

## ORIGINAL ARTICLE

# ACUTE TOXICITY OF SURFACTANTS AND DETERGENT-BASED DECONTAMINANTS IN MICE AND RATS

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### Summary

Detergent-based mixtures and pure surfactants used as decontaminants in mass casualty scenarios were investigated for their acute 24 h toxicity. Commercial detergents Neodekont<sup>TM</sup>, Argos<sup>TM</sup>, Dermogel<sup>TM</sup>, and FloraFree<sup>TM</sup> were tested in male Wistar rats after percutaneous, peroral and intramuscular administration. Pure surfactants althosan MB, sodium alkylbenzene sulphonate, triton X, benzyl dimethyl dodecyl ammonium chloride, and N-dodecyl pyridinium bromide were investigated in male ICR mice. Estimated LD<sub>50</sub> of surfactants varied from < 100 mg.kg<sup>-1</sup> (*i.m.*) to more than 2000 mg.kg<sup>-1</sup> (*p.o.*, *p.c.*) in mice. All detergents tested in rats were rather less toxic (LD<sub>50</sub> > 6 ml.kg<sup>-1</sup>), thus could be considered as safe if used as external skin decontaminant or if low volume is accidentally ingested.

*Key words: detergent; tenside; mass decontamination; surfactant; acute toxicity*

## INTRODUCTION

Mass casualty decontamination (MCD) implies first aid for large number of civilian casualties when chemical agents are involved due to industrial accidents or potential misuse of various chemicals (chemical warfare agents, pesticides, toxic industrial chemicals) by terrorist groups [1].

Within the frame of EU and US, common methods for MCD include washing of exposed body parts with water and/or detergent based washing

solutions [2, 3]. Such decontamination solutions operate on principle of detergency and washing abilities with no or minor reactive potential to agent detoxification. On the other hand, some detergents (surfactants), especially cationic compounds such as quarternary pyridinium salts, were proved as effective polyvalent decontamination catalysts [4-8].

In comparison with reactive decontaminants used in the army, washing detergents are usually considered as safe with minor toxic effects. The main toxic effect of detergents (surfactants) lies in cytolytic and haematolytic activity due to impairment of cell membrane integrity, mitochondrial functions and cellular metabolism [9-11]. Severity of toxic effect depends on agent concentration and its chemical characteristics, including length of alkane chain and unsaturations [9, 10, 12]. Acute and chronic detergent toxicities were investigated several times in cell cultures

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*in vitro* [10, 11, 13], *ex vivo* on tissues of experimental animals [e.g. 9, 14], or *in vivo* using various animal models [12]. Skin and eye irritation tests are common methods for safety assessment of detergents in drug research and cosmetics [12].

In this study, we focused on *in vivo* assessment of acute toxicity of detergent-based decontaminants used in MCD. The acute toxicity of four different commercial detergents used in EU - Neodekont™, Argos™, Dermogel™, and FloraFree™ was evaluated after percutaneous (*p.c.*), peroral (*p.o.*) and intramuscular (*i.m.*) administration. Furthermore, acute toxicity of pure single surfactants (althosan MB, sodium alkylbenzene sulphonate, triton X, benzyl dimethyl dodecyl ammonium chloride, and N-dodecyl pyridinium bromide), which are commonly contained in decontamination mixtures, was tested.

## MATERIAL AND METHODS

### Chemicals

Detergent means FloraFree™ (Deb Ltd., United Kingdom), Dermogel™ (KAO Corporation Espana, Spain) and Argos™ (Argos Hygiene, France) were obtained from Health Protection Agency (United Kingdom). Neodekont™ was produced by ChemProtect corp. (Czech Republic). FloraFree™ consists mainly of surfactants (e.g. potassium tallate, potassium cocoate etc.) and other compounds (e.g. isopropyl alcohol, sodium borate etc.) similarly as Argos™ (e.g. sodium alkylether sulfate, sodium alkylbenzene sulfonate, cocamide DEA etc.), Dermogel™ (sodium laureth sulphate, lauryl sulphate TEA, glycerin etc.) and Neodekont™ (pentasodium triphosphate, TEA-dodecylbenzene sulfonate, bentonite etc.).

Surfactant althosan MB (50% benzalkonium chloride) was purchased from ChemProtect corp. Sodium alkylbenzene sulphonate (80%), triton X (polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether), benzyl dimethyl dodecyl ammonium chloride and N-dodecyl pyridinium bromide as well as other chemicals used for histopathology were obtained from Sigma Aldrich Ltd. (Czech Republic).

