Health Council of the Netherlands

Trichloroacetic acid

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Trichloroacetic acid*
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Geachte staatssecretaris,

Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan trichloorazijnzuur.

Dit advies maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb het advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Subcommittee on the Classification of Carcinogenic Substances of
the Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2012/20, The Hague, November 13, 2012

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, Agriculture & Innovation, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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INAHTA

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie trichloroacetic acid onder de loep. Trichloorazijnzuur is een stof die voornamelijk wordt gebruikt als selectieve herbicide. Daarnaast wordt het ook gebruikt in metaal-, plastic-, en textiel industrie, als toevoeging in minerale smeerolie en als een analytisch reagens. Het wordt ook gebruikt voor de lokale behandeling van wratten, beschadigingen van de huid van hals of nek en andere dermatologische situaties. Trichloorazijnzuur is een belangrijke eind metaboliet van trichlooroethyleen en tetrachloorethyleen. Trichloorazijnzuur ontstaat ook bij chlorering van drinkwater en zwembaden.

Op basis van de beschikbare gegevens is de commissie van mening dat de gegevens over trichloorazijnzuur niet voldoende zijn om de kankerverwekkende eigenschappen te evalueren (categorie 3).*

* Volgens het classificatiesysteem van de Gezondheidsraad (zie bijlage F).

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the Committee. In this report, the Committee evaluates trichloroacetic acid. Trichloroacetic acid is mainly used as a selective herbicide. It also finds use in the metal, plastics and textile industries, as an additive in mineral lubricating oils and as an analytical reagent. It is used in the topical treatment of warts, cervical lesions and other dermatological conditions. Trichloroacetic acid is a major end metabolite of trichloroethylene and tetrachloroethylene. Trichloroacetic acid also arises as a result of chlorination or chloramination.

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of trichloroacetic acid (category 3).*

* According to the classification system of the Health Council (see Annex F).

Chapter 1

Scope

1.1 Background

In the Netherlands, a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex F).

This report contains the evaluation of the carcinogenicity of trichloroacetic acid.

1.2 Committee and procedures

The evaluation is performed by the subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B. The submission letter (in English) to the State Secretary can be found in Annex C.

In June 2012 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft

are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the Committee is based on scientific data, which are publicly available. The starting points of the Committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the Committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of trichloroacetic acid, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in Annex E.

More recently published data were retrieved from the online databases Medline, Toxline, and Chemical Abstracts using carcino*, cancer*, mutagen*, chromosom*, genotox* (*; wildcard character) and CAS no. 76-03-9 as key words. The last updated online search was in September 2012 and covered publications from 2004. The new relevant data were included in this report.

Chapter 2

General information

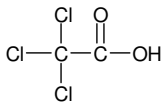
The data have been retrieved from the IARC evaluation of trichloroacetic acid[1] and the European Substance Information System (ESIS* and the INCHEM database of the International Programme on Chemical Safety (IPCS), which can be accessed via the inchem-site**.

2.1 Identity and physico-chemical properties

Chemical name	:	Trichloroacetic acid
CAS registry number	:	76-03-9
EINECS number	:	200-927-2
Synonyms	:	Trichloorazijnzuur, TCA, TCA (acid), trichloroethanoic acid, trichloro methane carboxylic acid
Appearance	:	Colourless to white deliquescent crystals with characteristic odour
Use	:	Trichloroacetic acid is mainly used as a selective herbicide. It also finds use in the metal, plastics and textile industries, as an additive in mineral lubricating oils and as an analytical reagent. It is used in the topical treatment of warts, cervical lesions and other dermatological conditions. Trichloroacetic acid is a major and final metabolite of trichloroethylene and tetrachloroethylene in humans and therefore used as a biomarker for exposure to these substances.
Chemical formula	:	$C_2HCl_3O_2$

* http://esis.jrc.ec.europa.eu (accessed October 11, 2012).

** www.inchem.org (accessed October 11, 2012), International Chemical Safety Card of trichloroacetic acid (0586). November 1998.

