Health Council of the Netherlands

Isoniazid

Evaluation of the carcinogenicity and genotoxicity

2013/33

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Isoniazid*
Uw kenmerk : DGV/BMO-U-932542
Ons kenmerk : U-7971/SV/fs/246-K19
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Geachte minister,

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

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 dit 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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Isoniazid

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 Minister of Social Affairs and Employment

No. 2013/34, The Hague, December 18, 2013

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, 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 de beroepsmatige uitoefening kunnen worden blootgesteld. In het voorliggende advies neemt de Subcommissie Classificatie van Carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, die deze evaluatie en beoordeling verricht, isoniazide onder de loep. Isoniazide is een stof die gebruikt wordt als medicijn voor de behandeling van tuberculose.

Op basis van de beschikbare gegevens concludeert de commissie dat isoniazide verdacht kankerverwekkend is voor de mens. Daarom beveelt de commissie aan de stof te classificeren in categorie 2*.

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

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 Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council. In this report, the Committee evaluated isoniazid. Isoniazid is a substance that is used as a medicine for treatment of tuberculosis.

Based on the available information, the Committee concludes that isoniazid is suspected to be carcinogenic to man. Therefore, the Committee recommends to classify the compound in category 2*.

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

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 proposal for a classification are expressed in the form of standard sentences (see Annex G).

This report contains the evaluation of the carcinogenicity of isoniazid.

1.2 Committee and procedures

The evaluation is performed by the Subcommittee on Classifying 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. A submission letter to the Minister can be found in Annex C.

In 2013 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 isoniazid, such an IARC-monograph and an update are available, of which the summaries and conclusions are inserted in Annex E.

Data published after 1987, when the IARC update was published, were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and the Registry of Toxic Effects of Chemical Substances (RTECS). The last updated online search was in December 2013. The new relevant data were included in this report.

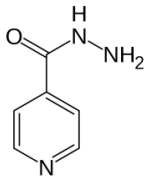
Chapter 2

General information

2.1 Identity and physico-chemical properties

The data have been retrieved from the European Substance Information System (ESIS*), an IUCLID chemical data sheet, which can be accessed via the same website, and the Hazardous Substances Data Bank (HSDB**).

Chemical name	:	Isoniazid
CAS registry number	:	54-85-3
EINECS number	:	200-214-6
Synonyms	:	Pyridinecarboxylic acid, hydrazine; 4-(hydrazinocarbonyl)pyridine; hydrazid; antimicina; izonicotinylhydrazine (IHN)
Appearance	:	Colourless or white crystals, or a white crystalline powder
Use	:	Medicine (an antibiotic used in tuberculosis treatment); organic intermediate
Chemical formula	:	$C_6H_7N_3O$



* ESIS can be accessed via the ECB-site: http://ecb.jrc.it

** HSDB can be assessed via http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Molecular weight	:	137.1 g/mol
Boiling point	:	-
Melting point	:	171.4 ˚C
Vapour pressure	:	4.6×10^{-5} mm Hg at 25 ˚C (estimated)
Vapour density (air = 1)	:	-
Solubility	:	In water: 140 g/L at 25 ˚C. Practically insoluble in ether and benzene
Conversion factor	:	1 mg/m^3 = 0.1750 × ppm
EU Classification (100% solution)	:	Not classified

2.2 IARC classification

Based on the evaluated data, the Working Group of IARC concluded in 1987 that there was limited evidence for carcinogenicity of isoniazid in animals, and inadequate evidence for classification of isoniazid as carcinogenic to humans (Group 3).[1]

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

Cohort studies

In the fourth monograph, and the update (supp. 7), IARC evaluated several epidemiological studies.[1,2]

A large prospective study of lung cancer in a population of more than 1 million by Hamond et al.[3] (cited in IARC[2]) showed a (not statistically significant) excess of deaths due to lung cancer (45 versus 36.5 expected) among 18,963 tuberculous patients, 10% of whom had received isoniazid treatment. ln another group of 311 patients treated with isoniazid between 1951 and 1956, 10 lung cancer deaths had been reported by 1966, whereas 6.3 were expected. This difference was not statistically significant.

