Warfarin

(CAS No: 81-81-2)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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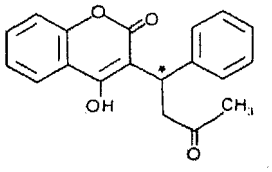
1 Introduction

The present document contains the assessment of the health hazard of warfarin by the Commitee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was written by AAE Wibowo, Ph.D. and MM Verberk, Ph.D. (Coronel Institute, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands).

The evaluation of the toxicity of warfarin has been based on the reviews by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACG98) and by Hall et al. (Hal80), Holbrook et al. (Hol96), Palareti and Legnani (Pal96), and Sutcliffe et al. (Sut87). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in September 1998, literature was searched in the databases Medline, Embase, Chemical Abstracts, starting from 1966, 1988, and 1970, respectively. HSELINE, CISDOC, MHIDAS, and NIOSHTIC (from 1985/1987-1998) and POLTOX (Toxline, Cambridge Scient Abstracts, FSTA; from 1990-1994), databases available on CD-ROM were also consulted. The following key words were used: warfarin and 81-81-2. The final literature search was carried out in Medline and Toxline in October 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. No comments were received.

2 Identity

name	:	warfarin
synonyms	:	(*RS*) 4-hydroxy-3-(3-oxo-1-phenylbutyl) coumarine (IUPAC name); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2*H*-1-benzopyran-2-one;(1-(4'-hydroxy-3'coumarinyl)-1-phenyl-3 butanone;3-(alpha-acetonylbenzyl)-4-hydroxycoumarin;coumadin; zoocoumarin
molecular formula	:	$C_{19}H_{16}O_4$
structural formula	:	
CAS number	:	81-81-2

* Chiral centre.

3 Physical and chemical properties

molecular weight	:	308.3
melting point	:	159-161°C
boiling point	:	decomposes
flash point	:	not available
vapour pressure	:	at 21.5°C: 9 Pa
solubility in water	:	not soluble (at 20°C: 1.7 mg/100 mL)
log $P_{octanol/water}$	:	2.52, 2.60 (experimental); 2.23 (estimated)
conversion factor	:	not applicable

Data from ACG98, NLM02, http://esc.syrres.com.

Warfarin is a colourless, odourless, and tasteless white crystalline powder. It is readily soluble in acetone and dioxane and moderately soluble in alcohols. It is acidic. The sodium salts are soluble in water. Contact with strong oxidisers may cause fire and explosions. Commercial warfarin is a racemic mixture of approximately equal amounts of the *R* and *S* enantiomers as either a potassium or a sodium salt (Hol96).

4 Uses

Warfarin is used as an anticoagulant drug as well as a rodenticide. As an oral anticoagulant drug, warfarin is effective in the prevention and treatment of deep vein thrombosis, and also in the prevention of thromboembolic disease. As a rodenticide, warfarin is applied to discrete sites in agriculture and urban rodent control in the form of baits containing 0.025% active ingredient. The sodium salt is available at 0.5% concentrate for use at a final concentration of 0.05% in liquid base (ACG98, Hol96, NLM02).

According to the database of the Dutch Pesticide Authorisation Board (CTB)*, warfarine is at present not permitted in the Netherlands for use as an active ingredient in pesticides. It is not registered for use as a drug.

* At: http://www.ctb-wageningen.nl.

5 Biotransformation and kinetics

Human data

In vivo studies

No qualitative or quantitative data were found on the percentage of pulmonary absorption of warfarin in humans. Warfarin caused intoxication in a worker following skin contact with the 0.5% concentrate. However, no quantitative data on the percentage absorption of warfarin was given (Fri65). Warfarin absorption following oral intake was studied in 4 patients on long-term treatment with warfarin. Subjects were given single doses of 4-[^{14}C]-racemic warfarin of 0.5 mg/kg bw. Four and 25 days after treatment, 60% and 92% of the radioactivity, respectively, were excreted in the urine. In another experiment by the same author, 8 human volunteers were given single oral doses of racemic (*rac*) warfarin of 0.5 mg /kg bw. To assess the percentage of the dose absorbed, the same volunteers received an intravenous injection of 0.5 mg/kg, 2 weeks later. The subjects were given vitamin K_1 prior to warfarin administration to avoid signs of toxicity. The percentage of the oral dose absorbed varied between 78 and 105%, and was complete by 120 minutes after administration. The compound reached maximal plasma concentrations (range: 4-7 mg/L) by 25 to 60 minutes after administration. The mean half-life of elimination from the plasma was about 36 hours. The conclusion of these studies was that warfarin was rapidly and extensively (>95%) absorbed from the gastrointestinal tract (Bre73a). In an earlier human volunteer study (n=15), a much longer time to reach peak concentrations (3 to 9 hours) was found following ingestion of single doses of 1.5 mg *rac*-warfarin/kg bw. The maximum plasma warfarin concentrations ranged from 8.6 to 17.5 mg/L (mean: 12 mg/L) and the plasma half-life ranged from 15 to 52 hours (mean: 42 hours) (ORe63). When 2 patients were given a single oral dose of 10 mg *rac*-warfarin (ca. 0.14 mg/kg bw), maximum plasma concentrations were reached by 30 to 60 minutes (McA92). Binding to albumin and extensive enterohepatic circulation contribute to the long plasma half-life (Jah77). The presence of food slowed the rate of warfarin absorption but did not affect its bioavailability (Mus76).

The relationship between dose levels of warfarin and steady-state warfarin concentrations in plasma or serum was reported in 2 studies. When 5 human volunteers were given therapeutic daily doses of 10 mg *rac*-warfarin (ca. 0.14 mg/kg bw/day) for 26 days, a steady-state plasma concentration (mean: 2.6

mg/L) was reached after 11 days (Jai79). Daily ingestion of non-therapeutic doses of 0.2 or 1.0 mg (ca. 0.003 and 0.015 mg/kg bw/day) *rac*-warfarin by human volunteers (n=7/group), for 3 weeks, resulted in steady-state serum concentrations of 0.060 or 0.231 mg/L after 2 weeks (Cho88).

The kinetics of the enantiomers *R*- and *S*-warfarin were also investigated in several human studies. When 10 human volunteers received single oral doses of *rac*-warfarin, *R*-warfarin, and *S*-warfarin of 1.5 mg/kg bw in separate experiments, *rac*-warfarin and its 2 enantiomers were absorbed from the gastrointestinal tract to the same extent. The mean half-lives of elimination from the plasma for *R*-warfarin, *S*-warfarin, and *rac*-warfarin were 58, 33, and 42 hours, respectively (ORe74). In another study, mean plasma half-lives of 45, 26, and 42 hours were found in 8 human volunteers receiving single oral doses of 0.75 mg/kg bw each of *R*-warfarin, *S*-warfarin, and *rac*-warfarin, respectively (Lew74). The plasma half-lives of the warfarin enantiomers were compared when single oral doses of 0.5 mg/kg bw of each enantiomer were given to 9 subjects or when daily oral doses of 5-17 mg (ca. 0.07-0.25 mg/kg bw/day) of each enantiomer were given to 8 subjects for 17 days. The mean plasma half-life of *R*-warfarin after a single dose was 35 hours and was significantly longer than that of *S*-warfarin (24 hours). After repeated doses to steady-state plasma concentrations, the mean plasma half-life of *R*-warfarin (54 hours) was also significantly longer than that of the *S*-isomer (32 hours). The plasma half-life of *R*- but not of *S*-warfarin was significantly longer after repeated than after single doses. Subjects (n=4) receiving daily doses of 5 mg/day of each enantiomer for 17 days had mean steady-state plasma concentrations of 1.1 and 0.97 mg/L for the *R*- and *S*-isomer, respectively (Bre73b). When 5 human volunteers took 1 mg of each enantiomer for 2 weeks, steady-state serum concentrations of 0.248 and 0.175 mg/L were measured for the *R*- and *S*-isomer, respectively (Cho86).

When absorbed, warfarin is bound to plasma proteins, mainly albumin (97.4 to 99.9%). The anticoagulant activity of warfarin is a function of the concentration of the unbound drug in plasma. Albumin-bound warfarin is pharmacologically inactive and is not biotransformed and excreted. The small apparent volume of distribution of warfarin reflects the high protein binding (Hol96). The binding of the *S*-enantiomer to albumin is greater than for the *R*-enantiomer (Par88). The unbound fraction is distributed mainly to the liver, where it binds strongly in a saturable way to the target enzyme, vitamin K-2,3-epoxide reductase, in liver microsomes (Pal96).

