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Trichloroethylene - Carcinogenicity dose-response analysis

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Contents

LIST OF ABBREVIATIONS

BMI	Body mass index
C	Chloral
CH	Chloral hydrate
CI	Confidence interval
CLP	Classification Labelling Packaging
CSR	Chemical safety report
CYP	Cytochrome P450
DCA	Dichloroacetic acid
DCVC	Dichlorovinyl cysteine
DCVCS	S-(1,2-dichlorovinyl)-L-cysteine sulfoxide
DCVG	Dichlorovinlyl glutathione
DMEL	Derived minimal effect level
DNA	Deoxyribonucleic acid
DNEL	Derived no effect level
ECHA	European chemicals agency
GSH	Glutathione
IRIS	Integrated Risk Information System
LMS	linearized multistage
NacDCVC	N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine
NAG	N- acetylglucosaminidase
NHL	Non-Hodgkin lymphoma
NOAEL	No observed adverse effect level
OEL	Occupational exposure limit
OR	Odds ratio
PBPK	Physiologically based pharmacokinetic
POD	Point of departure

RCC   Renal cell carcinoma

REACH   Registration, Evaluation, Authorisation and Restriction of Chemicals

RR   Relative risk

RRm   Summary relative risk

SCE   Sister chromatid exchange

SIR   Standardised incidence rate

SMR   Standardised mortality rate

SSB   Single strand break

TCA   Trichloroacetic acid

TCOH   Trichloroethanol

TWA   Time weighted average

UDS   Unscheduled DNA synthesis

VHL   von Hippel Landau tumour suppressor gene

EXECUTIVE SUMMARY

Executive Summary

The objective of this report is to present a dose-response analysis for the carcinogenic effects of trichloroethylene (CAS 79-01-06) for further use in risk and socio-economic assessment of applications for authorisation by ECHA's Scientific Committees.

The assessment in this report is primarily building upon a series of Expert assessments on trichloroethylene conducted since the year 2000. In all expert evaluations it is acknowledged that the carcinogenic response from trichloroethylene exposure is very complex, involving multiple genotoxic and cytotoxic metabolites acting in various manners.

Based on the review of these Expert asssessments, it is concluded that in terms of the REACH regulation trichloroethylene should be considered as a genotoxic **non-threshold carcinogen** due to data on the mutagenicity of trichloroethylene and the formation of several genotoxic metabolites, e.g DCVG; DCVC; DCA and Chloral hydrate.

The critical effect of trichloroethylene is considered to be the development of kidney cancer as evidenced by several epidemiological studies. In that respect IARC has recently (2012) evaluated trichloroethylene to be a group 1 carcinogen with sufficient evidence for carcinoigenic effects in humans.
In the epidemiological studies, increased risk of kidney cancer was found at relatively high occupational exposure, including very high peak exposures; altogether leading to cytotoxic responses noted as renal tubular damage in the kidneys. The cytotoxic effects are considered to enhance the carcinogenic response and thus, below cytotoxic levels, the risk for kidney cancer is considered to be considerably lower. Therefore, a linear dose-response relationship would overestimate the risk at low exposure levels.

The method for dose-response assessment by the German Committee on Hazardous Substances (AGS, 2008) was found to be the most scienticfically justified method, as this expert group used a sublinear method that at the same time took account of a non-threshold approach at low-level exposure and of a threshold approach for the the co-carcinogenic effect of cytotoxicity at the higher exposure levels.

The dose-response relationship for trichloroethylene was described by AGS (2008) in relation to 8h occupational exposure. As no specific human data were available concerning dose-response relationship from oral and dermal exposure, it was necessary to make route-to-route extrapolations from inhalation exposure applying relevant adjustment factors and absorption rates.

The derived dose-reponse relationships for carcinogenic effect in relation to inhalational, dermal and oral exposure to trichloroethylene are given in Chapter 7.

1. INTRODUCTION

Objective
The objective of this report is to present a dose-response analysis for the carcinogenic effects of trichloroethylene (CAS 79-01-06) for further use in risk and socio-economic assessment of applications for authorisation by ECHA's Scientific Committees. The analysis is based on recent reviews of relevant available scientific literature (including data from the REACH registration) on trichloroethylen and in particular risk assessments from international or national bodies.

Clear descriptions should be given in the report for the different mechanisms, which are considered to be important for development of trichloroethylene induced tumours. Furthermore, justifications should be given for the selection of the most relevant studies and exposure-related parameters for establishing a dose-response model. This should allow for quantitative cancer risk assessment at given exposure levels referred to in the future applications for authorisation.

Key data on trichloroethylene
From an initial literature search the following key expert evaluations were identified (in chronological order):

> WHO 2000. Chapter5.15 Trichloroethylene. Air quality guidelines-second edition.

> EU-RAR, 2004. European Union Risk Assessment Report on trichloroethylene. European Chemicals Bureau.

> WHO, 2005. Trichloroethylene in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/05.08/22

> AGS 2008 A. Exposure-risk relationship for trichloroethylene in BekGS 910 (English translation from 2012). Committee on Hazardous Substances (AGS). Published by Bundesanstalt für Arbeitzschutz und Arbeitsmedizin (BAuA).

> AGS 2008 B. Guide for the quantification of cancer risk figures after exposure to carcinogenic hazardous substances for establishing limit values at the workplace. Committee on Hazardous Substances (AGS). Published by Bundesanstalt für Arbeitzschutz und Arbeitsmedizin (BAuA)

> SCOEL, 2009. Recommendation from the Scientific Committee on Occupational Exposure Limits for Trichloroethylene SCOEL/SUM/142 April 2009

> WHO, 2010. WHO guidelines for indoor air quality: selected pollutants, World Health Organization, Regional Office for Europe p 377-414.

> US EPA (2011). TOXICOLOGICAL REVIEW OF TRICHLOROETHYLENE (CAS No. 79-01-6) In Support of Summary Information on the Integrated Risk Information System (IRIS) September 2011 EPA/635/R-09/011F

> HSE, 2012.The burden of occupational cancer in Great Britain. RR854 Research report.

IARC, 2012. IARC Monographs on the evaluation of carcinogenic risks to humans, vol 106. Some chlorinated solvents and their metabolites. Lyon: International Agency for Research on Cancer (in press).

Rusyn I, Chiu WA, Lash LH, Kromhout H, Hansen J, Guyton KZ. (2013). Trichloroethylene: Mechanistic, epidemiologic and other supporting evidence of carcinogenic hazard. Pharmacol Ther. 2013 Aug 23. pii: S0163-7258(13)00180-0. doi: 10.1016/j.pharmthera.2013.08.004 (extended summary of the IARC evaluation 2012).

Anses, 2013. Valeur toxicologique de reference du trichloroethylene. Avis de l´Anses Saisines no 2012-SA-0140 et 2012-SA-0141 based on Afsset, 2009. Proposition de valeurs guides de qualité de l'air intérieur. Trichloroéthylène (TCE). Auto-saisine.

After consultation with ECHA, further access was given to the data in the REACH registration of trichloroethylene in order to identify any additional relevant information.

Outline of the report
Chapters 2 and 3 of this report will provide an overview and background knowledge regarding trichloroethylene with respect to its physico-chemical properties, the pharmacokinetics and the data regarding carcinogenicity and mutagenicity of the substance. These chapters will mainly be based on the most updated in-depth expert evaluations of the substance among the references shown above.

In Chapter 3 (section 3.4) an overview of the discussions/ conclusions specifically with regard to the carcinogenic mode of action (threshold or non-threshold) of the substance will be given based on the expert evaluations.

In Chapter 4 a discussion and conclusion will be made by the contractor based on the background knowledge and the expert assessments, and the most appropriate scientifically based method (threshold / non-threshold method) for making a more detailed dose-response analysis of carcinogenicity of trichloroethylene will be presented.

Chapter 5 will further go into detail in the quantitative assumptions and calculations behind the dose-response estimates made by the various expert groups. With due consideration to the recommendations in the ECHA guidance R8 (on dose-response relationships), relevant dose metrics (or POD) will be identified as the basis for establishing relevant dose-response relationship for workers and consumers.

Chapter 6 will, based on the findings in chapter 5, adapt the relevant dose metrics to dose-response relationships for workers and general population in relation to relevant exposure routes (inhalation, dermal, oral). The assumptions with regard to necessary modifications and route-to-route extrapolations of the dose-metrics will be presented.

Chapter 7 will briefly summarise the contractor's proposal for the carcinogenic

dose-response relationships for trichloroethylene in relation to worker exposure and general population exposure via the relevant exposure routes.

2. IDENTITY AND PHYSICO-CHEMICAL PROPERTIES

Trichloroethylene (CAS number 79-01-6, EINECS number 201-167-4) is a colourless nonflammable liquid with a characteristic odour resembling that of chloroform. The odour is detectable at around 20 to 30 ppm.
The molecular formula of trichloroethylene is C_2HCl_3, and the structural formula is as follows (EU RAR, 2004):
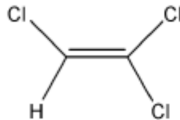
The physico-chemical properties of trichloroethylene are summarised in Table 2-1 below (EU RAR, 2004).

Table 2-1 Summary of physico-chemical properties of trichloroethylene (EU RAR, 2004)

Properties	Value
Molecular weight	131.5
Melting point	-84.8°C
Boiling point	86-88°C
Density	1.465 g/cm^3
Vapour pressure	86hPa at 20°C
Water solubility	1100 mg/l
Log octanol/water partition coefficient	2.29
Log sediment/water partition coefficient	2.1 (calculated)
Flammability	Lower limit 12.5%, upper limit 90%
Autoflammability	410°C
Vapour density	0.42 kg/m^3 (air=1)
Henry's law constant	1.03 x 10^{-2} atm m^3/mole
Suface tension	0.0293 N/m at 20°C
Conversion factor	1ppm=5.473 mg/m^3

3. HUMAN HEALTH HAZARD WITH FOCUS ON CANCER

This section will give an overall description regarding toxicokinetics, carcinogenicity and mutagenicity of trichloroethylene. These data will be used as a basis for further interpretation and evaluation in Chapter 4, where mechanistic considerations and conclusions of the expert groups will be given.

3.1 Toxicokinetics

3.1.1 Absorption and distribution

3.1.1.1 Human data

According to SCOEL (2009) trichloroethylene is well absorbed via all major routes of exposure in humans. Quantitative data are available for inhalation exposure, for which uptake was between 28% and 80%.The absolute uptake increased with increasing physical exercise.

Oral uptake has been demonstrated after accidental or deliberate ingestion of trichloroethylene. However, no quantitative information was available. Considerable percutaneous absorption has been demonstrated in human volunteers, however, with unknown rate.

Furthermore, trichloroethylene was shown to be rapidly distributed throughout the body, and trichloroethylene crosses the blood-brain barrier and the placental barrier and accumulates in fat tissue (SCOEL 2009).

3.1.1.2 Experimental animal data

SCOEL (2009) indicated that trichloroethylene was well absorbed by all major routes of exposure in animals. After short-term inhalation exposure of rats to very high concentrations of trichloroethylene, 31-79% was retained (exposure levels not specified). In mice, 40-54% uptake was shown. After oral uptake in rats, mice or rabbits, 80-98% of the activity of radiolabelled trichloroethylene was recovered in expired air or urine. Oral uptake in mice occurred faster than in rats and higher peak levels were reached.

Dermal absorption of liquid trichloroethylene through mouse skin has been found to be around 8 μg/cm^2 per minute and around 5.4 μg/cm^2 per minute for absorption through guinea pig skin with trichloroethylene in aqueous solutions. *In vitro* studies with rat skin indicated dermal uptake of 12 μg/cm^2 per minute.

Similar to humans, trichloroethylene was rapidly distributed throughout the body, and crossed the blood brain barrier and the placental barrier, and further tended to accumulate in fat tissues (SCOEL 2009).

It is noted that in the EU-RAR (2004), absorption rates of 100% were chosen (for both humans and animals) for all exposure routes (oral, dermal, inhalational) for the risk characterisation.

3.1.2 Metabolism and elimination

With respect to the metabolism, no relevant qualitative differences between experimental animals and humans have been observed, however attention should be paid to quantitative differences between species.

Figure 3-1 shows schematical the metabolic pathways and metabolites of trichloroethylene (WHO 2010; Lock and Reed 2006).

Figure 3-1 Metabolic pathways and metabolites of trichloroethylene
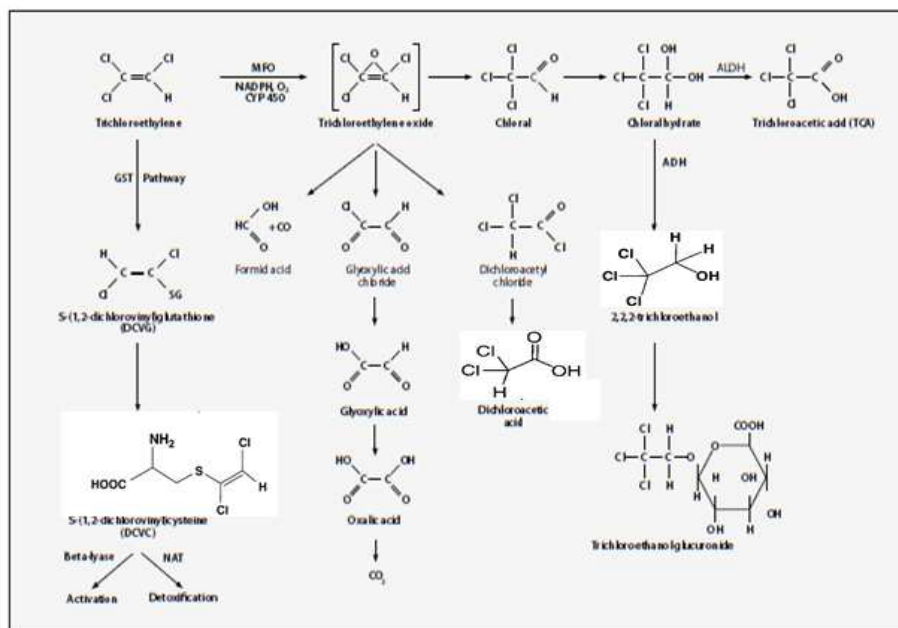
The CYP mediated *oxidative metabolic pathway* (arrow going to the right) has been shown to be the most dominant metabolic pathway, whereas the *reductive glutathione S-transferase pathway* (GST arrow going downwards) has been shown to be a minor pathway, especially operating when the oxidative pathway has been saturated.

3.1.2.1 Human data

SCOEL (2009) noted that free and conjugated trichloroethanol (TCOH) is found as the dominant excretion product from trichloroethylene exposure, as well as trichloroacetic acid (TCA). The relative amount of urinary TCA is higher in humans than in experimental animals. Furthermore, dichloroacetic acid (DCA) and monochloroacetic acid are formed (the latter to a larger extent than in animals).

In addition to the oxidative metabolism, a minor glutathione-dependent reductive pathway is also relevant for humans, as β-lyase activity has been demonstrated in human kidneys by the detection of N-acetyldichloro vinylcysteine in the urine

of workers.

A steady-state for trichloroethylene in blood is reached after continuous exposure after around two hours. Elimination is best described by a three-compartment model composed of richly perfused tissues (half-life of trichloroethylene 2-3 minutes), lean body mass (half-life trichloroethylene about 30 minutes) and fat-rich tissues (half-life trichloroethylene 3.5-5 hours). Blood concentrations of trichloroethylene increased during five consecutive days of exposure due to accumulation in fat tissue.

The half-life of TCOH in blood is 10-12 hours, leading to accumulation during the working week. Steady-state is reached by the fifth day after intermittent exposure to 250 ppm trichloroethylene (1770 mg/m^3). Following repeated exposure to 50 ppm (275 mg/m^3), elimination from blood was complete within 4 days after the last exposure.

For TCA, even longer half-lives in blood are reported, caused by its extensive plasma protein binding. Elimination from blood (half-life 70-100 hours) was nearly complete 13 days after the last exposure to 50 ppm trichloroethylene following repeated inhalation.

Trichloroethylene metabolites are mainly excreted via the urine, namely 29-50% of trichloroethylene as TCOH (free plus conjugated) and 10-24% of absorbed trichloroethylene as TCA. Other studies reported that up to 44% of trichloroethylene could be excreted as TCA.

Women have been found to eliminate less unchanged trichloroethylene than men (SCOEL 2009).

3.1.2.2 Experimental animal data

The major metabolic pathways are the same for different animal species and for different routes of exposure. However, quantitative differences exist between species and strains.

Trichloroethylene is rapidly oxidised by cytochrome P450 (mainly CYP2E1), via the respective epoxide, to trichloroacetaldehyde (chloral). This metabolite is further metabolised to TCOH or competitively to TCA, both of which are excreted in free or conjugated forms. Considerably higher levels of TCOH were found in mice compared to rats. A minor fraction appears as dichloro- and monochloroacetic acid. Other minor pathways result in the formation of carbon dioxide, carbon monoxide, oxalic acid, and glyoxylic acid. From the epoxide also N-hydroxy-acetylaminoethanol is formed (reaction with phosphatidylethanol-amine as a constituent of lipids).

Elevated formic acid levels were found in the urine of experimental animals after exposure to trichloroethylene. However, formic acid is not a trichloroethylene

metabolite. The trichloroethylene metabolites TCOH and TCA are suspected to interact with vitamin B12 through a free-radical mechanism inducing B12 deficiency and, as a consequence, also folate deficiency. As a result of folate deficiency, excess formic acid is excreted in urine.

Apart from oxidative metabolism, trichloroethylene is also metabolised via glutathione-S-transferase to S-1,2-dichlorovinyl glutathione and S-(1,2-dichlorovinyl)-L-cysteine (DCVC). This intermediate may be transformed by three metabolic pathways: either to N-acetyldichloro vinylcysteine by N-acetyltransferase or (by cleavage with β-lyase) to chlorothionoacetyl or chlorothioketene or by flavin monooxygenase to produce dichlorovinylcysteine sulphoxide. The mercapturic acids (N-acetyldichlorovinylcysteine) are eliminated by rats in minimal amounts. However, even if mercapturic acids are not detected in urine, the glutathione pathway may be relevant because of the mentioned metabolic activation via β-lyase leading to the toxicologically important chlorothioketenes, which may finally be hydrolysed to monochloroacetic acid.

Dichlorovinylcysteine sulphoxide may also be activated as it is a substrate for renal ß-lyase, but it is probably toxicologically active on its own. Thus, although the glutathione-pathway only contributes slightly to the total elimination of trichloroethylene, it may be important because of its toxicologically relevant metabolites.

The half-lives of TCOH in blood of 2-5.3 hours (Sprague-Dawley rats) or 0.5-2.7 hours (B6C3F1 mice) were considerably lower than in humans (10-12 hours). Similarly, the TCA half-lives of 5-7 and 4-7.7 hours in rats and mice, respectively, were much lower than in humans (70-100 hours).

Elimination after inhalation exposure of trichloroethylene is mainly via the urine after extensive metabolism. Other pathways of excretion are the faeces and exhaled air (carbon dioxide). As shown for the oral pathway, low inhalation exposure of both rats and mice resulted in approximately 65% of the dose being recovered as metabolites in urine, 7-23% as metabolites in faeces, 9-13% were eliminated as CO_2, and 1-4% as unchanged trichloroethylene in air. In rats, increasing fractions of trichloroethylene are excreted unchanged in expired air at higher concentrations. The major metabolite excreted in urine is (conjugated or free) TCOH, where relevant differences in the quantitative fractions and in the amount of the TCA are observed depending on species and strain. The fraction of TCOH excretion also changed (reduced) from short-term compared to subchronic or chronic exposure. Concentrations of DCA in the urine of rats were minimal after acute exposure to trichloroethylene. However, due to inhibition of its metabolism, the role of DCA after chronic exposure may change and this is currently insufficiently assessed (SCOEL 2009).

3.2 Carcinogenicity

The carcinogenicity of trichloroethylene has been studied in both carcinogenicity studies in experimental animal studies (all carcinogenicity studies made in the period of 1976-1990) and in epidemiological studies. Whereas no new experimental animal carcinogenicity studies have been performed since 1990, the knowledge regarding carcinogenicity from epidemiological studies on trichloroethylene has increased significantly in the past 10-15 years.