Structural formula	:	
Molecular weight	:	163.39
Boiling point	:	197.5 °C
Melting point	:	59 °C
Vapour pressure	:	1 mm/Hg at 51 °C
Vapour density (air = 1)	:	5.6
Solubility	:	Very soluble in water (1,306 g/100 g at 25 °C) and most organic solvents, including acetone, benzene ether, methanol and *ortho*-xylene
Conversion factor	:	1 mg/m^3 = 6.68 ppm 1 ppm = 0.1497 mg/m^3
EU Classification (100% solution)	:	Skin Corr. 1A: H314 (Causes severe skin burns and eye damage) STOT SE 3: H335 (May cause respiratory irritation); C ≥1%

2.2 IARC classification

In 2004, IARC[1] concluded that there is inadequate evidence in humans, and limited evidence in experimental animals for the carcinogenicity of trichloroacetic acid. Trichloroacetic acid was not classifiable as to its carcinogenicity to humans (group 3).[1]

Chapter 3

Carcinogenicity

3.1 Observations in humans

In its monograph of 2004 IARC[1] described quite a number of human studies (cohorts, as well as case-control studies) that analysed risk for cancer with respect to one or more measures of exposure to complex mixtures of disinfection by-products that are found in most chlorinated and chloraminated drinking-waters. Some of these studies show indications for increased relative risks for melanoma, and tumours of the urinary bladder, liver, colon and lung, i.e. for a diversity of tumours. However, no data specifically on trichloroacetic acid were available. Therefore, no conclusion could be drawn with respect to the carcinogenicity in humans by trichloroacetic acid specifically.

No additional human data have become available after the IARC evaluation of 2004.

3.2 Carcinogenicity studies in animals

Trichloroacetic acid has been evaluated previously (IARC 1995[2], 2004[1]) and was found to induce hepatocellular adenomas and carcinomas in male $B6C3F_1$ mice and to possess promoter activity. The previous evaluation of trichloroacetic acid indicated that there was limited evidence in experimental animals for its carcinogenicity.

3.2.1 *Carcinogenicity studies*

Oral exposure

In a study by Pereira (1996)[3] groups of 93, 46 and 38 female B6C3F1 mice, 7-8 weeks of age, were administered trichloroacetic acid in the drinking-water at concentrations of 2.0, 6.67 and 20.0 mmol/L (324, 1,080 or 3,240 mg/L), adjusted to pH 6.5-7.5 with sodium hydroxide. A control group received 20.0 mmol/L sodium chloride. Mice were killed after 360 or 576 days (when high-dose mice became moribund) of exposure and only livers were removed for histopathology. The livers were weighed and evaluated for foci of altered hepatocytes (basophillic and eosinophillic foci), adenomas and carcinomas. Data from mice administered trichloroacetic acid were compared with those from control mice using Fishers's exact test with a p-value < 0.05.

After 360 or 576 days of exposure, the liver-to-body weight ratio was increased dose-dependently following treatment with trichloroacetic acid. The high dose of trichloracetic acid (20.0 mmol/L(=3,240 mg/L)) increased, in comparison with controls, the incidence of foci (11/18 versus 10/90 at 576 days), adenomas (7/18 versus 2/90 at 576 days) and carcinomas (5/20 versus 0/40 at 360 days and 5/18 versus 2/90 at 576 days). The mid dose of trichloroacetic acid (6.67 mmol/L) increased the incidence of foci (9/27) and hepatocellular carcinomas (5/27) at 576 days, while the low dose of 2.0 mmol/L (324 mg/L) did not alter the incidence of any liver lesion. In control mice, the incidence was 1/40 adenoma (2.5%) at 360 days and 10/90 foci (11.1%), 2/90 adenomas (2.2%) and 2/90 carcinomas (2.2%) at 576 days.

In a study by DeAngelo et al. (1997)[4] groups of 50 male Fisher 344/N rats, 28-30 days of age, received 0.05, 0.5 and 5 g/L neutralized trichloroacetic acid, adjusted to pH 6.9-7.1 with sodium hydroxide, or 2 g/L sodium chloride in the drinking-water for a total 104 weeks. Interim sacrifices were made at 15, 30, 45 and 60 weeks. A complete necropsy of the animals was performed. The liver, kidney, spleen, testes and gross lesions were examined microscopically. A complete pathological examination was carried out on all tissues from all animals in the high-dose group.

The high dose of trichloroacetic acid but not the low or mid dose decreased body weight (~11%). Trichloroacetic acid did not affect the absolute or relative (to body weight) weights of the liver, kidneys, spleen or testes except for a decrease in the absolute liver weights in rats administered 5.0 g/L ($p \leq 0.05$). At 104 weeks, the number of animals per treatment group ranged form 20 to 24

including one rat that died after 76 weeks. The number of rats with hepatocellular adenomas varied between one and three among the treatment groups (4.2-15%). A single hepatocellular carcinoma (1/22, 4.6%) was found in the high-dose trichloroacetic acid-treated group. None of the treatment groups had a significant increase in the incidence of any tumour in other organs.

