Rhodium and compounds

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occupational Standards,

a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid
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Onderwerp : Aanbieding advies over 'Rhodium en verbindingen'
Uw kenmerk : DGV/BMO-U-932542
Ons kenmerk : U-801/JR/RA/459-T36
Bijlagen : 1
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Mijnheer de Staatssecretaris,

Bij Brief van 3 december, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In dat kader bied ik u hierbij een advies aan over de kankerverwekkende eigenschappen van 'Rhodium en verbindingen'. Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

prof. dr JA Knottnerus

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Dutch Expert Committee on Occupational Standards,
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to

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/08OSH, The Hague, 27 June 2002

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..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. 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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Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. In het voorliggende rapport neemt de Commissie WGD van de Raad, die deze beoordelingen verricht, rhodium en rhodiumverbindingen onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie concludeert dat wateroplosbaar rhodium(III)chloride onvoldoende is onderzocht. Hoewel de beschikbare gegevens het niet toelaten de stof te classificeren als 'kankerverwekkend voor de mens' of als 'moet beschouwd worden als kankerverwekkend voor de mens', is de commissie van mening dat waakzaamheid geboden is. De commissie adviseert daarom wateroplosbaar rhodium(III)chloride te classificeren als verdacht kankerverwekkend voor de mens (overeenkomend met EU categorie 3B). De commissie is verder van mening dat deze classificatie in 3B ook geldt voor alle rhodium(III)verbindingen, die rhodium(III)-ionen in oplossing genereren.

De commissie is van mening dat andere wateroplosbare en wateronoplosbare rhodiumverbindingen (inclusief de onoplosbare rhodium(III)verbindingen), evenals het metallisch rhodium onvoldoende zijn onderzocht. Zij adviseert daarom deze rhodium verbindingen en metallisch rhodium niet te classificeren.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the carcinogenic properties of substances at the workplace and proposes a classification with reference to the EU-directive. This evaluation is performed by the Dutch Expert Committee on Occupational Standards. The present report contains an evaluation by the committee on the carcinogenicity of rhodium and its compounds.

The committee concludes that water-soluble rhodium(III) chloride has been insufficiently investigated. While the available data do not warrant a classification as ‘carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is cause for concern for man. The committee recommends classifying water-soluble rhodium(III) chloride as suspected carcinogens to humans (comparable with EU category 3B). Furthermore, the committee is of the opinion that classification in category 3B should be applied to all rhodium(III) compounds, which generate rhodium(III)-ions in solution.

The committee concludes that the other water-soluble and -insoluble rhodium compounds (insoluble rhodium(III) compounds included) and metallic rhodium were insufficiently investigated. Therefore, the committee is of the opinion that these rhodium compounds and metallic rhodium cannot be classified.

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. The Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to study the carcinogenic properties of substances and to propose a classification with reference to an EU-directive (annex A and F). This task is carried out by the Council's Dutch Expert Committee on Occupational Standards, hereafter called the committee.

In addition to classifying substances with respect to their possible carcinogenicity according to the EU Guidelines, the committee also assesses the genotoxic properties of the substances in question. The committee expresses its conclusions in the form of standard sentences (annex E).

1.2 Committee and procedures

The present report contains evaluations by the committee of the carcinogenicity of rhodium and its compounds. The members of the committee are listed in annex B. The first draft of this report was prepared by MI Willems, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

In 2000 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 C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

No IARC evaluation of the carcinogenicity and genotoxicity of rhodium and its compounds is available.

The present evaluation has been based on literature retrieved from the on-line data bases Toxline, Cancerlit, and Medline, covering the period 1965 to March 1997, 1963 to April 1997, and 1966 to May 1997, respectively. An additional search up to May 2001 did not reveal relevant new literature.

Chapter **2**

Rhodium and its compounds

2.1 Introduction*

The element rhodium (atomic number 45) is a member of Group VIII of the Periodic Table of Elements. These elements are referred to as the platinum group. Rhodium possesses valence states ranging from +1 to +6.

