IN VIVO TOXICITY STUDY OF DIALKYL DISULPHIDES

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As a result of the industrial purification of hydrocarbons from mercaptans, tens of thousands of tons of dialkyl disulphides and their mixtures, the toxicity and hazard of which has not been fully understood, are accumulated annually. The exposure standards have been developed only for dimethyl disulphide. The study was aimed to define toxicometry parameters for diethyl disulphide, disulphide oil, and the mixture of dialkyl disulphides. Toxicology studies involving male outbred rats made it possible to define the median lethal doses and concentrations: diethyl disulphide — after intragastric injection DL_{50} = 1575 mg/kg, after the 4-hour inhalation exposure CL_{50} = 18,700 mg/m^3, after intraperitoneal injection DL_{50} = 1134 mg/kg, and after skin application DL_{50} > 2500 mg/kg; mixture of dialkyl disulphides — after intragastric injection DL50 = 428 mg/kg, after the 4-hour inhalation exposure CL_{50} = 4510 mg/m^3, after intraperitoneal injection DL_{50} = 212 mg/kg, and after skin application DL_{50} > 2500 mg/kg; disulphide oil — after intragastric injection DL_{50} = 448 mg/kg, after the 4-hour inhalation exposure CL_{50} = 4534 mg/m^3, after intraperitoneal injection DL_{50} = 156 mg/kg, and after skin application DL_{50} > 2500 mg/kg. The hazard assessment for dialkyl disulphides and their mixtures was performed.

Keywords: dialkyl disulphides, dimethyl disulphide, diethyl disulphide, methylethyl disulphide, disulphide oil, acute toxicity, LD_{50}, LC_{50}, hazard class

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Compliance with ethical standards: the study was approved by the Ethics Committee of the North-Western State Medical University named after I.I. Mechnikov (protocol № 8 dated November 11, 2020); laboratory animals were kept and fed in accordance with SP 2.2.1.3218-14 “Sanitary and Epidemiological Requirements for the Device, Equipment and Maintenance of Experimental Biological Clinics (Vivariums)”, as well as with the «Guide for Care and Use of Laboratory Animals» (USA).

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ТОКСИЧНОСТЬ ДИАЛКИЛДИСУЛЬФИДОВ В ЭКСПЕРИМЕНТАХ *IN VIVO*

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В результате промышленной очистки углеводородного сырья от меркаптанов ежегодно накапливаются десятки тысяч тонн диалкилдисульфидов и их смесей, токсичность и опасность которых в полной мере не изучена. Гигиенические нормативы разработаны только для диметилдисульфида. Целью исследования было установить параметры токсикометрии для диэтилдисульфида, «дисульфидного масла» и смеси диалкилдисульфидов. В токсикологических исследованиях на самцах беспородных крыс установлены среднесмертельные дозы и концентрации: диэтилдисульфида — при внутрижелудочном введении DL_{50} = 1575 мг/кг, при ингаляционном 4-часовом воздействии CL_{50} = 18 700 мг/м^3, при внутрибрюшинном введении DL_{50} = 1134 мг/кг, при накожном нанесении DL_{50} > 2500 мг/кг; смеси диалкилдисульфидов — при внутрижелудочном введении DL_{50} = 428 мг/кг, при ингаляционном 4-часовом воздействии CL_{50} = 4510 мг/м^3, при внутрибрюшинном введении DL_{50} = 212 мг/кг, при накожном нанесении DL_{50} > 2500 мг/кг; дисульфидного масла — при внутрижелудочном введении DL_{50} = 448 мг/кг, при ингаляционном 4-часовом воздействии CL_{50} = 4534 мг/м^3, при внутрибрюшинном введении DL_{50} = 156 мг/кг, при накожном нанесении DL_{50} > 2500 мг/кг. Проведена оценка опасности диалкилдисульфидов и их смесей.