The ability of mixtures of di- and trichloroacetic acid to induce liver tumours was studied in 6-week-old $B6C3F_1$ male mice (Bull et al., 2002[5]; IARC 2004[1]). As part of this experiment treatments included 0.5 and 2.0 g/L trichloroacetic acid. Twenty animals were assigned to each of the groups that received the above concentrations in their drinking-water for 52 weeks. Control animals were given the vehicle only.

The incidence of liver tumours (adenomas and carcinomas combined) was significantly increased ($p< 0.05$) in both treatment groups (11/20 in the 0.5 g/L and 9/20 in the 2 g/L group) compared to the incidence in the control group (1/20).

DeAngelo et al.[6] determined the prevalence and multiplicity (average number of tumours per animal) of hepatocellular neoplasia in the male B6C3F1 mouse exposed to trichloroacetic acid in the drinking-water. Male mice were exposed in study 1 to 0.05, 0.5, and 5 g/L trichloroacetic acid for 60 wk (50 animals/group, and in study 2 to 0.05 and 0.5 g/L trichloroacetic acid for 104 wk (low and mid-dose 58 animals/ group), and in study 3 to 4.5 g/L trichloroacetic acid for 104 wk (high dose 72 animals/group).

Time-weighted mean daily doses measured for the low, medium, and high dose groups were consistent over the three studies, 6-8, 58-68, and 572-602 mg/kg,day for the 0.05, 0.5, and the 4.5-5 g/L treatment groups, respectively. No significant changes in animal survival were noted across the studies. A significant increase in the prevalence and multiplicity of hepatocellular tumours was found in the 58-68 and 572-602 mg/kg/d trichloroacetic acid dose groups. The high dose group of the 60 wk study had a prevalence of 38% (versus 12% in controls), and a significant increase ($p \leq 0.03$) of the multiplicity of hepatocellular carcinoma from 0.07 ± 0.05 (control) to 0.42 ± 0.11. The 104 week study high dose group had a prevalence of 78% (versus 12% in controls), and showed a significant increase ($p \leq 0.03$) of the multiplicity of hepatocellular carcinoma from 0.20 ± 0.12 (control) to 1.50 ± 0.22.

3.2.2 *Tumour-promotion studies*

A couple of experimental studies were performed to investigate tumour promoting activity of trichloroacetic acid, using *N*-methyl-*N*-nitrosourea (MNU) as tumour initiating substance.

In one study (Pereira & Phelps, 1996[7]) groups of 6-40 female B6C3F_1 mice, 15 days of age, were initiated with an intraperitoneal injection of 25 mg/kg MNU. At 49 days of age, the animals received 2.0, 6.67 or 20.0 mmol/L (=324, 1080 or 3,240 mg/L) trichloroacetic acid, adjusted to pH 6.5-7.5 with sodium hydroxide, or 20.0 mmol/L sodium chloride as a control for the sodium salt in the drinking-water. Mice were killed after 31 or 52 weeks of exposure.

At 31 weeks, the high dose of trichloroacetic acid increased the incidence of hepatocellular adenomas in MNU-initiated mice from 0/10 to 6/10 and the multiplicity (average number of tumours per animal) from 0.00 to 1.30 ± 0.045. At 52 weeks, the mid and high doses of trichloroacetic acid significantly ($p < 0.01$) increased the incidence of carcinomas in MNU-initiated mice from 4/40 to 5/6 and 20/24, and multiplicity from 0.10 ± 0.05 to 1.33 ± 0.42 and 2.79 ± 0.48, respectively. At 52 weeks, the mid and high doses of trichloroacetic acid increased the incidence of adenomas from 7/40 to 16/24 and 5/6, respectively, and the multiplicity from 0.28 ± 0.11 to 2.00 ± 0.82 and 1.29 ± 0.24, respectively. In mice that were not administered MNU, the high dose of trichloroacetic acid significantly increased the incidence of carcinomas from 0/40 to 5/20.

In a second study by Pereira et al. (1997)[8] combinations of dichloroacetic acid and trichloroacetic acid have been evaluated for tumour-promoting activity. Female B6C3F_1 mice, 15 days of age, were initiated with MNU (25 mg/kg bw) followed by exposure to 0, 7.8, 15.6, and 25 mmol/L (=1,006, 2,012, 3,225 mg/L) dichloroacetic acid with or without 6.0 mmol/L (=972 mg/L) trichloroacetic acid or 0, 6.0 and 25 mmol/L (=0, 972 and 4,050 mg/L) trichloroacetic acid with or without 15.6 mmol/L (=2,012 mg/L) dichloroacetic acid. The pH of the dose solutions was adjusted to 6.5-7.5 with sodium hydroxide. Exposure was from week 4 to 48 of age, at which time the mice were killed.

