Health Council of the Netherlands

Phenacetin

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Phenacetin*
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Geachte staatssecretaris,

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

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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Evaluation of the carcinogenicity and genotoxicity

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. 2012/21, 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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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep 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 rapport neemt de Commissie fenacetine onder de loep. Fenacetine werd vanaf 1887 tot ongeveer 1980 gebruikt als pijnstiller. Omdat er steeds meer aanwijzingen kwamen dat chronisch gebruik van fenacetine vormen van nierproblemen kan veroorzaken, is de stof niet meer als geneesmiddel geregistreerd. Fenacetine wordt vaak versneden aangetroffen in illegaal verkrijgbare cocaïne.

Op basis van de beschikbare gegevens leidt de commissie af dat fenacetine kankerverwekkend is voor de mens. Zij beveelt aan om de stof te classificeren in categorie 1A.* De commissie concludeert verder dat de stof een stochastisch genotoxisch werkingsmechanisme heeft.

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

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 evaluated phenacetin. Phenacetin was after the introduction in 1887 up to the early 1980s used as an analgesic drug. Because chronic use of phenacetin is suspected to cause renal problems the registration of the drug has been discontinued. Phenacetin is being used as a cutting agent to adulterate illegally supplied cocaïne.

Based on the available information, the Committee is of the opinion that phenacetin is carcinogenic to humans and recommends to classify the substance in category 1A.* The Committee is furthermore of the opinion that phenacetin acts by a stochastic genotoxic mechanism.

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

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 I).

This report contains the evaluation of the carcinogenicity of phenacetin

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 Standards 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. No comments were received on the draft document.

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. The evaluation of the carcinogenicity of phenacetin has been based on IARC evaluations (IARC volume 13 (1977), IARC volume 24 (1980), IARC supplement 7 (1987) and IARC volume 100A (2011))[1-4] (in Annex E a summary is given of the IARC data) and additional scientific data, which are publicly available. Additional data were obtained from the online databases Toxline, Medline and Chemical Abstracts covering the period 1978 to September 2012 using phenacetin and CAS no 62-44-2 as key words in combination with key words representative for carcinogenesis and mutagenesis. The new relevant data were included in this report.

Chapter 2

General information

2.1 Identity and physicochemical properties

Chemical name	:	N-(4-ethoxyphenyl)acetamide[1]
CAS registry number	:	62-44-2
EINECS-number	:	200-533-0[5]
EEC-number	:	
RTECS-number	:	
Synonyms	:	N-(4-ethoxyphenyl), acetyl-phenetidine, 1-acetamido-4-ethoxybenzene
Appearance	:	odorless, white, glistening crystals, usally scales or as fine white, crystalline powder[6]
Occurrence	:	
Use	:	analgesic and antipyretic drug in human and veterinary medicine.[2]; registration in the Netherlands was discontinued in 1984 because of serious side effects on the kidney; illegal use as adulterant in cocaine powder
Molecular formula	:	C_{10}-H_{13}-N-O_2[6]
Structural formula	:	
Molecular weight	:	179.22[6]
Boiling point	:	242-245°C[6]
Melting point	:	134-135°C[6]
Vapour pressure	:	-
Vapour density (air = 1)	:	-
Solubility	:	Slightly soluble in water (1 in 1,300)[2]
Stability and reactivity	:	Unstable to oxidizing agents, iodine and nitrating agents[2]
EU Classification	:	Not classified in Annex I of Directive 67/548/EEC

2.2 IARC classification

In 2011, IARC concluded :

There is *sufficient evidence* in humans for the carcinogenicity of analgesic mixtures containing phenacetin. Analgesic mixtures containing phenacetin cause cancer of the renal pelvis, and of the ureter.

There is *limited evidence* in experimental animals for the carcinogenicity of analgesic mixtures containing phenacetin.

There is *sufficient evidence* in humans for the carcinogenicity of phenacetin. Phenacetin causes cancer of the renal pelvis, and of the ureter.

There is *sufficient evidence* in experimental animals for the carcinogenicity of phenacetin.

Analgesic mixtures containing phenacetin are *carcinogenic to humans (Group 1)*. Phenacetin is *carcinogenic to humans (Group 1)*.

Chapter 3

Carcinogenicity

3.1 Observations in humans

Many case report studies showed the existence of renal pelvic and other urothelial tumours in patients who have used large amounts of phenacetin-containing analgesics.[7-16, 17-22]

A vast amount of case-control studies[23-28, 29-44] have been published. These studies show that phenacetin-containing analgesics are part of the etiology of renal pelvic, urothelial and bladder cancer. Most of the exposed individuals in these case-control studies are exposed to phenacetin-containing analgesics, which makes it difficult to investigate the effect of exposure to phenacetin only. Most of the studies were published 15-20 years ago, due to the fact that phenacetin-containing products had been off the market in most countries for decades now. Recent studies were not published because the lack of long-time phenacetin users. The case-control studies have been summarized in the following paragraphs and in Annex F.

Renal pelvis cancer

McCredie et al. (1986) conducted a hospital based case-control study in New South Wales, Australia to investigate the risk factors for renal cancer. Sixty six cases of renal pelvis cancer, 86 cases of renal parenchyma cancer and 751 controls were collected between 1970 and 1982 in Sidney, Australia. Information

on consumption of phenacetin-containing analgesics was obtained through completion of a structured questionnaire at interview. Pathologists classified the tumours according to their histological appearances and sought evidence of 'intermediate' or 'advanced' renal papillary necrosis (RPN). Cases were excluded if the presence or absence of RPN could not be established. RPN and regular consumption of phenacetin both increased the risk for renal pelvis cancer. The risk of renal pelvis cancer increased nearly 4 times for regular consumers of phenacetin without RPN (RR: 3.6, 95% CI: 1.6-8.1) and 20 times for regular consumers of phenacetin with RPN (RR: 20, 95% CI: 12-34), compared to non-consumers without RPN.[36]

McCredie et al. (1988) also conducted a population-based case control study in New South Wales, Australia to investigate the risk of developing renal cancer papillary necrosis and cancer of the renal pelvis, ureter or bladder associated with consumption of either phenacetin or paracetamol. Data were acquired from 381 cases (identified between 1978 and 1982) and 808 controls. The risk of cancer of the renal pelvis was statistically significantly increased nearly 6 and 8-fold with a lifetime consumption of respectively, $\geq$ 0.1 kg (OR: 5.7, 95% CI: 3.2-10.0) and > 1 kg (OR: 7.9, 95% CI: 4.6-13.8) phenacetin.[37]

In another population-based case control study in New South Wales, Australia, McCredie et al. (1993) investigated the consumption of phenacetin and paracetamol and the risk of cancer of the kidney and renal pelvis, using data of 489 cases of renal-cell cancer and 147 cases of renal pelvic cancer diagnosed in 1989 and 1990, together with 523 controls from the electoral rolls. A dose-related increase in the risk of cancer of the renal pelvis was observed in consumers of phenacetin/aspirin compounds. When used according to the definition of "taken at least 20 times in lifetime" phenacetin/aspirin compounds increased the risk of renal pelvic cancer more than a 12-fold (RR: 12.2, 95% CI: 6.8-22.2).[39]