Warfarin is metabolised in the smooth endoplasmatic reticulum of the liver, involving stereospecific pathways catalysed by a variety of cytochrome P450 isoenzymes (Hol96, Pal96). The metabolism of *rac*-warfarin has been studied in

healthy human volunteers, who were given single oral doses of 1.5 mg/kg bw of sodium warfarin. Major metabolites excreted within 72 hours after administration were the regioisomers 6- and 7-hydroxywarfarin, formed by hydroxylation reactions, and 2 diastereoisomeric warfarin alcohols, formed by acetonyl (keto) reduction. No warfarin (<2% of the dose) was excreted in the urine. No quantitative data of the amount of excreted products were given (Lew70). In a subsequent study, single oral doses of 0.75 mg/kg bw of *rac*-warfarin were given to 3 volunteers, and as a single oral dose of 1.5 mg/kg bw to 1 subject. The percentage of the dose, excreted in urine collected for 7-10 days after administration, was in the range of 33-55%. The main metabolites were 7- and 6-hydroxywarfarin (47% and 32% of total urinary metabolites, respectively), while *RS*- and *SS*-warfarin alcohol were excreted in amounts of 11% and 7.5% of total urinary metabolites. Unchanged warfarin represented about 3.5% of total urinary metabolites (Lew74). It is likely that some warfarin is eliminated in the bile and undergoes enterohepatic circulation (Hol96). No warfarin was detected in the faeces of human volunteers given single oral doses of 1.5 mg/kg bw of sodium warfarin (ORe63).

The metabolism of *rac*-warfarin in humans is presented in Figure 1 (see Annex I).

An examination of the metabolic fate of the *R* and *S*-enantiomers of warfarin revealed that the 2 enantiomers were metabolised by different routes. In a human study, single oral doses of each enantiomer were given to 5 human volunteers in separate experiments, in the amount of 0.75 mg/kg bw. Following administration of *R*-warfarin and *S*-warfarin, 20-31% and 48-71% of the dose were excreted in the urine, respectively, within 7 to 10 days. Major metabolites of *R*-warfarin were 6-hydroxywarfarin and (*R,S*)-warfarin alcohol (50% and 30% of total urinary metabolites, respectively). The metabolite 7-hydroxywarfarin was formed to a smaller extent (13% of total urinary metabolites). In contrast, the main metabolite of *S*-warfarin was identified as 7-hydroxywarfarin (66% of total urinary metabolites). Other metabolites were 6-hydroxywarfarin and *S,S*-warfarin alcohol (Lew74).

Animal data

The committee did not find quantitative data on the percentage of pulmonary or skin absorption of warfarin in experimental animals. Indirectly, skin absorption has been demonstrated in rats and guinea pigs, by the measurement of the effects

of warfarin on blood coagulation (Fri65, Sag75) (see Section 6). In order to assess the amount of absorption via the oral route in rats (Sprague-Dawley), urinary excretion following either single oral doses or intravenous injections of 4-[^{14}C]-warfarin of 1 mg/kg bw was compared. At 100 hours after treatment, 43% or 41% of the dose were excreted in the urine after oral or intravenous administration, respectively. In faeces, 9.3% of the radioactivity could be isolated. The same experiment was conducted in rats, fitted with biliary fistula to prevent enterohepatic circulation. At 60 hours, 39.4 and 18.9% of the oral dose were found in the bile and the urine, respectively, while similar figures were obtained (47.5% in bile, and 21.3% in urine) after intravenous administration. Thus, elevated levels of radioactivity excreted in urine in animals without biliary fistula indicate the existence of an enterohepatic circulation for warfarin and metabolites in the rat (Los72).

The kinetics of 4-^{14}C-labelled *S*- and *R*-warfarin have been reported in rats, following a single intraperitoneal injection of 0.4 mg/kg bw of each enantiomer. At 150 hours after administration, ca. 65% of the *R*- and 50% of the *S*-warfarin dose were excreted in the urine, and 9% of the dose of either enantiomer in the faeces. No radioactivity was detectable in expired air (God69).

When absorbed, the liver showed the greatest affinity for warfarin in the rat. However, distribution and tissue uptake has also been demonstrated in extrahepatic tissues, e.g., heart and skeletal muscle, but probably reflected blood distribution rather than actual uptake of warfarin (And67). Binding of warfarin to plasma albumin is high in the rat, followed by sheep, dog, and horse (Sel77). A 6-fold greater binding to human, compared to canine plasma albumin has been demonstrated (ORe70). However, human and rat are more sensitive to the anticoagulant effects of warfarin than the other species investigated, suggesting that plasma albumin binding does not account for variations in the pharmacological response between species (Sut87). Following single intravenous administration of doses ranging from 1 to 12 mg/kg bw, the half-life of elimination of warfarin from plasma in rat, monkey, dog, and man was on average 9.9, 11.1, 22.5, and 36 hours, respectively (Nag69). The disappearance rate of unbound warfarin from plasma, compared to total warfarin, was faster in fasted rats (Lal77).

The metabolism of warfarin has been studied in the rabbit, the rat, and the guinea pig. When rabbits were given a single oral dose of racemic 4-[^{14}C]-warfarin (1 mg/kg bw), 82% or 90% of the radioactivity were excreted in the urine within 48 or 96 hours after administration, respectively. The major urinary metabolites were the warfarin alcohols (31% of the dose), 4'-hydroxycoumarin (11% of the dose), 6-and 7-hydroxycoumarin (7 and 3% of the dose,

respectively), and their glucuronides (7.5% of the dose). Unchanged warfarin (12% of the dose) was also excreted in the urine. Following an intravenous injection of 1 mg/kg bw, the bile was a minor excretory route for warfarin (6.2% of the dose after 6 hours), and most metabolites were glucuronide conjugates of warfarin and its hydroxylated metabolites (Won80).

When male Holtzman rats (n=13) were given a single intraperitoneal injection of an aqueous solution containing 1 mg of 4-[^{14}C]-warfarin, 67% of the dose was excreted in the urine and 33% in the faeces within 7 days after administration. The metabolites excreted in the urine were 7-hydroxywarfarin (23% of the dose), 4'-hydroxywarfarin (14% of the dose), 6-hydroxywarfarin (10% of the dose), 8-hydroxywarfarin (6% of the dose), a glucuronide of 7-hydroxywarfarin (2.6% of the dose), and an intramolecular condensation product: 2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(3,2-*c*)(1)benzopyran (4.4% of the dose). No warfarin alcohols were detected. Unchanged warfarin represented about 4.4% of the dose. Qualitatively, the same metabolites were identified in the faeces, but no quantification of metabolites was conducted. According to the authors, the similarities in urine and faeces metabolites suggest that enterohepatic circulation of metabolites did occur (Bar70).

Male guinea pigs received a single 1 to 2 mg/kg bw of 4-[^{14}C]-warfarin by intraperitoneal injection. About 86% of the administered radioactivity was excreted in the urine within 7 days after injection, most of it (50% of the dose) during the first 12 hours. Another 9% of the dose was excreted in the faeces within 7 days. The major metabolite excreted in the urine during 7 days after injection was 4'-hydroxywarfarin (24% of the dose). Other metabolites were 6-hydroxywarfarin (4% of the dose), salicylic acid (4% of the dose), 7 and 8-hydroxywarfarin (2% of the dose each), and 2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(3,2-*c*)(1)benzopyran (4% of the dose). Unchanged warfarin was excreted in about 11% of the administered dose. These metabolites were detected qualitatively in the faeces, but were not quantified (Dec73). In another study, male guinea pigs were given a single intraperitoneal injection of [^{14}C]-warfarin (1 mg/kg bw). The maximum blood level was reached within 1 hour, and the half-life of elimination from the blood was 9.6 hours. Radioactivity in tissues at 6 hours after administration was highest in the kidney, followed by bile, and liver. About 70% of the dose was excreted in the urine and 6% in the faeces at 48 hours after administration. However, 28% or 42% of the dose were excreted in the urine or the bile during the first 12 hours after administration. The major metabolite in urine and bile was 4'-hydroxywarfarin, followed by 7- and 8-hydroxywarfarin. A minor metabolite was 2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(3,2-*c*)(1)benzopyran. Wong et al. concluded that a majority of

warfarin metabolites, excreted in the bile, underwent enterohepatic circulation and was disposed of in the kidney in this species (Won78).

In vitro studies

The cytochrome P450 isoenzymes involved in the metabolism of warfarin have been studied in *in vitro* experiments, using either human cDNA-expressed cytochrome P450 or isoenzymes isolated from human liver microsomes. Cytochrome P450 2C9 has been claimed to be responsible for the metabolism of the *S*-isomer, whereas degradation of the *R*-isomer was determined mainly by cytochrome P450 1A2. Cytochrome P450 3A4 is involved in the metabolism of both isomers (Ret92, Wan83).

6 Effects and mechanism of action

Warfarin inhibits the synthesis of vitamin K-dependent clotting factors II (prothrombin), VII, IX, and X. The mechanism of this anticoagulant effect is based on interruption of the vitamin K recycling process in the liver, by inhibiting the activity of the enzymes vitamin K epoxide reductase and vitamin K quinone reductase. Vitamin K epoxide catalyses the conversion of vitamin K 2,3-epoxide to vitamin K quinone while vitamine K quinone reductase catalyses the subsequent conversion of the quinone to the active form of vitamin K, the hydroquinone. The vitamin K quinone can also be reduced to the hydroquinone form by a second NADPH-dependent enzyme, which is not inhibited by coumarin derivatives. Vitamin K hydroquinone is a substrate in the reaction that leads to the formation of γ-carboxyglutamyl residues in precursor proteins, to form active vitamin K-dependent clotting factors II, VII, IX, and X. This γ-carboxylation of glutamic acid residues at the *N*-terminal regions of precursor proteins, named PIVKAs (proteins induced by vitamin K antagonists), is catalysed by a vitamin K-dependent carboxylase enzyme, and requires molecular oxygen and carbon dioxide. Concomitantly to this process occuring in the liver only, vitamin K hydroquinone is epoxidised to vitamin K 2,3-epoxide. By the action of warfarin on the enzymes of the vitamin K cycle, vitamin K epoxide accumulates, leading to a reduced supply of vitamin K hydroquinone, and subsequently to inhibition of γ-carboxylation of PIVKAs, thereby interrupting the supply of functional vitamin K-dependent clotting factors. Depression of the plasma concentration of vitamin K-dependent clotting factors will produce a prolongation of the prothrombin time. Daily dosing with 2.5 to 15 mg warfarin, e.g., patients on long-term anticoagulant therapy, will result in low levels (10 to

30% of normal activity) of vitamin K-dependent clotting factors, with a danger to bleeding (Hir92, Hol96, Pal96, Sut87). In the occupational setting, activities below 70-80% of normal plasma activity are considered as adverse (Sit94).

