Alkyl Ethoxylates: An Assessment of Their Oral Safety Alone and in Mixtures

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Some alkyl ethoxylates (AEs), nonionic surfactants which are widely used in industry and in household cleaning products, have been demonstrated to produce acute neuropharmacologic effects when sufficient systemic exposure is achieved. An investigative program was undertaken to characterize these effects and to evaluate the neuropharmacologic potential of some AEs with specific chainlengths, both commercially available materials and a pure homologue, following accidental oral exposure. Results show that even these AEs can be used safely in household cleaning products when their pharmacological properties are taken into consideration.

Intraperitoneal administration of aqueous solutions of AEs to rats resulted in a progressive and reversible syndrome of central nervous system excitation and depression (ataxia, loss of righting) that resembled the induction of and recovery from general anesthesia. Oral administration to rats and mice of aqueous solutions of AEs, alone or in combination with anionic surfactants and ethanol, at concentrations that approach or exceed the maximum expected in household products (10–25%), rarely induced general anesthesia and then only at very high dose volumes (10 ml/kg and above).

Based on these results and the fact that products containing the AEs evaluated usually produce emesis in humans, it is concluded that: (i) AE-induced general anesthesia is not predicted to occur in humans following accidental oral exposure to products containing both the AEs and anionic surfactants, and (ii) general anesthetic effects in humans following accidental oral exposure to products containing fairly high levels of the specific AEs as the nonionic surfactant would be expected to occur only in cases of extremely high volumes of accidental ingestion (≥10 ml/kg).

The acute pharmacologic and toxic potential of certain alkyl ethoxylates (AEs), widely used nonionic surface active agents, have been described to some extent in the published scientific literature (1,2). The biological effects resulting from systemic (i.e. intravenous or intraperitoneal) administration of AEs have been described in rats, mice, rabbits and dogs. The pharmacologic effects following oral dosing in man with nonaethylene glycol mono-n-dodecyl ether ($C_{12}E_9$ or Thesit®) also have been described (3). Examination of the described signs of AE toxicity suggests that many of them are neuropharmacological in origin.

Since AEs are commonly used as nonionic surfactants in household cleaning products which have the potential for acute accidental ingestion by small children, a more thorough understanding of the potential for AEs to produce neuropharmacologic effects following oral exposure is desirable. In the present investigations, the effects of systemic exposure to a commercially available AE material were characterized and compared to the actions of other known neuropharmacologically active agents. Additionally, the neuropharmacologic potential of a pure AE homologue was evaluated when administered orally, either alone or with one of two anionic surfactants and the solvent ethanol, as would be usual in a typical liquid household cleaning product.

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EXPERIMENTAL PROCEDURES

Chemicals. The commercial alkyl ethoxylate material used in intraperitoneal and oral dosing experiments had an average alkyl chainlength of 12 to 13 and an average ethoxylate chainlength of 6.5 ($C_{12\text{-}13}E_{6.5}$). The nonaethylene glycol mono-n-tridecyl ether ($C_{13}E_9$) used in oral dosing experiments was synthesized by J. E. Thompson of the Procter & Gamble Company and had a minimum chemical purity of 96%. The anionic surfactants used in the AE mixtures oral dosing experiments were commercial preparations of linear alkylbenzene sulfonate with an average alkyl chainlength of 11.4 (LAS) and an alcohol ethoxy sulfate with an average alkyl chainlength of 14 to 15 and an average ethoxylate chainlength of 3 (AE_3S). Ethanol for use in the experiments was of >99% purity.

Animals and dose administration. Sprague-Dawley rats (175–225 g) or Swiss Webster mice (15–30 g) of both sexes obtained from Charles River Breeding Laboratories, Portage, Michigan, were used in all studies. Animals were acclimated for at least three days in the testing facility prior to use. The animals were food fasted for approximately 16–20 hr prior to treatment. The test materials were administered either intraperitoneally to rats using a 25 gauge, 3/4″ needle or orally to rats and mice by means of blunt-tipped gavage tubing.

