J Surfact Deterg (2020) **23:** 921–935
DOI 10.1002/jsde.12408

ORIGINAL ARTICLE

Mammalian Toxicity Testing of Semilinear and Branched Alcohol Ethoxylates

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Received: 16 September 2019 / Revised: 5 March 2020 / Accepted: 5 March 2020

Abstract Alcohol ethoxylates (AE) are commonly used nonionic surfactants with widespread adoption in consumer and industrial applications. The toxicology profile of this general class of molecules has been extensively reported previously. This report serves to make accessible previously unpublished toxicological data from 59 toxicology studies on AE produced from branched and semilinear alcohols produced by ExxonMobil, with alcohol backbones ranging from C9 to C15. Information on acute oral toxicity, acute dermal toxicity, genetic toxicity, skin irritation, eye irritation, skin sensitization, and oral repeat-dose toxicity are presented here. These data significantly enrich the database on the toxicity of branched AE and support that the degree of branching presents no unique toxicological hazards in relation to that of semilinear AE in this report, as well as in comparison with similar data published on a range of linear, semilinear, and branched AE.

Keywords Ethoxylates · Acute toxicity · Irritation · Branched chain alcohols · Straight chain alcohols · Surfactants

Supporting information Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Published online: 23 July 2020
J Surfact Deterg (2020) **23:** 921–935

J Surfact Deterg (2020) **23:** 921–935.

Introduction

The widespread use of chemicals requires the characterization and communication of their potential hazards. This is particularly important to ensure safe use of chemicals, such as surfactants, to which individuals may be exposed both in the workplace and at home. Several efforts have been made by the industry and regulators to promote and ensure the safe use of surfactant chemicals. In the cleaning products sector, the Human and Environmental Risk Assessment Project (HERA, 2005) is a notable voluntary effort that was initiated in 1999 with the objective to provide information to enable proper and scientifically sound judgments on the safe and effective domestic use of cleaning chemicals based on considerations of the potential hazards, as well as the risk of incurring those hazards, which may only occur with sufficient exposure. More recently, a similar effort to communicate the hazards and potential risks of chemicals has been made by the America Cleaning Institute in its Cleaning Products Ingredients Safety Initiative (CPISI) (DeLeo et al., 2017).

The work of HERA and CPISI has culminated in a series of assessments that describe in detail the inherent hazards of surfactant classes and the data underlying those assessments. Both initiatives go on to assess potential exposure to these chemicals in formulated products to determine the potential risk of harm. Considered together with other voluntary initiatives, such as the Screening Information Data Set (SIDS) program, the High Production Volume Chemicals Programs of the Organisation for Economic Cooperation and Development (OECD), the US Environmental Protection Agency (EPA), the International Council of

Chemical Associations (ICCA), and the Concise International Chemical Assessment Documents (CICAD) of the International Programme on Chemicals Safety (IPCS), the chemical, surfactants, and cleaning products industry has recognized the importance of making its hazard and risk assessments transparent to ensure the safe use of its products, as well as to instill confidence in its products. The need for such assessments and the data underlying them are increasing with the emergence of several regulatory and voluntary schemes across the world to characterize and manage chemical risks.

This report summarizes toxicological testing overseen by ExxonMobil on alcohol ethoxylates (AE) derived from highly branched alcohols manufactured by the Oxo process at ExxonMobil (Leonard W Russum & Robert J Hengstebeck, 1953). In addition, we present data on AE derived from semilinear alcohols formerly commercialized by the Exxon Chemical Company as a comparative toxicological dataset. Refer to Table 1 for detailed summaries of the alcohol feedstocks. Several of these studies have been previously reported in an unpublished report and referenced by the HERA risk assessment of AE (HERA, 2009). These data are now being published to support the various stakeholders who seek to manufacture and formulate with, or simply wish to better understand the toxicological properties of, surfactants derived from highly branched alcohol feedstocks.

Materials and Methods

Test materials were either obtained commercially or lab-synthesized. A full list of test materials may be found in Table 1. AE synthesis was carried out under a sodium hydroxide catalyst. Lab-synthesized samples were characterized by supercritical fluid chromatography to determine oligomer distributions.

All animal studies were conducted in accordance with ethical standards and guidelines in either the United States or the European Union at the time the studies were conducted (for complete list of studies, please refer Table S5). For a complete list of compliance details, including Good Laboratory Practice (GLP) standards for each study, as well as study details (animal species, gender, ages, and number used), please see Appendix (S1). For all studies specified as conducted with study designs "similar to" OECD test guideline study designs, all animals were treated humanely as described in the following references, with the aim of preventing injuries to the animals and technicians, minimizing stress to the animals, and reducing interference with study parameters (using guidelines endorsed by the Animal Welfare Institute (1970), Code of Federal Regulations (1966), American Veterinary Medical Association Council on Research (1978), Canadian Veterinary Medical Association (1978), and the United States Department of Health and Human Services (1985)). The study design and personnel training were sufficient to minimize animal suffering within the confines of the study objectives.

The level of study design detail and reporting in this manuscript was intended to achieve the highest Klimisch score possible (Klimisch et al., 1997) in order to maximize the utility of this report by stakeholders in their evaluation of individual study quality and results.

Acute Toxicity

Oral toxicity studies were conducted with rats and dermal studies with rabbits. For detailed information on animal

Table 1 Alcohol ethoxylate feedstocks used in this report

Sample name	Abbreviated name	Carbon number (%)							Average branches/ molecule	Details	Feedstock
		9	10	11	12	13	14	15			
Branched C11-rich oxo-alcohol	C11Br	0.1	8.5	85	6.4				2.23	Major isomers are dimethyl-1-nonanols and trimethyl-1-octanols.	Decenes (propylene/butene trimer)
Branched C12-rich oxo alcohol	C12Br		6	18	55	20	1		3.1	Major isomers are trimethyl-1-nonanols and tetramethyl-1-octanols.	Undecenes (propylene/ butene trimer)
Branched C13-rich oxo alcohol	C13Br			1	23	70	6		3.06	Major isomers are trimethyl-1-decanols and tetramethyl-1-nonanols.	Dodecenes (propylene-rich tetramer)
Semi-linear C9/11 oxo alcohol	C911	50		50					0.33	Linear C9 and C11 alcohols. Estimated 33% methyl and ethyl branching at α-Carbon	C8/10 linear α-olefins
Semi-linear C13/15 oxo alcohol	C1315					67		33	0.33	Linear C13 and C15 alcohols. Estimated 33% methyl and ethyl branching at α-Carbon	C12/14 linear α-olefins

strain, gender, age, number, and other study design specifics, please see Appendix S2. For studies that were determined to have been conducted with study designs "similar to" OECD 401 (acute oral toxicity) ((OECD), O.f.E.C.a.D, 1987b; OECD, 1981d), the studies followed the same basic study design as detailed in the OECD guideline: animals were acclimated for approximately 2 weeks, grouped by gender (five per cage), and fed and watered *ad libitum*. Temperature, humidity, and lighting (12-h light/dark cycle) were all monitored throughout the study duration. Animals were fasted for approximately 18 h prior to administration of the test material, and test material was administered *via* oral intubation, undiluted, at 2000 mg kg^{-1} fasted body weight. Animals were examined for viability throughout the study. In-life observations were made regarding the nature, onset, severity, and duration of toxicological signs (1, 2, 4, and 6 h after dosing) and once per day afterward for a total of 14 days. Body weights were recorded prior to dosing, at dosing initiation, at Day 7, and at Day 14, at which point animals were sacrificed and necropsies performed. LD_{50} values were either determined to be higher than the highest dose used in the study or were calculated using the standard Litchfield-Wilcoxon test to estimate a more precise value above the highest dose used.