Ключевые слова: диалкилдисульфид, диметилдисульфид, диэтилдисульфид, метилэтилдисульфид, дисульфидное масло, острая токсичность, LD_{50}, LC_{50}, класс опасности

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As a result of the industrial purification of hydrocarbons from mercaptans, tens of thousands of tons of dialkyl disulphides and their mixtures are being accumulated annually [1]. Dialkyl disulphides are used in oil industry as coke formation inhibitors in pyrolysis furnaces, and sulfiding agents, the hydrotreating and hydrocracking catalysts. In agriculture, dialkyl disulphides are used as insecticides, and in food industry, these are used as flavoring agents [2–7]. Despite the widespread industrial application of dialkyl disulphides, the exposure standards for diethyl disulphide, methylethyl disulphide, and disulphide oil have not been established. In order to establish the exposure standards and to evaluate the hazards of the substances, we conducted toxicology studies of the dialkyl disulphide effects in the acute experiment.

METHODS

Toxicity of dialkyl disulphides was assessed amidst single and repeated exposure, the study involved male outbred rats with initial weight of 220–250 g, and male mice with the weight of 20–25 g (nursery of laboratory animals "Rappolovo"; Leningrad Region). The delivered batches of animals had veterinary certificates specifying the animals' age and average weight, and indicating the absence of systemic diseases and parasitic infestation. The animals were taken to quarantine unit of the vivarium, where adaptation took place for 14 days. The animals were kept under standard housing conditions; they were given a standard diet, and had free access to water. During the quarantine period, each animal underwent daily examinations (behavior, overall condition, morbidity and mortality were evaluated). At the beginning of the experiments the animals, meeting the inclusion criteria, were randomized to groups. Animals, not meeting the inclusion criteria, were excluded from the study. Air change rate, room temperature and humidity were monitored daily. The temperature was maintained within the range of 20–24 °C, the relative humidity was within the range of 50–70%, the air change rate was 10 air changes per hour, and the lighting conditions were 12 h of light per day. The animals were euthanized in CO_2 chambers.

For the experiment the animals were divided into homogeneous groups based on the body weight (8–10 animals per group); laboratory animals were labeled on an individual basis.

Acute toxicity of dialkyl disulfides was assessed by intragastric injection, intraperitoneal injection, skin application, and inhalation [8].

The studied substances were as follows:

– diethyl disulphide (DEDS) with mass fraction of parent substance at least 99%, and mass fraction of dimethyl disulfide impurities at least 1%;

– disulphide oil (DSO) with mass fraction of dimethyl disulfide of 75.14%; mass fraction of diethyl disulphide of 2.08%; mass fraction of methylethyl disulphide of 21.69%; mass fraction of higher dialkyl disulphides C_4-$C_8S_2H_{10}$-H_{22} ≈1%.

– mixture of dialkyl disulphides with mass fraction of DMDS of 26.4%; mass fraction of MEDS of 53.0%; mass fraction of DEDS of 20.7%;

Physical and chemical properties of dialkyl disulphides are presented in Table 1 [9].

Intragastric injections of the substances (at a dose of 75–2000 mg/kg) were performed using the atraumatic probe; vegetable oil was used as a solvent.

The inhalation exposure modeling was performed in the chambers with a volume of 600 dm^3. The experimental animals were exposed to the following concentrations of dialkyl disulphide vapors: diethyl disulphide 10,000–22,000 mg/m^3; disulphide oil 4000–5000 mg/m^3; mixture of DADS 3800–6000 mg/m^3. The exposure time for a single inhalation exposure was 2 h in mice, and 4 h in rats.

The vapor concentrations in the air within the exposure chambers were controlled by gas chromatography with flame ionization detection.

During the acute experiments, the duration of observation after the exposure to the substance was 14 days. The overall condition, behavior, appearance, and response to external stimuli were evaluated in experimental animals. Clinical manifestations of poisoning were registered. In end of observation period necropsy was performed, the macroscopic examination of internal organs.

RESULTS

Intragastric injection of the mixture containing DADS and DSO resulted in the animals' death of pulmonary edema, mainly during the first day. Intragastric injection of DEDS resulted in the animals' death delayed until day 7. Clinical manifestations of acute dialkyl disulphide intoxication were similar: hypo- or adynamia of experimental animals, and decreased respiration rate. Macroscopic examination of the dead animals' internal organs revealed the following: brown lung induration and pulmonary hemorrhage, tracheal froth, dark brown spleen and kidneys, fine liver surface nodularity.