McLaughlin et al. (1985) conducted a population-based case-control study of renal cancer (495 cases of renal cell cancer, 74 cases of renal pelvis cancer and 697 controls) in Minneapolis, USA. Patients were collected in the period 1974-1979. Patients and the control group were interviewed in 1980 about the use of analgesic drugs. Information of different variables was obtained, including the use of analgesic drugs (phenacetin-containing, acetaminophen-containing and aspirin). A drug was considered phenacetin-containing if phenacetin was included in the formulation from 1955 to 1974. Exposures after 1973 were excluded for analysis. The groups were divided in male/female and in never, ever, irregular and regular (subdivided in $\leq$ 36 months and > 36 months) users. Long-term regular use of phenacetin-containing drugs was associated with an

increase in risk for renal pelvic cancer among males (OR: 8.1, 95% CI: 1.2-62), but not among females (4.2, 95% CI: 0.4-42).[41]

Pommer et al. (1999) conducted a case-control study in the area of the former West Berlin, including 647 new diagnosed cases of urethelial cancer (571 bladder, 25 ureter and 51 renal pelvis cancer cases) from eight hospitals of the study area between 1990 and 1995 and 647 population-based controls. Intake of more than 1 kg phenacetin in analgesic mixtures was associated with an increased risk (not significantly) of renal pelvic cancer (OR of 5.3, 95% CI: 0.3-81).[43]

Ureter cancer and/or renal pelvis cancer

Several of the case-control studies (including two studies which are already described above by McCredie et al.,1988, Pommer et al., 1999[37,43]) also analysed the risk of phenacetin-containing analgesics consumption for the development of ureter cancer (alone or together with renal pelvic cancer). In the population-based case-control study in New South Wales, Australia by McCredie et al. (1988)[37] no association was found between ureter cancer and a lifetime consumption of $\geq$ 0.1 kg (OR: 0.7, 95% CI: 0.3-2.2) or $\geq$ 1 kg phenacetin (OR: 1.2, 95% CI: 0.5-3.0).

In the case-control study in the area of the former West Berlin by Pommer et al. (1999)[43] no association was found between the number of renal pelvis cancer and ureter cancer combined and a lifetime intake of more than 1 kg phenacetin in analgesic mixtures (OR of 1.8, 95% CI: 0.2-13).

Jensen et al. (1989)[33] conducted a case-control study (96 cases and 294 controls, identified between 1979 and 1982) in Denmark to investigate the risk of analgesic intake (phenacetin and/or aspirin) and cancer of the renal pelvis and ureter. Seventy nine percent of the tumours were located in the renal pelvis (including calyces). There was an indication of a dose-effect relationship for phenacetin-containing analgesics and cancer of the renal pelvis and ureter. A statistically significant increase in relative risk (RR) was seen for female users of phenacetin-containing analgesics (RR: 4.2, 95% CI: 1.5-12.3), but not for male users (RR: 2.4, 95% CI: 0.9-6.8).[33]

Linet et al. (1995) investigated 502 cases (308 renal pelvis cancer and 194 ureter cancer, identified between 1983 and 1986) and 496 controls in a population-based case-control study in New Jersey, Iowa and Los Angeles, USA. Neither cumulative lifetime ingestion nor duration of regular use of phenacetin, whether alone or in combination with acetaminophen or aspirin, was associated with significantly increased risk of renal pelvis and ureter cancer. Although this

study contained a large amount of cases, it only contained small number of regular analgesic users.[35]

Renal cell cancer

Three case-control studies on renal pelvis cancer, which are already described above, also analysed the risk of phenacetin-containing analgesics consumption for the development of renal cell cancer.[36,41.]

In the population-based case-control study in Minneapolis, US of McLaughlin et al. (1985)[41] (described above), long-term regular use of phenacetin-containing drugs was associated with a statistically significant increase in risk for renal cell cancer in women (OR: 1.7, 95% CI: 1.1-2.7 for ever-users and OR: 1.7, 95% CI: 1.1-2.6 for irregular-users compared to never users).

In another population-based case-control study by McLaughlin et al.(1992)[42] in Shanghai, China (154 cases and 157 controls) regular use of phenacetin-containing analgesics (at least 2 times a week for a period of at least 2 weeks) was not associated with renal cell cancer (OR: 2.3, 95% CI: 0.7-7.0).

In the hospital based case-control study in New South Wales, Australia of McCredie et al. (1986)[36] (described above), regular use of phenacetin-containing analgesics increased the risk of cancer of the renal parenchyma (RR: 2.5, 95% CI: 1.3-4.9.), but was not increased by the presence of renal papillary necrosis (RPN). Thus, unlike renal pelvis cancer, the relationship between consumption of phenacetin-containing analgesics and renal parenchyma appears to be a direct one without any intervening effect of RPN.

In the population-based case-control study in New South Wales, Australia by McCredie et al. (1993) (described above), no association was found between the number of renal-cell cancers and consumption of phenacetin/aspirin compounds (RR: 1.4, 95% CI: 0.9-2.3).[39]

In another study McCredie et al. (1995)[40] pooled data from 1,313 cases and 1724 controls from Australia, Denmark, Germany, Sweden and the US, identified between 1989 and 1991. The role of phenacetin-containing and other types of analgesics in the development of renal-cell cancer was studied. Relative risks, adjusted for the effects of age, sex, body-mass index, tobacco smoking and study centre, were not statistically significantly increased with a lifetime consumption of $\geq$ 0.1 kg phenacetin (or when subjects were subdivided further by amount). According to the authors, these findings do not support the hypothesis that analgesics containing phenacetin increase the risk, although the

number of ‘regular’ users and the amount of analgesics consumed were too small to confidently rule out a minor carcinogenic effect of phenacetin.

Kreiger et al. (1993) performed a population-based case-control study in Ontario, Canada of risk factors for renal cell carcinoma. Data were collected on 518 case and 1,381 controls identified between 1986 and 1987. In this large study different risk factors for renal cell carcinoma were observed. No association was found between phenacetin-only use (5 cases, 9 controls) and the risk of renal cell carcinoma (OR: 2.5, 95% CI: 0.3-18.5 for males and OR: 1.8, 95% CI: 0.5-7.3 for females) or between acetaminophen-only use and the risk of renal cell carcinoma (OR: 0.8, 95% CI: 0.3-1.7 for males and OR: 0.9, 95% CI: 0.5-2.0 for females), although few subjects used either compound.[34]

Gago et al. (1999) conducted a population-based case-control study in Los Angeles, US (1,204 cases and equal number controls) to investigate the relationship between sustained use of analgesics and the risk of renal cell carcinoma. Regular use of analgesics (2 or more times a week for 1 months or longer) was a significant risk factor for renal cell carcinoma for all four major classes of analgesics (aspirin, non-steroidal anti-inflammatory agents other than aspirin, acetaminophen and phenacetin). Regular use of phenacetin containing analgesics was associated with an OR of 1.9 (95% CI: 1.3-2.7). A dose-related increase in risk of renal cell carcinoma was observed after further subdivision into different amounts of the maximum weekly dose.[32]