For studies that were determined to have been conducted with study designs "similar to" OECD 402 (acute dermal toxicity) (OECD, 1981b), the studies followed the same basic study design as detailed in the OECD guideline: animals were acclimated for approximately 2 weeks, individually housed, and fed and watered *ad libitum*. Temperature, humidity, and lighting (12-h light/dark cycle) were all monitored throughout the study duration. The day prior to topical administration of the test material, the hair on the dorsal surface of each rabbit, from the shoulder region to the lumbar region, was closely clipped with an electric clipper. The skin was left intact. Elizabethan-type collars were placed around the neck of each rabbit at this time to acclimate them to wearing collars. Animals were reclipped as needed for dermal evaluations. Test material was applied undiluted at 3160 mg kg^{-1} body weight. The test material was applied to the skin, covered with a gauze patch, and secured with tape and a plastic sleeve. After approximately 24-h exposure, the plastic sleeve, tape, and gauze patch were removed, and the amount of remaining material was estimated and recorded. Test material was then removed with distilled water and paper towels. Animals were examined for viability throughout the study. In-life observations were made regarding the nature, onset, severity, and duration of toxicological signs (2 and 4 h after dosing) and once per day afterward for a total of 14 days. Dermal responses were evaluated 24 h postdose and on Days 3, 7, 10, and 14 according to the Draize method of scoring. Body weights were recorded at dosing initiation, at Day 7, and at Day 14, at which point animals were sacrificed and necropsies performed.

Repeat-Dose Oral Toxicity

A non-OECD guideline oral gavage repeat-dose study was conducted for 28 days on C12Br/7EO in rats. Rats were exposed 7 days per week during the 28-day time frame, with the following dose groups: control (reverse osmosis water only), 100, 300, or 750 mg kg^{-1} day^{-1}. In the 750 mg kg^{-1} day^{-1} groups (high dose and satellite), the doses were lowered to 500 mg kg^{-1} day^{-1} on Day 5 due to excessive toxicity observed at the high dose level. In addition, a satellite group was dosed at the high dose level (750 mg kg^{-1} day^{-1} for Days 0–4; lowered to 500 mg kg^{-1}/d on Day 5 due to excessive toxicity observed at 750 mg kg^{-1} day^{-1}) 7 days per week for 28 days and was then observed for reversibility, persistence, or delayed occurrence of toxic effects for 14 days posttreatment. Animals were acclimated for approximately 1 week, single housed during the test period, and fed and watered *ad libitum*. Temperature was maintained at between 20 and 24.476°C with 40–70% relative humidity and a 12-h light/dark cycle. Daily observations of any toxicological signs were made (nature, onset, severity, duration), as well as weekly body weight and food consumption measurements. At study termination (Day 28), as well as Day 42 for the satellite group, the following were performed: hematology, serum chemistry, and necropsy (with organs and tissues being collected and weighed and with microscopic examination performed on tissues from control, high-dose, and any animals that perished prior to study termination). Organs and tissues subject to collection, weighing, gross evaluation, and microscopic analysis included: kidneys, liver, ovaries, testes, adrenals, and lungs with trachea (for weighing and fixation at termination), with all tissues that show abnormalities being preserved for all animals at sacrifice. Body weights were recorded prior to dosing; on day of dose initiation; and on Days 7, 14, 21, 27, 35, and 41, as well as on the day of scheduled sacrifice. Food consumption was recorded on Days 7, 14, 21, 27, 35, and 41. Hematology parameters measured during the study included: erythrocyte count, hematocrit, hemoglobin, leukocyte count (total and differential), platelet count, and reticulocyte count. Serum chemistry parameters measured during the study included: bilirubin, albumin, blood urea nitrogen, calcium, cholesterol, creatinine, electrolytes (Na^+, K^+, Cl^-), gamma glutamyl transferase, glucose, phosphorous, serum aspartate aminotransferase, serum alanine aminotransferase, total protein, and triacylglycerols. The following means were analyzed statistically: body weights, food consumption, hematology parameters, serum chemistry parameters, organ weights, and organ-to-body weight ratios. For

analysis, first, Bartlett's test (1% level of significance; all other tests at 1% and 5% significance) was performed for equal variances between dose groups. Parametric analyses included one-way Analysis of Variance (ANOVA), with Dunnett's test and standard linear regression analysis performed where appropriate; nonparametric analyses included the Kruskal-Wallis test followed by Dunn's Summed Rank test, performed where appropriate.

Two non-OECD guideline oral gavage repeat-dose studies were conducted on C13Br/9-10EO for approximately 2 weeks in rats. In the first study (Study ID 254270), rats were exposed 7 days per week during the 15-day time frame, with the following dose groups: vehicle control, 100, 500, and 1000 mg kg^{-1} day^{-1}. The study was terminated on the 15th day due to high mortality in the high-dose group; the study served as a probe to determine dose levels for the other repeat-dose study described here. Animals were acclimated for approximately 2 weeks, single housed during the test period, and fed and watered *ad libitum*. Temperature was maintained at between 20 and 24.4 °C, with 40–70% relative humidity and a 12 h light/dark cycle. Daily observations of any toxicological signs were made (nature, onset, severity, duration), as well as weekly body weight and food consumption measurements. Body weights were also recorded at death for animals that succumbed prior to study termination. At study termination (Day 15), gross necropsies were performed on all animals and included examination of the external surface; all orifices; carcass; cranial cavity; spinal cord; nasal and paranasal sinuses; thoracic, abdominal, and pelvic cavities with their associated organs and tissues; and organs and tissues of the neck. Mean body weights and mean food consumption were analyzed statistically for significant differences. For analysis, first, Bartlett's test (1% level of significance; all other tests at 1% and 5% significance) was performed for equal variances between dose groups. Parametric analyses included one-way ANOVA, with Dunnett's test and standard linear regression analysis performed where appropriate; nonparametric analyses included the Kruskal-Wallis test followed by Dunn's Summed Rank test, performed where appropriate. The second study (Study ID 254270a) was performed using the same conditions as Study ID 254270, with the following exceptions: dose groups included a vehicle control, 20, 100, and 500 mg kg^{-1} day^{-1}, and study termination commenced on Day 14.

Skin Irritation

Skin irritation studies described in this manuscript were either according or similar to OECD 404 (Acute Dermal Irritation/Corrosion) (OECD, 1981a). For studies that were determined to have been conducted with study designs "similar to" OECD 404, the studies followed the same basic study design as detailed in the OECD guideline: New Zealand white rabbits were quarantined and acclimated for approximately 2 weeks and were checked for viability twice daily during weekdays and once daily during weekends during the acclimation period and throughout the duration of the study. Animals were housed individually and fed and watered *ad libitum*. Temperature was monitored twice daily throughout the weekdays and once daily on weekends, maintained at a range of 18.3–21.1 °C; humidity was monitored once daily and maintained at a range of 40–60%; and lighting was maintained on a 12-h light/dark cycle. On the day prior to topical administration of the test material, the hair on the dorsal surface of each rabbit, from the shoulder to the lumbar region, was clipped and skin left intact. Elizabethan-type collars were placed around the neck of each rabbit to acclimate them to wearing collars. Test material was administered (0.5 mL) as a single dose under a gauze patch secured with tape. The patch was loosely held in contact with the skin by means of a semiocclusive dressing for 4 h, upon which the dressing and gauze patch were removed. Residual material was removed, as were the collars. Dermal responses were evaluated 45 min and 24, 48, and 72 h following patch removal (all scores according to Draize Method (Draize, 1959)). Body weights were recorded on the day of dosing and on Day 3. After the 72-h observations and terminal weights were recorded, all animals were sacrificed.