### Animals

Male Wistar rats (body weight 180-220 g) and male ICR mice (body weight 25-35 g) were purcha-

sed from Velaz corp (Czech Republic) and placed at an approved animal facility at the Faculty of Military Health Sciences (FMH). The animals were housed in Velaz breeding-boxes in groups of six individuals and kept under standard breeding conditions. The animals were given access to food (Cerea corp.) and drinking water *ad libitum*. The use of animals in this study was formally approved by the local ethical review committee of the Faculty of Military Health Sciences. All procedures involving animals were in accordance with extant legislation.

## EXPERIMENTS

Undiluted detergents (concentrated) or surfactants dissolved in physiological saline were administered to experimental animals via injections to the muscles of both hind limbs (*i.m.*), via gastric probe into the stomach (*p.o.*) or topically by a pipette (*p.c.*) as a continual layer across the clipped dorsal skin (5 x 7 cm). Side of application was un-occluded. Clipping was performed on the day before experiment to avoid skin irritation. Blank controls received the same procedure but instead of detergents, the same volume of physiological saline was administered. Animals that survived 24 h post-exposure were euthanized by CO<sub>2</sub>.

### Estimation of median lethal doses

Reduced numbers of experimental animals were used for estimation of median lethal dose (LD<sub>50</sub>, mg.kg<sup>-1</sup> for surfactants; ml.kg<sup>-1</sup> for mixtures) for each tested compound and way of administration based on the principle of Up-and-down procedure [15]. Due to expected low toxicity of detergents, a maximal applicable dose (max AD, Table 1) was estimated for each species and way of administration, subjectively according to personal experience. A dose equivalent to max AD was administered to group of 3 animals. If mortality occurred within 24 h, other 3 animals were administered to a semi-dose, etc., until estimation of probable LD<sub>50</sub> was reached. If no death occurred when max AD was administered, tested compound was considered as non-toxic under the tested dose and a lower dose was not administered.

If the data were sufficient, the acute toxicity was evaluated by the assessment of 24 h LD<sub>50</sub> and its confidence limits calculated by the probit analysis of deaths occurring within 24 h.

**Table 1.** Acute 24 h toxicity of commercial detergents used for MCD in laboratory rats. LD<sub>50</sub> (ml.kg<sup>-1</sup>) and confidence limit is shown where applicable. Maximal applicable dose (max AD) via *p.c.*, *p.o.* and *i.m.* administration is noted.

detergent mean	LD <sub>50</sub> (24 h; ml.kg <sup>-1</sup> )		
	<i>p.c.</i>	<i>p.o.</i>	<i>i.m.</i>
FloraFree™	> 10	> 6	> 6
Neodekont™	> 10	> 6	> 6
Dermogel™	> 10	> 6	> 6
Argos™	> 10	> 6	5.0 (3.6 – 6.6)
max AD (ml.kg <sup>-1</sup> )	10	6	6

### Histopathology

Additional histopathological examination was performed in mice perorally treated with triton X in the dose of 1000 mg.kg<sup>-1</sup>. All animals survived and were euthanized by CO<sub>2</sub> 24 h post-exposure. Samples of liver, lungs and colon were taken immediately after euthanasia of experimental animals, fixed in 10% buffered formalin, processed by standard histological paraffin technique and 4-5 µm sections were stained with haematoxylin and eosin. Samples were observed with the optical microscope Olympus BX 51 (Olympus, Aomori, Japan) with digital output (camera Olympus DP 71, Olympus, Aomori, Japan).

## RESULTS

Acute toxicity of detergents was beyond the max AD dose in all tested groups except for *i.m.* application of Argos [LD<sub>50</sub> = 5.0 (3.6 – 6.6), Table 1].

There was no evidence of toxic signs in *p.c.* treated animals. Perorally treated animals showed bristled hair and hunched back without any other negative signs. Uptake of food and drinking water decreased approximately by 50% in comparison to blank controls. Intramuscularly treated animals suffered from edema and inflammation of hind limbs with a negative impact on locomotive activity. Uptake of food and drinking water was not affected.

Acute toxicity of surfactants in mice was beyond the max AD in all tested compounds after *p.c.* administration as well as after *p.o.* and *i.m.* administration of sodium alkylbenzene sulphonate (Table 2). Remaining compounds did not show any skin irritation after *p.c.* treatment, nevertheless, *p.o.* and *i.m.* treated mice expressed the same signs as rats (bristled hair, hunched back and edema of hind limbs).