A cohort of 3,842 tuberculosis patients was followed for 16-24 years. (Stott et al.[4]; cited in IARC)[1] Patients (n = 2,041) treated with isoniazid during 1953-1957 and followed through to 1973, showed slight excesses of deaths from malignant neoplasms of the bronchus, lung and pleura (relative risk (RR) = 1.6 (95% confidence interval (CI), 1.2-2.1)). An increased risk was not observed for 655 treated for tuberculosis in 1950-1952, when isoniazid was not generally available (RR = 0.7 (95% CI, 0.1-1.5)). An excess risk of all malignant neoplasms was both seen in patients treated in 1953-1957 (RR = 1.4 (95% CI, 1.2-1.7)) and in 145 patients not treated with isoniazid over the same period (RR

= 1.8 (95% CI, 0.7-2.9)). Again, no excess was observed in those treated for tuberculosis in 1950-1952. IARC noted that no dose-response effect was seen either for total consumption or for maximum daily dose of isoniazid.

IARC also reported on a cancer incidence study in patients with tuberculosis – involving heavy smokers.(Clemmesen and Hjalgrim-Jensen[5]; cited in IARC)[1] In this study, an excess risk of lung cancer was observed among men exposed to isoniazid (RR = 3.4, based on 88 cases observed, 26.2 expected) but also among men not exposed (RR = 2.6, based on 18 cases observed, 7.0 expected). For women these figures were 4.6, based on 14 cases exposed, and 0.5, based on one case not exposed.

Campbell and Guilfoyle[6] (cited in IARC[2]) did not observe a statistically significant increase in cases of lung cancer among 129 isoniazid-treated tuberculosis patients, in a cohort of 3,064 tuberculosis patients.

A number of studies by Ferebee[7,8] (cited in IARC[2]) has been reported by IARC. In these studies, tuberculosis cases were given 5 mg isoniazid/kg bw daily for 1 year and observed for 10 years. In 10,531 patients treated in mental institutions and 12,439 household contacts treated prophylactically there was no indication of an increase in the frequency of cancer deaths after 10 years of observation, in comparison with similar numbers of controls given placebos.

Several additional cohort studies were reported by IARC in 1987, in which no excess cancer risk was observed among patients treated with isoniazid. (Glassroth et al.[9], Howe et al.[10], Boice and Fraumeni[11], and Costello and Snider[12]; all cited in IARC[1])

Selby et al.[13] tested the association of 215 drugs or drug groups, including isoniazid, with subsequent incidence of cancer at 56 sites, using computerized pharmacy records from 1969 to 1973 for a cohort of 143,574 members of the Kaiser Permanente Medical Care Program (KPMCP). The follow-up started in 1976 and continued through 1984. As of December 1984, 1,370,000 person-years of follow-up had been accumulated and 68,695 persons (48% of the original cohort) remained active KPMCP members. A total of 1,307,767 drug prescriptions were filled in the period of 1969-1973. Mean age at initial prescription was 31 years; 54% of the cohort was female. In total, 6,809 incident cancers have been verified in 6,382 cohort members.

For isoniazid, 665 users were identified in the cohort. A positive association was found between the isoniazid use and the relative risk of esophagus cancer (3 observed cases versus 0.6 expected; standardised morbidity ratio (SMR) = 5.07; $p < 0.05$). No other associations (positive or negative) between the use of isoniazid and cancer risk at other cancer sites were noted. However, the

conclusion was based on a small number of cases and possible confounding could not be excluded.

Continuing the work of Selby et al.[13], Van den Eeden and Friedman determined cancer occurrence through 1988 using the same cohort.[14] Cohort members were followed for an average of 11.6 years; during this time there were 8,058 incident cancers observed. The authors considered the possibility that in some cases, drugs might have been prescribed for symptoms related to cancer before it was diagnosed, as opposed to cancer being caused by the drug use. Therefore, analyses were routinely conducted that lagged the start of follow-up, until one or two years after the prescription were filled. In addition, SMRs were calculated for certain drugs when the analysis was restricted to individuals who received two or more, or three or more computer-recorded prescriptions. If the association increased with an increasing number of prescriptions, it was generally considered to carry more weight due to an apparent dose-response relationship.

The SMR for gallbladder cancer was statistically significantly elevated among isoniazid users (2 cases, versus 0.2 expected; SMR = 8.46; $p < 0.05$) and remained statistically significant after consideration of a 2-year lag (SMR = 9.66, $p < 0.05$). An increased risk of cancer of the esophagus was found, however, this was not statistically significant (3 cases versus 0.75 expected, SMR = 4.01, not statistically significant). No association was observed between isoniazid use and other types of cancer. There were too few subjects who had more than one prescription recorded to gain useful information from the dose analysis. Since the data were based solely on computer-recorded prescriptions during a certain period, no insight was available on drugs use prior to and following this time period, and no information was available on prescriptions filled other than by the participating pharmacy.