Eye Irritation

Eye irritation studies described in this manuscript were either according or similar to OECD 405 (Acute Eye Irritation/Corrosion) ((OECD), O.f.E.C.a.D, 1987a; OECD, 1981c). For studies that were determined to have been conducted with study designs "similar to" OECD 405, the studies followed the same basic study design as detailed in the OECD guideline: New Zealand white rabbits were quarantined and acclimated for approximately 2 weeks and were checked for viability twice daily during weekdays and once daily during weekends during the acclimation period and throughout the duration of the study. Animals were housed individually and fed and watered *ad libitum*. Temperature was monitored twice daily throughout the weekdays and once daily on weekends, maintained at a range of 18.3–21.1°C; humidity was monitored once daily and maintained at a range of 40–60%; and lighting was maintained on a 12-h light/dark cycle. Approximately 24 h before test substance application, both eyes of each animal were examined for the presence of corneal ulceration using 2% sodium fluorescein dye and ultraviolet (UV) light; immediately prior to test substance instillation, the eyes were reexamined without sodium fluorescein dye. Any

animals exhibiting any signs of corneal or conjunctival injury were excluded from the study. Test material was administered (0.1 mL) into the lower conjunctival sac of the right eye (without washing), with the contralateral eye serving as the control. Body weights were recorded on the day of dosing. Ocular irritation signs were observed at 1, 4, 24, 48, and 72 h postinstillation and once daily on Days 4, 7, 10, and 14 (some studies continued to 21 days). Treated and control eyes were examined and scored for ocular reactions according to the Draize scale. Observations for unusual effects (pannus, blistering, blistering of conjunctiva, ulceration, or other effects indicative of corrosion) were also noted, if present. Sodium fluorescein dye (2%) and UV light was used to confirm the presence or absence of corneal ulceration in treated eyes (starting at 24 h) until there was no dye retention for two consecutive observations. After terminal study day observations, all animals were sacrificed.

Skin Sensitization

Skin sensitization studies were performed according to OECD guideline 406 (Skin sensitization) (OECD, 1981e) to assess the contact allergenic potential of two AE substances (one branched and one linear). Albino guinea pigs were used, and the test followed an initial epidermal application of the test substance followed by an additional challenge application 2 weeks after the epidermal induction application, as per the OECD test guideline.

Genetic Toxicity

Bacterial Reverse Mutation Test

A mutagenesis assay similar to OECD 471 (OECD, 1983b) was performed on a branched ethoxylated alcohol and was based on the Ames assay (Ames et al., 1975). Five strains of *Salmonella typhurium*, which are sensitive to frameshift and base pair mutagens, were utilized to assess mutagenicity, and test doses used in the assay were based on a preliminary test for cytotoxicity that enabled the use of a top dose, which was cytotoxic but less than lethal for the strains used. Strains utilized in this study design were each sensitive to frameshifts or base pair substitutions (or both): TA98 (frameshift sensitive), TA100 (base pair substitution and frameshift sensitive), TA1535 (base pair substitution sensitive), and TA1537 and TA 1538 (both frameshift sensitive), and were all provided by Dr. B.N. Ames, University of California, Berkeley, and characterized previously published procedures (Maron and Ames, 1983). A vehicle control (dimethyl sulfoxide (DMSO) purity 99.9%) and a nontreated control were used, as well as positive control chemicals (2-Aminoanthracene, 2AA, purity 90%, at 5 μg plate^{-1}; 9-Aminocridine, 9AA, >98% purity at 100 μg plate^{-1}; *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, MNNG, purity 97% at 10 μg plate^{-1}; 2-Nitrofluorene, 2NF, purity 98% at 5 μg plate^{-1}). The test substance and positive controls were diluted with DMSO. Liver homogenate from Aroclor-pretreated Sprague Dawley rats (S9-R434, referred to as S9) was used for metabolic activation, and experimental groups were divided into those with metabolic activation (+S9) and those without (−S9). Doses of test substances were as follows: 10, 32, 100, 320, and 1000 μg plate^{-1} (this top dose was determined by the toxicity pretest mentioned above). Three experimental groups were used for each dose in each strain (both –S9 and +S9). Vehicle and nontreated controls were run for each strain (both –S9 and +S9). Positive controls for each type of mutation were run for the respective sensitive strains: 2AA (all strains under +S9 conditions), 9AA (TA1537, -S9), MNNG (TA100 and TA1535, −S9), and 2NF (TA98 and TA1538, -S9). To determine the highest dose of the test substance to be used in the assay, a dose range of 1–10,000 μg plate^{-1} was tested in TA98, with colony counts taken after 2 days of incubation. Because cytotoxicity was observed in both –S9 and +S9 plates, the 1000 μg plate^{-1} was selected as the high dose for the mutagenesis assay moving forward. For the mutagenesis assay, agar, tester strain, test or control material, and –S9 or +S9 conditions were combined on a plate. Plates were then stored at 37°C for 2 days and evaluated for toxic effects (read either manually or with automatic colony counter). Mean plate counts and standard deviations for each dose were determined, and any test value ≥3 times the mean of the concurrent vehicle control was considered to be a positive mutagenicity result.

Mammalian Erythrocyte Micronucleus Test

An *in vivo* micronucleus test, similar to OECD 474 (OECD, 1983a), was performed on a branched ethoxylated alcohol. CD-1 mice, approximately 7–8 weeks of age (both male and female), were used and were acclimated for 21 days at the testing facility, checked once per day for viability, and housed singly by Day 6. Animals had *ad libitum* access to food and water, with temperature maintained at between 20 and 24.4 °C, with 40–70% relative humidity and a 12-h light/dark cycle. Test substance dose volumes did not exceed 1 mL/100 g body weight and were prepared on the day of dosing. All animals were weighed one day prior to dosing and were administered a carrier (corn oil), positive control (cyclophosphamide, 40 mg kg^{-1} with water as carrier), or one of three doses of test material (0.625, 1.25, and 2.5 g kg^{-1} based on range finding assay) each and were sacrificed at 24, 48, or 72 h postdose (with the exception of the positive control, which only included the 24-h time

point). Ten animals per dose group and time point (five males, five females) were used, for a total of 130 animals. The test substance was administered as a single oral dose by gavage, and the positive control was administered by intraperitoneal injection as a single dose. Animals were examined for viability once daily. To determine the toxicity of the test material itself, two initial range-finding studies were performed: 2.0, 1.0, and 0.5 g kg^{-1} using four males for each group (all animals survived for 3 days after dosing); the second study used higher doses (2.5 and 5.0 g kg^{-1}), also using four males for each group (two males from the 5.0 g kg^{-1} dose group died within 24 h, determined to be due to test material toxicity). The range-finding studies informed the use of the doses for the micronucleus assay. For the micronucleus assay, immediately following the sacrifice of the animals at the appropriate time point, bone marrow from both femurs was removed, and smears were prepared (two slides per animal). Slides were evaluated for polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) by fluorescent staining, morphology, and diameter. A ratio of PCE to NCE was determined for each animal by counting a total of 1000 erythrocytes. Means and standard deviations were calculated on the micronuclei data, and a test of equality of group means by one-way ANOVA at each time point was also derived. If an ANOVA was significant, comparisons of carrier control to dosed group means were made (Duncan's Multiple Range Test). Standard regression analysis was performed to test for dose response. Residuals from the ANOVA were analyzed for normality (Wilk's Criterion); the residuals were normally distributed, so nonparametric analysis was not performed. Genders were analyzed separately.

Results

Acute Toxicity

Branched and semilinear AE exhibit overall low acute toxicity in rats and rabbits. Acute oral studies in rats on branched AE exhibit LD_{50} values in the range of 2000–8150 mg kg^{-1} (Table 2), including LD_{50} values derived as above using the top dose and those calculated using the standard Litchfield-Wilcoxon technique to estimate a more precise value outside the upper values of the dose range. Semilinear AE exhibit oral LD_{50} values >2000 mg kg^{-1} (2000 mg kg^{-1} being the highest dose tested) for alcohols within the carbon backbone range of C9–C11 and C13–15, with the exception of C1315/12EO, which resulted in 80% mortality at 2000 mg kg^{-1} in rats (Table 2) (Study ID 210374). Dermal acute toxicity LD_{50} values from studies on rabbits were > 3160 mg kg^{-1} (the highest dose tested for each substance) for branched AE ranging from C11 to C15 and 5–10 mol ethoxylate (Table 3). For detailed information on in-life observations and gross findings at necropsy, please see Table S3.