3.2.1 Experimental animal data

3.2.1.1 Inhalation studies

The inhalational carcinogenicity studies have been compiled and described in the following table as presented in the evaluations of WHO (2000) and WHO (2010):

Table 3-2 Review of inhalation carcinogenic assay (WHO, 2000 & 2010)

Species and strain	Treatment	Observed increase in tumour incidence	Reference
Mouse (m,f) B6C3F1	**0, 540, 1620 and 3240** mg/m^3, 7 hours/day, 5 days/week for 78 weeks; observation for rest of lifespan; trichloroethylene purity 99.9%, epoxide-free	**Pulmonary adenomas** in females only: 4/90, 6/90, 10/90 and 15/90; **hepatomas** in females: 3/90, 4/90, 4/90 and 9/90 **hepatomas** in males 14/90, 19/90, 27/90 and 21/90	Maltoni et. al.,1988.
Mouse (m, f) Swiss	**0, 540, 1620 and 3240** mg/m^3, 7 hours/day, 5 days/week for 78 weeks; observation for rest of lifespan; trichloroethylene purity 99.9%,.epoxide-free	**Pulmonary adenomas and carcinomas** in males only: 10/90, 11/90, 23/90 and 27/90 **hepatomas** in males: 4/90, 2/90, 8/90 and13/90	Maltoni,. et al, 1988.
Mouse (m, f) NMRI	**0, 540 and 2700** mg/m^3, 6 hours/day, 5 days/week for 78 weeks; observation until week 130; trichloroethylene purified, epoxide free	**Lymphomas** in females only: 9/29, 18/28 and 17/30	Henschler, et al, 1980
Mouse (f) ICR	**0, 270, 810 and 430** mg/m^3, 7 hours/day, 5 days/week for 104 weeks; observation until week 107; trichloroethylene purity 99.8% + 0.02% benzene + 0.02% epichlorohydrin	**Pulmonary adenocarcinomas**: 1/49, 3/50, 8/50 and 7/46	Fukuda, et al, 1983
Rat (m, f) Wistar	**0, 540 and 2700** mg/m^3, 6 hours/day, 5 days/week for 78 weeks; observation until week	No increase observed	Henschler, et al, 1980

Species and strain	Treatment	Observed increase in tumour incidence	Reference
	156; trichloroethylene purified, epoxide-free		
Rat (m, f) Sprague- Dawley	**0, 270, 810 and 430** mg/m^3, 7 hours/day, 5 days/week for 104 weeks; observation until week 107; trichloroethylene purity 99.8% + 0.02% benzene + 0.02% epichlorohydrin	No increase observed	Fukuda, et al, 1983
Rat (m, f) Sprague- Dawley	**0, 540, 1620 and 3240** mg/m^3 7 hours/day, 5 days/week for 104 weeks; observation for rest of lifespan; trichloroethylene purity 99.9% epoxide-free	**Renal adenocarcinomas** in males and at high dose only: 4/130 versus 1/130 in controls; **Leydig cell tumours in testis** 1/135, 16/130, 30/130 and 31/130	***Maltoni, et al, 1986***
Hamster (m, f) Syrian	**0, 540 and 2700** mg/m^3, 6 hours/day, 5 days/week for 78 weeks; observation until week 130; trichloroethylene purified, epoxide-free	No increase observed	Henschler, et al, 1980

Reference: used for dose-response assessment, see chapter 5

Overall, one inhalation study showed an increased incidence of lymphomas in female mice (Henschler *et al.*, 1980), two studies showed increased incidences of liver tumours in male B6C3F1 mice and male Swiss mice (Maltoni *et al.*, 1988), and three studies showed increased incidences of lung tumours in mice (Maltoni *et al.*,1988, Fukuda *et al.*, 1983). The lung tumours were found in female B6C3F1 mice, in female ICR mice and in male Swiss mice.One of three experiments with rats showed an increased incidence of interstitial testicular tumours and a marginal increase in renal cell tumours in males (Maltoni *et al.*,1988). No increased tumour incidence was observed in a study in hamsters.

3.2.1.2 Oral studies

The oral carcinogenicity studies as compiled and described below were included in the evaluation of WHO (2000) and WHO (2010):

Table 3-3 Review of oral carcinogenic assay (WHO 2000 & 2010)

Species and strain	Treatment	Observed increase in tumour incidence	Reference
Mouse (m, f) B6C3F1	**1169 and 2339** mg/kg bw (m), **869 and 1739** mg/kg bw (f) 5 days/week for 78 weeks; killed after 90 weeks; trichloroethylene + 0.09% epichlorohydrin and 0.19% epoxybutane	**Hepatocellular carcinomas**: in males 1/20, 26/50 and 31/48, and females 0/20, 4/50 and 11/40; lung adenomas: in males 0/20, 5/50 and 2/48 and females 1/20, 4/50 and 7/47	US NCI, 1976.
Mouse (m, f) B6C3F1	**0 and 1000** mg/kg bw, 5 days/week for 103 weeks; trichloroethylene epichlorohydrin-free	**Hepatocellular carcinomas**: in males (8/48 and 30/50) and females (2/48 and 13/49) (toxic effects: renal cytomegaly in all trichloroethylene -treated males and females)	NTP, 1990.
Mouse (m, f) Ha:ICR	**0, TWA 1900** mg/kg bw (m), **TWA 1400** mg/kg bw (f), 5 days/week for 78 wks; trichloroethylene with or without epichlorohydrin and epoxybutane	**Papillomas and carcinomas in forestomach** in groups given trichloroethylene + epichlorohydrin + epoxy- butane; no increase observed in groups that received pure trichloroethylene	Henschler, et al, 1984.
Rat (m, f) Osborne Mendel	**549 and 1098** mg/kg bw, 5 days/week for 78 weeks with observation until week 110	No increase observed but value of study reduced because survival was decreased owing to toxic nephropathy (both sexes, both dose levels)	US NCI, 1976.
Rat (m, f) F344/N	**0, 500 and 1000** mg/kg bw, 5 days/week for 104 weeks; trichloroethylene purity >99.9%, epoxide-free	**Renal tubular adenocarcinomas** in males only **0/33, 0/20 and 3/16**; nephropathy in all treated groups (m and f); NTP considers study inadequate (survival too low)	*NTP, 1990.*
Rat (m, f) Sprague - Dawley	**0, 50 and 250** mg/kg, 5 days/week for 52 weeks; observation for rest of lifespan; trichloroethylene purity >99.9%, epoxide free	No increase observed (karyomegaly in renaltubular cells at 250 mg/kg, males only)	Maltoni *et al.*, 1986
Rat (m, f) ACI, August, Osborne Mendel Marshall	**0, 500 and 1000** mg/kg bw, 5 days/week for 104 weeks; trichloroethylene purity >99.9%, epoxide-free	Study judged inadequate by NTP; nevertheless to be noted: in Osborne Mendel rats: **Renal tubular cell adenomas** in males only: 0/50, 6/50 and 1/50; in male Marshall rats: interstitial cell tumours 17/46, 21/48 and 32/48	NTP, 1988.

Reference:used for dose-response assessment, see chapter 5

Overall, as also indicated by SCOEL (2009), the studies in mice showed significant increases in benign and malign liver tumours (NCI, 1976; NTP, 1990).

In two rat studies, the incidence of renal cell tumours was significantly increased in males (NTP, 1988; NTP, 1990), and in one study an increased incidence of interstitial-cell testicular tumours (NTP, 1988) was observed. The renal cell tumours were induced in three rat strains: Fisher344, Marshal and Osborn-Mendel (in NTP 1988 and NTP 1990). These effects were only seen consistently in male animals and not in mice. Non-neoplastic nephrotoxicity was noted to occur in the animals that developed tumours.

3.2.1.3 Dermal studies

Only sparse data are available with regard to carcinogenicity after dermal application, as shortly described in the EU-RAR (2004).

The carcinogenicity of trichloroethylene following dermal application has been investigated in one study, using Ha:ICR Swiss mice. A group of thirty mice each received a thrice-weekly dermal application of 1 mg purified trichloroethylene (in acetone) until spontaneous death. The dose was described as being less than the maximum tolerated dose. No skin tumours were observed.
Further, an "initiation-promotion" study was also conducted, in which mice were given a single dermal application of 1 mg trichloroethylene followed by the three times weekly application of phorbol myristate acetate for life. Trichloroethylene showed no evidence of possessing initiating properties. Limitations of this study include the small group sizes and use of a single dose level that did not elicit toxicity (EU-RAR 2004).

3.2.2 Human data

The overview below is from the recent evaluations made by US-EPA (2011), HSE (2012), and IARC (2012). As the monograph from the IARC evaluation has not been published yet, the description of this relies on the rather detailed summary of the IARC expert group discussions and conclusions given by Rusyn *et al.* (2013).

3.2.2.1 Kidney cancer

The risk of kidney cancer from trichloroethylene exposure has been studied in cohort, case-control, and geographical/ecological studies. These studies have examined trichloroethylene in mixed exposures as well as alone (US-EPA 2011):.

Elevated risks were observed in many of the cohort and case-control studies examining kidney cancer incidence in industries or job titles with historical use of trichloroethylene, particularly among subjects ever exposed to trichloroethylene (Moore *et al.*, 2010; Brüning *et al.*, 2003; Raaschou-Nielsen *et al.*, 2003; Dosemeci *et al.*, 1999) or subjects with trichloroethylene surrogate for high exposure (Moore *et al.*, 2010; Charbotel *et al.*, 2006; Zhao *et al.*, 2005; Brüning *et al.*, 2003; Raaschou-Nielsen *et al.*, 2003). Greater susceptibility to trichloroethylene exposure and kidney cancer was observed among subjects with a functionally active GSTT1 (glutathione-S-transferase theta 1) polymorphism, particularly among those with certain alleles in single nucleotide

polymorphisms of the cysteine conjugation β-lyase gene region (Moore *et al.*, 2010).

In a meta-analysis of the overall effect of trichloroethylene exposure on kidney cancer, US-EPA (2011) found a small, statistically significant increase in summary relative risk (RRm) of 1.27 (95% CI: 1.13; 1.43) with an RRm estimate in the higher exposure group of 1.58, (95% CI: 1.28, 1.96).

The relative risk ratio estimates found by US-EPa (2011) for kidney cancer are presented in the figures below. (It should be noted that some of the studies presented below have been updated since this meta-analysis).

TCE Exposure and Kidney Cancer

Study	RR	LCL	UCL
Anttila (1995)	0.87	0.32	1.89
Axelson (1994)	1.16	0.42	2.52
Boice (1999)	0.99	0.40	2.04
Greenland (1994)	0.99	0.30	3.32
Hansen (2001)	1.10	0.30	2.80
Morgan (1998)	1.14	0.51	2.58
Raaschou-Nielsen (2003)	1.20	0.94	1.50
Radican (2008)	1.18	0.47	2.94
Zhao (2005)	1.70	0.38	7.90
Bruning (2003)	2.47	1.36	4.49
Charbotel (2006)	1.88	0.89	3.98
Dosemeci (1999)	1.30	0.90	1.90
Moore (2010)	2.05	1.13	3.73
Pesch (2000)	1.24	1.00	1.50
Siemiatycki (1991)	0.80	0.30	2.20
OVERALL	1.27	1.13	1.43

Figure C-5. Meta-analysis of kidney cancer and overall TCE exposure. Random-effects model; fixed-effect model same. Rectangle sizes reflect relative weights of the individual studies. The summary estimate is in the bottom row, represented by the diamond.

From US-EPA (2011)
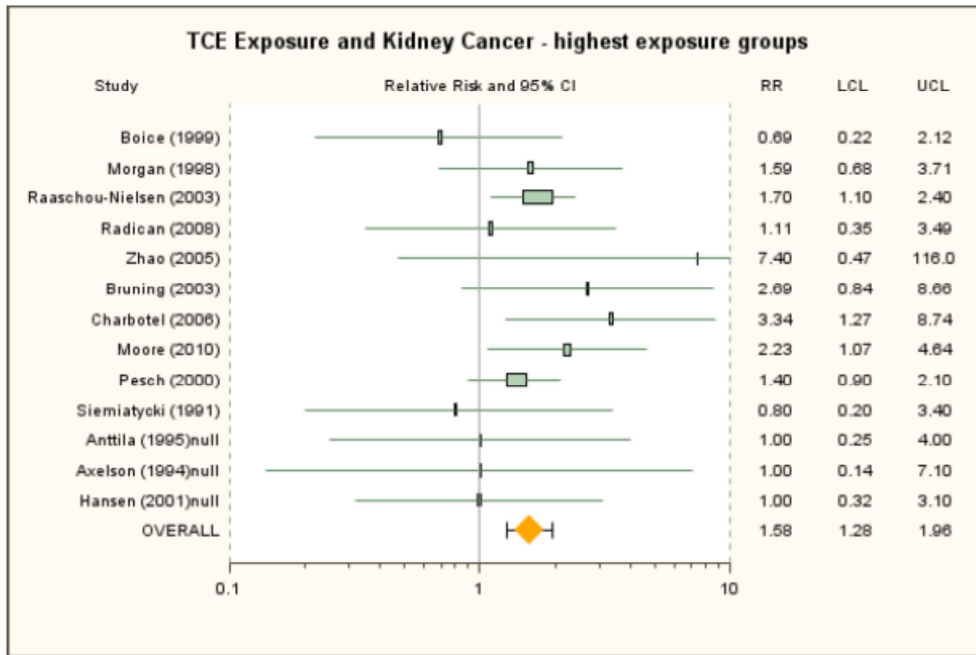
Figure C-7. Meta-analysis of kidney cancer and TCE exposure—highest
exposure groups, with assumed null RR estimates for Anttila et al. (1995),
Axelson et al. (1994), and Hansen et al. (2001) (see text). Random-effects
model; fixed-effect model same. Rectangle sizes reflect relative weights of the
individual studies. The summary estimate is in the bottom row, represented by
the diamond.

From US-EPA (2011)

In 2012, the Health and Safety Executive gave the following overview of
epidemiological studies regarding the association between kidney cancer and
trichloroethylene exposure (HSE 2012).

Table 3-4 Studies of trichloroethylene and kidney cancer (HSE, 2012)

Reference	Industry/ Product	Country	Design	Study size	Results#
Garabrant *et al.*, 1988	Aircraft manufacture	USA	Cohort	14,067 men & women	SMR=0.93 (95% CI 0.48-1.6, 12 obs.)
Spirtas *et al.*, 1991	Aircraft manufacture	USA	Cohort	7282 men & women	SMR=1.1 (95% CI 0.46-2.1, 8 obs.)
Axelson *et al.*, 1994	TCE use – biological monitoring	Sweden	Cohort	1421 men	SIR=1.2 (95%CI 0.42-2.5, 6 obs.)
Anttila *et al.*, 1995	TCE use – biological monitoring	Finland	Cohort	3089 men & women	SIR=0.87 (95% CI 0.32-1.9, 6 obs.)
Blair *et al., 1998* (update of Spirtas *et al., 1991*)	Aircraft maintenance	USA	Cohort	14,457 men & women	RR=1.6 (95% CI 0.5-5.1) * SMR=1.22 (95% CI 0.85-1.74, 30 obs.)

Morgan *et al.*, 1998	Aerospace workers	USA	Cohort	20,508 men & women [4733]	SMR=1.14 (95% CI 0.78 – 1.61, 32 obs.) [SMR=1.32 (95% CI 0.57-2.6, 8 obs)] *RCC and TCC*
Boice *et al.*, 1999	Aircraft manufacture	USA	Cohort	77,965 men & women	SMR=0.99 (95% CI 0.4-2.04, 7 obs) *SMR=0.92 (95% CI nr,
Hansen *et al.*, 2001	TCE use – biological monitoring	Denmark	Cohort	803 men & women	SIR(male)=0.9 (95% CI 0.2-2.6, 3 obs.) SIR(female)=2.4 (95% CI 0.03-14, 1 obs.)
Raaschou-Nielsen *et al.*, 2003	Industries using TCE	Denmark	Cohort [subcoh't of higher	40,049 men & women [14,360 subcohort]	SIR=1.2 (95% CI 0.94-1.50, 76 obs.) – RCC only SIR=1.2 (95% CI 0.8-1.8, 27 obs.) – TCC only [SIR=1.4 (95% CI 1.0-1.8,
Vamvakas *et al.*, 1998	Exposure derived from work history	Germany	Population Case- control	58 cases; 84 controls	OR=10.8 (95% CI 3.36-34.75) all exposures$
Bruning *et al.*, 2003	Exposure derived from work history	Germany	Population Case-control	134 cases; 401 controls	OR=2.47 (95% CI 1.36-4.49)
Henschler *et al.*, 1995	Cardboard manufacture	Germany	Retrospective cohort	169 men	SMR=3.3 (95% CI 0.40-12, 2 deaths)
Sharpe *et al.*, 1989	Work history (undefined degreasing solvents)	Canada	Case-control	164 cases, 161 controls	OR=3.4 (95% CI 0.92-13)
Charbotel *et al.*, 2006	Work history	France	Case-control	86 cases; 316 controls	OR=1.64 (95% CI 0.95-2.84) ever exposed OR=2.73 (95% CI 1.06-

References in bold: Studies used for dose-response estimations, see later in chapter 5

\# Considered to be renal cell cancers only unless otherwise specified
* Only workers with job descriptions indicating trichloroethylene exposure; all
 other workers included in second figure provided
nr = not reported
$ = Trichloroethylene and tetrachloroethylene combined
TCE= trichloroethylene

From this table the highest and/or significantly increased odds ratios (ORs in the range of 1.6-10.8) were observed in the case-control studies by Vamvakas *et al.* (1998), Brunning *et al.* (2003); Sharpe *et al.* (1989) and Charbotel *et al.* (2006). A significantly increased SMR of 3.3 was found in the cohort study by Henschler *et al.* (1995), whereas a significant SIR of 1.4 was found in a subcohort in the study by Raaschou-Nielsen *et al.* (2003).

According to the recent IARC evaluation from 2012 (Rusyn *et al.*,2013), the IARC working group also noted that the risk estimates from the case–control studies in general were stronger than those from the cohort studies. Two recent studies have provided detailed exposure assessments, one in France (Charbotel *et al.*, 2006) and one in Eastern Europe (Moore *et al.*, 2010). Both studies did demonstrate an exposure–response relationship. The French study was conducted in an area with high prevalence of occupational exposure to

trichloroethylene. An odds ratio of 1.64 (95% CI 0.95–2.84) was reported for the ever vs. never occupational trichloroethylene exposure, adjusted for tobacco smoking and body mass index. An odds ratio of 2.73 (95% CI 1.06–7.07) was reported for workers in the category with highest estimated trichloroethylene exposure. When the data were adjusted for exposure to cutting fluids and to other petroleum oils, the odds ratio was 2.63 (95% CI 0.79–8.83) suggesting no major confounding. The adjusted odds ratios in the East European study of any exposure to trichloroethylene were 1.6 (95% CI 1.04–2.54) and in the highest category of exposure intensity, it was 2.3 (95% CI 1.05–2.51).

Because the risk estimates for trichloroethylene exposure and kidney cancer were modest, and because most studies were small and had limited statistical power, the IARC Working Group also weighted two recent, meta-analyses based on virtually all existing studies of kidney cancer. Overall, they estimated statistically significant meta-relative risks of 1.3 to 1.4 for kidney cancer and trichloroethylene (Scott & Jinot, 2011; Karami *et al.*, 2012). One meta-analysis not only reported overall meta-RR, but also reported a meta-RR for highest exposure to trichloroethylene of 1.6 (95% CI 1.3–2.0), thus indicating some dose–response relationship (Scott & Jinot, 2011). Overall, the relative risk of kidney cancer was only modestly increased, but at the approximately same level as many, but not all, other occupational chemicals and exposure that IARC has classified as carcinogenic to humans (Group 1). The epidemiological evidence for the association with kidney cancer was, however, considered relatively robust because no single study appeared overly influential, the meta-RR estimates were not highly sensitive to alternate RR estimate selections, and there was no major heterogeneity across the studies (Rusyn *et al.*, 2013).

3.2.2.2 Non-Hodgkin lymphoma (NHL)
The meta-analysis on NHL made by US-EPA (2011) examined 17 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on trichloroethylene exposure. The meta-analyses of the overall effect of trichloroethylene exposure on NHL suggested a small, robust, and statistically significant increase in NHL risk. The summary estimate from the primary random effect meta-analysis (RRm) was 1.23 (95% CI: 1.07, 1.42). Meta-analysis of the highest exposure groups, either duration, intensity, or their product, cumulative exposure, results in an RRm of 1.43 (95% CI: 1.13, 1.82), which was greater than the RRm from the overall exposure analysis, and provides additional support for an association between NHL and trichloroethylene.

According to Rusyn *et al.* (2013), the IARC working group found that information on occupational trichloroethylene exposure and non-Hodgkin lymphoma (NHL) was provided from a total of 16 studies (8 cohort and 8 case–control studies). The majority of the studies have been published after the 1995 IARC evaluation. A large cohort study of trichloroethylene–exposed workers from Denmark (Raaschou-Nielsen *et al.*, 2003), as well as most cohort studies of aircraft and aerospace workers in the United States, reported modestly elevated relative

risks for NHL (Boice *et al.*, 1999; Radican *et al.*, 2008; Lipworth *et al.*, 2011). The three cohort studies of biologically monitored workers from Nordic countries show evidence of increased risk of NHL ranging from 1.5 to 3.1, based on a total of 21 cases (Axelson *et al.*, 1994; Anttila *et al.*, 1995; Hansen *et al.*, 2001). Several case–control studies showed modestly increased odds ratios, but were limited in interpretation due to the use of different classification systems for NHL. A recent meta-analysis of existing studies of NHL (Scott & Jinot, 2011) reported meta-relative risks of 1.2 (95% CI 1.1–1.4) for any exposure to trichloroethylene and 1.4 (95% CI 1.1–1.8) for higher exposure. There was heterogeneity between studies, and also some indication of publication bias. The overall epidemiologic evidence was less strong and consistent than for kidney cancer.

3.2.2.3 Liver cancer

US EPA (2011) found that observations from several studies provided some evidence of susceptibility of liver, gallbladder, and biliary tract; these observations were consistent with pharmacokinetic processing of trichloroethylene and the extensive intra- and extrahepatic recirculation of metabolites. Magnitude of risk of gallbladder and biliary tract cancer is slightly higher than the risk of primary liver cancer in Raaschou-Nielsen *et al.* (2003), the study with the most cases. Observations in Blair *et al.* (1998), Hansen *et al.* (2001), and Radican *et al.* (2008), three smaller studies, suggested slightly larger risk ratios for primary liver cancer compared to gallbladder and biliary tract cancer. Overall, these studies were not found to be highly informative for cross-organ comparison of relative magnitude of susceptibility.

The meta-analyses of the overall effect of trichloroethylene exposure on liver (and gall bladder/biliary passages) cancer suggested a small, statistically significant increase in risk. The summary risk estimate (RRm) from the meta-analysis of the 9 (all cohort) studies was 1.29 (95% CI: 1.07, 1.56). According to Rusyn *et al.* (2013), the IARC working group referred to nine cohort studies that had examined the relationship between occupational trichloroethylene exposure and risk of liver cancer. A majority of the cohorts only reported results for the combination of cancer of the liver and gall bladder or biliary passages. Although positive associations were observed in some studies, the results were somewhat inconsistent; there was no overall indication of an exposure–response relationship, and none of the studies provided information on potential confounders, such as alcohol consumption. The only case–control study available had only one exposed case. A recent meta-analysis reported a meta-RR of 1.3 (95% CI 1.1–1.6) for the overall trichloroethylene exposure based on the nine cohorts. The RR was similar when results from eight studies that provided information on high exposure were analysed (1.3, 95% CI 0.9–1.8) (Scott & Jinot, 2011).
Recently, Hansen *et al.* (2013) among cohorts of 5553 workers in the Nordic countries found increased standardised increased incidence ratios of 1.93 (CI 95%: 1.19-2.95) for liver cancer and 2.13 (CI 95%: 1.32-3.75) for cervical cancer, whereas no significantly increased ratios were found for kidney cancer or NHL.

3.2.2.4 Lung cancer

US EPA (2011) evaluated cancer of the respiratory tract including lungs, bronchus, and trachea from 25 cohort, community studies and case-control

studies of trichloroethylene. Twelve studies from the period of 1991 to 2008 of the 25 studies approached standards of epidemiologic design and analysis identified in the review of the epidemiologic body of literature on trichloroethylene and cancer.

A qualitative assessment of the epidemiological literature did not provide strong evidence for any association between trichloroethylene exposure and lung cancer. The limited epidemiological literature on lung and laryngeal cancer in trichloroethylene-exposed groups was inconclusive due to study limitations (low power, null associations, CIs on RRs that include 1.0).

The IARC (2012) working group also evaluated the association to trichloroethylene and lung cancer and cancer of other target sites. According to Rusyn (2013), the working group noted statistically significant excess risks of cancer of the lung, cervix and esophagus and multiple myeloma and leukemia were observed in isolated studies. However, due to relatively few observations for each site and due to inconsistencies in reported results, the database was considered to be inadequate with respect to evidence of human carcinogenicity.

3.2.2.5 Recent studies on occupational TCE exposure and risk of cancer
New and updated epidemiological studies of cancer risk after exposure to TCE have been published after the IARC evaluation in October 2012. Most importantly, the three Nordic cohort studies based on routine measurements of a urinary metabolite (Hansen *et al.* 2001; Axelson *et al.*1994; Anttila *et al.*1995) have been pooled, and the follow-up for cancer has been extended (Hansen *et al.* 2013). Overall, this pooled analysis finds a significantly increase in risk of liver cancer (1.93; 1.19-2.95) and cervical cancer (2.31; 1.32-3.75). Based on 32 cases of kidney cancer, the relative risk was 1.01 (0.70-1.42). The relative risk of non-Hodgkin's lymphoma was 1.26 (0.89-1.73). In general, the updated results were in line with previous results reported from the three individual studies, and given the relative small numbers of cancer from this pooled study, it is unlikely that these findings would have changed the conclusion of the IARC expert panel (McNeil 2013 and Purdue 2013). Finally, Charbotel *et al.* (2013) found no association between occupational TCE exposure and cervical cancer risk in a case-control study from France.

3.2.3 Conclusion on cancer effects of trichloroethylene
The experimental animal data show carcinogenic effects associated with exposure to trichloroethylene mainly via inhalation and oral routes. The carcinogenic effects mostly observed in animals were hepatocellular carcinomas (hepatomas) in mice (oral and inhalational exposure), pulmonary adenomas and carcinomas in mice (oral and inhalation exposure), renal adenocarcinomas in rats (oral and inhalational exposure), Leydig cell tumours in rats (inhalational exposure) and lymphomas in mice (inhalational exposure).

The association between trichloroethylene exposure and increased cancer risk has been studied in several epidemiological studies. Most clearly, there is an

association between increased risk of kidney cancer and humans occupationally exposed to trichloroethylene. The effects of trichloroethylene exposure on the development of Non-Hodgkin lymphoma have also been evaluated, and a significant increase in NHL risk has been identified in humans exposed to trichloroethylene. Regarding the liver cancer risk, the exposure to trichloroethylene suggests a modest increase in relative risk. The strength of the studies evaluating the effect of trichloroethylene exposure on lung cancer risk is low, so the association between trichloroethylene exposure and lung cancer cannot be concluded.

Recently, IARC (2012) has concluded trichloroethylene to be carcinogenic to humans (IARC group 1): IARC concluded that there was sufficient evidence for the carcinogenicity of trichloroethylene from both experimental animal data as well as from human data, and from mechanistic data.

In the EU, trichloroethylene has been classified as carcinogenic (Carc. 1B, H350 (CLP)).

3.3   Mutagenicity
This section especially focuses on the outcome of the most recent expert evaluations, i.e. US-EPA 2011 and the IARC evaluation from 2012.

Trichloroethylene
Trichloroethylene has a limited ability to induce mutation in bacterial systems, but greater evidence of potential to bind or to induce damage in the structure of DNA or the chromosome in a number of targets. A series of carefully controlled studies evaluating trichloroethylene (without mutagenic stabilisers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays.Therefore, it appears unlikely that trichloroethylene is a direct-acting mutagen, though trichloroethylene has shown potential to affect DNA and chromosomal structure. Trichloroethylene was also positive in some, but not all, fungal and yeast systems (US-EPA 2011).

Trichloroethylene may bind to nucleic acids and proteins, and such binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bio-activated with mouse and rat microsomal fractions. DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies reported the induction of micronuclei *in vitro* and *in vivo* from trichloroethylene exposure. Reports of SCE induction in some studies were consistent with DNA effects, but would require further study (US-EPA 2011).

Trichloroacetic acid (TCA)
Trichloroacetic acid (TCA), an oxidative metabolite of trichloroethylene, exhibited little, if any, genotoxic activity *in vitro*. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system, but a mutagenic response was induced in

TA100 in the Ames fluctuation test. Measures of DNA-repair responses in bacterial systems had shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations. TCA was positive in some genotoxicity studies *in vivo* mouse, newt, and chick test systems. DNA unwinding assays either showed TCA to be much less potent than DCA or negative. Due to limitations in the genotoxicity database, the possible contribution of TCA to trichloroethylene genotoxicity was considered to be unclear by the US-EPA (2011).

Dichloroacetic acid (DCA)
Dichloroacetic acid (DCA), a chloroacid metabolite of trichloroethylene, has been studied using different types of genotoxicity assays, although limited studies were conducted for different genetic endpoints. DCA has been demonstrated to be mutagenic in *in vitro S. typhimurium* assays, in mouse lymphoma assay, and further in *in vivo* cytogenetic tests, in the micronucleus test, and in the Big Blue mouse system. DCA caused DNA strand breaks in mouse and rat liver cells following *in vivo* exposure in mice and rats. Because of uncertainties as to the extent of DCA formed from trichloroethylene exposure, further conclusions as to the possible implication from DCA genotoxicity to trichloroethylene toxicity were considered to be difficult by US-EPA (2011).

Chloral hydrate (CH)
Chloral hydrate (CH) was mutagenic in the standard battery of screening assays. Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for mutagenicity at the Tk locus. *In vitro* tests showed that CH also induced micronuclei and aneuploidy in human peripheral blood lymphocytes and Chinese hamster pulmonary cell lines. Micronuclei were also induced in Chinese hamster embryonic fibroblasts. Several studies demonstrate that CH induced aneuploidy (loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast, cultured mammalian somatic cells, and spermatocytes of mice. CH was negative for sex-linked recessive lethal mutations in *Drosophila*. CH induces SSBs in hepatic DNA of mice and rats and mitotic gene conversion in yeast. CH affected centrosome structure, which resulted in the inability to reform normal microtubule formations and caused abnormal fertilisation and mitosis of sea urchin embryos. Based on the existing data, US-EPA (2011) found that CH had the potential to be genotoxic, particularly when aneuploidy was considered in the weight of evidence for genotoxic potential. CH appeared to act through a mechanism of spindle poisoning, resulting in numerical changes in the chromosomes (US-EPA 2011).

Dichlorovinyl cysteine (DCVC)
Dichlorovinyl cysteine (DCVC), and to a lesser degree dichlorovinlyl glutathione (DCVG), caused mutagenicity in bacteria based on consistent results in a number of available studies. DCVC was a strong, direct-acting mutagen both with and without the presence of mammalian activation enzymes. The lack of

similar response in bacterial assays with trichloroethylene was likely the result of the small yield (if any) of DCVC under *in vitro* conditions, since *in vivo,* DCVC is likely formed predominantly *in situ* in the kidney (S9 fractions are typically derived from the liver). DCVC and DCVG have not been evaluated extensively in other genotoxicity assays, but the available *in vitro* and *in vivo* data were predominantly positive. For instance, several studies have reported that DCVC induced primary DNA damage in mammalian cells *in vitro* and *in vivo*. Long-term exposure to DCVC-induced de-differentiation of cells. It has been shown to induce expression of the protooncogene c-Fos and cause cell transformation in rat kidney cells. In LLC-PK1 cell clones, DCVC was reported to induce UDS, but not micronuclei. Finally, DCVC induced transformation in kidney epithelial cells isolated from Eker rats carrying the heterozygous Tsc-2 mutations. Moreover, the lack of loss of heterozygosity (LOH) at the Tsc-2 locus observed in exposed cells does not constitute negative evidence of DCVC genotoxicity, as none of the renal tumours induced in Eker rats by the genotoxic carcinogen N-ethyl-N-nitrosourea showed loss of heterozygosity (LOH) (US-EPA 2011).

Furthermore, US-EPA (2011) found support of the importance of metabolism, as there is some concordance between effects observed from trichloroethylene and those from several metabolites. For instance, both trichloroethylene and CH have been shown to induce micronuclei in mammalian systems, but chromosomal aberrations have been more consistently observed with CH than with trichloroethylene. The role of TCA in trichloroehylene genotoxicity was considered less clear, as there was less concordance between the results from these two compounds. Finally, several other trichloroethylene metabolites showed at least some genotoxic activity, with the strongest data from DCA, DCVG, and DCVC. Even though the quantity of DCA, DCVG and DCVC were smaller compared to TCA and TCOH (for which there were almost no genotoxicity data), these metabolites might still be toxicologically important.

US-EPA (2011) found that uncertainties with regard to the characterisation of trichloroethylene genotoxicity still remained, particularly because not all trichloroethylene metabolites have been sufficiently tested in the standard genotoxicity screening battery to derive a comprehensive conclusion. However, the metabolites that have been tested, particularly DCVC, have predominantly resulted in positive data, although to a lesser extent DCVG and N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC). This supports that these compounds are genotoxic, particularly in the kidney, where *in situ* metabolism produces and/or bioactivates these trichloroethylene metabolites (US-EPA 2011).

This interpretation of the genotoxicity data by the US-EPA (2011) is in line with the recent evaluation of IARC in 2012 where the mutagenicity of trichloroethylene and its metabolites were evaluated. Overall, the IARC expert group found that there is strong evidence from trichloroethylene itself and from its metabolites to conclude that, following metabolism, trichloroethylene can be genotoxic, particularly in the kidney where *in situ* metabolism occurs and where

glutathione conjugation metabolites of trichloroethylene are generated (Rusyn 2013).

In EU, the mutagenicity of trichloroethylene has been recognised for many years as trichloroethylene since 2001 has been classified as a mutagen (Muta cat 3;R68 (DSD) and Muta 2;H341 (CLP)).

3.4 Mode of action (threshold/ non threshold)

In this section, the conclusions (regarding mode of action and threshold/non-threshold considerations) from the recent expert evaluations (as listed in the introduction) will be relatively briefly described.

In the sections below, focus is on the conclusions by the expert groups in relation to:

- Carcinogenic mode of action
- Use of threshold/non-threshold approach
- Identification of the most appropriate data sets for dose-response extrapolations

3.4.1 WHO 2000 evaluation

The purpose of this evaluation on trichloroethylene was to establish a WHO ambient air quality guideline. The WHO expert group put emphasis on the experimental animal inhalation and oral carcinogenicity studies: Maltoni *et al.* 1988; Henschler *et al.* 1980; Fukuda *et al.*1983; Maltoni *et al.* (1986); US NCI 1976; NTP 1990; Henschler *et al.* (1984) and NTP (1988). Epidemiological studies were only mentioned from secondary references and not from original literature and the IARC conclusion from 1995 was emphasised (limited human evidence for carcinogenicity).

From the mechanistic discussions, it was concluded that the observed increase of malignant lymphomas and liver tumours in mice may be of limited relevance to humans, whereas the data did not exclude the human relevance of lung tumours in mice and testicular tumours in rats. The evidence for kidney tumours in rats was considered to be weak.

Also, it was acknowledged that trichloroethylene may have a weak genotoxic action *in vivo*.

From epidemiological studies, positive associations between exposure to trichloroethylene and risks for cancer of the liver and biliary tract and non-Hodgkin lymphomas were observed. However, confounding in these studies could not be ruled out, and a quantitative risk estimate could therefore not be made from these data.

Overall, WHO (2000) considered the increase in lungs tumours in mice and in testes tumours in rats to be the most appropriate basis for a risk evaluation. Due

to the data on genotoxicity, it was not possible for WHO (2000) to conclude on a threshold with regard to carcinogenicity, and a linear low dose extrapolation (i.e. a non-threshold approach) from the animal data was used in order to provide a conservative approach for the estimation of the human cancer risk.

For estimating a unit risk, WHO (2000) applied a linearised multistage model on the incidence of Leydig cell tumours in rats (Maltoni *et al.* 1986) as this was found to be the most sensitive study. A unit risk estimate of 4.3 x 10^{-7} (μg/m^3)$^{-1}$ for trichloroethylene was derived and concluded.

3.4.2 EU-RAR (2004)

This risk assessment report was performed as part of the previous EU risk assessment program on existing high production volume chemicals. All relevant experimental animal data as well as human data at that time were referenced and described.The conclusion very much reflected the conclusions made by the Specialised Experts (March 2000) and the conclusion by the EC working Group on the Classification and labelling of Dangerous Substances (April 2000), where it was decided that trichloroethylene should be classified as Carc cat 2; R45 and Mut cat 3; R40.

The EU-RAR (2004) noted that trichloroethylene has been shown to induce apparent species and strain specific toxicity in mouse liver, mouse lung and the rat kidney.

With respect to liver toxicity in mice, the EU-RAR (2004) found a growing body of evidence that showed that development of liver tumours in mice was linked to the way mice metabolises trichloroethylene. In this species, trichloroethylene was much more readily metabolised to trichloroacetic acid, a metabolite which had also been shown to induce peroxisome proliferation and to cause sustained cell proliferation in mice. It was thought that these effects in combination lead to the development of liver tumours in mice. Studies *in vitro* had further showed that the metabolism of trichloroethylene in human hepatocytes was closer to that found in rat hepatocytes (i.e. much less than in mouse cells), and rat was a species which does not develop liver tumours following exposure to trichloroethylene. In addition, human hepatocytes had been shown not to undergo peroxisome proliferation in response to trichloroacetic acid, whereas both mouse and rat hepatocytes did. It therefore seemed reasonable to conclude that the findings in mice were unlikely to be of significance for humans.

The EU-RAR (2004) also found evidence linking the development of tumours in the mouse lung with metabolism of trichloroethylene in mouse Clara cells. It had been demonstrated *in vitro* that mouse Clara cells metabolise trichloroethylene to chloral hydrate, but are then inefficient at detoxifying this metabolite. This meant that chloral hydrate would build up within the Clara cell, and it was thought that the buildup of chloral hydrate within Clara cells resulted in cytotoxicity and repeated cycles of cell destruction and replication leading to

tumour formation. In contrast, perfused rat lungs exposed to trichloroethylene *in vitro* did not accumulate chloral hydrate and human lung tissue appeared to possess a negligible capability to metabolise trichloroethylene to chloral hydrate. This suggested that the lung tumours seen in mice were caused by a mode of action that was not relevant to humans.

The mode of action by which rats developed kidney toxicity and kidney tumours was, however, considered as less clear. There was evidence to show that hyaline droplet nephropathy was not involved. Instead, it has been suggested that the kidney tumours in rats arised as a result of repeated cytotoxicity. One proposed mode of action involved metabolism via the glutathione conjugation pathway to form DCVC,which could be activated by renal β–lyase to reactive metabolites, known to be mutagenic and nephrotoxic. It was mentioned that the metabolites from the glutathione pathway had also been detected in humans; however, it seemed that this was qualitatively a very minor pathway in all species. A second proposed mode of action noted involved the trichloroethylene induced increased excretion of formic acid, possibly resulting from an inhibition of the methionine salvage pathway.

The EU-RAR (2004) noted the uncertainty surrounding the mode of action by which nephrotoxicity may occur in rats and the relevance to humans, but concluded that the findings in the kidneys of the rat were to be considered of concern for human health.

The EU-RAR (2004) especially took note of the epidemiological studies by Henschler *et al.* (1995) and Vamvakas *et al.* (1998) that found increased risk of kidney cancer in trichloroethylene exposed workers. Although the EU-RAR (2004) mentioned uncertainties related to these studies, it was concluded (also considering the rat data) that there was 'some concern for the potential carcinogenicity to humans'.

The EU-RAR (2004) in their summary specifically referred to the evaluation by the Specialised Experts in March 2000 that considered four plausible, not mutually exclusive mechanisms for kidney tumour formation, possibly relevant for humans. However, they agreed that there was insufficient evidence for any of them to be considered as proven. One mechanism involved the formation of reactive intermediates locally in the kidney by beta lyase following metabolism of trichloroethylene via a glutathione pathway. A second mechanism involved renal toxicity via the accumulation of formic acid and, potentially, a perturbation of the methylation status. The other two plausible mechanisms involved genotoxicity. The Specialised Experts considered that trichloroethylene might pose a carcinogenic hazard through either a pathway involving induction of aneuploidy, or one involving mitotic recombination or point mutations.

Overall, carcinogenicity was in the EU-RAR (2004) regarded as a critical health effect in the risk characterisation for human health. Because of uncertainties

about the mode of action, it was not possible to draw any conclusions with regard to the presence of an identifiable threshold level of exposure below which there was no increased risk.

Consequently, no threshold was assumed for mutagenicity and carcinogenicity in the risk characterisation part of the EU-RAR (2004) report, and all exposure scenarios with trichloroethylene exposure led to a conclusion (iii), indicating concern for mutagenicity and carcinogenicity.

Thus, the EU-RAR (2004) concluded on a non-threshold mode of action for trichloroethylene due to uncertainties about the mode of action and due to the concern for the formation of known genotoxic metabolites. No specific data set or dose metric for point of departure were identified for a further dose response analysis of the carcinogenicity of trichloroethylene.

3.4.3 WHO 2005 evaluation

The purpose of this evaluation was to derive a WHO drinking-water quality guideline on trichloroethylene.

The basis for evaluating the carcinogenicity in experimental animals was to a great extent the same as in the WHO (2000) evaluation, as no newer studies were included; however, emphasis was put on the oral studies (NTP 1983; NTP 1988 and NTP 1990). WHO (2005) put further emphasis on the findings of kidney tumours in rats to be relevant for humans compared to the WHO (2000) evaluation. Also the section regarding epidemiological data was much more extended than the WHO (2000) evaluation, and considerations regarding carcinogenic mode of action were described in more detail.

The experimental animal results considered most pertinent by WHO (2005) in assessing the weight of evidence of carcinogenicity of trichloroethylene in humans were principally the significant increases in kidney tumours in rats (NTP, 1983, 1990), pulmonary tumours in mice (Fukuda *et al.*, 1983; Maltoni *et al.*, 1986, 1988; NTP, 1988) and testicular tumours in rats (Maltoni *et al.*, 1986, 1988; NTP, 1988). Although there was some doubt about the human relevance of pulmonary tumours in mice, the potential for lung tumours in humans could not be ruled out.

Various mode of actions were considered e.g.: non-genotoxic processes related to cytotoxicity, peroxisome proliferation and altered cell signalling; genotoxic processes, such as the production of genotoxic metabolites (e.g., chloral and DCVC); or the production of reactive oxygen species related to peroxisomal induction in the liver. As trichloroethylene appeared to be weakly genotoxic in *in vitro* and *in vivo* assays, and as several mutagenic or carcinogenic metabolites were formed, the genotoxic mode of action was not to be ignored.

The evidence surrounding kidney tumours in rats after oral exposure was

especially considered. Although it was noted that the tumours were few, the finding was repeatable in Sprague-Dawley rats exposed to trichloroethylene by the inhalational route.The tumours are historically rare in rats, so their appearance among dosed animals was considered biologically significant.

Also, there were similarities between sites and histopathological characteristics of the tumours observed in human patients and in rat bioassays. The metabolites derived from trichloroethylene were noted to be identical in humans and in experimental animals. So, small increases in renal tumours in male rats at doses inducing renal damage were found to support the epidemiological evidence.

Further support regarding mode of action was noted from the data on multiple mutations of the von Hippel Landau tumour suppressor genes followed by renal neoplasia that has been found in renal carcinoma patients with high prolonged trichloroethylene exposure.