Subsequently, Friedman et al.[15] evaluated the association between the risk of breast cancer and use of antibiotics, including isoniazid, in 2,130,829 adult female subscribers to KPMCP. Women aged ≥ 20 years were identified and followed up, starting in August 1994. Only invasive breast cancer was considered for follow-up, and patients diagnosed before the antibiotic dispensing was first recorded were excluded. Follow-up ended when breast cancer was diagnosed, the subject left KPMCP or at the end of 2003, whichever occurred first. Use of hormones, including estrogens, progestins, preparations for birth control, menopausal hormonal therapy, and other indications, plus tamoxifen and raloxifene, all of which may affect risk of breast cancer, was included as a time-

dependent variable in the analytical model used. Use was defined as receiving at least 2 prescriptions for this particular category of hormones.

Out of a total of 2,130,829 women included in the group, 1,469,680 received at least one antibiotic and 18,251 developed breast cancer. The risk of breast cancer was slightly elevated for all users of any antibiotic (hazard risk (HR) = 1.14 (95% CI, 1.10-1.18)), but there was little, if any, dose response relationship (HR = 1.17 (95% CI, 0.97-1.42)) for > 1000 days of use compared with no use. For isoniazid alone, no statistically significant increase in risk of breast cancer was observed after > 100 days of use (62.4 thousands of person-years, 34 cases; HR = 0.72 (95% CI, 0.51-1.00)).

Case control studies

In the 1987 update, IARC has evaluated four case-control studies concerning bladder and kidney cancers (Glassroth et al.[16]), bladder cancer (Miller et al.[17]; Kantor et al.[18]) and cancer in children (Sanders and Draper[19]) which provided no conclusive evidence of a cancer risk associated with isoniazid therapy.[1]

Friedman et al. performed nested case-control analyses in two cohorts from the KPMCP program: 78,118 female members of the who received prescriptions in 1969-1973, and 3,289,408 female members who received prescriptions in 1994-2006.[20] Out of the first cohort, 2,467 members in total developed cancer; in the second cohort, the number of cancer patients was 24,528. In both cohorts, follow up ended when breast cancer was diagnosed, when the subject left KPMCP for any reason, or at the end of June 2006. Longest follow-up was until June 30, 2006. Ten randomly selected concurrent control women were age-matched to almost every case. Women first diagnosed with breast cancer before a study period were excluded from the respective cohorts. To rule out pre-diagnostic prescription for symptoms possibly related to breast cancer, a 2-year lag of follow-up was introduced after the prescriptions were filled. In addition, the number of prescriptions (one, two and at least three) was considered to ascertain possibly greater risk of longer use. The hormone use was also taken into account, as reported above. For isoniazid, no increased breast cancer risks were observed in the two cohorts.

Conclusion

One epidemiological study found an association of isoniazid with lung cancer, and another with cancers of the esophagus and gallbladder. These studies

however, show several limitations – most notable a small number of cases. In the other available studies, no increased cancer risks were observed. The Committee concludes that the available epidemiological studies do not allow conclusions on the carcinogenicity of isoniazid in humans.

3.2 Carcinogenicity studies in animals

IARC evaluated several carcinogenicity studies, in mice, rats and hamsters, and noted positive findings in mice after exposure to isoniazid via different routes.[1,2] The animal studies evaluated by IARC are summarised in Annex E.

Additional studies do not involve the inhalatory route and have a number of methodological limitations, such as use of partially hepatectomised animals, application of a single dose level and/or inclusion of insufficient numbers of animals.

Oral administration

Studies with rats

Gershbein and Rao administered a diet containing 0.030% isoniazid to 13 young adult male Sprague-Dawley rats for 87 weeks.[21] Two rats died during the course of the study. At necropsy, chronic nephritis was observed in two of the survivors, of which one displayed panniculitis. Tumours were absent throughout the gastrointestinal tract of the animals treated with isoniazid. Also no subcutaneous tumours were noted. Clinical chemistry revealed no remarkable differences compared to the control animals that received a plain diet.

