A., Feron, V.J. (1986) Effect of variable versus fixed exosure levels on the toxicity of acetaldehyde in rats. J. Appl. Toxicol. 6:331-336

Atkinson, R. (1987): Intern. J. Chem. Kinetics, 19, 799-828

Atkinson (1995) cited in Toxic Air Contaminant Identification List Summaries of the California Air Resources Board, http://www.arb.ca.gov/

ASG/CEFIC (2003a): Vinyl acetate monomer: An acute toxicity study with the Daphnid, Daphnia magna, Straus Study ID: 031038, Performing Laboratory: Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Michigan, USA

ASG/CEFIC (2003b):Vinyl acetate monomer (VAM): Toxicity to the early life stages of the fathead minnow, Pimephales promelas Rafinesque. Study ID: 031056 Performing Laboratory: Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Michigan, USA

ASG/CEFIC (2003c): Vinyl acetate monomer: Growth inhibition test with the freshwater green alga, Pseudokirchneriella subcapitata. Study ID: 031041, Performing Laboratory: Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Michigan, USA

AUS (1994): MAK-Liste, Amtliche Nachrichten Arbeit-Gesundheit-Soziales, 48. Jh., Sondernummer 2/1993, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

AUH (1995): Ausschuss für Umwelthygiene, Arbeitsgemeinschaft der leitenden Medizinalbeamtinnen und -beamten der Länder. Standards zur Expositionsabschätzung. Behörde für Arbeit, Gesundheit und Soziales, Hamburg (AUH-Bericht)

B (1998): Exposure Limit Values, Koninklijk Besluit van 10 augustus 1998, Moniteur Belge/Belgisch Staatsblad number 176, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

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

BASF AG (1967): Vinylacetat. Ergebnis der gewerbetoxikologischen Vorprüfung. Report No. XVI/354, 28.03.1967

BASF AG (1999): Statement from 06.06.1999

Basuroy, S., Sheth, P., Mansbach, C.M., Rao, R.K. (2005) Acetaldehyde disrupts tight junctions and adherens junctions in human colonic mucosa: protection by EGF and L-gluamine. Am J Physiol Gastrointzest Liver Physiol 289: G367-75

BfG (1995): "Bundesanstalt für Gewässerkunde", selected sites of chemical industry and attached water flows in the Rhein-area, Koblenz, 19 may 1995.

BG Chemie 2002): Statement from 10.06.2002 (Brock, T.H.)

BGAA (1996): Berufsgenossenschaftlicher Arbeitskreis Altstoffe: Vinylacetat, Exposition am Arbeitsplatz

Blasiak, J., Gloc-Fudala, E., Trzeciak, A. (1999): Formation of DNA crosslinks in human lymphocytes by acetaldehyde revealed by the comet assay. CELLULAR and MOLECULAR BIOLOGY LETTERS 4, 181-187

Blasiak, J., Trzeciak, A., Malecka-Panas, E., Drzewoski, J., Wojewodzka, M. (2000): In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells. Toxicol Vitro 14: 287-295

Bogdanffy, M.S. (1990): Biotransformation enzymes in the rodent nasal mucosa: The value of a histochemical approach. Environ. Health Persp. 85: 177-186

Bogdanffy, M.S. (2002): Vinyl acetate-induced intracellular acidification: implications for risk assessment. Toxicol Science 66: 320-326

Bogdanffy, M.S., Dreef-van der Meulen, H.C., Beems, R.B., Feron, V.J., Cascieri, T.C., Tyler, T.R., Vinegar, M.B., Rickard, R.W. (1994b): Chronic toxicity and oncogenicity inhalation study with vinyl acetate in the rat and mouse. Fund Appl Toxicol 23: 215-229

Bogdanffy, M.S., Gladnick, N.L., Kegelman, T., Frame, S.R. (1997): Four-week inhalation cell proliferation study of the effects of vinyl acetate on rat nasal epithelium. Inhalation Toxicology 9: 331-350

Bogdanffy, M.S., Kee, C.R., Hinchman, C.A., Trela, B.A. (1991): Metabolism of dibasic esters by rat nasal mucosal carboxylesterase. Drug Metab Dispos 19: 124-129

Bogdanffy, M.S., Manning, L.A., Sarangapani, R. (1999a): High-affinity nasal extraction of vinyl acetate vapor is carboxylesterase dependent. Inhal Toxicol 11: 927-941

Bogdanffy, M.S., Plowchalk, D.R. (2001) Mode-of-action-based dosimeters for interspecies extrapolation of vinyl acetate inhalation risk. Inhal Toxicol 13:377-396

Bogdanffy, M.S., Plowchalk, D.R., Harman, A.E., Taylor, M.L., Keller, D.A. (1995): Dosimetry of vinyl acetate in the nasal passages of rats: Comparison of in vitro and in vivo methods of quantifying metabolism. Inhalation Toxicol. 6: 409-412

Bogdanffy, M.S., Randall, H.W., Morgan, K.T. (1986): Histochemical localization of aldehyde dehydrogenase in the respiratory tract of the Fischer-344 rat. Toxicol. Appl. Pharmacol. 82: 560-567

Bogdanffy, M.S., Randall, H.W., Morgan, K.T. (1987): Biochemical quantitation and histochemical localization of carboxylesterase in the nasal passages of the Fischer-344 rat and B6C3F1 mouse. Toxicol Appl Pharmacol 88: 183-194

Bogdanffy, M.S., Sarangapani, R., Andersen, M., Jarabeck, A. and Dellarco, V.L. (1998): Inhalation hazard identification and dose-response characterization for vinyl acetate. Draft report, 1998

Bogdanffy, M.S., Sarangapani, R., Kimbell, J.S., Frame, S.R., Plowchalk, D.R. (1998): Analysis of vinyl acetate metabolism in rat and human nasal tissues by an in vitro gas uptake technique. Toxicol. Sci. 46: 235-246

Bogdanffy, M.S., Sarangapani, R., Plowchalk, D.R., Jarabek, A., Andersen, M.E. (1999b): A biologically based risk assessment for vinyl acetate –induced cancer and noncancer inhalation toxicity. Toxicol Sci 51: 19-35

Bogdanffy, M.S., Taylor, M.L. (1993): Kinetics of nasal carboxylesterase-mediated metabolism of vinyl acetate. Drug Metabolism Disposition 21: 1107-1111

Bogdanffy, M.S., Tyler, T.R., Vinegar, M.B., Rickard, R.W., Carpanini, F.M.B., Cascieri, T.C. (1994a): Chronic toxicity and oncogenicity study with vinyl acetate in the rat: In utero exposure in drinking water. Fund Appl Toxicol 23: 206-214

Borelli, S. (1988): Dermatologischer Noxen-Katalog. Krankheiten der Haut und Schleimhaut durch Kontakte in Beruf und Umwelt. Band 3: Kontakt - Beruf - Diagnose (Erfassungszeitraum bis 1970), 2211

Boyland, E., Chasseaud, L.F. (1970): The effect of some carbonyl compounds on rat liver glutathione levels. Biochem Pharmacol 19: 1526-1528

Brams, A., Buchet, J.P., Crutzen-Fayt, M.C., De Meester, C., Lauwerys, R., Leonard, A. (1987) A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). Toxicol Letters 38:123-133

Bringmann, G., Kühn, R. (1976): Vergleichende Befunde der Schadwirkung wassergefährdender Stoffe gegen Bakterien (Pseudomonas putida) und Blaualgen (Microcystis aeruginosa), GWF, Wasser / Abwasser 117, 410-413

Bringmann, G, Kühn, R. (1977a): Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Bakterien (Pseudomonas putida) und Grünalgen (Scenedesmus quadricauda) im Zellvermehrungshemmtest, Z. Wasser Abwasser Forsch. 10, 87-98

Bringmann, G. Kühn, R. (1978a): Testing of substances for their toxicity threshold: Model organisms Microcystis aeruginosa and Scenedesmus quadricauda. Mitt. Internat. Verein. Limnol. 21, 275-284

Bringmann, G. Kühn, R. (1978b): Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen (Microcystis aeruginos und Blaualgen Scenedesmus quadricauda. Vom Wasser 50, 45-60

Bringmann G., et al. (1980): Bestimmung der biologischen Schadwirkung wassergefährdender Stoffe gegen Protozoen III: Saprozoische Flagellaten. Z. Wasser Abwasser Forsch. 13, 170-173

Bringmann, G. Kühn, R. (1982): Ergebnisse der Schadwirkung wassergefährdender Stoffe gegen Daphnia magna in einem weiterentwickelten standardisiertem Testverfahren, Z. Wasser Abwasser Forsch. 15, 1-6

Brown, H.P., Monticello, T.M., Maronpot, R.R., Randall, H.W., Hotchkiss, J.R., Morgan, K.T. (1991): Proliferative and neoplastic lesions in the rodent nasal cavity. Toxicologic Pathology 19: 358-372

Burditt et al.(1963): Screening of fumigants for toxicity to eggs and larvae of the oriental fruir fly and mediterranean fruit fly. J. Econ. Entomol. 56, 261-265

BUA (1993): BUA-Stoffbericht: Vinylacetat, No. 116, Beratergremium für umweltrelevante Altstoffe (BUA) der Gesellschaft Deutscher Chemiker, S. Hirzel Wissenschaftliche Verlagsgesellschaft

Butterworth, B.E., Bogdanffy, M.S. (1999): A comprehensive approach for integration of toxicity and cancer risk assessments. Regulatory Toxicology and Pharmacology 29: 23-26

Calderón-Garcideuñas, L., Rodríguez-Alcaraz, A., Garcia, R., Barragan, G., Villarreal-Calderón, A., Madden, M.C. (1999): Cell proliferation in nasal respiratory epithelium of people exposed to urban pollution. Carcinogenesis 20: 383-389

Calderón-Garcideuñas, L., Rodríguez-Alcaraz, A., Villarreal-Calderón, Lyght, O., Janszen, D., Morgan, K.T. (1998): Nasal epithelium as a sentinel for airborne environmental pollution. Toxicological Sciences 46: 352-364

Carpenter, C.P., Smyth, H.F., Pozzani, U.C. (1949): The assay of acute vapour toxicity, and the grading and interpretation of results on 96 chemical compounds. J Ind Hyg Toxicol 31: 343-346

Casanova-Schmitz, M., David, R.M., Heck, H.A. (1984): Oxidation for formaldehyde and acetaldehyde by NAD^+-dependent dehydrogenases in rat nasal mucosal homogenates. Biochem Pharmacol 33: 1137-1142

Cassee, F.R., Gropten, J.P., Feron, V.J. (1996) Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fund Appl Toxicol 29:208-218

CEC - Comission of the European Communities (2003): Technical Guidance Document in support of Commission Directive93/67/EEC on Risk Assessment for new notified substances: Commission Regulation (EC) No. 1488/94 on Risk Assessment forexisting substances: Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market (Parts I-IV) Joint Research Centre, European Chemicals Bureau, Ipra, Italy

Celanese (1998): Attachment 1 of personal communication on Japanese oral carcinogenicity studies in rats and mice

Celanese (2008): Information provided by the company, Industry Comment to the Human Health Section of the RAR (11.02.2008)

Chemicals Inspection and Testing Institute (Ed.) (1992): Biodegradation and bioakkumulation of existing chemicals based on the Chemical Substances Control Law (CSCL) Japan. Japan Chemical Industry Ecology-Toxicology and Information Center 14-19, 2-17.

