Section 6.1.4 Annex Point IIA6.4	Acute eye and dermal irritation	
	JUSTIFICATION FOR CHOICE OF KEY STUDY	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	
Detailed justification:	***Statement***	

L(+) lactic acid as active substance in biocides: Doc. III-A

Section 6.1.4 Skin and eye irritation

Introduction: Lactic acid is widely occurring in nature as metabolic substance in all living cells. As such, it is a natural constituent of many foods, such as meat, dairy products and fruits. Since 1885, it has been manufactured for industrial purposes in Europe.

Lactic acid is approved in the EU (E270) as food additive, and has a GRAS (Generally Recognized as Safe) status in the USA. It is used in many foodstuffs, cosmetics, pharmaceuticals, feed, and technical applications, such as in biocides.

This statement has been written to evaluate the dermal irritation/corrosion properties of lactic acid in man. Several skin irritation tests were performed with lactic acid (88%) with different animals to establish the properties of lactic acid on the skin. I discuss below the preferred choice of the pig as animal model to predict the potential risks of lactic acid to human skin.

Skin irritation

1. Introduction

Standard testing methods for skin irritation/corrosion properties are described in OECD guideline 404 (Acute Dermal Irritation/Corrosion).

In the description of the testing strategy, evaluation of existing human studies or occupational reports should be considered first. Concerning the occupational experience with lactic acid, there is a long history of working with lactic acid and using lactic acid: there was no incidence reported with an irreversible adverse effect on the skin.

Further in the testing strategy, concerning the physicochemical properties it is mentioned for substances exhibiting pH $\leq$ 2.0, as lactic acid, these can be considered corrosive, and then its acid reserve (or buffering capacity) may also be taken into consideration. If there is a buffering capacity, it may that a substance is not corrosive to the skin, then further testing should be undertaken to confirm this. We will show that lactic acid, as weak acid, is having a buffering capacity. X

The albino rabbit is described in OECD 404 as the preferred laboratory animal. Testing on other species is permitted, when a rationale for using

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other species is provided. The reason why rabbit is mostly used as animal model for skin tolerance tests, is that it is more responsive than human skin and by this animal model, it is believed that very sensitive individuals or local reactions are taken into consideration.

Based on the dermal irritation studies with albino rabbits, one could suggest that lactic acid should be classified as severely irritating and corrosive. The rabbit however appeared to be extra sensitive to lactic acid.

Based on significant similarities between human and pig skin, the domestic pig was proposed as a valuable animal model for human skin (Bisset, 1985) and it has been suggested that the domestic pig offers the most appropriate model for all types of dermatological and surgical wound investigations (Vardaxis, 1997).

Therefore it was decided to use pigs in another dermal testing, because the emphasized anatomical similarities of pig skin to human skin, and the demonstrated similarity of skin penetration in pig and man.

2. Studies

Study 1 (TNO report V 87.405/270419): X

Summary of the study:

A sample of lactic acid (88%) was examined for acute dermal irritating/corrosive properties in an experiment with three pigs.
In each test animal, the test substance was brought into contact with three separate areas of shaved dorsal skin for 3 and 60 minutes and for 4 hours, respectively.

The test sample did not cause any skin irritation when it was brought into contact with the dorsal skin of pigs for 3 or 60 minutes or for 4 hours.

The test sample did not cause any skin irritation.
On the basis of the results obtained in the present study with pigs, it was concluded that, according to EEC standards, lactic acid (88%) is not irritating or corrosive to skin.

Study 2 (TNO Report V 87.406/270419).

Summary of the study:

A sample of lactic acid (50%) was examined for acute dermal irritating/corrosive properties in an experiment with three pigs.
In each test animal, the test substance was brought into contact with three separate areas of shaved dorsal skin for 3 and 60 minutes and for 4 hours, respectively.

The test sample did not cause any skin irritation when it was brought into contact with the dorsal skin of pigs for 3 or 60 minutes or for 4 hours.

On the basis of the results obtained in the present study with pigs, it was concluded that, according to EEC standards, lactic acid (50%) is not

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irritating or corrosive to skin.

Study 3 (IRI report 235943 dated September 1986)

Summary of the study:

The corrosivity potential of a test material, Lactic Acid Q88, was investigated by means of a test in guinea pigs. The test was designed to assess irritancy and/or corrosivity by means of topical application of the test material to intact skin under semi occlusive conditions for various exposure times viz: 3 min, l h and 4 h. Two groups of 3 animals were used. In Group l exposures of 3 min and l h were investigated while 4 h exposures were investigated in Group 2.
No irritation or corrosion were noted in animals exposed to Lactic Acid Q88 for 3 min and l h. Responses in animals exposed to the test material for 4 h were limited to very slight erythema which was noted at patch removal and l h after patch removal only. Skin appeared normal at 24 h after patch removal.