In this study, also the effect of isoniazid in the diet on 1,2-dimethylhydrazine induced tumourigenesis was studied. Ten male weanling rats were fed a plain and an isoniazid-containing diet, respectively, for 15 days, after which 1,2-dimethylhydrazine was injected subcutaneously at a dosage of 9.0 mg/kg bw once per week for 7 weeks, then twice weekly for a total of 23 injections. Colon adenocarcinomas occurred in 80-100% of the animals (22 in the plain diet group; 28 in the isoniazid group). Adenocarcinomas of the small intestine also occurred in 70-80% of the animals of the plain diet and isoniazid group (total number of tumors 15 in the controls and 16 in the isoniazid group). The number of colon adenocarcinomas in the group receiving isoniazid-containing diet was statistically significantly increased in comparison to the group which received a plain diet ($p < 0.05$).

Subcutaneous injection

Studies with rats

Gershbein and Rao also treated 9 intact and 11 partially hepatectomised young male Sprague-Dawley rats with weekly subcutaneous injections of isoniazid at a dose level of 83 mg/kg bw for 87 weeks.[21] It was thought that the removal of two-thirds of the liver prior to continued injections might predispose to possible lesions. The control animals (8 intact and 7 partially hepatectomised) received weekly subcutaneous injections of saliva. Five rats from the intact group and 8 from the partially hepatectomised group died before the end of the treatment, deaths occurring on average after 49 (intact group) and 45 (partially hepatectomised group) weeks. Tumours were absent throughout the gastrointestinal tract of the animals injected with isoniazid. Two subcutaneous tumours occurred in partially hepatectomised rats, versus 0 in intact groups and 1 in both control groups. They were fibrous, generally benign and also presenting fibroangiomatous proliferation sarcomas with necrosis (dermatofibrosarcoma).

Studies with mice

Fujii reported the results of a newborn mouse tumourigenesis assay (NMTA) on 45 chemicals, including isoniazid.[22] Fifty-three male and female newborn CDF1 mice were injected subcutaneously, within 24 hours of birth, with 4 injections of 410 µg/kg bw isoniazid. The treated animals were weaned at 1 month, separated by sex and observed for 1 year. Animals were necropsied completely when moribund or dead. After 1 year the numbers of survivals were 23 males and 27 females. In total, 3 males and 2 females developed tumours (tumour incidence of 12% and 7%, respectively). Out of these, three were lung tumours (2 in males, 8% tumour incidence; 1 in females (4% tumour incidence)); one was a liver tumour (male, 4% tumour incidence) and one was a granulosa cell tumour of the ovary (female, 4% tumour incidence). The observed incidences were not statistically significantly different from those observed in 1% gelatin-treated controls.

3.3 Conclusion

Several types of tumours have been reported in different strains of mice. Although these studies show limitations and the results are not consistent, these

findings cannot be ignored. The Committee concludes that there is limited evidence of carcinogenicity in animals.

Chapter 4

Genotoxicity

4.1 Gene mutation assays

4.1.1 In vitro

IARC reports positive results in several gene mutation assays with *S. typhimurium*. A study with *E. coli* gave negative results. Isoniazid did not induce gene conversion in yeast.[23]

Jung et al. and Müller et al. studied the mutagenicity of 30 compounds of various chemical classes, including isoniazid, in *S. typhimurium* strain TA102, with and without rat S9 metabolic activation.[24,25] The tests were performed in 3 independent laboratories, in at least 2 independent experiments using 5 doses (up to 5,000 µg/plate) and 3 plates per dose. In all 3 laboratories negative results were obtained for isoniazid; however, no details on the results were reported.

Blanco et al. studied the mutagenicity of isoniazid in *E. coli* WP2 tester strain IC203.[26] This strain is deficient in OxyR, which is a transcriptional activator of peroxide-inducible genes and allows detecting reactive oxygen species-dependent mutagenicity. Concentration levels of 0, 1, 2.5, 5, 7.5 and 10 mg/plate isoniazid were tested with and without S9 metabolic activation. Plates were incubated for 2 days and the experiments were performed at least in triplicates. For comparison, a parent strain WP2 *uvrA*/pKM101 and two strains IC204 (a derivative of WP2