Name: *metallic rhodium*		
CAS no	:	7440-16-6
EINECS no	:	231-125-0
Description	:	a silvery white, soft, ductile, and malleable metal
Occurrence	:	as one of the rarest elements, it occurs naturally. It constitutes about $1x10^{-7}$% of the earth's crust, and is found in small amounts associated with all native platinum, in the minerals rhodite, sperrylite, iridosmine, and in some nickel-copper ores
Use	:	in rhodium-platinum alloys for spinnerets and bushings for synthetic fiber extrusion, high-temperature furnace windings, laboratory crucibles, and as catalysts for various organic and inorganic reactions including the oxidation of ammonia in the manufacture of nitric acid; its capacity for making a highly resistant coating is utilised in the surfacting of reflectors, electric contacts, scientific instruments, and jewelry.

* Data from ACG91, Bud89

Chem formula	:	Rh
Atomic mass	:	102.9
Boiling point (104.5 kPa)	:	3727 °C
Melting point (101.3 kPa)	:	1966 °C
Relative density (20 °/4 °C)	:	(water=1) : 12.41 g/cm^3
Solubility	:	insoluble in water and all acids; finely divided material is slowly soluble in aqua regia and concentrated sulphuric acid

Rhodium forms a variety of salts with valence states of +1 (I), +2 (II), +3 (III) and +4 (IV). Some of these salts are water soluble, such as rhodium trichloride ($RhCl_3$) when hydrated, sodium chlorodite (Na_3RhCl_6), and chloropentaamine rhodium chloride(III) ($[Rh(NH_3)_5Cl]Cl_2$), whereas others are not, such as rhodium dioxide, rhodium sulphide, and nonhydrated rhodium trichloride.

The physical and chemical properties of rhodium trichloride, a rhodium(III) compound, are shown below.

Name: *rhodium(III) chloride*

CAS no	:	trihydrate: 13569-65-8; hydrate: 20765-98-4; anhydrous: 10049-07-7
RTECS no	:	hydrate: VI9290000
EINECS no	:	anhydrous: 233-165-4
synonyms	:	rhodium trichloride, rhodium chloride
Description	:	a ruby-red crystalline powder, with a chlorine odour
Use	:	catalyst for the direct conversion of methane to acetic acid
Chemical formula	:	trihydrate: $Cl_3Rh{\cdot}3H_2O$; hydrate: $Cl_3Rh{\cdot}xH_2O$; anhydrous: Cl_3Rh
Molecular mass	:	trihydrate: 263.3 g/mol; anhydrous: 209.26 g/mol
Boiling point (104.5 kPa)	:	trihydrate: - ; anhydrous: 717 °C
Melting point	:	trihydrate: 100 °C (decomposes); anhydrous: 450 °C (decomposes)
Relative density	:	trihydrate: (water=1): > 1
solubility	:	hydrated forms: solubility in water is very good; anhydrous: insoluble in water, soluble in alkali hydroxide or cyanide solutions;
EC classification anhydrous	:	R 20/21/22: harmful by inhalation, in contact with skin and if swallowed R 36: irritating to eyes R 40: possible risk of irreversible effects

Various rhodium(I) and -(III) organometallic complexes and some inorganic rhodium(III) compounds have been investigated for their antitumour properties, which was initiated by the success with platinum compounds. But at present, however, no rhodium-based antitumour drugs have yet been approved for use in humans.

2.2 IARC conclusion

There is no IARC evaluation on the carcinogenicity of rhodium or its compounds available.

2.3 Human data

No data were found.

2.4 Animal data

Currently, one study is available in which the carcinogenicity of a rhodium compound was investigated. In this study, rhodium chloride (not further defined; probably soluble $RhCl_3.xH_2O$) was administered at a dose of 5 µg/mL (5 ppm) in the drinking water to random-bred Swiss mice (n=54/sex) from weaning until natural death. Treatment induced a statistically significant increase in the incidence of malignant tumours, predominantly lymphomas-leukaemias and adenocarcinomas or papillary adenocarcinomas of the lung (17/59 versus 11/80 in controls). Body weights were lower in 6 out of the 16 intervals at which these were analysed for both sexes, but terminal body weights did not differ from those from controls. Longevity defined as the mean age of the last 10% to survive was 708±16.5 and 818±25.4 days in exposed male and female animals, respectively (versus 696±19.2 and 817±18.8 in male and female controls, respectively) (Sch71). The validity of this study is questioned by the committee, because of the methodological deficiences in the study (*e.g.* only one dose applied and tumours were pooled despite sex-specific differences).

