Attachment B

to Pending Petition of The Dow Chemical Company to Remove 2-BEB from CAA Section 112(b)(3) List of Hazardous Air Pollutants

Comparative Assessment of Metabolism of 2-BEB between Human and Rat

Fagen Zhang and Matthew LeBaron

Table of Contents

This addresses EPA's information request number 2: **"References that demonstrate the rapid hydrolysis of 2-Butoxyethylbenzoate (2-BEB) to benzoic acid and ethylene glycol butyl ether following inhalation exposure."** (Subparts 2(a)-(d) are set out and addressed below).

Introduction

2-Butoxyethylbenzoate (2-BEB) is a carboxylate ester that is synthesized from 2-butoxyethanol (EGBE) and benzoic acid (BA). Following systemic absorption, it is expected that 2-BEB will be rapidly metabolized (hydrolyzed) by the numerous systemic carboxylesterases (CES) in mammalian systems (*e.g.*, esterases in the intestinal, liver, lung, nasal mucosa, skin, and/or blood compartments) into BA and EGBE. BA and EGBE will be subsequently metabolized to their corresponding metabolites. Therefore, the toxicity of 2-BEB in mammalian systems can be evaluated based on the esterase hydrolysis products (EGBE and BA), which are dependent on the CESs present in various tissues from the corresponding exposure routes such as oral, inhalation, or dermal exposure. As explained in the discussion of subparts 2(a) and (b) below, CES activity can be found in human lungs, nasal mucosa, liver, blood/plasma, and skin, supporting that the low levels of 2-BEB that would be released into the environment would be rapidly hydrolyzed to EGBE and BA in humans via oral, inhalation, and/or dermal exposure as has been seen in oral studies with rats.

Brief review of data supporting the rapid carboxylesterase hydrolysis metabolic pathways of 2-BEB in rats via the oral route

***In vivo* metabolism study via oral gavage administration in rats**

- *No 2-BEB (parent) was detected in C_{max} rat blood samples from oral gavage administration at dose levels of 25 mg/kg or 250 mg/kg 2-BEB.*

  An assessment of pharmacokinetics and metabolism was performed with radiolabeled 2-BEB (where the radiolabeled position was on the glycol ether portion of 2-BEB) to evaluate absorption, distribution, metabolism, and excretion (ADME) (Zhang 2016). In that study, animals were dosed by oral gavage either once (25 and 250 mg/kg body weight (bw)) or daily for 14 days (25 mg/kg bw). C_{max} blood samples were collected from the study and analyzed by high-performance liquid chromatography (HPLC) separation with in-line radiochemical detection (RAM). In addition, collection of fractions with liquid scintillation spectrometry (LSS) followed by HPLC with electrospray ionization and accurate mass/time-of-flight mass spectrometry detection (LC/ESI/TOF-MS) was used for metabolite identification. The results indicated that no detectable parent (2-BEB) was present in any of the C_{max} blood samples. The most abundant C_{max} blood metabolite was identified as 2-butoxyacetic acid, which was formed from the glycol ether (EGBE) portion of 2-BEB.

  The absence of 2-BEB and presence of the most abundant 2-butoxyacetic acid in C_{max} blood samples clearly indicated that 2-BEB was initially quickly hydrolyzed via CESs to BA and EGBE once it was absorbed. The formed EGBE was then subsequently metabolized to 2-butoxyacetic acid (the most abundant metabolite) and other metabolites.

- *Similar oral gavage dose recoveries were observed in excreta and CO_2 from ADME studies at equal molar dose levels in rats for both ^{14}C-2-BEB (radiolabeled position was on the 2-butoxyethanol portion) and ^{14}C-2-butoxyethanol (radiolabeled position was the same as in ^{14}C-BEB).*

  The percentages (based on total radioactivity) of the dose recovered in excreta and CO_2 from the ADME study on 2-BEB [dose level 250 mg/kg (1.1 mmol/kg) (Zhang 2016)] and a previous ADME study on 2-butoxyethanol [dose level 125 mg/kg (1.1 mmol/kg) (Ghanayem, Burka et al. 1987)] are summarized in **Table 1**, below.