Bladder cancer

Several epidemiological studies [23,25,27,29-31,43] have examined phenacetin and bladder cancer. Two of the case-control studies on renal pelvis and ureter cancer which are already described above, also analysed the risk of phenacetin-containing analgesics consumption for the development of bladder cancer (McCredie et al., 1988; Pommer et al., 1999).[37,43]

In the population-based case-control study in New South Wales, Australia by McCredie et al. (1988)[37](described above), risk for cancer of the bladder was doubled by the consumption of phenacetin (OR: 2.0, 95% CI: 1.1-3.5 for subjects with a lifetime consumption of $\geq$ 1 kg phenacetin and OR: 2.1, 95% CI: 1.3-3.5 for subjects with a lifetime consumption of $\geq$ 0.1 kg phenacetin).

In the case-control study in Berlin, Germany by Pommer et al. (1999)[43] (described above), no association was observed between a lifetime intake of more than 1 kg phenacetin in analgesic mixtures and bladder cancer (OR: 0.75, 95% CI: 0.39-1.43).

In a population-based case-control study conducted in Los Angeles, California, US by Castelao et al. (2000), 1,514 cases of bladder cancer and an equal number of controls, identified between 1987-1996 were investigated. Regular use of analgesics was not associated with an increased risk of bladder cancer in either man or women. The intake of phenacetin-containing analgesics was positively related to bladder cancer risk in a dose-dependent manner, while the intake of its major metabolite in humans, acetaminophen, was unrelated to risk. Regular use of phenacetin-containing analgesics was not associated with an increased risk of bladder cancer (OR: 1.5, 95% CI: 0.85-2.73).[29]

In a hospital based case-control study conducted in Spain by Fortuny et al. (2006), the use of non-aspirin non-steroidal anti-inflammatory drugs (NSAID), aspirin, paracetamol (acetaminophen), phenacetin, and metamizol (dipyrone) and risk of bladder cancers was assessed. Data on 958 cases and 1,029 controls, identified between 1997 and 2000 was analysed. A significant reduction in bladder cancer risk was observed for regular users of non-aspirin NSAIDs compared with never users. No evidence of an overall effect for regular use paracetamol or aspirin was observed. Regular use of phenacetin was not associated with an increased risk of bladder cancer (OR: 1.3, 95% CI: 0.3-4.5). However, this estimate was based on only 7 cases and 12 controls.[30]

In a population-based case-control study conducted in New Hampshire, UK by Fortuny et al. (2007), the influence of phenacetin, other analgesics and NSAID use on the risk of bladder cancer was investigated. Data from 376 cases and 463 controls, identified between 1998 and 2001 was analysed. Elevated OR's were associated with reported use of phenacetin-containing medications (OR: 2.2, 95% CI: 1.3-3.8 for ever compared to never users), especially with longer duration of use (OR: 3.0, 95% CI: 1.4-6.5 for > 8 years of use).[31]

3.2 Carcinogenicity studies in animals

A group of 30 BD I and BD III rats (age, 100 d) received phenacetin (40-50 mg) daily in the diet (average total, dose 22g). One rat died after a total dose of 10 g and was found to have an osteochondroma. The mean age of death of the treated animals was 770 days, the control animals 750 days. No tumours related to treatment were observed.[45]

Four groups of 15, 20, 20, and 24 male albino rats were fed with diets containing 0, 0.05, 0.1 or 0.5 % N-hydroxyphenacetin (metabolite of phenacetin) during 73 weeks. Assuming a body weight of 400 grams and a daily food intake of 20 grams, the exposure of N-hydroxyphenacetin was 25, 50, and 250 mg/kg bw/day respectively. Of treated animals 11, 13 and 15 rats were still alive at the

time of appearance of the first tumour after 45, 45 and 38 weeks. Of these animals 8/11, 13/13 and 15/15 developed liver tumours (described as hepatocellular carcinomas). None of the control group animals developed tumours. One of the animals fed with 0.1% diet developed a transitional cell carcinoma of the renal pelvis.[46]

Female SD rats were given 0 or 0.535% phenacetin in the diet for 86 or 110 weeks. Assuming a body weight of 400 grams and a daily food intake of 20 grams the exposure of phenacetin was 268 mg/kg bw/day. In the 86-week study, epithelial hyperplasia of renal papillae was found in 2/24 controls and 21/38 treated animals. In the 110 week study the following changes were observed: Urothelial hyperplasia of the renal papillae in 26 animals, dilatation of vasa recta in 28, and epithelial hyperplasia in 1 animal. In addition, carcinomas of the mammary gland (5/30) and ear duct (4/30; $P>0.05$) were found in the treated group. In the control group, uroepithelial hyperplasia was found in 5 animals, dilatation of vasa recta in 8 and mammary carcinoma in 1 animal.[47]

Two groups of SD rats (50 male, 50 female, age 9 wks) were fed a diet containing 1.25 or 2.5% phenacetin for 18 months, followed by a basal diet for 6 months. Assuming a body weight of 400 grams and a daily food intake of 20 grams the exposure of phenacetin was 625 and 1,250 mg/kg bw/day respectively. The control group (65 male and 65 female) were fed with the same basal diet. Among animals surviving for 24 months or dying within 24 months with tumour(s), neoplasms were detected in 27/27 males and 21/27 females fed 2.5%, in 20/22 males and 19/25 females fed 1.25% and in 1/19 males and 6/25 females in the control group. Tumours (benign and malignant) of the nasal cavity were found in 16/27 males and 7/27 females fed 2.5% and in 16/22 males and 6/25 females fed 1.25%. Malignant tumours of the urinary tract were detected in 13/27 males and 4/27 females fed with the high dose and in 1/22 males and 0/25 females fed with the low dose; 2 papillomas were found in females given the high dose. No nasal cavity or urinary tract tumours were seen in controls.[48]

Two groups of B6C3F1 mice (52 male and female, age 6 weeks) were fed for 96 weeks a diet containing 1.25 or 0.6% phenacetin followed by a basal diet for 8 weeks. Assuming a body weight of 20 grams and a daily food intake of 3 grams the exposure of phenacetin was 1,875 and 900 mg/kg bw/day respectively. The control group of animals (50 mice of each sex) was fed the same basal diet for 104 weeks. All animals were killed at the end of the experiment. The organs were examined histopathologically. Mice that died during the experiment were also autopsied.