uvrA carrying a deletion of the *umuDC* genes) and IC206 (a derivative of IC204 carrying a deletion of *mutY* genes) were tested as well. In the absence of metabolic activation, isoniazid induced a positive SOS-dependent mutagenic response in strain IC203 (698 ± 137 revertants/plate at the highest concentration, versus 144 ± 16 in controls), but not in the parent WP2 *uvrA*/pKM101 strain. It also induced a moderate level of SOS-independent revertants in strain IC206 (119 ± 58 revertants/plate at the highest dose level, versus 32 ± 4 in controls). Mutagenic potency of isoniazid in the absence of metabolic activation was 12.4, 1.5, 0.6 and 0.06 induced revertants/µmol for strains IC203, IC206, WP2 *uvrA*/ pKM101 and IC204, respectively. In the presence of a metabolic activation system, mutagenicity of isoniazid was suppressed in IC203 strain (~154 revertants/plate at the highest concentration) and was comparable to the results in WP2 *uvrA*/pKM101. No data on mutagenicity of isoniazid in IC204 and IC206 strains in the presence of metabolic activation were reported.

Oberly et al. studied the mutagenicity of 66 compounds, including isoniazid, in the L5178 $tk^{+/-}$ mouse lymphoma assay (MLA), with and without S9 metabolic activation.[27] Concentrations of 625 and 2,500 µg/mL and 250 and 2,500 µg/mL were tested (up to the limit of solubility), with and without metabolic activation, respectively. In the absence of metabolic activation, the observed mutant frequencies were 3.4×10^{-5} and 2.4×10^{-5}, respectively, while mutation indices (the folds increase in induced mutant frequencies over background mutant frequencies) were 1.5 and 1.1. In the presence of a metabolic activation system, induced mutant frequencies were 3.3×10^{-5} in both cases, while mutation indices were 1.1 in both cases. Based on the results, isoniazid was concluded to give negative results in the study.

4.1.2 In vivo assays

IARC did not evaluate any in vivo gene mutation assays with isoniazide.[23]

The ability of isoniazid to induce somatic mutations in mice was studied by Neuhäuser-Klaus and Chauhan using the mammalian spot test.[28] Male HT- and T-stock and female C57BL/6JHan and T-stock mice were used. In none of the treatment schedules, an increase in frequency of offspring with spots of genetic relevance was observed compared to controls.

4.2 Cytogenetic assays

4.2.1 In vitro

IARC concluded that results from chromosomal aberrations and sister chromatid exchanges in human cells in vitro were inconclusive. In cultured rodent cells, isoniazid induced chromosomal aberrations and sister chromatid exchanges, according to IARC.[23]

No additional in vitro chromosome aberration tests or micronucleus assays are available.

4.2.2 In vivo

Human data

Three studies (Gopal Rao et al.[29], Masjedi et al.[30] and Ekmekçi and Şayli[31]) evaluated the influence of anti-tuberculosis drugs, including isoniazid, on the chromosome aberration and/or micronuclei and/or sister chromatid exchange frequencies in pulmonary tuberculosis patients.

The Committee notes that in all cases, treatment involved a combination of at least three drug types (isoniazid, streptomycin, refampicin, pyrazinamide, ethambutol). Due to the co-exposure to other drugs, no conclusions on isoniazid can be drawn based on these studies.

Animal data

In 1987, IARC concluded that isoniazid did not induce chromosomal aberrations, sister chromatid exchanges in rodents. Also, isoniazid did not induce dominant lethal mutations in mice.[23]

Takasawa and co-workers[32] tested a number of chemicals, including isoniazid, in liver and peripheral blood micronucleus assays with 4-week-old male F344 rats. Isoniazid was administered as a single dose in aqueous solution to groups of 5 animals by oral gavage at dose levels of 0 (water), 125, 250 and 500 mg/kg bw. Diethylnitrosamine was used as a positive control. For the liver micronucleus assay, hepatocyte suspensions were prepared 3, 4 or 5 days after dosing. Two thousand parenchymal hepatocytes were scored for micronuclei and the mitotic

index. For the peripheral blood micronucleus assay, the blood was collected at the same days post-dosing. Two thousand reticulocytes were scored for micronuclei for each animal.

Isoniazid gave negative results in the liver micronucleus assay, but induced a statistically significant, although weak, response in the peripheral blood micronucleus assay. A statistically significant increase in the number of micronucleated reticulocytes was seen only on day 5 at the highest dose level of 500 mg/kg bw. However, the observed statistical increase was minimal and the value of only one animal was higher than usual. The ratio of reticulocytes to total erythrocytes was not scored.