  As shown in **Table 1**, very similar percentages of dose recoveries in excreta and CO_2 were observed for 2-BEB and EGBE at the same mmol/kg dose levels and collection time points after oral gavage dosing. As the same ^{14}C-radiolabeled positions were labeled in both test substances, the results in **Table 1** clearly indicate that 2-BEB was rapidly hydrolyzed via CESs to EGBE and BA after dosing, and subsequently the formed EGBE exhibited the similar toxicokinetics to the directly administered EGBE in rats.

- *Similar major metabolite profiles were observed in urine from both oral gavage ADME studies of ^{14}C-2-BEB (radiolabeled position was on the 2-butoxyethanol portion) and ^{14}C-2-EGBE (radiolabeled position was the same as in ^{14}C-2-BEB) at equal molar dose levels in rats.*

  According to the urinary metabolite profiling results on the 2-BEB ADME study in rats at the dose level of 250 mg/kg (1.1 mmol/kg, (Zhang 2016)), no parent 2-BEB was detected in the urine samples, and three major metabolites were detected and identified as 2-butoxyacetic acid (BAA, most abundant), 2-butoxyethanol glucuronide (the second most abundant), ethylene glycol (EG, the third most abundant peak; this peak was eluted in solvent front and had the same retention time as radiolabeled ^{14}C-EG). Similarly, three major metabolites were detected in the urinary samples from an oral gavage ADME study in rats at a dose level of 125 mg/kg [(1.1 mmol/kg) on 2-butoxyethanol (EGBE), (Ghanayem, Burka et al. 1987)]. These three metabolites were identified as BAA (most abundant), 2-butoxyethanol glucuronide (the second most abundant), and a third most abundant peak, which was eluted in solvent front and was not identified in the Ghanayem *et al.* (1987) study. Dow assumes that this unidentified third peak was not discerned due to the state of analytical methodology and instrumentation at the time of the study (*i.e.*, 1987). A much more recent study by Zhang *et al.* (2016), using current state of the art instrumentation and methodology, identified a similar early eluting peak as EG, assumed to be the unidentified peak seen in the Ghanayem *et al.* (1987) study (Zhang 2016).

  Based on the major urinary metabolite profiling results, the proposed metabolic pathways of both 2-BEB and EGBE are depicted in **Figure 1**, below (which can be found following Tables 1 and 2).

  As shown in **Figure 1**, the similar major urinary metabolite formation patterns as between 2-BEB and EGBE further indicate that 2-BEB was quickly hydrolyzed via CESs to form EGBE and BA after oral gavage administration in rats. The resulting EGBE was subsequently further metabolized to the major metabolites as directly orally administered EGBE in rats.

EPA information request 2(a): "Enzymes involved, levels of these enzymes in lungs and blood and a comparison of these levels to levels in the liver (for oral exposure) and skin (for dermal exposure)."

EPA information request 2(b): "Any differences in metabolism of 2-BEB that are expected following oral versus inhalation exposure."

Dow response: *Information requests 2(a) and 2(b) are addressed together, as follows:*

Numerous studies have shown that various CESs are present in a wide variety of organs and tissues in many mammalian species, including human and rat (Satoh and Hosokawa

1998). These CESs are present not only in tissues related to oral exposure (such as liver, blood, and plasma) but also in tissues (such as lung, nasal mucosa, and skin, along with blood and plasma) relevant to other routes of exposure (inhalation or dermal). Such CESs will rapidly hydrolyze 2-BEB to EGBE and BA regardless of exposure route (*i.e.*, oral, inhalation, or dermal).

CESs are a large family of enzymes that are responsible for the metabolism of various xenobiotics. Several members of the large family of enzymes, including CES1, CES2, CES3, CES4A, and CES5A have been detected in various human tissues. According to the Human Protein Atlas, CES1 is a major metabolism enzyme and is enriched in liver and lung.[1] CES2 is enriched in the gastrointestinal tract and also expressed at high levels in liver, kidney, testis, and bone marrow and lymphoid tissues.[2] CES3 is expressed in colon, trachea, and brain and also expressed at high levels in liver, kidney, and bone marrow and lymphoid tissues.[3] The CES activities have been assayed with various ester substrates, including *p*-nitrophenyl acetate, 1-naphthyl acetate, procaine, alkylparaben, and phenyl salicylate. Based on published data, CES hydrolytic activity in various tissues (such as lung, nasal mucosa, liver, and skin) of both human and rat are summarized in **Table 2**. As shown in **Table 2**, the CES activities were approximately 630 and 250 nmol/min/mg protein in human lung microsomes and cytosol, respectively, around 3,720 and 430 nmol/min/mg protein in human liver microsomes and cytosol, respectively, and 84 – 99 and 52 – 120 nmol/min/mg protein in human skin microsomes and cytosol, respectively, based on the hydrolysis of *p*-nitrophenyl acetate. The levels of CES activities in human blood and plasma were approximately 230 and 46 nmol/min/ml, respectively, based on the hydrolysis of 1-naphthyl acetate. In addition, as shown in **Table 2**, numerous carboxylate esters have been shown to be hydrolyzed by these esterases to form the corresponding alcohol and acid (Bogdanffy and Taylor 1993, Bogdanffy, Randall et al. 1987, Mattes and Mattes 1992, Nambu, Miyazaki et al. 1987, Gaustad, Sletten et al. 1991, McCracken, Blain et al. 1993, Dean, Zhang et al. 1995, Satoh and Hosokawa 1998, Prusakiewicz, Ackermann et al. 2006, Jewell, Ackermann et al. 2007, Rudakova, Boltneva et al. 2011, Imai, Takase et al. 2013, Ozaki, Sugihara et al. 2013, Ozaki, Sugihara et al. 2015, Tokudome, Katayanagi et al. 2015, Gabriele, Puccini et al. 2018). The presence of esterases in blood, plasma, lung, nasal mucosa, and skin clearly indicates that once carboxylate esters are exposed to humans or rats via dermal and inhalation exposures, they will be quickly hydrolyzed via these CESs similar to oral exposure scenarios. Therefore, it is expected that 2-BEB will be quickly hydrolyzed to EGBE and BA once humans or rats are exposed to 2-BEB, regardless of the route of exposure.

EPA information request 2(c): "Any differences in metabolism of 2-BEB in rat versus human."

Dow response: *Based on the available data, there are no predicted qualitative differences in the metabolism of 2-BEB in rat versus human or between the different organs.*

---

[1] Human Protein Atlas -- CES1, available at https://www.proteinatlas.org/ENSG00000198848-CES1/tissue.

[2] Human Protein Atlas -- CES2, available at https://www.proteinatlas.org/ENSG00000172831-CES2/tissue.

[3] Human Protein Atlas -- CES3, available at https://www.proteinatlas.org/ENSG00000172828-CES3/tissue.

As discussed above (referencing **Table 2**), CESs are active in blood (plasma), liver, skin, nasal mucosa, and lung of both human and rat. Carboxylesterases, whether human or rat or found in different organs, have the same metabolic hydrolysis reaction (Figure 1):

Ester (2-BEB) + Water (H_2O) → Alcohol (EGBE) + Carboxylate (BA)

Therefore, these CESs will rapidly hydrolyze 2-BEB to EGBE and BA, following absorption in either human or rat via oral, inhalation, or dermal exposures. The formed EGBE and BA will then be converted to the corresponding metabolites. BA has been shown to be metabolized primarily to hippuric acid in both rat and human (Panel 2001). EGBE has also been demonstrated to be metabolized to primarily 2-butoxyacetic acid in both humans and rats (Ghanayem, Burka et al. 1987, Corley, Bormett et al. 1994, Gift 2005). Based on the ester hydrolysis of 2-BEB followed by the further metabolism of benzoic acid and EGBE, it is to be expected that 2-BEB will be metabolized similarly in both humans and rats. The proposed metabolic pathways of 2-BEB are shown in **Figure 1**.

EPA Request 2(d): "Evidence of rapid hydrolysis following exposure to structurally similar esters (for read across)."