Chemical Week (2003): Product focus vinyl acetate monomer, June 25, p. 31.

Chemsafe (1994): national database for safety data of the Physikalisch-technische Bundesanstalt, Braunschweig, established by expert judgement

Chou W.L., Speece R.E., Siddiqi R.H. (1979) Acclimation and degradation of petrochemical wastewater components by methane fermentation. Biotechnol. Bioeng. Symp. 8: 391-865

Ciccioli, P., Cecinato A., Brancaleoni, E. Brachetti , A. Frattoni M. (1994): Polar organic compounds (VOC) of natural origin as precursors of ozone. Environmental Monitoring and Assessment 31, 211-217

Cicciolo, P., Brancaleoni, E. Brachetti , A. Frattoni M. Cecinato A., Oinciarelli L.(2001): Determination of volatile organic compounds (VOC) emitted from biomass burning of Mediterranean vegetation species by GC-MS, Analytical Letters 34(6) 937-955

Clark, N.W.E., Scott, R.C., Blain, P.G. and Williams, F.M. (1993): Fate of fluazifop butyl in rat and human skin in vitro. Arch. Toxicol. 67, 44 – 48.

Colipa (1989): The European Cosmetic Toiletry and Perfumery Association 1989; Annex 1, Use levels of cosmetics

Coutelle, C., Ward, P.J., Fleury, B., Ouattrocch, P., Chambrin, H., Iron, A., Couzigou, P., Cassaigne, A. (1997). Laryngeal and oropharyngel cancer and alcohol dehydrogenase 3 and glutathione S-transferase M1 polymorphisms. Hum. Genet. 99: 319-325, 1997

Cresswell, D.G., Strong, H.A., Hopkins, R. (1979): Investigations into the metabolic fate of vinyl acetate in the rat and mouse. Report No. 2511-51/11-14, Part 2 Hazleton Laboratories Europe; cited in: ECETOC Joint Assessment of Commodity Chemicals 1991, No 18

D (1998): Technische Regeln für Gefahrstoffe (TRGS) 900, Grenzwerte in der Luft am Arbeitsplatz „Luftgrenzwerte“. Ausgabe: Oktober 1996, zuletzt geändert BArBl. Heft 6/1999

Dahl, A.R., Miller, S.C., Petridou-Fischer, J. (1987): Carboxylesterases in the respiratory tracts of rabbits, rats and syrian hamsters. Toxicology Letters 36: 129-136

Daniels, W. (1983) Poly(vinyl acetat). in: Grayson M., ed. Kirk-Othmer Encyclopedia of Chemical Technology, Vol.23, 3rd ed. New York, NY, John Wiley and Sons, 817-847

DECOS (1995): Dutch expert committee on occupational standards: Calculating cancer risk. No. 1995/06 WGD, The Hague

Deese, D.E., Joyner, R.E. (1969): Vinyl acetate: A study of chronic human exposure. Am Ind Hyg Assoc J 30: 449-457

de Heer et al. (1999): Principles for study protocols addressing the dermal absorption of pestices). Zeist, The Netherlands: TNO, 1999. (TNO-report V98.356)

DEN (1999): Dermal exposure network: Issues relating to the risk assessment of dermal exposures; An output of the EU dermal exposure network, compiled on behalf of the network by J. Jackson (1999)

Deutsches Teppichforschungsinstitut E.V (1992):. Report No. 92625, 4th Nov 1992

DK (1996): Exposure Limit Values for Substance and Materials: Arbejdstilsynet Graensvaerdier for Stoffer og Materialer. At-Anvisning. Nr. 3.1.0.2. Dec. 1996, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Dong, Y., Peng, T., Yin, S, (1996): Expression and activities of class IV alcohol dehydrogenase and class III aldehyde dehydrogenase in human mouth. Alcohol 13: 257-262

DuPont (2000): Vinyl Acetate: Subchronic Toxicity 90-Day Drinking Water in Rats and Mice, Bamberger, J.R., Laboratory Project ID: DuPont-3978, September 7, 2000

Dybing, E., Sanner, T., Roelfzema, H., Kroese, D. and Tennant, R. W. (1997): T25: A simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specifity and mutagenicity. Pharmacology and Toxicology, 80, 272 - 279

EC (European Comission) (1993): Risk Assessment of Notified New Substances; Technical Guidance Document

EC (European Comission) (1994): Risk Assessment of Existing Substances; Technical Guidance Document; Doc XI/919/94

EC (European Comission) (1995): Risk Assessment of New and Existing Substances; Technical Guidance Document

Ellenhorn, M.J., Barceloux, D.G. (1988): Medical Toxicology; Elsevier New York, 906

Eriksson, C.J.P. (1985) Endogenous acetaldehyde in rats. Effects of exogenous ethanol, pyrazole, cyanamide and disulfiram. Biochemical Pharmacology 34:3979-3982

Eriksson, C.J.P. (1987) Human acetaldehyde levels: aspects of current interest. ICPEMC Working Paper Nr. 15/3. Mutat Res 186:235-240

F (1996): Valeurs limites d'exposition professionelle aux agents chimiques en France 1996: INRS - Cahiers de Notes Documentaires ND 1945-153-93, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Farbman, A.I. (1992) Structure in olfactory mucous membrane. In : Cell biology of olfaction. Cambridge University Press, 1992.

Farkas, D., Tannenbaum, S.R. (2005) Characterization of chemically induced hepatoxicity in collagen sandwiches of rat hepatocytes. Tox Sciences 85: 927-934

Fedtke, N., Wiegand, H.-J. (1990): Hydrolysis of vinyl acetate in human blood. Arch Toxicol 64: 428-429

FEICA (2001): Information on exposure, Association of European Adhesive Manufactures

Feron, V.J., Kruysse, A., Woutersoen, R.A. (1982): Respiratory tract tumors in hamsters exposed to acetaldehyde vopour alone or simultaneously to benzo(a)pyrene or diethyl-nitrosame. Eur J Cancer Clin Oncol 18: 13-31

FIN (1996): Workplace Exposure Limits: HTP-Arvot 1996, Turvallisunstiedote 25, Työministerö, Kemian Työsuojeluneuvottelukunta, Tampere 1996, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Finley, B.I., Proctor, D., Scott, P., Harrinton, N., Paustenbach, D., Price, P. (1994): Recommended distributions for exposure factors frequently used in health risk assessment. Risk Anal 14: 533-553

Florin, I., Rutberg, L., Curvall, M., Enzell, C.R. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. Toxicol 18:219-232

Frederick, C.B., Bush, M.L., Lomax, L.G., Black, K.A., Finch, L. et al. (1998): Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharm 152: 211-231

Frederick, C.B., Potter, D.W., Chang-Mateu, M.I., and Anderson, M.E. (1992): A physiologically based pharmacokinetic and pharmacodynamic model to describe the oral dosing of rats with ethyl acrylate and its implications for risk assessment. Toxicol. Appl. PPharmacol. 114, 246-260.

Freiwillige Meldungen an das BgVV (Stand 1996)

Freudenheim, JL, Ambroson, D.B., Moysich, K.B., Vena, J.E., Marshall, J.R., Graham, S., Laughlin, R., Nemoto, T., Shields, P.G. (1997)Alcohol intake and breast cancer risk: effect of alchohol metabolism by alcohol dehydrogenase (Abstract No. 4153). Proc Am Assoc Caner Ris 38:619

Fukunaga, T., Sillanaukee, P., Eriksson, C.J.P. (1993) Problems involved in the determination of endogenous acetaldehyde in human blood. Alcohol & Alcoholism 28:535-541

Gage, J.C. (1970): The subacute inhalation toxicity of 109 industrial chemicals. Brit J Industr Med 27: 1-18

Gale, E.P. (1979): Vinyl acetate. Hazleton Rep. 1979; No. 1840-51/2

Gale, E.P. (1980a): Vinyl acetate. 13-week oral (drinking water) toxicity study in the rat. Hazleton UK; Report No. 2146-51/4

Gale, E.P. (1980b): Vinyl acetate. 13-week oral (drinking water) toxicity study in the mouse. Hazleton UK; Report No. 2146-51/5

Garcia Trejo, A. (1988): Fungal degradation of polyvinyl acetate. Ecotoxicology and Environmental Safety, 16(1), 25-35.