4.3 Miscellaneous

In 1987, IARC concluded that isoniazid did not induce DNA damage or unscheduled synthesis in vivo.[23]

Maru and co-workers[33] studied the formation and persistence of isoniazid-DNA adducts in male Swiss mice. Tissue DNA of mice (number of animals not reported) was prelabeled by the neonatal subcutaneous administration of [^{3}H]-deoxycytidine daily from the 2nd to the 21st day of life. At 9 weeks of age, each animal was administered 1.1 mg isoniazid by gavage and killed 2, 5, 12, 24, or 72 hours later. DNA was extracted from liver, lung, spleen and kidney. Labeling of DNA in spleen and kidney was insufficient to detect adduct formation in vivo. In liver and lung, three different isoniazid-DNA adducts were detected.

Saffhill et al.[34] investigated the ability of isoniazid to methylate tissue DNA by administering a single dose of 1.1 mg isoniazid* by gavage to groups of 6 male Swiss mice. The mice were sacrificed after different time intervals (from 5 hours to 5 days), and DNA from the lungs and liver was prepared and analysed for alkylated products. The liver DNA contained readily detectable amounts of *O*^{6}-methylguanine by 5 hours, which increased several fold by 24 hours and reached a level of ~ 0.3 µmol/mol guanine, but was no longer detectable at 2 days. In lung DNA, a maximum amount of *O*^{6}-methylguanine (~ 0.5 µmol/mol guanine) was reached at 1 day and then was lost progressively, until it was no longer detectable at 3 days.

* A dose that, if administered continuously, is reported to result in a 50% incidence of lung tumours.[2]

For comparison, a similar experiment was performed in Wistar rats. The dose employed (6.9 mg per rat) was calculated on a body weight basis to correspond to those used in mice. In contrast to the findings in mice, O^6-methylguanine levels in rats were at the limits of detection in lung and only slightly higher in liver.

4.4 Conclusion

Positive results have been obtained in vitro, i.e. in bacterial gene mutation tests and chromosomal aberration tests. The in vivo data, however, are consistently negative (i.e., a mouse spot test, several chromosomal aberration assays and sister chromatid exchange tests and a dominant lethal mutation assay). Therefore, the Committee concludes that there is no evidence that isoniazid is genotoxic.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenesis and genotoxicity

In two epidemiological studies associations have been found between isoniazid and cancer. One was related to lung cancer, the other to cancers of the esophagus and gallbladder. These studies, however, show several limitations – most notably, a small number of cases. In the other available studies, no increased cancer risks were observed. The Committee concludes that the available epidemiological studies do not allow conclusions on the carcinogenicity of isoniazid in humans.

Several types of tumours have been reported in different strains of mice. Although these studies show limitations and the overall results in animal studies are not consistent, these findings cannot be ignored. The Committee concludes that there is limited evidence of carcinogenicity in animals.

Positive results have been obtained in in vitro genotoxicity assays, i.e. in bacterial gene mutation tests and chromosomal aberration tests. In vivo assays, however, are consistently negative (i.e., a mouse spot test, several chromosomal aberration assays and sister chromatid exchange tests and a dominant lethal mutation assay). The Committee concludes that there is no evidence that isoniazid is genotoxic.

5.2 Recommendation for classification

The Committee concludes that isoniazid is suspected to be carcinogenic to man, and recommends to classify the compound in category 2*.

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

References

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A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	IARC evaluation and conclusion
F	Animal studies
G	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 *(until April 1, 2013)*; Exponent, Menlo Park, United States *(from August 15, 2013)*
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- S.R. Vink, *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 (in English)

Subject : Submission of the advisory report *Isoniazid*
Your Reference : DGV/MBO/U-932542
Our reference : U-7971/SV/fs/246-K19
Enclosed : 1
Date : December 18, 2013

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to isoniazid.

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. 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 2013 for public review. The following organisation and person has commented on the draft document:

- Professor D. Coggon, Southampton, United Kingdom.

Annex **E**

IARC evaluation and conclusion

ISONICOTINIC ACID HYDRAZIDE (ISONIAZID)

VOL.: 4 (1974) (p. 159)

Summary of Data Reported and Evaluation

Animal carcinogenicity data

Isonicotinic acid hydrazide (INH) is carcinogenic in mice after oral, subcutaneous and intraperitoneal administration. The observation of tumours in rats in only one of several oral studies is inconclusive. INH failed to produce tumours in hamsters when given orally.