The high dose of dichloroacetic acid (25 mmol/L(=3,225 mg/L)) and trichlorocacetic acid (25 mmol/L(=4,050 mg/L)) significantly increased ($p < 0.05$) the multiplicity of hepatocellular adenomas from 0.07 $\pm$ 0.05 (no dichloroacetic acid or trichloroacetic acid) to 1.79 $\pm$ 0.29 and 0.52 $\pm$ 0.11, respectively. The lower doses of dichloroacetic acid and trichloracetic acid did not significantly increase the incidence or multiplicity of adenomas).

In a third study (Pereira et al., 2001[9]) the effect of chloroform on liver tumours promotion by trichloroacetic acid has been investigated. Groups of male and female $B6C3F_1$ mice, 15 days of age, were initiated with 30 mg/kg MNU. At 5 weeks of age, the mice started to receive in the drinking-water 4.0 g/L trichloroacetic acid neutralized with sodium hydroxide and 0, 800 or 1,600 mg/L chloroform and were killed at 36 weeks of age. The results were analysed for statistical significane by a one-way ANOVA followed by the Tukey test with p-value < 0.05.

In MNU-initiated mice that did not receive trichloroacetic acid, hepatocellular adenomas were found in 2/29 females and 2/8 males, while no hepatocellular carcinomas were found. Trichloroacetic acid increased the incidence of liver carcinomas (10/16) and adenomas (12/16) in male mice. In female mice administered trichloroacetic acid, the incidence of mice with hepatocellular adenocarcinomas and adenomas was not significantly altered: 4/14 and 2/14. In male mice administered trichloroacetic acid plus 0, 800, and 1,600 mg/L chloroform, the incidence of hepatocellular adenocarcinomas was 10/16, 7/9 and 6/8 and that of hepatocellular adenomas was 12/16, 6/9 and 1/8, respectively. The incidence of mice with hepatocellular adenomas was significantly lower in mice administered trichloroacetic acid plus 1600 mg/L chloroform than in mice administered trichloroacetic acid (< 0.05). No altered hepacotcyte foci, adenomas or adenocarcinomas were found in six MNU-initiated male mice that were administered 1600 mg/L chloroform. Multiplicity of tumours (adenomas plus adenocarcinomas) was increased in male mice from 0.25 ± 0.16 to 3.18 ± 0.82 ($p < 0.001$), but not in female mice, with 0.07 ± 0.04 and 0.64 ± 0.22 for control and trichloroacetic acid-exposed mice, respectively. Sixty per cent of the tumours were adenocarcinomas, indicating that multiplicity of adenocarcinomas was significantly increased in male mice exposed to trichloroacetic acid.

Chapter 4

Mode of action

4.1 Genotoxic mode of action

4.1.1 Gene mutation assays

In vitro

Trichloroacetic acid was repeatedly shown to be not mutagenic to *Salmonella typhimurium* in a couple of studies including strains TA98, TA100, TA1535, and TA1538, in the presence or absence of metabolic activation. Trichloroacetic acid was weakly mutagenic in mouse lymphoma cells at high dose (3,000 µg/mL) (Harrington-Brock et al., 1998[10]).

In vivo

Point mutations in exons 1, 2 and 3 of K- and H-*ras* proto-oncogenes were studied in trichloroacetic acid-induced liver tumours of male $B6C3F_1$ mice (104-week treatment with 4.5 g/L in drinking-water). Trichloroacetic did not modify the incidence of mutations in exon 2 of H-*ras* in carcinomas (45% versus 58% for control). Only four carcinomas showed mutations in the other exons of H-*ras* or in K-*ras*. In tumours with mutation in exon 2 of H-*ras*, treatment with trichloroacetic acid dit not modify the mutational spectrum compared with that of spontaneous liver tumours, that is to say 80% of the mutations in codon 61

were CAA-> AAA, and 20% were CAA->CGA (Ferreira-Gonzalez et al., 1995[11]).

4.1.2 *Cytogenetic assays*

In vitro

In human cells in vitro, trichloroacetic acid did not induce chromosomal aberrations (Mackay et al., 1995[12]).