Ginsberg, G., Smolenski, S., Hattis, D., Sonawane, B. (2002) Population distribution of aldehyde dehydrogenase-2 genetic polymorphism: Implications for risk assesment. Regulat Toxicol Pharmacol 36: 297-309

Goldsmith, D.J., Hilton, P.J. (1992): Relationship between intracellular proton buffering capacity and intracellular pH. Kidney Int. 41: 43-49

Green, T., Toghill, A., Lee, R., Moore, R., Foster, J. (2002) The development of forestomach tumors in the mouse following exposure to 2-butoxyethanol by inhalation: studies on the mode of action and relevance to humans. Toxicology 180:257-273

Grosjean E., Grosjean D. (1998): Rate constants for the gas-phase reaction of ozone with unsaturated oxygenates. J. of Chemical Kinetics 30, 21-29. Handbook of Chemistry and Physics (1980 – 1981): 61 ed.

Halvorson, M.R., Noffsinger, J.K., Peterson, C.M. (1993) Studies of whole blood-associated acetaldehyde levels in teetotalers. Alcohol 10:409-413

Hardisty, J.F., Garman, R.H., Harkema, J.R., Lomax, L.G., Morgan, K.T. (1999) Histopathology of nasal olfactory mucosa from selected inhalation toxicity studies conducted with volatile chemicals. Toxicologic Pathology 27:618-627

He, S.M., Lambert, B. (1985): Induction and persistence of SCE-inducing damage in human lymphocytes exposed to vinyl acetate and acetaldehyde in vitro. Mutat Res 158: 201-208

Hedberg, J.J., Hoog, J.O., Nilsson, J.A., Xi, Z., Elfwing, A., Grafstrom, R.C. (2000) Expression of alcohol dehydrogenase 3 in tissue and cultured cells from human oral mucosa. Am J Pathol 157:1745-1755

Hill Top Biolabs. Inc (1995): Delayed contact hypersensitivity study in Guinea pigs (Buehler technique) of vinyl acetate. Project No. 94-8486-21, 01.03.1995

Hinderliter, P.M., Thrall, K.D., Corley, R.A., Bloemen, L.J., Bogdanffy, M.S. (2005): Validation of human Physiologically-Based Pharmacokinetic Model for vinyl acetate against human nasal dosimetry data. Toxicol Science 85: 460-467

Hodgson, A.T., Wooley, J.D., Daisey, J.M. (1992a): Volatile organic chemical emissions from carpets; Indoor Environment Program, Energy and Environment Division, Lawrence Berkeley Laboratory, University of California 1992a

Hodgson, A.T., Wooley, J.D., Daisey, J.M. (1992b): Emission of volatile organic compounds from new carpets measured in a large-scale environmental chamber, Proc Ann Meet - Air waste Manag Assoc; Vol. 5, Paper No 92/79.15

Hoechst AG (1970) Scholz and Weigand. Unpublished report No. 0091/70. 27.2.1970

Hoechst AG (1988): Vinyl acetate, Study of the mutagenic potential in strain TA 102 of Salmonella typhimurium, Hoechst Report No 88.0313, March 28, 1988.

Hoechst AG (1992): Internal calculation of the dep. Product Development /Ecology

Hoechst AG (1993a): Statement from 21.07.1993, Prüfbericht des Deutschen Teppich-Forschungsinstituts (TFI), Aachen, Nr.92/625, 1992

Hoechst AG (1993b): Unpublished data July 21, 1993

Hoechst AG (1994a): Safety Data Sheet, February 15, 1994

Hoechst AG (1994b): Produktinformation Vinylacetat der Abt. Marketing Chemikalien, April, cited in IUCLID "Vinyl acetate"

Hoechst AG (1994c): Product information Vinylacetat of the dep. Marketing Chemicals

Hoechst AG (1995a): Stofferfassungsbogen, März 1995

Hoechst AG (1995b): Occupational health department, personal communication

Holma, B., Lindegren, M., Andersen, J.M., (1977) pH effects on ciliomotility and morphology of respiratory mucosa. Arch Envionmen Health 32: 216-226

Holub, J., Tarkowski, S. (1982): Hepatic content of free sulfhydryl compounds in animals exposed to vinyl acetate. Int Arch Occup Environ Health 51: 185-189

Homann, N., Kärkkäinen, P., Koivisto, T., Nosowa, T., Jokelainen, K., Salaspuro, M., (1997) Effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal tract mucosa. JNCI 89:1692-1697

Hude, W. von der, Behm, C., Gürtler, R., Basler, R. (1988): Evaluation of the SOS chromotest. Mutat Research 203, 81-94

Huff, J.E., Haseman, J.K., Demarini, D.M., Eustis, S., Maronpot, R.R., Peters, A.C., Persing, R.L., Chrisp, C.D., Jacobs, A.C. (1989): Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F1 mice. Envion Health Persp 82: 125-163

Hurtt, M.E., Vinegar, M.B., Rickard, R.W., Cascieri, T.C., Tyler, T.R. (1995): Developmental toxicity of oral and inhaled vinyl acetate in the rat. Fund Appl Toxicol 24: 198-205

Industrial BIO-TEST Labs Inc (1972): Primary skin irritation tests with eighteen materials in albino rabbits with cover letter dated 061589. Report IBT 1972; No. A1854

IPCS (1999): Final draft report of the IPCS workshop on developing a conceptual framework for cancer risk assessment, 16-18 February 1999, Lyon, France, IPCS/99.6

IRL (1997): Irish Exposure Limits: Code of Practice for the Safety, Health and Welfare of Work (Chemical Agents) Regulations, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Irvine, L.F.H. (1980): Vinyl acetate: oral and inhalation teratology studies in the rat. Hazleton UK 1980; Report-No. 2195-51/6-7

Ishidate Jr, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. (1984): Primary mutagenicity screening of food additives currently used in Japan. Fd Chem Toxic 22, 623-636

Ishidate, M. (1988): Data book of chromosomal aberration test in vitro (revised edition). Elsevier, Amsterdam

Isse, T., Oyama, T., Matsuno, K., Ogawa, N., Narai-Suzuki, R., Yamaguchi, T., Murakami, T., Kinaga, T., Uchiyama, I. Kawamoto, T. (2005) Paired acute inhalation test reveals that acetaldehyde toxicity is higher in aldehyde dehydrogenase2 knockout mice than in wild-type mice. J Toxicol Sci 30:329-37

Ittah and Zisman (1992): Evalutation of volatile Allyl Alcohol Derivates for control of snails on cut roses for export. Pestic. Sci. 35, 183-186

IUCLID-Data Set "Vinyl acetate" (Hoechst) 1994

Jantunen, K., Mäki-Paakkanen, J., Norppa, H. (1986): Induction of chromosome aberrations by styrene and vinyl acetate in cultured human lymphocytes: dependence on erythrocytes. Mutation Res 159: 109-116

Jayanth, V.R., Bayne, M.T., Varnes, M.E. (1994) Effects of extracellular and intracellular pH on repair of potentially lethal damage, chromosome abberations and DNA double-strand breaks in irradiated plateau-phase A549 cells. Radiat Res 139:152-162.

JETOC (2004): Mutagenicity Test Data of Existing Chemical Substances. Japan Chemical Industry Ecology-Toxicology and Information Center, Supplement 3, 2004

Joshi S.B., Dodge M.C., Bufalini J.J. (1982). Reactivities of selected organic compounds and contamination effects. Atmos. Environ 16: 1301-1310

Jung, R., Engelhart, G., Herbolt, B., Jäckh, R., Müller, W. (1992): Collaborative study of mutagenicity with Salmonalla typhimurium TA 102. Mutat Res 278: 265-270

Kari, F.W., Bucher, J., Eustis, S.L., Haseman, J.K., Huff, J.E. (1992): Toxicity and carcinogenicity of hydroquinone in F344/N rats and B6C3F1 mice. Fd Chem Toxic 30: 737-747

Kemper, F. (1964): Schädigung des Zahnarztes durch Medikamente und Werkstoffe. DDZ 7: 187-191

Kilgour, J.D., Simpson, S.A., Alexander, D.J., Reed, C.J. (2000) A rat nasal epithelial model for predicting upper respiratory tract toxicity: in vivo-in vitro correlations. Toxicology 145:39-49

Kimbell, J.S., Gross, E.A., Joyner, D.R., Godo, M.N., Morgan, K.T. (1993): Application of computational fluid dynamics to regional dosimetry of inhaled chemicals in the upper respiratory tract of the rat. Toxicol Appl Pharmacol 121: 253-263

Kirby, P.E. (1983): Mouse lymphoma mutagenesis assay with 40171 (ML-NCI 78) Microbiological Associates; NO1-CP-15739

Klaschka, F,, Vossmann, D. (1994): Kontaktallergene: Chemische, klinische und experimentelle Daten (Allergen-Liste). Erich Schmidt Verlag, Berlin 1994

Koivisto, T., Salaspuro, M. (2003) Aldehyde dehydrogenase of the rat colon: Comparison with other tissues of the almintary tract and the liver. Alcoholism: Clinical and Experimental Research 20:551-555

Knowles, S.E., Jarret, I.G., Owen, H.F., Ballard, F.J. (1974): Production and utilization of acetate in mammals. Biochem J 142: 401-411

Kominsky, J.R., Freyberg, R.W. (1992): Exposure to volatile components of polyvinyl acetate (PVA) emulsion paints during application and drying. Prepared for: The National Paint and Coatings Association (NPCA); February 14th, 1992

Korte, A., Obe, G., Ingwersen, I., Rueckert, G. (1981): Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. Mutat Res 88: 389

Kuykendall, J.R., Bogdanffy, M.S. (1992): Reaction kinetics of DNA-histone crosslinking by vinyl acetate and acetaldehyde. Carcinogenesis 13: 2095-2100

Kuykendall, J.R., Taylor, M.L., Bogdanffy, M.S. (1993): Cytotoxicity and DNA-protein crosslink formation in rat nasal tissues exposed to vinyl acetate are carboxylesterase-mediated. Toxicol Appl Pharmacol 123: 283-292

Lähdetie, J. (1988): Effects of vinyl acetate and acetaldehyde on sperm morphology and meiotic micronuclei in mice. Mutat Res 202, 171-178

Laib, R.J., Bolt, H.M. (1986): Vinyl acetate, a structural analog of vinyl carbamate, fails to induce enzyme-altered foci in rat liver. Carcinogenesis 7: 841-843

Lam, C.W., Casanova, M., Heck, H.D. (1986): Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure. Fundam. Appl. Toxicol. 6: 541-550

Lambert, B., Chen, Y., He, S.M., Sten, M. (1985): DNA cross-links in human leucocytes treated with vinyl acetate and acetaldehyde in vitro. Mutat. Res. 146: 301-303

Lantz, R.D., Orozco, J. Bogdanffy, M.S. (2003) Vinyl acetate decreases intracellular pH in rat nasal epithelial cells. Toxicol Sciences 75:423-431

Lewis, J.L., Nikula, J.K., Nowak, R., Dahl, A.R. (1994): Comparative localization of carboxylesterase in F344 rat, Beagle dog, and human nasal tissue. The Anatomical Record 239: 55-64

Lijinsky, W., Andrews, A.W. (1980) Mutagenicity of vinyl compounds in Salmonella typhimurium. Tertatog Carcinog Mutagen 1:259-267

Lijinsky, W., Reuber, M.D. (1983): Chronic toxicity studies of vinyl acetate in Fischer rats. Toxicol Appl Pharmacol 68: 43-53

Lobemeier, C., Tschoetschel, C., Westie, S. and heymann, E. (1996): Hydrolysis of Parabenes by extracts from different layers of human skin. Biol. Chem 377, 647- 651.