Dow response: *Several glycol ethers have an acetate attached via an ester bond, which is rapidly hydrolyzed by CESs to form the glycol ether alone and acetic acid. This occurs through the same CES hydrolysis that occurs with 2-BEB to form EGBE and BA. The glycol ether, once the acetate is hydrolyzed to acetic acid, is further metabolized to the acid metabolite, as is seen with both EGBE and 2-BEB (Figure 1).*

a. *Glycol ether acetate metabolism comparison between rat and human via various routes/matrices.*

Ethylene glycol ethyl ether acetate (EGEEA) has been shown to be rapidly metabolized *in vitro* using rat plasma to ethylene glycol ethyl ether (EGEE) (Hoffmann and Jäckh 1985). An inhalation study with humans has shown that EGEEA is metabolized to the acid metabolite (ethoxyacetic acid) (Groeseneken *et al.* 1987a, b), which would have occurred through the same metabolic pathway, via CESs, that 2-BEB is metabolized to BAA in rats, thus supporting the existence of this pathway via inhalation in humans. Another glycol ether acetate, propylene glycol methyl ether acetate (PGMEA), was shown to be rapidly hydrolyzed *in vitro* with rat and human blood and liver homogenates (Domoradzki *et al.* 2003). This further supports the rapid CES hydrolysis of glycol ethers and an acid linked via an ester bond in both rats and humans.

b. *The major toxicity data for 2-butoxyethyl acetate was established based on EGBE and acetic acid (AA) formed from hydrolysis of 2-butoxyethanol acetate via CESs in both human and rat.*

As a carboxylate ester, 2-butoxyethyl acetate (EGBEA) is expected to be rapidly hydrolyzed to 2-butoxyethanol (EGBE) and AA via CESs in both humans and rats. The rapid hydrolysis of EGBEA to EGBE was the basis of the read-across for EGBEA registration (and lack of associated toxicity of AA) (Bureau 2006). Specifically, EGBE data were used for:

i. Repeated dose toxicity of EGBEA (oral, inhalation, or dermal exposures) in both humans and animals (such as rats);

ii. Mutagenicity data of EGBEA;

iii. Carcinogenicity data of EGBEA; and

iv. Toxicity for reproduction of EGBEA.

Conclusions

Based on the discussion above and the supporting study data, 2-BEB is clearly rapidly hydrolyzed to EGBE and BA in rats after oral administration. Furthermore, as shown by the evidence referenced in this response to information request #2, it is expected that 2-BEB will be similarly metabolized via the CESs present in various tissues (*i.e.*, blood, liver, lung, and skin) after inhalation or dermal exposures in humans. As BA has been shown to have minimal toxicity (BA is widely used as a preservative in the food industry), it is expected that the toxicity of 2-BEB is driven solely by the hydrolysis product EGBE. It should also be noted that based on the conservative estimates in The Dow Chemical Company's delisting petition (highest daily exposures of 1.82E-5 mg/m^3, 6.01E-14 mg/kg bw/day and 4.92E-14 mg/kg bw/day by inhalation, dermal and oral routes, respectively), these very low concentrations of 2-BEB would be metabolized rapidly by CESs in humans.

Table 1. Percentages of Dose Recoveries in Excreta and CO_2

Test Material	Dose Level		Percentage of Dose Recoveries in Excreta and CO_2				Minimal Absorption	
	mg/kg	mmol/kg	In 24-hr urine	In 24-hr CO_2	In 48-hr CO_2	In 48-hr Feces	% of the Dose	References
2-BEB[a]	250	1.1	61-62	11-14	13-15	2	88-95	Zhang *et al.* 2016
EGBE[b]	125	1.1	64	14	18	2	>88	Ghanayem *et al.* 1987

[a]Both excreta and CO_2 were collected through 168 hr after dosing.
[b]Both excreta and CO_2 were collected through 48 hr after dosing.

Table 2. Carboxylesterase Activity in Blood, Liver, Lung, and Skin of Both Human and Rat

Tissue Types	Carboxylesterase Activity		References
	nmol/min/mg protein	µmol/min/mL	
Human blood		0.23 ± 0.11 (based on hydrolysis of 1-naphthylacetate)	(Rudakova, Boltneva et al. 2011)
Rat blood		3.59 ± 0.43 (based on hydrolysis of 1-naphthylacetate)	(Rudakova, Boltneva et al. 2011)
Human plasma	44 ± 1 (based on naphthyl acetate hydrolysis)		(Prusakiewicz, Ackermann et al. 2006)
		0.046 ± 0.002 (based on hydrolysis of 1-naphthylacetate)	(Rudakova, Boltneva et al. 2011)
Rat plasma		3.69 ± 0.15 (based on hydrolysis of 1-naphthylacetate)	(Rudakova, Boltneva et al. 2011)
	0.02-0.15 (based on 4-hydroxybenzoic acid formation from alkylparaben)		(Ozaki, Sugihara et al. 2013)
	230 ± 10 (based on para-nitrophenylacetate hydrolysis		(Prusakiewicz, Ackermann et al. 2006)
	190 ± 10 (based on naphthyl acetate hydrolysis)		(Prusakiewicz, Ackermann et al. 2006)
	1.2 (based on phenyl salicylate)		(Ozaki, Sugihara et al. 2015)

Table 2. Carboxylesterase Activity in Blood, Liver, Lung, and Skin of Both Human and Rat (*continued*)

Tissue Types	Carboxylesterase Activity		References
	picomol/min/g tissue	nmol/min/mg protein	
Human liver microsomes		2 to 95 (based on 4-hydroxybenzoic acid formation from alkylparaben)	(Ozaki, Sugihara et al. 2013)
		3,720 ± 90 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
	6.99 ± 2.56 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
Human liver cytosol	0.39 ± 0.15 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
		430 ± 30 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
Rat liver microsomes		8,800 ± 300 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
		1.2 (based on phenyl salicylate)	(Ozaki, Sugihara et al. 2015)
		0.2 - 0.8 (based on 4-hydroxybenzoic acid formation from alkylparaben)	(Ozaki, Sugihara et al. 2013)
	52.9 ± 5.6 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
Rat liver cytosol	3.49 ± 0.31 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
		660 ± 30 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)

Table 2. Carboxylesterase Activity in Blood, Liver, Lung, and Skin of Both Human and Rat (*continued*)

Tissue Types	Carboxylesterase Activity		References
	µmol/min/g tissue	µmol/min/mg protein	
Human lung microsomes		0.63 ± 0.12 (based on substrate *p*-nitrophenyl acetate)	(Gabriele, Puccini et al. 2018)
Human lung cytosol		0.25 ± 0.07 (based on substrate *p*-nitrophenyl acetate)	(Gabriele, Puccini et al. 2018)
Rat lung microsomes		64 ± 3 (using 4-nitrophenyl butyrate)	(Gaustad, Sletten et al. 1991)
	4.86 ± 3.1 (based on phenylacetate hydrolysis		(McCracken, Blain et al. 1993)
		0.003 - 0.013 (based on 4-hydroxybenzoic acid formation from alkylparaben)	(Ozaki, Sugihara et al. 2013)
Rat lung cytosol	14.2 ± 5.5 (based on phenylacetate hydrolysis		(McCracken, Blain et al. 1993)
Dark human skin microsomes		84 ± 6 (based on the hydrolysis of p-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)