Study 4 (PURAC report: Acid Reserve at pH 2, 21 August 2006)

In the Annex, the acid reserve at pH 2 and pH 4 is calculated for some weak acids and strong acids. The acid reserve is expressed as gram NaOH needed to raise the pH of 100 ml of the acid, to a pH of 2. Also the acid reserve, as gram NaOH, to raise the pH to pH 4, is calculated.

As expected, for strong acids, much NaOH is needed to raise the pH of < 2, to a pH 2. For weak acids, only small amounts are needed to raise to pH 2. For lactic acid only 0.6 g. This means that only a small amount of neutralizing agent is needed to raise the pH to pH of 2. The skin is having a neutralizing capacity to handle this.

Comparing the acid reserve at pH 2 with that of pH 4, it can be seen, that for the strong acids, there is practically no difference, while weak acids have their strongest buffering capacity close to pH 4. The skin is having a pH > 4, and when coming in contact with lactic acid, the local skin pH will decrease and skin fluids will start neutralizing, and as lactic acid is buffering strongly at pH 4, the local skin pH will not rapidly decrease to pH below 3.5 or to pH 2.

It can be concluded that the buffering capacity of lactic acid is making this substance not corrosive. X

3. Evaluation
Comparative evaluations studies are published of skin irritation in rabbits and humans, which have shown that rabbit skin is far more sensitive than human skin under similar testing conditions. A comparative study with several animal models (Motoyoshi, 1987) on the skin irritancy of twenty oils and twenty synthetic perfumes, indicates that the skin sensitivity decreases in the following order: rabbit, guinea pig, rat, man and miniature swine. The rabbit skin is more permeable than human skin, which may account for a significant part, for the

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increased irritation observed in rabbits.

In this same study, 6 compounds that produced the least reaction in the human closed patch test, produced the most severe responses in the rabbit skin, and would be classified as severe irritants. Histologically, the skin of the miniature swine closely resembles that of human skin and was considered to be suitable for investigating skin irritancy.

Considerable inter-species variability's concerning the anatomy, physiology, and biochemistry of the skin are known. The structure of human skin consists of the epidermis (the superficial portion), and the dermis, which is the deeper, thicker portion of the skin that is composed of connective tissue, blood vessels, glands and nerves. Hair follicles and sweat glands are epidermal appendages. The human epidermis is composed of 5 strata (Klaassen, 2001), of which the stratum corneum acts as the absorption rate-limiting barrier of the skin. Human skin thickness ranges from 0.5 mm (eyelid) to 4.0 mm (palm and sole), over the various regions of the body.

In general, small mammals (rabbits, rats, mice, etc.) have a dense layer of body hair, and a thin epidermis and dermis, relative to the humans. The epidermis is only 2-4 cell layers in most mammals compared to 6-10 cell layers in the human and porcine epidermis (Vardaxis, 1997). The stratum corneum is also in general much thicker in humans than in animals, resulting in a lower percutaneous permeability in humans. Another major difference between most furry mammals and human skin is that these mammals lack eccrine sweat glands.

The use of the pig as animal model for human skin is based on the fact that porcine skin and human skin have several similar characteristics in contrast to small mammals. In the literature (Simon and Maibach, 2000) it is well-recognized that pig skin is an appropriate animal model for human skin, in terms of anatomy, physiology, biochemistry and absorption characteristics.

These similarities (Bissett, 1985) include hair density, skin surface structure, epidermal structure, sebum composition, epidermal turnover rate, epidermal lipid composition, and the use of fat for insulation (in contrast to fur in many small mammals).

A significant difference (Vardaxis, 1997) between porcine and human skin, is that the pig possesses apocrine glands instead of eccrine sweat glands present in human skin. However, it is unlikely that this difference in glands would have any impact on the predictive potential of a dermis irritation reaction in the pig.

For many chemicals, the skin permeability characteristics of pig skin resemble those of human skin, and the pig is a representative animal model for humans in skin permeation studies.

Remark: PURAC is producing lactic acid since 1936 at a high volume, during many years producing more than 100.000 ton per year. During all these years producing such high amounts of lactic acid, there was no incidence reported of operators in the factory, with an irreversible adverse effect on the skin, in the cases of direct dermal contact with lactic acid, after a spill / leakage incident.