Overall, WHO (2005) found use of a non-threshold approach most appropriate for describing the carcinogenic risks in relation to trichloroethylene. Use of a linearised multistage (LMS) approach was supported by the possible genotoxicity associated with some trichloroethylene metabolites, particularly DCVC and DCVG. Although not used, the WHO (2005) noted that a non-linear approach could be argued due to a possible mixed mode of action (mutagenicity and cytogenicity) of trichloroethylene and enhanced susceptibility of the rat to nephropathy.

Applying the linearised multistage model on the pooled combined tubular cell adenomas and adenocarcinomas of the kidneys in male rats in the oral study, WHO concluded on a cancer unit risk estimate of 7.80×10^{-4} (mg/ kg body weight per day)$^{-1}$ (see section 4.3).

3.4.4 AGS (2008) evaluation

The purpose of this evaluation on trichloroethylene was to determine dose-response relationship and establish tolerable/acceptable risk levels for workers. The focus in this evaluation was on the dose-response relationship for renal cancers in humans (especially the epidemiological studies by Henschler *et al.* 1995; Vamvakas *et al.* 1998, and Brüning *et al.* 2003).

Overall, AGS (2008) considered it doubtful whether trichloroethylene could also cause hepatic cancer and non-Hodgkin lymphomas in humans. In comparison to the triggering of renal tumours, the dose-response analyses performed by AGS indicated a low probability of higher cancer risks for these other organs.

AGS (2008) found trichloroethylene to be a complete carcinogen for the kidney, i.e. involving both initiation and promotion/progression processes. It was noted that reactive metabolites (chlorothioketenes) were formed in the target tissue of the proximal tubulus due to the reductive, glutathione-dependent metabolism,

which was mediated by glutathione- transferase(s) and beta-lyase. Furthermore, gender- and species-related differences were noted for this metabolic pathway among rats, mice and humans. In case of the further beta-lyase-dependent metabolism, there was an inter-species difference with higher beta-lyase activity in rats compared to humans (*in vivo*). The glutathione-dependent pathway was found to have greater weight, when the oxidative main metabolic pathway was saturated at higher trichloroethylene exposure levels.

Due to the metabolic activation (via DCVC/thioketene) of trichloroethylene and the observation of specific VHL mutations in humans, the genotoxic mechanisms were considered probable for the development of renal tumours.

It was noted that in cases where renal carcinoma occurred after a high occupational exposure to trichloroethylene, mutations of the VHL tumour suppressor gene have more frequently been found. The VHL gene and its coded gene products (pVHL) are involved in regulating the cellular metabolism under oxygen-deprived conditions and in stabilising micro-tubular structures. However, the mechanistic background of the relationship between VHL mutations and the occurrence of renal cancer appeared not to have been clarified in all its details.

The dose-effect characteristics of the genotoxic effects of trichloroethylene on the kidney was by the AGS expert group considered to be most adequately described by a non-linear approach. Peak exposure was considered important for the occurrence of kidney cancer, and it was noted that the dose-dependent shift of the metabolic pathways most probably would lead to an overestimation of the actual risk when assessing average low level trichloroethylene exposure. Because of the species-related differences in beta-lyase activity, the risk extrapolated on the basis of animal studies would tend to overestimate the risk to humans.

It was described that the nephrotoxic effect of trichloroethylene in the relevant range of exposure is considered to be mediated by the metabolites trichloroethanol and trichloroacetic acid. The quantities of the metabolites were significant and they interact with the vitamin B12-dependent C1 metabolism. This interaction further results in a folic acid deficit and an excess of formic acid leading to an acidification of the cytoplasm and consequently to cytotoxicity at the higher dose levels. This has been proven in animal studies with subacute exposure (28 days, 6 h/day) of rats to 250 or 500 ppm trichloroethylene, in which no morphological renal damage was found, but there was an increase of formic acid in the urine, and this was associated with a decreasing pH-value.

AGS (2008) noted that Green *et al.* (2004) had further elaborated on the cytotoxic metchanism and carried out an occupational medical field study with 70 workers with trichloroethylene exposure and 54 controls. The average trichloroethylene exposure calculated on the basis of trichloroacetic acid excretion was 32 ppm (total range: 0.5 - 252 ppm) with an average length of exposure of 4.1 years (total range: 1 - 20 years). Significant differences between

the exposed persons and the controls were found in the excretion of biomarkers for subclinical nephrotoxicity (N- acetylglucosaminidase (NAG) and albumine as well as formic acid). Associations were also found between the excretion of trichloroacetic acid and formic acid on the one hand and methylmalonic acid and glutathione-transferase alpha on the other, but these were generally within the range in controls. Clinically manifest renal damage was not found in this study, but the authors found that there were dose-dependent subclinical effects in the studied dose range (up to 250 ppm trichloroethylene). Manifest clinical effects of renal damage only occur above this range. This view was consistent with the data from animal studies.

Also, in another study with Scandinavian workers in which the majority of the employees (25 of 29) had been exposed to less than 6 to 10 ppm trichloroethylene, it was noted that there was no increased excretion of the N-acetyl-ß-D-glucosamine (NAG) biomarker (Selden *et al.* 1993). Although, this was a small study cohort, a threshold for nephrotoxicity was noted at approx. 6 ppm (33 mg/m^3), especially since the effects were not pronounced at 32 ppm.

Overall, AGS (2008) concluded that a clear threshold for the effects of trichloroethylene could not be established, as genotoxicity was observed in the kidneys. Also, local genotoxicity could not either be excluded for the development of liver cancer and non-Hogdkin lymphoma.

Based on the epidemiological studies performed in Germany (Henschler *et al.* 1995); Vamvakas *et al.* 1998; Brüning *et al.* 2003), an excess lifetime risk of renal cancer amounting to 5% after an assumed cumulative exposure of 3000 ppm-years was estimated.

A sublinear dose-response curve was considered most appropriate for low dose extrapolation from the human data on kidney tumours. A break point for nephtotoxicity of 6 ppm was determined based on the biomarker studies. Thus, a linear extrapolation for excess risk was performed for exposures above 6 ppm based on the dose-response data from (Henschler *et al.* 1995;d Vamvakas *et al.* 1998, and Brüning *et al.* 2003), and another linear extrapolation was used from the extrapolation of excess risk from 6 ppm and down to 0 ppm exposure. Below 6 ppm the slope factor was determined as significantly lower (risk downscaled with a factor of 10) as no cancer-enhancing effect (nephrotoxicity) was expected to occur at these lower levels (further details in section 4).

3.4.5 SCOEL 2009 evaluation
The purpose of this evaluation on trichloroethylene was to determine dose-response relationship and establish a tolerable exposure level for workers. SCOEL (2009) put emphasis on the human data on renal cell carcinoma and refered to animal data and genotoxicity data as supporting data important for mechanistic considerations.

For the epidemiological studies, the evidence for kidney cancer was considered much stronger compared to the human data on non-Hodgkin's lymphoma and

liver tumours. This was especially supported by the most recent studies by Brüning *et al.* (2003), Raaschou-Nielsen *et al.* (2003) and Charbotel *et al.* (2006).

SCOEL found that data for trichloroethylene indicated a weak mutagenic effect *in vitro*. Further evidence existed that trichloroethylene led to specific mutations in the kidney at the von-Hippel-Lindau (VHL) tumour suppressor gene.

SCOEL (2009) also recognised that some of the metabolites of trichloroethylene were genotoxic, especially those formed via the minor reductive pathway of metabolism, if not eliminated as mercapturic acids. DCVC are to be considered as genotoxic that can possibly react with DNA. In addition, dichloroacetic acid (DCA) (assumed to be one of the minor oxidative metabolites) was genotoxic in several *in vitro* and *in vivo* assays, but this was considered probably to be of minor relevance in trichloroethylene metabolism in humans.

Therefore, SCOEL considered it plausible that genotoxicity may contribute to the genesis of specific local tumours, but that genotoxicity was probably not the general major driving force for carcinogenesis of trichloroethylene as with classic DNA-alkylating agents. In general, trichloroethylene presumably may act via a number of different mechanisms in parallel, some of which result in tumours in experimental animals and in humans.

In relation to renal cell carcinoma, SCOEL found that chronic progressive nephropathy could be a mechanism of tumour formation. Rats exposed to trichloroethylene have been shown to excrete elevated amounts of formic acid. This intermediate is formed via the oxidative pathway by the interaction of TCOH and/or trichloroacetic acid with the vitamin B12-dependent C1 metabolism). Formic acid was nephrotoxic by induction of cellular acidosis and was already elevated in the urine of rats after subacute exposure to 250 to 500 ppm (1,365-2,730 mg/m^3) (Green *et al.*, 1998). Mice eliminated much less formic acid than rats. This could explain the differences in sensitivity between rats and mice with regard to nephrotoxicity and also to secondary carcinogenesis via this tumour-promoting mechanism. However, after chronic intake of high amounts of TCOH via drinking water only a moderate increase in renal cell turnover could be demonstrated, which was reversible. On the other hand, inhalation exposure of workers to low concentrations of trichloroethylene apparently has led to disturbances of vitamin B12 metabolism, elevated formate excretion and first indications of renal injury.

SCOEL found the results of animal studies in the male rat and data on metabolism and modes of action supportive for the renal carcinogenic effects of trichloroethylene. Genotoxicity may contribute to the mechanism because DCVC and its respective β-lyase products have a genotoxic potential. Moreover, mutations of the VHL gene in the kidney tumours of trichloroethylene-exposed humans were plausibly associated with a loss of tumour suppression and subsequent growth of renal cell tumours. In general, a mechanism was assumed in which kidney toxicity combined with genotoxicity in the kidney were

considered as co-carcinogenic factors.

SCOEL found it important to note that tumours in human kidneys were only observed after occupational trichloroethylene exposure to very high concentrations, which were clearly nephrotoxic. Such exposures clearly exceeded former exposure limits of 50 ppm, and peak exposures of several hundred ppm were very likely involved. At these high dose ranges, it is known that the toxification of trichloroethylene via the reductive glutathione-pathway is proportionally increased, compared to lower doses where the glutathione-dependent metabolism is only marginal. This, because the oxidative CYP-dependent metabolism is saturable. Both aspects, the impact of cytotoxicity and the relative increase of glutathione-dependent metabolism at high doses of trichloroethylene, make a sub-linear dose-response relationship at lower exposure concentrations highly plausible.

Therefore, according to SCOEL (2009) a linear extrapolation of kidney tumour risks should be limited to clearly nephrotoxic concentrations. Even this approach is still considered conservative by SCOEL, as the relative influence of glutathione-dependent metabolism at slightly nephrotoxic exposure concentrations has probably already decreased, compared to the much higher concentrations at which tumours have been observed.

The observations in experimental systems, as well as in occupationally exposed and diseased persons, led SCOEL to conclude that human renal cell cancer risk would be avoided if exposure to nephrotoxic concentrations of trichloroethylene does not occur, including trichloroethylene concentrations leading to sub-clinical renal changes that can be monitored by urinary excretion of suitable marker proteins. In a cohort study by Green *et al.* (2004) covering 70 workers some minor sub-clinical alterations in renal functional parameters were observed at mean trichloroethylene levels of 32 ppm (range 0.5-252 ppm). At lower exposure levels Seldén *et al.* (1993), found no increase in urinary excretion of the NAG marker protein in workers exposed in the range of 6-10 ppm trichloroethylene.

On this background and with reference to the SCOEL strategy for derivation of OELs for carcinogens and mutagens (Bolt and Huici-Montagud, 2008), SCOEL concluded trichloroethylene to be a *"genotoxic carcinogen, for which a practical threshold* is supported by studies on mechanisms and/or toxicokinetics"*.

Hence, a health-based occupational exposure limit (OEL) could be established for trichloroethylene based on a NOAEL in exposed humans related to the avoidance of renal toxicity. From the data from the studies of Green *et al.* (2004) and Seldén *et al.* (1993), an OEL (TWA) of 10 ppm was therefore proposed for trichloroethylene.

Overall, SCOEL (2009) concluded on a <u>practical</u>* threshold for the carcinogenic effects of thrichloroethylene due the findings from worker exposure and due to mechanistic considerations. The genotoxic potential of DCVC was

assumed to contribute (but not to be the primary course) to the development of renal cell carcinoma after high levels of trichloroethylene exposure. The cytotoxic action of trichloroethylene metabolites was considered as the driving force for the development of renal cancer, especially the formation of the metabolite formic acid as a nephrotoxic substance was mentioned and also the formation of DCVC causing mutation in the VHL gene (leading to loss of tumour suppression and subsequent growth of renal cell tumours). Therefore, the derivation of an OEL was based on a NOAEL for cytotoxic effects on the kidney. This level was also considered to be a practical threshold for the carcinogenic effects.

*The term "**practical threshold**" refers to the publication by Bolt & Huici-Montagud (2008): 'Strategy of the scientific committee on occupational exposure limits (SCOEL) in the derivation of occupational exposure limits for carcinogens and mutagens'. Here carcinogens are grouped into four different groups according to their mode of action and considerations regarding threshold level/ non threshold levels:

(A) Non-threshold genotoxic carcinogens; for low-dose assessment of risk, the LNT model appears appropriate. For these chemicals, regulations (risk management) may be based on the ALARA principle ("as low as reasonably achievable"), technical feasibility, and other socio-political considerations.

(B) Genotoxic carcinogens, for which the existence of a threshold cannot be sufficiently supported at present. In these cases, the LNT model may be used as a default assumption, based on the scientific uncertainty.

(C) Genotoxic carcinogens with a **practical threshold**, as supported by studies on mechanisms and/or toxicokinetics; health-based exposure limits may be based on an established NOAEL (no observed adverse effect level).

(D) Non-genotoxic carcinogens and non-DNA-reactive carcinogens; for these compounds a true ("perfect") threshold is associated with a clearly founded NOAEL. The mechanisms shown by tumour promoters, spindle poisons, topoisomerase II poisons and hormones are typical examples of this category.

3.4.6 WHO 2010 evaluation

The purpose of this WHO evaluation of trichloroethylene was to establish a WHO indoor air quality guideline for the substance. The carcinogenicity studies for evaluation of the experimental animal evidence are the same as in WHO (2000), however, now supported by further mechanistic data. The evaluation of the human data on carcinogenicity now refers to studies up to 2009, however, still the overall human evidence is considered to be limited and considered comparable to the IARC conclusion from 1995 (limited human evidence).

WHO (2010) found that the metabolism of trichloroethylene without any doubt plays a very important role in its mechanism of carcinogenic action. However, the mechanisms behind trichloroethylene-induced carcinogenesis were considered to be complex, involving multiple carcinogenic metabolites acting in various ways. Past explanations, such as the hypothesis linking mouse liver tumours to peroxisome proliferation, were not considered consistent. A more plausible mode of action could be that trichloroethylene induced liver tumours through trichloroacetic acid and dichloroacetic acid leading to modification of the cell signalling systems that control cell division rate and cell death. This hypothesis suggested that humans are likely to be much less responsive than

mice, and that carcinogenic effects are unlikely to occur at low environmental exposures.

WHO (2010) noted that the induction of pulmonary tumours in mice could be linked to the fact that Clara cells rapidly metabolise trichloroethylene into chloral hydrate, via CYP4502E1, leading to pulmonary accumulation of this metabolite, which then produces cell changes and compensatory proliferation. But other mechanisms of action may be involved, particularly since chloral hydrate is probably genotoxic and, at high doses, clastogenic. In rats, Clara cells are capable of metabolising chloral hydrate into trichloroethanol. In humans, the capacity of the lungs to transform trichloroethylene into chloral hydrate was thought to be negligible and, consequently, the mechanism of pulmonary carcinogenesis demonstrated in mice may be specific to mice.

Furthermore, renal tumours in male rats could be linked to cytotoxicity and persistent cell regeneration. Conjugation to glutathione and the involvement of beta-lyase in the renal tubules may lead to the formation of nephrotoxic and probably genotoxic reactive metabolites, in particular DCVC and DCVG. Studies have demonstrated that trichloroethylene induces mutations in the VHL tumour suppressor gene in the cells of renal carcinomas in patients with this form of cancer. A second possible and relevant mechanism involved increased secretion of formic acid, leading to a disruption in the detoxification mechanism by methionine. However, it was emphasised that the mechanisms behind kidney tumours in rats were not fully clarified.

These considerations, and the effects observed in humans, justified according to WHO (2010) a cautious attitude regarding the extrapolation to humans of results observed in animals.

Overall, WHO (2010) found that animal evidence is sufficient to demonstrate carcinogenic effects of trichloroethylene by both oral and inhalation routes, and there is sufficient evidence to conclude that trichloroethylene is at least weakly genotoxic. Positive associations had been established between occupational exposure and risks for cancer of the liver, kidney and bile duct and non-Hodgkin's lymphoma. Although lung and testis tumours observed in rodents have not been reported in humans, they cannot be excluded. The presence of possible exposure misclassification or co-exposure in occupational cohort studies were found to somewhat weaken the confidence in the association. Overall, it was concluded that sufficient evidence exists to suggest an association between trichloroethylene exposure and cancer (liver and kidney).

WHO (2010) applied a non-threshold approach with a risk estimate rather than a safe level. This was based on recent data on a mechanism of action that was not species-specific, the evidence for weak genotoxicity, and the consistency between certain cancers observed in animals and in humans (in particular liver cancer).

Thus, carcinogenicity (with the assumption of genotoxicity) was selected as the end-point for setting the guideline value in indoor air.

Overall, WHO (2010) found the use of a non-threshold approach most appropriate for describing the carcinogenic risks in relation to trichloroethylene, and the expert group concluded on a unit risk estimate of 4.3 x 10-7 $(\mu g/m^3)^{-1}$, applying a linearised multistage model on the incidence of the Leydig cell tumours in rats in the Maltoni *et al.* (1986) study. *This was also the conclusion for the unit risk estimate in the WHO (2000) evaluation.*

3.4.7 US EPA 2011 evaluation

In 2011, the US EPA finalised a very comprehensive health assessment report on trichloroethylene for the update of the Integrated Risk Information System (IRIS) database. The report contains a thorough evaluation of the toxicological and epidemiological data on trichloroethylene in relation to both carcinogenic and non-carcinogenic end-points. As also indicated in Section 3.2.2, US-EPA (2011) found the strongest and most convincing data for an association between trichloroethylene and kidney cancer, which will be further described below. Furthermore, the carcinogenic dose-response relationship for the development of kidney cancer was determined based on the epidemiological data by Charbotel *et al.* (2006) on trichloroethylene exposed workers.

US EPA (2011) found that the positive genotoxicity data in the database of available studies of trichloroethylene metabolites derived from GSH conjugation (in particular DCVC), together with toxicokinetic data consistent with their systemic delivery to and *in situ* formation in the kidney, supported the conclusion that a mutagenic mode of action is operative in trichloroethylene-induced kidney tumours. While supporting the biological plausibility of this hypothesised mode of action, available data on the VHL gene in humans or transgenic animals did not conclusively elucidate the role of VHL mutation in trichloroethylene-induced renal carcinogenesis. Cytotoxicity and compensatory cell proliferation, similarly presumed to be mediated through metabolites formed after GSH-conjugation of trichloroethylene, had also been suggested to play a role in the mode of action for renal carcinogenesis, as high incidences of nephrotoxicity had been observed in animals at doses that induce kidney tumours. Human studies had reported markers for nephrotoxicity at current occupational exposures, although data were lacking at lower exposures. Nephrotoxicity was observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumours were only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appeared to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity were reached before the onset of tumours. In addition, nephrotoxicity had not been shown to be necessary for kidney tumour induction by trichloroethylene in rodents. In particular, there was a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumours induced by trichloroethylene.

Furthermore, it was not clear if nephrotoxicity is one of several key events in a mode of action, if it was a marker for an upstream key event (such as oxidative stress) that could contribute independently to both nephrotoxicity and renal carcinogenesis, or if it was incidental to kidney tumour induction. Therefore, although the data were consistent with the hypothesis that cytotoxicity and regenerative proliferation contribute to trichloroethylene-induced kidney tumours, the weight of evidence was not as strong as the support for a mutagenic mode of action. Moreover, while toxicokinetic differences in the GSH conjugation pathway along with their uncertainty were addressed through PBPK modelling, no data suggest that any of the proposed key events for trichloroethylene-induced kidney tumours in rats were to be ruled out for humans. Therefore, trichloroethylene-induced rat kidney tumours provided additional support for the convincing human evidence of trichloroethylene-induced kidney cancer, with mechanistic data supportive of a mutagenic mode of action.

Further, US-EPA (2011) found trichloroethylene characterised as carcinogenic to humans by all routes of exposure. This conclusion was based on convincing evidence of a causal association between trichloroethylene exposure in humans and kidney cancer. The consistency of increased kidney cancer RR estimates across a large number of independent studies of different designs and populations from different countries and industries provided compelling evidence given the difficulty, in detecting effects in epidemiologic studies when the RRs were modest and the cancers were relatively rare, and therefore, the individual studies had limited statistical power. The strong consistency of the epidemiologic data on trichloroethylene and kidney cancer argued against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends were observed in high-quality studies. These studies were conducted in populations with high trichloroethylene exposure intensity or had the ability to identify trichloroethylene-exposed subjects with high confidence. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use was the mode(s) of action. However, mode-of-action data were lacking or limited for each of the cancer responses associated with trichloroethylene exposure, with the exception of the kidney tumours. For the kidney tumours, the weight of the available evidence supported the conclusion that a mutagenic mode of action was operative; this mode of action supported linear low-dose extrapolation. The weight of evidence also supported involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of trichloroethylene, although not with the extent of support as for a mutagenic mode of action. In particular, data linking trichloroethylene-induced proliferation to increased mutation or clonal expansion were lacking, as

were data on the quantitative contribution of cytotoxicity. Moreover, it was considered unlikely that any contribution from cytotoxicity led to a non-linear dose-response relationship near the PODs. In the case of the rodent bioassays, maximal levels of toxicity were reached before the onset of tumours. Finally, because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action did not provide evidence against the use of linear extrapolation from the POD.