Neuropharmacologic effects of commercial AE. A freshly prepared 8% (w/v) aqueous solution of $C_{12\text{-}13}E_{6.5}$ was used to establish a dose-response relationship for this

TABLE 1

Criteria for AE-Induced Neuropharmacologic Effects

Grade 1	Ataxia (incoordinated gait; "wobbling").
Grade 2	Loss of righting reaction (animal unable to maintain an upright position but reacts visibly to external stimulation).
Grade 3	"Severe loss of righting reaction"—depressed state with little or no visible reaction to external stimulation.
Grade 4	Impaired respiration—gasping breaths, cyanosis; hypothermia. Death associated with significant prior neurologic dysfunction.

material following intraperitoneal administration to rats. The concentration of aqueous dosing solutions of $C_{12\text{-}13}E_{6.5}$ used for oral studies conducted in mice was 24% (w/v) and in rats was varied from 5–24% (w/v) in order to assess the effects of concentration and dose volume on the production of any neuropharmacologic effects. After dosing, animals were placed in individual plastic shoebox cages for observation. Animals were observed closely for the first 3–4 hr after dosing and once every 30 min thereafter for the remainder of the day. Animals were graded for neuropharmacologic effects using the criteria shown in Table 1, with time of onset and duration of specific neuropharmacological effects being noted. An attempt was made to establish dose levels for $C_{12\text{-}13}E_{6.5}$ which produced toxic effects ranging from minimal neuropharmacologic disturbance (i.e. ataxia) to 100% lethality via both intraperitoneal and oral administration.

Neuropharmacologic and plasma evaluation of $C_{13}E_9$. A 25% (w/v) aqueous solution of pure $C_{13}E_9$ homologue was administered orally to rats at a dose of 10 ml/kg, and the animals were then observed as described above to establish the time course for the appearance of any neuropharmacologic effects. In subsequent experiments, 15 rats were dosed orally with 10 ml/kg of either (i) a 25% (w/v) aqueous solution of $C_{13}E_9$, (ii) an aqueous solution consisting of 25% $C_{13}E_9$/12% LAS/15% EtOH (w/v), or (iii) an aqueous solution consisting of 25% $C_{13}E_9$/30% $NaAE_3S$/15% EtOH (w/v), and then divided into five groups of three rats each. After noting the presence of any neurologic effects, these groups were killed at 0.5, 1, 1.5, 2 and 2.5 hr post-dosing, respectively, using an overdose of ether anesthetic. At the time of death, blood was collected from the vena cava using a heparinized needle and syringe. Blood samples from individual rats were centrifuged and resulting plasma samples collected for determination of plasma levels of $C_{13}E_9$. Plasma levels of $C_{13}E_9$ from individual rats were determined by HPLC-UV and values averaged for each timepoint.

RESULTS AND DISCUSSION

The dose-response relationship for neuropharmacologic effects produced following intraperitoneal administration of $C_{12\text{-}13}E_{6.5}$ to rats is shown in Table 2. Increasing doses of $C_{12\text{-}13}E_{6.5}$ produced a temporal progression of increasingly severe neuropharmacologic effects (ataxia → loss

