Safety Assessment of Acetyl Trialkyl Citrates as Used in Cosmetics

Status:	Re-Review for Panel Review
Release Date:	May 10, 2019
Panel Meeting Date:	June 6-7, 2019

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Wilbur Johnson, Jr., Senior Scientific Analyst.

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Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr., Senior Scientific Analyst
Date: May 10, 2019
Subject: Re-Review of the Safety Assessment of Acetyl Alkyl Citrates

The CIR Expert Panel first published the safety assessment of Acetyl Trialkyl Citrates in 2002. The Panel concluded that Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trioctyl Citrate (now known as Acetyl Triethylhexyl Citrate), and Acetyl Trihexyl Citrate are safe as used in cosmetics, as described in that report (identified as *acetyl062019orig* in the pdf). Minutes from the deliberations of the original review are also available (identified as *acetyl062019min*).

Because it has been at least 15 years since the first report was published, in accord with CIR Procedures, the Panel should consider whether the safety assessment of Acetyl Trialkyl Citrates should be re-opened. An exhaustive search of the world's literature was performed for studies dated 1996 forward. A synopsis of the relevant data is enclosed (*acetyl062019new data*).

Also included for your review are current and historical use data (*acetyl042019use tbl*). The current data indicate that only Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are used in cosmetic products. The frequency of use has increased for both ingredients since the final report was issued. According to VCRP data, Acetyl Triethyl Citrate and Acetyl Tributyl Citrate were reported to be used in 9 and 27 formulations, respectively, in 1998. In 2019, the VCRP data indicate that Acetyl Triethyl Citrate is used in 22 formulations, and Acetyl Tributyl Citrate is used in 438 formulations (*acetyl062019FDA*). There were no reported uses of Acetyl Trihexyl Citrate or Acetyl Triethylhexyl Citrate (formerly Acetyl Trioctyl Citrate) in 1998 or in 2019. For Acetyl Triethyl Citrate, the maximum concentration of use was 7% in nail products in 1999; however, according to a recent survey provided by the Council, current use concentration data on this ingredient were not submitted. For Acetyl Tributyl Citrate, the maximum concentrations of use have increased slightly since the original report was issued. In 1999, Acetyl Tributyl Citrate was used at up to 7% in nail products and up to 3% in products (i.e., eyeliners) that resulted in dermal contact; data collected in 2018 indicate that the maximum concentrations of use are 8.9% in nail products and 7% in eye products that result in dermal contact (*acetyl042019conc1 and acetyl042019conc2*).

A data profile is included; data in the original (2002) report, as well as new data, are identified therein (*acetyl062019prof*). New data that were not included in the original report include: animal dermal irritation and sensitization data on Acetyl Triethylhexyl Citrate; animal ocular irritation data on Acetyl Triethylhexyl Citrate; animal carcinogenicity data on triethyl citrate for Acetyl Triethylhexyl Citrate read-across; and other potentially relevant studies on Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, and Acetyl Trihexyl Citrate. Also included are the FDA VCRP data (acetyl*062019FDA*) and the concentration of use data that were submitted in response to a Council survey (*acetyl062019conc1 and acetyl062019conc2*).

If, upon review of the new studies and updated use data, the Panel determines that a re-review is warranted, a full draft amended report will be presented at an upcoming meeting.

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Acetyl Trialkyl Citrates Data Profile* – June 6-7, 2019 Panel – Wilbur Johnson																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Acetyl Triethyl Citrate	X	X	O					O			O				X			X		O	O		O	O			O		
Acetyl Tributyl Citrate	X	X	O			O		O		O	O X			O	O X	X		O X		O X	O		O	O			O		
Acetyl Triethylhexyl Citrate																		X		O X			X				X		
Acetyl Trihexyl Citrate			O			O	O								O	O				O							O		

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

[Acetyl Trialkyl Citrates (years 1996 forward) – 3/26-27/2019]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECET-OC	Web
Acetyl Triethyl Citrate	77-89-4	Yes	41/0	5/0	5/0	Yes	No	Yes	No	No	No	No	No	No	No	No	No	
Acetyl Tributyl Citrate	77-90-7	Yes	149/7	52/7	66/3	Yes	No	Yes	No	No	No	No	No	No	Yes	No	No	
Acetyl Triethylhexyl Citrate (Acetyl Trioctyl Citrate)	126-40-9 144-15-0	Yes	192/0 135/0	1/0	0/0 1/0	Yes	No	Yes	No	No	No	No	No	No	No	No	No	
Acetyl Trihexyl Citrate	24817-92-3	Yes	205/0	1/0	1/0	No	No	No	No	No	No	No	No	No	No	No	No	

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - https://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable

Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable

IFRA (International Fragrance Association) – http://www.ifraorg.org/

RIFM (the Research Institute for Fragrance Materials) should be contacted

Qualifiers
Absorption
Acute
Allergy
Allergic
Allergenic
Cancer
Carcinogen
Chronic
Development
Developmental
Excretion
Genotoxic
Irritation
Metabolism
Mutagen
Mutagenic
Penetration
Percutaneous
Pharmacokinetic
Repeated dose
Reproduction
Reproductive
Sensitization
Skin
Subchronic
Teratogen
Teratogenic
Toxic
Toxicity
Toxicokinetic
Toxicology
Tumor

March 3-4, 1999 CIR Expert Panel Meeting (70th)

Acetyl Triethyl Citrate, Acetyl Tributyl Citrate,
Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate

Unpublished data that were received just prior to the Panel meeting will be incorporated into the Draft Report after the Panel's review.

The Teams issued the following informal data requests:

Dr. Schroeter's Team

1. UV absorption spectrum
2. Reproductive and developmental toxicity study

Dr. Belsito'sTeam

1. UV absorption spectrum. If absorption occurs, then photosensitization data will be needed
2. If there is evidence of dermal absorption, then a reproductive and developmental toxicity study will be needed.
3. Irritation and sensitization data at use concentrations

June 14-15, 1999 CIR Expert Panel Meeting (71st)

Acetyl Triethyl Citrate, Acetyl Tributyl Citrate,
Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate

Dr. Belsito noted that his Team issued the following informal data request on this group of ingredients at the March 3-4, 1999 Panel meeting:

(1) UV absorption spectrum. If absorption occurs, then photosensitization data will be needed
(2) If there is evidence of dermal absorption, then a reproductive and developmental toxicity study will be needed.
(3) Irritation and sensitization data at use concentrations

He also recalled that a large submission of unpublished data on these ingredients was received, including, chemical characterization, concentration of use, metabolism, acute oral and dermal toxicity, short-term dermal toxicity, chronic oral toxicity, ocular irritation, skin irritation and sensitization (animals and humans), mutagenicity, and carcinogenicity. Dr. Belsito said that, based on these data, his Team determined that it could be concluded that these ingredients are safe as used. He also noted, however, that his Team expressed concern over the observation that a large number of the references included in the Draft Report were incomplete (without dates or journal names), and requested verification of these references. Dr. Belsito emphasized that his Team's safe as used conclusion is contingent on the ability of the CIR staff to verify these references.

Dr. Schroeter said that his Team concurs with the Belsito Team's proposal. He also said that it is his understanding that UV absorption data will be submitted.

The Panel voted unanimously in favor of issuing a Tentative Report with a safe as used conclusion on Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate.

Dr. Andersen asked Dr. Belsito to explain exactly what he meant by verification of references.

Dr. Belsito said that he was referring to the ability of the staff to obtain dates and sources for the incomplete references that are cited in text.

Dr. Andersen concurred with Dr. Bergfeld's suggestion that the date on which unpublished data were received, clearly identified as the actual date on which data were received, should be used when the actual date on which the study was conducted is not included.

December 20-21, 1999 CIR Expert Panel Meeting (73rd)

Acetyl Triethyl Citrate, Acetyl Tributyl Citrate,
Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate

Dr. Schroeter stated that his Team had expressed concern over the sensitization potential of Acetyl Triethyl Citrate based on positive results in the guinea pig maximization test, but can now conclude, based on the negative human skin sensitization test data on Acetyl Triethyl Citrate and Acetyl Tributyl Citrate and other data in the Final Report, that all four ingredients are safe as used.

Dr. Belsito said that it should be stated in the report discussion that the human sensitization test data are on 100% Acetyl Triethyl Citrate, but that this ingredient is used at concentrations of 4 to 7% primarily in hair and nail products. Dr. Belsito also noted that while it is assumed that the test concentration in the human study is 100%, no specific test concentration is stated. He asked for clarification of the test concentration of Acetyl Triethyl Citrate that was applied so that this information can be incorporated into the Final Report.

Dr. Bergfeld wanted to know whether the possibility that the actual test concentration will not be provided will have any impact on the proposed safe as used conclusion.

Dr. Belsito said that it would be nice to have the information, but, without it, no change in the safe as used conclusion is necessary.

The Panel concluded that Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate are safe as used in cosmetic formulations and voted unanimously in favor of issuing a Final Report.

Table 1. Current and historical frequency and concentration of use of Acetyl Trialkyl Citrates according to duration and exposure.

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Acetyl Triethyl Citrate				**Acetyl Tributyl Citrate**			
	2019[1]	**1998[1]**	**2018[2]**	**1999[1]**	**2019[3]**	**1998[1]**	**2018[2]**	**1999[1]**
Totals*	**22**	**9**	**NR**	**4-7**	**438**	**27**	**0.09-8.9**	**0.8-7**
Duration of Use								
Leave-On	*21*	*9*	*NR*	*4-7*	*437*	*26*	*6-8.9*	*0.8-7*
Rinse-Off	*1*	*NR*	*NR*	*NR*	*1*	*1*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	5	3	7.5	3
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	10	NR	NR	NR	1	NR	0.0015 - 0.09	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	5	3	0.0015 – 7.5	3
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	11	1	NR	NR	3	1	0.09	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	11	8	NR	4-7	428	23	6-8.9	0.8-7
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

References

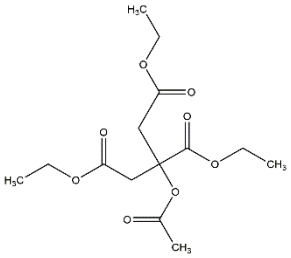
1. Andersen F. Final report on the safety assessment of acetyl triethyl citrate, acetyl tributyl citrate, acetyl trihexyl citrate, and acetyl trioctyl citrate. *International journal of toxicology* 2002;21 Suppl 2:1-17.

2. Personal Care Products Council. 2019. Council Concentration of Use by FDA Product Category: Acetyl Trialkyl Citrates (Unpublished data submitted by the Personal Care Products Council on January 8, 2019).

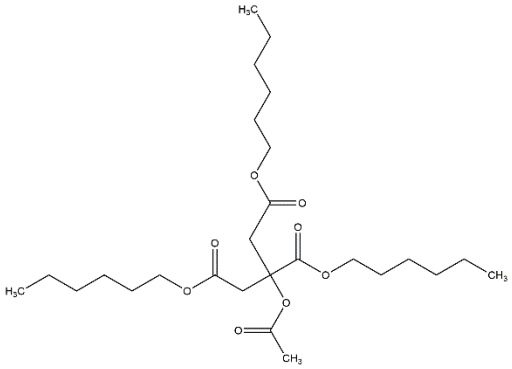
3. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2019. Voluntary Cosmetic Registration Program - Frequency of use of Cosmetic Ingredients. College Park, MD:2019. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3, 2019; received February 13, 2019.

New Data – Acetyl Trialkyl Citrates

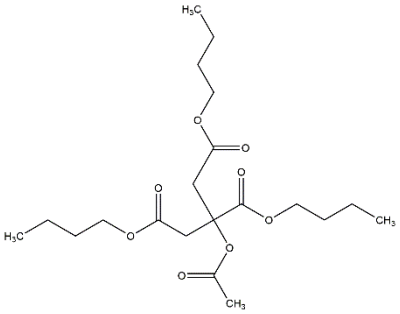
Structures



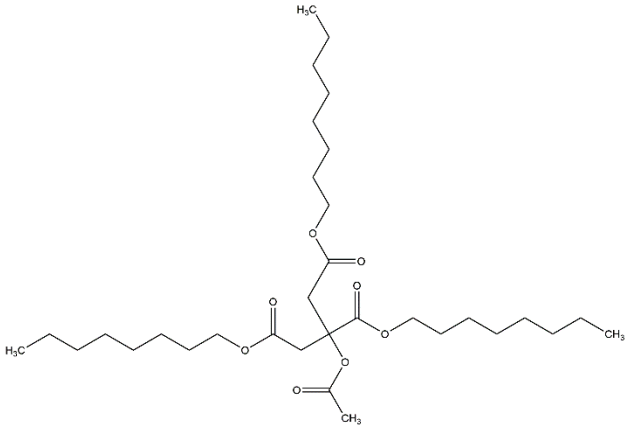
Acetyl Triethyl Citrate



Acetyl Trihexyl Citrate



Acetyl Tributyl Citrate



Acetyl Triethylhexyl Citrate

(previously known as Acetyl Trioctyl Citrate)

Figure 1. Acetyl Trialkyl Citrates

Subchronic Oral Toxicity

Acetyl Tributyl Citrate

Prior to a subchronic toxicity evaluation, groups of 50 Wistar rats (25 males, 25 females per group) were dosed continuously (in diet) with Acetyl Tributyl Citrate at target doses of 0, 100, 300, and 1000 mg/kg/day.[1,2] The animals were dosed for 4 weeks before pairing and then throughout mating. F_0 males were killed after mating. Dosing of the F_0 females was continued throughout gestation, littering, and lactation until they were killed (postnatal day 21). The F_1 offspring were exposed in utero and from birth until initiation of the 13-week study. In the subchronic (13-week) study, the F_1 animals (20 males, 20 females per group) were fed Acetyl Tributyl Citrate (in diet) at same target doses of 0,100, 300, and 1000 mg/kg/day. Ten F_1 males and 10 F_1 females were assigned to the control and high dose group for a 4-week recovery period, after the 13-week dosing period. Dosing with 1000 mg/kg/day caused a slight reduction in body weight gain in both sexes (F_1). Liver weights (absolute or relative, not specified) were increased, and hepatic hypertrophy occurred at 1000 mg/kg/day in both sexes (F_1). Hepatic hypertrophy resulting from an induction of metabolizing enzymes as an adaptive response to treatment is a common finding following administration of high doses of xenobiotics (according to the study authors), and is not considered to be toxicologically significant. Weak peroxisome proliferation, recognized as a rodent-specific effect, was measured in males of the 300 mg/kg/day group and in both sexes dosed with 1000 mg/kg/day. Slight variations in urinary composition and in plasma electrolyte concentrations suggested an effect on renal function at doses of 300 and 1000 mg/kg/day. These changes

were all shown to be reversible and within normal historical control ranges, and there were no histopathological changes in the kidneys. The authors considered this possible effect on the kidneys to be due to adaptation to the excretion of high levels of Acetyl Tributyl Citrate and/or metabolites, and agreed that it is not a toxicologically significant finding. The no-observed-adverse-effect-level (NOAEL) for Acetyl Tributyl Citrate systemic toxicity were considered to be 100 mg/kg/day (males) and 300 mg/kg/day (females). Results relating to reproductive toxicity in this study are summarized under the Developmental and Reproductive Toxicity heading below.

Relative to the finding of increased liver weight above, it should be noted that the US Consumer Product Safety Commission has stated that the increased liver weight is most likely an adaptive change occurring as a consequence of metabolic load.[3,4] Furthermore, it was noted that the increased relative organ weights are not adverse and that they might be related to enzyme induction, possibly with a contribution from the slight decrease in body weight.

Developmental and Reproductive Toxicity

Animal

Acetyl Tributyl Citrate

A study was performed to evaluate the effects of Acetyl Tributyl Citrate exposures on female reproduction using groups of 7 to 8 adult female CD-1 mice.[7] Two groups were dosed orally (pipette tip placed in check pouch) with Acetyl Tributyl Citrate (in tocopherol-stripped corn oil) at doses of 5 mg/kg/day and 10 mg/kg/day, respectively, for 15 days. The control group was dosed orally with vehicle only. The female mice were then bred with a proven breeder male. Acetyl Tributyl Citrate exposure prior to mating did not alter body weights, estrous cyclicity, or gestational and litter parameters. Relative spleen weights were slightly increased in the 5 mg/kg/day group. Dosing with 10 mg/kg/day targeted ovarian follicles and decreased the number of primordial, primary, and secondary follicles present in the ovary. The authors noted that these findings suggest that low levels of Acetyl Tributyl Citrate may be detrimental to ovarian function.

In a two-generation reproductive toxicity study, groups of 50 Wistar rats (25 males, 25 females per group) were dosed continuously (in diet) with Acetyl Tributyl Citrate at target doses of 0, 100, 300, and 1000 mg/kg/day.[1,2] The animals were dosed for 4 weeks before pairing and then throughout mating. F_0 males were killed after mating. Dosing of the F_0 females was continued throughout gestation, littering, and lactation until they were killed (postnatal day 21). The F_1 offspring were exposed in utero and from birth until initiation of the 13-week study. The F_1 animals (20 males, 20 females per group) were then fed Acetyl Tributyl Citrate (in diet) at same target doses of 0,100, 300, and 1000 mg/kg/day for 13 weeks (as described earlier in the Subchronic Oral Toxicity section). In parental animals, the following parameters were unaffected by treatment: estrous cycles, mating performance, fertility, gestation length, and parturition. At a dose of 1000 mg/kg/day, litter size and numbers of implantations were lower than in controls, but were within the laboratory's historical control values. Litter size, survival, and growth were similar in all dose groups. In the offspring, anogenital distance and sexual maturation in both sexes, and retention areolae in males, were unaffected by dosing with Acetyl Tributyl Citrate. There were no effects of Acetyl Tributyl Citrate on sperm motility, counts, or morphology. There also were no effects of the test substance on any of the reproductive endpoints. There were no findings at necropsy of parental animals or surplus offspring that were considered treatment-related. The NOAELs for reproductive and developmental toxicity were considered to be 300 mg/kg/day for parental animals and 1000 mg/kg/day for offspring.

Genotoxicity

In Vitro

Acetyl Tributyl Citrate

Ames test results were negative for Acetyl Tributyl Citrate at the following doses in *Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1537 (50 to 5,000 µg/plate, with and without metabolic activation).[8] Results were negative for Acetyl Tributyl Citrate in the in vitro chromosomal aberrations assay: rat lymphocytes (4 to 400 µg/ml, with and without metabolic activation).[8] Results were negative for Acetyl Tributyl Citrate in the forward mutation assay: L5178Y (TK+/TK-) mouse lymphoma cells (200 to 4580 µg/ml, with metabolic activation; 10 to 230 µg/ml, without metabolic activation).[8] Results for another forward mutation assay on Acetyl Tributyl Citrate were also negative: CHO/HGPRT (25 to 400 µg/ml, with and without metabolic activation).[8]

Acetyl Triethyl Citrate

Ames test results were negative for Acetyl Triethyl Citrate. The following doses were tested using *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538 (333 to 10,000 µg/plate, with and without metabolic activation).[9]

In Vivo

Acetyl Tributyl Citrate

Results were negative for Acetyl Tributyl Citrate in the in vivo/in vitro unscheduled DNA synthesis assay. The assay involved male rat primary cultures of hepatocytes obtained from Han-Wistar rats that received single oral doses of 800 or 2000 mg/kg.[2,8]

A chromosomal aberration assay on Acetyl Tributyl Citrate (in PEG 400) was performed using 10 Wistar rats that received a single oral dose of 2000 mg/kg.[10] A vehicle control group was also used. Bone marrow cells were collected and 100 well-spread metaphases/animal were scored for chromosomal aberrations. The mitotic index was slightly reduced after 24 h, but the test substance did not induce chromosomal aberrations at any time point that was investigated.

Carcinogenicity

Acetyl Tributyl Citrate

Groups of up to 100 Wistar rats were dosed orally (via diet) with Acetyl Tributyl Citrate in a combined 2-year chronic/carcinogenicity study.[2,10] The intended daily intake in dosed groups was 0, 100, 300, and 1000 mg/kg/day. The chronic toxicity evaluation (after 52 weeks of dosing) in this study involved groups of 40 rats (20 males, 20 females per group), and the carcinogenicity evaluation (after 104 weeks of dosing) involved groups of 100 (50 males and 50 females per group). In both the chronic and carcinogenicity evaluation, gross examination was performed on all animals, and histopathological examination was performed on: control animals, animals of the highest dose group, all animals that died spontaneously or were killed in extremis, and on all gross lesions from all animals. The following results were obtained after 52 weeks (for groups of 40 rats): Acetyl Tributyl Citrate induced slight reductions in body weight and food consumption. Changes in clinical chemistry parameters were defined to various parameters indicating adaptive changes of metabolic activation, which were not considered to be of primary toxicological relevance. The adaptive changes were expressed by few macroscopically discernible liver changes, liver weight increase, and minimal hepatocellular hypertrophy in individual animals. Macroscopic findings were restricted to the liver of the high dose group, and consisted of an accentuated lobular pattern in 5 males and enlargement of the liver in 5 males and 5 females. At histopathologic examination (non-neoplastic), a minimal centrilobular hepatocellular hypertrophy was diagnosed in 2 males and 1 female of the high dose group. The NOAEL was 300 mg/kg/day for males and 1000 mg/kg/day for females. The NOEL was defined to be at 100 mg/kg/day for males and 300 mg/kg/day for females.

After 104 weeks of dosing (groups of 100 rats), mean body weights and mean body weight gains reduction were observed in males of the 300 and 1000 mg/kg/day dose groups, and in females of the 1000 mg/kg/day dose group. Absolute and relative liver weight increased significantly in males and females dosed with 1000 mg/kg/day. An increased incidence of hepatocellular hypertrophy was observed at 1000 mg/kg/day in males (5/50 males versus 0/50 in control). Dosing with 1000 mg/kg/day also caused minimal to moderate single cell necrosis of hepatocytes in males (7/50 versus 0/50 in control) and in females (1/50 versus 0/50 in control). The authors noted that the relative liver weight increase, along with an increased incidence of hepatocellular hypertrophy at 1000 mg/kg/day in males, was considered to be the morphologic expression of an adaptive metabolic response rather than a toxic effect. However, they also noted that it is not possible to affirm and reject the possibility of a pathological effect. The origin of single cell necrosis in hepatocytes of males or females dosed with 1000 mg/kg/day remained unclear. The authors proposed a NOAEL of 300 mg/kg/day for males and 1000 mg/kg/day for females based on effects on body weights at 1000 mg/kg/day in males and on the slight increase in liver weights and on centrilobular hypertrophy in the liver at 1000 mg/kg/day in both sexes. Acetyl Tributyl Citrate did not induce test substance-related neoplastic lesions in any of the dose groups.

Triethyl Citrate (potential read-across for Acetyl Triethylhexyl Citrate, as proposed in the ECHA dossier)

Rats (strain not specified; 15 males, 15 females) were fed triethyl citrate in the diet at a concentration of 3% (~ 1.5 g/kg/day) for 2 year.[11] Additional details relating to the test protocol are not included. There was no evidence of carcinogenic activity in any of the animals tested.

Dermal Irritation and Sensitization

Irritation

Acetyl Tributyl Citrate

The skin irritation potential of Acetyl Tributyl Citrate was evaluated using albino rabbits (2 to 3 animals).[8] The test substance was applied to intact/abraded skin at a dose of 1000 mg/kg (1 ml/kg), repeatedly for 4 to 18 applications. The last application was followed by a 36-h to 2-week observation period. Skin irritation was not observed.

Acetyl Triethylhexyl Citrate

The skin irritation potential of Acetyl Triethylhexyl Citrate was evaluated using 3 male albino rats.[11] Three semiocclusive patches, each containing the test substance (0.5 ml), were applied to different regions of the back. Exposure durations initially were 3 min and 1 h. The test substance was also applied (right side of back) for 4 h to 2 animals because reactions at 3 min and 1 h were negative. Reactions were scored at 1 h, 24 h, 48 h, and 72 h after the end of exposure. Very slight erythema was observed in 1 animal after the 4-h exposure, but was fully reversible within 48 h. It was concluded that Acetyl Triethylhexyl Citrate was non-irritating to the skin of rabbits.

Sensitization

Acetyl Triethylhexyl l Citrate

Acetyl Triethylhexyl Citrate was evaluated for skin sensitization in the maximization test, using 10 Pirbright white guinea pigs (5 males, 5 females).[11] The induction phased involved intradermal injection and dermal application of 5% Acetyl Triethylhexyl Citrate. Induction was followed by a 2-week non-treatment period. The challenge phase involved a 24-h application of the undiluted test substance (0.5 ml, under occlusive patch) to a 5 x 5 cm^2 area on each flank. Reactions were scored at 24 h and 48 h post-application. No reactions were observed during induction, and there was no evidence of allergic skin reactions at 24 h or 48 h after challenge patch application. Acetyl Triethylhexyl Citrate was classified as a non-sensitizer.

Ocular Irritation

Acetyl Triethylhexyl Citrate

The ocular irritation potential of Acetyl Triethylhexyl Citrate was evaluated using 3 albino rabbits. [11] The test substance (0.1 ml) was instilled into the conjunctival sac (left eye). Untreated right eyes served as controls. Reactions were scored at 1 h, 24 h, 48 h, and 72 h post-instillation. Slight conjunctival redness and discharge were observed in all animals at 1 h post-instillation. In 1 animal, slight redness was also observed up to 48 h post-instillation. All ocular irritation reactions observed were fully reversible within 72 h post-instillation. Acetyl Triethylhexyl Citrate was classified as non-irritating to the eyes of rabbits.

Other Relevant Studies

Acetyl Tributyl Citrate, Acetyl Triethyl Citrate,

and Acetyl Trihexyl Citrate

The effect of Acetyl Tributyl Citrate on in vitro growth and viability of mouse ovarian antral follicles was evaluated.[5] Antral follicles were isolated from female CD-1 female mice (32 - 37 days old) and treated with Acetyl Tributyl Citrate (0.001 to 1000 µg/ml) for 24 to 72 h. Follicle diameter, ATP production, quantitative polymerase chain reaction (qPCR), and the terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling assay (TUNEL) were used to measure follicle growth, viability, cell cycle and apoptosis gene expression, and cell death-associated DNA fragmentation, respectively. Acetyl Tributyl Citrate increased (statistically significant) the number of nongrowing follicles at 0.01 µg/ml and did not affect ATP production, but increased TUNEL positive area in treated follicles. Thus, Acetyl Tributyl Citrate exposure resulted in dose-specific follicle growth inhibition

In a frog embryo teratogenesis assay-Xenopus (FETAX), the 96-h lowest observed effective concentrations (LOECs) for survival, developmental abnormality, and growth effects on *Xenopus laevis* embryos (amphibian embryos) were: 5.3 mg/L (Acetyl Tributyl Citrate), 90.9 mg/L (Acetyl Triethyl Citrate) and 14.9 mg/L (Acetyl Trihexyl Citrate).[6] Embryos were incubated with Acetyl Tributyl Citrate at concentrations ranging from 5.3 to 31.5 mg/L, and with Acetyl Triethyl Citrate at concentrations ranging from 90.9 to 681.6 mg/L. Embryos were incubated with Acetyl Trihexyl Citrate at concentrations ranging from 14.9 to 75.4 mg/L. These results suggest that the developmental toxicity of Acetyl Triethyl Citrate is low, when compared to Acetyl Tributyl Citrate and Acetyl Trihexyl Citrate.

Acetyl Tributyl Citrate

Steroid and xenobiotic receptor (SXR) is activated by endogenous and exogenous chemicals. and is highly expressed in the liver and intestine, where it regulates cytochrome P450 3A4 (CYP3A4), which in turn controls xenobiotic and endogenous steroid hormone metabolism.[12] To obtain in vivo evidence for the intestine-specific effects of Acetyl Tributyl Citrate, groups of 3 Wistar rats were treated with Acetyl Tributyl Citrate (5 or 50 mg/kg) by intraperitoneal injection for three consecutive days, and the animals were evaluated for the expression of CYP3A1 (which corresponds to human CYP3A4) in liver, duodenum, and ileum using quantitative real-time reverse transcription polymerase chain reaction (qRTPCR). Acetyl Tributyl

Citrate increased CYP3A1 expression in the duodenum and ileum of rats. However, Acetyl Tributyl Citrate did not induce CYP3A1 in the liver. These results suggest that Acetyl Tributyl Citrate specifically induces CYP3A in the intestine by activating SXR. The authors suggested that Acetyl Tributyl Citrate-containing products be used cautiously because they may alter metabolism of endogenous steroid hormones.

To determine whether oral exposure of Acetyl Tributyl Citrate induces CYP3A1 gene induction, groups of 3 rats were treated with Acetyl Tributyl Citrate (50 mg/kg) daily by gavage for 2 days.[12] As seen in the intraperitoneal injection study, Acetyl Tributyl Citrate did not induce CYP3A1 expression in the liver. Although CYP3A1 induction by Acetyl Tributyl Citrate was not observed in the duodenum, significant CYP3A1 induction by ATBC was observed in the ileum, indicating that Acetyl Tributyl Citrate is likely to be absorbed in the distal portion of the intestine (where Acetyl Tributyl Citrate induces CYP3A1 expression.) Taken together, these findings indicate that Acetyl Tributyl Citrate is an intestine-specific inducer of CYP3A.

A study was performed to establish optimal conditions for using human adrenal H295R cells to detect chemicals interfering with the production of key adrenal steroids.[13] The study was performed in accordance with Organization for Economic Co-operation and Development Test Guideline (OECD TG) 456. This validated test guideline, based on H295R cells, promotes measurement of testosterone and estradiol production as read-out. H295R cells' supernatants were characterized by liquid chromatography–mass spectrometry (LC–MS)-based steroid profiling. Steroid profiles were determined before and after 48 h of incubation with Acetyl Tributyl Citrate (10 mM). Exposure of H295R cells to Acetyl Tributyl Citrate tended to increase the synthesis of the corticosteroids, corticosterone and aldosterone, and increased 11-deoxycorticosterone production. As seen with torcetrapib, Acetyl Tributyl Citrate enhanced progesterone and 17α-hydroxyprogesterone levels. Hence, these results suggest that Acetyl Tributyl Citrate increases mineralocorticoid production at a concentration of 10 mM, while none of these steroid metabolites were altered at lower concentrations.

The effect of Acetyl Tributyl Citrate on the expression of key steroidogenic genes was also determined in this study.[13] As the positive control, torcetrapib led to a profound up regulation of the expression of CYP11B2 and 3β-HSD2 and a more moderate induction of CYP11B1, CYP21A2, StAR, CYP11A1 and CYP17A1. The elevated expression of 3β-HSD2 explains the increased production of progesterone and 17α-hydroxyprogesterone, whereas the enhanced CYP11B1, CYP11B2, and CYP21A2 are responsible for the observed increase in aldosterone, cortisol, corticosterone and 11β-hydroxyandrostenedione. At concentrations of 10 mM, Acetyl Tributyl Citrate increased CYP11B2, 3β-HSD2, and CYP21A2 expression. It also increased, or tended to increase, CYP11B1 expression. Thus, Acetyl Tributyl Citrate resembled the steroid profile of torcetrapib. The authors noted that this torcetrapib-like steroid pattern with increased corticosteroids could be explained by elevated expression of CYP11B2 and 3β-HSD2 mRNA levels. Thus, Acetyl Tributyl Citrate does not directly modulate the activity of these enzymes but rather alter their expression levels.

The estrogen receptor (ER) activity of Acetyl Tributyl Citrate was evaluated using HeLa9903 and VM7-Luc cell cultures.[14] Both cell lines stably express ER-α gene containing a firefly luciferase gene as a reporter gene. Estrogenic and anti-estrogenic activities were evaluated in accordance with OECD TG 455. In the test for estrogenic activity, HeLa 9903 cells were treated for 24 h with Acetyl Tributyl Citrate concentrations ranging from 1 nM to 1 mM. Estrogenic activity was assessed by increasing ER transcriptional activity of the test substance using 17-β-estradiol (E2)-enhanced ER transcriptional activity. Antiestrogenic activity was assessed using VM7-luc cells treated for 24 h with 500 pM E2 and Acetyl Tributyl Citrate (9.7 nM to 10 µM). Results indicated that Acetyl Tributyl Citrate had no estrogenic activity, but demonstrated anti-estrogenic activity.

Androgen receptor (AR) activity was also evaluated.[14] The androgenic and anti-androgenic activities assessment used AR-EcoScreen™ cells, and the protocol was in accordance with OECD TG 458. These cells stably express an AR reporter gene containing a firefly luciferase. AR-EcoScreen™ cells were treated for 24 h with Acetyl Tributyl Citrate (10 pM to 10 µM). Androgenic activity was assessed by increasing AR transcriptional activity of the test substance using 5α-dihhydrotestosterone (DHT)-enhanced AR transcriptional activity. Results indicated anti-androgenic activity.

A purely in silico study was performed evaluating the sex hormone binding globulin (SHBG) binding-potential of Acetyl Tributyl Citrate.[15] Results from a Schrodinger's Induced Fit Docking (IFD) protocol predicted that that Acetyl Tributyl Citrate would bind in the ligand binding pocket of SHBG.

Acetyl Triethyl Citrate and Acetyl Tributyl Citrate

The effects of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate on steroid and xenobiotic receptor (SXR)-mediated transcription in vitro was evaluated using a luciferase reporter, SXR-coactivator interaction, quantitative real-time PCR analysis of CYP3A4 expression, CYP3A4 enzyme activity assays, and SXR knockdown.[12] SXR is activated by endogenous and exogenous chemicals including steroids, bile acids, and prescription drugs. SXR is highly expressed in the liver and intestine, where it regulates cytochrome P450 3A4 (CYP3A4), which in turn controls xenobiotic and endogenous steroid hormone metabolism. Acetyl Triethyl Citrate and Acetyl Tributyl Citrate activated SXR (i.e., increased SXR-mediated

transcription) at concentrations of 1 µM and 10 µM, and Acetyl Tributyl Citrate was more potent at both concentrations. Acetyl Triethyl Citrate (10 µM) and Acetyl Tributyl Citrate (10 µM) were both substantially more potent at this concentration than at the 1 µM. Acetyl Tributyl Citrate dose-dependently activated SXR, producing a greater effect than the antibiotic rifampicin, known to be a strong activator of SXR. Acetyl Tributyl Citrate did not stimulate the following nuclear receptors: human estrogen receptor α and β, human PPARγ, human thyroid hormone receptor β, human progesterone receptor 1, and human androgen receptor, or human glucocorticoid receptor-mediated transcription, as measured using these luciferase reporter assays.

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Final Report on the Safety Assessment of Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate[1]

Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate all function as plasticizers in cosmetics. Additionally, the Trihexyl and Trioctyl forms are described as skin-conditioning agents—emollients, although there are currently no reported uses of Acetyl Trihexyl Citrate or Acetyl Trioctyl Citrate. Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are used in nail products at concentrations up to 7%. Recognizing that there are no reported uses of Acetyl Trihexyl or Trioctyl Citrate, if they were to be used in the future, their concentration of use is expected to be no higher than that reported for Acetyl Triethyl and Tributyl Citrate. These ingredients were sufficiently similar in structure that safety test data on one were considered applicable to all. Approximately 99% of orally administered Acetyl Tributyl Citrate is excreted—intermediate metabolites include acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate, and acetyl dibutyl citrate. In acute, short-term, subchronic, and chronic feeding studies, these ingredients were relatively nontoxic. Differences from controls were either not statistically significant or not related to any organ toxicity. Ocular exposures produced moderate reactions that cleared by 48 hours after instillation. Dermal application was not toxic in rabbits. In a guinea pig maximization test, Acetyl Triethyl Citrate was a sensitizer whereas Acetyl Tributyl Citrate was not. Limited clinical testing of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate was negative for both skin irritation and sensitization. These clinical data were considered more relevant than the guinea pig maximization data, suggesting to the Cosmetic Ingredient Review Expert Panel that none of these ingredients would be a sensitizer. Physiologic effects noted with intravenous delivery of Acetyl Triethyl Citrate or Acetyl Tributyl Citrate include dose-related decreases in blood pressure and intestinal muscular spasms. These ingredients were not genotoxic in bacterial or mammalian test systems. No significant differences in tumor induction (lymphomas) were noted in rats fed Acetyl Tributyl Citrate for 2 year. Acetyl Tributyl Citrate was not a developmental or reproductive toxicant in studies in mice and rats. Based on all the available data, these ingredients were considered safe as used in cosmetics.

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Wilbur Johnson, Jr., Senior Scientific Analyst and Writer. Address correspondence to Wilbur Johnson, Jr., Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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INTRODUCTION

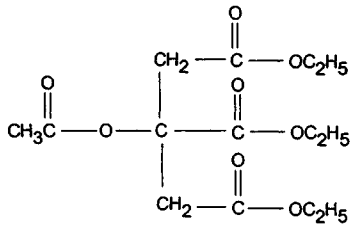
The safety of Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate in cosmetics is reviewed in this report. Reportedly, all four ingredients function as plasticizers in cosmetic products. Acetyl Trihexyl Citrate and Acetyl Trioctyl Citrate also function as skin-conditioning agents—emollients. Current frequency of use data submitted to the Food and Drug Administration (FDA) indicate that of the four ingredients being reviewed, only Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are being used in cosmetics.

CHEMISTRY

Chemical and Physical Properties

Properties of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are included in Tables 1 and 2, and properties of Acetyl Trihexyl Citrate are included in Table 2. Data from the published literature and from chemical suppliers are summarized in Tables 1 and 2, respectively.

Acetyl Triethyl Citrate (CAS No. 77-89-4) is the aliphatic ester that conforms to the following formula (Wenninger et al. 2000):



It is described as a clear oily, essentially odorless liquid that is soluble in common organic solvents (Nikitakis and McEwen 1990). Other names for this chemical are as follows: 2-(Acetyloxy)-1,2,3-Propanetricarboxylic Acid, Triethyl Ester; 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Triethyl Ester (Wenninger et al. 2000), and ATEC; Citric Acid, Acetyl Triethyl Ester; Tricarballylic Acid, Beta-Acetoxytributyl Ester; Triethyl Acetylcitrate; and Triethyl Citrate, Acetate (Registry of Toxic Effects of Chemical Substances [RTECS] 1998).

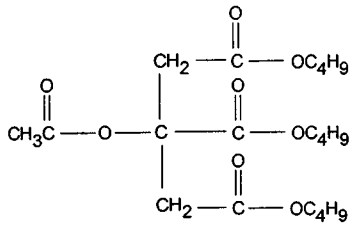
TABLE 1
Properties of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate

Properties	Acetyl Triethyl Citrate	Acetyl Tributyl Citrate	References
Form	Clear oily, essentially odorless liquid	Colorless to white, oily liquid with faint odor	Nikitakis and McEwen 1990; MSDS-OHS 1998
Molecular weight	318.31 Da	402.49 Da	HODOC 1998; MSDS-OHS 1998
Solubility	Soluble in common organic solvents; slightly soluble in H_2O	Soluble in alcohol and ether and insoluble in H_2O; slightly soluble in chloroform	Nikitakis and McEwen 1990; Lewis 1993; MSDS-OHS 1998; HODOC 1998
Viscosity	53.7 cps at 25°C	42.7 cps at 25°C	Lewis 1993
Density	1.135 g/cm^3 at 25°C	1.046 g/cm^2 at 25°C	Lewis 1993
Specific gravity	1.135 to 1.39 at 25°/25°C	1.048 at 25°C (specific gravity of H_2O = 1)	Nikitakis and McEwen 1990; MSDS-OHS 1998
Refractive index	1.4386 at 25°C	1.4408 at 25°C	Lewis 1993
Vapor pressure	—	1 mm Hg at 173°C	HSDB 1998
Vapor density	—	14.1 (vapor density of air = 1)	MSDS-OHS 1998
Volatility	—	<0.1%	MSDS-OHS 1998
Evaporation rate	—	<1 (evaporation rate of butyl acetate = 1)	MSDS-OHS 1998
Boiling point	131–132°C at 760 mm Hg	172–174°C at 1 mm Hg	HODOC 1998
Distillation range	131–132°C at 1 mm Hg	172–174°C at 1 mm Hg	Lewis 1993
Melting point	−45°C	−75°C	Beilstein 1998
Freezing point	—	−80°C	MSDS-OHS 1998
Pour point	−47°C	−60°C	Lewis 1993
Flash point	370°F (187°C)	400°F (204°C)	Lewis 1993
Combustibility	Combustible	Combustible	Lewis 1993

TABLE 2
Properties of Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, and Acetyl Trihexyl Citrate (Morflex, Inc. 1998; Soeler et al. 1998)

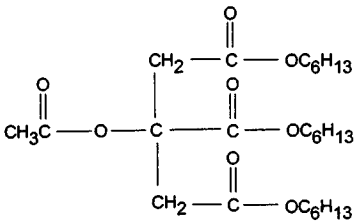
Properties	Acetyl Triethyl Citrate	Acetyl Tributyl Citrate	Acetyl Trihexyl Citrate
Appearance	Clear liquid	Clear liquid colorless	Clear liquid
Odor	Essentially odorless	Essentially odorless	Essentially odorless
Molecular weight	318.3 Da	402.5 Da	486 Da
Water solubility	0.72 g/100 ml at 25°C	<0.1 g/100 ml at 25°C; 0.002%	<0.1 g/100 ml at 25°C
Acetone solubility	∞	∞	∞
Ethanol solubility	∞	∞	∞
Heptane solubility	Insoluble	∞	∞
Isopropanol solubility	∞	∞	∞
Toluene solubility	∞	∞	∞
Viscosity	54 cps at 25°C	33 cps at 25°C	36 cps at 25°C
Specific gravity (specifications)	1.135–1.139 at 25/25°C	1.045–1.055 at 25/25°C	1.003–1.007 at 25/25°C
Refractive index	1.432–1.441 at 25°C/D	1.4410–1.4425 at 25°C/D (Specification); 1.4417 at 25°C	1.445–1.449 at 25°C/D
Vapor pressure	5.7×10^{-3} mm Hg at 20°C	5.2×10^{-2} mm Hg at 20°C	—
Pour point	−43°C	−59°C	−57°C
Flash point	188°C (closed cup)	204°C (closed cup)	240°C (closed cup)
Neutralization number (specifications)	0.2 mg KOH/g max	0.2 mg KOH/g max	0.2 mg KOH/g max

Acetyl Tributyl Citrate (CAS No. 77-90-7) is the aliphatic ester that conforms to the following formula (Wenninger et al. 2000):



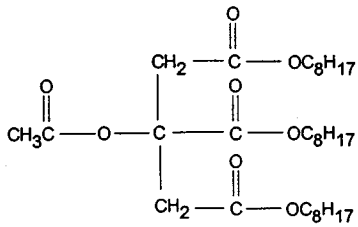
Other names for this chemical are as follows (Wenninger et al. 2000): 2-(Acetyloxy)-1,2,3-Propanetricarboxylic Acid, Tributyl Ester; 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Tributyl Ester (Wenninger et al. 2000), and Acetyl Butyl Citrate; Acetylcitric Acid, Tributyl Ester; Citric Acid, Tributyl Ester, Acetate; Tributyl Acetylcitrate; Tributyl *O*-acetylcitrate; Tributyl 2-(acetyloxy)-1,2,3-Propanetricarboxylate; and Tributyl Citrate Acetate (RTECS 1998).

Acetyl Trihexyl Citrate (CAS No. 24817-92-3) is the organic compound that conforms to the following formula (Wenninger et al. 2000):



Other names for this chemical include 2-(Acetyloxy)-1,2,3-Propanetricarboxylic Acid, Trihexyl Ester and 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Trihexyl Ester (Wenninger et al. 2000).

Acetyl Trioctyl Citrate (CAS No. 144-15-0) is the aliphatic ester that conforms to the following formula (Wenninger et al. 2000):



Other names for this chemical are as follows: 2-(Acetyloxy)-1,2,3-Propanetricarboxylic Acid, Tris(2-Ethylhexyl) Ester; 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Tris(2-Ethylhexyl) Ester (Wenninger et al. 2000); and Tris(2-Ethylhexyl) 2-(Acetyloxy)propane-1,2,3-Tricarboxylate (Chemical Identification [ChemID®] 1998).

Methods of Production

Acetyl Triethyl Citrate is produced via the reaction of citric acid triethyl ester with acetic acid anhydride (80°C; H_2SO_4 catalyst) (Beilstein 1998) and Acetyl Tributyl Citrate is produced via the reaction of tri-n-butyl citrate with acetic anhydride (acetylation) (Hazardous Substances Databank [HSDB] 1998). According to another source, Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are produced via the esterification and acetylation of citric acid (Lewis 1993).

Analytical Methods

Acetyl Triethyl Citrate has been analyzed by infrared spectroscopy and nuclear magnetic resonance (NMR) spectroscopy (CRC Handbook of Data on Organic Compounds [HODOC] 1998), and mass spectroscopy (Pfannhauser, Eberhardt, and Woidich 1982). Positive identification of Acetyl Triethyl Citrate is defined as a close match to a standard infrared (IR) spectrum with no indication of foreign materials (Nikitakis and McEwen 1990). It has also been analyzed by thin-layer chromatography (Thoma and Heckenmueller 1986), gas chromatography (Gutierrez-Rocca and McGinity 1994), and high-performance liquid chromatography (Bodmeier and Paeratakul 1997).

Acetyl Tributyl Citrate has been analyzed by IR spectroscopy (Heath and Reilly 1981; HODOC 1998), NMR spectroscopy, and mass spectroscopy (HODOC 1998). It has also been analyzed by gas chromatography, thin-layer chromatography (Heath and Reilly 1981; Cosmetic, Toiletry, and Fragrance Association [CTFA] 1998a; Gutierrez-Rocca and McGinity 1994), and high-performance liquid chromatography (Bodmeier and Paeratakul 1997).

Impurities

Acetyl Triethyl Citrate

The composition of Acetyl Triethyl Citrate is defined as follows: $C_{14}H_{22}O_8$, empirical formula for Acetyl Triethyl Citrate (99% minimum); acidity, as citric acid (0.02% maximum), and moisture (0.3% maximum) (Nikitakis and McEwen 1990). Volatiles account for 1.3% of the composition of Acetyl Triethyl Citrate (Morflex, Inc. 1998).

The chemical specifications for Acetyl Triethyl Citrate are as follows: ester content (99% minimum), identification, IR (meets test), color, American Public Health Association (APHA) (50 maximum), water (0.3% maximum), and heavy metals (10 ppm maximum) (Morflex, Inc. 1998). Specifications for the specific gravity and refractive index are included in Table 2.

Acetyl Tributyl Citrate

The purity of Acetyl Tributyl Citrate is >99% (CTFA 1998a), and, reportedly, volatiles account for 0.2% of its composition (Morflex, Inc. 1998). According to another source, the components of Acetyl Tributyl Citrate are as follows: Acetyl Tributyl Citrate (99.4%); ash (0%); moisture (0.14%); acidity, as citric acid, before heating (0.003%); acidity, as citric acid, after

heating (0.027%); heavy metals (3 ppm); and butyl acetate (trace) (Soeler et al. 1998).

In an analysis reported by Castle et al. (1988), using NMR spectroscopy, commercial Acetyl Tributyl Citrate accounted for 97% of the sample. Furthermore, no impurities were detectable during gas chromatography analysis. Small amounts of tributyl citrate and tributylpropene 1,2,3-tricarboxylate impurities, detected by gas chromatography–mass spectrometry, resulted from the production of [$^2H_4$] Acetyl Tributyl Citrate (deuterated compound). Synthesis was based on initial deuterium exchange between ^2H_2O and citric acid during heating under basic conditions. The deuterated citric acid was esterified with n-butanol and acetylated to produce [$^2H_4$] Acetyl Tributyl Citrate, accompanied by material that was incompletely deuterated. The [$^2H_4$] Acetyl Tributyl Citrate produced (1.8 g) was 79% chemically pure (13.5% yield from citric acid) (Castle et al. 1988).

The chemical specifications for Acetyl Tributyl Citrate are as follows: ester content (99% minimum); identification, IR (meets test); color, APHA (30 maximum); water (0.25% maximum); and heavy metals (10 ppm maximum) (Morflex, Inc. 1998). Specifications for the specific gravity and refractive index are included in Table 2.

According to another source, Acetyl Tributyl Citrate is supplied according to the following purity requirements: Characteristics (clear, oily liquid free of suspended solids or foreign matter; essentially odorless), assay (gas chromatography) (>99.0%), acidity (as citric acid) (0.02%), color (30 APHA), turbidity (5 NTU), specific gravity (d) 25°/25°C (1.045 to 1.055), refractive index (n) 25°C (1.441 to 1.4425), water (0.25%), and heavy metals (as Pb) (10 mg/kg) (CTFA 1999b).

Acetyl Trihexyl Citrate

Volatiles account for 1.4% of the composition of Acetyl Trihexyl Citrate (Morflex, Inc. 1998). Chemical specifications for this ingredient are as follows: ester content (99% minimum); identification, IR (meets test); color, APHA (100 maximum); water (0.15% maximum); and heavy metals (5 ppm maximum) (Morflex, Inc. 1998). Specifications for the specific gravity and refractive index are included in Table 2.

Reactivity

Acetyl Tributyl Citrate is stable at normal temperatures and pressure, and, also, will not polymerize. Reportedly, Acetyl Tributyl Citrate or Acetyl Triethyl Citrate in the presence of strong oxidizers is considered a fire and explosion hazard. The Material Safety Data Sheets–Occupational Health and Safety (MSDS-OHS) states that the potential for an explosive reaction also exists for either ingredient in the presence of nitrates (MSDS-OHS 1998).

The saponification of Acetyl Tributyl Citrate to 1-butanol has been demonstrated (Heath and Reilly 1981). According to another study, citric acid is the theoretical hydrolysis product of Acetyl Tributyl Citrate (Hollingsworth 1975).

USE

Purpose in Cosmetics

Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate function as plasticizers in cosmetic products. Acetyl Trihexyl Citrate and Acetyl Trioctyl Citrate also function as skin-conditioning agents—emollients (Wenninger et al. 2000).

Scope and Extent of Use in Cosmetics

Frequency of use data submitted to FDA in 1998 are presented in Table 3. Acetyl Triethyl Citrate and Acetyl Tributyl Citrate were used in 9 and 27 cosmetic products, respectively. No uses were reported for Acetyl Trihexyl Citrate or Acetyl Trioctyl Citrate (FDA 1998).

Concentration of use values are no longer reported to FDA by the cosmetics industry (FDA 1992). However, concentration of use data received from CTFA are included in Table 3 (CTFA 1999a).

Cosmetic products containing Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are applied to the hair, nails, and eye area, and could come in contact with ocular and nasal mucosae. These products could be used on a daily basis and potentially can be applied frequently over a period of several years.

The use of these ingredients in cosmetics is not restricted in Japan (Japan Ministry of Health, Labor, and Welfare 2000). None of these ingredients is restricted in any way under the rules governing cosmetic products in the European Union (European Commission 2002).

Noncosmetic Use

Acetyl Triethyl Citrate is used as a plasticizer for cellulosics, particularly ethyl cellulose; Acetyl Tributyl Citrate is used as a plasticizer for vinyls, adhesives, and coatings; and Acetyl Trihexyl Citrate is used as a plasticizer in polymeric medical articles (Lewis 1993).

Acetyl Triethyl Citrate and Acetyl Tributyl Citrate have been approved for use as components of adhesives that can be safely used as components of articles intended for use in packaging, transporting, or holding food (21CFR175.105). Both ingredients also have been approved for use as components of resinous and polymeric coatings (coatings for polyolefin films included) that can be safely used as the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (21CFR175.300; 21CFR175.320). Acetyl Tributyl Citrate has been approved by the FDA for use as a synthetic flavoring substance in food (21Code of Federal Regulations [CFR] 172.515).

Other indirect food additive uses of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate that have been approved by FDA include components of surface lubricants used in the manufacture of metallic articles that contact food (21CFR178.3910) and plasticizers that migrate from food-packaging material

TABLE 3
Product formulation data on Acetyl Triethyl Citrate and Acetyl Tributyl Citrate

Product category	Total in category (FDA 1998)	Total with ingredient (FDA 1998)	Concentration of use (CTFA 1999a)
	Acetyl Triethyl Citrate		
Other hair preparations	276	1	
Manicuring preparations—basecoats and undercoats	48	1	4%
Nail polish and enamel	80	6	4%–7%
Other manicuring preparations	61	1	
1998 totals/ranges for Acetyl Triethyl Citrate		**9**	**4%–7%**
	Acetyl Tributyl Citrate		
Eyebrow pencil	99	—	0.8%
Eyeliner	514	2	3%
Mascara	187	—	1%
Other eye makeup preparations	120	1	
Other hair preparations	276	1	
Manicuring preparations—basecoats and undercoats	48	4	4%–6%
Nail polish and enamel	80	15	0.8%–7%
Nail polish and enamel removers	34	1	
Other manicuring preparations	61	3	
Paste masks (mud packs)	269	—	0.7%
1998 totals/ranges for Acetyl Tributyl Citrate		**27**	**0.7%–7%**

(21CFR181.27). Good manufacturing practice for food packaging materials includes the restriction that the quantity of any substance that becomes a component of food as a result of use in food-packaging material shall not be intended to accomplish any physical or technical effect in the food itself and shall be reduced to the least amount reasonably possible (21CFR181.22).

BIOLOGICAL PROPERTIES

Absorption, Metabolism, and Excretion

Acetyl Tributyl Citrate

The metabolism of Acetyl Tributyl Citrate was evaluated using groups of male rats (number of animals, weights, and strain not stated). Each animal received a single oral dose of ^{14}C-Acetyl Tributyl Citrate (dose not stated). At 48 hours post dosing, approximately 99% of the administered dose had been excreted either in the urine (59% to 70%), feces (25% to 36%), or in the expired air (2%). Only 0.36% to 1.26% of the dose remained in the tissues or carcass. Both the absorption and metabolism of ^{14}C-Acetyl Tributyl Citrate proceeded rapidly, and the following metabolites were identified: acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate, and acetyl dibutyl citrate. The data on metabolites were not quantified (CTFA 1998a).

Acetyl Trihexyl Citrate

Fouda (1982) hydrolyzed Acetyl Trihexyl Citrate during incubation with rat serum, liver, or intestinal enzyme preparations. With rat serum, at a concentration of 50 nmol/ml of serum, the half-life for hydrolysis of Acetyl Trihexyl Citrate was 0.02 hour. Hexanol was a product of this process. In another experiment, the preincubation of rat serum with hexanol (50 nmol/ml) for 30 minutes had no effect on the rate of hydrolysis of Acetyl Trihexyl Citrate.

The hydrolysis of Acetyl Trihexyl Citrate during incubation with rat intestinal cytosolic fraction was also evaluated. Compared to the preceding experiment (incubation with rat serum), the hydrolysis of Acetyl Trihexyl Citrate by rat intestinal enzymes proceeded at a slower rate.

In another experiment, Acetyl Trihexyl Citrate was incubated with rat liver cytosolic fraction. At test concentrations of 50 and 1000 nmol/ml, the half-life for ester hydrolysis was 6 and 11 hours, respectively, and the half-life for hexanol (hydrolysis product) input was 0.4 and 5.6 hours, respectively. Thus, ester hydrolysis rates and hexanol input were concentration dependent. Intermediate products were also identified. Acetyl diethyl citrate was formed after 1 and 24 hours of incubation of rat liver cytosolic fraction with 1000 nmols Acetyl Triethyl Citrate/ml. Dihexyl citrate was detected only after 24 hours of incubation (Fouda 1982).

Percutaneous Absorption

Data on the percutaneous absorption of Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, or Acetyl Trioctyl Citrate were not found in the published literature.

TOXICOLOGY

Acute Oral Toxicity

Acetyl Triethyl Citrate

The acute oral toxicity of Acetyl Triethyl Citrate was evaluated using 63 rats (species and weights not stated). The number of animals tested per dose group (total of eight groups) ranged from 1 to 20, and doses ranged from 5 to 15 cc/kg. The following signs were noted after dosing: weakness, depression, ataxia, hyperexcitability, unrest, urinary dribbling, and irregular and labored respiration. In the advanced stage of poisoning, convulsions were observed in some of the animals. Absorption of the test substance was described as rapid, with signs appearing within a few minutes of dosing. The LD_{50} for Acetyl Triethyl Citrate was approximately 7 cc/kg (Finklestein and Gold 1959).

The acute oral toxicity of Acetyl Triethyl Citrate was also evaluated using 22 cats (weights not stated). The number of animals tested per dose group (total of seven groups) ranged from two to four, and doses ranged from 1 to 9.55 cc/kg. The following signs were noted after dosing: nausea, vomiting, ataxia, weakness, muscle twitching, tremors, reflex hyperexcitability, lowering of body temperature, a gasping and shallow respiration, prostration, convulsions, respiratory failure, and death. The absorption of the test substance was described as rapid, with signs appearing within minutes of dosing. The LD_{50} for Acetyl Triethyl Citrate was approximately 7 cc/kg (same value reported for Acetyl Triethyl Citrate in rats). At gross examination, abnormalities in organs that could have accounted for the observed toxic effects were not found. At hematological examination (one cat dosed with 5 cc/kg and evaluated over a 2-month period), no effects on blood count, blood sugar, or blood nitrogen were noted (Finklestein and Gold 1959).

In another experiment, Acetyl Triethyl Citrate was administered by stomach tube to three cats (weights not stated) at doses of 6, 9, and 12 cc/kg, respectively. An electrocardiogram was performed on each animal, and blood pressure recordings were taken from the carotid artery. Results indicated that Acetyl Triethyl Citrate caused progressive lowering of the blood pressure, which led to the development of shock. Frequently, blood pressure was <100 mm Hg for 1 or 2 hours before death. Electrocardiogram results were progressive slowing of the heart rate from 200 beats per minute or greater (controls) to rates approaching 150 beats per minute. The effect of Acetyl Triethyl Citrate on neuromuscular conduction also was evaluated in three cats. The responses to stimulation of the phrenic and sciatic nerves indicated the absence of any material interference with neuromuscular transmission after dosing with Acetyl Triethyl Citrate (Finklestein and Gold 1959).

Acetyl Tributyl Citrate

The acute oral toxicity of Acetyl Tributyl Citrate was evaluated using five rats (strain and weights not stated). The test substance was administered at doses ranging from 10 to 30 cc/kg, and animals were observed for 3 weeks. Signs of systemic toxicity were not observed, and none of the animals died; however, transient sluggishness was reported. The LD_{50} was not achieved at doses up to 30 cc/kg (Gold, Modell, and Finkelstein 1998).

Mortality was not observed in groups of mice or rats (males and females) dosed intragastrically (25,000 mg/kg body weight) with undiluted Acetyl Tributyl Citrate (Larionov and Cherkasova 1998).

A single dose of Acetyl Tributyl Citrate (30 to 50 cc/kg) was administered by stomach tube to each of four fasted cats (weights not stated), and animals were observed for 2 months. Two additional cats served as controls. Signs of nausea were observed in test animals, and, within a few hours of dosing, diarrhea (oozing of oily material) was noted. The diarrhea subsided within 24 hours of dosing. The behavior and general appearance of animals indicated systemic toxicity. Two cats dosed with 50 cc/kg were used for hematological evaluations and no effects on the following blood parameters were found: blood cell counts, hemoglobin, sugar, nonprotein nitrogen, or creatinine. Results from urinalyses indicated no abnormalities in specific gravity, albumin, sugar, pH, or microscopic formed elements (Finklestein and Gold 1959).

Acute Dermal Toxicity

The acute dermal toxicity of Acetyl Trihexyl Citrate was evaluated using five male and five female albino rabbits. The animals were divided equally into two groups (intact and abraded test sites, respectively) and a single dose (2 g/kg) of undiluted test substance was applied topically to each animal. The mean body weight was 2.29 kg for one group of rabbits (intact sites) and 2.81 kg (abraded sites) for the other group. Test sites were covered with an occlusive dressing for 24 hours. The animals were killed at the end of a 14-day observation period. Acetyl Trihexyl Citrate induced neither clinical signs of systemic toxicity nor mortality during the observation period. At necropsy, no test substance–related gross changes were observed in any of the animals tested. Acetyl Trihexyl Citrate was not toxic when administered dermally to rabbits ($LD_{50} > 2$ g/kg). Results concerning the skin irritation potential of Acetyl Trihexyl Citrate in this study are included in "Skin Irritation" later in the text of this report (CTFA 1982a).

Acute Intraperitoneal Toxicity

The acute intraperitoneal (IP) toxicity of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate (each in 3% acacia) was evaluated using Swiss albino mice (number not stated; weights between 16 and 20 g). All deaths that resulted from dosing with Acetyl Triethyl Citrate occurred within the first hour after dosing ($LD_{50} = 1150 \pm 185$ mg/kg). