



Original Article

Derivation of acceptable daily exposure value for alanine, *N,N*-bis(carboxymethyl)-, trisodium salt

Masayuki Mishima¹, Dana Hoffmann², Gaku Ichihara³, Satoshi Kitajima⁴,
Makoto Shibutani⁵, Satoshi Furukawa⁶ and Akihiko Hirose⁷

¹Research Division, Chugai Pharmaceutical Co., Ltd., Shizuoka, Japan

²Group Safety, Security, Health and Environmental Protection, F. Hoffmann - La Roche Ltd., Basel, Switzerland

³Department of Occupational and Environmental Health, Tokyo University of Science, Chiba, Japan

⁴Division of Cellular and Molecular Toxicology, Center for Biological Safety and Research,
National Institute of Health Sciences, Kanagawa, Japan

⁵Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo, Japan

⁶Biological Research Laboratories, Nissan Chemical Corporation, Saitama, Japan

⁷Division of Risk Assessment, Center for Biological Safety and Research, National Institute of Health Sciences,
Kanagawa, Japan

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ABSTRACT — Use of a non-phosphate detergent builder, alanine, *N,N*-bis(carboxymethyl)-trisodium salt (ABCT), has been expanded to wide range of washing and cleaning products for consumer uses and industrial applications including cleaning agents in food or pharmaceutical factories. Therefore, determination of acceptable daily exposure (ADE) of ABCT by oral, parenteral or inhalation route based on updated toxicity database could provide valuable information on the risk management for protection of consumers, patients and workers. Here, we proposed the ADEs based on the toxicological information of various *in vivo* and *in vitro* non-human studies. Because the full report of each toxicity study was not disclosed, derivation of the ADE was done based on available information mainly from ECHA database. ABCT exhibited renal toxicity as a main effect; however, ABCT did not exhibit carcinogenicity, genotoxicity, reproductive toxicity, irritation, and sensitization. Applying modification factors to the NOAEL of the animal study of longest treatment period, oral ADE was determined as 260 mg/person/day. Taking the oral bioavailability into the consideration of conversion to other routes, parenteral and inhalation ADEs were determined as 50 mg/person/day.

Key words: Alanine, *N,N*-bis(carboxymethyl), Detergent builder, Chelate, Cleaning agent, ADE

INTRODUCTION

Alanine, *N,N*-bis(carboxymethyl)-trisodium salt (ABCT) is a chelating builder to increase cleansing action of detergents. ABCT is a chemical of low environmental impact and used as an alternative for traditional phosphate builders that cause eutrophication and environmental disruption. According to the information of ECHA,

total production of the compound was categorized in the annum tonnage banding of 10,000-100,000 tones. ABCT is contained in wide range of washing and cleaning products for consumer uses and industrial applications including cleaning agents in food or pharmaceutical factories. Therefore, determination of acceptable daily exposure (ADE) of ABCT for oral, parenteral and inhalation route could provide valuable information on the risk manage-

Correspondence: Masayuki Mishima (E-mail: mishimamsy@chugai-pharm.co.jp)

ment for protection of consumers, patients and workers.

Here, we proposed ADE for ABCT based on the open access information of ECHA website. Many of the ECHA registered studies were performed in accordance with OECD guidelines, often by the company participating business with the test compound and were not necessarily reviewed for its scientific appropriateness. However, we considered that the ECHA database was the most useful in the currently available information of this compound. Because the full report of each toxicology study was not disclosed, derivation of the ADE was done based on available information mainly from ECHA database.

MATERIALS AND METHODS

Substance information

Identification

| | |
|-------------------|--|
| IUPAC Name: | (2S)-2-(bis-carboxymethyl-amino)-propionic acid, trisodium salt |
| Synonyms: | Alanine, <i>N,N</i> -bis(carboxymethyl)-, trisodium salt, ABCT, Trisodium 2-[bis(carboxymethyl)amino]propanoate, Methyl glycine diacetic acid trisodium salt |
| Trade Names: | Trilon M Liquid, Trilon ES9964 Pulver |
| CAS Number: | 164462-16-2 |
| EC Number: | 423-270-5 |
| Formula: | C ₇ H ₈ NNa ₃ O ₆ (Fig.1) |
| Molecular Weight: | 271.11 |

Chemical and Physical Properties

| | |
|------------------------|--|
| Appearance: | White powder |
| Melting Point: | > 390°C |
| Solubility 24°C: | Water: > 500 g/L pH 10 - 12 (Trilon M Liquid) |
| Partition coefficient: | Log Pow ≤ -4 |

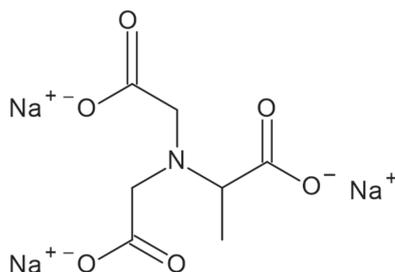


Fig. 1. (2S)-2-(bis-carboxymethyl-amino)-propionic acid, trisodium salt.

