Policy Forum

Establishment of Occupational Exposure Limit for Warfarin in China*

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This study aims to establish the occupational exposure limit (OEL) in the air for workplace of warfarin based on the available toxicological studies and field investigations by using questionnaire and air monitoring. The clinical therapeutic dose was used as lowest observed effect level (LOEL), and no observed effect level (NOEL) was achieved by using a safety factor. The highest concentration of warfarin monitored in the worksite of centrifuge washing, drying and packing were 0.029 mg/m^3, 0.051 mg/m^3 respectively, which did not exceed the OEL 0.1 mg/m^3 recommended by NIOSH and ACGIH. Considering its feasibility for enforcement and protection for workers, we recommend OEL 0.1 mg/m^3 of warfarin in China.

Warfarin was firstly synthetically derived from coumarin and registered for use in the United States. Currently, it was used therapeutically as an anticoagulant[1] and commercially as a rodenticide[2]. This chemical acts via inhibiting the synthesis of vitamin K-dependent clotting factors, including Factors II, VII, IX, and X, as well as the regulatory factors for protein C, protein S, and protein Z. The inhibition of warfarin occurs through competition with Vitamin K, for this reason, coumarin derivatives are also referred to vitamin K antagonists[3].

Warfarin is the most common oral anticoagulant used today. The dosage and administration is individualized based on the patient's prothrombin time (PT). The typical therapeutic dose range of warfarin is 2-10 mg/day by oral administration, which induces a 1.2 to 2-fold increase of PT for majority of patients as compared with the normal values (MediText Database).

As for the OEL for warfarin in the air of workplace, a TLV-TWA of 0.1 mg/m^3 is recommended by ACGIH , a REL of 0.1 mg/m^3 and the determination method (NAMAM, Method 5002) by NIOSH, a MAK of 0.5 mg/m^3 (Inhalable fraction) by DFG[4] . China is one of the largest warfarin producers and consumers in the world but no OEL is available for this coumarin derivative[5]. This study filled the gap and set an OEL for warfarin as well as its determination method in China.

Summary of Toxicology Warfarin makes effect on the liver by inhibiting prothrombin formation and damaging the blood vessels directly. In rats, the lowest reported oral LD_{50} is 1.6 mg/kg, the lowest intraperitoneal lethal dose (LDL_0) is 420 mg/kg, the inhalation LC_{50} is 320 mg/m^3 and the dermal LD_{50} is 1400 mg/kg[6]. Either a large single dose or a small dose of warfarin was in capable of causing intoxication. Rats and mice died after ingesting 1 mg/kg/day for 6 days. The major signs of poisoning include massive hemorrhages, visible hematomas under the skin or around joints, bleeding from body orifices. Symptoms as shock, weakness, and labored breathing may also occur. It was reported that intramuscular injection of warfarin 10 mg/kg to pregnant rabbits on day 8 to day 28 would cause stillbirths or fetal malformations[7]. It also caused birth defects and fetal toxicity for animals (TERIS Database). But no exact data were identified to evaluate warfarin's reproductive toxicity.

An anticoagulant effect may occur or the risk of hemorrhage could strengthen in human when exposure to warfarin. It was reported that warfarin has few toxic effects in human, although anorexia, nausea, vomiting, diarrhea, and skin lesions may occur except for abnormal bleeding. The reported lowest lethal dose in human was 6667 μg/kg (RTECS Database). However, warfarin is more toxic in animal when ingested in small doses over a period of 5-6 days, and 1-2 mg/kg/day for 6 days was considered to be lethal (ReproTox Database). The oral TDL_0 of warfarin for children is 4 mg/kg, and for women is 300 μg/kg/2day. Warfarin sodium was also proved to be toxic in animals. Different LD_{50} were for different animals by different administrations. The LD_{50} of warfarin sodium by intravenous were 200 mg/kg, 200 mg/kg, 160 mg/kg, 100 mg/kg, and 25 mg/kg for

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chick, dog, mouse, rabbit and rat respectively; and 182 mg/kg, 200 mg/kg, 374 mg/kg, 800 mg/kg, and 8.7 mg/kg for guinea pig, dog, mouse, rabbit, and rat by oral respectively (ReproText Database). It was reported that severe poisoning, congenial malformation and death could occurred in humans when exposure to warfarin.

No pharmacokinetic information was available after inhalation of warfarin. It was well absorbed (70%-100%) via oral administration[8]. It also could be absorbed via skin contact and caused systemic toxicity when the amount was sufficient[9]. Once warfarin was absorbed, it will be converted predominantly by liver cytochrome P450 to inactive hydroxylated metabolites, which are excreted mainly through urinary system. The elimination half-life of warfarin is about 7 days, its therapeutically effective half-life is about 40 h, and the half-life of the R-enantiomer is about twice longer than the S-enantiomer.

Occupational Exposure Limit The traditional approach for setting an OEL is to identify a no-observed-effect-level (NOEL) based on animal or human studies and then to apply appropriate uncertainty, or safety factors, when necessary[10-12]. The typical equation is:

$$OEL = [(NOEL)(BW)] / [(SF)_n (BR)]$$

Where:

NOEL = no-observed-effect-level for the most sensitive adverse effect;

BW = body weight of an adult worker, typically assumed by default to be 70 kg;

$(SF)_n$ = a number of safety factors including uncertainties such as animal-to-human variability in response, human-to-human variability in response, bioavailability by different routes of exposure, biological half-life, quality of the available data;

BR = breathing rate of an adult worker, typically the default values for an adult were 70 kg weight and 10 m^3/8-h work in a day.

If an appropriate NOEL can not be identified, then an appropriate lowest-observed-effect-level (LOEL) may be used. This LOEL is adjusted by a safety factor depending on the severity of the adverse effects. The main adverse effects of warfarin include hemorrhage from mucous membranes, gastrointestinal and genitourinary tract, and the results of inhibition of liver synthesis of prothombin and clotting factors. Vitamin K1 is a specific and effective antidote for both animals and humans in case of warfarin poisoning. Considering the severity of main adverse effects for warfarin, a safety factor of 3 was assumed to adjust the LOEL to a NOEL. In some cases, the drug's therapeutic dose could be used as a LOEL[13].

The loading dosage of warfarin used as an anticoagulant is 10 mg/day, and the maintenance dose range of 2-10 mg/day is used. For health protection, the high end of the therapeutic dose range of 10 mg/day can be used as LOEL.

Because of the reported bioavailability of warfarin via oral administration was about 70%, a 0.7 adjustment factor (in the numerator of the equation below) for bioavailability differences between the oral and inhalation routes was assumed. A safety factor of 2 for human-to-human variability in response was also assumed.

Finally, the worker inhalation rate of 10 m^3/8-h workday is usually assumed. Using these assumptions, the estimated OEL for warfarin is:

$$OEL = (10\ mg/day)(0.7) / (3)(2)(10\ m^3/day) = 0.1167\ mg/m^3$$

Rounded to one significant figure, the estimated OEL for warfarin becomes 0.1 mg/m^3.

The Determination Method of Warfarin in the Air of Workplace The NIOSH method (5002) of warfarin was used in this study. The warfarin was collected by PTFE filters with the pore size of 1 μm, and a 5 mL of methanol was used for desorption. The desorption liquid of 20 μL was injected into HPLC System. A C_{18} column and a UV detector were used for the purposes of separation and detection. According to the production procedure for warfarin, the most possible interfering chemicals were identified as Benzylideneacetone and 4-Hydroxycoumarin. These chemicals do not interfere with the determination of warfarin.

The warfarin concentrations in the air of workplace using personal sampling and area sampling were shown in Table 1 and Table 2.

Field Investigations The selected company producing anticoagulant warfarin was located at Jiangsu province, south China. There were 18 employees who are exposed to warfarin directly for 8 h per day for 26 day a month. Information such as occupation, sex, work years and symptoms occurred was included in the field questionnaire. Only 1 employee (4.3%) had the symptoms of ecchymosis and gingiva bleeding during last 3 months. Personal protective equipments(PPE) were provided, and ventilation equipment was also well operated. The results were shown in Table 3.

Table 1. The Warfarin Concentrations in the Air of Workplace (Personal Sampling)

Occupation	Personal Sampling (PC-TWA, mg/m^3)			
	1d	2d	3d	4d
Feeding	0.00073 ND	0.0016 0.00086	0.0014 0.0012	0.0024 0.0024
Discharging	ND	0.0045 0.0070	ND	0.0043 0.0087
Centrifuge washing	ND	0.0025	0.029	ND
Drying and packing	ND	0.0072	0.051	ND
Management office	ND	ND	ND	ND

Note. d: a shift (8-h work day); ND: concentrations determined were below the LOD (0.00063 mg/m^3).

Table 2. The Warfarin Concentrations in the Air of Workplace (Area Sampling)

Occupation	Area Sampling (mg/m^3)			
	1d	2d	3d	4d
Feeding	ND	ND	ND	ND
Discharging	ND	0.0045 0.0070	ND	ND
Centrifuge washing	ND	ND	0.047	ND
Drying	ND	ND	ND	0.037
Packing	ND	ND	ND	ND
Management office	ND	ND	ND	ND

Note. d: a shift (8-h work day); ND: means that concentrations determined were below the LOD (0.00063 mg/m^3).

Table 3. The Results of Occupational Health Investigations

Occupation	Sex	Symtoms	Work Years
		Ecchymosis and Gingiva Bleeding	
Operators	18[a]	1	≥3
Administrative Staffs	3[a], 2[b]	ND	≥3
Total	23	1	

Note. a: man; b: woman; ND: no symtoms were observed; All staffs in this company have been working over three years.

The relatively high concentrations of warfarin determined in the air of workplace were 0.051 mg/m^3 and 0.029 mg/m^3, which correspond to the worksite of centrifuge washing, drying and packing. Both of these values did not exceed the OEL 0.1 mg/m^3 recommended by NIOSH and ACGIH. Compared to previous studies, the bioavailability of warfarin was considered and a 0.7 adjustment factor for bioavailability differences between the oral and inhalation routes was assumed, and the possible interference chemicals were also identified in this study.

Based on the available toxicological document review and field investigation, an OEL in the air of 0.1 mg/m^3 is recommended for workplace of warfarin in China, which is feasible to compliance for warfarin manufacturing company employers and more health protective for the employees. And with OEL 0.1 mg/m^3, no specific PPE are needed in workplace of warfarin in China.

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