Histopatological examination in mice *p.o.* treated with triton X showed mild morphological changes such as liver vacuolar degeneration (Figure 1), lung

**Table 2.** Acute 24 h toxicity of surfactants in laboratory mice. Estimation of LD<sub>50</sub> (mg.kg<sup>-1</sup>) and maximal applicable dose (max AD) via *p.c.*, *p.o.* and *i.m.* administration are shown. The EEC hazard classification system designed for acute oral toxicity in rat is shown (*tox. cat.*). Toxic categories are LD<sub>50</sub> ≤ 25 mg.kg<sup>-1</sup> (very toxic), LD<sub>50</sub> = 25-200 mg.kg<sup>-1</sup> (toxic), LD<sub>50</sub> = 200-2000 mg.kg<sup>-1</sup> (harmful) and LD<sub>50</sub> > 2000 mg.kg<sup>-1</sup> (unclassified).

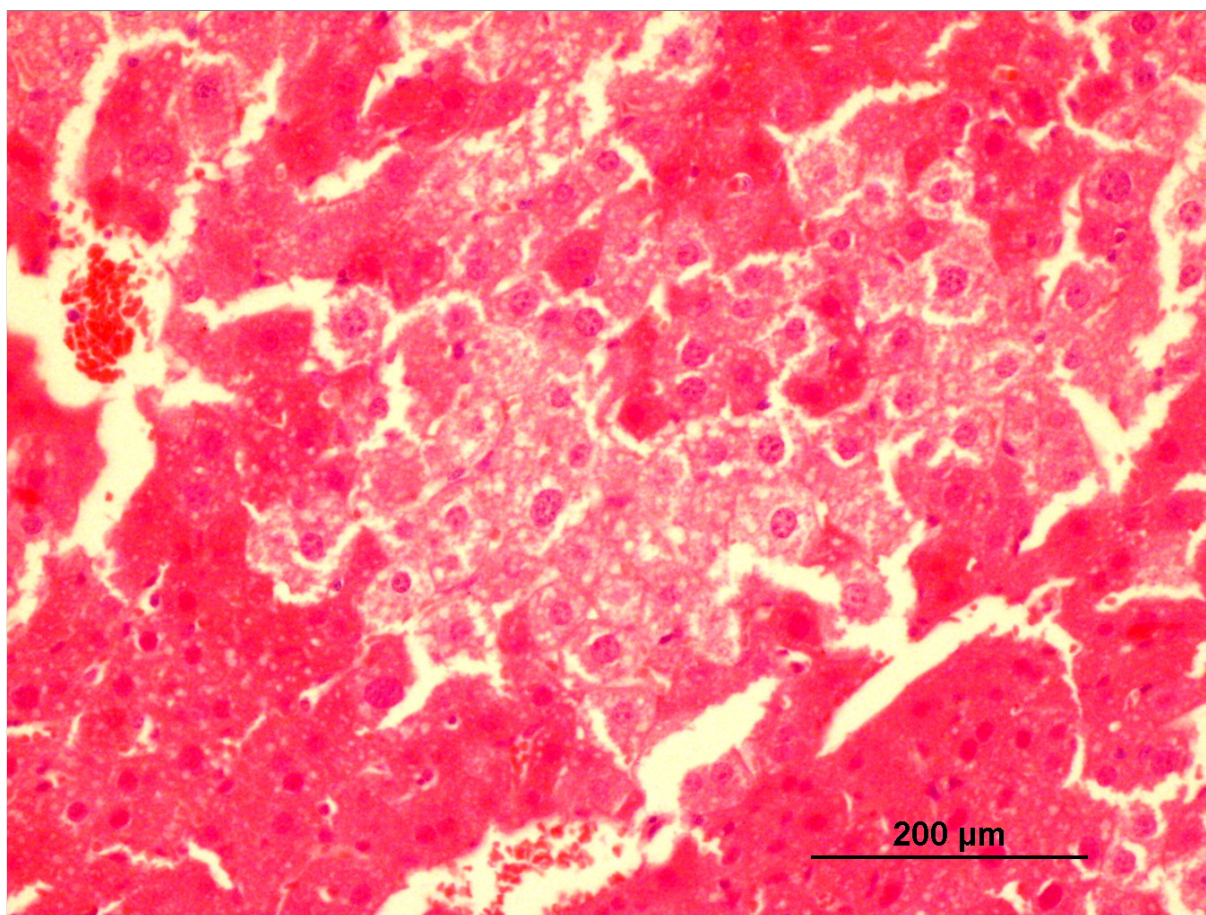
surfactant	LD <sub>50</sub> (24 h; mg.kg <sup>-1</sup> )			
	<i>p.c.</i>	<i>i.m.</i>	<i>p.o.</i>	<i>tox. cat.</i>
althosan MB 50%	> 5000	< 1000	< 1000	harmful
sodium alkylbenzene sulphonate 80%	> 5000	> 2000	> 2000	unclassified
triton X	> 5000	< 1000	> 1000	harmful
benzyl dimethyl dodecyl ammonium chloride	> 5000	< 100	> 500	harmful
N-dodecyl pyridinium bromide	> 5000	< 100	> 200	harmful
max AD (mg.kg <sup>-1</sup> )	5000	2000	2000	