For the other trichloroethylene -induced cancers, the mode(s) of action was unknown. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk.

Using the dose-response relationship in the Charbotel *et al.* (2006) study an *inhalation unit risk* for trichloroethylene was estimated to 1×10^{-6} per µg/m^3 for development of renal cell carcinoma and of 4×10^{-6} per µg/m^3 for development of cancer on multiple sites (adjusted for the potential risk for NHL and liver cancer).

The oral slope factor (unit risk) was estimated to 1.0×10^{-2} per mg/kg/day, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate.

Thus, US EPA (2011) with respect to low-dose extrapolation for carcinogenic effects clearly concluded on a non-threshold approach with linear extrapolation due to the genotoxic mode of action of the metabolites of trichloroethylene. Any possible involvement of a cytotoxicity mode of action was considered to be additional to the mutagenic mode of action and was not considered to be the primary cause. Also, cytotoxicity was not considered to affect the dose-response significantly even at high exposure levels. The Charbotel *et al.* (2006) study was evaluated as a high quality study and adequate for describing the dose-reponse relationship for trichloroethylene exposure and renal cancer risk in humans.

3.4.8 IARC 2012 evaluation

The purpose of this evaluation was to update the previous IARC (1995) evaluation where trichloroethylene was concluded to be a group 2A carcinogen (limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals). New epidemiological and mechanistic data, supported by animal data, led IARC 2012 to conclude trichloroethylene to be a Group 1 carcinogen with sufficient evidence of carcinogenic effects in humans.

The following description of the IARC (2012) evaluation is from Rusyn *et al.* (2013), as IARC has not finalised and released the IARC Monograph of the evaluation.

The IARC expert group recognised that trichloroethylene is metabolised via two

main pathways to multiple toxic, mutagenic and carcinogenic metabolites that likely contributed to the carcinogenicity of the parent compound. Supporting mechanistic findings included evidence of genotoxicity of trichloroethylene and its metabolites, with the strongest evidence of metabolites formed from the glutathione pathway in the kidney. A recent epidemiologic study reported that kidney cancer risk was attenuated in individuals lacking the GSH conjugation gene GSTT1. This study provided evidence in support of the hypothesis that GSH conjugation plays a critical role, and contributes significantly to an overall cohesive mechanistic database.

With regard to the IARC evaluation of genotoxicity, the strongest evidence was found for the following metabolites: chloral (C) and chloral hydrate (CH) and DCVG and DCVC.

The IARC expert group found strong evidence to suggest that C/CH may cause genotoxicity. Numerous studies had shown that C/CH was genotoxic both *in vivo* and *in vitro* in mammalian and other test systems, including studies with and without metabolic activation. The types of genotoxic damage detected encompass mutations, chromosomal aberrations, micronuclei, and cell transformation.

DCVG has not been evaluated in most recommended genotoxicity screening assays. The mutagenicity of DCVG had been evaluated in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and a beta-lyase inhibitor. DCVG exhibited direct-acting mutagenicity with kidney mitochondria, cytosol, or microsomes enhancing the effects and a beta-lyase inhibitor diminishing, but not abolishing the effects. Addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with *in situ* metabolism playing a significant role in the genotoxicity of DCVG-derived species in the kidney. DCVC has demonstrated a strong, direct-acting mutagenicity both with and without the presence of mammalian activation enzymes, including those derived from the kidney, in bacterial mutagenesis tests.

The genotoxicity of DCVC was supported by the predominantly positive results in other available *in vitro* and *in vivo* assays. The observed effects included DNA strand breaks and unscheduled DNA synthesis, but not micronuclei. S-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS), a product of sulfoxidation of DCVC, had been found to be mutagenic in the Ames *S. typhimurium TA100* strain. NAcDCVC was also shown to exhibit direct-acting mutagenicity in the absence of exogenous metabolic activation, with kidney cytosol enhancing the effects and a beta-lyase inhibitor diminishing, but not abolishing the effects.

The IARC expert group also discussed the several (non-genotoxic) mechanisms that have been suggested to account for cytotoxicity of trichloroethylene in the kidneys.Thus, i*n vitro* studies with GSH conjugates of trichloroethylene showed

unequivocally that DCVC and its metabolites (e.g., DCVCS) were cytotoxic to primary human proximal tubular cells. Similar observations were made in rodent. In addition, increased formation and urinary excretion of formic acid mediated by the oxidative metabolites TCA or TCOH had also been suggested to contribute to the observed nephrotoxicity of trichloroethylene. However, these oxidative metabolites did not appear sufficient to explain the range of renal effects observed after trichloroethylene exposure. Other mechanisms of cytotoxicity, including alteration of calcium ion homeostasis and mitochondrial dysfunction, had been identified *in vitro* in kidney cells. The primary limitation to the evidence supporting the role of this mechanism in kidney cancer was that nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumours were only observed at low incidences in rats at the highest tested doses (NCI, 1976; NTP, 1990). Therefore, data demonstrating a causal link between compensatory proliferation and the induction of kidney tumours were considered to be lacking.

Other non-genotoxic mechanisms such as peroxisome proliferation in the kidneys and α2µ-Globulin-associated nephropathy were discussed by the IARC expert group as well. These mechanisms were not considered as plausible mechanisms for the development of kidney cancer.

Overall, the IARC expert group found that the occupational studies provided sufficient evidence of an association between trichloroethylene exposure and kidney cancer.

Positive associations, supported by a meta-analysis, have also been observed between occupational trichloroethylene exposure and risks for non-Hodgkin lymphoma and cancer of the liver, but this epidemiological evidence was characterised as limited.

Thus, IARC (2012) put strong emphasis on the formation of genotoxic metabolites of trichloroethylene especially DCVC, as a mechanistic support for their conclusion. At the same time the data on non-genotoxic mechanisms, e.g. cytotoxicity were not considered sufficient for the development of cancer, as data demonstrating a causal link between compensatory proliferation in kidneys and induction of tumours were found to be lacking for humans as well as animals.

Discussions and conclusions regarding carcinogenic threshold / non threshold or in relation to identification of a POD for risk assessment or unit risk estimates were *not addressed* by IARC, as these elements are not part of the task of the IARC expert group that focuses on the hazard identification and the degree of evidence for carcinogenicity of a substance.

3.4.9 HSE 2012

The purpose of this evaluation was to produce an updated estimate of the current burden of cancer for Great Britain resulting from occupational exposure to carcinogenic agents including trichloroethylene.

In this evaluation, HSE as a basis for their evaluation used an average SMR for kidney cancer of 1.2 (95% CI 0.8-1.7) reported in the review from Wartenberg *et al.* (2000). This was considered as a SMR for 'higher exposures' groups selected from the available epidemiological studies at that time (year 2000) even though more recent epidemiological studies were presented by the HSE (2012) (see Table 3-3).

Based on estimates of the number of highly exposed workers in Great Britain and the back ground incidence for kidney cancer, HSE (2012) concluded that the estimated total (male and female) attributable fraction for kidney cancer associated with occupational exposure to trichloroethylene was 0.04% (95% Confidence Interval (CI)=0.00-0.15), which corresponded to 1 (95%CI=0-5) death, and 3 (95%CI =0-10) registrations.

Discussions and conclusions regarding carcinogenic mode of action; threshold / non threshold; identification of a specific POD for dose-response estimations or unit risk estimates were not addressed in the HSE (2012) evaluation.

3.4.10 Afsset 2009 and Anses 2013 evaluations

In 2009, the French agency Afsset established indoor air quality guidelines values (Afsset, 2009), and the conclusion was to use the unit risk estimate provided by WHO (2000). The purpose of the Anses (2013) evaluation was to evaluate whether the unit risk estimate recently provided by US EPA (2011) should result in an adaption of the previous Afsset (2009) conclusion.

The mechanistic consideration by Afsset (2009) is given below (translated from French text):

Affset (2009)
The mode of carcinogenic action of trichloroethylene can be attributed to mechanisms involving non-genotoxic actions (cytotoxicity, peroxisome proliferation, altered cell signaling) as well as genotoxic modes of action (chloral hydrate and DCVC).

However, with the current state of knowledge, various assumptions fail to accurately identify key events responsible for the development of cancer at different sites (lungs, liver, kidneys). The ambiguity about the role of active metabolites and the different mode of action should lead to great caution when making extrapolation from animal data to humans (differences in sensitivity, quantitative differences in the kinetics between species and also according to the differences in levels of exposure). Trichloroethylene should be considered to pose a cancer risk to humans; however, the interspecies differences in

metabolic capacity make it difficult to predict with confidence the risks to humans.

Thus, the Afsset working group (2009) suggested the carcinogenic effects of trichloroethylene to be without threshold. It was concluded that the development of cancer, demonstrated in animals, could also occur in humans. The working group considered the WHO (2000) assessment for an ambient air quality guideline to be of high quality. Although it was based solely on animal data, the cancer risk estimate developed by WHO (2000) was accepted by the working group.

Anses (2013)

Anses evaluated in 2013 the new units risk estimate provided by US EPA (2011) based on the epidemiological data from the Charbotal *et al.* (2006) study. Anses identified several limitations of the US EPA evaluation:

- The methodology used by US EPA by defining reference concentrations from all the (experimental animal) studies and then provide arguments for supporting the most sensitive value seems not to follow the US EPA guidelines (or the methodology proposed by Anses) for establishing toxicological reference values.

- The key study (Charbotel *et al.*, 2006) seems to be well performed with a good retrospective exposure assessment and in particular the achievement of a cumulative exposure index that takes into account both the dermal and inhalational exposure. The results show a strong association between cumulative exposure over a working period and the risk of kidney cancer that remained significant after adjustment for smoking and body mass index. However, the significance disappears when the model takes into account exposure to cutting oils and oil. However, the odds ratio remains high. It cannot be excluded that an increased strength of the study could have led to a statistically significant result even with the mixed oil exposure. However, due to concomitant trichloroethylene and cutting oils and oil exposure, the authors acknowledge that the role of confounding factors cannot be excluded.

- The reconstruction of the exposure is well documented but remains a difficult task, complicated by the fact that exposure data in some cases could be very old and because the quality of the trichloroethylene used might have changed during time. The use of combined exposure (inhalation and dermal) makes it difficult to use these figures to establish a toxicological reference value by inhalation alone.

- Regarding the method of estimating the unit risk, the US EPA application of American mortality tables associated with French incidence data is questionable.

- Finally, the adjustment of the calculated unit risk for kidney cancer to the potential risk of multi-site tumours by applying a multiplicative factor is questionable as it is unusual in the construction of toxicological reference values. The pooling of tumour risk in different organs is unusual and not recommended by the experts' Panel of Anses: CES "chemistry" and Working group "VTR 2."

Thus, based on this the Expert Panel of Anses (2013) recommended *not* to use the specific unit risk estimate derived by US EPA in 2011. The Anses expert group did not in its evaluation question the use of a non-threshold approach for trichloroethylene as used by Afsset (2009) and US-EPA (2011).

3.4.11 REACH registration of Trichloroethylene

From the REACH registration dossier on trichloroethylene available at the ECHA web-site (January 2014), it can be seen that the registrant uses a DNEL value of 54.7 mg/m^3 for long-term occupational exposure. Thus the registrant considers that a threshold exists for the carcinogenic effects. As justification the registrant refers to the evaluation of SCOEL (2009).

In addition to this information the contractor has via ECHA got access to three Chemical safety reports (CSR) from the registrants.

Two of the CSRs (dated 2010-09-20 and 2013-09-27) consider trichloroethylene as a threshold carcinogen and used the OEL of 54.7 mg/m^3 by SCOEL (2009) as a DNEL for worker exposure. The CSRs made reference to the SCOEL (2009) evaluation for using this threshold approach. The 8-h DNEL for workers was further converted to a 24-h inhalational DNEL for the general population. Also DNEL for dermal exposure (workers and general population) was derived based on route-to-route extrapolation of the inhalation DNEL. A NOAEL with respect to kidney toxicity in experimental animals was used as a starting point to derive an oral DNEL for the general population.

In the third CSR (dated 2010-08-04), reference to the classification as Carc 1B; H350 and Muta2; H341 was given, and a *DMEL* value for inhalation to workers was set to 54.7 mg/m^3 with reference to an OEL of 54.7 mg/m^3 given by ACGIH (no further reference is given to this, but the most recent ACGIH evaluation on trichloroethylene has been made in the 2007 supplement to the 7th edition of the ACGIH Documentation of the Threshold limit values).
No DMEL is set for the general population as no exposure is expected for consumers. The document does not include any data under the section carcinogenicity, but only a reference to the classification as mutagenic and carcinogenic.

Overall, the REACH registration dossier and its CSRs do not bring any new data or justification into the discussion regarding the carcinogenic mode of

action of trichloroethylene compared to the data and argumentations referred to by the expert groups.

4. OVERVIEW AND CONCLUSIONS REGARDING CARCINOGENIC MODE OF ACTION AND THRESHOLD/ NON-THRESHOLD

An overall and more general description of trichloroethylene regarding toxicokinetics, mutagenicity data and carcinogenicity (experimental animal data as well as human epidemiological data) has been given in Chapter 2.

With this overall description as a background, the key expert group evaluations during the period 2000-2013 have been scrutinised with regard to conclusions regarding mechanistic mode of action and conclusions regarding threshold/ non-threshold approach for low exposure extrapolation in Chapter 3.

The table below gives a short overview of these findings.

Table 4-1 Overview of the findings on the carcinogenic mode of action of trichloroethylene

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies/ effects of most concern for POD	Unit risk/ Slope factor / Threshold dose
WHO (2000)	Genotox.	Non-threshold linear approach	Maltoni et al (1986). -Rats -Inhalation. -Leydig tumours	Unit risk,24 hr exp. 4.3×10^{-4} $(mg/m^3)^{-1}$
EU-RAR (2004)	Genotox + cytotox	Non-threshold	Focus on exp. animal studies. -kidney cancer POD not defined	Not addressed
WHO (2005)	Genotox	Non-threshold linear approach	NTP (1990) -rats -oral -kidney cancer	Unit risk,24 hr exp. 7.8×10^{-4} (mg/kg bw d)$^{-1}$
AGS (2008)	Genotox + cytotox	Non-threshold Sublinear approach	Henschler *et al.* (1995) Vamvakas *et al.* (1998) Brüning *et al.* (2003) Green *et al.* (2004) Seldén *et al.* (1993) -humans	Slope factors,8 hr exp*. Above 6 ppm: 1.31×10^{-4} (mg/m^3)$^{-1}$ Below 6 ppm: 1.22×10^{-5} (mg/m^3) $^{-}$

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies/ effects of most concern for POD	Unit risk/ Slope factor / Threshold dose
			-inhalation -kidney cancer+ cytotox	[1]
SCOEL (2009)	Cytotox+ genotox	Practical threshold	Brüning *et al.* (2003) Raashcou-Nielsen *et al.* (2003) Charbotel *et al.* (2006) Green *et al.* (2004) Seldén *et al.* (1993) -humans -inhalation -kidney cancer+ cytotox	Threshold, 8 hr exp: 57 mg/m^3 (as NOAEL and OEL)
WHO (2010)	Genotox	Non-threshold linear approach	Maltoni et al (1986). -Rats -Inhalation. -Leydig tumours	Unit risk,24 hr exp. $4.3 \times 10\text{-}4 \ (\text{mg/m}^3)^{-1}$
US-EPA (2011)	genotox	Non-threshold linear approach	Charbotel *et al.* (2006) -humans -inhalation -kidney cancer	Unit risk, 24 hr exp. Inhalation: $1 \times 10^{-3} \ (\text{mg/m}^3)^{-1}$ Oral: $1,0 \times 10^{-2} \ (\text{mg/ kg bw d})^{-1}$
IARC (2012)*	genotox	Not stated	Overall epidemiological evidence with focus on kidney cancer No POD identified	Not addressed
HSE (2012)	Not addressed	Cancer incidences only estimated considered existing high occupational exposures	review paper by Wartenberg *et al.* (2000) -humans -inhalation -kidney cancer	Not addressed
Afsset (2009) /	genotox	Non threshold linear approach	WHO (2000) Maltoni et al (1986).	Unit risk,24 hr exp. $4.3 \times 10^{-4} \ (\text{mg/m}^3)^{-1}$

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies/ effects of most concern for POD	Unit risk/ Slope factor / Threshold dose
Anses (2013)			-Rats -Inhalation. -Leydig tumours	

calculated from the presented dose response curves

4.1 Discussion

From these findings an overall interpretation will be made in the following. The discussion below does not include the assessment of HSE (2012) as the scope of this expert evaluation is considered outsite the scope of this project.

In all the other expert evaluations it is acknowledged that the carcinogenic response from trichloroethyelene exposure is very complex, involving multiple genotoxic and cytotoxic metabolites acting in various manners.

The metabolic pathway of trichloroethylene is by all considered a key for understanding the mechanistic actions regarding the carcinogenicity of trichloroethylene.

In animals as well as in humans, trichloroethylene is metabolised in the body either by the oxidative CYP-mediated pathway – which is the dominant metabolic pathway- or by the minor reductive glutathione dependent pathway. The reductive pathway especially operates at high trichloroethylene levels when the oxidative pathway has been saturated. Metabolism appears to be qualitatively identical, irrespective of species and exposure route. However, great quantitative differences exist between the metabolic capacity between experimental animals and humans, where in general animals are considered to have higher metabolic capacity.

The **oxidative CYP-mediated pathway** leads to formation of oxidation products such as chloral (C), chloral hydrate(CH), trichloroacetic acid (TCA), trichloroethanol (TCOH), trichloroethanol glucuronide, dichloroacetyl chloride (DCAC), dichloroacetic acid (DCA), formic acid and oxalic acid. Most of these metabolites are considered to possess a cytotoxic potential, but especially chloral hydrate and dichloroacetic acid are as well considered as having a genotoxic potential.

The **reductive glutathione mediated pathway** leads to the formation of dichlorovinlyglutathione (DCVG), which is further converted to dichlorovinlycysteine (DCVC). DCVC is then further converted to several reactive metabolites by beta-lyase conversion or deactivated by N-acetylation to form NAcDCVC. The metabolites DCVG, DCVC and the further activated metabolites of DCVC are considered as cytotoxic substances as well as direct acting genotoxic substances.

For the various tumour forms, several modes of action have been considered:

Liver tumours

For liver tumours occurring in mice, especially TCA and DCA are considered important in the development of liver cancer as TCA and DCA may modify the cell signalling system that control cell division and cell death. Furthermore, they may induce cytotoxicity and secondary oxidative stress. However, the exact mechanism has not been clarified and in general the expert evaluations express concern towards the genotoxic mode of action as well.

Kidney tumours

The metabolites DCVG and DCVC are considered closely linked to the development of tumours in the kidneys observed in rats and humans. This is supported by data on a human subpopulation in which the GHS conjugation gene was lacking (leading to the blockage of formation of DCVG and DCVC metabolites) where an attenuated response for kidney cancer was found.

DCVC and its metabolites have been found to be cytotoxic to primary human proximal cells. As the metabolites may also cause mutations of the VHL tumour suppressor gene this may increase the potential for neoplastic lesions. Also the formation of formic acid has been suggested to play a role as formic acid may disrupt the detoxification mechanism by methionine.

It is far from clear whether the cytotoxic effects on its own is the primary mechanism for development of kidney tumours as this seems contradicted by rat data showing nearly 100% nephrotoxic effects at a dose level which was associated with only a very low incidence of kidney cancer.

Thus overall, the metabolites DCVG and DCVC and their genotoxic mode of action are by most of the expert groups considered as most plausible primary explanation for the kidney tumor formation. This has been decisive for choosing a non-threshold approach for low dose-extrapolation.

Lung tumours

For lung tumours in mice the formation of chloral hydrate has been suggested to be important, as the Clara cells in mice rapidly metabolise trichloroethylene to chloral hydrate leading to pulmonary accumulation of this metabolite. This may produce cell changes and compensatory cell proliferation. However, concern is also expressed about a genotoxic mode of action of chloral hydrate.

In humans the capacity of the lungs cells to transform trichloroethylene to chloral hydrate is considered as negligible.

Other tumours

For tumours such as malignant lymphoma in mice and Leydig cell tumours in rats there are no specific hypotheses in relation to the carcinogenic mode of

action that may operate, and thus concern towards genotoxic mode of action has been expressed.

Conclusion from discussion

So overall, taking into account the formation of several genotoxic metabolites and the lack of adequate explanation for a non-genotoxic mode of action, all expert groups (with the exception of SCOEL (2009)) find a non-threshold approach for most appropriate for cancer risk estimations.

Before making a final conclusion on which expert approach shall be used for the assessment of the carcinogenic potential of trichloroethylene, the evaluation of SCOEL (2009) shall be looked at more thoroughly, as this is the only evaluation that considers trichloroethylene to have a practical threshold for carcinogenicity.