In vivo

In one study (Mackay et al., 1995[12]), trichloroacetic acid induced micronuclei and chromosomal aberrations in bone-marrow cells and abnormal sperm morphology after injection into Swiss mice in vivo.[13] In another study, in which a 10-fold higher dose was injected to C57BL/6jfBL10/-Alpk mice, no micronucleus formation was observed. Trichloroacetic acid induced the formation of micronuclei in erythrocytes of newt larvae in vivo (Giller et al., 1997).[14] It also induced chromosomal aberrations in vivo in the bone marrow of the chicken *Gallus domesticus* (Bhunya et al., 1996).[15]

4.1.3 *Miscellaneous*

In vitro

Trichloroacetic acid did not induce λ prophage or SOS repair in *Escherichia coli* (DeMarini et al., 1994[16]; Giller et al., 1997[14]).

Trichloroacetic acid did not induce DNA strand breaks or DNA damage in mouse, rat or hamster cells in vitro (Chang et al., 1992[17]; Plewa et al., 2002[18]; Stauber et al., 1998[19]).

After treatment with trichloroacetic acid, the level of malondialdehyde-derived adducts was increased in vitro (Beland, 1999[20]).

In vivo

The level of 8-hydroxyguanosine-DNA adducts in the liver of $B6C3F_1$ mice was not modified after administration of trichloroacetic acid through drinking-water (Parrish et al., 1996[21]), was slightly increased after administration by gavage (Austin et al., 1996[22]) and was clearly increased after intraperitoneal injection

(Von Tungeln et al., 2002[23]). After treatment with trichloroacetic acid, the level of malondialdehyde-derived adducts was increased in vivo (Von Tungeln et al., 2002[23]).

4.2 Non-genotoxic mode of action

4.2.1 Peroxisome proliferation

In vitro

An in-vitro study of COS-1 cells transiently co-transfected with a peroxisome proliferator-activated receptor (PPAR) expression plasmid, pCMV-mPPARalpha, together with a reporter plasmid containing a peroxisome proliferator response element, Pluc4A6-880, clearly demonstrated that trichloroacetate directly activates PPARalpha (Zhou & Waxman, 1998[24]). In other studies, trichloroacetic acid induced peroxisome proliferation in primary cultures of hepatocytes from rats and mice but not in those from humans (Elcombe, 1985[25]; Walgren et al., 2000a[26]). In addition, human hepatocytes that expressed endogenous human PPARalpha did not respond to trichloroacetic acid, whereas human cells co-transfected with mouse PPARalpha and mouse retinoid X receptor plasmids displayed increased activity of the peroxisome proliferator response element reporter after treatment with trichloroacetic acid and other peroxisome proliferators. Retinoid X receptor that forms a heterodimer with PPAR enhanced PPAR-DNA binding and transcriptional activation (retinoid X receptor is a common partner for many steroid receptors) (Walgren et al., 2000b[27]).

Smith et al.[28,29] examined the induction of DNA synthesis and apoptosis by trichloroacetic acid in hepatocytes from B6C3F1, PPARalpha knockout and 129/Sv wildtype mouse strains. The carcinogenic effect of trichloroacetic acid in mice, both belonging to the peroxisome proliferator class, is believed to involve agonist binding to the peroxisome proliferator activated receptor alpha (PPARalpha).

Trichloroacetic acid (0.1-5.0 mM (=16.2-810 mg/L) produced a concentration-related increased DNA synthesis in both B6C3F1 and 129/Sv hepatocytes at 24, 48 and 72 hrs. In hepatocytes from PPARalpha knock-out mice it failed to increase DNA synthesis at any time point examined. In human hepatocytes, trichloroacetic acid decreased DNA synthesis. Apoptosis was increased by 2.5 (=405 mg/L) and 5.0 mM (=810 mg/L) trichloroacetic acid (~2-fold) in B6C3F1 hepatocytes after 48 and 72 hrs and by 5.0 mM (=810 mg/L) trichloroacetic acid (1.5-2.5-fold) in 129/Sv hepatocytes at 48 and 72 hr

exposure. No changes in apoptosis were seen in PPARalpha null or human hepatocytes. In addition, peroxisomal beta oxidation, a measure of peroxisome proliferation, was increased by trichloroacetic acid in hepatocytes from B6C3F1 (~2-4-fold over control), and 129/Sv (~2-fold) mice, whereas no induction was seen in hepatocytes from PPARalpha null mice or in human hepatocytes.