Phenacetin at a dose of 0.6% induced a significant increased incidence of renal cell adenoma in male mice only. A dose of 1.25% was induced a significant

increase in both renal cell adenoma and carcinoma in male mice. A clear dose-response relationship was seen between the doses of phenacetin and the induction of renal cell carcinoma. A statistically significant increased incidence of tumours was found in the liver, lung, skin, hematopoietic system (leukaemia or lymphoma) and occasionally in some other organs.[49]

Four groups of twenty rats (male Sprague-Dawley, age 6 weeks) were given phenacetin (0, 0.5, 1.0 or 1.5 %) in the diet for 6 or 12 weeks. The 0.5, 1.0 and 1.5 % groups had a real phenacetin intake of 0.78, 1.28 and 1.77 g/kg bw (at week 2 of the experiment) and this intake decreased to 0.31, 0.65 and 1.18 g/kg bw (at week 12).Ten rats of each group were killed at 6 and 12 weeks. One hour before killing a single i.p injection of labelled thymidine was given. To determine to which extent the labelled thymidine was incorporated in the DNA of various tissues, the labelling index was measured. A high labelling index indicates a high cell proliferation. There was a dose-related increase in the labelling index in the urothelium of the bladder and kidney (especially after 6 weeks and 1.0% and 1.5% dose). After 6 weeks the labelling indices were increased in the bladder. After 12 weeks the labelling indices in the bladder were only increased numerically but not statistically significant. In the renal pelvic the labelling index was significantly increased at doses of 1.0 and 1.5 %. At week 12 the majority of rats treated with 1.5% had labelling indices $\geq$ 2-fold than the control both in kidney and bladder. The increased labelling indices were associated with urothelial hyperplasia (in particular after 6 weeks).[50]

Twenty male Crl:CDBR rats were treated by gavage with phenacetin during 7 or 14 days. The rats were divided in 4 groups: a control, a low-dose (100 mg/kg bw/day), an intermediate (625 mg/kg bw/day) and a high-dose group (1,250 mg/kg bw/day). One week of phenacetin treatment resulted in dose-related increases in DNA synthesis in both respiratory and olfactory mucosa. The increase observed in the respiratory mucosa was due to inflammatory cells in the lamina propria and not to proliferation of the respiratory epithelial cells. One or two weeks of daily phenacetin treatment resulted in degenerative changes in the olfactory epithelium and necrosis of Bowman's glands. These changes were associated with increases in cell proliferation in the olfactory epithelium only. Two-week daily gavage treatment of rats with phenacetin at 100, 625 and 1,250 mg/kg/day increased olfactory epithelial cell replication by 62.1, 174 and 763%, respectively.[51]

Phenacetin was mixed in the feed at a concentration of 0.7 or 1.4% and administered to transgenic CB6F1-rasH2 mice and non-transgenic, wildtype (non-Tg, WT) mice during 24 weeks. Assuming a body weight of 20 grams and a daily food intake of 3 grams the exposure to phenacetin was 1,050 and 2,100

mg/kg bw/day respectively. Phenacetin induced spleen haemangiosarcoma and lung adenomas in the rasH2mice but not in the non-Tg mice. Lung adenomas (12 in exposed versus 2 in control) and spleen hemangiosarcomas (6/0) were found in male rasH2 treated with 1.4% phenacetin in the feed. This incidence was significant higher than in the corresponding non-Tg mice.[52]

P53$^{+/-}$ transgenic mice were given phenacetin by daily gavage with dose of 100, 200 and 350 mg/kg bw/day suspended in 0.5% methylcellulose during 26 weeks. In a separate study the mice were given a dose of 0.14, 0.7 and 1.4% phenacetin in the diet. Control and high-dose groups of wild-type mice were included in both studies. No increase in treatment-related tumour incidence was found after 26 week of treatment.[53]

The transgenic Tg.AC mice strain is able to respond to dermal application with development of squamous-cell papillomas of the skin. Phenacetin was administered topically (0, 0.08, 0.4 and 2 mg, daily) and in the diet (0, 12, 60, 300 ppm) during 26 weeks. Phenacetin was negative by both routes of exposure.[54]

Phenacetin was administered in the feed (0, 0.1, 0.25, 0.5, or 0.75% w/w) to transgenic Xpa$^{-/-}$ mice (15 male, 15 female), to double transgenic Xpa$^{-/-}$/p53$^{+/-}$ mice (15 male, 15 female) and to wild type (WT) C57BL/6 mice (15 male, 15 female). Assuming a body weight of 20 grams and a daily food intake of 3 grams the exposure of phenacetin was 150, 375, 750, 1,125 mg/kg bw/day respectively. The exposure to phenacetin was 39 weeks for all groups. At the end of the experiment renal proximal tubular hyperplasia was observed in two high-dose Xpa$^{-/-}$ males and in one Xpa$^{-/-}$/p53$^{+/-}$ male mouse. A tubular adenoma was found in a Xpa$^{-/-}$/p53$^{+/-}$ female mouse. In all male and female transgenic, but not the WT mice, multifocal karyomegaly in the proximal renal tubules was found. In addition, olfactory epithelial degeneration was observed in the nose of most male and female transgenic and WT mice of the high-dose groups.[55]

Phenacetin had the ability to induce morphological transformation in cultured

C3H/10T$^{1/2}$ clone 8 mouse embryo cells (10T$^{1/2}$ cells). Treatment of the 10T$^{1/2}$ cells with 0.5, 1.0, and 2.0 mg/ml phenacetin caused a dose-dependent decrease in plating efficiency and a dose-dependent increase in type II morphologically transformed foci.[56]

Phenacetin tested in the Syrian hamster embryo transformation assay gave negative results. The highest concentration phenacetin tested was 500 µg/ml phenacetin. Phenacetin above a concentration level of 500 µg/ml was insoluble in the medium with DMSO.[57]

In an initiation-promotion experiment male F344 rats (6 weeks of age) were divided in two groups of 20 and one of 10 rats. The two groups of 20 rats were pretreated with 0.1% DHPN in drinking water and 3.0% uracil in the diet during 4 weeks. DHPN (dihydroxy-di-N-propylnitrosamine) is a carcinogen which is known to induce tumours of the renal pelvis, renal tubular cells and urinary bladder in rats. One week after cessation, one group received basal diet and one group received a diet containing 2.0% phenacetin (average intake 1,145 mg/kg/day) during the following 35 weeks. The group of 10 animals was given, during the same period, a diet with 2.0% phenacetin (average intake 1,068 mg/kg/day) without the initial combination treatment of DHPN and uracil. The occurrence of renal cell tumours was increased in the group given phenacetin (9/20) as compared with the DHPN + uracil alone control (1/19). In the urinary bladder, phenacetin treatment was associated with increased incidence of preneoplastic or neoplastic lesions. The group of animals, treated with phenacetin alone, without the pretreatment, induced simple hyperplasias of the urinary bladder at high incidence.[58]

Chapter 4

Mode of action

4.1 Genotoxic mode of action

More details of these studies have been summarized in Annex H.