Human carcinogenicity data

Available evidence from the first 15 years of human exposure has not suggested that INH is carcinogenic in man in the doses applicable to treatment and prophylaxis of tuberculosis.

Supplement 7 (1987), (p. 227)

ISONICOTINIC ACID HYDRAZIDE (ISONIAZID) (Group 3)

A. Evidence for carcinogenicity to humans (inadequate)

Several early studies showed no significant excess of cancer among patients treated with isoniazid. A study of 3842 tuberculosis patients followed for 16-24 years showed slight excesses of deaths from malignant neoplasms of the bronchus, lung and pleura in 2041 patients treated with isoniazid during 1953-1957 and followed through to 1973 (relative risk, 1.6; 95% confidence interval, 1.2-2.1), but none in 655 treated for tuberculosis in 1950-1952 when isoniazid was not generally available (0.7; 0.1- 1.5). An excess of all malignant neoplasms was seen in patients treated in 1953-1957 (1.4; 1.2-1.7), but also in 145 patients not treated with isoniazid over the same period (1.8; 0.7-2.9). Again, no excess was observed in those treated for tuberculosis in 1950-1952. No dose-response effect was seen either for total consumption or for maximum daily dose of isoniazid. Additional studies of cancer incidence and mortality among patients treated with isoniazid have shown no excess of lung cancer, or of cancer as a whole, that could be attributed to treatment. A cancer incidence study in patients with tuberculosis, involving heavy smokers, showed an excess of lung cancer among men exposed to isoniazid (3.4, based on 88 cases observed, 26.2 expected) but also among those not exposed (2.6, based on 18 cases observed, 7.0 expected). The difference between the two ratios was not statistically significant. The corresponding figures for women were 4.6, based on 14 cases exposed, and 0.5, based on one case not exposed. ln a preliminary analysis of one-year case records, 72 (4.9%) cancer patients had healed tuberculosis compared with 26 (2%) noncancer patients. Four case-control studies concerning bladder and kidney cancers, bladder cancer, and cancer in children have provided no conclusive evidence of a risk associated with isoniazid therapy. A single case of mesothelioma has been reported in a nine-year-old child whose mother was treated with isoniazid for a positive tuberculin skin test in the second and third trimesters of pregnancy.

B. Evidence for carcinogenicity to animals (limited)

Isoniazid produced lung tumours in mice after oral, intraperitoneal or subcutaneous administration. Studies in rats were considered inadequate for

evaluation. No tumour was produced in hamsters after oral administration of isoniazid.

C. Other relevant data

ln the one available study, isoniazid did not induce chromosomal aberrations in lymphocytes of treated patients.

Isoniazid did not induce dominant lethal mutations in mice, or chromosomal aberrations, sister chromatid exchanges or DNA damage in rodents treated in vivo. Results for chromosomal aberrations and sister chromatid exchanges in human cells in vitro were inconclusive; it did not induce unscheduled DNA synthesis. ln cultured rodent cells, it induced chromosomal aberrations and sister chromatid exchanges, but not DNA damage.

It did not induce transformation of Syrian hamster embryo cells. It did not induce gene conversion in yeast. Isoniazid was mutagenic to *Salmonella typhimurium* but not, in a single study, to *Escherichia coli*.

Overall evaluation

Isonicotinic acid hydrazide (Isoniazid) is not classifiable as to its carcinogenicity to humans (Group 3).

Annex **F**

Animal data

Animal carcinogenicity data reported by IARC.[1,2]

Species, strain (sex)	Dose	Exposure duration/ frequency	Carcinogenic effects	References cited in IARC
Oral administration				
Mouse, 'dd'	0.25, 0.125, 0.1, 0.06 and 0.01% isoniazid in diet	7 months	Pulmonary tumours at 7 months: • 100% (0.25% isoniazid) • 70 0% (125% isoniazid) • 60% (0.1% isoniazid) • 50% (0.06% isoniazid) • 8% (0.01% isoniazid)	(Mori and Yasumo (1959) Mori et al. (1960))[a]
Mouse, 'other' N=20/group	0.25% isoniazid in diet	2 months 3 months 4 months 7 months Control groups	40% 50% 50% 100% Negative and 3%	
Rat, albino N=60	30 mg/kg bw daily in drinking water	290 or 355 days	No tumours were observed	Loscalzo (1964)
Rat, several strains	NS	NS	No tumours were observed	Peacock and Peacock (1966), Lucchesi et al. (1967); Toth and Toth (1970)