congestion and presence of siderophages, which were not present in control samples. We found pinworms gen. *Syphacia* in colon sections of both triton X administered and control animals in low to moderate numbers.

## DISCUSSION

Toxic effects of surfactants are usually presented after a direct contact with eyes and skin, mainly characterised by irritation, redness, itching, scaling (eyes), erythema, contact dermatitis (skin), or harmful effects if swallowed e.g. damage of intestinal wall and

internal organs (liver, kidneys etc.). Inhalation of aerosols leads to respiratory tract irritation. Carcinogenic, teratogenic and mutagenic effects are considered after chronic exposition but there is usually a lack of experimental data (material safety data sheets). Surfactants act as deleterious agents mainly in microorganisms and water organisms, thus are chiefly used as disinfections or inhibitors of the fungal or algae growth [6]. Birds and mammals are usually less sensitive to their effects. Accordingly, all tested detergents and surfactants were non-toxic after *p.c.* administration in rat and mice without any evident skin irritation except for group *p.c.* administered with benzyl dimethyl dodecyl ammonium chloride showing slight erythema.



**Figure 1.** Liver vacuolar degeneration in mice perorally treated with triton X (1000 mg.kg<sup>-1</sup>). Haematoxylin and eosin. Foto by L. Novotny.

Detergents were also non-toxic under *p.o.* and *i.m.* administrations except for Argos<sup>TM</sup> with LD<sub>50</sub> estimated to be 5 ml.kg<sup>-1</sup>. Estimated toxicity of

surfactants varied from non- (sodium alkylbenzene sulphonate, LD<sub>50</sub> > 2000 mg.kg<sup>-1</sup>) or low-toxic agents (althosan MB and triton X, LD<sub>50</sub> ± 1000 mg.kg<sup>-1</sup>) to



more toxic benzyl dimethyl dodecyl ammonium chloride and N-dodecyl pyridinium bromide with *i.m.* toxicity less than 100 mg.kg<sup>-1</sup>. Thus, all surfactants with the exception of sodium alkylbenzene sulphonate were classified as harmful according to EEC hazard classification system designed for rats, here related to equivalent doses in mice. Results for detergents and surfactants are not directly comparable due to a different animal model. For instance, mice in this study were evidently more resistant to perorally administered triton X (LD<sub>50</sub> > 1000 mg.kg<sup>-1</sup>) in comparison with rats (male and female rat LD<sub>50</sub> are 500 mg.kg<sup>-1</sup> and 707 mg.kg<sup>-1</sup>, respectively, according to material safety data sheet (triton X-100, available on-line: [http://www.dnr-is.com/src/EZ%20Precipitation%20MSDS\(3\).pdf](http://www.dnr-is.com/src/EZ%20Precipitation%20MSDS(3).pdf)). However, the referred data are generally inconsistent presenting also 1800 mg.kg<sup>-1</sup> (triton X, *p.o.*; sciencelab.com). Histopathological examination of mice treated with triton X proved only a mild organ damage. Moreover, no serious health complications or even deaths were observed after the dose of 1000 mg.kg<sup>-1</sup>.

In summary, acute toxicity of all tested detergents (Argos™, FloraFree™, Dermogel™ and Neodekont™) and surfactants sodium alkylbenzene sulphonate, althosan MB and triton X was generally low. All these compounds could be considered safe if used as MCD means or even if low volumes are accidentally ingested, although the chronic impact of detergents was not evaluated in this study. Health risk of a single accidental ingestion of surfactants benzyl dimethyl dodecyl ammonium chloride and N-dodecyl pyridinium bromide should be considered.

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