TABLE 2

Intraperitoneal Dosing of $C_{12\text{-}13}E_{6.5}$ to Sprague Dawley Rats

	Ataxia			Loss of Righting			Severe Loss of Righting			Death
Dose (g/kg)	Incidence[a]	Onset (min)	Duration (min)	Incidence[a]	Onset (min)	Duration (min)	Incidence[a]	Onset (min)	Duration (min)	Incidence[a]
0.11	3/6	15.0 ± 3.5[b]	44.0 ± 13	1/6	15.0	32.0	0/6	—	—	0/6
0.15	3/6	18.0 ± 1.5	2.0 ± <0.5	3/6	20.0 ± 1.5	12.5 ± 0.5	3/6	29.0 ± 2.5	27.0 ± 6.5	0/6
0.18	5/5	13.5 ± 0.5	2.5 ± 0.5	5/5	16.0 ± 1.0	6.0 ± 1.0	5/5	22.5 ± 1.5	35.9 ± 9.0	0/5
0.22	6/6	13.0 ± 1.5	2.0 ± <0.5	6/6	15.0 ± 2.0	4.0 ± 0.5	6/6	19.0 ± 2.0	70.0 ± 12.5	0/6
0.25	5/5	11.0 ± 1.5	2.0 ± 0.5	5/5	11.5 ± 2.0	5.5 ± 0.5	5/5	16.5 ± 2.5	69.5 ± 2.5	0/5
0.29	6/6	11.0 ± 0.5	1.5 ± 0.5	5/6	12.5 ± 1.0	3.0 ± 0.5	5/6	15.5 ± 0.5	110.0 ± 8.0	3/6
0.32	5/5	7.0 ± 1.0	4.5 ± 1.0	5/5	11.5 ± 1.0	3.0 ± 0.5	5/5	14.0 ± 1.0	(6.0–18.5)[c]	4/5

[a]n/n, number animals affected/number animals treated.

[b]Values shown are mean ± S.E.M.

[c]Range of times until immediate death.

TABLE 3

Comparison of General Anesthesia Induction and Acute Alkyl Ethoxylate Toxicity

Induction of General Anesthesia	Acute AE Toxicity
Stage 1. Analgesia	Stage 1. "Numbness"; decreased locomotor activity.
Stage 2. Excitement Struggling; dilated pupils; increased muscle tone; vomiting; lid reflexes present, respiration irregular with breath holding.	Stage 2. Ataxia; dilated pupils; vomiting; lid reflexes present; irregular breathing (inspiratory biased, apneustic or normal in character).
Stage 3. Surgical anesthesia Slow, regular breathing; analgesia; disappearance of lid reflexes; loss of righting reaction.	Stage 3. Slow regular breathing (normal, inspiratory biased or apneustic in character); absence of lid reflex; analgesia; loss of righting reaction.
Stage 4. Medullary paralysis Apnea.	Stage 4. Apnea.

TABLE 4

$C_{13}E_9$ Plasma Levels and Neuropharmacologic Effects in Sprague Dawley Rats

Oral treatment (10 ml/kg)	Time post-administration (hr)	$C_{13}E_9$ Plasma level[a] (μg/ml)	Observed neuropharmacologic effects (Incidence[b], effect)
25% $C_{13}E_9$	0.5	11.0	NOE[c]
	1.0	11.0	NOE
	1.5	14.5	1/3, ataxia
	2.0	8.7	NOE
	2.5	25.3	3/3, loss of righting reflex
25% $C_{13}E_9$/ 12% LAS/15% EtOH	0.5	3.2	NOE
	1.0	0.9	NOE
	1.5	2.7	NOE
	2.0	5.2	NOE
	2.5	2.7	NOE
25% $C_{13}E_9$/ 30% $NaAE_3S$/15% EtOH	0.5	5.9	NOE
	1.0	6.7	NOE
	1.5	11.9	NOE
	2.0	21.8	1/3, ataxia
	2.5	18.0	NOE

[a]Values shown are averages of samples from three individual animals.
[b]n/n, number of animals affected/number of animals treated.
[c]NOE, No observed effect.

of righting reflex → respiratory depression) that resulted in death at the highest doses. The neuropharmacologic effects in those animals receiving less than lethal doses were completely reversible, with recovering animals exhibiting a temporal regression of neuropharmacologic effects. A comparison of the time course and character of neuropharmacologic effects produced by systemic administration of AEs to the well-known stages of the induction of general anesthesia, illustrated in Table 3, suggests that these nonionic surfactants can act as general anesthetics following acute systemic exposure to sufficiently high levels.