4.2 Threshold approach by SCOEL 2009

SCOEL (2009) consider cytotoxicity as a prerequisite for the formation of kidney cancer in humans, and states that:

"Tumours in human kidneys were only observed after occupational trichloroethylene exposure to very high concentrations, which are clearly nephrotoxic. Such exposures clearly exceeded former exposure limits of 50 ppm, and peak exposures of several hundred ppm very likely involved. At these high dose ranges, it is known that the toxification of trichloroethylene via the reductive glutathione-pathway is proportionally increased, compared to lower doses where glutathione-dependent metabolism is only marginal. This is because the oxidative CYP-dependent metabolism is saturable. Both aspects, the impact of cytotoxicity and the relative increase of glutathione-dependent metabolism at high doses of trichloroethylene, make a sub-linear dose-response relationship at lower exposure concentrations highly plausible. Therefore, a linear extrapolation of kidney tumour risks should be limited to clearly nephrotoxic concentrations. Even this approach is still conservative, as the relative influence of glutathione-dependent metabolism at slightly nephrotoxic exposure concentrations has probably already decreased, compared to the much higher concentrations at which tumours have been observed."

"Observations in experimental systems, as well as in occupationally exposed and diseased persons, lead to the conclusion that human renal cell cancer risk is avoided if exposure to nephrotoxic concentrations of trichloroethylene does not occur, including trichloroethylene concentrations leading to sub-clinical renal changes that can be monitored by urinary excretion of suitable marker proteins. In the occupational field study by Green et al. (2004) on 70 workers, the mean trichloroethylene exposure was 32 ppm (range 0.5-252 ppm). In this cohort some minor sub-clinical alterations in renal functional parameters were observed. This is corroborated by data of Seldén et al. (1993), who found no increase in urinary excretion of the NAG marker protein in workers exposed to a range of 6-10 ppm trichloroethylene.

Against this background and with reference to the SCOEL strategy in the derivation of OELs for carcinogens and mutagens (Bolt and Huici-Montagud, 2008), SCOEL regards trichloroethylene as a "genotoxic carcinogen, for which

a practical threshold is supported by studies on mechanisms and/or toxicokinetics" (group C)."

Thus, SCOEL identify and base their choice of a practical threshold level on the sensitivity of the epidemiological studies, i.e. the level of cancer incidence or level of biomarker that can be determined at the level of significance in the available epidemiological studies. This may be a practical threshold, however not a real threshold, as effects may still occur at lower levels, however below a significant detectable level in epidemiological studies. These lower and un-detectable levels of cancer are still to be considered associated to the exposures to trichloroethylene and thus, it may still be relevant to extrapolate the risk at these lower levels when assessing the health impact and socio-economic impact of low level trichloroethylene exposure.

Furthermore, the mechanistic evidence for the cytotoxic mode of action as the crucial mode of action as stated by SCOEL, and consequently the use of a threshold approach is not supported by other recent expert groups evaluations (AGS 2008, US-EPA 2011, IARC 2012) that do not find the hypothesis of cytotoxic mode of action (and a threshold approach) as strong enough for rejecting the possible genotoxic mode of action (and a non-threshold approach).

In addition to this and as discussed below, it has to be acknowledged that the criteria for using threshold/ non-threshold approach by SCOEL are not quite the same as the criteria/ recommendations used in relation to REACH.

The strategy of SCOEL for deriving OELs for carcinogens and mutagens has been described by Bolt & Huici-Montagud (2008). Here carcinogenic substances are differentiated into four classes in relation to methods for the OEL derviation:

(A) *Non-threshold genotoxic carcinogens; for low-dose assessment of risk, the LNT model appears appropriate. For these chemicals, regulations (risk management) may be based on the ALARA principle ("as low as reasonably achievable"), technical feasibility, and other socio-political considerations.*
(B) *Genotoxic carcinogens, for which the existence of a threshold cannot be sufficiently supported at present. In these cases, the LNT model may be used as a default assumption, based on the scientific uncertainty.*
(C) *Genotoxic carcinogens with a practical threshold, as supported by studies on mechanisms and/or toxicokinetics; health-based exposure limits may be based on an established NOAEL (no observed adverse effect level).*
(D) *Non-genotoxic carcinogens and non-DNA-reactive carcinogens; for these compounds a true ("perfect") threshold is associated with a clearly founded NOAEL. The mechanisms shown by tumour promoters, spindle poisons, topoisomerase II poisons and hormones are typical examples of this category.*

Using this approach SCOEL (2009) concluded trichloroethylene to belong to group C with a *practical* threshold; i.e. no *true* or *"perfect"* threshold (group D) was concluded.

In relation to decisions regarding threshold/ non-threshold in REACH, guidance on this can be found in the REACH R8 guidance document (2012) where the following is expressed:

"No DNEL can be derived for non-threshold mutagens/carcinogens as it is assumed that a no-effect-level cannot be established for these substances (either because there is no threshold or the threshold level cannot be determined). In such cases, and assuming that there are data allowing it, the registrant should develop a DMEL (derived minimal effect level), a reference risk level which is considered to be of very low concern."

….

"It is to be noted that the decision on a threshold and a non-threshold mode of action may not always be easy to make, especially when, although a biological threshold may be postulated, the data do not allow identification of it. If not clear, the assumption of a non-threshold mode of action would be the prudent choice."

Thus, the REACH guidance does *not* speak of a *practical threshold*, but in fact encourage use of a non-threshold approach as it is emphasised that in cases where mode of action is not clear a non-threshold approach should be preferred as a prudent choice, i.e. when no *perfect* threshold can be concluded a non-linear approach should be used.

Thus, in the case of trichloroethylene where SCOEL cannot define a *perfect* threshold, such a case has to be dealt with as a non-threshold approach in the REACH regulation as no option for a practical threshold is mentioned.

The different wordings used in connection with SCOEL's procedure and in connection with the REACH guidance may be seen in a different context for the need of a specific limit value in the occupational environment and the need for a more risk driven approach represented by the REACH regulation.

For the occupational environment, more pragmatism may be needed in order to achieve practical solutions for limiting the exposure. In that respect a concrete figure representing a defined tolerable level as the OEL may be more practical to use as documentation and for enforcement than using a non-threshold dose-response association for a chemical.

In REACH and in relation to the authorisation procedure there is more concern on actual or remaining risks, and when the scientific data allow for this, a risk based approach is preferred for assessing human health impact. Also for further socio-economic assessment, a risk based approach is more useful for assessing the cost/benefits.

4.3 Conclusion

Trichloroethylene should in terms of the REACH regulation be considered as a **non-threshold carcinogen** due to data on the mutagenicity of trichloroethylene itself and the formation of several genotoxic metabolites e.g DCVG; DCVC; DCA and Chloral hydrate.

Convincing data on cytotoxic modes of action as the primary cause for the formation of kidney cancer and other tumour forms are lacking.

Also, it should be noted that due to the increase in the epidemiological evidence during the latest 15 years there has been a clear shift towards relying more on these human data when assessing the evidence for the carcinogenicity of trichloroethylene and also in relation to the evaluation of the dose-response relationship for the substance. Experimental animal data are increasingly used today as supporting data and as data for mechanistic considerations. Especially the formation of the same genotoxic metabolites dichlorovinlyglutathione (DCVG) and dichlorovinlycysteine (DCVC) in the kidneys in rats and humans has been a crucial finding. Also, further human investigations showed an attenuated cancer risk among individuals lacking the GSH conjugation gene responsible for formation of these metabolites.

A specific advantage of using the human data is the identification of a threshold limit in humans for the cytotoxic (nephrotoxic) effects of the substance, as the carcinogenic potency of the substance by the majority of the expert groups are considered to be enhanced at cytotoxic exposure levels, whereas in general a more flat dose-response curve is assumed at exposure level below cytotoxic levels.

In that respect the evaluation and approach used by AGS (2008) is the only expert evaluation that at the same time use a non-threshold approach for low level exposure as well as a threshold approach for the cytotoxicity for the higher exposure levels.

Thus, the AGS (2008) approach for estimating the cancer risk from trichlorpoethylene exposure may be the preferred approach to use in the context of this report, and a further and more detailed quantitative analysis of this approach will be given in Chapter 5.

5. DOSE-RESPONSE ANALYSIS AND QUANTITATIVE CANCER RISK ASSESSMENTS

In Chapter 4 it was concluded that cancer risk of trichloroethylene should be performed with a non-threshold approach, i.e. the cancer risk from trichloroethylene exposure at high as well as at low dose levels is to be assessed based on estimated quantitative risk levels. Also, it was concluded that the cancer potency of the substance is enhanced at cytotoxic exposure levels. The AGS (2008) dose-response assessment is the only assessment that considers both of these aspects and may therefore at this stage seem to be the most scientific suitable approach for assessing the dose-response relationship for human exposure to trichloroethylene at both high and low dose exposure.

In this chapter, however, **all** the non-threshold quantitative dose-response relationship found in Chapter 3 will be further described in order to be more clear on how the unit risks have been derived and also for giving more background for comparing or using various elements from the approaches.

Based on this more in-depth evaluation, the most scientifically justified and appropriate dose-response function will be recommended for use in the context of health impact assessment and socio-economic assessment of industrial uses and exposure scenarios of trichloroethylene.

Thus the following expert assessments using a quantitative non-threshold approach will be further scrutinised (see Table 4-1).

WHO(2000)

WHO (2005)

WHO (2010)

AGS (2008)

Afsset (2009)

US-EPA (2011)

Anses (2013)

5.1 Inhalation exposure

5.1.1 WHO (2000) and WHO (2010)*
The WHO evaluations for air guidelines for indoor air (WHO 2010) and ambient air (WHO 2000) both conclude on the same cancer unit risk estimate for **trichloroethylene of 4.3 x 10^{-7} (µg/m^3)$^{-1}$** for 24 h inhalation exposure to the public.

This unit risk estimate was derived by WHO (2000) from the data on increased Leydig cell tumours in Sprague Dawley rats in the study by Maltoni (1986) and applying a linearised multistage model for low dose extrapolation.

Table 5-1

Species and strain	Treatment	Observed increase in tumour incidence	Reference
Mouse (m,f) B6C3F1	**0, 540, 1620 and 3240** mg/m³, 7 hours/day, 5 days/week for 78 weeks; observation for rest of lifespan; trichloroethylene purity 99.9%, epoxide-free	Pulmonary adenomas in females only: **4/90, 6/90, 10/90 and 15/90**; hepatomas in females: **3/90, 4/90, 4/90 and 9/90** hepatomas in males **14/90, 19/90, 27/90 and 21/90**	Maltoni et. al.,1988.
Mouse (m, f) Swiss	**0, 540, 1620 and 3240** mg/m³, 7 hours/day, 5 days/week for 78 weeks; observation for rest of lifespan; trichloroethylene purity 99.9%,.epoxide-free	Pulmonary adenomas and carcinomas in males only: **10/90, 11/90, 23/90 and 27/90** hepatomas in males: **4/90, 2/90, 8/90 and13/90**	Maltoni,. et al, 1988.
Rat (m, f) Sprague-Dawley	**0, 540, 1620 and 3240** mg/m³ 7 hours/day, 5 days/week for 104 weeks; observation for rest of lifespan; trichloroethylene purity 99.9% epoxide-free	Renal adenocarcinomas in males and at high dose only: **4/130 versus 1/130 in controls**; Leydig cell tumours in testis **1/135, 16/130, 30/130 and 31/130**	Maltoni, et al 1986

Also unit risk estimates of 9.3×10^{-8} and 1.6×10^{-7}, respectively, were calculated by applying the same linearised multistage mode on the incidence of pulmonary adenomas in B3C6F1 mice (Maltoni 1988) and on pulmonary adenomas/carcinomas in Swiss mice (Maltoni 1988).

However, the most conservative (potent) **unit risk of 4.3 x 10⁻⁷ (µg/m³)⁻¹ in relation to Leidig-cell tumours** in rats was chosen as the basis for the air quality guideline for trichloroethylene. The following dose-reponse associations were given:

2.3 µg/m³ corresponding to	1:1 000 000 excess lifetime risk
23 µg/m³ corresponding to	1:100 000 excess lifetime risk
230 µg/m³ corresponding to	1:10 000 excess lifetime risk

It is not possible to make a further in-depth evaluation of the derivation of the unit risk, as WHO (2000) did not further describe exactly how the linearised multistage model was applied. Also, the descriptions did not refer to the type of

dose metric adjustments or intra-species extrapolations applied when going from experimental animal conditions and exposure durations to human 24-hour life-time exposure.

This section also applies for the Afsset (2009) evaluation as this evaluation refers back to and concludes as the WHO evaluations. Further Anses (2013) concluded not to recommend revision of this evaluation based on the US-EPA (2011) evaluation.

5.1.2 AGS (2008)

AGS (2008) made their dose-response assessment from the combined data from the epidemiological studies performed in Germany (Henschler *et al.* 1995; Vamvakas *et al.* 1998; Brüning *et al.* 2003).

Henschler *et al.* (1995) performed a retrospective cohort study on cardboard workers in Germany comprising of 169 men, who had been exposed to trichloroethylene for a least 1 year in the period 1956-1975 and 190 unexposed workers from the same factory. By the end of this period five of the exposed workers had been diagnosed with kidney cancer, and just after this period further 2 kidney cancer cases were observed. The workers that developed kidney cancer had on average been exposed to trichloroethylene for 15.2 years. No kidney cancers were observed among the controls. Trichloroethylene was the only solvent used in the factory, and the solvent was heavily used for all types of cleaning and degreasing of the machines (often on hot surfaces) and also used for cleaning clothes and hands. All exposed workers enrolled in the study had been continuously exposed to trichloroethylene over a long period of time with extremely high exposure levels at regular weekly or biweekly intervals during major cleaning operations. Data on regularly occurring sub-anesthetic symptoms (headaches, drowsiness, dizziness and vertigo) indicated exposure levels well above 200 ppm (1094 mg/m3). However, no quantitative estimates on exposure levels or cumulated dose levels were developed in the study.

The five cancer cases resulted in a standardised incidence rate (SIR) of 7.97 (95%CI 2.59-18.59) in relation to the background incidence rate of kidney cancer in Denmark (data from the Danish Cancer Registry). An even higher SIR of 9.66 (95%CI 3.14-22.55) was found when using data from the former cancer registry from East Germany.

Vamvakas *et al.* (1998) performed a hospital-based case-control study investigating the occupational exposure on 58 patients diagnosed with renal cell cancer in the period of 1987-1992. The hospital was located in a German area (same area as the Henschler *et al.* 1995 study) with a large number of small plants manufacturing metal and electric devices. A group of 84 patients from accident wards were selected as controls. Air and biomonitoring data were not available. The occupational histories was evaluated by personal interviews using specifically designed questionnaires concerning job tasks and allowing for a semi-quantitative exposure assessment of each individual. Symptoms related to acute neurotoxic/prenarcotic effects were graded in none, slight, moderate

and severe degree. Exposure ratings as +, ++ and +++ were given based on the symptoms grading and on further information on the frequency of symptoms during a week, and for how long a period these occurred (years). Further information of duration of the work (years) and total time (hours) of exposure were included in the exposure scoring. Of the 58 cases, 19 had histories of occupational exposure to trichloroethylene of at least 2 years, whereas only 5 controls had a history of this type of exposure.

The study demonstrated an association of renal cell cancer with long-term exposure to trichloroethylene with a significantly increased overall odds ratio, OR of 10.80 (95% CI: 3.36-34.75) (OR adjusted for age, gender, smoking, BMI, blood pressure, and intake of diuretics).

Furthermore, the impact of the intensity of exposure was analysed, and increased ORs as given below in Table 5-2 were obtained (OR adjusted for age and blood pressure).

Table 5-2

Exposure category	Cases	Controls	OR (95% CI)
+++	8	2	11.42 (1.96-66.79)
++	9	3	11.92 (2.55-55.60)
+	2	2	6.61 (0.50-87.76)
No exposure	39	77	1.0

Here a significant (P<0.05) increase in the ORs was found with increasing exposure levels.

Brüning *et al.* (2003) made a case-control study covering the same area as Vamvakas *et al.* (1998) but for the follow-up period of 1992-2000. In this study 134 persons diagnosed with renal cell cancer and 401 controls were enrolled. All persons (or next of kin) were interviewed using the same questionnaire as Vamvakas *et al.* (1998). The same poor occupational conditions when working with trichloroethylene was emphasised; however, it was noted that since the 1980'es continuous reduction was to be assumed due to increased enforcement of the OEL value of 50 ppm. Thus, it was expected that lower risk estimates for renal cell cancer would occur for this update period.

An OR of 5.57 (95%CI: 2.33-13.32) for renal cancer was obtained when working with industrial metal degreasing based on 15 cases and 11 controls occupied within this area. No significantly increased OR was obtained for other parts of the metal industry, e.g. in metal processing, metal working or in the steel industry.

An OR of 2.47 (95%CI: 1.36-4.49) for the development of renal cell cancer was obtained for self-assessed exposure to trichloroethylene from 25 cases and 38 controls.

When addressing the intensity of trichloroethylene exposure in relation to increased frequency of narcotic symptoms, significantly increased ORs were obtained in a dose-related manner, see Table 5-3 below:

Table 5-3

Narcotic symptoms if exposed to TRI	Cases	Controls	OR* (95% CI)
Any occurrence of symptoms	19	18	3.71 (1.80-7.54)
Non-daily occurrence of symptoms	13	10	4.60 (1.87-11.30)
Daily occurrence of symptoms	5	4	5.91 (1.46-23.99)

*The ORs adjusted for age, gender and smooking

A significantly increased OR of 7.25 (95%CI: 1.96-26.78) was found for 10-20 years since last exposure and an increased OR of 2.86 (95%CI: 1.49-5.49) was found for more than 20 years since the first exposure, indicating a certain lag time for the development of renal cell cancer in relation to trichloroethylene exposure.

From the combined data of these epidemiological studies, AGS (2008) concluded an excess lifetime risk of renal cancer to 5 % in relation to cumulative exposure of 3000 ppm-years. The dose-response assessments for this conclusion were presented in an unpublished document to the German CMR working group. The value was derived from a 1.6% baseline-incidence for renal cancer in Germany and an overall average relative risk of 3 at the dose level of 3000 ppm-years.

Table 5-4

Exposure concentration [ppm]	Length of exposure	Cumulative exposure [ppm-years]	Excess risk	Comment
500 and 100	18 y, 2 h/d, 3 d/w, peak exposure; otherwise approx. 100 ppm	3000	5 %	Point of reference according to the studies by Henschler *et al.* (1995), Vamvakas et al (1998), Brüning et al (2003) from Germany

Assuming 40 years of exposure, 3000 ppm-years correspond to an average dose-level of 75 ppm. However, it was noted that the risk most probably was driven by high peak exposures, and therefore when converting these to an overall average exposure level, this may result in overestimating of the risk at

this (considerable) lower average exposure.

With respect to dose-response relation at various concentrations the expert group acknowledged that the development of renal cancer followed several years of high trichloroethylene exposure (generally accompanied by pre-narcotic episodes (Brüning *et al.* 2003) and seems to be related to tubular renal damage. Promotion/progression seems to be essential for promoting the development of renal cancer. This view is supported by biomarker studies in humans such as the excretion of glutathione-transferase alpha or alpha1- microglobulin in urine.

Furthermore, in a study with Scandinavian workers in which the majority of the employees (25 of 29) had been exposed to less than 6 to 10 ppm trichloroethylene, there was no increased excretion of the N-acetyl-ß-D-glucosamine (NAG) biomarker (Selden *et al.* 1993). The level of 6 ppm (33 mg/m3) was by the AGS expert group considered a threshold for nephrotoxicity, especially since nephrotoxicity at 32 ppm in another study was not considered as pronounced.

AGS (2008) considered 6 ppm as a threshold for cytotoxic (and co-carcinogenic) effects in the kidneys and down-scaled the risk at 6 ppm and below with a factor of ten in order to consider the lowered risk below the cytotoxic levels. Using this approach, a sublinear non-threshold approach was obtained that took into account the genotoxic mechanism as well as the cytotoxic co-carcinogenic mechanism that operates at the higher dose levels. Thus, the dose-response curve becomes steeper above the threshold level of 6 ppm for the cytotoxic effects, see red line in Figure 5-1 below.

Based on these considerations and PODs of 6 ppm and 75 ppm, the following dose-response curve could be made, see Figure 5-1.

Figure 5-1. Excess risk for carcinogenic effects-Working lifetime exposure
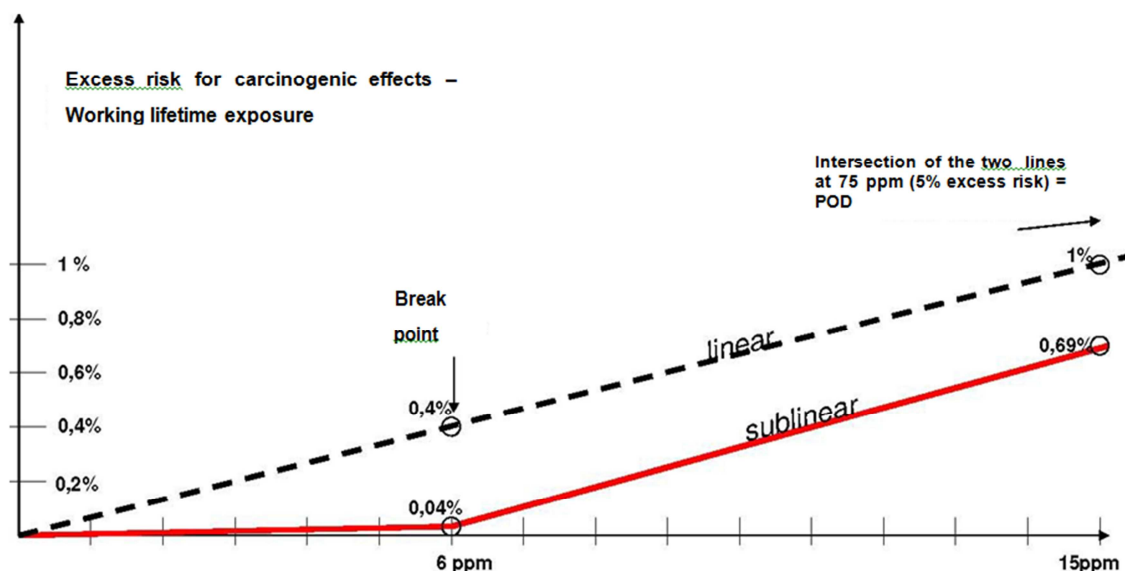
The figure both contains a linear extrapolation from the POD of 75 ppm down to a threshold concentration of 6 ppm for a cancer-enhancing effect (nephrotoxicity) and a further linear extrapolation from 6 ppm to 0 ppm. This results in a sublinear dose-response curve with 6 ppm as a break point.