2.5 Mutagenicity and genotoxicity

Currently, a number of data on the mutagenicity and genotoxicity of various water-soluble rhodium(III) compounds and a few water-soluble rhodium(I) compounds are available. Most of these data were derived from studies investigating the antitumour properties of the rhodium compounds.

In annex D, data of the studies evaluated on the next page are given in detail.

Rhodium(I) complexes. Information on rhodium(I) compounds is limited.

In one study, four rhodium(I) complexes (25-100 µg/plate) were tested. When dissolved in acetone without adding a metabolic activation system in *Salmonella typhimurium* strains TA92, TA2410, TA100, and TA98, negative results were obtained; when dissolved in DMSO weakly positive results were observed in some of these strains for some of these complexes (Are85).

In another study, all seven rhodium(I) complexes tested (0-160 µg/plate) induced mutations in *Escherichia coli* and *Salmonella typhimurium* strains (Mon87).

Rhodium(III) complexes. In a number of studies, positive results were reported for several rhodium(III) complexes in the Ames test (Bün96, LaV86).

A series of 19 hexacoordinate rhodium(III) complexes, with systematic variations in complex charge, ligand composition, and stereochemistry, were evaluated for their DNA-damaging and mutagenic activities in a battery of *Escherichia coli* K12 and *Salmonella typhimurium* strains. Results showed that plasmid pKM101 - a plasmid with excision repair activity - in for instance strains TA92, TA98, TA100, or *umu* SOS repair were required for expression of mutagenicity by these complexes. The genetic activity was profoundly affected by the composition of the ligands as well as the three-dimensional structures of the coordination complexes (War81).

Two rhodium(III) complexes (K_2RhCl_5 and $(NH_4)_3RhCl_6$, solvent H_20) were positive in the SOS chromotest using strain *Escherichia coli* PQ37 (Lan97).

Rhodium trichloride ($RhCl_3 \cdot 3H_2O$) was positive in *Bacillus subtilis* (recombination-repair assay), in *Escherichia coli* WP2 strains (reversion assay), and in *Salmonella typhimurium* strain TA98 (reversion assay; not in TA100, TA1535, TA1537, TA1538) (Kan80).

Rhodium trichloride ($RhCl_3 \cdot 3H_2O$) was positive when tested - in probably one single trial - in cultured Chinese hamster V79 lung fibroblasts increasing forward mutation frequencies by approximately 2 and 4 times at concentrations of 150 and 300 µmol/L, respectively, when compared with control rates (Kan90).

In one study, cultured human lymphocytes obtained from two healthy non-smoking male volunteers showed chromosomal aberrations when incubated with *cis*-[Rh(2,2'-biquinoline)$_2$Cl$_2$]Cl (Sad00).

Results of spectroscopic studies of the interaction of rhodium trichloride ($RhCl_3 \cdot xH_2O$) with isolated calf thymus DNA suggested binding of rhodium(III) with both the phosphates and the bases of DNA (Sas79).

2.6 Evaluation

No data on humans were available.

When administered in the drinking water from weaning until death, water-soluble rhodium chloride increased the incidence of malignant tumours in mice. Although the quality of this study is poor and the validity may be questioned, the committee is of the opinion that this study is reason for concern.

In vitro, several water-soluble rhodium(III) compounds were mutagenic in bacteria. In mammalian cells, water-soluble rhodium trichloride induced mutations in Chinese hamster V79 lung fibroblast cells, and *cis*-[Rh(2,2'-biquinoline)$_2$Cl$_2$]Cl induced chromosomal aberrations in primary human lymphocytes.

In one study a few rhodium(I) compounds were weakly mutagenic in bacteria. Because the data of this study were limited, the committee cannot make a conclusion for these rhodium(I) compounds.

No *in vivo* mutagenicity or genotoxicity tests were available.

2.7 Recommendation for classification

The committee is of the opinion that water-soluble rhodium(III) chloride has been insufficiently investigated. While the available data do not warrant a classification as 'carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern for man. The committee recommends classifying water-soluble rhodium(III) chloride as suspected carcinogens (comparable with EU category 3B). Furthermore, the committee is of the opinion that classification in category 3B should be applied to all rhodium(III) compounds, which generate rhodium(III)-ions in solution.