Source of toxicological information

Many of the ECHA registered studies were performed in accordance with OECD guidelines, often by the company participating business with the test compound and were not necessarily reviewed for its scientific appropriateness. However, we considered that the ECHA database was the most useful in the currently available information of this compound. Because the full report of each toxicology study was not disclosed, derivation of the ADE was done based on available information mainly from ECHA database.

Derivation of ADE

The concept of ADE derivation described in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q3C and Q3D guidelines (ICH, 2014) was applied.

RESULTS AND DISCUSSION

Acute toxicity

Female rats gavaged with 2,000 mg/kg ABCT displayed impaired general state, dyspnea, staggering and piloerection. All symptoms were reversible until 3 days post administration. Male rats did not show any signs of abnormalities (ECHA, 2017). Oral LD50 was > 2,000 mg/kg in rats (ECHA, 2017).

Repeated dose toxicity (Table 1)

Repeated dose toxicity studies of 28 days, 90 days and 24 months in rats were reported. The study of longest treatment period was a combined chronic toxicity / carcinogenicity study for 24 months fed with 1,000, 5,000 and 19,200 ppm ABCT that corresponded to 54, 262 and 1132 mg/kg/d for males, and 66, 334 and 1317 mg/kg/d for females, respectively (ECHA, 2017). The studies included clinical pathology, ophthalmoscopy and histopathology analysis, and were recognized pivotal. These studies were done in accordance with OECD guideline 453 and GLP compliance.

Genotoxicity

ABCT was negative in Ames test, gene mutation test with CHO cells and *in vivo* mouse erythrocyte micronucleus test in mice up to 2,000 mg/kg (ECHA, 2017). Ambiguous result came from *in vitro* chromosomal aberration test with V79 cells (ECHA, 2017). Taken together, the results suggested no genotoxic concern on the test article.

ADE for alanine,N,N-bis(carboxymethyl)

Table 1. Pivotal Repeated Dose Toxicity Studies with ABCT.

| Study type | Species | Doses [mg/kg/d] | NOAEL ¹ [mg/kg/d] | Main Findings |
|--|---------|--|------------------------------|---|
| 1 month repeated dose toxicity (ECHA, 2017) | Rats | 0, 76/88, 315/378, 1,313/1,504 ²⁾ (in the drinking water) | 76/88 ²⁾ | 315/378 mg/kg/d: -kidney: slight tubular vacuolization 1,313/1,504 mg/kg/d: -kidney: focal or multifocal vacuolization of the tubular epithelia in the renal cortex and focal calcification in the cortico-medullary area |
| 3 months sub-chronic toxicity (ECHA, 2017) | Rats | 0, 170/204, 874/1056, 1325/1588, 1774/2097 ²⁾ (in the diet) | 170/204 ²⁾ | ≥ 874/1056 mg/kg/d: -kidney: focal hyperplasia of the urothelium in the renal pelvis/ureter, increased absolute and relative weight-liver: reduced zonal fatty infiltration ≥ 1325/1588 mg/kg/d: -liver: increased relative weight ≥ 1774/2097 mg/kg/d: -kidney: focal tubular vacuolization (unilateral) |
| 24 months combined chronic toxicity / carcinogenicity (ECHA, 2017) | Rats | 0, 54/66, 262/334, 1132/1317 ²⁾ (in the diet) | 262/334 ²⁾ | 1132/1317 mg/kg/d: -increased total protein and globulin in blood -increased erythrocytes in urine -kidney: increased absolute and relative weight, cytoplasmic vacuolization and pigment storage in tubular epithelial cells, (multi) focal tubular hyperplasia, (multi) focal ectasia of ducts or vessels in papillar regions, cyst(s) in the kidneys, pelvic dilation, chronic fibrotic, partly necrotizing pyelitis, mineralization in the papilla, nodular or papillary hyperplasia of the transitional cell epithelium in the renal pelvis |

¹⁾ NOAEL values are reported in study report summaries. However, no effect was observed at this dose level. Thus, values were taken as NOEL.

²⁾ male/female

Carcinogenicity (Table 1)

In a combined chronic toxicity/carcinogenicity study, daily oral treatment of rats with ABCT for 24 months did not result in treatment-related changes in the incidence of neoplastic lesions (ECHA, 2017).

Reproductive toxicity (Table 2)

No changes in reproduction and fertility have been observed in rats following oral ABCT treatment. No indications of developmentally toxic/ teratogenic effects were seen (ECHA, 2017).

Skin/eye irritation

Results of rabbit skin (OECD guideline 404) and eye irritation test (OECD guideline 405) with ABCT solution (Trilon M liquid) suggested non-irritant (ECHA, 2017; BASF, 2015).

Sensitization

Guinea pig maximization test (OECD guideline 406) suggested no skin sensitization (ECHA, 2017; BASF, 2015).

Safety pharmacology

No relevant data was available.

Bioavailability

Rat oral bioavailability ranged from 17-39%, 20.94% after single oral administration with 465.2 mg/kg, 16.98% with 24.1 mg/kg, and 38.74% with 512.5 mg/kg in rats. Repeated oral administration achieved 32.94% of bioavailability with 475.8 mg/kg (ECHA, 2017).

Other *in vitro* safety data

No relevant data was available.

Experiences of human exposure

No relevant data was available.

Information from other sources

No relevant data was available.

Derivation of ADE

Critical effects

Renal toxicity was the critical leading effect of ABCT for the purpose of this assessment and was used as the basis for setting ADE value.

ADE for oral route

Chronic nephropathy was observed in a 24-month feeding study in rats (ICH, 2014). A NOAEL of 262 mg/kg/d

Table 2. Pivotal Reproductive Toxicity Studies with ABCT.

| Study Type | Species | Doses [mg/kg/d] | NOAEL [mg/kg/d] | Main Findings |
|-------------------------------------|---------|----------------------------|--|---|
| Reproductive toxicity (ECHA, 2017) | Rats | 0, 50, 200, 1000 (gavage) | ≥ 1000 (reproductive performance, fertility and pups) 200 (general toxicity) | -no reproductive and fertility effects observed -no offspring effects observed |
| Developmental toxicity (ECHA, 2017) | Rats | 0, 100, 300, 1000 (gavage) | ≥ 1000 (dam) ≥ 1000 (fetus) | -no embryonic/teratogenic effects observed |

for males and 334 mg/kg/d for females (= 5000 ppm) was observed. However, no effect was reported at this dose, thus 262 mg/kg/d is taken as NOEL. Chronic nephropathy occurs spontaneously in aged rats. ABCT increase the severity of this finding but is not considered to be the cause of the effect. Therefore it is not relevant for humans.

$$\text{ADE} = 262 \text{ mg/kg/d} \times 50 \text{ kg} / (\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}) = 262 \sim 260 \text{ mg/person/day}$$

Modification factors applied:

F1 = 5 for extrapolation from rat to human

F2 = 10 for variability between individuals

F3 = 1 for study duration of 2 years with rats

F4 = 1 for severity of systemic toxicity

F5 = 1 for conversion of the starting point to NOEL

ADE for parenteral and inhalation routes

Absolute bioavailability (BA) of ABCT in rats has been determined to be 17-39%. Therefore, the ADE for the parenteral route is considered to be approximately 5-fold lower than the oral route. Because the available toxicological data did not indicate irritating or sensitizing activity, the parenteral ADE can be applied to inhalation route.

$$\text{ADE} = 260 / 5 = 52 \sim 50 \text{ mg/person/day}$$

In conclusion, because toxicological data did not suggest genotoxicity or carcinogenicity of ABCT, oral ADE could be determined as 260 mg/person/day based on the NOAEL from the 24-month rat study. Applying an additional factor for the oral BA, parenteral and inhalation ADEs were proposed as 50 mg/person/day.

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Conflict of interest---- MM and DF are employees of the pharmaceutical companies that intend to use ABCT. Other authors declare that there is no conflict of interest. Because the full report of each toxicology study was not disclosed, the authors considered the appropriateness of NOAEL based on the open access information of ECHA website. The ECHA registered studies were often performed by the company that participates business with the test compound.

REFERENCES

- ICH (2014): Quality Guidelines, Q3C and Q3D. <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html>
- ECHA (2017): information, EC number: 423-270-5. Trisodium 2-[bis(carboxylatomethyl)amino]propanoate. <https://echa.europa.eu/registration-dossier/-/registered-dossier/15592/1>
- BASF (2015): Safety Data Sheet Trilon M Liquid, version 2.1, 2015/05/27. http://worldaccount.basf.com/wa/NAFTA~fr_FR/Catalog/Detergents/doc4/BASF/PRD/30043459/pdf?asset_type=msds/pdf&language=EN&validArea=CA&urn=urn:documentum:ProductBase_EU:09007af8802b9c29.pdf#search=%27trilon+sds%27