The dose-response could mathematically be expressed as:

At 6 ppm and above:
Excess risk (kidney cancer) = 7.2×10^{-4} ppm^{-1} x concentration (ppm) − 0.0039

Below 6 ppm:
Excess risk (kidney cancer) = 6.7×10^{-5} ppm^{-1} x concentration (ppm)

The mathematical expressions above were calculated as follow:

At 6ppm and above:
5% = a x 75ppm + b
0.04% = a x 6ppm + b
Then a = $(0.05 − 0.0004) / (75 − 6) = 7.2 \times 10^{-4}$ and b= $0.05 - (7.2 \times 10^{-4} \times 75)$ = 0.0039

Below 6 ppm:
0.04% = a x 6ppm
Then a = $0.0004/ 6 = 6.7 \times 10^{-5}$

The following dose-reponse relationship was presented, Table 5-5:

Table 5-5 AGS (2008)

Average ppm	ppm-years	Excess risk	Remarks
75 ppm	3000	5 %	POD; German epidemiological studies of kidney cancer
19.3 ppm	772	1 %	linearised ("steep" part)
6.8 ppm	272	0.1 %	linearised ("steep" part)
6 ppm	240	0.04 %	"Break point"; at threshold for non-carcinogenic nephrotoxicity after exposure to trichloroethylene
1.5 ppm	60	0.01 %	linearised ("flat" part)
0.6 ppm	24	0.004 %	linearised ("flat" part)

Comments
The AGS (2008) approach may be considered as a bridging between the SCOEL threshold approach and a linear non-threshold approach, as a threshold

of 6 ppm is used for nephrotoxicity and a break point for the sublinear dose-response curve.

Expressed in mg/m^3 (1 ppm = 5.47 mg/m^3), the dose response-relation found by AGS (2008) can be transferred to:

At 33 mg/m^3 above:
Excess risk (kidney cancer) = 1.3 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) – 0.0039

Below 33 mg/m^3:
Excess risk (kidney cancer) = 1.2 x 10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

5.1.3 US EPA (2011)

In the US-EPA (2011) document, cancer potency estimates were derived from data on experimental animals as well as from human data from epidemiological studies.

Cancer unit risks were calculated on each tumour type in each of the experimental animal studies (covering the studies presented in Table 3-1). Modelling was performed using either the applied dose/exposure (default dosimetry) or by using several internal dose-metrics for transformation to a human dose metric. A total of 7 different PBPK-models were used for these assessments (see Appendix A) and were combined with the US EPA preferred multistage model for the dose-response modelling.

The following unit risk estimates were calculated from the most sensitive inhalation tests:

Table 5-6   Inhalation: Most sensitive tests for each sex/species (US-EPA 2011)

Sex/species	Endpoint (study)	Unit risk per ppm		
		Preferred dose-metric	Default methodology	Alternative dose-metrics, studies, or endpoints
Female mouse	Lymphoma (Henschler et al., 1980)	1.0×10^{-2}	9.1×10^{-3}	$1 \times 10^{-3} \sim 4 \times 10^{-3}$
Male mouse	Liver hepatoma (Maltoni et al., 1986)	2.6×10^{-3}	2.9×10^{-3}	2×10^{-3}
Female rat	-	-	-	-
Male rat	Leukemia+ Kidney adenoma and carcinoma+ Leydig cell tumors (Maltoni et al., 1986)	8.3×10^{-2}	7.0×10^{-3}	$4 \times 10^{-4} \sim 5 \times 10^{-2}$ [individual site results]

In mice, the highest unit risk of 0.01 ppm^{-1} (corresponding to 0.0018 (mg/m^3)$^{-1}$ as 1 ppm = 5.47 mg/m^3 trichloroethylene) was derived based on the data on

lymphoma in female mice.

In rats, the highest unit risk of 0.083 ppm^{-1} (corresponding to 0.015 (mg/m^3)$^{-1}$) was derived based on the findings of combined tumours (leukemia+kidney adenoma and carcinoma +Leydig cell tumours) in male rats.

In relation to the human data, US-EPA (2011) found that only the studies by Charbotel *et al.* (2006) and Moore *et al.* (2010) were considered to have sufficient exposure-response information for a dose-response analysis. The Charbotel *et al.* (2006) study was, however, preferred as the only data set to be used for the quantitative dose-response analysis. The data in this study relied on a task-exposure matrix based on decades of measurements from the specific workshops in the Arve Valley, whereas the Moore *et al.* (2010) exposure assessment of trichloroethylene was less detailed and considered more applicable for a more general ranking of exposures.

Charbotel *et al.* (2006) made a case-control study on renal cell cancer in the Arve Valley, France. The valley is known for its widely developed screw-cutting industry in which trichloroethylene is used for degreasing. In the study, 86 cases with renal cell cancer were selected from local general practitioners and urologists and from hospitals in the area. Also 316 controls were randomly selected as being residents in the same geographical area at the time of the diagnosis of the case disease. Persons with a history of kidney diseases were excluded from the control group. Telephone interviews were used to obtain occupational information for the individuals and information on medical conditions. Next of kin was interviewed to obtain information for individuals that had died. The occupational questionnaire was one questionnaire devoted for the screw cutting industry and one for other jobs. In the Arve Vally, occupational measurements of trichloroethylene in the srew-cutting industry have been performed since 1960es, and based on these experiences a detailed task-exposure matrix for trichloroethylene exposure was developed for various degreasing operations, types of machines and operational conditions and the workers distance from the degreasing process. Also specific tasks related to peak exposure reaching 200 ppm or more were identified (Fevotte *et al.* 2006). Based on this matrix, all the trichloroethylene exposed cases and controls were allocated to one of three exposure levels:

> Low exposure: a cumulative average exposure level in the range of 1-150 ppm x years;

> Medium exposure: a cumulative average exposure level in the range of 155-335 ppm x years;

> High exposure: above 335 ppm x years*).*

Based on this, the following odds ratios for renal cell cancer could be derived for the various exposure groups:

Table 5-7 ORs for development of renal cell cancer among trichloroethylene exposed workers

	Cases n=86	Controls n=316	Crude OR (95% CI)	Adjusted OR (95% CI)
Cumulative dose				
Non-exposed	49 (57.0%)	206 (65.2%)	1	1
Low	12 (14.0%)	37 (11.7%)	1.51 (0.71–3.17)	1.62 (0.75–3.47)
Medium	9 (10.5%)	36 (11.4%)	1.16 (0.51–2.65)	1.15 (0.47–2.77)
High	16 (18.6%)	37 (11.7%)	2.23 (1.09–4.57)	2.16 (1.02–4.60)
Cumulative dose plus peaks				
Non-exposed	49 (57.0%)	206 (65.2%)	1	1
Low/medium No peaks	18 (20.9%)	65 (20.6%)	1.27 (0.68–2.39)	1.35 (0.69–2.63)
Low/medium + peaks	3 (3.5%)	8 (2.5%)	1.88 (0.44–8.08)	1.61 (0.36–7.30)
High No peaks	8 (9.3%)	23 (7.3%)	1.84 (0.73–4.69)	1.76 (0.65–4.73)
High + peaks	8 (9.3%)	14 (4.4%)	2.70 (1.09–6.67)	2.73 (1.06–7.07)

The adjusted ORs were matched for sex and age and also further adjusted for tobacco smoking and BMI.

Furthermore, a significant dose-response (P=0.04) trend for cumulative dose was observed. Charbotel *et al.* (2006) noted that the OR was higher when peak exposure was included in the analysis. When the same analysis was performed on the sub-set of living persons below 80 years (60 cases and 225 controls), even higher ORs were obtained in the 'high' and 'high + peaks' groups.

The following results from the Charbotel *et al.* (2006) study were used for the unit risk calculations by US-EPA (2011).

Table 5-8 Relationship between trichloroethylene exposure and renal cell carcinoma (from US-EPA 2011)

Cumulative exposure category	Mean cumulative exposure (ppm × yrs)	Adjusted OR (95% CI)
Nonexposed		1
Low	62.4	1.62 (0.75, 3.47)
Medium	253.2	1.15 (0.47, 2.77)
High	925.0	2.16 (1.02, 4.60)

The results from the table were used for predicting the extra risk of renal cell cancer incidence from continuous environmental exposure to trichloroethylene.

Extra risk was defined as: Extra risk = $(Rx – Ro)/(1 – Ro)$,

where Rx is the lifetime risk in the exposed population and Ro is the lifetime risk

in an unexposed population (i.e., the background risk). Because kidney cancer is a rare event, the ORs in the table were used as estimates of the RR ratio = Rx/Ro.

In addition, it was generally assumed that RR estimates transfer across populations, independent of background disease rates. In this case, the RR estimates based on the Charbotel *et al.* (2006) study, which was conducted in France, were assumed to apply to the U.S. population.

A linear regression coefficient of 0.001205 per ppm × year (SE = 0.0008195 per ppm × year) was obtained from the results in Table 5-5.

The risks were computed up to age *85 years* for continuous exposures to trichloroethylene. Conversions between occupational trichloroethylene exposures and continuous environmental exposures were made to account for differences in the number of days exposed per year (240 vs. 365 days) and in the amount of air inhaled per day (10 *vs.* 20 m^3).

Using 1% extra risk as point of departure, a linear approach was applied giving the following dose-response association, see Table 5-9:

Table 5-9

Exposure concentration (ppm)	MLE of extra risk	95% UCL on extra risk
0.001	2.603×10^{-6}	5.514×10^{-6}
0.01	2.603×10^{-5}	5.514×10^{-5}
0.1	2.602×10^{-4}	5.512×10^{-4}
1.0	2.598×10^{-3}	5.496×10^{-3}
10.0	2.562×10^{-2}	5.333×10^{-2}

From this the US-EPA (2011) chose the higher 95% confidence unit risk value of

5.5×10^{-3} ppm^{-1} or 1×10^{-3} (mg/m^3)$^{-1}$

as the preferred unit risk estimate for excess risk of **<u>kidney cancer</u>.**

Also US-EPA calculated a unit risk estimate for the combined risk of kidney cancer + non-Hodgkin lymphoma + liver cancer using the RRs for all three tumour types in the Raashou *et al.* (2002) study. From this, an adjustment factor of 4 was established and thus the unit risk for **combined cancers (kidney cancer + non-Hodgkin lymphoma + liver cancer)** was estimated 4 times higher to **0.022 ppm^{-1} or 0.0040 (mg/m^3)$^{-1}$**

5.2 Dermal exposure

No data on unit risk in relation to dermal exposure have been presented.

5.3   Oral exposure

5.3.1 WHO (2005)

WHO (2005) used the data on the development of kidney tumours from the oral studies in rat as the basis for the evaluation of the cancer risk for oral exposure to trichloroethylene.

In a NTP (1988) carcinogenicity study, four strains of male and female rats were dosed with trichloroethylene by gavage at dose levels of 0, 500 and 1000 mg/kg bw/d for 103 weeks. In male Osborne-Mendel rats, increases in the incidence of renal cell adenomas and adenocarcinomas were observed (0, 6/44 and 2/33 in control and low and high dose, respectively).

In a NTP (1990) 2-year carcinogenicity study, F344/N rats were dosed with trichloroethylene by gavage at dose levels of 0, 500 and 1000 mg/kg bw/d. Significantly increased incidences of kidney tumours (after adjustment for reduced survival) were found (0, 2/46 and 3/33 rats having kidney tumours in the control and low and high dose groups, respectively).

(It should be noted that these results as described by WHO (2005) seem to differ from the results as described by WHO 2000 and 2010, see Table 3-2).

WHO (2005) then refers to the calculations made by Health Canada (2003a) that applied the linearised multistage (LMS) model on the pooled combined tubular cell adenomas and adenocarcinomas of the kidneys in rats from the NTP 1988 and NTP 1990 studies following oral exposure. Based on this a **unit risk of 7.80 × 10^{-4} (mg/kg of body weight per day)$^{-1}$ for kidney tumours** was calculated.

No further description on the calculations by Health Canada (2003a) was given by WHO (2005); however, it was stated that an animal-to-human kinetic adjustment factor, expressed as $(0.35/60)^{1/4}$, was applied to the dose metrics, assuming a rat weighs 0.35 kg and a human weighs 60 kg.

It was not possible to retrieve the Health Canada (2003a) reference.

5.3.2 US-EPA (2011)

US-EPA (2011) calculated using an oral unit risk estimate of **1.01 x 10^{-2} (mg/kg bw/d)$^{-1}$** in relation to excess kidney cancer risk by using two PBPK models for extrapolation from inhalational exposure to oral exposure, see Table 5-10**.**

Table 5-10 (from US-EPA 2011)

	Kidney
Inhalation unit risk (risk per ppm)	5.49×10^{-3}
Primary dose-metric	ABioactDCVCBW34
ppm per mg/kg/d	1.70
Oral slope factor (risk per mg/kg/d)	9.33×10^{-3}
Alternative dose-metric	TotMetabBW34
ppm per mg/kg/d	1.97
Oral slope factor (risk per mg/kg/d)	1.08×10^{-2}

ABioactDCVCBW34 = Amount of DCVC bioactivated in the kidney per unit body weight¾ (mg DCVC/kg¾/week).

TotMetabBW34 = Total amount of trichloroethylene metabolized per unit body weight¾ (mg TCE/kg¾/week).

The oral unit risk estimate derived by US-EPA (2011) may be compared to estimates when making more simplistic route to route extrapolations using standard procedures as described in the REACH R8 guidance document and using data on absorption rates, e.g. from the EU-RAR (2004) or from the US.EPA (2011) document.

Absorption by inhalational exposure

1)
In the EU-RAR (2004), an inhalational absorption rate of 100% was concluded for the risk characterisation. This value may therefore be used for a route-to-route extrapolation.

2)
The US-EPA (2011) reported a retention rate of 40% from a volunteer study by Jakubowski and Wieczorek (1988) in relation to exposure at 49 mg/m^3 during 2 hours at rest. Other older studies reported retention rates in the range of 25-70% at concentrations in the range of 509 to 1080 mg/m3 either during rest or during light exercise. From this, it seems reasonable to use an absorption rate of 40% as a conservative estimate for inhalational exposure to trichloroethylene.

Absorption by oral exposure
1)
In the EU-RAR (2004), an oral absorption rate of 100% was concluded for the risk characterisation. This value may therefore be used to a route-to-route extrapolation.

2)

The US-EPA (2011) document referred to data showing a bioavailability between 60-80% for unfasted rats and 90% for fasted rats. Other studies found up to 98% of a dose given by gavage to rat and mice to be expired in air and excreted in urine. Thus, absorption of 90% may be used as a suitable value for route-to route extrapolations here.

Route-to-route extrapolations

In the following, route-to-route extrapolations are made from 1 mg/m^3 trichloroethylene exposure to an external dose expressed as mg/kg bw/d. (1 mg/m^3 corresponds to an excess risk level of 0.001 using the US-EPAs unit risk estimate of 0.001 $(mg/m^3)^{-1}$).

Route to route extrapolation (1)

Here the starting asumption is 100% absorption by both the inhalational and the oral route (or 1:1 absorption).

If a person weighing 60 kg inhales 20m^3 air per day, the internal dose at 1 mg/m^3 trichloroethylene would be:

1mg/m^3 x 20m^3 / 60 kg = 0.333 mg/kg bw/d

Thus an oral dose of **0.333 mg/kg bw/d** can, using these assumptions, be considered equipotent to continuous inhalation at 1 mg/m^3 corresponding to a risk level of 0.001 (as indicated from the unit risk value given below table 5-9). Using a conversion factor of **0.333**, the concentrations in mg/m^3 can be transferred into equipotent oral doses.

This then results in an oral unit risk estimate of :

Risk = Unit risk x Dose

Unit risk = Risk / Dose = 0.001 / 0.333 mg/kg bw/d = **0.003 (mg/kg bw/d)$^{-1}$**

Route to route extrapolation (2)

Here the starting asumptions are 40% absorption by the inhalational route and 90% absorption by the the oral route.

If a person weighing 60 kg inhales 20m^3 air per day, the internal dose at 1 mg/m^3 trichloroethylene would be:

(1mg/m^3 x 20m^3 / 60 kg) x 0.4 (inh. abs rate) = 0.133 mg/kg bw/d

To obtain this internal dose from oral ingestion, it would require ingestion of

0.133 mg/kg bw/d / 0.90 (oral abs rate) = 0.148 mg/kg bw/d.

Thus an oral dose of **0.148 mg/kg bw/d** can, using these assumptions, be considered equipotent to continuous inhalation at 1 mg/m^3 corresponding to a risk level of 0.001.

Using a conversion factor of **0.148** the concentrations in mg/m^3 can be transferred to equipotent oral doses

This results in an oral unit risk estimate of:

Risk = Unit risk x Dose

Unit risk = Risk / Dose = 0.001 / 0.148 mg/kg bw/d = **0.00675 (mg/kg bw/d)$^{-1}$**

(That is approximately twice as high a unit risk as in calculation 1).

.

US-EPA (2011) Route to route extrapolation

According the US-EPA (2011), PBPK-modelling a risk level at 0.001 (at 1 mg/m^3) corresponds to an oral unit risk of **0.01 (mg/kgbw/d)$^{-1}$** .

Thus the oral dose at the risk level of 0.001 would be:

Risk = Unit risk x Dose

Dose = Risk / unit risk = 0.001 / 0.01 (mg/kg bw/d)$^{-1}$ = **0.10 mg/kg bw/d**

Thus an oral dose of **0.10 mg/kg bw/d** is considered equipotent to continuous inhalation to 1 mg/m^3 corresponding to a risk level of 0.001.

Using a conversion factor of **0.10,** the concentrations in mg/m^3 can be transferred to equipotent oral doses.

Preferred route-to-route extrapolation

It is not possible from the US-EPA (2011) document to obtain the specific details in the PBPK-modelling. However, it may be a somewhat surprising that a higher unit risk estimate is calculated using the PBPK modelling compared to more conventional route-to-route predictions. As the more simplistic calculation methods do not take into account the first pass metabolism in the liver that may occur after oral exposure, these methods should in fact overestimate the risk by oral exposure, because when taking into account of first pass metabolism, this would reduce the amount of trichloroethylene (due to the high oxidative pathway metabolic capacity of the liver) reaching the kidneys, which then would lead to a lower risk. However, this seems not to be the case as the unit risks for the PBPK modelling actually lead to a higher unit risk estimate compared to the simplistic calculations.

As calculation 1 may underestimate the risk (when assuming 100% absorption by inhalation), it seems most appropriate to use extrapolation 2, as this method takes into account differences in the absorption rates for inhalational and oral exposure. Further, this calculation is more transparent compared to the PBPK modelling, as it is not quite clear why a higher unit risk is obtained with this model.

5.4. Overall Conclusion

The following unit risks/slope factors have been found for describing the dose-response relationship of the carcinogenicity for trichloroethylene:

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies/ effects of most concern for POD	Unit risk/ slope factor or Threshold dose
WHO (2000) **WHO (2010)** **Afsset (2009)/ Anses (2013)**	Genotox.	Non-threshold linear approach	Maltoni et al (1986). -Rats -Inhalation. -Leydig tumours	Unit risk,24 hr exp. 4.3×10^{-4} $(mg/m^3)^{-1}$
WHO (2005)	Genotox	Non-threshold linear approach	NTP (1990) -rats -oral -kidney cancer	Unit risk,24 hr exp. 7.8×10^{-4} $(mg/kg\ bw\ d)^{-1}$
AGS (2008)	Genotox + cytotox	Non-threshold Sublinear approach	Henschler *et al.* (1995) Vamvakas *et al.* (1998) Brüning *et al.* (2003) Green *et al.* (2004) Seldén *et al.* (1993) -humans -inhalation -kidney cancer + cytotox	Slope factors 8 hr exp*. Above 6 ppm: 1.31×10^{-4} $(mg/m^3)^{-1}$ Below 6 ppm: 1.22×10^{-5} $(mg/m^3)^{-1}$
WHO (2010)	Genotox	Non-threshold linear approach	Maltoni et al (1986). -Rats -Inhalation. -Leydig tumours	Unit risk, 24 hr exp. $4.3 \times 10\text{-}4$ $(mg/m^3)^{-1}$

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies/ effects of most concern for POD	Unit risk/ slope factor or Threshold dose
US-EPA (2011)	genotox	Non-threshold linear approach	Charbotel *et al.* (2006) -humans -inhalation -kidney cancer	Unit risk, 24 hr exp. Inhalation: 1×10^{-3} $(mg/m^3)^{-1}$ Oral: 1.0×10^{-2} $(mg/ kg\ bw\ d)^{-1}$

calculated from the presented dose response curves

As discussed in Chapter 3, it seems most appropriate and justified to use dose-response estimates from human studies when making human health impact assessment of trichloroethylene exposure.