In addition, the committee concludes that the other water-soluble and -insoluble rhodium compounds (insoluble rhodium(III) compounds included) and metallic rhodium were insufficiently investigated. The committee, therefore, recommends not classifying these compounds.

References

ACG91 American Conference of Governmental Industrial Hygienists (ACGIH). Rhodium and compounds. In: Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Cincinnati OH, USA: ACGIH, 1991: 1337-9.

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Bün96 Bünger J, Stork J and Stalder K. Cyto- and genotoxic effects of coordination complexes of platinum, palladium and rhodium *in vitro*. Int Arch Occup Health, 1996; 69: 33-38.

Are85 Aresta M, Treglia S, Collucia M, *et al.* Mutagenic activity of transition-metal complexes: relation structure-mutagenic and antibacterial activity for some Pd(II), Pt(II) and Rh(I) complexes. Curr Top Environ Toxicol Chem, 1985; 8: 453-66.

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Kan80 Kanematsu N, Hara M, Kada T. Rec assay and mutagenicity studies on metal compounds. Mutat Res 1980; 77: 109-16.

Kan90 Kanematsu N, Nakamine H, Fukuta Y, *et al.* Mutagenicity of cadmium, platinum and rhodium compounds in cultured mammalian cells. J Gifu Dent Soc 1990: 17: 575-82.

Mon87 Mutagenic effects of rhodium(I) and rhuthenium(II) organometallic complexes. Inorganic Chimica Acta, 1987; 31-41.

Lan97 Lantzsch H, Gebel T. Genotoxicity of selected metal compounds in the SOS chromotest. Mutat Res 1997; 389: 191-7.

Sad97 Sadiq MF, Zaghal MH. Mutagenic activity of rhodium(III) complexes with 2,2'-biquinoline and 2-(2'-pryridyl)quinoline. Polyherdon, 1997; 16: 1483-1486.

Sad00 Sadiq MF, Zaghal MH and El-Shanti HE. Induction of chromosomal aberrations by the rhodium(III) complex *cis*-[$Rh(biq)_2Cl_2$]Cl in cultured human lymphocytes. Mutagenesis, 2000; 15: 375-378.

Sas79 Sasi R, Nandi US. Interaction of rhodium(III) with DNA. Biochim Biophys Acta 1979; 563: 527-33.

Sch71 Schroeder HA, Mitchener M. Scandium, chromium (VI), gallium, yttrium, rhodium, palladium, indium in mice: effects on growth and life span. J Nutrition 1971; 101: 1431-8.

War81 Warren G, Abbott E, Schultz P, *et al*. Mutagenicity of a series of hexacoordinate rhodium(III) compounds. Mutat Res 1981; 88: 165-73.

A Request for advice

B The committee

C Comments on the public review draft

D Mutagenic and genotoxic data

E Classification of substances with respect to carcinogenicity

F Guideline 93/21/EEG of the European Union

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

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- PJ Boogaard
  toxicologist; Shell International Petroleum Company, The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; IRAS, Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen.
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by A van der Klugt.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. No organisations and persons have commented on the draft document.

Annex **D**

Mutagenic and genotoxic data

See next pages.

Table 1 In vitro mutagenicity and genotoxicity testing of various water-soluble rhodium compounds.