The studies of Walgren et al. (2004)[30] were undertaken to determine whether a primary rat hepatocyte model system could be used to examine structure-activity relationships of haloacetates for the induction of peroxisomal palmitoyl-CoA oxidation. The haloacetates tested differed in both type (iodo, bromo, chloro and fluoro) and extent (mono, di and tri) of substitution. Significant differences were observed in both potency and efficacy. Potency varied over about two orders of magnitude, in the order of mono > di = tri. Within the monohalo-substituted series, the order of potency was iodo > bromo > chloro, with the fluoro analog being essentially inactive.

In vivo

As reported in the monograph on trichloroacetic acid (IARC, 1995[2]), short-term treatment (≤ 14 days) resulted in increases in cell replication rates in the liver of mice. The elevated rates of replication were not sustained and became substantially reduced compared with controls with and without chronic pretreatment (Pereira, 1996[3]; Stauber & Bull, 1997[31]). In an experiment in which treatment of male $B6C3F_1$ mice with 2 g/L trichloroacetic acid was terminated after 1 year (50 weeks), cell replication rates within tumours were not dependent upon continued treatment (for an additional 2 weeks). Trichloroacetic acid did not stimulate replication of initiated cells. As only one time-point was measured, the possibility that trichloroacetic acid affected replication rates of preneoplastic lesions cannot be ruled out (Stauber & Bull, 1997[31]).

In a 7 day in vivo study performed by Smith et al.[28,29], trichloroacetic acid (0.5 and 2.0 g/L) increased DNA synthesis (2.0-5.7-fold) and peroxisomal beta oxidation in the 129/Sv mouse, while no changes in these endpoints were seen in the PPARalpha null mice. Trichloroacetic acid did not alter levels of apoptosis in either strain of mice.

Non-hepatoproliferative changes (cytoplasmic alterations, inflammation, and necrosis) in mice treated with trichloroacetic acid were mild and dose related in the earlier described carcinogenicity study of DeAngelo et al.[32] A TCA-induced increase in liver palmitoyl CoA oxidase activity, a marker of peroxisome proliferation, correlated with tumor induction. A linear association (r^2 = .984 and

r^2 = .987 for 60 and 104 weeks respectively) was found between peroxisome proliferation and tumour induction.

4.2.2 *Methylation*

In vitro

No assays with trichloroacetic acid were available to the Committee.

In vivo

Short-term oral treatment (11 days) of mice with trichloroacetate (25 mmol/L) inhibited methylation of DNA in liver, an effect that was not observed with long-term treatment (44 weeks) (Tao et al., 1998[33]). However, methylation of DNA was depressed in trichloroacetate-promoted liver tumours at 44 weeks and termination of treatment 1 week prior to sacrifice did not reverse this effect. An increased expression of c-*jun* and c-*myc* proto-oncogenes was observed when the 5-methylcytosine levels in their respective promoter regions decreased (Tao et al., 2000a[34]) and administration of methionine 30 min after trichloroacetate inhibited expression of both proto-oncogenes (Tao et al., 2000b[35]). Increased cell replication rates and decreased methylation of the c-*myc* gene were first observed simultaneously in mice 72 h after the start of exposure to trichloroacetic acid. Trichloroacetic acid induced DNA hypomethylation by inducing DNA replication and preventing the methylation of the newly synthesized strands of DNA (Ge et al., 2001[36]). The authors speculated that trichloroacetate depleted *S*-adenosylmethionine levels. Depressed levels of 5-methylcytosine were observed in the kidney and bladder as well as the liver.

Li et al. (abstract)[37] determined the methylation status the regulatory region of 17 and 30 CpG sites in the tumour suppressor genes estrogen receptor (ER)-alpha and p16. DNA was isolated from mouse liver tumours induced by dichloroacetic acid and trichloroacetic acid, treated with bisulfite, PCR-amplified for the genes, and sequenced. The percentage of the CpG sites that were methylated in the ER-alpha gene was 3.9 ± 1.9% in normal liver tissue, while in dichloroacetic acid and trichloroacetic acid -promoted tumours the percentages was increased to 42.3 ± 10.9% and 23.5 ± 7.9%, respectively. The extent to which the CpG sites in the p16 gene were methylated in normal liver ranged from 0 to 1 site, while dichloroacetic acid and trichloroacetic acid -promoted tumours contained 2-3 methylated CpG sites. Hence, dichloroacetic acid and trichloroacetic acid increased the methylation of the two tumour suppressor genes in liver tumours.