The vitamin K cycle in the liver and its inhibition by coumarins (e.g., warfarin) is shown in Figure 2 (see Annex II).

In extrahepatic tissues (lung, kidney, pancreas, spleen, bone, placenta), vitamin K-dependent proteins containing glutamic acid residues, other than clotting factors, have been found. Consequently, warfarin may have an effect on these proteins by antagonising the action of vitamin K in these tissues (Sut87).

Human data

Irritation and sensitisation

The most important non-haemorrhagic side effect of warfarin is skin necrosis. Warfarin-induced skin necrosis occurs predominantly in females (85% of the patients), usually in areas rich in subcutaneous fat tissues (breasts, thighs, and buttocks) with a sudden onset of painful or cold sensation in the affected areas, and the appearance of well-demarcated erythematous lesions that progressed to purpuric and haemorrhagic areas. In males with skin necrosis, the distribution of lesions was similar to that in females, except that the breasts were usually spared, while involvement of the penis was common (Sal97). The first symptoms of skin injury usually appear within 10 days of therapy with a peak incidence being between days 3 and 6. Cases of rare late-onset warfarin-induced skin necrosis have been reported after 15, 16, and 17 days, 3 months, 17 months, and 3 years of warfarin therapy (Ess98). Some cases of warfarin-induced skin necrosis are reported below. A 64-year-old female patient developed ecchymoses on the forearms, hands, left breast, and right thigh and hip, after a total dose of 35 mg warfarin during 3 days. The lesions progressed through a blistering stage to a dry gangrene with eschar formation. Microscopic examination of the skin showed areas of vascular congestion and multiple platelet thrombi in the small arterioles and veins (Lac75). Another case dealt with a 53-year-old man who experienced 2 episodes of skin necrosis on his left flank and buttock, 5 days after initiation of warfarin therapy for thrombophlebitis. The lesions formed multiple haemorrhagic bullae that ruptured and an eschar that did not heal and eventually required skin grafting (Hor81). The same author reported on a 79-year-old woman, who developed an area of erythema surrounded by a halo on her left

thigh, 7 days after initiation of warfarin therapy. The erythematic area turned into a blue-black colour and rapidly formed an eschar deep into the subcutaneous tissue. A 37-year-old woman was admitted with an erythematous area on her right thigh that turned black and subsequently formed an eschar. Her prothrombin time was 2-fold the control level (Hor81). Late-onset warfarin-induced skin necrosis, 16 days after the initiation of warfarin therapy, was reported in a 34-year-old female patient. She was treated with 10 mg warfarin/day for 4 days, which was decreased to 5 mg/day before discharge from the hospital. Petechiae developed in thighs, legs, and left under arm. Lesions progressed to blue-black ecchymotic areas with several bullae. Microscopic examination revealed subepidermal haemorrhage with adjacent epidermal necrosis. Unlike other cases of warfarin-induced skin necrosis, the skin lesion was not associated with either a deficiency of protein C, or resistance to activated protein C (Ess98).

Cases of dermatitis have been reported in the older literature. A 50-year-old man developed transient urticaria, 40 minutes after the oral administration of 50 mg of warfarin sodium. The rash completely subsided in 2 days, following treatment with diphenhydramine (She59). In another case, a 63-year-old male patient developed pruritic, maculopapular, erythematous eruption on the face, neck, hands, and forearms after treatment with an initial dose of 75 mg sodium warfarin, followed by a daily maintenance dose of 7.5 mg for 27 days. Lesions disappeared with steroid therapy, but recurred upon further treatment with warfarin (Cra60).

Short-term toxicity

There is very little information on health effects of workers engaged in the manufacture or use of warfarin. One case has been reported, in which a 23-year-old farmer developed signs of poisoning following regular skin contact with a 0.5% warfarin solution, used for the preparation of baits, during a 4-week period. Two days after the last skin contact with warfarin, gross haematuria appeared. The next day haematoma were noticed on arms and legs. There was a dull pain in both groins. Haematuria subsided after 3 days of rest, but recurred along with epistaxis, haemorrhages from the mucosa of palate and mouth, and bleeding from the lower lip, 1 day after he returned to work. When admitted to hospital, prothrombin time was increased, and haemoglobin and red blood cell count decreased. Following treatment with vitamin K_1 (phytonadione), the subject responded promptly. Two days after treatment, haematology and urine parameters did not show abnormalities (Fri65). Cases of a warfarin-induced

haemorrhagic syndrome were reported in 741 Vietnamese infants. The cause was identified as dermal exposure to talcum powder, contaminated with 1.7% to 6.5% warfarin. 177 out of the 741 children died (Mar83).

Several reports have been published on poisoning cases, when warfarin bait, used as a rodenticide, was accidentally or deliberately mixed with food, or taken in an attempt to commit suicide. In Korea, a family of 14 persons lived for a period of 15 days on a diet consisting almost entirely of corn meal-containing warfarin. The first symptoms appeared 7-10 days after the eating of warfarin-contaminated food began. A 19-year-old man and a 3-year-old girl died after 15 days, after having ingested an estimated total warfarin amount of 12.5 and 31 mg/kg bw, respectively. The other 12 persons, aged between 8 and 70 years, survived after having received vitamin K_1 therapy. The ingested total amounts of warfarin ranged from 4 to 22 mg/kg bw. Symptoms of toxicity were ecchymosis, epistaxis, and gum haemorrhages (Lan54). One case dealt with murder on a 32-year-old man, who was given an estimated daily amount of 60-90 mg warfarin in his food for 15 days. On the 4th day after intake started, the victim developed severe nosebleeds. Later, he bled from the mouth. Two days before death, he complained of pain in his limbs. He died of circulatory failure on day 15. Macroscopic examination revealed haemorrhages in muscles, intestine, lungs, heart, and kidneys. Microscopic examination showed congestion and oedema in the lungs and liver injury (Pri66). In another case, a 73-year-old woman developed episodes of hypoprothrombinaemia each time she had ingested a cough syrup, which had been deliberately contaminated by her daughter-in-law with a warfarin-containing rat killer. Symptoms of toxicity were backache, haematuria, epistaxis, and bruises on the arms and the legs. The estimated ingested amount of warfarin was 20-40 mg/day. Recovery occurred after vitamin K treatment (Nil57). In a suicide attempt, a 22-year-old man had consumed daily amounts of approximately 20 g of a 0.5% warfarin formulation, in the form of a dry powder, over a 6-day period. The total ingested warfarin dose was estimated approximately 600 mg. Symptoms of toxicity were back pain, abdominal pain, epistaxis, and haematuria. Prothrombin time and Lee-White coagulation time were prolonged, but no abnormal haemoglobin level was found. Following treatment with vitamin K_1, he recovered 14 days after having taken the last dose (Hag53, Hol52).

The above poisoning cases have given some insight in the dose-response relationship following ingestion of warfarin. The relationship between warfarin dose or plasma warfarin concentration and anticoagulant activity has been examined in a series of human volunteer studies and in patients receiving therapeutical doses of warfarin. In a human volunteer study, 14 subjects received