Rat, Cb/Se (males and females)	35 mg daily in drinking water	48 weeks	Males: • 1/49 liver tumour • 2/49 lung tumour Females: • 11/40 fibroadenomas of the mammary gland • No tumours were observed in controls	Severi and Biancifiori (1968)
Hamster (7 males and 9 females)	0.25% isoniazid in drinking water	Up to 90 weeks	No pulmonary tumours were observed 1 hepatoma and 1 haemangioma in females No control data available	Peacock and Peacock (1966)
Hamster, Syrian Gold, 50 males and 50 females; 35 males and 36 females	0.1% and 0.2% isoniazid	For life	No carcinogenic effects were observed	Toth and Boreisha, (1969); Toth and Shubik (1969)
	0.3% isoniazid	42 weeks	No carcinogenic effects were observed	
Subcutaneous and/or intramuscular administration				
Mouse, 'dd', 15 surviving	2 mg s.c. every 2 days	18 weeks	Pulmonary tumours at 7 months: • 53% in 15 treated animals • 11% in 9 control animals	Mori et al. (1960)
Mouse, Strong A and BALB/c	2 mg s.c. every 2 days	18 weeks	Lung tumours: • 52%; 51/99 (males; Strong A) • 61%; 58/95 (females; Strong A) • 39%; 81/210 (controls, males) • 36%; 76/209 (controls, females) • 39%; 28/71 (males; BALB/c) • 40%; 21/53 (females; BALB/c) • 31%; 32/102 (controls, males) • 22%; 20/90 (controls, males)	Jones et al. (1971)
Mouse, 'white',	30 doses i.p., 82 mg/animal	Within 3 months	Tumours at 7.5 months: 14/45, including 7 lung adenomas, 6 leukaemias and 1 reticulum cells sarcoma of the liver. No tumours were observed in 50 controls	Juhász et al. (1957)
	Total dose of 40-55 mg		Tumours at 13 months: • 15/50 (mainly mediastinal lymphosarcomas and myeloid leukaemias) • 1/50 controls developed myeloid leukaemia	Juhász et al. (1963)
Mouse, R_3	1.25 mg i.p. daily	87 days	17/45 treated animals developed lung tumours. None were observed in 45 controls	Schwan (1962)

perinatal administration

Mouse, Swiss, males and females	F0: 1.1 mg/day by gastric intubation F1 mice: 0, 0.55, 1.1 and 2.2 mg/day	F0 and F1: During life. Pregnant females received isoniazid from day 1 gestation throughout, during lactation until death.	Lung tumours in F0: • 50% (males) • 67% (females) • 5% (controls) Lung tumours in F1: • 13/30; 43% (0.55 mg) • 14/30; 47% (1.1 mg) • 3/4; 75% (2.2 mg) • 7/20; 35% (control) Lung tumours in F2: 7/10; 70% (F2 from F1 0.55 mg) 1/9; 11% (F2 from F1 control)	Malini et al. (1983)
Mouse, Swiss, males and females	1.1 and 0.55 mg/ kg bw by gastric intubation	Female mice received isoniazid from the 5th day the vaginal plug was seen. The litter received isoniazid 12 weeks after birth until 18-24 months	Liver and lung tumours: • 16/29; 55% (1.1 mg) • 6/19; 32% (0.55 mg) • 1/20; 5% (control group) 8/9; 89% (1.1 mg litters) 7/14; 50% (0.55 mg litters)	Bhide et al. (1978); Bhide et al. (1981); Maru and Bhide (1982)
Rats, Wistar	4 and 6 mg/kg bw		No lung and liver tumours were observed	Bhide et al. (1981)

[a] IARC notes that similar results were found in various strains of mice, by Biancifiori and Ribacchi (1962a,b); Biacifiori et al. (1963); Ribacchi et al. (1963); Weinstein and Kinosita (1963); Toth and Shubik (1966a,b); Kelly et al. (1969); Toth and Toth (1970), Yamamoto and Weisburger (1970); Jones et al. (1971) and Linnik (1972).

Annex **G**

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 as 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.[35]

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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