Chapter 5

Classification

5.1 Evaluation and conclusion

The Committee is of the opinion that there is insufficient evidence for the carcinogenicity of trichloroacetic acid in humans. The basis for this conclusion was the observation that in all human studies that analysed the risk for cancer, the investigated population was exposed to complex mixtures, i.e. disinfection by-products that are found in most chlorinated and chloraminated drinking-waters, and none of them specifically to trichloroacetic acid. Although some of these studies showed indications of increased relative risks for some tumours, i.e. melanoma and tumours of the urinary bladder, liver, colon and lung, no conclusion could be drawn with respect to the carcinogenicity in humans by trichloroacetic acid specifically. The Committee shares the opinion of IARC (2004)[1] that the human data do not allow a conclusion on the carcinogenicity ot trichloroacetic acid. The Committee is aware that since this 2004 evaluation by IARC no additional human studies have appeared.

With regard to the evidence from animal carcinogenicity data, the Committee observed increased incidences of hepatocellular adenomas and carcinomas in mice: in both male and female mice, after prolonged exposure to (neutralized) trichloroacetic acid via drinking-water. No increase in incidence of liver tumours or tumours at any other site was observed in a 2-year study with male rats. The Committee shares the opinion of IARC that trichloroacetic acid induces

peroxisome proliferation in the livers of mice at doses within the same range as those that induce hepatic tumours.

Peroxisome proliferators cause proliferation of peroxisomes and hepatocarcinogenesis in rodent liver. This is mediated by peroxisome proliferator-activated receptor-alpha (PPARalpha), a nuclear receptor protein functioning as transcription factor.

A number of other effects of trichloroacetic acid were observed: in in vitro transfection experiments it was demonstrated that trichloroacetate directly activates PPAR-alpha in primary cultures of hepatocytes from rats and mice, but not from humans, whereas human hepatocytes co-transfected with mouse PPAR-alpha did respond to trichloroacetate. This strongly suggested peroxisome proliferation as underlying mechanism for liver tumour-induction in these rodent species. Also, brief stimulation of cell division in the liver during the first days of treatment, and depressed cell replication upon chronic treatment were observed. The initial increase in cell proliferation correlated with a decreased methylation of the promoter regions of the c-*jun* and c-*myc* proto-oncogenes coupled with an increased expression of these genes.

A small number of recent mechanistic animal studies have appeared. These studies confirm the potential of trichloroacetic acid to induce peroxisome proliferation in livers of mice and rats[4,6]. Smith et al.[28,29] showed that induction of peroxisome proliferation by trichloroacetic acid occurred in primary cultures of hepatocytes from rats and mice but not in those from humans: the data demonstrate that human hepatocytes are refractory to the induction of DNA synthesis and apoptosis by trichloroacetic acid and that the PPAR-alpha is required for the induction of DNA synthesis observed following trichloroacetic acid exposure in B6C3F1 and 129/Sv wildtype mice. Accordingly, these data support the notion that the induction of liver tumours in mice via this mechanism is unlikely to be of relevance to human health.[38]

A critical question is whether there is any role for direct DNA interactions of trichloroacetic acid that could underly part of the liver tumours observed. From the available in vitro data on mutagenicity and genotoxicity it is clear that trichloroacetic acid is not a directly acting mutagenic chemical, either with or without metabolic activation. The positive in vivo data apparently are due to indirect effects induced by high doses of trichloroacetic acid and relate to its peroxisome proliferating effects, e.g. the production of superoxide anions, lipid peroxidation, DNA adducts by malondialdehyde, and DNA single-strand breaks.[1] As also demonstrated by the data of Smith et al.[28,29], of Walgren et al. (2000[26,27]; cited in IARC, 2004[1]), and the arguments indicated above, these effects are not expected to occur in humans.

5.2 Recommendation for classification

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of trichloroacetic acid (category 3).*

* According to the classification system of the Health Council (see Annex F).

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A	Request for advice
B	The Committee
C	The submission letter
D	Comments on the public review draft
E	IARC Monograph
F	Carcinogenic classification of substances by the Committee

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, Zeist; Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- J. van Benthem
  Genetic Toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- Ms M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- G.B. van der Voet, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter

Subject	: Submission of the advisory report *Trichloroacetic acid*
Our reference	: U-7411/BvdV/fs/246-B17
Your Reference	: DGV/MBO/U-932342
Enclosed	: 1
Date	: November 13, 2012

Dear State Secretary,

I hereby submit the advisory report on the effects of occupational exposure to *Trichloroacetic acid*.