a single oral dose of *rac*-warfarin of 1.5 mg/kg bw (see Section 5). The anticoagulant action of warfarin, i.e., inhibition of hepatic synthesis of clotting factors II, VII, IX, and X (the prothrombin complex), was determined by the measurement of the plasma prothrombin time (PT) by the one-stage prothrombin method of Quick. In all subjects, PTs were significantly increased (i.e., prothrombin complex activity significantly depressed) within 24 hours. The maximal PT increment was reached between 36 and 72 hours after treatment. The average PT was still abnormal at 144 hours after dosing. There was a significant correlation between the plasma warfarin levels at 48, 72, and 96 hours, and prothrombin complex activity depression. For example, at 48 or 96 hours after warfarin dosing, average prothrombin complex activities were depressed by 70% or 50%, respectively, at an average plasma warfarin concentration of 1 mg/L (ORe63). In another study, 5 human volunteers received a daily therapeutical oral dose of 10 mg *rac*-warfarin (ca. 0.14 mg/kg bw) for 26 days (see Section 5). The mean steady state plasma warfarin concentration (2.6 mg/L) was associated with a mean PT of 20.9 seconds (normal range: 12-14 seconds) (Jai79). The effect of low dose administration of *rac*-warfarin on clotting factor activity and vitamin K_1 metabolism was studied in groups of 7 human volunteers, who were given daily oral doses of 0.2 or 1.0 mg (ca. 0.003 or 0.015 mg/kg bw/day) for 3 weeks (see Section 5). In the 1.0-mg group, at a mean steady state plasma warfarin concentration of 0.231 mg/L, there was a statistically, but not biologically significant prolongation of the mean PT of 0.9 seconds. This prolongation was mainly due to one volunteer, who showed a PT increment of 2.5 seconds and had a significant decrease in individual clotting factor activity. No statistically significant change in mean PT or any clotting factor activity was observed in the 0.2-mg group (steady state plasma warfarin concentration: 0.06 mg/L). However, when vitamin K_1 was given to all 7 volunteers in both groups, detectable levels of vitamin K 2,3-epoxide were observed that peaked approximately 2 hours after treatment. This indicates that warfarin inhibits the enzyme vitamin K epoxide reductase, even at 0.003 mg/kg bw/day (Cho88). The NOAEL for a biological significant inhibition of clotting factor activity was set at 0.015 mg/kg bw/day. In a group of 15 patients receiving warfarin at a mean daily dose of 4.6 mg (range: 2 to 7.5 mg) for 3 weeks, a mean steady state plasma warfarin concentration of 0.67 mg/L was required to achieve a PT ratio of 1.8. The mean free plasma warfarin concentration was 0.014 mg/L (Rou79). A clinical study in patients with metastatic breast cancer was conducted to investigate which daily warfarin dose was required to maintain a target international normalised ratio (INR) of 1.3-1.9. INR is the ratio of the PT in the patient to that in a normal person not treated with anticoagulant. This range of

INRs, which is equivalent to only a 1-3 second prolongation of the PT, is effective in the prevention of thromboembolic disease and is associated with less bleeding. The dose needed to achieve this target INR was 2.6 mg warfarin daily for on average 181 days (Lev94). Other therapeutical maintenance doses of warfarin as an anticoagulant were reported to be 3-9 mg/day, to keep the INR in the range of 2.0-4.5 (Lau92) or 2-10 mg/day (Maj95). It has been reported that low-intensity warfarin therapy with an INR of 2.0-3.0, which corresponds with a PT ration of 1.35-1.6, is as effective as high-intensity warfarin (INR>3.0) in the prevention of thromboembolism and is associated with less bleeding (Hir92).

The enantiomers of warfarin differ in their anticoagulant potency in man. Following single high dose levels (0.75 and 1.5 mg/kg bw), *S*-warfarin has been reported to have 1.6 (Lew74) and 3.4 (ORe74) times the potency of *R*-warfarin, respectively. At steady state therapeutical doses, *S*-warfarin was 1.6 (Bre73b) or 2.7 (Win78) times more active as an anticoagulant than the *R*-enantiomer. Both enantiomers produced a significant increase in prothrombin time, when given to 5 human volunteers at a daily dose of 1 mg each, for 2 weeks. At steady state, the increase in prothrombin time with *S*-warfarin (1.8 seconds) was statistically significantly higher than with *R*-warfarin (1.0 second). The greater anticoagulant potency of *S*-warfarin was reflected by a greater degree of inhibition of the enzyme vitamin K epoxide reductase, measured indirectly by increased levels of vitamin K 2,3-epoxide following treatment with vitamin K_1 (Cho86). The LOAEL for a biological significant increase of prothrombin time was set 0.015 mg/kg bw/day for *S*-warfarin. For *R*-warfarin, the NOAEL was 0.015 mg/kg bw/day.

The committee concludes that measurement of enantiomer concentrations may better predict anticoagulation than measurement of racemate concentrations.

A study has been reported on a group of 36 patients receiving long-term *rac*-warfarin therapy at daily doses of 2.5 to 12 mg (mean: 6.1 mg). The PT ratio was in the range of 2.0-3.3, at mean steady-state plasma concentrations of 0.48 and 0.87 mg/L for *S*- and *R*-warfarin, respectively. Chan et al. suggested that the degree of anticoagulation is best predicted by the concentration of the free (unbound) *S*-isomer of warfarin (Cha94).

The warfarin metabolites (*RS*)- and (*SS*)-warfarin alcohol showed anticoagulant activity. However, their potency was much less than for *rac*-warfarin (Lew73).

Reproduction toxicity

Coumarin derivatives, including warfarin, are teratogenic in man. Warfarin therapy specifically between weeks 6 and 9 of gestation may result in ‘warfarin embryopathy’, i.e., fetal malformations characterised by nasal hypoplasia and stippled epiphyses, while exposure during the second and third trimester is associated with disruptional abnormalities of the central nervous system (Hal80, Pau93). In a review concerning 418 reported pregnancies with coumarin derivative (including warfarin) therapy, one-sixth of these had resulted in abnormal liveborn infants, one-sixth in abortion or stillbirth, and two-thirds in apparently normal infants. A total of 11 (3%) liveborn infants had primarily haemorrhagic manifestations, 16 (4%) had findings consistent with warfarin embryopathy (i.e., nasal hypoplasia, with or without stippled epiphyseal calcifications that resemble chondrodysplasia punctata as well as defects of the bones and deformities of the limbs) whereas 11 (3%) had significant warfarin-induced central nervous system abnormalities. Maternal daily doses of warfarin, given between 6 and 9 weeks of gestation, ranged between 2.5 and 15 mg. In the 11 cases with central nervous system abnormalities, not clearly caused by intrauterine or perinatal haemorrhage, brain malformation, such as hydrocephalus, meningocele, and microcephaly, ophthalmological abnormalities, such as microphthalmia and optic atrophy, and other abnormalities, such as developmental retardation, and deafness were reported (Hal80). In later report, 15 studies published between 1980 and 1989 the effects of warfarine on pregnancy outcome were reviewed. Among the total of 635 pregnancies and 485 liveborns discussed in these studies, 8 (1.3% of the pregnancies; 1.6% of the liveborns) certainly had features of the ‘warfarin embryopathy’ while broad inclusion criteria resulted in 20 possibly affected infants, and 4 (0.6 or 0.8%, respectively) had central nervous system and/or ophthalmological abnormalities. From the aforementioned figures of Hall et al. (Hal80) and their own figures, Pauli and Haun thought that reasonable ranges of risk for ‘warfarin embryopathy’ would be 1.5-5% of exposed infants, only at exposure between gestational weeks 6 and 9, and for central nervous system effects 0.5-2%, most often resulting from second trimester exposure (Pau93). The mechanism giving rise to warfarin embryopathy may be related to the reduced γ-carboxyglutamate content of the bone osteocalcin. Inhibition of the synthesis of these proteins by coumarin derivatives during a critical embryological period of ossification could explain the teratogenic effects seen in warfarin embryopathy (Hal80, Hol96, Pau93).

No cases of teratogenicity have been reported following the use of warfarin as a rodenticide (WHO95).

Animal data

Irritation and sensitisation

The committee did not find data from experimental animal studies on the skin- or eye-irritating or sensitising properties of warfarin.

Acute toxicity

The results of acute lethal toxicity tests are summarised in Table 1.

Table 1 Summary of acute toxicity studies for warfarin in experimental animals

exposure route	vehicle	species (sex)	strain	LC_{50}/LD_{50}	reference
sodium warfarin					
inhalation		rat		320 mg/m^3 [a]	ACG98
dermal		rat		1400 mg/kg bw	ACG98
oral	water	rat (male)	Sprague-Dawley	323 mg/kg bw	Hag53
	water	rat (female)	Sprague-Dawley	58 mg/kg bw	Hag53
	CMC	rat (male)	Sprague-Dawley	100 mg/kg bw	Bac78
	CMC	rat (female)	Sprague-Dawley	8.7 mg/kg bw	Bac78
	peanut oil	rat (male)	Sherman	3.0 mg/kg bw	Gai60
		rat	Sherman	1.6 mg/kg bw	Hay67
		rat	AW 49	3.4 mg/kg bw	Nie73
	water	mouse (male, female)		374 mg/kg bw	Hag53
		mouse	NMRI	640 mg/kg bw	Nie73
	water	guinea pig (male, female)		182 mg/kg bw	Hag53
	water	rabbit (male, female)		800 mg/kg bw	Hag53
	water	dog (male, female)		200-300 mg/kg bw	Hag53
intravenous	water	rat (male, female)	Sprague-Dawley	186 mg/kg bw	Hag53
	water	rabbit (male, female)		100-200 mg/kg bw	Hag53
	water	dog (male, female)		200-300 mg/kg bw	Hag53
acid warfarin					
oral	CMC	rat (male)	Sprague-Dawley	112 mg/kg bw	Bac78
	CMC	rat (female)	Sprague-Dawley	10 mg/kg bw	Bac78

[a] Exposure time not given.
Abbreviations: CMC= carboxymethylcellulose.