Lund, V.J. (1994): Toxicity of nasal respiratory mucosa in humans. Inhalation Toxicol 6: Suppl., 277-287

Mabey W., Mill T. (1978). Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. data 7: 383-415

Mainwaring, G., Foster, J., Lund, V., Green, T. (2001) Methyl methacrylate toxicity in rat nasal epithelium: studies of the mechanism of action and comparisons between species. Toxicology 158: 109-118

Mäki-Paakanen, J., Norppa, H. (1987): Induction of micronuclei by vinyl acetate in mouse bone marrow cells and cultured human lymphocytes. Mutation Res 190: 41-45

Maltoni, C., Ciliberti, A., Cotti, G., Conti, B., Belpoggi, T. (1989): Benzene, an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Persp 82: 109-124

Maltoni, C., Ciliberti, A., Lefemine, G., Soffritti, M. (1997): Results of a long-term experimental study on the carcinogenicity of vinyl acetate monomer in mice. Annals New York Academy of Sciences 837: 209-38

Mattes, P.M., Mattes, W.B. (1992) α-Naphthyl butyrate carboxyesterase activity in human and rat nasal tissue. Toxicol Appl Pharmacol 114:71-76

McCann, J., Choi, E., Yamasaki, E., Ames, B.N. (1975): Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci 72: 5135-5139

McCracken, N.W., Blain, P.G., and Williams, F.M. (1993): Nature and role of xenobiotic-metabolizing esterases in rat liver, lung, skin and blood. Biochem. Pharmacol. 45, 31 – 36.

Mebus, C.A., Carpanini, F.M.B., Rickard, R.W., Cascieri, T.C., Tyler, T.R., Vinegar, M.B. (1995): A two-generation reproduction study in rats receiving drinking water containing vinyl acetate. Fund Appl Toxicol 24: 206-216

Mellon Institute (1969): Range finding toxicity studies. Report 32-99, Sponsor: Union Carbide

Merck Index (1996): Encyclopedia of chemicals, drugs and biologicals; twelfth ed.; Merck & Co., Inc. Whitehouse Station, NJ

Migliore, L., Cocchi, L., Scarpato, R. (1996): Detection of the centromere in micronuclei by fluorescence in situ hybridization: its application to the human lymphocyte micronucleus assay after treatment with four suspected aneugens. Mutagenesis 11, 285-290

Miller, R.R., Ayres, J.A., Jersey, G.C., McKenna, M.J. (1981): Inhalation toxicity of acrylic acid. Fundamental and Applied Pharmacology 1: 271-277

Minardi, F., Belpoggi, B., Soffritti, M. Ciliberti, A., Lauriola, M. Cattin, E., Maltoni, C. (2002) Results of long-term carcinogenicity bioassay on vinyl acetate monomer in Sprague-Dawley rats. Ann NY Acad Sci 982:106-122

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S.-I., Shimada, H., Sutou, S., Suzuki, T., Wakata, A., Sofuni, T., Hayashi, M. (1997): Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Group 1,2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS·MMS. Mutation Res 389: 3-122

Morita, T., Takeda, K., Okumura, K. (1990): Evaluation of clastogenicity of formic acid, acetic acid and lactic acid on cultured mammalian cells. Mutation Res 240: 195-202

Morris, J.B. (1997): Uptake of acetaldehyde vapour and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster, and guinea pig. Fundam Appl Toxicol 35: 91-100

Morris, J.B., Hassett, N.D., Blanchard, K.T. (1993): A physiologically based pharmacokinetic model for nasal uptake and metabolism of nonreactive vapours. Toxicol Appl Pharmacol 123: 120-129

Mukerjee N, Pietruszko R (1992) Human mitochondrial aldehyde dehydrogenase substrate specificity: comparison of esterase with dehydrogenase reaction. Arch. Biochem. Biophys. 299: 23-29.

Morris, J.B., Symanowicz, P., Sarangapani, R. (2002): Regional distribution and kinetics of vinyl acetate hydrolysis in oral cavity of the rat and mouse. Toxicol. Lett. 126: 31-39

Mustonen, R., Kangas, J., Vuojolahti, P., Linnainmaa, K. (1986): Effects of phenoxyacetic acids on the induction of chromosome aberration in vitro and in vivo. Mutagenesis 1: 241-245

Myhr, B.C. (1991): Validation studies with Muta™ mouse: A transgenic mouse model for detecting mutations in vivo. Environ Molec Mutag 19 (Suppl. 2), 2-141

Nakamoto, T., Wagner, M., Melvin, J.E., Bogdanffy, M.S. (2005) Vinyl acetate induces intracellular acidification inmouse oral buccal epithelial cells. Tox Letters 158:116-121

Nersesyan, A.K., Kukumadzhyan, V.A., Zil'fyan, V.N. (1990): Evaluation of activity of some chemical substances being in use in the industry of Armenia. Biol Zh Arm 9: 796-797

Nieder et al (1990) Degradation of vinyl acetate by soil, sewage sludge, and the newly isolated aerobic bacterium V2. Appl. Environ. Microbial 56: 3023-3028

NL (1999): De Nationale MAC-lijst 1999: Ministerie van Sociale Zaken en Werkgelegenheid, Sdu Uitgevers, Den Haag 1999, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

NO (1996): Administrative Norms for Contaminations in the Workplace 1996: Arbeidstilsynet best. nr. 361, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Norppa, H., Tursi, F., Pfäffli, P., Mäki-Paakkanen, J., Järventaus, H. (1985): Chromosome damage induced by vinyl acetate through in vitro formation of acetaldehyde in human lymhocytes and Chinese hamster ovary cells. Cancer Res 45: 4816-4821

Norwegian Product Register (from 16.12.1994)

NTP (1986): National Toxicology Program. Toxicology and carcinogenesis studies of benzene (CASl No. 71-43-2) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park, North Carolina, U.S. Department of Health and Human Services (NTP-TR 289; NIH Publication 86-2545)

Obe, G., Natarajan, A.T., Meyers, M., Den Hertog, A. (1979) Induction of chromosomal aberrations in peripheral lymphocytes of human blood in vitro, and of SCEs in bone-marrow cells of mice in vivo by ethanol and its metabolite acetaldehyde. Mutation Res. 68: 291-294

Ohta, Y., Ichimura, K.(2001) Globosal basal cells are identified as proliferating cells in mouse olfactory epithelium. Ann Otol Thinol Laryngol 110: 53-55

Olson, M.J., Martin, J.L., Larosa, A.C., Brady, A.N., Pohl, L.R. (1993) Immunohistochemical localisation of carboxylesterase in the nasal mucosa of rats. J Histochem Cytochem 41:307-311.

Ott, M.G., Teta, M.J., Greenberg, H.L. (1989): Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. Am J Ind Med 16: 631-643

Owen, P.E. (1979a): Vinyl acetate: 4 week inhalation dose range finding study in the mouse. Hazleton Lab Europe; Report-No. 1884-51/3

Owen, P.E. (1979b): Vinyl acetate: 4 week inhalation dose range finding study in the rat. Hazleton Lab Europe; Report-No. 1835-51/3

Owen, P.E. (1980a): Vinyl acetate: 3 month inhalation toxicity study in the mouse. Hazelton Lab Europe; Report No. 2303-51/5

Owen, P.E. (1980b): Vinyl acetate: 3 month inhalation toxicity study in the rat. Hazleton Lab Europe; Report-No. 2286-51/5

Owen, P.E. (1988): Vinyl acetate: 104 week inhalation combined chronic toxicity and carcinogenicity study in the rat and mouse. Hazleton UK; Report-No. 5547-51/15

Pahren H.R., Bloodgood D.E. (1961) Biological oxidation of several vinyl compounds. J. Water Pollut. Contr. Fed. 33: 233-238

Parker (1983): The effects of selected chemicals and water quality on the marine polychaete (Ophryotrocha diadema). Water Research 18, 865-868

Pickering and Henderson (1966): Acute toxicity of some important petrochemicals to fish.
J. Water Pollut. Contr. Fed. 38, 1419-1429

Plowchalk, D.R., Andersen, M.B., Bogdanffy, M.S. (1996): Biologically-based modeling of intracellular adidification of nasal epithelial cells induced by vinyl acetate exposure. The Toxicologist 15: 250

Plowchalk, D.R., Andersen, M.E., Bogdanffy, M.S. (1997): Physiologically based modelling of vinyl acetate uptake, metabolism, and intracellulur pH changes in the rat nasal cavity. Toxicol Appl Pharmacol 142: 386-400

Potter, P.M., Wolverton, J.S., Morton, C.L., Whipple, D.O., Danks, M.K.(1998) In situ subcellular localization of epitope-tagged human and rabbit carboxylesterases. Cytometry 32:223-232

Pronko, P., Bardina, L., Satanovskaya, V., Kuzmich, A., Zimatkin, S. (2002) Effect of chronich alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. Alcohol and Alcoholism 37:229-235