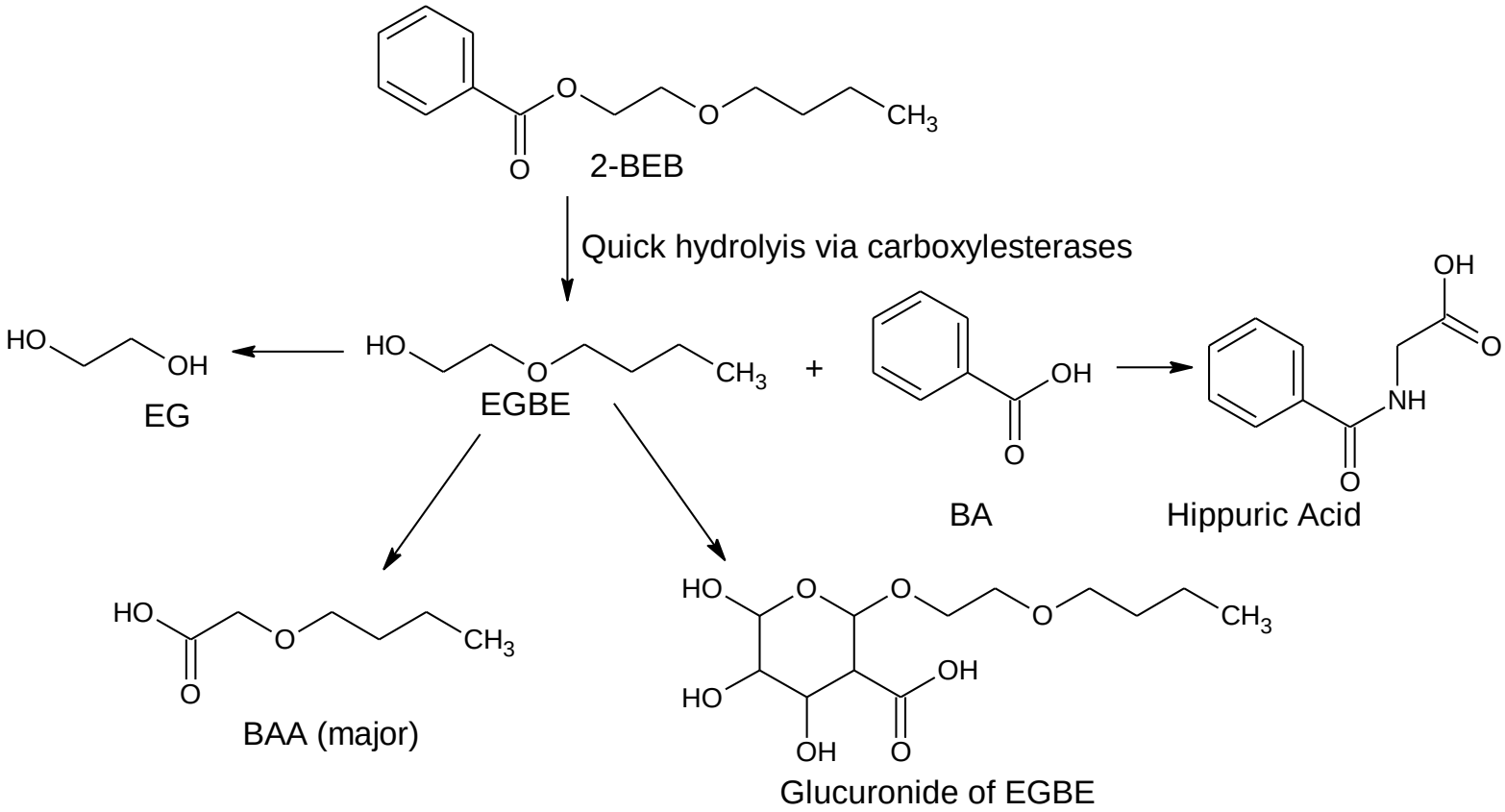
Table 2. Carboxylesterase Activity in Blood, Liver, Lung, and Skin of Both Human and Rat (*continued*)

Tissue Types	Carboxylesterase Activity		References
	picomol/min/g tissue	nmol/min/mg protein	
Light human skin microsomes		99 ± 6 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
Human skin microsomes	1.79 ± 0.51 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
Human skin cytosol		2.1 ± 4.3 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Tokudome, Katayanagi et al. 2015)
Dark human skin cytosol		120 ± 6 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
Light human skin cytosol		52 ± 2 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
Human skin cytosol	1.35 ± 0.35 (based on hydrolysis of procaine)		(Jewell, Ackermann et al. 2007)
Rat skin microsomes		0.5 - 2 (based on 4-hydroxybenzoic acid formation from alkylparaben)	(Ozaki, Sugihara et al. 2013)
		84-99 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Ozaki, Sugihara et al. 2013)
		5.5 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Tokudome, Katayanagi et al. 2015)
		6 (based on phenyl salicylate)	(Ozaki, Sugihara et al. 2015)
Rat skin cytosol		29.9 ± 4.3 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Tokudome, Katayanagi et al. 2015)
		380 ± 20 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Prusakiewicz, Ackermann et al. 2006)
Rat skin S9		188 ± 30.3 (based on the hydrolysis of *p*-nitrophenyl acetate)	(Imai, Takase et al. 2013)