There are large differences in lethality of *rac*-warfarin between species, sexes, and strains. In summary, rats were the most sensitive and mice and rabbits the

least sensitive species. In rats, oral LD_{50}s varied from 1.6 to 323 mg/kg bw, dependent on sex, strain, and mode of application. Female rats were 5 to 10 times more susceptible than males and Sprague-Dawley rats were less susceptible than other strains tested. The acute oral toxicity of *rac*-warfarin in Sprague-Dawley rats was higher when the compound was administered in carboxymethylcellulose than in water. Sodium warfarin and acid (enol) warfarin exhibited the same range of toxicity. When *rac*-warfarin was intravenously administered to rats or dogs, the acute toxicities were in the same range of toxicity.

When rats were treated with enzyme inducers phenobarbital, chlordane, or DDT for 4 days prior to administration of warfarin, a 10-fold increase in the LD_{50} of warfarin was observed (Ike68).

Signs of intoxication were convulsions in animals that succumbed within several hours after dosing. Haemorrhages were not observed in this group. Conversely, in animals dying delayedly, convulsions were not observed and haemorrhages were found in several tissues. In addition, the animals' appearance was marked by ruffled coat, pallor, and extreme lassitude (Hag53). Autopsy revealed haemorrhages in the intestine, thoracic cavity, peritoneal cavity, and urinary bladder (Bac78).

The committee concluded that the differences in acute toxicity of *rac*-warfarin between species, sexes, and strains, as well as differences in acute toxicity between enantiomers might be explained by differences in kinetics and metabolism on the one hand and by a difference in the inhibition of the synthesis of vitamin K-dependent clotting factors on the other hand.

Several studies have been reported on the anticoagulant effects of sodium warfarin following single application via different routes.

Rabbits or guinea pigs, treated dermally with aqueous sodium warfarin at single doses of 0.25 or 1.7 mg a.i./kg bw, respectively, showed a maximum inhibition of 32% or 42% of the activity of the prothrombin complex, respectively, at day 2 after administration. In guinea pigs, the same anticoagulant effect was observed following a single oral dose of 2 mg/kg bw (Fri65). In another study, Sprague-Dawley rats (n=4-5/group) were given sodium warfarin via the food at doses of 0, 1.3, 4.8, or 32 mg/kg bw for 1 day. On average, mean PTs were increased 1.7, 3.8, or 4.9-fold, respectively, compared to the control value. No mortality was observed at these doses (Hag53). The anticoagulant effect of warfarin was studied in AW49 rats and NMRI mice. A 3-fold increase in PT, compared to control animals, was found at a single oral dose of 1 mg/kg bw for rats and at 10 mg/kg for mice (Nie73).

The effects of organic solvents on the anticoagulant response to warfarin treatment were investigated in male Sprague-Dawley rats. A 2.8 to 3.6-fold increase in PT compared to control rats was found, 24 hours after a single subcutaneous injection of 1 mg/kg bw sodium warfarin in corn oil. Significant increments in PT were seen when warfarin (1 mg/kg bw) was administered simultaneously with hepatotoxic doses of styrene (4.4- to 6.9-fold) or trichloroethylene (4.8- to 7.4-fold). However, no statistically significant changes in PT were observed following the simultaneous injection with hepatotoxic doses of carbon tetrachloride. Pre-treatment of rats with either styrene, trichloroethylene, or carbon tetrachloride, at 24 hours prior to subcutaneous injection with warfarin (1 mg/kg bw), also caused an increment of PT compared to treatment with warfarin alone. Solvents alone had no effect on PT. The author concludes that acute exposure to organic solvents may lead to enhanced anticoagulant response to warfarin (Cha86).

Studies on species differences in anticoagulant response to warfarin showed that the rat, mouse, and human are most sensitive, guinea pigs, cats, and dogs intermediate, and the rabbit and cow least affected. It was suggested that the difference in affinity at the site of action between species and not plasma protein binding accounts for differences in pharmacological response (Sut87).

Differences in the anticoagulant potency of *S*-warfarin and *R*-warfarin were demonstrated in the rat prothrombin-time assay. A single oral dose of *S*-warfarin was 5.5 times as active as a single oral dose of *R*-warfarin, as measured at 24 hours after dosing. Single oral doses of 0.375 mg/kg bw of *S*-warfarin and 1.9 mg/kg bw of *R*-warfarin showed parallel time–response curves between 0 to 48 hours after treatment. Maximum prothrombin times were increased approximately 3.5-fold, compared to control values at 36 hours after dosing (Ebl66). In another study, it was demonstrated that following a single intravenous administration, *S*-warfarin was 2 times more potent than *R*-warfarin in its ability to inhibit clotting factor synthesis (Bre72). In rabbits, following a single intravenous administration, the minimum plasma concentrations to achieve complete inhibition of clotting factor synthesis were 103, 99, and 25 mg/L for *rac*-warfarin, *R*-warfarin, and *S*-warfarin, respectively, indicating that the *S*-enantiomer is the most potent anticoagulant (Bre85).

Short-term toxicity

When a single dose of 0.4 mg/kg bw of an aqueous solution of sodium warfarin was applied to the skin of rabbits for 2 consecutive days, the activity of the prothrombin complex was decreased by 62% at post-treatment day 2. At day 5,

the activity was returned to normal (Fri65). In another dermal study, an aqueous solution of sodium warfarin was administered to the skin of female Wistar rats (n=7/group) at doses of 0,10, 50, or 100 mg a.i./kg bw/day, for 3 consecutive days. On the basis of statistically significant increased clotting times at the 2 higher dose levels, compared to control animals, it was concluded that skin absorption did occur. The anticoagulant effect of 3 topical applications at 50 mg/kg bw/day was about the same as that of 3 oral doses of 0.6 mg/kg bw/day (Sag75).

Sprague-Dawley rats (n=4-6/group) were given sodium warfarin via the diet at doses equivalent to approximately 0.13, 0.35, 0.4, 1.0, 1.5, 3.0, 6.0, or 18 mg/kg bw/day for various time periods. Doses of 0.4 mg/kg bw/day and above caused death of all animals within about 7 to 11 days. One out of 4 rats given 0.35 mg/kg/day for 6 months died, but all 4 rats given 0.13 mg/kg bw for 8 months survived. In another experiment by the same authors, rats (n=10/group) received daily doses of sodium warfarin via the diet, varying from 1.2, 6.9, or 28 mg/kg bw for 2 days to 1.1, 4.4, or 17 mg/kg bw for 5 days. Mortality was 50% or more in the high-dose group after 2 to 5 days feeding and in the mid-dose group after 3 to 5 days feeding. In the low-dose group, 2 or 1 out of 10 animals died after 4 or 5 days feeding respectively. In a separate experiment, the authors also determined the PTs of animals (n=2-4/group) treated with the same doses for 1 to 5 days. After 2 days of feeding, mean PT values were increased 2.8, 4.9, or 5.7-fold and after 4 days feeding 7.7, 23, or 33-fold, respectively, compared to non-treated animals (Hag53). In another study, 5-day cumulative oral LD_{50}s of 2.1 and 25 mg/kg/day were found in AW49 rats and NMRI mice, respectively (Nie73). When groups of Sprague-Dawley rats (n=110) were given warfarin via the diet for 90 days, a cumulative oral LD_{50} of 0.077 mg/kg/day was found (Hay67). For *R*- and *S*-warfarin, 10-day cumulative oral (diet) LD_{50} values of 17.7 and 2 mg/kg diet (i.e., ca. 0.9 and 0.1 mg/kg bw/day, assuming a 200-g rat consumes 10 g diet/day), respectively, were calculated in male Sprague-Dawley rats (Elb66).

Dogs (greyhound; n=3/sex) were given warfarin via the diet at a dose equivalent to 10 mg/kg bw/day for 4 or 5 days. Signs of intoxication were apathy and reduced food intake by day 4 or 5 and anorexia and vomiting by day 7 or 8. Two dogs developed severe respiratory distress and 2 died suddenly from intraperitoneal bleeding. Blood urea levels were significantly increased after treatment, but no changes were observed in liver function tests. None of the animals showed evidence of gastro-intestinal bleeding. At the end of treatment, the average PT was increased more than 18-fold compared to pre-exposure values. Levels of factors II, IX, and X at the end of treatment were reduced to

7%, 3.5%, and 3% of pre-exposure levels. Platelet count, platelet aggregation, and platelet adhesiveness were not changed significantly after treatment. Average warfarin concentrations in plasma were 32.6 and 11.9 mg/L at days 4 and 7, respectively (For73).

Long-term toxicity and carcinogenicity

The committee did not find data from studies on the long-term toxicity, including carcinogenicity, of warfarin.

Mutagenicity and genotoxicity

The committee did not find data from studies on the mutagenicity or genotoxicity of warfarin

Reproduction toxicity

In a developmental toxicity study, pregnant F_1 mice (n=8-14/group) were intraperitoneally given doses of sodium warfarin of 0, 1, 2, or 4 mg/kg bw/day on days 3 to 11 of gestation. The mice were killed on gestational days 12 through 17. In the groups treated with 2 and 4 mg/kg bw/day, there was a very high incidence of haemorrhaged placentas and fetal deaths. Maternal deaths were also increased in these groups compared with the control group. PTs were 3.5- to 5-fold the control values when measured 24 hours after the final injection. No significant effects were found in mice treated with 1 mg/kg bw/day. No increase in the frequency of malformations of offspring was observed in any of the treated groups. When pregnant mice were given a single intraperitoneal dose of warfarin of 4 mg/kg bw on gestational day 10 or 11 and killed on day 18, the incidence of fetal deaths and the frequency of minor fetal malformations was significantly increased. Malformations included open eyelid and minor skeleton and ossification abnormalities, particularly of the sternum. Administration of vitamin K_1 to mice treated with warfarin on day 10 reduced the incidence of fetal deaths to that of the control mice. It was suggested that the embryotoxicity of warfarin is related to its vitamin K antagonism, rather than to its direct toxicity (Kro74).

In another study, Sprague-Dawley rats (n=5) were given warfarin at daily oral doses of 0 or 100 mg/kg bw on gestational days 1 through 12. Concurrently, daily intramuscular injections of 10 mg/kg bw of vitamin K_1 were given. Other groups of rats (n=6/group) received oral doses of warfarin of 0, 1, 3, 6, 12, 25, 50, or 100 mg/kg bw and concurrent daily intramuscular injections of 10 mg/kg

bw of vitamin K_1, on gestational days 9 through 20. The concurrent administration of vitamin K_1 creates an extrahepatic vitamin K deficiency while it allows normal synthesis of vitamin K-dependent clotting factors in the liver. Therefore, the experimental design is an animal model of the warfarin embryopathy observed in humans. None of these dosing regimens had any apparent deleterious effect on the dams. Maternal PTs were the similar among exposed and control animals. There were no haemorrhages in any of the dams, and all treated animals maintained their pregnancies. No significant abnormalities were observed in the fetuses when warfarin and vitamin K_1 were administered from day 1 to day 12 of pregnancy. However, similar treatment from day 9 to day 20 of gestation caused a significant decrease in mean litter size and an increase in resorptions at 100 mg/kg bw/day and a high incidence (28-37%) of haemorrhages in the fetuses examined on day 21 of gestation at 3 mg/kg bw/day and above. There were no haemorrhages in the control fetuses from dams receiving vitamin K_1 only and at 1 mg/kg bw, the incidence of haemorrhages was not significantly different from controls. Haemorrhages affected the fetal brain, face, eyes, and ears, and, occasionally, the limbs. Brain haemorrhages were often intraventricular and caused various degrees of hydrocephaly. Bony defects were not a feature of prenatal exposure to warfarin, probably because the vitamin K-dependent components of bone development occur post-natally in the rat. The NOAEL was estimated at 1 mg/kg bw/day (How90).

In a consecutive study, the same authors gave daily subcutaneous doses of sodium warfarin (100 mg/kg bw) and vitamin K_1 (10 mg/kg bw) for up to 12 weeks to Sprague-Dawley rats, starting on the day after birth. All rats survived without any sign of haemorrhage. However, the warfarin-treated rats showed a marked maxillonasal hypoplasia associated with an 11-13% reduction in the length of the nasal bones compared with controls, large calcified areas in the septal cartilage of the nasal septum (not present in the controls), and abnormal calcium bridges in the epiphysial cartilages of the vertebrae and long bones. The authors suggested that the septal growth retardation occurs because the warfarin-induced extrahepatic vitamin K deficiency prevents the normal formation of the vitamin K-dependent matrix carboxyglutamylic acid (GLA) protein in the embryo (How92).

The committee concludes that with the above rat model, most of the features of warfarin teratogenicity in the human have been demonstrated.

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for warfarin in the Netherlands is 0.1 mg/m^3, 8-hour TWA.

Existing occupational exposure limits for warfarin in some European countries and the USA are summarised in Annex III.

8 Assessment of health hazard

The major use of warfarin is as an anticoagulant drug in the prevention and/or treatment of thromboembolic disease. As a rodenticide, it is used in agriculture as a tracking dust and in urban rodent control as a bait, containing 0.025-0.05% active ingredient. Warfarin consists of a racemic mixture of equal amounts of 2 distinct enantiomers (*S* and *R*). Occupational exposure may occur during manufacture, formulation, and bait application. Workers can be exposed to warfarin through inhalation of dust or by direct skin contact with the compound. The committee did not find qualitative or quantitative data on the uptake of the compound through the lungs. In one occupational worker, signs of poisoning and prolongation of the prothrombin time indirectly demonstrated skin absorption of warfarin, but there were no quantitative data of the percentage of dermal absorption. In a human study, an oral dose of 4-[^{14}C]-*rac*-warfarin of 0.5 mg/kg bw was completely absorbed within 120 minutes after administration. *Rac*-, *S*-, and *R*-warfarin are absorbed to the same extent. When absorbed, warfarin is bound to plasma proteins, mainly albumin. The binding of *S*-warfarin to albumin is greater than that of *R*-warfarin. The elimination half-life of *rac*- warfarin from human plasma varies greatly among individuals and ranges from 15 to 52 hours (mean: 42 hours). The average plasma half-life of the *S*-enantiomer is shorter (33 hours) than that of the *R*-enantiomer (58 hours). Steady-state blood levels are reached after 7-10-day daily warfarin administration. The committee concludes that warfarin is a cumulative agent due to its long half-life. Binding to albumin and extensive enterohepatic circulation contribute to the long half-life. Warfarin is extensively, but slowly metabolised in the smooth endoplasmatic reticulum of liver cells and slowly excreted in the urine. In man, only 60% of total urinary metabolites were excreted within the first 4 days after a single oral administration. Major urinary metabolites of *rac*-warfarin are the regioisomers 6- and 7-hydroxywarfarin, and 2 diastereoisomeric warfarin alcohols. No warfarin was detected in the faeces. Metabolic patterns of the enantiomers differ in that the major metabolite of *S*-warfarin is 7-hydroxywarfarin and major

metabolites of *R*-warfarin are 6-hydroxywarfarin and (*R,S*) warfarin alcohol. Substantial differences in the metabolic pathways exist in various species, including rat, guinea pig, rabbit, and man.

Cases of warfarin-induced skin effects, including skin necrosis and dermatitis, have been reported when the drug was used as an anticoagulant in the clinical treatment of patients. Warfarin is highly toxic for mammalian species, including humans. The primary mechanism of warfarin toxicity is inhibition of the synthesis of vitamin K-dependent blood clotting factors. A cumulative total dose of about 1 g of warfarin consumed in 15 days, equivalent to about 60-90 mg/kg bw/day, has been reported to be fatal to a 32-year-old man. The main symptoms of warfarin poisoning in less severe cases are excessive bruising, nose and gum bleeding, pallor, haematomas around joints and on the buttocks, and blood in the urine and faeces. Bleeding from several organs within the body, leading to shock and possibly death, occurs in the more severe cases. The onset of the symptoms of warfarin poisoning may not be evident until a few days after exposure. A clinical study revealed that the lowest maintenance dose used in the prevention of thromboembolic disease, associated with minimal bleeding, was 2.5 mg warfarin/day (ca. 0.04 mg/kg bw), for on average 181 days. In a 3-week toxicity study in human volunteers taking low, non-therapeutical daily doses of *rac*-warfarin of 0.2 and 1 mg (ca. 0.003 and 0.015 mg/kg bw), no biologically significant inhibition of the activity of vitamin-K dependent clotting factors, i.e., below 70 to 80% of normal plasma activity, was observed. Numerous cases on developmental effects, the so-called ‘warfarin embryopathy’, which is characterised by nasal hypoplasia and stippled epiphyses, as well as disruptional abnormalities of the central nervous system, have been reported from the use as a therapeutical drug at daily doses between 2.5 and 15 mg. From reviews comprising 418 and 635 pregnancies, respectively, Pauli and Haun (Pau93) estimated that ‘warfarin embryopathy’ could occur in 1.5-5% of infants if only exposed during gestational weeks 6 to 9, and central nervous system effects in 0.5-2% infants if exposed in the second trimester.

In experimental animals, the compound is not irritating to the skin. The committee did not find data on the eye-irritation or sensitisation potential of warfarin. Based on the results of acute lethal oral toxicity studies in the rat, the committee considers the compound as very toxic. Large variations in the acute oral toxicity of warfarin between species were found. No reliable data on the acute inhalation or dermal toxicity of warfarin were available. Embryotoxicity and developmental effects were found in one study in rats following oral exposure to warfarin. The NOAEL was 1 mg/kg bw/day. No information could

be found on standard short- or long-term toxicity, including carcinogenicity, or mutagenicity or genotoxicity studies of warfarin.

The committee considers that the teratogenic effects reported in women treated therapeutically with warfarin doses of 2.5 to 15 mg are the critical findings in deriving a health-based recommended occupational exposure limit (HBROEL) and that 2.5 mg or 0.04 mg/kg bw is the LOAEL to be used as a starting point. For extrapolation to a HBROEL, the committee applies an assessment factor of 30, taking into account the absence of a NOAEL and the type and severity of the effects. Assuming a worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred-value approach, a health-based occupational exposure limit of 0.01 mg/m^3 is recommended for warfarin.

The committee recommends a health-based occupational exposure limit for warfarin of 0.01 mg/m^3, as inhalable dust, as an 8-hour time-weighed average (TWA). In view of the potential for dermal penetration, a skin notation is recommended.

References

ACG98 American Conference of Governmental Industrial Hygienists (ACGIH). Warfarin. In: TLVs and other occupational exposure values - 1998. [CD ROM]. Cincinnati OH, USA: ACGIH®, Inc, 1998.