The overall appearance of animals, which survived acute intoxication, was the same as of controls throughout the observation period.

Based on the acute toxicity parameters defined for intragastric injection, the studied dialkyl disulphides are moderately hazardous substances (hazard class 3 [10]). DEDS is assigned hazard class 4, and the mixture of DADS and disulphide oil is assigned hazard class 3 [11] (Table 2).

The studied substances are supported as mildly hazardous by the species ratio values:

DEDS species ratio: 1575/1565 = 1.006.

Table 1. Physical and chemical properties of dialkyl disulphide samples

Indicator	Dialkyl disulphides		
	DMDS	MEDS	DEDS
Chemical formula	CH_3SSCH_3	$CH_3SSC_2H_5$	$C_2H_5SSC_2H_5$
№ CAS	624-92-0	20333-39-5	110-81-6
Physical appearance	pale yellow transparent liquid	oleaginous fluid	oleaginous fluid
Molecular mass, g/mol	94.2	108.23	122.25
Density, g/cm^3	1.057	1.022	0.993
Boiling point, °C	109.7	131.6	154.1
Refractive index	1.5259	1.5146	1.506
Sulphur content, wt%	68.09	59.26	52.46
Solubility			
Water	insoluble	insoluble	sparingly soluble
Diethyl ether	soluble	soluble	mixing
Ethanol	soluble	soluble	mixing

Note: DMDS — dimethyl disulphide; MEDS — methylethyl disulphide; DEDS — diethyl disulphide.

Table 2. Dialkyl disulphide acute toxicity parameters with intragastric injection

Animal species	Lethal doses, mg/kg		
	LD_{16}	LD_{50}	LD_{84}
	Diethyl disulphide		
Male mice	942	1565 ± 370	2601
Male rats	1384	1575 ± 91	1793
Mixture of DADS			
Male mice	244	435 ± 118	775
Male rats	307	428 ± 83	597
Disulphide oil			
Male mice	276	381 ± 56	527
Male rats	265	448 ± 142	759

Mixture of DADS species ratio: 428/435 = 0.98.

DSO species ratio: 448/381 = 1.17.

Upon intraperitoneal injection in rats the acute toxicity parameters has been defined (Table 3), supporting these compounds as being moderately hazardous.

After intraperitoneal injection of the mixture containing DADS and DSO, adynamia occurred within 5 minutes, and the respiration rate decreased in experimental animals. The animals died of respiratory arrest 30–60 min after administration of the substance.

After intraperitoneal injection of DEDS, the experimental animals exhibited psychomotor retardation during one hour, they did not respond to external stimuli. After 4–5 h the overall appearance of experimental animals was the same as of controls. The animals died of pulmonary edema during the first day after administration of DEDS.

Masroscopic features of internal organs were the same as in case of intragastric injection.

Based on the acute toxicity parameters defined for inhalation exposure, the mixture of DADS and disulphide oil are assigned hazard class 2 [10] and 3 [11]. Based on the CL_{50} value, DEDS is assigned hazard class 3 [10] and 4 [11] (Table 4).

No significant species differences were defined for dialkyl disulphide inhalation poisoning.

The index of potential inhalation toxicity (IPITac) indicated low hazard upon single inhalation exposure to DEDS (IPITac = 24999.3/18,700 = 1.33), and moderate hazard upon exposure to the mixture of DADS (IPITac = 74004.5/4534 = 16.3), and disulphide oil (IPITac = 105336.5/4510 = 23.3).

Clinical manidestations of acute inhalation poisoning with dialkyl disulphide vapors were as follows: hypo- or adynamia of experimental animals, signs of hypoxia (moderate cyanosis of faces and paws), and breathing problems. The periods of decreased motor activity were followed by periods of increased motor activity. The animals died of pulmonary edema due to exposure to the mixture of DADS and DSO within 24 h of inhalation exposure; when exposed to DEDS, the animals died mostly on day 3–5 of observation. Masroscopic features of internal organs were the same as in case of intragastric injection.

The overall appearance and behavior of animal survivors being monitored for 14 days after exposure were the same as those of controls.