Repeat-Dose Toxicity

Repeat-dose toxicity studies on branched AE resulted in a range of No Observed Adverse Effect Level (NOAEL) values between 100 and 300 mg kg^{-1} day^{-1} (Study IDs 254270, 254270a, 189470). C13Br/9-10EO was administered by oral gavage to male and female rats (dose range 0, 100, 500, and 1000 mg kg^{-1} day^{-1}, with 20 of each gender per group) (Study ID 254270). Twenty-six test animals died prior to study termination: one female at 100 mg kg^{-1} day^{-1} was found dead on day 10. At the 1000 mg kg^{-1} day^{-1} dose level, eight males were found dead between Days 3 and 7, whereas only 3 of 20 females survived to 14 days, with all either being found dead or moribund and sacrificed between Days 1 and 4. There was no mortality in the 500 mg kg^{-1} day^{-1} dose group. Clinical in-life observations were limited to an overall low incidence of findings in the 0, 100, and 500 mg kg^{-1} day^{-1} groups and were limited to the increased incidence of wet rales in the 500 and 1000 mg kg^{-1} day^{-1} dose groups in males (up to 5/20 and 7/20, respectively, for a single day) and at the 100 and 500 mg kg^{-1} day^{-1} dose level in females (up to 2/20 and 7/20, respectively). Dry rales were observed with less frequency and generally at higher doses. In addition, there was an increased incidence of urine and fecal staining and soft stool in males and females in the 1000 mg kg^{-1} day^{-1} dose group (Table S4). Of note are statistically significant, dose-related decreases in food consumption and mean body weight in males (500 and 1000 mg kg^{-1} day^{-1} dose groups); no such findings were noted in females at the 500 mg kg^{-1} day^{-1} dose level. All animals were subjected to necropsy entailing a physical examination of all orifices; the carcass; cranial cavity; spinal cord; the nasal and perinasal sinuses; the thoracic, abdominal, and pelvic cavities (and their associated organs and tissues); and the tissues and organs of the neck. Organs were not weighed nor subject to histopathological examination. There were no significantly different necropsy findings in males or females at the 100 mg kg^{-1} day^{-1} dose level. At higher dose levels, there were increased incidences of liver (slightly accentuated lobular pattern), kidney (discoloration, dilated renal pelvis), and stomach (tan raised embedded nodules) findings in both genders. A NOAEL of 100 mg kg^{-1} day^{-1} was established based on the decreases in mean body weight and food consumption in males at 500 mg kg^{-1} day^{-1}, as well as the increased incidence of necropsy findings in both genders. The study

Table 2 Median lethal dose (LD_{50}) values and mortality observations in rats corresponding to acute oral toxicity studies on alcohol ethoxylates

Substance (study number)	Dose levels (mg kg^{-1})	LD_{50} (mg kg^{-1})	Observed mortality
C11Br/5EO (214101)	2000	>2000	0/10
C11Br/10EO (213801)	2000	>2000	2/10: 1 male (Day 1) 1 female (4 h)
C12Br/6.7EO (269802)	Group I: 3160 Group II: 3730 Group III: 4400 Group IV: 5190	4050.5[a]	20/40: Group I: 2 1 male (Day 1) 1 female (6 h) Group II: 3 2 males: 1 (Day 1); 1 (Day 2) 1 female (6 h) Group III: 7 4 males: 2 (6 h); 2 (Day 1); 1 (Day 3) 2 females (Day 2) Group IV: 8 4 males: 2 (Day 1); 2 (Day 3) 4 females: 2 (6 h); 1 (Day 2); 1 (Day 3)
C12Br/7EO (211487)	2000	>2000	0/10
C12Br/9EO (211522)	2000	>2000	0/10
C13Br/7EO (269902)	Group I: 2000 Group II: 3160 Group III: 5010	8150[a]	3/30: Group III: 3 1 male: 1 (Day 3) 2 females: 1 (Day 0; 1 (Day 2)
C13Br/9EO (210464)	2000	>2000	1/10: 1 female (Day 1)
C13Br/11EO (210497)	2000	2000	5/10: 1 male (Day 9) 4 females (Day 2)
C911/6EO (210407)	2000	>2000	2/10: 2 females (Day 2)
C911/8EO (210431)	2000	>2000	4/10: 2 males (Day 2) 2 females (Day 2)
C1315/7EO (210341)	2000	>2000	1/10: 1 female (Day 2)
C1315/7EO (217181)	2000	>2000	1/10: 1 female (Day 3)
C1315/7.4EO (270102)	Group I: 2000 Group II: 3160 Group III: 5010	2643.9[a]	19/30: Group I: 2 2 females (Day 1) Group II: 7 4 males: 1 (Day 1); 2 (Day 2); 1 (Day 3) 3 females: 1 (Day 1); 1 (Day 3); 1 Day 4 Group III: 10 All males and females by Day 3
C1315/12EO (210374)	2000	<2000	8/10: 3 males: 1 (Day 2); 1 (Day 6) 5 females: (Day 2)

No corresponding unexposed control group was included in the studies.

[a] Calculated using the standard Litchfield-Wilcoxon technique for combined genders.

was terminated after 15 days due to high mortality levels observed in the 1000 mg kg^{-1} day^{-1} group.

In a follow-up study, C13Br/9-10EO was then administered *via* oral gavage to rats (doses of 0, 20, 100, and 500 mg kg^{-1} day^{-1}, 20 of each gender per group) (Study ID 254270a) for 14 days. Only one high-dose female was sacrificed prior to study termination in moribund condition (Table S4). Clinical in-life observations were limited to wet rales and soft stool in high-dose males only (up to 5/20 animals on Day 12; 0/20 and 1/20 in low and medium dose, respectively), where high-dose females showed an increased incidence of alopecia (up to 5/20 animals on

Table 3 Median lethal dose (LD_{50}) values and mortality observations in rabbits corresponding to acute dermal toxicity studies on alcohol ethoxylates

Substance (study number)	Dose levels (mg/kg)	LD_{50} (mg/kg)	Observed mortality
C11Br/5EO (214106)	3160	>3160	1/10: 1 male (Day 2)
C11Br/10EO (213806)	3160	>3160	0/10
C12Br/6.7EO (269807)	Group I: 1590 Group II: 2000 Group III: 2510 Group IV: 3160	>3160	1/8: Group IV: 1 female (Day 5)
C13Br/7EO (269907)	3160	>3160	0/6
C1315/7.4EO (270107)	3160	>3160	0/6

No corresponding unexposed control group was included in the studies reported in Table 3.

Days 9–13; 0/20 for both low- and medium-dose levels); all other dose levels showed a low incidence of abnormalities. Food consumption was measured during the first week of the study and showed a statistically significant, dose-related decrease in high-dose males compared to the controls. No necropsies were performed, and body weights were only measured at Days 0 and 7. Based on the limited information collected in this study, a NOAEL of 100 mg kg^{-1} day^{-1} was established based on the decrease in food consumption in males at 500 mg kg^{-1} day^{-1} during the first week of the study.

A 28-day oral study in rats was performed on C12Br/7EO in which the NOAEL was determined to be 300 mg kg^{-1} day^{-1} (dose range 0, 100, 300, and 500 mg kg^{-1} day^{-1}, five of each gender per group) (Study ID 189470). Two females receiving 750 mg kg^{-1} day^{-1} were euthanized in moribund condition on Day 4. Because of this and the clinical findings at this dose, the high-dose level was reduced to 500 mg kg^{-1} day^{-1} on study Day 5. Subsequently, one female each in the high-dose group was either found dead or was sacrificed moribund on Days 18 and 20, respectively. One male from the mid-dose group was found dead on Day 8, and necropsy found a torn esophagus, indicative of a gavage dosing error, as the likely cause of death. One male each from the high-dose group died on Days 13 and 14. Necropsy of the Day 13 death found an esophageal tear, a likely cause of death, although this was also accompanied by gastrointestinal abnormalities also found in other animals of this dose group. No treatment-related microscopic changes were observed in any male or female rats at the low- or mid-dose levels.