ACG03a American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2003. Cincinnati OH, USA: ACGIH®, Inc, 2003: 140.

ACG03b American Conference of Governmental Industrial Hygienists (ACGIH). 2003 TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, Inc, 2003: 59.

And67 Anderson GF. The distribution of warfarin (coumadin) in the rat. Thromb Diath Haemorrh 1967; 18:754-8.

Arb02 Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2002: 40 (At-vejledning C.0.1).

Bac78 Back N, Steger R, Glassman JM. Comparative acute oral toxicity of sodium warfarin and microcrystalline warfarin in the Sprague-Dawley rat. Pharmacol Res Commun 1978; 10: 445-52.

Bar70 Barker WM, Hermodson MA, Link KP. The metabolism of 4-C^{14}-warfarin sodium by the rat. J Pharmacol Exp Ther 1970; 171: 307-13.

Bre72 Breckenridge A, L'E Orme M. The plasma halflives and the pharmacological effect of the enantiomers of warfarin in rats. Life Sci 1972; 11: 337-45.

Bre73a Breckenridge A, Orme M. Kinetics of warfarin absorption in man. Clin Pharmacol Ther 1973; 14: 955-61.

Bre73b Beckenridge AM, Orme M, Wesselng H, et al. Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clin Pharmacol Ther 1973; 15: 424-30.

Bre85 Breckenridge AM, Cholerton S, Hart JAD, et al. A sudy of the relationship between the pharmacokinetics and the pharmacodynamics of the 4-hydroxycoumarin anticoagulants warfarin, difenacoum and brodifacoum in the rabbit. Br J Pharmacol 1985; 84: 81-91.

Cha86 Chakrabarti S. Influence of organic solvents on the anticoagulant response to warfarin in rats. Res Com Chem Pathol Pharmacol 1986; 53: 203-11.

Cha94 Chan E, McLachlan AJ, Pegg M, et al. Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. Br J Clin Pharmacol 1994; 37: 563-9.

Cho86 Choonara IA, Haynes BP, Cholerton S, et al. Enantiomers of warfarin and vitamin K1 metabolism. Br J Clin Pharmacol 1986 ; 22: 729-32.

Cho88 Choonara IA, Malia RG, Haynes BP. The relationship between inhibition of vitamin K_1 2,3-epoxide reductase and reduction of clotting factor activity with warfarin. Br J Clin Pharmacol 1988; 25: 1-7.

Cra60 Crawford W, Adams W, Pass BJ . Extensive dermatitis due to warfarin sodium (Coumadin). Circulation 1960; 22: 947-8.

Dec73 Deckert FW. Warfarin metabolism in the guinea pig. I. Pharmacological studies. Drug Metab Dispos 1973; 1: 704-10.

DFG03 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT values 2003. Maximum concentrations and biological tolerance values at the workplace. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 2003: 114 (rep no 39).

Ebl66 Eble JN, West BD, Link KP. A comparison of the isomers of warfarin. Biochem Pharmacol 1966; 15: 1003-6

EC04 European Commission: Directorate General of Employment and Social Affairs. Occupational exposure limits (OELs). http://europe.eu.int/comm/employment_social/h&s/areas/oels_en.htm.

Ess98 Essex DW, Wynn SS, Jin DK. Late-onset warfarin-induced skin necrosis: case report and review of the literature. Am J Hematol 1998; 57: 233-7.

For73 Forbes CD, Thomson C, Prentice CRM, et al. Experimental warfarin poisening in the dog. Platelet function, coagulation and fibrinolysis. J Comp Pathol 1973; 83: 173-80.

Fri65 Fristedt B, Sterner N. Warfarin intoxication from percutaneous absorption. Arch Environ Health 1965; 11: 205-8.

Gai60 Gaines TB. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 1969; 2:88-99

God69 Goding LA, West BD. Synthesis and relative urinary excretion rates of the enantiomers of warfarin-4-^{14}C and phenprocoumon-2-^{14}C. J Med Chem 1969; 12: 517-8.

Hag53 Hagan EC, Radomski JL. The toxicity of 3-(acetonylbenzyl)-4-hydroxycoumarin (warfarin) to laboratory animals. J Am Pharm Assoc 1953; 42: 379-82.

Hal80 Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy. Am J Med 1980; 68: 122-40.

Hay67 Hayes WJ Jr. The 90-dose LD_{50} and a chronicity factor as a measure of toxicity. Toxicol Appl Pharmacol 1967; 11: 327-35.

Hir92 Hirsh J, Dalen JE, Deykin D, et al. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. Chest 1992; 102 (suppl): 312-26.

Hol52 Holmes RW, Love J. Suicide attemp with warfarin, a bishydroxycoumarin-like rodenticide. JAMA 1952; 148: 935-7.

Hol96 Holbrook AM, Wells PS, Crowther NR. Pharmacokinetics and drug interactions with warfarin. In: Poller L, ed. Oral anticoagulants. London, UK: Arnold Publ. Co, 1996: 30-48 (Chapter 3).

Hor81 Horn J, Danziger L, David R. Warfarin-induced skin necrosis: Report of four cases. Am J Hosp Pharm 1981; 38: 1763-8.

How90 Howe AM, Webster WS. Exposure of the pregnant rat to warfarin and vitamin K1: an animal model of intraventricular hemorrhage in the fetus. Teratology 1990; 42: 413-20.

How92 Howe AM, Webster WS. The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology 1992; 46: 379-90.

HSE02 Health and Safety Executive (HSE). EH40/2002. Occupational exposure limits 2002. Sudbury (Suffolk), England: HSE Books, 2002: 28.

Ike68 Ikeda M, Conney AH, Burns JJ. Stimulatory effect of phenobarbital and insecticides on warfarin metabolism in the rat. J Pharmacol Exp Ther 1968; 162: 338-43.

Jai79 Jain A, McMahon FG, Slattery JT, et al. Effect of naproxen on the steady state serum concentration and anticoagulant activity of warfarin. Clin Pharmacol Ther 1979; 8: 243-7.

Jah77 Jähnchen E, Meinertz T. Pharmakokinetische Aspekte der Überdosierung und Intoxikation mit oralen Antikoagulantien. Arzneimittelforschung 1977; 27: 1849-55.

Kro74 Kronick J, Phelps NE, McCallion DJ, et al. Effects of sodium warfarin administered during pregnancy in mice. Am J Obstet Gynecol 1974; 118: 819-23.

Lac75 Lacy JP, Goodin RR. Warfarin-induced necrosis of skin. Ann Intern Med 1975; 82: 381-2.

Lal77 Laliberte R, Chakrabarti S, Brodeur J. The influence of fasting on the disposition of warfarin in rats. J Pharmacol Exp Ther 1977; 200: 44-51.

Lan54 Lange PF, Terveer J. Warfarin poisening. Report of fourteen cases. US Armed Forces Med J 1954; 5: 872-7.

Lau92 Laurence DR, Bennett PN , eds. In: Clinical pharmacology. 7th ed. Edinburgh, Scotland: Churchill Livingstone, 1992.

Lev94 Levine M, Hirsh J, Gent M, et al. Double-blind randomised trial of very-low-dose warfarin for prevention of thromboembolism in stage IV breast cancer. Lancet 1994; 343 (8902): 886-9.

Lew70 Lewis RJ, Trager WF. Warfarin metabolism in man: identification of metabolites in urine. J Clin Invest 1970; 49: 907-13.

Lew73 Lewis RJ, Trager WF, Robinson J. Warfarin metabolites : activity and pharmacology of warfain alcohols. J. Lab Clin Med 1973 ; 81: 925-31.

Lew74 Lewis RJ, Trager WF, Chan KK, et al. Warfarin. Stereochemical aspects of its metabolism and the interaction with phenylbutazone. J Clin Invest 1974 ; 53 :1607-17.

Los72 Losito R, Rousseau MA. 14C-warfarin excretion in the rat. Thromb Diath Haemorrh 1972 ; 27: 300-8.

McA92 McAleer SD, Chrystyn H, Foondun AS. Measurement of the (R)- and (S)-isomers of warfarin in patients undergoing anticoagulant therapy. Chirality 1992; 4: 488-93.

Maj95 Majerus PW, Broze GJ Jr, Miletich JP, et al. Anticoagulant, thrombolytic, and antiplatelet drugs. In: Hardman JG, Limbird LE, eds(-in –chief). Goodman & Gilman‘s the pharmacological basis of therapeutics. 9th ed. New York, USA: McGraw-Hill, 1995: 1341-59 (Chapter 54).

Mar83 Martin-Bouyer G, Linh PD, Tuan LC, et al. Epidemic of haemorrhagic disease in Vietnamese infants caused by warfarin-contaminated talcs. Lancet 1983; 1 (8318): 230-2.

Mus76 Musa MN, Lyons LL. Absorption and disposition of warfarin: effects of food and liquids. Curr Ther Res Clin Exp 1976; 20: 630-3.

Nag69 Nagashima R, Levy G. Comparative pharmacokinetics of coumarin anticoagulants V: Kinetics of warfarin elimination in the rat, dog, and rhesus monkey compared to man. J Pharm Sci 1969; 58: 845-9.

Nie73 Niedner R, Kayser M, Reuter, et al. Toxizität von Warfarin und Einfluβ auf die Blutgerinnung von Ratten und Mäusen. Arzneimittelforschung 1973; 23: 102.

Nil57 Nilsson IM. Recurrent hypoprothrombinaemia due to poisoning with a dicumarol-containing rat-killer. Acta Haematol 1957; 17: 176-82 .

NLM02 US National Library of Medicine (NLM), ed. Warfarin. In: Hazardous substances data bank (HSDB) (last revision date warfarin file: 13 May 2002; last review date: 11 January 1994); http://toxnet/nlm.nih.gov.

ORe63 O'Reilly RA, Aggeler P, Leong LS. Studies on the coumarin anticoagulant drugs: the pharmacodynamics of warfarin in man. J Clin Invest 1963; 42: 1542-51.

ORe70 O’Reilly RA. Interaction of several coumarin compounds with human and canine plasma albumin. Mol Pharmacol 1970; 7: 209-18.

ORe74 O’Reilly RA. Studies on the optical enantiomorphs of warfarin in man. Clin Pharmacol Ther 1974; 16: 348-54.

Pal96 Palareti G, Legnani C. Warfarin withdrawal. Pharmacokinetic-pharmacodynamic considerations. Clin Pharmacokinet 1996; 30: 300-13.

Par88 Park BK. Warfarin : metabolism and mode of action. Biochem Pharmacol 1988 ; 37: 19-27.

Pau93 Pauli RM, Haun JM. Intrauterine effects of coumarin derivatives. Dev Brain Dysfunct 1993; 6: 229-47.

Pri66 Pribilla O. Mord durch Warfarin. Arch Toxikol 1966; 21: 235-49.

Ret92 Rettie AE, Korzewka RR, Kunze KL, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P450: a role of P450 2C9 in the etiology of (S)-warfarin-drug interactions. Chem Res Toxicol 1992; 5: 54-9.

Rou79 Routledge PA, Chapman PH, Davies DM, et al. Pharmacokinetics and pharmacodynamics of warfarin at steady state. Br J Clin Pharmacol 1979; 8: 243-7.

Sag75 Sagner G, Becker K. Hautresorption von Warfarin bei Mensch und Ratte. Umwelthygiene 1975; 6: 156-8.

Sal97 Sallah S, Thomas DP, Roberts HR. Warfarin and heparin-induced skin necrosis and the purple toe syndrome: infrequent complications of anticoagulant treatment. Thromb Haemost 1997; 78: 785-90.

Sel77 Seller EM, Lang-Sellers ML, Koch-Wester J. Comparative warfarin binding to albumin from various species. Biochem Pharmacol 1970; 6: 1-12.

She59 Sheps SG, Gifford RW. Urticaria after administration of warfarin sodium. Am J Cardiol 1959; 3: 118.

Sit94 Sittert van NJ, Tuinman CP. Coumarin derivatives (rodenticides). Toxicology 1994; 91: 71-6.

Sut87 Sutcliffe FA, MacNicoll AD, Gibson GG. Aspects of anticoagulant action: a review of the pharmacology, metabolism and toxicology of warfarin and congeners. Quart Rev Drug Metab Drug Interact 1987; 5: 225-72.

Swe00 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. Solna, Sweden: National Board of Occupational Safety and Health, 2000; Ordinance AFS 2000:3.

SZW03 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2003. The Hague, the Netherlands: Sdu, Servicecentrum Uitgevers, 2003: 43.

TRG00 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. BArbBl 2000; 2.

Wan83 Wang PP, Beaune P, Kaminsky LS, et al. Purification and characterisation of six cytochrome P450 isoenzymes from human liver microsomes. Biochemistry 1983; 22: 5375-85.

WHO95 World Health Organization (WHO): International Programme on Chemical Safety (IPCS). Warfarin. Geneva, Switzerland: World Health Organization, 1995; Health and Safety Guide No 96.

Win78 Wingard LB, O'Reilly RA, Levy G. Pharmacokinetics of warfarinenantiomers: A search for intrasubject correlations. Clin Pharmacol Ther 1978; 23: 212-6.

Won78 Wong LT, Solomonraj G, Thomas BH. Fate of [^{14}C] warfarin in guinea pigs: effect of a concomitant single dose of salicylate. J Pharm Pharmacol 1978; 30: 240-3.

Won80 Wong LT, Solomonraj G. Biliary and urinary excretion of [^{14}C] warfarin in rabbits. Xenobiotica 1980; 10: 201-10.

Annex I

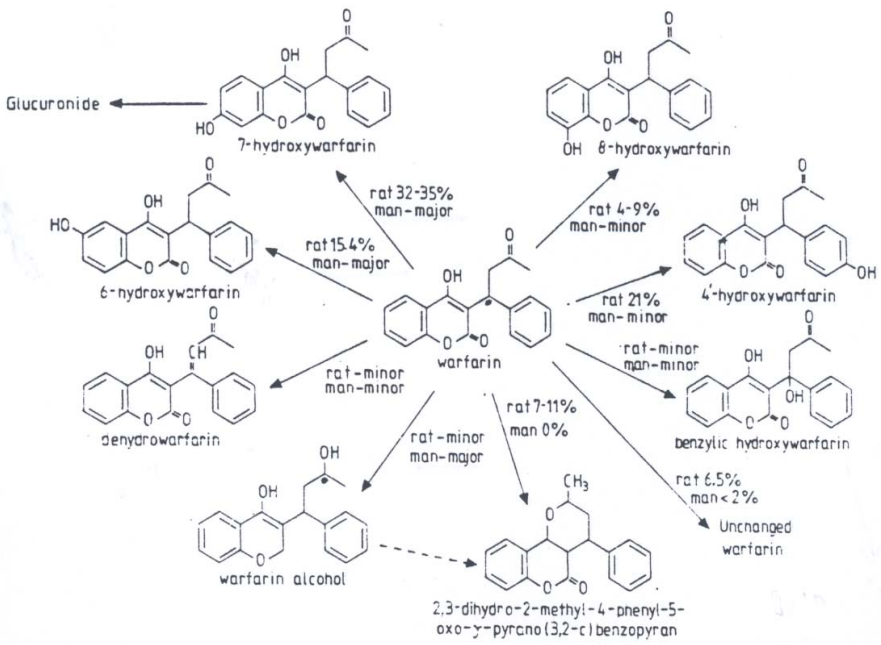


Figure 1 Metabolic pathways for warfarin in the rat and man (from Sut87).
Indicated figures are the percentage of dose excreted.
* = chiral centre

Annex II

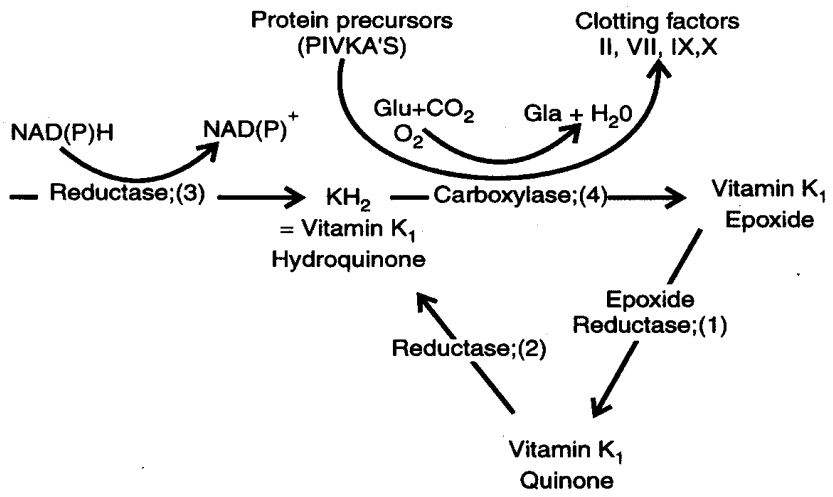


Figure 2 The vitamin K cycle (from Hol96).
(1) and (2) are dithiol-dependent reductase enzymes that are inhibited by warfarin. In the presence of warfarin, vitamin K epoxide accumulates.
(3) is an NADPH-dependent reductase that is relatively insensitive to the effects of warfarin.
The carboxylase enzyme (4) is responsible for the γ-carboxylation of glutamine residues to γ-carboxyglutamyl residues.

Annex III

Occupational exposure limits for warfarin in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	0.1	8 hour	administrative		SZW03
Germany						
- AGS	-	0.5[c]	8 hour			TRG00
	-	2.0[c]	15 min			
- DFG MAK-Kommission	-	0.5[c]	8 h			DFG03
	-	1.0[c]	15 min[d]			
Great Britain						
- HSE	-	0.1	8 hour	OES		HSE02
		0.3	15 min			
Sweden	-	-				Swe00
Denmark	-	0.1	8 hour	OEL		Arb02
USA						
- ACGIH	-	0.1	8 hour	TLV		ACG03b
- OSHA	-	0.1	8 hour	PEL		ACG03a
- NIOSH	-	0.1	10 hour	REL		ACG03a
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] As inhalable fraction.

[d] Maximum number per shift: 4, with a minimum interval between peaks of 1 hour.