4.1.1 Gene mutation assays

In vitro

Phenacetin was not mutagenic in several bacterial models in the presence or absence of rat or mouse liver microsome preparations: the models included a repair test in *Bacillus subtillus*[59] and reverse mutation test in *Salmonella typhimurium* TA1535, TA 1537, TA98 and TA 100[60,61], *Escherichia coli* K 12/343/13[61], and *B. subtilis* TKJ 5211.[59] Positive bacterial mutagenic results have been obtained in *S. typhimurium* TA 100 in the presence of hamster, but not rat, liver post-mitochondrial supernatant of Aroclor-treated animals.[62-64] Phenacetin led to an increase in the mutant frequency in *Salmonella typhimurium* TA 100 in the presence of a hamster liver metabolic activation.[65,66]

In the *hprt* test phenacetin induced an increase in the mutant frequency in V79 Chinese hamster cells in vitro in the presence of hamster liver microsome preparations.[65,67.]

In vivo

Phenacetin was negative in an intrasanguineous host-mediated assay with *E.coli* K 12 in NMRI mice given 2 mmol/kg intraperitoneally. Phenacetin did not induce an increased frequency of sex-linked recessive lethals in *Drosophila melanogaster.*

Phenacetin was given in the feed of DNA repair deficient ($Xpa^{-/-}$ and $Xpa^{-/-}/Trp53^{+/-}$) mice and wild type (WT) carrying the *IacZ* (0.75% w/w, during 0, 4, 8, or 12 weeks). $Xpa^{-/-}$ mice lack the normal nucleotide excision repair pathway. Due to this deficiency, these mice are more sensitive to genotoxic compounds than wild type mice. Phenacetin exposure induced an increase in the *lac*Z mutant frequency in the kidney of WT, $Xpa^{-/-}$ and $Xpa^{-/-}/Trp53^{+/-}$ mice as compared with concurrent untreated controls of the wild type C57BL/6 mice. The increase in $Xpa^{-/-}$ and $Xpa^{-/-}/Trp53^{+/-}$ mice was stronger than in WT mice. A minor and negative response was found in the liver and the spleen, respectively. The observed phenacetin-induced mutant frequency was higher in male than in female mice.[68]

4.1.2 *Cytogenetic assays*

In vitro

Phenacetin induced DNA fragmentations in an acellulair test-system with λ DNA but not with calf thymus DNA.[69]

In vivo

No data were available on the genetic and related effects of phenacetin in humans.

The results of studies on the induction of chromosomal aberrations, sister chromatid exchanges and micronuclei in rodents treated with phenacetin in vivo were equivocal.[61,70] Phenacetin exposure did not result in an enhanced number of micronucleated erythrocytes in the bone marrow of NMRI mice given 2 x 5 mmol/kg bw intraperitoneally.[61]

Following in vivo treatment, the alkaline elution assay showed no increase of DNA damage in bone-marrow cells of i.p-treated mice or in liver cells of rats treated by gavage. However, an increase of DNA damage was observed in liver of rats after i.p. administration of phenacetin and in kidney of rats receiving

phenacetin by gavage.[65] Sister chromatid exchanges were seen in mice (i.p, 330 mg/kg bw) treated with phenacetin. This increase of SCE was weak but statistically significant.[65]

The micronucleus bone marrow test showed a positive response in mice given phenacetin i.p. Phenacetin doses of 37.5, 75, 150, 300, 400 and 600 mg/kg bw/day were administered only once or multiple times (2-4) to CD-1 mice. Positive responses were seen at 600 mg/kg/day after single and triple dosing and at 400 and 600 mg/kg/day after double dosing.[71,72] A single dose of phenacetin of 0, 2, 5, 50 and 100 mg/kg given i.p to SJL Swiss mice resulted in a moderate but significant increase of cells with micronuclei compared with the control group.[73]

The micronucleus assay with peripheral reticulocytes from phenacetin-treated mice (CD-1 and MS/Ae strain) was negative after a single dose of 400, 600 and 800 mg/kg bw(24 h after i.p). Positive results were obtained with 600 and 800 mg/kg bw after 48 h. Double treatment (24 h between treatments) enhanced the responses. A dose response was obtained for all different sample times. In this same experiment CD-1 mice treated with phenacetin (i.p, 600 mg/kg bw, single and double treatment) gave a positive result in the micronucleus test in bone marrow cells.[74]

Phenacetin was administered to rats (Sprague-Dawley) with doses of 500, 1,000 and 2,000 mg/kg bw/day during 2 days or 250, 500, 750, 1,000 mg/kg bw/day during 14 days. Blood samples were taken on day 1, 3, 6, 9, 12 and 15 for the micronucleus assay with peripheral reticulocytes. In the 14-day test, phenacetin increased the frequency of micronucleated reticulocytes in peripheral blood at 500 mg/kg bw/day starting from day 9, and at 750 and 1,500 mg/kg bw/day starting from day 6. In the test with 2 days application the frequencies of micronucleated reticulocytes increased at 1,000 and 2,000 mg/kg bw/day. In the test with 14 days application the micronucleus assay in the bone marrow showed a positive dose-related response.[75]

4.1.3 Miscellaneous

In vitro

Hepatocytes isolated from mouse, hamster, rat and guinea pig showed no marked increase in unscheduled DNA synthesis (UDS) after exposure to phenacetin.[76] After treatment with phenacetin, mouse L-cells gave positive results using a DNA-synthesis inhibition test system.[77] An increase in DNA damage measured by the alkaline elution assay was not observed when human and rat hepatocytes were treated with phenacetin in vitro.[78]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

The Committee is aware that in most of the epidemiological studies described above the effect of phenacetin may be influenced by other analgetic comedications, by selection bias, especially in the hospital-based case-control studies, and recall bias. However, the Committee is also of the opinion that the epidemiological evidence cannot exclude that phenacetin-containing analgetics are part of the etiology of renal pelvic, urothelial and bladder cancer. However, the evidence is considered sufficient by the Committee. For bladder cancer the evidence does not support such a relationship. Based on the available information the Committee concludes that there is sufficient evidence for carcinogenicity of phenacetin to humans.

Phenacetin induced tumours of the urinary tract (in mice and rats) and nasal cavity (in rat) when given orally. New published data consisted of 9 not standard carcinogenicity studies, which support this conclusion. Three of these studies with rats gave insight in the mechanism of the damage induced by phenacetin. They gave evidence of DNA damage in the bladder or nasal mucosa. Four other studies used transgenic mice. In two of these studies, the transgenic mice showed increased lung, spleen and kidney tumours compared to wild type mouse. The two other studies are transformation tests with mouse-embryo and hamster embryo cells, of which only the study in mouse-embryo showed increased transformation. Considering the available animal data, the Committee concludes

that there is sufficient evidence for carcinogenicity of phenacetin to animals. In addition, the Committee is aware that both animal data and the human data show a relationship beween phenacetin and cancer of the kidney. This relationship was even more supported by the observation that phenacetin increased the *lac*Z mutant frequency in kidney of transgenic mice. Such an analogy in cancer development in man and animal on the level of a specific organ supports the role of phenacetin as a carcinogen.