This advisory report is part of an extensive series in which carcinogenic substances are classified in accordance with European Union guidelines. This involves substances to which people can be exposed while pursuing their occupation.

The advisory report was prepared by the Subcommittee on the Classification of Carcinogenic Substances, a permanent subcommittee of the Health Council's Dutch Expert Committee on Occupational Safety (DECOS). The advisory report has been assessed by the Health Council's Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,

(signed)
Professor W.A. van Gool
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in June 2012 for public review. The following organisations and persons have commented on the draft document:

- National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA.

Annex **E**

IARC Monograph

Volume 84, (excerpt from Trichloroacetic acid, pp. 403-440)

Summary of Data Reported and Evaluation

1 Exposure data

Trichloroacetic acid is mainly used as a selective herbicide. It also finds use in the metal, plastics and textile industries and as an analytical reagent. It is used in the topical treatment of warts, cervical lesions and other dermatological conditions. Trichloroacetic acid is a major end metabolite of trichloroethylene and tetrachloroethylene. Wider exposure to trichloroacetic acid occurs at microgram-per-litre levels in drinking-water and swimming pools as a result of chlorination or chloramination.

2 Human carcinogenicity data

Several studies analysed risk with respect to one or more measures of exposure to complex mixtures of disinfection by-products that are found in most chlorinated and chloraminated drinking-water. No data specifically on trichloroacetic acid were available to the Working Group.

3 Animal carcinogenicity data

In four studies, neutralized trichloroacetic acid, when administered in the drinking-water to female and/or male mice, increased the incidences of hepatocellular adenomas and carcinomas. In a study in male rats, trichloroacetic acid did not increase the incidence of liver tumours or tumours at any other site. When administered in the drinking-water, trichloroacetic acid promoted the induction of hepatocellular adenomas and/or carcinomas in carcinogen-initiated male and female mice and of kidney tumours in male mice.

4 Other relevant data

The half-life of trichloroacetic acid, given orally or formed as a metabolite of trichloroethylene or trichloroethanol, is longer in humans than in rodents. Trichloroacetic acid may be reduced in vivo to dichloroacetic acid, but the artefactual conversion of trichloroacetic acid to dichloroacetic acid hinders any clear conclusions. A fraction of trichloroacetic acid is metabolized to carbon dioxide.

Trichloroacetic acid induces peroxisome proliferation in the livers of mice at doses within the same range as those that induce hepatic tumours. A brief stimulation of cell division is observed in the liver during the first days of treatment, but depressed cell replication results from chronic treatment. The initial increase in cell proliferation was correlated with decreased methylation of the promoter regions of the c-*jun* and c-*myc* proto-oncogenes and increased expression of these genes.

Effects of trichloroacetic acid on reproduction and development in rats have been reported, but were not confirmed in a subsequent study. In-vitro results suggest that trichloroacetic acid can produce teratogenic effects at high doses.

In male mice, trichloroacetic acid modified neither the incidence of mutations in exon 2 of H-*ras* in carcinomas, nor the mutational spectrum observed in tumours that bore a mutation in exon 2. In female mice, 27% of tumours promoted by trichloroacetic acid exhibited loss of heterozygosity at a minimum of two loci on chromosome 6.

In mouse liver in vivo, measurements of trichloroacetic acid-induced 8-hydroxydeoxyguanosine DNA adducts gave different results depending on the route of administration. Trichloroacetic acid induced abnormal sperm in mice in vivo in one study and chromosomal aberrations in mouse and chicken bone marrow in vivo. The results of in vivo studies in rodents on the induction of DNA strand breaks and micronuclei were inconsistent. It induced the formation of micronuclei in newt larvae in vivo.

In human cells in vitro, trichloroacetic acid did not induce chromosomal aberrations or DNA strand breaks in single studies. In single studies on cultured rodent cells, trichloroacetic acid was weakly mutagenic; no effect was observed in a DNA strand-break assay or a single-cell gel assay. It also inhibited intercellular communication in cultured rodent cells. Trichloroacetic acid caused neither mutation in bacteria nor SOS repair.

5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of trichloroacetic acid.

There is *limited evidence* in experimental animals for the carcinogenicity of trichloroacetic acid.

Overall evaluation

Trichloroacetic acid is not classifiable as to its carcinogenicity to humans (Group 3).

For definition of the italicized terms and definition of Groups, see Preamble Evaluation.

Previous evaluation: Vol. 63 (1995)

Last updated: 29 September 2004.

Annex **F**

Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[39]