RCC (2003a) Vinylacetate (stab.): Primary skin irritation study in rabbits (4-hour semi-occlusive application). Unpublished RCC report No. 846923, 29 April 2003

RCC (2003b) Reference Vinylacetate (CAS 108-05-4): Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study Number 84925; February 2003

RCC (2003c) Vinylacetate (Stab.): Primary eye irritation study in rabbits. Unpublished RCC report No. 846924, May 2003

Price et al. (1974): Brine shrimp bioassay and seawater BOD of petrochemicals, J. Water Pollut. Contr. Fed. 46, 63-77

Price K.S., Waggy G.T., Conwa R.A. (1974) Brine shrimp bioassay and seawater BOD of petrochemicals. J. Water Pollut. Contr. Fed. 46: 63-77

Reed, C.J. (1997): In vitro models of nasal cavity toxicity. Mut Res 380: 97-111

Regulations to amend the Regulations on Specific Commodities under the Foods Act, (1994): Bundesgesetzblatt I: 775

Ristow, H., Obe, G. (1975): Acetaldehyde induces cross-links in DNA and causes sister-chromatid exchanges in human cells. Mutation Res 58: 115-119

Robinson, D.A., Bogdanffy, M.S., Reed, C.J. (2002) Histochemical localisation of carboxylesterase activity in rat and mouse oral cavity mucosa. Toxicology 180:209-220

S (1999): Occupational Exposure Limit Values 1996: Arbetarskyddsstyrelsen Hygieniska Gränsvärden, AFS 1996:2, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Sarangapani, R. (2001): A biologically-based risk assessment for vinyl acetate from oral exposure. A status report of the research conducted by the K.S. Crump Group Inc., ICF Consulting, Inc., Research Triangle Park, NC

Satoh, T., Hosokawa, M. (1995) Molecular aspects of carboxylesterase isoforms in comparison with other esterases. Tox Letters 83/83:439-445

Saunders, S. M., Baulch, D. L., Cooke, K. M., Pilling, M. J., and Smurthwaite, P. I. (1993): Kinetics and mechanisms of the reactions of OH with some oxygenated compounds of importance in tropospheric chemistry. International Journal of Chemical Kinetics 26(1): 113-30.

SCCNFP (2004): Opinion of the Scientific Committee on cosmetic products and non-food products intended for consumers concerning acetaldehyde. Adopted by the SCCNFP during the 28th plenary meeting of 25 May 2004, SCCNFP/0821/04

Sreerama, L., Hedge, M., and Sladek, N. (1995): Identification of a class 3 aldehyde dehydrogenase in human saliva and increased levels of this enzyme, glutathione S-transferases, and DT-diaphorases in the saliva of subjects who continually ingest large quantities of coffee or broccoli. Clin. Cancer Res.1, 1153-1163.

Shirinian, G.S., Arutyunyan, R.M. (1980) Study of cytogenetic change levels under PVA production. Biol Z Armenii XXXIII, 7: 748-752

Schwob, J.E., Youngentob, S.L., Mezza, R.C. (1995) Reconstitution of the rat olfactory epithelium after methyl bromide-induced lesion. J Comp Neurol 359:15-37

Scientific Committee (1994): General scheme for determining the safety margin of preservatives used in cosmetic products

Shaw, D.C. (1987): Vinyl acetate: (drinking water) 2-generation reproduction study in the rat. Hazleton UK; Report-No. 4661-51/17a

Shaw, D.C. (1988): Vinyl acetate: 104 week oral (drinking water) combined chronic toxicity and carcinogenicity study in the rat following in utero exposure, Vol. I, II, III. Hazleton UK; Report-No. 5531-51/16

SIDS Dossier (1994): (Written Review Procedure for SIDS Dossier with Testing Plan), US EPA, 13.10.1994.

Simon, P., Filser, J.G., Bolt, H.M. (1985a): Metabolism and pharmacokinetics of vinyl acetate. Arch Toxicol 57: 191-195

Simon, P., Ottenwälder, H., Bolt, H.M. (1985b): Vinyl acetate: DNA-binding assay in vivo. Toxicol; Lett 27: 115-120

Sipi, P., Järventaus, H., Norppa, H. (1992): Sister-chromatid exchanges induced by vinyl esters and respective carboxylic acids in cultured human lymphocytes. Mutation Res 279: 75-82

Soffritti, M., Belpoggi, F., Lambertini, L., Lauriola, M., Padovani, M., Maltoni, C. (2002): Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann NY Acad Sci 982:87-105

Souza, R.F., Shewmake, K., Terada, L.S., Spechler, S.J. (2002) Acid exposure activates the mitogen-activated protein kinase pathways in Barrett's esophagus. Gastroenterology 122: 299-307

SRI International (1997/98): Directory of Chemical Producers Western Europe, Volume 2

St. Clair, M.B.G., Morgan, K.T. (1992): Changes in the upper respiratory tract. In: Pathobiology of the aging rat. Mohr U, Dungworth DL, Capen CC (eds) ILSI Press 1992; 111-127

Stanek, J.J., Morris, J.B. (1999) The effect of inhibition of aldehyde dehydrogenase on nasal uptake of inspired acetaldehyde. Toxicol Sciences 49:225-231

Stott, W.T., Dryzga, M.D., Ramsey, J.C. (1983): Blood-flow distribution in the mouse. J Appl Toxicol 3: 310-312

Strong, H.A., Cresswell, D.G., Hopkins, R. (1980): Investigations into the metabolic fate of vinyl acetate in the rat and mouse. Report No. 2511-51/11-14, Part 2, Hazleton Laboratories Europe

Stuckey D.C., Owen W.F., McCarty P.L., et al. (1980). Anaerobic toxicity evaluation by batch and semi-continuous assays. J. Water Poll. Control fed. 52: 720-729

Swedish Product Register (February 1995)

Tabata, T., Katoh, M., Tokudome, S., Nakajima, M., Yokoi, T. (2004) Identification of the cytosolic carboxylesterase catalyzing the 5'-deoxy-5-fluorocytidine formation from capecitabine in human liver. Drug Metabolism and Disposition 32:1103-1110

Takeshita, T., Iijima, S., Higurashi, M. (1986): Vinyl-acetate induced sister chromatid exchanges in murine bone marrow cells. Proc Japan Acad 62: 239-242

Thomson, P.J., Potten, C.S., Appleton, D.R. (1999) Mapping dynamic epithelial cell proliferative activity within the oral cavity of man: a new insight into carcinogenesis? British Journal of Oral and Maxillofacial Surgery 37: 377-383

Til, H.P., Woutersen, R.A., Feron, V.J. (1988): Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. Fd Chem Toxic 26: 447-452

UK (1999): Occupational Exposure Limits 1999: HSE, Health and Saftey Executive EH 40/99, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Ullmann (1998): Ullmann's Encyclopedia of industrial chemistry, Sixth Ed., Electronic Rel., Wiley-VCH, Weinheim, 1998

Umbach, W. (1995): Kosmetik, Thieme-Verlag Stuttgart, 282-283

Umeda, Y., Matsumoto, M., Yamazaki, K., Ohnishi, M., Arito, H., Nagano, K., Yamamoto, S., Matsushim, T., (2004a) Carcinogenicity and chronic toxicity in mice and rats administered vinyl acetate monomer in drinking water. J. Occup. Health 46:87-99

Umeda, Y., Matsumoto, M., Yamazaki, K., Ohnishi, M., Arito, H., Nagano, K., Yamamoto, S., Matsushim, T., (2004b) Response to Dr. Bogdanffy et al.'s comments on our paper. J. Occup. Health 46:250-251

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and S-formylglutathione hydrolase activities in different rat tissues. Adv. Exp. Med. Biol. 414: 365-371.

USA (1997): TLVs (Threshold Limit Values) and BEIs (Biological Exposure Indices): ACGIH, Cincinnati, 1997, cited in Ariel Insight V.1.2 Database, Ariel Research Corporation, updated Aug. 1999

Valentine, R., Bamberger, J.R., Szostek, S.R., Frame, J.F., Hansen, J.F., Bogdanffy, M.S. (2002): Time- and concentration-dependent increases in cell proliferation in rats and mice administered vinyl acetate in drinking water. Toxicol. Sci. 67: 190-197

VATG (1994): Vinyl Acetate Toxicology Group, OECD SIDS Dossier "Vinyl Acetate" 1994

Velvart, J. (1993): Toxikologie der Haushaltsprodukte, 3. Auflage, Verlag Hans Huber, Bern

Voullaire, E., Kliemt, G. (1995): Gefahrstoffe in Klein- und Mittelbetrieben: Neue Wege überbetrieblicher Unterstützung, Fb 703, Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Wirtschaftsverlag NW, Bremerhaven

Wacker Chemie (2002): Vinyl acetat in the value chain.