Effects on body weight (high-dose males at end of Week 1) and food consumption (statistically significant decrease in high-dose males and females at the end of Week 1) were observed at 750 mg kg^{-1} day^{-1}; upon decreasing the dose to 500 mg kg^{-1} day^{-1}, these metrics showed an apparent recovery, and no differences were observed for the duration of the study. There were no statistically significant differences in absolute organ weights; however, relative kidney and liver weights were increased in the mid- and high-dose males, and adrenals were increased in the high-dose males only. Only the liver changes in the high-dose group were associated with histopathological findings (centrilobular hepatocyte hypertrophy). Relative organ weight increases were not observed in the satellite group, and neither were microscopic lesions of the liver, indicating that these findings were reversible. Other treatment-related postmortem findings were limited to the high-dose and satellite animals that died prior to study termination (intestinal distention in two males and two females in the high-dose group and two females in satellite group; hyperplasia/hyperkeratosis, necrosis, ulcers in gastric mucosa, and/or edema at the limiting ridge were observed in three high-dose female rats sacrificed in moribund condition). An equivocal finding in the kidneys of male rats in this study, including hyaline droplets in renal cortical tubules, was observed at a slightly increased incidence in the mid- (3/5) and high- (3/5) dose groups but was also observed in a control animal and two satellite group males, but was not considered significant. Detailed summaries of organ weights, clinical and pathological parameters, in-life observations, and other study findings can be found in Table S4. A NOAEL of 300 mg kg^{-1} day^{-1} was established based on findings in the 500 mg kg^{-1} day^{-1} dose group: treatment-related mortality, adverse clinical signs during the exposure period (including dyspnea, emaciation, reduced stool, reduced food consumption, abdominal/anogenital staining, hypoactivity, and general poor condition), and increased relative liver weights associated with histopathological changes.

Skin Irritation

Branched and semilinear AE produce a range of erythema and edema scores across all time points in primary skin irritation studies in the rabbit, as well as a range of qualitative findings by the study termination date (Table 4). Overall, branched AE had relatively lower erythema and edema scores (averaged over 24-h, 48-h, and 72-h time points) and fewer qualitative findings by study termination than semilinear AE of a similar carbon number and degree of ethoxylation (Table 4). More specifically, for branched AE, 18 of 48 total animals tested exhibited no signs of erythema (Draize score: 0), and 40 of 48 total animals exhibited no signs of edema (Draize score: 0) (Table 4). For semilinear AE, 1 of 48 animals tested exhibited no signs of erythema (Draize score: 0), and 19 of 48 total animals exhibited no

Table 4 Individual animal average skin irritation scores (reported averages over 24, 48, and 72 h per animal) and qualitative observations from studies on alcohol ethoxylates. Unless noted otherwise, the test substance was applied undiluted

Substance (study number)	Average erythema scores [maximum score]	Average edema scores [maximum score]	Notable qualitative observations (study duration)
C11Br/5EO (214104)	(1, 1, 1.7, 0.7, 0.7, 0) [4]	(0, 0, 0, 0, 0, 0) [4]	Desquamation[a] in six animals at study termination (7 days)
C11Br/10EO[b] (213804)	(0, 0, 0, 0, 0, 0) [4]	(0, 0, 0, 0, 0, 0) [4]	No irreversible alterations were evident on the skin by study termination (3 days)
C12Br/6.7EO[c] (269804)	(1.7, 1, 1.7, 1.7, 0.3, 1) [4]	(0, 1, 1.7, 1.3, 0, 1) [4]	Desquamation[a] in four animals at study termination (7 days)
C12Br/7EO (211498)	(0, 1, 0, 1, 0, 1) [4]	(0, 0, 0, 0, 0, 0) [4]	No irreversible alterations or corrosive effects were evident on the skin (14 days)
C12Br/9EO (211533)	(0, 0.7, 0.7, 0.7, 0.7, 0.3) [4]	(0, 0, 0, 0, 0, 0) [4]	No irreversible alterations were evident on the skin by study termination (3 days)
C13Br/7EO[d] (269904)	(1.3, 0.7, 2, 1, 1, 1) [4]	(0.3, 0.3, 0, 0, 0.3, 0) [4]	Desquamation[a] in one animal at study termination (7 days)
C13Br/9EO (210475)	(1, 0, 0.3, 1, 0, 0.7) [4]	(0, 0, 0, 0, 0, 0) [4]	Slight scales in two animals at study termination (7 days)
C13Br/11EO (210508)	(0, 0, 0, 0, 0, 0) [4]	(0, 0, 0, 0, 0, 0) [4]	No irreversible alterations were evident on the skin by study termination (3 days)
C911/6EO (210418)	(1.3, 0.3, 1, 1, 1, 0.7) [4]	(0, 0, 0, 0, 0, 0) [4]	Slight to moderate scales in two animals at study termination (7 days)
C911/8EO (210442)	(0.7, 1, 0.7, 0.3, 0.3, 0) [4]	(0, 0, 0, 0, 0, 0) [4]	No irreversible alterations or corrosive effects were evident on the skin (7 days)
C1315/7EO (210352)	(2.7, 2, 2.7, 2.7, 2.7, 2.7) [4]	(1.7, 0.7, 1.7, 1.7, 1.3, 1) [4]	Slight to severe scales in six animals at study termination (7 days)
C1315/7EO[e] (216551)	(1.7, 1.7, 2, 1.7, 1.7, 1.7) [4]	(0.3, 0.3, 0, 0.7, 0, 0.3) [4]	Slight to severe scales in three animals at study termination (14 days)
C1315/7EO (217192)	(2, 2, 1.7, 2, 2, 2) [4]	(1, 1, 0.7, 0.7, 1, 1) [4]	Moderate to severe scales in six animals at study termination (7 days)
C1315/7EO (224190)	(1, 2, 0.3, 2, 1.7, 2) [4]	(0, 0, 0, 1, 0, 0) [4]	Slight to moderate scales in six animals at study termination (7 days)
C1315/7.4EO[f] (270104)	(3, 2, 2, 3.3, 2, 2.7) [4]	(2, 0.7, 0.3, 2, 0.3, 0) [4]	Desiccation in two animals at 72 h, but was reversible Desquamation[a] in five animals at study termination (7 days) Atonia[g] in six animals at study termination (7 days) Eschar in two animals at study termination (7 days) Leathery skin in three animals at study termination (7 days)
C1315/12EO (210385)	(0.7, 0.3, 0.7, 1.3, 1, 1) [4]	(0, 0, 0, 0, 0, 0) [4]	Slight scales in one animal at study termination (7 days)

Note: No control/unexposed comparative groups used in the studies listed in Table 4.

[a] Desquamation was defined in the study report as "small flakes of skin coming off."

[b] Clinical observations noted for two animals: poor food consumption, abnormal stool, distended abdomen, emaciation, hypoactivity, and anogenital staining. Affected animals were given saline and alfalfa to replace fluids lost and stimulate increased food consumption.

[c] One animal exhibited a decrease in body weight from its initial value and exhibited poor food consumption, soft stool, small amount of stool, and anogenital staining.

[d] One animal exhibited a decrease in body weight from its initial value, but no associated clinical signs were recorded.

[e] Test substance applied at 20% dilution.

[f] Preparation of test material included placing the test material in a beaker of warm tap water prior to dosing in order for the test material to completely liquify.

[g] Atonia was defined in the study report as "lack of resiliency of skin".

signs of edema (Draize score: 0) (Table 4). Thus, the average, or composite score, for edema and erythema across branched AE is appreciably lower than that of semilinear AE. The maximum erythema score observed in branched AE was 2; for semilinear AE, it was 3.3 (Table 4). The maximum edema score observed in branched AE was 1.7; for semi-linear AE, it was 2.0 (Table 4). As far as qualitative effects were observed and recorded, "no irreversible alterations were evident on the skin" in four studies (of eight total studies) on branched AE examining six

animals each; this observation was only recorded for one study (of eight total studies) on semilinear AE examining six animals each. A range of qualitative observations was recorded in the remaining studies for both branched and semilinear AE (see Table 4 for further details).