	type of assay and doses used	results (+, positive; -, negative)	ref.
Rhodium(I) compounds:			
[#]RhCOD, RhCODA, RhNAA, and RhCAT	1) Ames test (*S. typhimurium* TA92, TA98, TA100, TA2410 reversion assays): 25-50-100 µg/plate dissolved in DMSO or acetone, without metabolic activitation	1) In acetone: - (all strains tested). In DMSO: RhCAT was negative in all strains tested; RhCOD, RhCODA and RhNAA were positive (weak)	Are85
[#][Rh(phen)COD]Cl, [Rh(phen)-NBD]Cl, [Rh(4,7-dimethyl-phen)-COD]Cl, [Rh(4,7-dimethyl-phen)-NBD]Cl, [Rh(3,4,7,8-tetramethyl-phen) COD]Cl, [Rh(3,4,7,8-tetramethyl-phen) NBD]Cl.	1) Mutagenic activity in *E. coli B* strains (WP2, WP2uvrA, WP2/TM1, WP2/TM2) and *S. typhimurium* strains (TA92, TA98, TA100): up to 40-50 µg/plate and up to 100-160 µg/plate for [Rh(phen)COD]Cl, all without metabolic activation	1) All compounds were positive in *E.coli* and *S.tyhimurium*. *E.coli* strains carrying the pKM101 plasmid were more prone to be mutated. A higher number of revertants were found in strains lacking excision repair activity.	Mon87
[#]COD=cis,cis-1,5-cyclooctadiene; CODA=COD-aniline; NBD=1,5-norbornadiene; phen=1,10-phenanthroline			
Anorganic rhodium(III) compounds:			
$RhCl_3.3H_2O$	1) recombination-repair assay (*B. subtilis):* 5 µM in water	1) + (strong)	Kan80
	2) Spot test (*E. coli* and *S. typhimurium*): dose not given	2) +: *E. coli* B/rWP2 *try*$^-$ and WP2 *hcr*$^-$*try*$^-$; TA98 -: TA100, TA1535, TA1537, TA1538	Kan80
	3) Quantitative mutation induction (*E. coli*): 0.1-1 mg/mL in water	3) +: dose-dependent	Kan80
	4) HGPRT mutagenicity test (V79 cells): 0-150-300 µM in water	4) 300 µM: 4-fold increased mutation frequency	Kan90
	5) Ames test (*S.typhimurium*): dose not given[(*)]	5) +: TA92, TA98, TA100, -: *his*G46, TA1535, TA1537, TA1538	War81
$RhNO_3$	1) recombination-repair assay (*B. subtilis):* 5 µM in water	1) +	Kan80
K_2RhCl_5	1) SOS chromotest (*E. coli* PQ37): 0-1812 µM in water	1) +: for genotoxicity	Lan97
	2) Ames test (*S. typhimurium* TA97a, TA98, TA100, TA102): 0-500 µg/plate in culture medium, with and without metabolic activiation (S9)	2) +: TA97a (only with S9), TA98, TA100 (only with S9) and TA102	Bün96

(*) TA92, TA98 and TA100 contain plasmid pKM101.

Table 1 Continued (1)

	type of assay and doses used	results (+, positive; -, negative)	ref.
Rhodium(III) amine-derivate compounds:			
$(NH_4)_3RhCl_6$	1) SOS chromotest (*E. coli* PQ37): 7-1802 µM in water	1) +: for genotoxicity	Lan97
	2) Ames test (*S. typhimurium* TA97a, TA98, TA100, TA102): 0-500 µg/plate in culture medium, with and without metabolic activiation (S9)	2) +: TA97a, TA98, TA100 (only with S9) and TA102	Bün96
trans-$[Rh(NH_3)_4Cl_2]Cl$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) + (strong): TA100 and TA102 when incubated in light +: TA100 and TA102 when incubated in dark	LaV86
	2) Ames test (*S.typhimurium*): dose not given(*)	2) +: TA92, TA100; -: TA98, *his*G46, TA1535, TA1537, TA1538	War81
$[Rh(NH_3)_5Cl]Cl_2$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) +: only in TA 102 when incubated in dark	LaV86
	2) Ames test (*S.typhimurium*): dose not given(*)	2) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
trans-$[Rh(NH_3)_4Br_2]Br$,	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) + (strong): TA100 and TA102 when incubated in dark or light	LaV86
$[Rh(NH_3)_5I](ClO_4)_2$	1) the same	1) + (weak): only in TA100 when incubated in dark	LaV86
$[Rh(NH_3)_5H_2O](ClO_4)_3$	1) the same	1) +: only in TA100 when incubated in light	LaV86
Rhodium(III) 1,10-phenanthroline-derivate compounds:			
$[Rh(phen)_3]Cl_3$	1) Ames test (*S.typhimurium*): dose not given(*)	1) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
cis-$[Rh(phen)_2Cl_2]Cl$	1) The same	1) +: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81

(*) TA92, TA98 and TA100 contain plasmid pKM101.