Wacker-Chemie (1995b): Department L-Q, Erhebung zum Kontakt mit Vinylacetat (VAM). Personal Communication (13. Oktober 1995)

WackerChemie (1995a): Department L-Q, Personal Communication (1. Juni 1995)

WackerChemie (1993): Laborbericht, Abt. L 1993; Nr. 415

Waxweiler, R.J., Smith, A.H., Falk, H., Tyroler, H.A. (1981): Excess lung cancer risk in a synthetic chemicals plant. Environ Health Perspect 41: 159-165

Wouterson, R.A., Appelman, L.M., Feron, V.J., Van der Heijden, C.A. (1984): Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: interim results after 15 months. Toxicology 31: 123-133

Wouterson, R.A., Appelman, L.M., Van Garderen-Hoetmer, A., Feron, V.J. (1986): Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41: 213-231

Wouterson, R.A., Feron, V.J. (1987): Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47: 295-305

Yamahara, H., Lee, V.H.L. (1993): Drug metabolism in the oral cavity. Advanced Drug Delivery Reviews 12: 25-40

Yin, S., Liaou, C., Wu, C. et al. (1997): Human stomach alcohol and aldehyde dehydrogenases: comparison of expression pattern and activities in alimentary tract. Gastroenterology 112: 766-775

Yokoyama, A., Muramatsu, T., Ohmori, T., Higuchi, S., Hayashida, M., Ishii, H. (1996) Esophageal cancer and aldehyde dehydrogenase-2 genotypes in Japanese males. Cancer Epidemiol Biomarkers Prev 5:99-102

Zeh, H., Kohlhammer, K, Krell, M (1994): VOC-emissions from latex paints and plasters during application. Surface Coatings International 4: 142-152

Zeh, H. (2000): The determination of VOC emisions from latex paints during and after application; methods, performance and precision. Surface coatings International 3: 111.118

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. (1992): Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Molec Mutag 19 (Suppl. 2), 2-141

Zenz, C. Cordaso, E.M. (1994): Vinyl acetate, Other important and widely encountered chemicals; Occupational medicine; 3rd ed.; C. Zenz, O.B. Dickerson, E.P. Horvath, Jr., Editors; Mosby-Year Book, St. Louis, page 710in

Zhang, X., Zhang, Q-Y., Liu, D., Su, T., Weng, Y., Ling, G., Chen, Y., Gu, J., Schilling, B. Ding, X. (2005) Expression of cytochrome P450 and other biotransformation genes in fetal and adult human nasal mucosa. Drug Metabolism and Disposition 33: 1423-1428

Zissu, D. (1995) Histopathological changes in the respiratory tract of mice exposed to ten families of airborne chemicals. J Appl Toxicol 15:207

7 ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient

Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration

pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum

UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

The report provides the comprehensive risk assessment of the substance vinyl acetate. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified

There is concern for the terrestrial environment due to processing of vinyl acetate. There is no concern for the other environmental compartments due to the production and use of vinyl acetate.

For human health, there is concern for workers, in terms of cancer risks and repeated dose toxicity, but neither for consumers, nor for humans exposed via the environment

Appendix I

of the Risk Assessment Report

Vinyl acetate

CAS-No.: 108-05-4

Estimation of Abiotic Degradation

April 2005

AOP V1.91 ESTIMATION OF ABIOTIC DEGRADATION

EstimateSMILES : C=COC(=O)C
CHEM : C:\EPISUITE\VAM.mol
MOL FOR: C4 H6 O2
MOL WT : 86.09

SUMMARY (AOP v1.91): HYDROXYL RADICALS
Hydrogen Abstraction = 0.0422 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds = 26.3000 E-12 cm3/molecule-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 26.3422 E-12 cm3/molecule-sec
HALF-LIFE = 1.218 Days (12-hr day; 0.5E6 OH/cm3)
HALF-LIFE = 14.617 Hrs
** Designates Estimation(s) Using ASSUMED Value(s)

SUMMARY (AOP v1.91): OZONE REACTION
OVERALL OZONE Rate Constant = 0.175000 E-17 cm3/molecule-sec
HALF-LIFE = 6.549 Days (at 7E11 mol/cm3)

Experimental Database Structure Match:
Chem Name : Vinyl acetate
CAS Number: 000108-05-4
Exper OH rate constant : 25 E-12 cm3/molecule-sec
Exper OH Reference: (Saunders,S.M. et al.,1993)
Exper Ozone rate constant: 3.2 E-18 cm3/molecule-sec
Exper NO3 rate constant : --- cm3/molecule-sec

Hydrogen Abstraction Calculation:
Kprim = 0.136 F(-C(=O)OR)=0.136(0.310)= 0.042
H Abstraction TOTAL = 0.042 E-12 cm3/molecule-sec

OH Addition to Olefinic Bonds Calculation:
Kd = K(CH2=CH-)C(-O **)= 26.300(1.00) = 26.300 E-12 cm3/molecule-sec
ASSUMED Values designated by: **

Ozone Reaction with Olefins Calculation:
Ko = K(CH2=CH-R)O x(-Default Value **)= 0.175000(1.000) = 0.175000 E-17 cm3/molecule-sec
ASSUMED Values designated by: **

Appendix II

of the Risk Assessment Report

Vinyl acetate

CAS-No.: 108-05-4

Estimation of Biodegradability

April 2005

BIOWIN V4.00 ESTIMATION OF BIODEGRADABILITY

SMILES : C=COC(=O)C
CHEM : vinyl acetate
---------- BIOWIN v4.00 Results ----------
Linear Model Prediction: **Biodegrades Fast**
Non-Linear Model Prediction**: Biodegrades Fast**
Ultimate Biodegradation Timeframe: Weeks
Primary Biodegradation Timeframe: Days
MITI Linear Model Prediction: **Readily Degradable**
MITI Non-Linear Model Prediction: **Readily Degradable**

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	0.1742	0.1742
MolWt	*	Molecular Weight Parameter		-0.0410
Const	*	Equation Constant		0.7475
RESULT		LINEAR BIODEGRADATION PROBABILITY		0.8807

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	4.0795	4.0795
MolWt	*	Molecular Weight Parameter		-1.2225
RESULT		NON-LINEAR BIODEGRADATION PROBABILITY		0.9972

A Probability Greater Than or Equal to 0.5 indicates: Biodegrades Fast
A Probability Less Than 0.5 indicates: Does NOT Biodegrade Fast

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	0.1402	0.1402
MolWt	*	Molecular Weight Parameter		-0.1903
Const	*	Equation Constant		3.1992
RESULT		SURVEY MODEL - ULTIMATE BIODEGRADATION		3.1491

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	0.2290	0.2290
MolWt	*	Molecular Weight Parameter		-0.1242
Const	*	Equation Constant		3.8477

RESULT | SURVEY MODEL - PRIMARY BIODEGRADATION | | 3.9525

Result Classification: 5.00 -> hours 4.00 -> days 3.00 -> weeks

(Primary & Ultimate) 2.00 -> months 1.00 -> longer

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	0.3437	0.3437
Frag	1	Methyl [-CH3]	0.0004	0.0004
Frag	3	-C=CH [alkenyl hydrogen]	0.0062	0.0186
MolWt	*	Molecular Weight Parameter		-0.2561
Const	*	Equation Constant		0.7121

RESULT | MITI LINEAR BIODEGRADATION PROBABILITY | | 0.8187

TYPE	NUM	BIOWIN FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Ester [-C(=O)-O-C]	2.4462	2.4462
Frag	1	Methyl [-CH3]	0.0194	0.0194
Frag	3	-C=CH [alkenyl hydrogen]	0.0285	0.0855
MolWt	*	Molecular Weight Parameter		-2.4853

RESULT | MITI NON-LINEAR BIODEGRADATION PROBABILITY | | 0.9303

A Probability Greater Than or Equal to 0.5 indicates --> Readily Degradable

A Probability Less Than 0.5 indicates --> NOT Readily Degradable

Appendix III

of the Risk Assessment Report

Vinyl acetate

CAS-No.: 108-05-4

Distribution and fate

April 2005

Distribution and Fate

time	$d := Tag$
melting point:	$MP := 180 \cdot K$
vapour pressure:	$VP := 12000 \cdot Pa$
water solubility:	$SOL := 20000 \cdot mg \cdot l^{-1}$
part. coefficient octanol/water:	$LOGP_{OW} := 0.7$
molecular weight:	$MOLW := 0.086 \cdot kg \cdot Mol^{-1}$
gas constant:	$R := 8.3143 J \cdot Mol^{-1} \cdot K^{-1}$
temperature:	$T := 293 \cdot K$
conc. of suspended matter in the river:	$SUSP_{water} := 15 \cdot mg \cdot l^{-1}$
density of the solid phase:	$RHO_{solid} := 2500 \cdot kg \cdot m^{-3}$
volume fraction water in susp. matter:	$Fwater_{susp} := 0.9$
volume fraction solids in susp.matter:	$Fsolid_{susp} := 0.1$
volume fraction of water in sediment:	$Fwater_{sed} := 0.8$
volume fraction of solids in sediment:	$Fsolid_{sed} := 0.2$
volume fraction of air in soil:	$Fair_{soil} := 0.2$
volume fraction of water in soil:	$Fwater_{soil} := 0.2$
volume fraction of solids in soil:	$Fsolid_{soil} := 0.6$
aerobic fraction of the sediment comp.:	$Faer_{sed} := 0.1$
product of CONjunge and $SURF_{air}$:	$product := 10^{-4} \cdot Pa$

Distribution air / water: Henry-constant

$$HENRY := \frac{VP \cdot MOLW}{SOL} \qquad HENRY = 51.6 \cdot Pa \cdot m^3 \cdot Mol^{-1}$$

$$\log\left(\frac{HENRY}{Pa \cdot m^3 \cdot Mol^{-1}}\right) = 1.713$$

$$K_{air\_water} := \frac{HENRY}{R \cdot T} \qquad K_{air\_water} = 0.021$$

Solid / water-partition coefficient Kpcomp and
total compartment/water-partition coefficient Kcomp_water

$a := 0.52$ (a,b from TGD, p. 541 for nonhydrophobics)

$b := 1.02$ $K_{OC} := 10^{a \cdot LOGP_{OW} + b} \cdot l \cdot kg^{-1}$ $K_{OC} = 24.21 \cdot l \cdot kg^{-1}$

Suspended matter

$Kp_{susp} := 0.1 \cdot K_{OC}$ $Kp_{susp} = 2.421 \cdot l \cdot kg^{-1}$

$K_{susp\_water} := Fwater_{susp} + Fsolid_{susp} \cdot Kp_{susp} \cdot RHO_{solid}$ $K_{susp\_water} = 1.505$

factor for the calculation of $Clocal_{water}$:

$faktor := 1 + Kp_{susp} \cdot SUSP_{water}$ $faktor = 1$

Sediment

$Kp_{sed} := 0.05 \cdot K_{OC}$ $Kp_{sed} = 1.211 \cdot l \cdot kg^{-1}$

$K_{sed\_water} := Fwater_{sed} + Fsolid_{sed} \cdot Kp_{sed} \cdot RHO_{solid}$ $K_{sed\_water} = 1.405$