Oral dosing of $C_{12\text{-}13}E_{6.5}$ to mice produced a dose-related progression of neuropharmacologic effects, but the incidence, onset and duration of these toxic signs occurred with tremendous variability. Oral doses of $C_{12\text{-}13}E_{6.5}$ which resulted in clearly defined incidences, onsets and durations of specific neuropharmacological effects in mice represented volumes of administration judged to be nonphysiological in mice (i.e. greater than 10 ml/kg body weight) and higher than the volumes of household cleaning products containing AEs which likely would be ingested accidentally by children. Consistent production of neuropharmacologic effects in rats following oral administration of various concentrations at several dosing volumes also proved difficult to achieve. The usual spectrum of AE-induced neuropharmacologic effects was observed only when concentrations of the AE were around 10–15% and the volume of administration greatly exceeded what would be considered normal accidental ingestion conditions (i.e. 30 ml/kg). Even under these conditions, the onset of neuropharmacologic effects occurred at very long latencies (3–4 hr) after administration of material. It is possible that, in general, concentrated aqueous solutions of AEs inhibit their own systemic absorption by a local anesthetic action on the stomach and small intestine.

Based on previous structure-activity relationship work in our laboratory which suggested that pure AE homologues of certain alkyl and ethoxylate chainlength (i.e. possessing an appropriate relative hydrophilicity/lipophilicity) could be absorbed following oral exposure in rats, the ability of $C_{13}E_9$ to produce neuropharmacologic effects when administered orally to rats was examined. A 25% aqueous solution of $C_{13}E_9$ dosed orally at 10 ml/kg (i.e. the upper range of expected volumes of accidental ingestion of household cleaning products by children) did produce some neuropharmacologic effects (ataxia and loss of righting reflex) in some or most animals starting at 1.5 hr post-dosing. These specific neuropharmacologic effects correlated to achievable plasma levels of $C_{13}E_9$, as shown in Table 4. The effect of oral coadministration of either of two anionic surfactants and ethanol with $C_{13}E_9$ on achievable plasma levels of that AE, and consequently production of $C_{13}E_9$-induced neuropharmacologic effects in rats, is also presented in Table 4. The mixtures of 25% $C_{13}E_9$/12% LAS/15% EtOH and 25% $C_{12}E_9$/30% $NaAE_3S$/15% EtOH were considered to contain maximal or exaggerated levels of AE, common anionic surfactants and solvent which might be found in typical liquid household cleaning products. The oral coadministration of LAS and ethanol with $C_{13}E_9$ lowered the achievable plasma level of that AE, and neuropharmacologic effects were not elicited. It is possible that LAS inhibits the systemic absorption of $C_{13}E_9$ from the gastrointestinal tract. Oral coadministration of high levels of $NaAE_3S$ and ethanol with $C_{13}E_9$ did not markedly decrease the achievable plasma levels of $C_{13}E_9$ as did LAS. However, $NaAE_3S$ did not markedly increase the incidence or severity of $C_{13}E_9$-induced neuropharmacologic effects as might be expected if the two materials have competitive metabolic (detoxification) pathways.

Of note in the above studies is the fact that they were conducted in species not capable of exhibiting emesis. As

general anesthetics commonly produce emesis during the induction of anesthesia, species capable of this response (including humans) are likely to exhibit emesis following acute oral exposure to household cleaning products containing sufficient levels of alkyl ethoxylates. With less AE available for systemic absorption, the likelihood for AE-induced neuropharmacologic effects to be observed following accidental ingestion is decreased.

When all the above data are considered, it can be seen that some AEs do possess the ability to produce acute, reversible neuropharmacological effects following sufficient exposure. However, these AEs clearly can be formulated safely into household cleaning products which might be accidentally ingested by children if their pharmacological properties are understood and taken into account. The level and characteristics of the AE used, the presence of certain anionic surfactants in the product formulation and even the ability of AEs to cause emesis in humans are factors which are likely to mitigate the potential for neuropharmacologic effects being produced following accicental ingestion of liquid household cleaning products containing these alkyl ethoxylates.

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REFERENCES

1. Soehring, K., K. Scriba, M. Frahm and G. Zeollner, *Arch. Int. Pharmacodyn 87:*301 (1951).
2. Benke, G.M., N.M. Brown, M.J. Walsh and R.B. Drotman, *Food Cosmet. Toxicol. 15:*309 (1977).
3. Hochrein, M., and I. Schleicher, *Duet. Med. Wschr. 76:*736 (1951).

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