Phenacetin was negative in almost all in vitro bacterial mutagenicity tests. On the other hand, DNA damage was observed in mammalian cells in vitro and in vivo. Phenacetin induced inhibition of DNA synthesis and an increase in the mutant frequency in a gene mutation assay with mammalian cells when hamster but not rat S9 mix was used as metabolic activation. The positive findings in vitro were confirmed in in vivo genotoxicity tests. Phenacetin was positive in several micronucleus tests as well as in a gene mutation test with transgenic animals; in several studies a clear dose-response relationship was observed.

Therefore, it can be concluded that phenacetin is a stochastic genotoxic compound.

5.2 Recommendation for classification

The Committee concludes that phenacetin is carcinogenic to humans and recommends classifying the substance in category 1A.*

Moreover, the Committee concludes that phenacetin has a stochastic genotoxic working mechanism.

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

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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	Human data
G	Animal data
H	Genotoxicity data
I	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 *Phenacetin*
Our reference	: U-7412/BvdV/fs/246-C17
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 *Phenacetin*.

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. No comments were received on the draft document.

Annex **E**

IARC Monograph

Volume 100A, 2011 (excerpt from Phenacetin, pp397-400)

Phenacetin was considered by previous IARC Working Groups in 1976 and 1980. Analgesic mixtures containing phenacetin were considered by a previous IARC Working Group in 1987. Since that time, new data have become available, these have been incorporated in the Monograph, and taken into consideration in the present evaluation.

5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of analgesic mixtures containing phenacetin. Analgesic mixtures containing phenacetin cause cancer of the renal pelvis, and of the ureter.

There is *limited evidence* in experimental animals for the carcinogenicity of analgesic mixtures containing phenacetin.

There is *sufficient evidence* in humans for the carcinogenicity of phenacetin. Phenacetin causes cancer of the renal pelvis, and of the ureter.

There is *sufficient evidence* in experimental animals for the carcinogenicity of phenacetin.

Analgesic mixtures containing phenacetin are *carcinogenic to humans (Group 1)*.

Phenacetin is *carcinogenic to humans (Group 1)*.

For the overall evaluation of phenacetin, the Working Group took into consideration that tumours of the renal pelvis and ureter are not known to result from the other components of the analgesic mixtures used in most countries; namely, aspirin, codeine phosphate, and caffeine.

Annex **F**

Human data

Human case-control studies of phenacetin exposure and different forms of cancer (published after the IARC publication of 1987).

reference	design/population	results				confounding factors	remarks
		exposure phenacetin containing drugs		cases / control[a]	risk ratio(95% CI)		
renal pelvic cancer							
McLaughlin et al., 1985[41]	population-based case-control, Minneapolis, US	never	m	24/232	OR 1	adjusted for age and cigarette smoking.	the separate effects of the analgesics could not be adequately assessed because most long-term users took both phenacetin and acetaminophen-containing products
			f	12/147	OR 1		
		ever	m	26/196	OR 1.2 (0.6-2.4)		
			f	9/100	OR 1.3 (0.5-3.4)		
	(74 cases and 697 controls, identified between 1974-1979)	irregular	m	21/175	OR 1.1 (0.6-2.3)		
			f	12/122	OR 1.1 (0.4-3.2)		
		regular ≤36 mo	m	1/17	OR 0.5 (0.02-3.9)		
			f	1/12	OR 1.8 (0.4-22.0)		
		regular>36 mo	m	4/4	OR 8.1 (1.2-62.2)		
			f	2/10	OR 4.2 (0.4-42.0)		
McCredie et al., 1986[36]	hospital-based case-control, Sidney, New South Wales, Australia	no consumption (lifetime exposure < 1kg)		32/672		adjusted for sex	
	(66 cases and 751 controls, identified between 1970-1982)	lifetime exposure ≥ 1 kg with RPN		27/35	RR 20 (12-34)		
		lifetime exposure ≥ 1 kg absence of RPN		7/44	RR 3.6 (1.6-8.1)		

McCredie et al., 1988[37]	population-based case-control, New South Wales, Australia (73 cases and 688 controls, identified between 1980-1982)	≥ 1 kg / lifetime ≥ 0.1 kg / lifetime	33/54 40/636	OR 7.9 (4.6-13.8) OR 5.7 (3.2-10.0)	adjusted for sex and exposure to paracetamol and tobacco	most cases were included in previous studies
McCredie et al., 1993[39]	population-based case-control, New South Wales, Australia (147 cases and 523 controls identified in 1989-1990)	non-consumers ≤ 2.04 kg/ lifetime 2.04-6.87 kg/ lifetime ≥ 6.88 kg/ lifetime consumption of aspirin or phenacetin	76/474 12/16 16/16 42/17	OR 1 OR 5.2 (2.2-12.4) OR 8.3 (3.4-20.5) OR 18.5 (8.7-39.9)	adjusted for age, sex method of interview, cigarette smoking, paracetamol in any form and educational level	
Stewart et al., 1999[44]	"blinded" histopathological review of cases from population- based case-control study, New South Wales, Australia	< 1 kg / lifetime 1.0-4.9 kg / lifetime 5.0-9.9 kg / lifetime ≥ 10.0 kg / lifetime	20/37 6/5 5/4 17/5	RR 1.0 RR 1.9 (0.5-7.3) RR 2.1 (0.5-8.9) RR 5.6 (1.8-18)	adjusted for age and smoking	this study used the same cases as McCredie et al., 1993
Pommer et al., 1999[43]	hospital-based and population-based case-control, (former) West Berlin, Germany (51 cases and 647 controls)	no/rare analgesic intake > 1.0 kg / lifetime	20/19 7/2	OR 1.0 OR 5.3 (0.3-81)	adjusted for socioeconomic status, cigarette smoking and laxative intake	
Jensen et al., 1989[33]	hospital-based case-control, Copenhagen, the island of Sjaelland, Denmark (96 cases and 294 controls, identified between 1979 and 1982)	adjusted never used ever used crude: never used ever used 1-749 g > 750 g dose unknown	m 31/113 f 9/55 m 13/12 f 17/15 m 31/113 f 9/55 m 13/12 f 17/15 m 6/7 f 2/3 m 5/2 f 7/7 m 4/4 f 6/4	RR 1.0 RR 1.0 RR 2.4 (0.9-6.8) RR 4.2 (1.5-12.3) RR 1.0 RR 1.0 RR 3.9 (1.7-9.1) RR 6.9 (2.7-17.7) RR 3.1 (1.0-9.6) RR 6.1 (1.5-25.6) RR 9.1 (2.2-38) RR 6.1 (1.9-20.0) RR 2.4 (0.4-14.5) RR 9.2 (2.5-33)	adjusted for age, sex, tobacco smoking and occupational exposures known to be associated with high risks of these cancers	79% of the tumours were located in the renal pelvis including calyces
Linet et al., 1995[35]	population-based case-control, New Jersey, Iowa and Los Angeles, US (502 cases and 496 controls identified between 1983-1986)	no regular use ≤ 1.0 kg / lifetime > 1.0 kg / lifetime	385/369 21/23 9/12	OR 1.0 OR 0.8 (0.4-1.6) OR 0.3 (0.3-2.1)	adjusted for age, sex, geographic area and cigarette smoking	308 cases with renal pelvis cancer and 194 cases with ureter cancer This study only contained small number of regular analgesic users and no analgesic abusers.