Soil

$Kp_{soil} := 0.02 \cdot K_{OC}$ $Kp_{soil} = 0.484 \cdot l \cdot kg^{-1}$

$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + Fwater_{soil} + Fsolid_{soil} \cdot Kp_{soil} \cdot RHO_{solid}$

$K_{soil\_water} = 0.931$

Sludge (activated sludge)

$K_{p\_sludge} := 0.37 \cdot K_{OC}$ $K_{p\_sludge} = 8.958 \cdot l \cdot kg^{-1}$

Raw sewage

$K_{p\_sewage} := 0.30 \cdot K_{OC}$ $K_{p\_sewage} = 7.263 \cdot l \cdot kg^{-1}$

Elimination in STPs

rate constant in STP: k = 1 h^{-1}

elimination P = f (k, logpow, logH) = 90 %

fraction directed to surface water $Fstp_{water}$ = 0,1

Biodegradation in different compartments

surface water

$kbio_{water} := 0.047 \cdot d^{-1}$ (TGD, table 5)

soil

$DT50bio_{soil} := 30 \cdot d$ (TGD, table 6)

$kbio_{soil} := \frac{\ln(2)}{DT50bio_{soil}}$ $kbio_{soil} = 0.023 \cdot d^{-1}$

sediment

$kbio_{sed} := \frac{\ln(2)}{DT50bio_{soil}} \cdot Faer_{sed}$ $kbio_{sed} = 2.31 \cdot 10^{-3} \cdot d^{-1}$

Degradation in surface waters

$khydr_{water} := 0.0407 \cdot d^{-1}$

$kphoto_{water} := 1 \cdot 10^{-10} \cdot d^{-1}$

$kdeg_{water} := khydr_{water} + kphoto_{water} + kbio_{water}$

$kdeg_{water} = 0.088 \cdot d^{-1}$

Atmosphere

calculation of CONjunge * SURFaer for the OPS-model

$VPL := \frac{VP}{\exp\left[6.79 \cdot \left(1 - \frac{MP}{285 \cdot K}\right)\right]}$ $VP := wenn(MP > 285 \cdot K, VPL, VP)$ $VP = 1.2 \cdot 10^{4} \cdot Pa$

$Fass_{aer} := \frac{product}{VP + product}$

$Fass_{aer} = 8.333 \cdot 10^{-9}$

degradation in the atmosphere

$kdeg_{air}$ = 0,0475 h^{-1} (see AOP-calculation)

Appendix IV

of the Risk Assessment Report

Vinyl acetate

CAS-No.: 108-05-4

Continental and regional exposure

May 2006

SimpleBox2.0a INPUT - Vinyl acetate

	Unit	Input	
Physicochemical properties			
COMPOUND NAME	[-]	Vinyl acetate	Substance
MOL WEIGHT	[g.mol^{-1}]	86	Molecular weight
MELTING POINT	[° C]	-93.2	Melting Point
VAPOR PRESSURE(25)	[Pa]	12000	Vapour pressure at 25°C
log Kow	[log10]	0.7	Octanol-water partition coefficient
SOLUBILITY(25)	[mg.l^{-1}]	20000	Water solubility
Distribution - Partition coefficients			
- Solids water partitioning (derived from K_{oc})			
Kp(soil)	[l.kg$_d^{-1}$]	0.484	Solids-water partitioning in soil
Kp(sed)	[l.kg$_d^{-1}$]	1.211	Solids-water partitioning in sediment
Kp(susp)	[l.kg$_d^{-1}$]	2.421	Solids-water partitioning in sudpended matter
- Biota-water			
BCF(fish)	[l.kg$_w^{-1}$]	3.16	Biocentration factor for aquatic biota
Degradation and Transfromation rates			
- Characterisation and STP			
PASSreadytest	[y / n]	y	Characterization of biodegradability
- Environmental Total Degradation			
kdeg(air)	[d^{-1}]	1.14E+00	Rate constant for degradation in air
kdeg(water)	[d^{-1}]	8.80E-02	Rate constant for degradation in bulk surface water
kdeg(soil)	[d^{-1}]	2.30E-02	Rate constant for degradation in bulk soil
kdeg(sed)	[d^{-1}]	2.31E-03	Rate constant for degradation in bulk sediment
Sewage treatment (e.g. calculated by SimpleTreat)			
- Continental			
FR(volatstp) [C]	[-]	1.16E-01	Fraction of emission directed to air (STPcont)
FR(effstp) [C]	[-]	1.00E-01	Fraction of emission directed to water (STPcont)
FR(sludgestp) [C]	[-]	2.00E-03	Fraction of emission directed to sludge (STPcont)
- Regional			
FR(volatstp) [R]	[-]	1.16E-01	Fraction of emission directed to air (STPreg)
FR(effstp) [R]	[-]	1.00E-01	Fraction of emission directed to water (STPreg)
FR(sludgestp) [R]	[-]	2.00E-03	Fraction of emission directed to sludge (STPreg)
Release estimation			
- Continental			
Edirect(air) [C]	[t.y^{-1}]	2879,52	Total continental emission to air
STPload [C]	[t.y^{-1}]	364.78	Total continental emission to wastewater
Edirect(water1) [C]	[t.y^{-1}]	0	Total continental emission to surface water
Edirect(soil3) [C]	[t.y^{-1}]	0	Total continental emission to industrial soil
Edirect(soil2) [C]	[t.y^{-1}]	0	Total continental emission to agricultural soil
Edirect(air) [R]	[t.y^{-1}]	319,95	Total regional emission to air
STPload [R]	[t.y^{-1}]	40.53	Total regional emission to wastewater
Edirect(water1) [R]	[t.y^{-1}]	0	Total regional emission to surface water
Edirect(soil3) [R]	[t.y^{-1}]	0	Total regional emission to industrial soil

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Edirect(soil2) [R]	[$t.y^{-1}$]	0	Total regional emission to agricultural soil

SimpleBox2.0a OUTPUT - Vinyl acetate

Physicochemical properties

COMPOUND NAME	[-]	Vinyl acetate	Substance

Output

- Continental

PECsurfacewater (total)	[$mg.l^{-1}$]	9,22E-07	Continental PEC in surface water (total)
PECsurfacewater (dissolved)	[$mg.l^{-1}$]	9,22E-07	Continental PEC in surface water (dissolved)
PECair	[$mg.m^{-3}$]	1,87E-06	Continental PEC in air (total)
PECagr.soil	[$mg.kg_{wwt}^{-1}$]	1,10E-07	Continental PEC in agricultural soil (total)
PECporewater agr.soil	[$mg.l^{-1}$]	2,02E-07	Continental PEC in pore water of agricultural soils
PECnat.soil	[$mg.kg_{wwt}^{-1}$]	4,64E-08	Continental PEC in natural soil (total)
PECind.soil	[$mg.kg_{wwt}^{-1}$]	4,64E-08	Continental PEC in industrial soil (total)
PECsediment	[$mg.kg_{wwt}^{-1}$]	9,51E-07	Continental PEC in sediment (total)

- Regional

PECsurfacewater (total)	[$mg.l^{-1}$]	8,33E-06	Regional PEC in surface water (total)
PECsurfacewater (dissolved)	[$mg.l^{-1}$]	8,33E-06	Regional PEC in surface water (dissolved)
PECair	[$mg.m^{-3}$]	9,67E-06	Regional PEC in air (total)
PECagr.soil	[$mg.kg_{wwt}^{-1}$]	9,05E-07	Regional PEC in agricultural soil (total)
PECporewater agr.soil	[$mg.l^{-1}$]	1,66E-06	Regional PEC in pore water of agricultural soils
PECnat.soil	[$mg.kg_{wwt}^{-1}$]	2,40E-07	Regional PEC in natural soil (total)
PECind.soil	[$mg.kg_{wwt}^{-1}$]	2,40E-07	Regional PEC in industrial soil (total)
PECsediment	[$mg.kg_{wwt}^{-1}$]	8,62E-06	Regional PEC in sediment (total)

Appendix:

MTD exceeded in vinyl acetate studies?

The question has been raised whether the highest concentrations/doses tested exceed the maximum tolerated dose (MTD).

The MTD has been exceeded if a test concentration induce the following effects:
Significantly reduced body weight (BW) gain (reduction of ≥10%) as sign of nonspecific toxicity or as consequence of systemic/specific toxic effects

- Treatment-related mortalities, associated with significantly reduced life spans in long-term studies
- Indications on severe systemic toxicity from clinical, functional, laboratory or morphological examinations

The following repeated dose studies with inhalation and oral exposure to vinyl acetate have been analysed:
(Sequences of studies analogue to RAR Chapters on Repeated dose toxicity/Inhalation studies and Carcinogenicity/Inhalation studies)

Reference	**Treatment-related Mortalities**	**Mean BW/gain reduction**	**% Decrease in final BW or BW gain**	**Food and water consumption estimated/ reduced**	**Respiratory tract toxicity**	**Clinical signs of toxicity**	**Organ toxicity other than Resp Tract**	**Indicated that doses exceed MTD?**
				Inhalation route				
Gage, 1970 3 w study on <u>rats</u> 0, 100, 250, 630, 2000 ppm (shortly summerized data only)	No incidental deaths in rats	Low BW gain in M/f 2000 ppm & F≥250 ppm	No quantitative data	No data	2000 ppm alveolar histiocytosis	2000 ppm: eye/nose irritation, respiratory difficulties	No indication from necropsy (organs examined histopathologically unknown)	No (no indication on systemic toxic effects, degree & relevance of low BW gain unknown, no data on food consumption)
Owen, 1979a,b 4 wk range finding studies on <u>mice & rats</u> 0, 150, 500,	No incidental deaths in rats and mice	Significant lower BW gain in m/f mice at 1000 ppm Non-significant	No tables or figures available	No data	No data	Mice ≥150 ppm & rats ≥500 ppm intermittent respir distress , hunched	No indication from necropsy, lower spleen weights in male mice at 1500	No (no indication on systemic toxicity, relevance of