Table 1 Continued (2) ...

	type of assay and doses used	results (+, positive; -, negative)	ref.
Rhodium(III) bipyridyl-derivate compounds:			
$[Rh(bpy)_3]Cl_3$	1) Ames test (*S.typhimurium*): dose not given(*)	1) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
cis-$[Rh(bpy)_2Cl_2]Cl$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) + (strong): only in TA100 when incubated in light	LaV86
	2) Ames test (*S.typhimurium*): dose not given(*)	2) +: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81
cis-$[Rh(bpy)_2Br_2]Br$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) + (strong): TA100 when incubated in light, +: TA102 (in light); TA100 and TA102 (in dark)	LaV86
cis-$[Rh(bpy)_2I_2]ClO_4$	1) the same	1) + (strong): TA100 (in light), +:TA102 (in light) -: when incubated in dark	LaV86
Rhodium(III) pyridyl-derivate compounds:			
$[Rh(py)_3Cl_3]$, $[Rh(py)_3(SCN)_3]$	1) Ames test (*S.typhimurium*): dose not given(*)	1) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
trans-$[Rh(py)_4Cl_2]Cl$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) + (strong): TA102 when incubated in dark or light -: TA100 when incubated in dark or light	LaV86
	2) Ames test (*S.typhimurium*): dose not given(*)	2) +: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81
trans-$[Rh(py)_4Br_2]Br$	1) Spot test (*S. typhimurium* TA100, TA102): 0.06 mL of a 2 mg/mL solution in growth medium, without metabolic activation	1) +: only in TA100 when incubated in light	LaV86
	2) Ames test (*S.typhimurium*): dose not given(*)	2) +: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81

(*) TA92, TA98 and TA100 contain plasmid pKM101.

Table 1 Continued (3) ...

	type of assay and doses used	results (+, positive; -, negative)	ref.
Rhodium(III) quinoline-derivate compounds:			
[#]*cis*-[Rh(biq)$_2$Cl$_2$]Cl, [#]*cis*-[Rh(pq)$_2$-Cl$_2$]Cl, *mer*-[Rh(pq)Cl$_3$(H$_2$O)], *fac*-[Rh(pq)Cl$_3$(H$_2$O)], *cis*-[Rh(pq)$_2$-Cl$_2$][Rh(CO)$_2$Cl$_2$]H$_2$O, *cis*-[Rh(biq)$_2$- Br$_2$]Br, *cis*-[Rh(pq)$_2$Br$_2$]Br.	1) Ames test (*S. typhymurium* TA1535, TA98): 10-400 µg/plate, without metabolic activation	1) All compounds were postive, of which *cis*-[Rh(biq)$_2$Cl$_2$]Cl was the most active compound.	Sad97
	2) only *cis*-[Rh(biq)$_2$Cl$_2$]Cl: chromosome aberration assay in lymphocytes, derived from two healthy non-smoking male volunteers: 0.1, 0.5, 1.0 and 20 µg/mL, exposure duration 3 or 20 hours, no metabolic activation system added	2) *cis*-[Rh(biq)$_2$Cl$_2$]Cl showed to be clastinogenic	Sad00
[#] biq=2,2'-biquinoline; pq=2-(2'-pyridyl)quinoline			
Rhodium(III) ethylenediamine-derivate compounds:			
cis-[Rh(en)$_2$Cl$_2$]Cl	1) Ames test (*S.typhimurium*): dose not given(*)	1)+: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81
trans-[Rh(en)$_2$Cl$_2$]Cl, [Rh(en)$_3$]Cl$_3$	1) The same	1) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
Other organometallic rhodium(III) compounds:			
[Rh(1,2-propanediamine)$_3$]Cl$_3$	1) Ames test (*S.typhimurium*): dose not given(*)	1) -: TA92, TA98, TA100, *his*G46, TA1535, TA1537, TA1538	War81
trans-[Rh(3-picoline)$_4$Cl$_2$]Cl, *cis*-[Rh(triethylenetetramine)Cl$_2$]Cl, [Rh(CH$_3$CN)$_3$Cl$_3$]	1) The same	1) +: TA92, TA89, TA100; -: *his*G46, TA1535, TA1537, TA1538	War81

(*) TA92, TA98 and TA100 contain plasmid pKM101.

Annex **E**

Classification of substances with respect to carcinogenicity

See next page.

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

Judgement of the committee	Comparable with EU class
This compound is known to be carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen.	3
This compound has been extensively investigated. Although there is insufficient evidence of a carcinogenic effect to warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern.	(A)
This compound has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is a cause for concern.	(B)
This compound cannot be classified	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Limited evidence of a carcinogenic effect

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds); if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.