Pommer et al., 1999[43]	hospital-based and population-based case-control, West Berlin, Germany (76 cases and 647 controls)	> 1.0 kg / lifetime	7/3	OR 1.8 (0.2-13)	adjusted for socioeconomic status, cigarette smoking and laxative intake.	51 cases with renal pelvis and 25 cases with ureter cancer
ureter cancer						
McCredie et al., 1988[37]	population-based case-control, New South Wales, Australia (55 cases and 688 controls, identified between 1980-1982)	≥ 1 kg / lifetime ≥ 0.1 kg / lifetime	6/54 49/636	OR 1.2 (0.5-3.0) OR 0.7 (0.3-2.2)	adjusted for sex and exposure to paracetamol and tobacco	
renal cell cancer						
McLaughlin et al., 1985[41]	population-based case-control, Minneapolis, US (495 cases and 697 controls, identified between 1974-1979)	never	m 188/232 f 74/147	OR 1.0 OR 1.0	adjusted for age and cigarette smoking.	
		ever	m 125/196 f 108/122	OR 0.7 (0.5-1.0) OR 1.7 (1.1-2.7)		
		irregular	m 99/175 f 86/100	OR 0.7 (0.5-0.9) OR 1.7 (1.1-2.6)		
		regular ≤36 mo	m 18/17 f 10/12	OR 1.3 (0.6-2.7) OR 1.9 (0.7-5.6)		
		regular>36 mo	m 8/4 f 12/10	OR 2.2 (0.6-8.9) OR 2.4 (0.8-6.7)		
McCredie et al., 1986[36]	hospital-based case-control, Sidney, New South Wales, Australia (86 cases and 751 controls, identified between 1970-1982)	no consumption (lifetime exposure < 1kg)	72/672		adjusted for sex	
		lifetime exposure ≥ 1 kg with RPN	1/35	RR 2.5 (1.3-4.9)		
		lifetime exposure ≥ 1 kg absence of RPN	13/44	RR 0.4 (0.1-2.7)		
McLaughlin et al., 1985[41]	population-based case-control, Shanghai, China (154 cases and 157 controls, identified between 1978-1989)	regular use (at least 2 times/week for 2 weeks or longer)	154/157	OR 2.3 (0.7-7.0)	adjusted for age, sex, education, BMI and cigarette smoking.	
McCredie et al., 1993[39]	population-based case-control, New South Wales, Australia (489 cases and 523 controls identified in 1989-1990)	non-consumers ≤ 2.04 kg/ lifetime 2.04-6.87 kg/ lifetime ≥ 6.88 kg/ lifetime consumption of aspirin or phenacetin	420/474 21/16 24/16 17/17	OR 1 OR 1.4 (0.7-2.9) OR 1.8 (0.9-3.5) OR 1.0 (0.5-2.1)	adjusted for age, sex method of interview, cigarette smoking, paracetamol in any form and obesity	

Kreiger et al., 1993[34]	population-based case-control, Ontario, Canada (490 cases and 1351 controls, identified between 1986-1987)	no phenacetin or acetaminophen	m	265/578	OR 1.0	adjusted for age, active cigarette smoking and combined Quetelet index	this study included only a small amount of phenacetin users
			f	166/580	OR 1.0		
		phenacetin only	m	2/2	OR 2.5 (0.3-18.5)		
			f	3/7	OR 1.8 (0.5-7.3)		
		phenacetin and acetaminophen	m	3/4	OR 1.4 (0.3-6.7)		
			f	0/8	-		
		any phenacetin	m	5/6	OR 1.7 (0.5-5.9)		
			f	3/15	OR 0.8 (0.2-2.7)		
McCredie et al., 1995[40]	case-control, data pooled from studies in Australia, Denmark, Germany, Sweden and US (1313 cases and 1724 controls, identified between 1989-1991)	reference group	m	839/1094	RR 1.0	adjusted for centre, age, sex, BMI, cigarette smoking the RR as not changed by additional adjustment for consumption of paracetamol or other analides	this study only contained a small number of regular analgesics users and the amount of consumed analgesics was also small
			f	474/630	RR 1.0		
		< 0.1 kg	m	14/28	RR 0.6 (0.3-1.2)		
			f	17/22	RR 1.1 (0.6-2.3)		
		≥ 0.1 kg	m	46/67	RR 0.9 (0.6-1.4)		
			f	51/58	RR 1.4 (0.9-2.1)		
		0.1-1.0 kg	m	25/48	RR 0.7 (0.4-1.2)		
			f	26/32	RR 1.3 (0.7-2.3)		
		1.1-5.0 kg	m	16/17	RR 1.3 (0.6-2.7)		
			f	20/14	RR 2.1 (1.0-4.4)		
		> 5 kg	m	5/2	RR 2.6 (0.5-14.2)		
			f	5/12	RR 0.6 (0.2-1.8)		
Gago-Dominguez et al., 1999[32]	population-based case control, Los Angeles, California, US (1204 cases and 1204 controls, identified between 1986-1994)	non/irregular use analgesics		616/744	OR 1.0	adjusted for level of education, BMI, cigarette smoking, hypertension, use amphetamines.	
		regular use		86/55	OR 1.9 (1.3-2.7)		
		max weekly dose <2 g		41/37	OR 1.3 (0.8-2.2)		
		max weekly dose 2-<4 g		22/6	OR 4.1 (1.5-10.8)		
		max weekly dose 4-<8 g		23/12	OR 2.3 (1.0-5.0)		
bladder cancer							
McCredie et al., 1988[37]	population-based case-control, New South Wales, Australia (162 cases and 688 controls, identified between 1980-1982)	≥ 1 kg / lifetime		27/54	OR 2.0 (1.1-3.5)	adjusted for sex and exposure to paracetamol and tobacco	most cases were included in previous studies
		≥ 0.1 kg / lifetime		135/636	OR 2.1 (1.3-3.5)		
Pommer et al., 1999[43]	hospital-based and population-based case-control, (former) West Berlin, Germany (571 cases and 647 controls, identified between 1990-1994)	> 1.0 kg / lifetime		23/23	OR 0.7 (0.4-1.4)	adjusted for socioeconomic status, cigarette smoking and laxative intake.	
Castelao et al., 2000[29]	population-based case-control, Los Angeles, USA (1514 cases and 1514 controls, 1987-1996)	non/irregular use analgesics		961/920	OR 1.0	adjusted for level of education, cigarette smoking, NSAID use, use other analgesics, employment as hairdresser	
		regular use		82/64	OR 1.5 (0.9-2.7)		
		< 46 g / lifetime		25/18	OR 1.4 (0.6-3.1)		
		46-250 g / lifetime		27/20	OR 1.6 (0.7-3.7)		
		>250 g / lifetime		21/20	OR 1.9 (0.8-4.4)		

Fortuny et al., 2006[30]	hospital-based case-control, Spain (958 case and 1029 controls, identified between 1997-2000)	nonusers	848/893	OR 1.0	adjusted for age, sex, region, cigarette smoking, use other NSAID or analgesics
		ever use	59/67	OR 1.1 (0.7-2.0)	
		non regular use (> 20 times lifelong and < 2 times/week for 1 month)	52/55	OR 1.1 (0.6-2.0)	
		regular use (≥ 2 times/week for ≥ 1 month)	7/12	OR 1.3 (0.3-4.5)	
Fortuny et al., 2007[31]	population-based case control, New Hampshire, UK (376 cases and 463 controls, identified between 1998-2001)	never use	313/421	OR 1.0	adjusted for age, sex, region, cigarette smoking, use other NSAID or analgesics
		ever use	53/35	OR 2.2 (1.3-3.8)	
		duration 4 yr	22/14	OR 2.2 (1.0-4.7)	
		duration 4-8 yr	6/9	OR 1.1 (0.4-3.5)	
		duration > 8 yr	25/12	OR 3.0 (1.4-6.5)	

[a] The number of cases and controls do not necessarily add up to the total number of cases and controls of the whole study (as mentioned in the second column), since in many studies also exposure to other (non-phenacetin-containing) analgesics are studied.

Annex **G**

Animal data

animal species, (number, sex, age)	dose, route of exposure	duration	carcinogenic effects	ref.
RAT, BD I & III 30, sex unspecified, 100 d	40-50 mg phenacetin oral (diet) (average total dose, 22 g)	2 yr	no tumours observed	2
RAT, albino, 15-24, male	0.05, 0.1 or 0.5 % N-hydroxyphenacetin oral (diet)	1.5 yr	hepatocellular carcinomas	2
RAT, S-D, female	0.535% phenacetin oral (diet)	1.5-2 y	carcinomas of the mammary gland and ear duct	3
RAT, S-D, 50 male, 50 female, 9 wk	1.25-2.5% phenacetin oral (diet)	1.5 yr	tumours in nasal cavity tumours in the urinary tract papillomas (only in female)	3
MICE, B6C3F1, 52 m+f,	0.6-1.25 % phenacetin oral (diet)	2 yr	renal cell adenoma kidney, liver, lung, skin and hemapotopoietic tumours	1
RAT, S-D, 20, m, 6 wk	0.05, 1.0 and 1.5% oral (diet)	6-12 wk	increased labeling index kidney and bladder	50
RAT, F344, 10-20, m, 6 wk	pretreatment 0.1% DHPN and 3.0% uracil +phenacetin 2.0% oral (diet) (1068-1145 mg/kg/d)	35 wk	renal cell tumours in the pre-treated rats. no tumours in the non pre-treated rats.	58
RAT, Crl:CDBR	100, 625 and 1250 mg/kg, oral (gavage)	7-14 d	increased DNA synthesis in respiratory and olfactory mucosa	51

Annex **H**

Genotoxicity data

In vitro assays.

test	cell line/species	concentration	results		remarks	reference
			- act.	+ act.		
DNA fragmentation	Calf thymus DNA	0.2-2.5 mM	-			Adams et al., 1996[69]
	λ DNA	0.1010 mM	-	NT		
	λ DNA	0.25-2.5 mM	NT	+		
gene mutation test in bacteria reverse mutation test	S.thyphimurium TA97, TA98, TA100 and TA102	sublethal doses <10 mg/plate	-	- + TA100		De Flora et al., 1985[65]
gene mutation test in bacteria reverse mutation test	TA98, TA 100, TA1,535, TA1,537, TA1,538	5,50,500, 1,000, 2,500 and 5,000 µg/plate	-	- + TA100		Oldham et al., 1986[66]
DNA-repair test	E.coli strains: WP2uvrA, WP67, TM1,080, TM1,080	0.3, 1, 3 mg/plate	-	NT		De Flora et al., 1985[65]
DNA synthesis inhibition test	mouse L-cells	1 mM	NT	+ (rat-S9)		Goto et al., 1983[77]
alkaline elution assay	rat hepatocytes	0, 1, 1.8, 3.2 mM	-	NT		Robbiano et al., 1994[78]
	human hepatocytes	0, 1, 1.8, 3.2 mM	-	NT		
unscheduled DNA synthesis (UDS) test	liver-hepatocytes, mouse, rat, guina pig or hamster	0.1, 0.5, 1, 2.5, 5, 10 mM, 18-19 h	-		UDS measured by scintillation counting	Holme et al., 1986[76]

gene mutation test in mammalian cells *Hprt*-test	V79	0, 1 and 5 mM	-	- (rat-S9) ± (hamster-S9)		De Flora et al., 1985[65]
gene mutation test in mammalian cells *Hprt*-test	V79	0, 1, 1.5, 5, 7.5 mM	-	- (rat-S9) + (hamster-S9)		Fassina et al., 1990[67]

In vivo mutation assays.

test	species	route of administration	dose	results	remarks	reference
alkaline elution assay	rat, liver cells	i.p	330 mg/kg	+		De Flora et al., 1985[65]
		gavage		-		
	rat, kidney cells	gavage		+		
	mouse, bone marrow	i.p		-		
sister chromatid exchange test (SCE)	mouse	i.p	330 mg/kg	+		De Flora et al., 1985[65]
micronucleus test in bone marrow cells	CD-1 mice	i.p	37.5, 75, 150, 300, 400 and 600 mg/kg; 1, 2, 3 or 4 times	+		Sutou et al., 1990[71]
micronucleus test in bone marrow cells	SJL Swiss mice	i.p	0, 2, 5, 50, 100 mg/kg, 1 dose	+		Sicardi et al., 1991[73]
micronucleus test in peripheral blood cells	CD-1 mice	i.p	400, 600, 800 mg/kg	-	single treatment	Higashikuni et al., 1992[74]
		i.p	400, 600, 800 mg/kg	+	double treatment	
		i.p	400, 600, 800 + 300, 400, 600, 800 mg/kg	+		
micronucleus test in peripheral blood cells	MS/Ae mice	i.p	400, 600 mg/kg	+	single treatment	
		i.p		+	double treatment	
micronucleus test in bone marrow cells	CD-1 mice	i.p	600 mg/kg	+	single treatment	
		i.p		+	double treatment	
micronucleus test in peripheral blood cells	Sprague-Dawley rats	gavage	500, 1,000, 2,000 mg/ml during 2 days	+		Asanami et al., 1995[75]
			250, 500, 750, 1,000 mg/ml during 14 days	+	sample times on day 1,3,6,9,12 and 15.	
micronucleus test in bone marrow cells			250, 500, 750, 1,000 mg/ml during 14 days	+		
in vivo gen- mutation assay with lacZ transgenic mice	C57BL/6 mice	oral, in feed	0.75% w/w, 4, 8 and 12 weeks	+	sample times 4, 8 or 12 weeks	Luijten et al., 2006[68]

Annex I

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.[79]