1000, (50/)1500 ppm		decrease in BW gain in rats up to 1500 ppm				posture	ppm and male rats at ≥1000 ppm (no histopathology)	lower BW gain questionable since no data on food consumption, Low BW gain at concentration above those inducing respiratory distress)
Bogdanffy et al., 1997 1,5 or 20 d (cell proliferation) studies on <u>rats</u> 0, 50, 200, 600, 1000 ppm	No incidental deaths	Reduced mean BW at 1000 ppm day 3 to day 26	-11% at the end of study, max –14% in week 5	No data	Olfactory degeneration ≥600 ppm, minimal degenration in respiratory mucosa at 1000 ppm	No effect observed/reported	No data	No (absence of mortalities, no indication of systemic toxicity, reduced BW gain at dose above those inducing nasal toxicity, no data on food consumption)
Owen, 1980 a,b 90 day studies on <u>rats & mice</u> 0, 50, 200, 1000 ppm	*<u>Rat</u>*: One incidental death in a control rat, *<u>Mic</u>*e: in 6 f and 3 m of high dose, 2 m controls, all died during blood sampling procedure	*<u>Rats & mice</u>*: Lower mean final BW and significantly educed BW gain at 1000 ppm	*<u>Rats:</u>* Mean final BW in m and f -19% Mean BW <u>gain</u> – 38% (m), -54% (f) lower in 1000 ppm rats *<u>Mice</u>*: Mean final BW in m –18% and in f-17%, Mean BW <u>gain</u>	No data	*<u>Rats:</u>* Lung effects (related to parasitic infection), *<u>Mice</u>* at 1000 ppm meta-hyperplasia, inflammation in nasal mucosa (type of epithelium not identified),	Mice ≥200 ppm and rats 1000 ppm intermittent respiratory distress, hunched posture, ruffled fur	No data	No (no treatment-related mortalities, no indication on systemic toxicity, lower BW gain at concentration above or equal to those inducing

			– 60% (m) – 50% (f) in 1000 ppm mice		trachea, bronchia			respiratory symptoms, no data on food consumption)
Owen, 1988, Bodanffy et al., 1994b 53 wk, 83 wk, 2 years, or 70 wk plus 16 wk recovery on rats and mice 0, 50, 200, 600 ppm	No treatment related mortalities	*Rats & mice*: End of study: significantly lower BW gain at 600 ppm and in mice at 200 ppm:	*Rats & Mice*: mean final BW at the end of study rats: (m) –10% (f) –15% (no data specified), mice at 600 ppm (m&f) –15%), not quantified for 200 ppm	*Rats:* Decreased blood glucose (f at 600 ppm) and reduced urine volume (m & f at 600 ppm) are contributed to the nonmeasured reduced food and water consumption	*Rats & Mice*: ≥200 ppm olfactory degeneration, metaplasia Trachea (in mice at 600 ppm) meta-hyperplasia rough haircoat, hunched posture, lung (600 ppm rats & mice) increased weight, degeneration of bronchiolar epithelium, alveolar histiocytosis	*Rats & Mice*: ≥50 ppm rough haircoat, hunched posture	No toxic effect in other organ system	No (The authors interpreted lower glucose levels and oligurie as indicative for reduced consumption of water/food, no treatmen-related mortalities, no indication on systemic toxicity, no data on food and water consumption)

Oral administration								
Reference	**Treatment-related Mortalities**	**Mean BW/gain reduction**	**% Decrease in final BW or BW gain**	**Food and water consumption estimated/ reduced**	**Gastro-intestinal tract toxicity**	**Clinical signs of toxicity**	**Organ toxicity other than gastrointestinal tract**	**Indicated that doses exceed MTD**
Gale, 1979 4-wk dose range-finding studies on rats & mice 0, 50 (to 10000 ppm in wk 4), 150, 1000 or 5000 ppm in water	No mortalities	Reduced BW gain in rats f≥1000 ppm and m ≥5000 ppm and in male mice at 5000 ppm	No data	*Rats:* Reduced water consumption f≥1000 ppm and m≥5000 ppm, reduced food consumption f at 10000 ppm (-11%) *Mice:* Reduced water consumption in m at ≥1000 ppm and f≥5000 ppm, reduced food consumption in f at 5000 ppm	-	*Mice*: tremor and hypothermia in one f at 5000 ppm	*Mice* at 5000 ppm: Reduced thymus weight *Rats & mice* at all doses: lower liver weight without corresponding alterations (significance?)	No (no mortalities, no systemic toxic effect, tremor and hypothermia in a female mice might indicate nonspecific toxicity, reduced BW only at doses equal or above those with reduced water & food consumption)
Gale, 1980 13-wk studies (OECD 408) in rats & mice, o, 200, 1000, 5000 ppm	No treatment-related mortalities	Non-significantly lower mean BW in m rats at 5000 ppm (-8%) *Mice*: no effect on overall BW gain	-	*Rats*: significantly reduced water consumption in m & f at 5000 ppm (-23%, -25%) , transiently reduced in m&f at 1000 ppm *Mice*: no change of water and food consumption	-	No effect observed	No effect observed	No (no mortalities, no systemic/specific or nonspecific toxic effects in rats up to 5000 ppm, slightly lower BW associated to reduced water consumption)

DuPont, 2000 92-day study on cell proliferation of oral cavity in rats 0, 1000, 5000, 10000, 24000 ppm, males only	No mortalities	lower mean BW ≥5000 ppm & lower BW gain ≥5000	lower mean BW -8.8, -7.4, -6.1% lower BW gain ppm -13.4, -11, -9.2%	significantly lower water consumption ≥5000 ppm, (-28, -37, -40.3%) lower overall food consumption nonsign. At 5000 ppm (-7.2%), signif. At ≥10000 ppm (-6%, - 7.4%) , no effect on food efficiency	Oral cavity: Increased proliferation rates ≥10000 ppm thoughout & at the end of the study	No clinical sign of toxicity or gross lesions	No data	No (no mortalities, no systemic/specific or nonspecific effects in rats up to 24000 ppm, lower BW associated to lower water/food consumption)
DuPont 2000 92-day study on cell proliferation in the oral cavity in mice 0, 1000, 5000, 10000, 24000 ppm, males only	No mortalities	no effect on BW/BW gain	-	significantly lower water consumption at ≥1000 ppm (-23, -30.5, -31.4, -35%), no effect on overall food consumption & efficiency	Oral cav ity: Increased cell proliferation at day 92 only	No clinical sign of toxicity or gross lesions	No data	No (no mortalities, no systemic/specific or nonspecific effects in mice up to 24000 ppm, no effect on BW)
Bogdanffy et al., 1994a 2 years, interim sacrifices at wk 52, 78 rats 0, 200, 1000, 5000 ppm	No treatment-related mortalities	Reduced BW gain at 5000 ppm m –11% in 1st year, -17% 2nd year, f -11% 2nd year		Significantly lower water consumption at 1000 ppm (m -4.5% to max. –11%, f –5%-max. 16% at various intervals), at 5000 ppm (m –18% to max. – 34%, f –20% to max. 39%) reduced food consumption in m	No tissues from oral cavity examined for histopathology, no effect on tongue and stomach	No clinical sign of toxicity or treatment-related gross lesions	No toxic effect	No (no mortalities, no systemic/specific or nonspecific toxic effects in rats up to 5000 ppm, effect on BW in males at 5000 related to reduced water consumption ≥1000 ppm & to lower food

				at 5000 ppm (-3% to max. –12%)				consumption at 5000 ppm)
Lijinsky and Reuber, 1983 130 wk, rats at 0, 1000 or 2500 ppm	No treatment-related mortalities	No data	-	No data	No data	No data	No data	Study insufficient
Celanese, 1998 (=Japanese Bioassay) 104 wk rats & mice 0, 400, 2000, 10000 ppm	No treatment-related mortalities	*Rats:* lower mean in m& f at 10000 ppm at the end of study *Mice:* Lower mean BW in 2nd year in m &f at 10000 ppm	*Rats*: about 6-7% below controls (calculated from graphics) *Mice*: m 37 g versus 52 g in controls, about –20%(f) to 30% (m) at the end of study	*Rats:* lower water consumption at 10000 ppm throughout the stud, about –27% to –30% at the end of study (calculated from graphics) *Mice*: Low water consumption in 2nd year at 10000 ppm, at the end of study about –18% (f) to –35% (m) (calculated from graphics), No effect on food consumption	No data on toxic effects	No data on clinical signs	No data	No (no indication on systemic/specific toxic effects, low BW in mice & rats related to reduction in water consumption)

Conclusion:

1. From inhalation studies on repeat-dose toxicity (subacute, subchronic, chronic) or carcinogenicity (2 years)

- there were no treatment-related mortalities,
- no indication on systemic toxicity
- significantly reduced body weigth gain and final body weight

Reduced body weight gain is one of the critieria indicative for an excess of MTD. In inhalation studies on vinyl acetate , a (significant) reduction in mean final body weight or mean body weight gain was seen at concentrations of 1000 ppm in rats and mice exposed for 4 weeks until 90 days. When rats and mice were exposed to vinyl acetate during 2 years, lower BW gain was observed at 600 ppm, which is the dose inducing a significant increase in nasal tumors in rats and the highest dose tested. Significantly reduced urine volume and glucose levels during and at the end of study indicated that lower body weight gain can be attributed to reduction in the water and food consumption that was not measured in this and all other studies.

Although not examined, it is likely, that reduced BW gain and lower bw gain was interpreted to be related to reductions in water and/or food consumption.

Therefore, for concentrations up to 600 ppm exposed during 2 years the MTD was not exceeded.

2. For the oral studies on repeat-dose toxicity (subacute and subchronic)

- There were no treatment-related mortalities,
- no indication on nonspecific or systemic/specific toxicity
- and reduced mean BW (gain) was interpreted to be related to the lower water/food consumption.

Presumably due to a bad palatibility in drinking water, rats and mice of the oral studies had a reduced water or water and food consuption. Because of the absence of other toxic effects and the consistency of dose-responses, the lower mean BW or BW gain was attributed to the reduced consumption of water and food.

Therefore, for doses up to 24000 ppm administered up to 2 years, the MTD was not exceeded.