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4. Summary

The rabbit is more sensitive to skin irritants than other test species or humans. Although the rabbit is currently used as the most conservative animal in standard irritancy tests, the literature provides strong evidence that the pig is a more relevant animal model for human skin than the rabbit. Thus, in case of L(+) lactic acid for which there are irritancy/corrosion data in the guinea pig, domestic pig, and rabbit, greater emphasis should be given to findings in the guinea pig and pig irritancy tests, as these are more relevant models for predicting dermal effects in humans, than findings in the rabbit study.

Eye irritation

1. Introduction

Standard testing methods for eye irritation/corrosion properties are described in OECD guideline 405 (Acute eye Irritation/Corrosion). The rabbit is described in OECD 404 as the preferable laboratory animal. Testing on other species is permitted, provided that a rationale for using these other species is provided.

2. Studies

Study 1 (TNO report V96.157 dated March 1996)

Three different forms of **lactic acid,** i.e. a powder (sample code H60) and two liquids (sample code HS88 and sample code BF S36), were examined undiluted for eye irritating/corrosive potential in an ex vivo bioassay, namely the Enucleated Eye Test with chicken eyes (CEET) (The Chicken Enucleated Eye Test (CEET) is an alternative to the Draize eye irritation test with albino rabbits)

The eyes were collected as waste material from a slaughter-house for chickens, which were killed for human consumption.

The three lactic acid samples caused quite different corneal effects in the CEET. Generally, sample H60 induced moderate corneal effects, sample HS88 severe corneal effects and sample BF S36 slight corneal effects.

On the basis of the results obtained with this *in vitro (ex vivo)* assay and according to the

scheme for (EC-)classification applied, the following was concluded:

- the buffered lactic acid sample (BF S36) can be considered not irritating to eyes,

- the powder sample (H60) can be considered irritating to eyes (R36), and

- the lactic acid sample (HS88) can be considered severely irritating to eyes (R41)

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Publication 1

Ocular tolerance studies were carried out with a group of humectants and moisturizers used in cosmetics (Guillot, 1982) including tests with lactic acid. Eyes of rabbits were examined after 1 and 24 h and after 2, 3, 4 and 7 days, with fluorescein staining. The ocular irritation index (OII) was determined and evaluated on a scale from 0 to 110. A compound does not provoke any significant injury to the eye mucous membrane when no opacity of the cornea and when OII is less than 15. Lactic acid instilled at 20% and 10% provoked a significant ocular irritation: OII was 39.5 resp. 31.2. Only for the 10% dilution, these lesions were reversible 7 days after instillation.

3. Evaluation

The results clearly demonstrate that Lactic Acid must be considered severely irritating to the eyes.

4. Summary:

L(+) lactic acid is severely irritating to the eyes

References:

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IRI (1986) Lactic acid Q88: a skin corrosivity test in Guinea Pigs. *Inveresk Research International,* Report 3625.

Klaassen, C.D. (ed.) (2001) *Casarett and Doull's Toxicology: the Basic Science of Poisons (6th ed.). McGraw-Hill Health Professionals Division; Chapter 5, pages 117-119 and Chapter 19, pages 657-658.*

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TNO (1987). Acute dermal irritation/corrosion study with lactic acid (88%) in pigs. L.van Beek, *TNO Report V 87.405 / 270419.*

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Vardaxis, N.J., et al., (1997). Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. *Journal Anat.,* ***190****(4): 601-611.*

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	JUSTIFICATION FOR CHOICE OF KEY STUDY Annex: PURAC (2006). Acid Reserve at pH 2. G. Nanninga. *Report 21 Aug. 2006.*	Official use only
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/07/16	
Evaluation of applicant's justification	Applicant's justification is acceptable with amendments: Relevant species: L(+) lactic acid proved to be corrosive in *in vitro* and in in *vivo* rabbit dermal irritation tests; and it was irritating in patch tests in humans (York et al. 1996). The participant proposed classification with R38. Since the studies with pigs do not support the classification with R38, it doesn't seem to be adequate to use these studies as sole dermal irritation key study (as proposed by the participant) to provide information on the irritating properties of L(+) lactic acid. From the human patch tests it is likely that dermal irritation studies in pigs underestimate the irritating potential of L(+) lactic acid for human skin while rabbit skin seems to be much more sensitive than human skin. Thus, the human patch test data should be used as key study (York et al. 1996) showing adequate results for classification and labelling. Acid reserve at pH2: L(+) lactic acid revealed skin corrosive properties in several studies (in vitro: Corrositex assay (Harbell 1994), TER assay (York et al. 1996), rabbit in vivo: Barnes 1983; van Beek 1986). These studies show that the acid reserve of L(+) lactic acid at < pH 2 is high enough to display corrosive properties dependent on the test system used (e.g. the buffering capacity of the skin).	
Conclusion	Applicant's justification is acceptable with amendments (see Evaluation)	
Remarks	None	

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	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		