Skin Sensitization

Limited data are available on the skin sensitization potential of AE in this dataset; however, two *in vivo* studies do exist and indicate that these substances are not sensitizers. C12Br/7EO was negative for skin sensitization (contact allergenic) potential by assessment of erythema and edema in a hypersensitivity assay 24 and 48 h following an epicutaneous challenge application (Study ID 211511). One animal was found dead during the course of the study, but this was unrelated to substance administration, and the animal exhibited no findings of note at necropsy. Based on these data, C12Br/7EO is not considered to have skin sensitization potential. In a similar study, C1315/7EO was also negative for skin sensitization (contact allergenic) potential and is also not considered to have skin sensitization potential (Study ID 211408).

Eye Irritation

Branched and semilinear AE produce a range of corneal opacity, iritis, conjunctival redness, and chemosis scores across all time points in primary ocular irritation studies in rabbit, as well as a range of qualitative findings by the study termination date (Table 5). Generally, branched and semilinear AE presented here are considered to have some degree of an irritating effect on the eye based on average individual animal scores (averaged over 24, 48, and 72 h time points), qualitative observations throughout the study period, or a combination of both (Table 5). This observation remains true even if the test material was diluted to 20% (Study IDs 211544, 216628, 216663). There does not appear to be a distinct pattern differentiating branched from semilinear AE in this dataset for the degree or presence of eye irritation, nor does there appear to be an impact on this metric based on degree of ethoxylation.

Genetic Toxicity

An *in vitro* Ames test for mutagenicity was performed on a branched AE (C12Br/7EO), as was an *in vivo* micronucleus test in mouse. The Ames test yielded neither a positive response for mutagenicity, nor was there a dose-related increase in revertants for any of the tester strains, and this was verified by the performance of a repeat assay (Study ID 189425). Cytotoxicity was observed at the 1000 μg plate^{-1} dose for tester strain TA100 (+S9) and at 1000 and 320 μg plate^{-1} for the same strain (-S9). Positive and negative controls responded as expected. C12Br/7EO did not induce a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes at any dose (0.625, 1.25, or 2.5 g kg^{-1}) or time point (24, 48, and 72 h after dosing) in an assay similar to OECD 474 and is thus not considered clastogenic in mouse bone marrow (Study ID 189430). C12Br7/EO did not induce a statistically significant decrease in the mean percentage of polychromatic erythrocytes (a measure of bone marrow toxicity) outside the normal carrier control range in this same test.

Discussion

The widespread adoption of the Globally Harmonized System of classification and labelling, as well as the proliferation of voluntary initiatives emphasizing safer chemicals, has increased the need for toxicity data for product risk assessment. To date, a broad database has been published on the mammalian toxicology properties of AE; however, the majority of the reported studies have been conducted on linear, or semilinear, AE (HERA, 2009). In this publication, we make accessible high-quality summaries of 59 toxicity studies on branched and semilinear AE coordinated by ExxonMobil, significantly expanding the public database for this class of substances.

When reviewed together with the studies summarized by HERA and in the Recommendations for the Harmonized Classification and Labelling put forth by the European Committee of Organic Surfactants and Intermediates (CESIO) report (CESIO, 2017), the data presented in this report support that there is no evidence of any unique effect of alcohol branching on the degree of toxicity for a variety of endpoints. Branched AE with a predominant alcohol carbon chain length of 11–13 carbons and ranging from 5 to 11 mol ethoxylate present the same low oral and dermal acute toxicity as semilinear AE reported here (Tables 2, 3, S2, and S3), as well as other AE of similar chain length and degree of ethoxylation (HERA, 2009). With the exception of C1315/12EO (Study ID 210374, Table 2), the oral LD_{50} values were ≥ 2000 mg kg^{-1}. Dermal LD_{50} values exceeded the highest dose tested (3160 mg kg^{-1}) in all five studies. The experimental LD_{50} values detailed in this report are essentially consistent with the recommended classifications reflected in the CESIO report for the respective AE (CESIO, 2017). The only notable exception in the oral toxicity study dataset presented in these reports is that of C1315/12EO, a semilinear AE, which exhibited 80% mortality at 2000 mg kg^{-1} (Study ID 210374). A general trend of the higher degree of acute oral toxicity with a

Table 5 Individual animal average eye irritation scores (reported averages over 24, 48, and 72 h per animal) and qualitative observations from studies on alcohol ethoxylates. Unless noted in footnotes below, the test substance was applied undiluted

Substance (study number)	Average corneal opacity scores [maximum score]	Average iritis scores [maximum score]	Average conjunctival redness scores [maximum score]	Average conjunctival chemosis scores [maximum score]	Notable qualitative observations
C11Br/5EO (214113)	(1, 1, 1, 1, 1, 1.7) [4]	(1, 1, 1, 1, 1, 1) [2]	(3, 3, 3, 3, 3, 3) [3]	(3, 3, 3, 3, 3, 3) [4]	Corneal opacity in six animals at study termination (14 days) Corneal ulceration in four animals through 14 days Corneal pannus in six animals from 7 through 14 days Corneal stippling in one animal through 14 days Corneal ulceration in five animals through Day 14 Conjunctival necrosis in 5 animals through Day 14
C11Br/10EO[b,c] (213813)	(1, 1, 1, 1.3, 1.3, 2) [4]	(1, 0.7, 1, 0.7, 0.3, 1) [2]	(3, 3, 3, 3, 3, 3) [3]	(2, 2, 2.3, 2, 2.3, 2) [4]	Corneal opacity in three animals at study termination (14 days) Corneal ulceration in three animals at 14 days Corneal pannus in four animals from 7 through 14 days Transient corneal stippling observed for five animals throughout study
C12Br/6.7EO[c] (269813)	(1, 1, 1, 1, 1, 1) [4]	(1, 0.3, 0.3, 0, 1, 1) [2]	(2, 2, 2, 2, 2, 2.3) [3]	(1.7, 1.7, 2, 2, 2, 1.7) [4]	Corneal opacity in one animal at study termination (14 days) Corneal ulceration in two animals at 14 days Corneal pannus in three animals from 7 through 14 days Protruding cornea in one animal (14 days) Alopecia around eye in two animals (14 days)
C12Br/7EO (211500)	(1, 1, 1, 1, 1, 1) [4]	(0, 0.7, 0, 0.7, 0, 0) [2]	(2, 2, 1, 2, 2, 2) [3]	(1.3, 0.7, 1, 0.7, 0.7, 0.7) [4]	Corneal opacity in five animals at study termination (21 days)
C12Br/9EO (211544)	(1, 1, 1, 1, 1, 1) [4]	(0.3, 0.7, 1, 0, 1, 0.3) [2]	(2, 2, 2, 2, 2, 2) [3]	(2, 1.7, 1.7, 1, 2, 1.7) [4]	Corneal opacity in six animals at study termination (21 days)
C12Br/9EO[a] (216641)	(1, 1, 1, 1, 1, 1) [4]	(0, 0, 0, 0, 0, 0) [2]	(2, 2, 2, 2, 2, 2) [3]	(0.3, 1, 1.3, 0.3, 1.7, 0.3) [4]	Corneal opacity in five animals at study termination (21 days)
C13Br/7EO[c] (269913)	(1, 1, 1, 1, 1, 1) [4]	(0.7, 0.3, 1, 1, 1, 1) [2]	(2, 2.7, 2.3, 2.3, 2, 2.3) [3]	(2.3, 1.7, 2, 2, 1.3, 2.3) [4]	Corneal opacity in one animal at study termination (14 days) Corneal ulceration in three animals to 14 days Corneal pannus in two animals through 14 days Alopecia around treated eye (two animals) throughout course of study; persisted to 14 days in one animal
C13Br/9EO (210486)	(1, 1, 1, 1, 1, 1) [4]	(0, 0, 0, 0.3, 0, 0.3) [2]	(1.3, 2, 1, 1, 1.3, 2) [3]	(0.3, 1, 1, 1, 0.7, 2) [4]	Corneal opacity in two animals at study termination (21 days)
C13Br/11EO[a] (216628)	(1, 1, 1, 1, 1, 1) [4]	(0.3, 0.3, 0.3, 0, 0, 0) [2]	(2, 2, 2, 1.3, 1.7, 2) [3]	(1.7, 1.3, 1.3, 1.3, 1.3, 1.7) [4]	Corneal opacity in six animals at 7 days (study termination 14 days)

(Continues)

Table 5 Continued

Substance (study number)	Average corneal opacity scores [maximum score]	Average iritis scores [maximum score]	Average conjunctival redness scores [maximum score]	Average conjunctival chemosis scores [maximum score]	Notable qualitative observations
C13Br/11EO (210510)	(1.7, 1, 1, 1, 1, 1) [4]	(0.3, 0, 0.3, 0, 0.3, 0) [2]	(2, 2, 2, 2, 2, 2) [3]	(1.3, 1.7, 2, 1.3, 1.3, 1.3) [4]	Corneal opacity in five animals at study termination (21 days)
C911/6EO[a] (216663)	(1, 1, 1, 1, 1, 1) [4]	(0, 1, 0, 0, 0, 0.7) [2]	(2, 2, 2, 2, 2, 1.7) [3]	(1, 0.3, 0.3, 1.3, 1, 1.3) [4]	Corneal opacity in one animal at study termination (21 days)
C911/6EO (210420)	(1, 1, 1, 1, 1, 1) [4]	(0, 0, 0.7, 0, 0, 1) [2]	(2, 2, 2, 2, 2, 2) [3]	(0.7, 0.7, 1, 1, 1, 1) [4]	Corneal opacity in five animals at study termination (21 days) Scleral erythema in three animals at study termination (21 days)
C911/8EO (210453)	(1.7, 1.7, 1, 1, 1, 1.7) [4]	(0.3, 0, 0, 0.7, 0, 0.3) [2]	(1.3, 2, 2, 1.7, 2, 1) [3]	(1, 1.7, 1.3, 1, 1, 1) [4]	Corneal opacity in six animals at study termination (21 days)
C1315/7EO (217203)	(1, 1, 1, 1, 1, 1) [4]	(0, 0, 0, 0, 0, 0) [2]	(2, 2, 2, 2, 1.7, 2) [3]	(1, 0.7, 1, 0.7, 0.3, 0.7) [4]	Corneal opacity observed (6/6 animals) between 24 h and 72 h (study termination 7 days)
C1315/7EO (210363)	(1, 1, 0.7, 0.3, 0, 0.7) [4]	(0.7, 0.7, 0, 0, 0, 0) [2]	(2.7, 2.7, 1.7, 1, 0.7, 1) [3]	(2.7, 2, 0.7, 0, 0, 0) [4]	Corneal opacity with different intensities observed (3/6 animals) between 24 h and 7 days (study termination 14 days)
C1315/7.4EO[d] (270113)	(0, 0, 0, 0, 0.7, 0) [4]	(0, 1, 1, 0.7, 0.3, 0.7) [2]	(2.3, 3, 2.7, 3, 3, 3) [3]	(1.3, 1, 1, 1, 1.3, 1) [4]	Corneal opacity in one animal up to 48 h (study termination 14 days) Corneal stippling in two animals up to 4 h
C1315/12EO (210396)	(1, 1, 2, 1, 1, 1) [4]	(0, 0, 0.3, 0, 0, 0) [2]	(2, 2, 2, 1, 2, 1.7) [3]	(1, 0.3, 1.7, 1, 1, 1) [4]	Corneal opacity in three animals at study termination (21 days)

While there was no designated control group of animals, the right eye of each treated animal remained untreated and served as the reference control.

[a] Test substance applied at 20% dilution.

[b] Clinical observations noted for three animals: poor food consumption, abnormal stool production, distended abdomen, unthrifty coat, and anogenital staining. Affected animals were given saline and alfalfa to replace fluids lost and stimulate increased food consumption.

[c] Where study report indicates "slight dulling of normal luster" (designated as a "+" rather than a numerical Draize score) at any time point, corneal opacity scores are reported in this table as "1" for each animal for numerical reporting and averaging purposes.

[d] One animal died prior to study termination (does not appear to be test substance related); animal exhibited poor food consumption, distended abdomen, and no stool on Days 8 and 9 of the study (all tissues and organs showed moderate postmortem changes).

longer ethoxylate chain has been reported (Talmage, 1994), with it generally leveling off around 12EO. It is possible that the distribution of ethoxylation in this sample was particularly broad or that this study—the only AE in this dataset with 12EO or higher—was on the cusp of the biological effects of this noted range. In-life observations were limited mainly to any combination of the following: abdominal staining, anogenital staining, nasal discharge, oral discharge, unthrifty coat, wet and/or dry rales, abdominal griping, a range of abnormal stool findings, hypoactivity or hyperactivity, and hypothermia, and were generally observed in less than 50% of animals at varying time points of observation (Tables S2 and S3). Gross postmortem examinations in acute oral studies were associated with findings in the gastrointestinal tract (Table S2). GI effects were among the effects observed in repeat-dose oral toxicity studies with C12Br/7EO and C13Br/9-10EO. These results are generally consistent with reported data on linear or semilinear AE (HERA, 2009), and as they are generally considered irritants to the eye, this would correspondingly lead one to expect irritative and other associated impacts on other mucous membranes in the body, especially in oral gavage studies, which deliver the test substance directly to the lining of the GI tract. Similarly, gross postmortem findings in dermal studies were associated mainly with findings at the site of administration on the skin (Table S3).

Three repeat-dose studies are available on branched AE in this dataset, and the NOAEL derived from these studies (100–300 mg kg^{-1} day^{-1}) are within the range of previously published, extensive datasets on AE (HERA, 2009). Oral exposure to C12Br/7EO for 28 days resulted in a NOAEL of 300 mg kg day^{-1} (doses of 0, 100, 300, and 500 mg kg^{-1} day^{-1}) (Study ID 189470) and was driven primarily by treatment-related mortality, in-life clinical observations throughout the exposure period, and associated postmortem findings at 500 mg kg^{-1} day^{-1}. The maximum tolerated dose was exceeded at 750 mg kg^{-1} day^{-1}, resulting in a dose adjustment to the high-dose and satellite groups at Day 5. Limited studies with C13/9-10Br by the oral route for either 14 or 15 days resulted in a NOAEL of 100 mg kg^{-1} day^{-1} (Study IDs 254270, 254270a), which was based on statistically significant decreases in mean body weight and food consumption in males at 500 mg kg^{-1} day^{-1} (Study ID 254270) and statistically significant decreases in food consumption in males after 7 days at 500 mg kg^{-1} day^{-1} (Study ID 254270a). One female receiving 100 mg kg day^{-1} was found dead on Day 10, with wet rales observed the day prior. The lungs were found to be mottled red and dark red, and the liver was found to be dark red. Ingesta were found present in the esophagus. Due to the isolated nature of this death, with no deaths observed in the 500 mg kg^{-1} day^{-1} dose group, this was determined not to be test material-related.

Including the above animal, wet rales were observed in four different females of the low-dose group and three during the final two days of the study. Of these three, only one displayed any lung abnormalities during necropsy. Specifically, of the two displaying these symptoms during the final observation, one had no abnormalities during necropsy, and the other—which had wet rales observed on Days 13–15—presented with slightly reddened lungs (all lobes, all surfaces). The nature and prevalence of this finding was similar between control animals (5/20 males and 6/20 females) and low-dose animals (7/20 males, 4/20 females). The female that had wet rales on the penultimate day presented no abnormalities during necropsy. At the higher sublethal dose level of 500 mg kg^{-1} day^{-1}, rales were more prevalent, and lung abnormalities were somewhat correlated with rale incidence (9/20 males overall, 6/13 showing symptoms; 6/20 females overall, 4/15 showing symptoms). We conclude that the incidence of rales in females at 100 mg kg^{-1} day^{-1}, and both genders at 500 mg kg^{-1} day^{-1}, was treatment related but, due to a lack of correlation with necropsy findings, was nonadverse at the low-dose level.