It was found that the mixture of DADS and DSO was fatal for a part of mice during the 2-hour exposure with the 2/3 of the tail length placed into test tubes filled with substances; however, DEDS was not fatal for experimental mice.

Upon dermal exposure, the following median lethal doses (DL_{50}) were defined for experimental rats: mixture of DADS — 7400 (5690; 9620) mg/kg, DSO — 3400 (2345; 4930) mg/kg. Based on the median lethal doses upon skin application, the studied substances were assigned hazard class 4 [10]. Skin application of DEDS for 4 h was never fatal for experimental rats. No signs of skin irritation were observed. Clinical manifestations of acute poisoning upon dermal exposure of experimental animals to dialkyl disulfides were the same as in case of intragastric injection.

The study results were indicative of the substances being hazardous in contact with skin.

After application of one drop of the studied substance on the mucous membrane of the rat's eye, the irritant effect in the form of hyperemia was observed. Hyperemia vanished 1–2 days later, and further observation demonstrated that the experimental rats had the same overall appearance and showed the same dynamic changes of body weight as the controls.

DISCUSSION

The results obtained for acute oral toxicity of disulfide oil are within a factor of three of the literature data (1590 mg/kg reported by Morgott et al., and 428 mg/kg in our studies) [5], which could indicate different composition of disulphide oil, the use of different experimental animal species, or the use of different solvent for intragastric injection. The article by Morgott et al. [5] refers to the unpublished results obtained at the IIT Research Institute. There is no information about this source in the Scopus, and PubMed databases. The inhalation toxicity data also differ: Morgott et al. [5] report it to be greater than 4840 mg/m^3; in our studies CL_{50} of 4534 mg/m^3 was

Table 3. Dialkyl disulphide acute toxicity parameters with single intraperitoneal injection in male rats (n = 10)

Substances	Lethal doses, mg/kg		
	LD_{16}	LD_{50}	LD_{84}
Diethyl disulphide	1384	1575 ± 91	1793
Mixture of DADS	187	212 ± 11	240
Disulphide oil	98	156 ± 33	248

Table 4. Dialkyl disulphide acute toxicity parameters with single inhalation exposure

Animal species	Lethal concentrations, mg/m^3		
	CL_{16}	CL_{50}	CL_{84}
Diethyl disulphide			
Male mice	17 630	18125 ± 515	19 930
Male rats	17 800	18700 ± 556	19 730
Mixture of DADS			
Male mice	3900	4200 ± 190	4750
Male rats	3300	4534 ± 519	5120
Disulphide oil			
Male mice	3900	4200 ± 180	4700
Male rats	4400	4510 ± 60	4600

established. However, the acute toxicity data for disulfide oil are close to data obtained by Morgott et al. for dimethyl disulfide.

CONCLUSION

As a result of the experimental studies, the acute toxicity parameters were defined for intragastric injection and inhalation exposure: for DEDS, DL_{50} was 1575 mg/kg, and CL_{50} was 18,700 mg/m^3; for the mixture of DADS, DL_{50} was 448 mg/kg, and CL_{50} = 4510 mg/m^3; for DSO, DL_{50} was 428 mg/kg, and CL_{50} = 4534 mg/m^3. The median lethal dose (DL_{50}) for dermal exposure to the mixture of DADS was 7400 mg/kg, and for DSO it was 3400 mg/kg; DEDS was not fatal in contact with skin. The data obtained show that dialkyl disulfides are the moderately hazardous substances upon single intragastric injection, inhalation exposure, and skin application. Comparison of the studied substances with dimethyl disulfide (DMDS) by toxicity has shown that based on the toxicometry parameters, DEDS is about 9 times less toxic upon intragastric injection, and 3 times less toxic upon single inhalation exposure, compared to DMDS. Comparison of the mixture of DADS and DSO with DMDS has revealed similar inhalation toxicity. The mixture of DADS and disulphide oil is 2 times less toxic upon intragastric injection compared to DMDS. Comparison of DSO and DADS mixture toxicity has shown that impurities do not affect the toxicometry parameters. These toxicometry parameters would be used to define the hazard class and to establish the dialkyl disulfide exposure standards. To date, the exposure standards have been developed only for dimethyl disulphide.

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