Regarding the threshold level for cytotoxic effects in the kidneys, AGS (2008) concluded to use a level of 6 ppm, and SCOEL (2007) concluded to use of level of 10 ppm. Both expert groups referred to a study by Selden *et al.* (1993) that found no increased level of the biomarker N-acetyl-ß-D-glucosaminidase (NAG) in urine among 29 workers exposed to relatively low levels of trichloroethylene (NAG in urine was used as an indicator for subclinical kidney damage). Both AGS (2008) and SCOEL (2007) made their conclusions on the no effect level for cytotoxicity with reference to the majority of the data-points/workers in the study. Thus, AGS (2008) referred to 23 of the lowest exposed workers, and SCOEL (2007) referred to 25 of the lowest exposed workers among the total of 29 workers. The Selden *et al.* (1993) study provided a table showing the average exposure level measured by air sampling for a one week of working period of the workers.

Exposure level (mg/m3)	Number of samples	%
≤ 9	6	21
10 – 19	10	34
20 – 29	7	24
30 – 39	2	7
40 – 49	0	0
50 – 99	3	10
≥ 100	1	4
Total	29	100

The table actually showed that 25 of the workers were at or below exposures up to 30-39 mg/m3 (average of this range is 34.5 mg/m3 or approximately equivalent to 6 ppm). No workers were exposed at the next exposure range

from 40 and up to 49 mg/m3 (9 ppm).

Furthermore, three workers were exposed at levels in the range of 50-99 mg/m^3, and one worker above 100 mg/m^3, but these four data points were not specifically addressed by AGS (2008) and SCOEL (2007) as they made their conclusions based on the majority of the data-points below these levels.

In conclusion, no sign of subclinical kidney toxicity was noted in this study. When deciding on a no effect level for subclinical effects in the kidneys, a level of 6 ppm is the most relevant and justified figure to use as this reflects the upper average exposure level for 25 of the 29 workers.

The dose-response relationship presented by AGS (2008) should be the preferred dose-response relationship for inhalational exposure as this approach take into account the sublinear dose-response relationship for the substance. The AGS (2008) approach in fact makes a bridging between the SCOEL approach and the non-threshold approaches, as a threshold of 6 ppm-10 ppm (as defined by SCOEL also) is used as the break point for the dose response curve.

The AGS (2008) slope factor at exposure levels above 33 mg/m^3 of 1.31×10^{-4} (mg/m^3)$^{-1}$ derived from the German studies for 8 h occupational exposure may be converted to continuous lifetime exposure (general population) by multiplying with a factor of:

$$20\text{m}^3/\text{d} / 10\text{m}^3/\text{d} \times 7\text{d}/5\text{d} \times 52\text{w}/48\text{w} \times 70\text{y}/40\text{y} = 5.3 \text{ (see Section 5.1.2)}$$

This results in a unit risk estimate for the general population of 0.69×10^{-3} (mg/m^3)$^{-1}$, which is very close to the US-EPA (2011) unit risk estimate of 1.0×10^{-3} (mg/m^3)$^{-1}$ based on the Charbotel *et al.* (2006) study.

Below exposure levels of 33 mg/m^3, the AGS unit risk of 1.2×10^{-5} (mg/m^3)$^{-1}$ converted to continuous exposure would be 6.4×10^{-5} (mg/m^3)$^{-1}$, which is a factor of 15 lower than the US-EPA (2011) unit risk estimate.

When calculating the risk for oral exposure, it seems appropriate to use the AGS (2008) inhalational dose-response curve and transfer this to inhalational dose-response for the general population. This inhalational dose-response can then be transferred to the oral dose metrics. This last step can be obtained by the use of a conversion factor of 0.148 when transferring the exposure from *mg/m^3* to *mg/kg/d*.

In the following Chapter 6 the contractor will - based on the analysis in the Chapters 3 and 4 - provide recommendations for dose response-relationships for inhalational, dermal and oral exposure of workers as well as of the general population.

6. DERIVATION OF REFERENCE EXPOSURE METRICS (DOSE-RESPONSE RELATIONSHIP AND UNIT RISKS)

(Contractors proposal taking into account the REACH R8 guidance for metric modification and low dose extrapolation)

6.1 Inhalation

Starting point is AGS (2008) Unit risk for 8 hr worker exposure (see Section 5.1.2):

At 6 ppm and above:
Excess risk = 7.2×10^{-4} ppm^{-1} x concentration (ppm) $-$ 0.0039

Below 6 ppm:
Excess risk = 6.7×10^{-5} ppm^{-1} x concentration (ppm)

Expressed in *mg/m^3* (1 ppm = 5.47 mg/m^3) this corresponds to

At 33 mg/m^3 and above:
Excess risk = 1.3×10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) $-$ 0.0039

Below 33 mg/m^3:
Excess risk = 1.2×10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

6.1.1 Worker exposure

Based on the dose-response equations above, the following excess risk can be calculated for various exposure levels.

Table 6-1 Excess lifetime kidney cancer risk estimated for workers exposed at different 8h-TWA concentrations of trichloroethylene for 40 years

TWA trichloroethylene concentration (mg/m^3)	Excess kidney cancer risk in EU workers (x10^{-4})
400	481
300	351
100	91.0
60	39.0
40	13.0
33 (6ppm)*	**4.0**
20	2.4
10	1.2
5	0.6
1	0.12
0.1	0.012

*break-point for the sublinear dose-response curve

6.1.2 General population

The trichloroethylene exposure levels for workers have to be adjusted to average exposure for the general population. In workers' lifetime exposure, it is considered that workers are exposed from inhalation of 10 m^3 per day, 5 days a week, and 48 weeks per year for 40 years. In the general population, the lifetime continuous exposure is in relation to inhalation of 20m^3 per day, 7 days a week for 70 years.

The adjustment factor between workers' exposure and general population exposure is then (according to the factors in Table R8-18 of the REACH R8 guideline):

Adjustment factor = 20m^3/d /10m^3/d x 7d/5d x 52w/48 w x 70y/40y = **5.3**

Considering this adjustment of exposure, the break point of the dose-response curve at the risk level of 0.0004 for worker lifetime exposure has to be adjusted to by this factor:

33 mg/m^3 / 5.3 = 6.2 mg/m^3 for an excess risk of 0.0004

Thus using this factor the worker dose-response relationship can be converted to a dose-response relationship for the general population:

Workers:
At 33 mg/m^3 and above
Excess risk = 1.3 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) – 0.0039

Below 33 mg/m^3:
Excess risk = 1.2 x 10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

General population (using the conversion factor of 5.3 for the slopes):

At 6.2 mg/m^3 and above:
Excess risk = 6.9 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) – 0.0039

Below 6.2 mg/m^3:
Excess risk = 6.4 x 10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

Based on these dose-response equations above, the following excess risk can be calculated for various exposure levels for the general population.

Table 6-2 Excess lifetime kidney cancer risk estimated for the general population exposed at different 24-h average concentrations of trichloroethylene for 70 years

Trichloroethylene 24-h concentration (mg/m^3)	Excess kidney cancer risk in EU general population (x10^{-4})
60	375.0
30	168.0
20	99.0
10	30.0
6.2*	**4.0**
3	1.9
1	0.6
0.1	0.06
0.01	0.006

*break-point for the sublinear dose-response curve

6.2 Dermal exposure

6.2.1 Worker exposure
No data on unit risk in relation to dermal exposure have been presented. For the risk characterisation, it will, as a conservative approach, be considered that the dermal absorption rate is equivalent to the inhalation absorption rate for trichloroethylene.

Then, the following route-to-route extrapolations are made from 1 mg/m^3 exposure (corresponding to an excess risk level of 0.001) to a dermal dose expressed as mg/kg bw/d.

For the workers' exposure:
If a person weighing 70 kg bw inhales 10m^3 air per day, the dose at 1 mg/m^3 trichloroethylene would be:

(1mg/m^3 x 10m^3 / 70kg) = 0.143 mg/kg bw/d

Thus a dermal dose of **0.143 mg/kg bw/d** can, using these assumptions, be considered equipotent to continuous inhalation at 1 mg/m^3.

Considering this conversion factor, the break point of the dose-response curve at the risk level of 0.0004 for workers' lifetime exposure has to be adjusted by this factor:

33 mg/m^3 x 0.143 = 4.72 mg/kg bw/d for an excess risk of 0.0004

Thus using this factor, the workers dose-response relationship by inhalation exposure can be converted to a dose-response relationship for the workers by dermal exposure:

Workers inhalation exposure:

At 33 mg/m^3 and above:
Excess risk = 1.3 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) − 0.0039

Below 33 mg/m^3:
Excess risk = 1.2 x 10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

Workers dermal exposure (using the conversion factor of 1/ 0.143 for the slopes):

At 4.72 mg/kg bw/d and above:
Excess risk = 9.09 x 10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) − 0.0039

Below 4.72 mg/kg bw/d:
Excess risk = 8.4 x 10^{-5} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Based on the dose-response equations above, the following excess risk can be calculated for various dermal dose levels.

Table 6-3 Excess lifetime kidney cancer risk estimated for workers exposed at different dermal dose levels of trichloroethylene for 40 years

Trichloroethylene dermal dose (mg/kg bw/d)	Excess kidney cancer risk in EU workers ($x10^{-4}$)
50	416
30	234
10	52
4.72*	**4.0**
1	0.84
0.5	0.42
0.1	0.084
0.01	0.0084
0.001	0.00084

*break-point for the sublinear dose-response curve

6.2.2 General population

The adjustment factor between the daily dermal exposure levels for workers' and general population exposure is:

Adjustment factor = 7d/5d x 52w/48 w x 70y/40y x 60kg/70kg= **2.3**

Considering this adjustment of exposure, the break point of the dose-response curve at the risk level of 0.0004 for worker lifetime exposure has to be adjusted to by this factor:

4.72 mg/kg bw/d / 2.3 = 2.05 mg/kg bw/d for an excess risk of 0.0004

Thus using this factor of 2.3, the worker dose-response relationship for dermal exposure can be converted to a dose-response relationship for the general population for dermal exposure:

Workers dermal exposure:

At 4.72 mg/kg bw/d and above:
Excess risk = 9.09×10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 4.72 mg/kg bw/d:
Excess risk = 8.4×10^{-5} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

General population dermal exposure (using the conversion factor of 2.3 for the slopes):

At 2.05 mg/kg bw/d and above:
Excess risk = 2.09 x 10^{-3} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 2.05 mg/kg bw/d:
Excess risk = 1.9 x 10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Based on the dose-response equations above, the following excess risk can be calculated for various dermal dose levels.

Table 6-4 Excess lifetime kidney cancer risk estimated for general population exposed at different daily dermal dose levels of trichloroethylene for 70 years

Trichloroethylene dermal dose (mg/kg bw/d)	Excess kidney cancer risk in EU general population (x10^{-4})
30	588
10	170
5	65.5
3	23.7
2.05*	**4.0**
1	1.9
0.1	0.19
0.01	0.019
0.001	0.0019

*break-point for the sublinear dose-response curve

6.3   Oral exposure
Not relevant for workers (convention that have been made in REACH).

6.3.1 General population
As explained in Chapter 4, when calculating the risk of oral exposure it seems appropriate to use the inhalational dose-response curve , but transferred to the oral dose metrics. This can be obtained by the use of a conversion factor of 0.148 when transferring the exposure from *mg/m^3* to *mg/kg/d*.

Considering this conversion factor, the break point of the dose-response curve at the risk level of 0.0004 for general population lifetime exposure has to be adjusted to by this factor:

6.2 mg/m^3 x 0.148 = 0.92 mg/kg bw/d for an excess risk of 0.0004

Thus using this factor, the general population dose-response relationship by inhalation exposure can be converted to a dose-response relationship for the general population by oral exposure.

General population inhalation exposure:
At 6.2 mg/m^3 and above:
Excess risk = 6.9 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) – 0.0039

Below 6.2 mg/m^3:
Excess risk = 6.4 x 10^{-5}(mg/m^3)$^{-1}$ x concentration (mg/m^3)

General population oral exposure (using the conversion factor of 1 / 0.148 for the slopes):
At 0.92 mg/kg bw/d and above:
Excess risk = 4.66 x 10^{-3} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 0.92 mg/kg bw/d:
Excess risk = 4.32 x 10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Based on the dose-response equations above, the following excess risk can be calculated for various oral dose levels.

Table 6-5 Excess lifetime kidney cancer risk estimated for the general population exposed at different oral daily doses of trichloroethylene for 70 years

Trichloroethylene oral dose (mg/kg bw/d)	Excess kidney cancer risk in EU general population (x10^{-4})
30	1359
10	427
1	7.6
0.92*	**4.0**
0.5	2.16
0.1	0.43
0.01	0.043
0.001	0.0043

*break-point for the sublinear dose-response curve

7. RECOMMENDATION OF REFERENCE EXPOSURE METRICS (DOSE-RESPONSE RELATIONSHIP AND UNIT RISKS)

7.1 Inhalation

7.1.1 Worker exposure

Worker (8-h) dose–response relationship as concluded by AGS (2008):

At 6 ppm and above:
Excess risk = 7.2×10^{-4} ppm^{-1} x concentration (ppm) $-$ 0.0039

Below 6 ppm:
Excess risk = 6.7×10^{-5} ppm^{-1} x concentration (ppm)

Expressed in *mg/m^3* (1 ppm = 5.47 mg/m^3) this corresponds to

At 33 mg/m^3 and above:
Excess risk = 1.3×10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) $-$ 0.0039

Below 33 mg/m^3:
Excess risk = 1.2×10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

Table 7-1 Excess lifetime kidney cancer risk estimated for workers exposed at different 8h-TWA concentrations of trichloroethylene for 40 years

TWA trichloroethylene concentration (mg/m^3)	Excess kidney cancer risk in EU workers (x10^{-4})
400	481
300	351
100	91.0
60	39.0
40	13.0
*33 (6ppm)**	*4.0*
20	2.4
10	1.2
5	0.6
1	0.12

TWA trichloroethylene concentration (mg/m^3)	Excess kidney cancer risk in EU workers (x10^{-4})
0.1	0.012

*break-point for the sublinear dose-response curve

7.1.2 General population

General population 24-h dose–response relationship:

At 6.2 mg/m^3 and above:
Excess risk = 6.9 x 10^{-4} (mg/m^3)$^{-1}$ x concentration (mg/m^3) – 0.0039

Below 6.2 mg/m^3:
Excess risk = 6.4 x 10^{-5} (mg/m^3)$^{-1}$ x concentration (mg/m^3)

Table 7-2 Excess lifetime kidney cancer risk estimated for the general population exposed at different 24-h average concentrations of trichloroethylene for 70 years

Trichloroethylene 24-h concentration (mg/m^3)	Excess kidney cancer risk in EU general population (x10^{-4})
60	375
30	168
20	99
10	30
*6.2**	*4.0*
3	1.9
1	0.6
0.1	0.06
0.01	0.006

*break-point for the sublinear dose-response curve

7.2 Dermal exposure

7.2.1 Worker exposure

Worker dose-reponse relationship for dermal exposure:

At 4.72 mg/kg bw/d and above:
Excess risk = 9.09 x 10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 4.72 mg/kg bw/d:
Excess risk = 8.4 x 10^{-5} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Table 7-3 Excess lifetime kidney cancer risk estimated for workers exposed at different dermal dose levels of trichloroethylene for 40 years

Trichloroethylene dermal dose (mg/kg bw/d)	Excess kidney cancer risk in EU workers (x10^{-4})
50	416
30	234
10	52
*4.72**	*4.0*
1	0.84
0.5	0.42
0.1	0.084
0.01	0.0084
0.001	0.00084

*break-point for the sublinear dose-response curve

7.2.2 General population

General population dose-response relationship for dermal exposure:

At 2.05 mg/kg bw/d and above:
Excess risk = 2.09 x 10^{-3} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 2.05 mg/kg bw/d:
Excess risk = 1.9 x 10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Table 7-4 Proposed excess lifetime kidney cancer risk estimated for general population exposed at different daily dermal dose levels of trichloroethylene for 70 years

Trichloroethylene dermal dose (mg/kg bw/d)	Excess kidney cancer risk in EU general population (x10^{-4})
30	588
10	170
5	65.5
3	23.7
*2.05**	*4.0*
1	1.9
0.1	0.19
0.01	0.019
0.001	0.0019

*break-point for the sublinear dose-response curve

7.3   Oral exposure

Not relevant for workers (convention that have been made in REACH).

7.3.1 General population

General population dose-response relationship for oral exposure:

At 0.92 mg/kg bw/d and above:
Excess risk = 5.43×10^{-3} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d) – 0.0039

Below 0.92 mg/kg bw/d:
Excess risk = 4.32×10^{-4} (mg/kg bw/d)$^{-1}$ x dose (mg/kg bw/d)

Table 7-5 Excess lifetime kidney cancer risk estimated for the general population exposed at different oral daily doses of trichloroethylene for 70 years

Trichloroethylene oral dose (mg/kg bw/d)	Excess kidney cancer risk in EU general population (x10^{-4})
30	1359
10	427
1	7.6
*0.92**	*4.0*
0.5	2.16
0.1	0.43
0.01	0.043
0.001	0.0043

*break-point for the sublinear dose-response curve

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Appendix A

Experimental animal studies and PBPK models used by US-EPA (2011) for the calculations of cancer unit risk estimates:

Bioassay	Strain	Endpoint	Applied dose	PBPK-based— primary dose-metric	PBPK-based— alternative dose- metric(s)	Time-to-tumour
ORAL						
Female mice						
NCI (1976)	B6C3F₁	Liver carcinomas	√	AMetLiv1BW34	TotOxMetabBW34	
		Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
		Multiple	√	TotMetabBW34	AUCCBld	
		Combined risk	√			
Male mice						
NCI (1976)	B6C3F₁	Liver carcinomas	√	AMetLiv1BW34	TotOxMetabBW34	
Female rats						
NTP (1988)	August	Leukemia	√	TotMetabBW34	AUCCBld	
Male rats						
NTP (1988)	August	Subcutaneous tissue	√	TotMetabBW34	AUCCBld	
NTP (1988)	Marshall	Testicular interstitial cell tumours	√	TotMetabBW34	AUCCBld	√
NTP (1988)	Osborne-Mendel	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	√
NTP (1990)	F344/N	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	√

PBPK-based dose-metric abbreviations:

ABioactDCVCBW34 = Amount of DCVC bioactivated in the kidney per unit body weight¾ (mg DCVC/kg¾/week).

AMetGSHBW34 = Amount of TCE conjugated with GSH per unit body weight¾ (mg TCE/kg¾/week).

AMetLiv1BW34 = Amount of TCE oxidized per unit body weight¾ (mg TCE/kg¾/week).

AMetLngBW34 = Amount of TCE oxidized in the respiratory tract per unit body weight¾ (mg TCE/kg¾/week).

AUCCBld = Area under the curve of the venous blood concentration of TCE (mg-hr/L/week).

TotMetabBW34 = Total amount of TCE metabolized per unit body weight¾ (mg TCE/kg¾/week).

TotOxMetabBW34 = Total amount of TCE oxidized per unit body weight¾ (mg TCE/kg¾/week).

Bioassay	Strain	Endpoint	Applied dose	PBPK-based— primary dose-metrica	PBPK-based— alternative dose-metric(s)a	Time-to-tumour
INHALATION						
Female mice						
Fukuda *et al.* (1983)	Crj:CD-1 (ICR)	Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
Henschler *et al.* (1980)	Han:NMRI	Lymphoma	√	TotMetabBW34	AUCCBld	
Maltoni *et al.* (1986)	B6C3F$_1$	Liver hepatomas	√	AMetLiv1BW34	TotOxMetabBW34	
		Lung adenomas and carcinomas	√	AMetLngBW34	TotOxMetabBW34 AUCCBld	
		Combined risk	√			
Male mice						
Maltoni *et al.* (1986)	Swiss	Liver hepatomas	√	AMetLiv1BW34	TotOxMetabBW34	
Female rats						
None selected						
Male rats						
Maltoni *et al.* (1986)	Sprague-Dawley	Kidney adenomas and carcinomas	√	ABioactDCVCBW34	AMetGSHBW34 TotMetabBW34	
		Leydig cell tumours	√	TotMetabBW34	AUCCBld	
		Leukemias	√	TotMetabBW34	AUCCBld	
		Combined risk	√			

PBPK-based dose-metric abbreviations:

ABioactDCVCBW34 = Amount of DCVC bioactivated in the kidney per unit body weight¾ (mg DCVC/kg¾/week).

AMetGSHBW34 = Amount of TCE conjugated with GSH per unit body weight¾ (mg TCE/kg¾/week).

AMetLiv1BW34 = Amount of TCE oxidized per unit body weight¾ (mg TCE/kg¾/week).

AMetLngBW34 = Amount of TCE oxidized in the respiratory tract per unit body weight¾ (mg TCE/kg¾/week).

AUCCBld = Area under the curve of the venous blood concentration of TCE (mg-hr/L/week).

TotMetabBW34 = Total amount of TCE metabolized per unit body weight¾ (mg TCE/kg¾/week).

TotOxMetabBW34 = Total amount of TCE oxidized per unit body weight¾ (mg TCE/kg¾/week).