The NOAEL between C12Br/7EO and C13Br/9-10EO were based on different findings; this could be more closely related to the differences in dose duration (4 weeks *vs* 2 weeks) rather than reflective of inherently different patterns of physiological responses to the substances themselves. This supposition is further supported by the wide range of largely nonspecific gross postmortem findings associated with exposure to both substances, resulting in findings of effects on the liver and kidney and a range of abnormalities in the GI tract (Table S4), with additional changes to white blood cell, alanine aminotransferase, adrenal weight, urinary bladder, ovarian sac, and adrenals at low incidences associated with exposure to C12Br/7EO (Study ID 189470) and lung, esophagus, brain, pituitary, and thymus findings at low incidences associated with exposure to C13/9-10EO (Study ID 254270).

Sixteen separate 4-h, semiocclusive dermal irritation studies were performed on branched (eight studies) and semilinear AE (eight studies), with quantitative erythema and edema scores (according to the Draize method) and notable qualitative observations recorded (Table 4). Branched AE elicited lower overall average erythema scores than semilinear AE, even when diluted to 20% prior to administration. There was not necessarily a discernable pattern when it came to differences in edema scores resulting from differences in branching, alcohol carbon chain length, or degree of ethoxylation. Of note, the following branched AE would not be considered irritating to the skin: C11Br10EO and C13Br/11EO, based on considerations of both quantitative and qualitative outcomes included in the study reports (average erythema and edema scores of 0 for all six animals and no irreversible alterations

evident on skin by study termination, Table 4). Comparatively speaking, these results are within the range of findings for linear and semilinear AE previously summarized (HERA, 2009). Included in these reports, however, are studies performed at different study lengths (i.e., 4, 6, 24 h, up to several days), differing levels of occlusion, and some including a repeat-dose paradigm. Thus, these results are more widely varied; however, a pattern did emerge: a lower degree of ethoxylation (<4 ethoxylate units) appeared to be associated with higher skin irritation potential in 4-h studies (HERA, 2009).

Two skin sensitization tests in guinea pigs according to the Magnusson Kligman protocol (one branched and one semilinear AE: C12Br/7EO and C1315/7EO, respectively) yielded negative results for skin sensitization potential, with zero positive results in challenged animals exposed to the AE described (Study IDs 211511, 211408). Of 25 total tests (including those performed according to Magnusson Kligman and Buehler protocols) previously conducted on a range of linear and semilinear AE (C9-C21, 2–21 mol ethoxylate), 22 studies resulted in a conclusion of "no evidence" for skin sensitization, 2 studies found "essentially no evidence" for skin sensitization, and 1 study found weak sensitization potential (HERA, 2009). Of note is that one of the studies on linear and semilinear AE that found "essentially no evidence" for skin sensitization was in the same alcohol carbon chain length range and degree of ethoxylation as the substances studied in this report (C12-15, 7 mol ethoxylate) (HERA, 2009). The study that found weak sensitization potential used a C7-9 alcohol with 6 mol ethoxylate. This is consistent with the understanding that AE can undergo autoxidation, resulting in the formation of peroxides and hydroperoxides, which are known skin sensitizers (Bodin et al., 2003). Because shorter-chain-length AE are more hydrophilic, it is logical that the C7-9 6EO exhibits weak sensitization potential.

Data presented here on branched AE (Table 5) aligns with previously reported profiles of eye irritation findings, including both quantitative scores and qualitative observations throughout the study period (HERA, 2009). Branched and semilinear AE (either applied undiluted or at 20% dilution) typically resulted in average corneal opacity scores of 1 (individual animal scores averaged over 24, 48, and 72 h according to Draize scale); average iritis scores ranging between 0 and 1; and average conjunctival redness and chemosis scores typically between 2 and 3 and 1 and 3, respectively (Table 5). No overall pattern was observed in ocular irritation potential for either alcohol carbon chain length (11–13 carbons) or degree of ethoxylation (5–-12 mol) for either branched or semilinear AE. Both quantitative Draize scoring and qualitative observations for irreversible effects on the eye are considered for globally harmonized system (GHS) classification purposes, and irreversible qualitative effects on the eye were observed across studies on the branched and semilinear AE presented here (Table 5). Taking this into consideration, the eye irritation data presented here are in general agreement with the classification, labelling, and packaging classifications outlined in the CESIO report (CESIO, 2017). Generally, AE are considered moderate to severe eye irritants if applied undiluted to the eye, whereas AE diluted to 0.1% are generally not irritating in a range of both GLP and non-GLP studies (Talmage, 1994). The variability observed across studies reported in the HERA document and the review cited here was, at least in part, attributed to variations in study design. Overall, when tested at concentrations that would be found in finished products (i.e., household cleaning products), the irritation potential of AE decreases to mildly irritating to the eyes (HERA, 2009).

The current dataset includes a limited amount of both *in vitro* and *in vivo* genetic test data on a branched AE (C12Br/7EO) and yielded no indications of genetic toxicity or clastogenicity (Study IDs 189425, 189430). These data align with a broader understanding of a lack of genotoxic potential of AE: More than a dozen tests in bacteria, multiple *in vitro* tests in mammalian and yeast systems, and multiple *in vivo* mammalian assays for genetic toxicity yielded no indications that AE exposure causes damage to genetic material (HERA, 2009). Based on the lack of an association between genetic toxicity and exposure to AE, both in the literature and in these reports (in addition to a lack of carcinogenic potential in the literature on AE (HERA, 2009)), it is reasonable to expect that the branched and semilinear AE presented here are unlikely to pose a carcinogenic risk.

Our data are in general agreement with previously published reports on branched and semilinear AE in that they confer no appreciable or particularly differentiating toxicological properties unique to the degree of branching. A range of primary toxicological endpoints useful for hazard and risk assessment, as well as to support product registrations, are included for a level of detail that has been specifically tailored to achieve maximum reliability (Klimisch et al., 1997) (Table S1). Together with the environmental data published in a companion article of this journal, we seek to enrich the publicly available dataset on human and environmental safety data to the various stakeholders who may wish to understand the inherent toxicological hazards of surfactants derived from highly branched alcohol feedstocks.

Conclusions

Previously unpublished data on branched and semilinear AE are presented in this manuscript and cover alcohol carbon chains ranging from C9 to C15 and 5–12 mol ethoxylate. The data presented here cover multiple toxicological endpoints useful for risk assessment: skin irritation,

eye irritation, skin sensitization, genetic toxicity, oral and dermal acute toxicity, and oral repeat-dose toxicity. The skin irritation data presented here include both quantitative and qualitative metrics. Across the 16 total studies on skin irritation on branched and semilinear AE, trends were observed where generally higher degrees of ethoxylation were associated with decreased irritation potential, similar to a previously reported trend (HERA, 2009). Two branched AE presented here would not be considered irritating to the skin under GHS (C11Br10EO and C13Br/11EO). Also similar to previous reports, no overall pattern was observed in ocular irritation potential for the alcohol dataset presented in this manuscript (based on either degree of ethoxylation, hydrophobe branching, or carbon chain length (HERA, 2009)). Two skin sensitization studies and two genetic toxicity studies yielded negative findings. Overall, all AE acute toxicity data presented here (both oral and dermal routes) follow a low order of toxicity, with 18 of 19 studies reporting LD_{50} values of ≥2000 mg kg^{-1} (Tables 2 and 3). This report contains NOAEL from three oral repeat-dose studies on branched AE, published here for the first time (100–300 mg kg^{-1} day^{-1}), and are within the NOAEL range of previously published, extensive datasets on AE. Findings from the studies listed here align with previously summarized information (HERA, 2009) on AE and add to the evidence supporting the established toxicological hazard profiles of these intermediate chemicals.

Conflict of Interest The authors declare that they have no conflict of interest.

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