Table 2. Carboxylesterases in nasal mucosa of both human and rat (*continued*)

Tissue types	Carboxylesterase Activity		References
	K_m (µM)	V_{max} (nmol/min/mg protein)	
Human nasal mucosa	60.8 ± 7.9 (based on substrate α-naphthyl butyrate)	145.5 ±11.3 (based on substrate α-naphthyl butyrate)	(Mattes and Mattes 1992)
Rat nasal mucosa	50.0 ± 2.9 (based on substrate α-naphthyl butyrate)	5007 ± 377 (based on substrate α-naphthyl butyrate)	(Mattes and Mattes 1992)
	34100 ± 3900 (based on substrate *p*-nitrophenyl butyrate)	605 ± 58 (based on substrate p-nitrophenyl butyrate)	(Bogdanffy, Randall et al. 1987)
	330 ± 50 (based on substrate vinylacetate)	88810 ± 3390 (based on substrate vinylacetate)	(Bogdanffy and Taylor 1993)

Figure 1. Proposed metabolic pathways of 2-BEB and EGBE in human and rat.



References

Bogdanffy, M. S., H. W. Randall and K. T. Morgan (1987). "Biochemical quantitation and histochemical localization of carboxylesterase in the nasal passages of the Fischer-344 rat and B6C3F1 mouse." *Toxicol. Appl. Pharmacol.* **88**(2): 183-194.

Bogdanffy, M. S. and M. L. Taylor (1993). "Kinetics of nasal carboxylesterase-mediated metabolism of vinyl acetate." *Drug Metab. Dispos.* **21**(6): 1107-1111.

Bureau, E. C. (2006). "2-Butoxyethanol Acetate (EGBEA)." European Union Risk Assessment Report **69**(4th Priority List).

Corley, R. A., G. A. Bormett, and B. I. Ghanayem (1994). "Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans." *Toxicol. Appl. Pharmacol.* **129**(1): 61-79.

Cosmetic Ingredient Review (2001). "Final Report ion the Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate." *Int. J. Toxicol.* **20** (Suppl. 3): 23-50.

Dean, R. A., J. Zhang, M. R. Brzezinski, and W. F. Bosron (1995). "Tissue distribution of cocaine methyl esterase and ethyl transferase activities: Correlation with carboxylesterase protein." *J. Pharmacol. Exp. Therap.* **275**(2): 965-971.

Domoradzki, J. Y., K. A. Brzak, and C. M. Thornton (2003). "Hydrolysis kinetics of propylene glycol monomethyl ether acetate in rats *in vivo* and in rat and hyman tissues *in vitro*." *Toxicol. Sci.* **75**(1): 31-39.

Gabriele, M., P. Puccini, M. Lucchi, A. Vizziello, P. G. Gervasi, and V. Longo (2018). "Presence and inter-individual variability of carboxylesterases (CES1 and CES2) in human lung." *Biochem. Pharmacol.* **150**: 64-71.

Gaustad, R., K. Sletten, D. Lovhaug, and F. Fonnum (1991). "Purification and characterization of carboxylesterases from rat lung." *Biochem. J.* **274 (Pt 3)**: 693-697.

Ghanayem, B. I., L. T. Burka, J. M. Sanders, and H. B. Matthews (1987). "Metabolism and disposition of ethylene glycol monobutyl ether (2-butoxyethanol) in rats." *Drug Metab. Dispos.* **15**(4): 478-484.

Gift, J. S. (2005). "U.S. EPA's IRIS assessment of 2-butoxyethanol: The relationship of noncancer to cancer effects." *Toxicol. Lett.* **156**(1): 163-178.

Groeseneken, D., H. Veulemans, R. Masschelein, and E. Van Vlem (1987a). "Ethoxyacetic acid: A metabolite of ethylene glycol monoethyl ether acetate in man." *Br. J. Ind. Med.* **44**: 488-493.

Groeseneken, D., H. Veulemans, R. Masschelein, and E. Van Vlem (1987b). "Pulmonary absorption and elimination of ethylene glycol monoethyl ether acetate in man." *Br. J. Ind. Med.* **44**: 309-316.

Hoffmann, H. D., and R. Jäckh (1985). "Cleavage of glycol ether acetates by rat plasma *in vitro*." Unpublished manucript. Dept. Toxicol. BASF, Ludwigshafen, Germany.

Human Protein Atlas, available at http://www.proteinatlas.org and the primary publication cited, Uhlén, M., *et al.* (2015). "Tissue-based map of the human proteome." *Science* **347**(6220): 1260419.

Imai, T., Y. Takase, H. Iwase, and M. Hashimoto (2013). "Involvement of Carboxylesterase in Hydrolysis of Propranolol Prodrug during Permeation across Rat Skin." *Pharmaceutics* **5**(3): 371-384.

Jewell, C., C. Ackermann, N. A. Payne, G. Fate, R. Voorman, and F. M. Williams (2007). "Specificity of procaine and ester hydrolysis by human, minipig, and rat skin and liver." *Drug Metab. Dispos.* **35**(11): 2015-2022.

Mattes, P. M. and W. B. Mattes (1992). "alpha-Naphthyl butyrate carboxylesterase activity in human and rat nasal tissue." *Toxicol. Appl. Pharmacol.* **114**(1): 71-76.

McCracken, N. W., P. G. Blain, and F. M. Williams (1993). "Nature and role of xenobiotic metabolizing esterases in rat liver, lung, skin and blood." *Biochem. Pharmacol.* **45**(1): 31-36.

Nambu, K., H. Miyazaki, Y. Nakanishi, Y. Oh-e, Y. Matsunaga, and M. Hashimoto (1987). "Enzymatic hydrolysis of haloperidol decanoate and its inhibition by proteins." *Biochem. Pharmacol.* **36**(10): 1715-1722.

Ozaki, H., K. Sugihara, Y. Tamura, C. Fujino, Y. Watanabe, N. Uramaru, T. Sone, S. Ohta, and S. Kitamura (2015). "Hydrolytic metabolism of phenyl and benzyl salicylates, fragrances and flavoring agents in foods, by microsomes of rat and human tissues." *Food Chem. Toxicol.* **86**: 116-123.

Ozaki, H., K. Sugihara, Y. Watanabe, C. Fujino, N. Uramaru, T. Sone, S. Ohta, and S. Kitamura (2013). "Comparative study of the hydrolytic metabolism of methyl-, ethyl-, propyl-, butyl-, heptyl- and dodecylparaben by microsomes of various rat and human tissues." *Xenobiotica* **43**(12): 1064-1072.

Prusakiewicz, J. J., C. Ackermann, and R. Voorman (2006). "Comparison of skin esterase activities from different species." *Pharm. Res.* **23**(7): 1517-1524.

Rudakova, E. V., N. P. Boltneva, and G. F. Makhaeva (2011). "Comparative analysis of esterase activities of human, mouse, and rat blood." *Bull. Exp. Biol. Med.* **152**(1): 73-75.

Satoh, T., and M. Hosokawa (1998). "The mammalian carboxylesterases: From molecules to functions." *Annu. Rev. Pharmacol. Toxicol.* **38**: 257-288.

Tokudome, Y., M. Katayanagi, and F. Hashimoto (2015). “Esterase Activity and Intracellular Localization in Reconstructed Human Epidermal Cultured Skin Models.” *Ann. Dermatol.* **27**(3): 269-274.

Zhang, F., A. J. Clark, E. L. McClymont, and T. C. Erskine (2016). “2-Butoxyethyl benzoate: Pharmacokinetics and metabolism in F344/DuCrl Rats.” The Dow Chemical Company Internal Report.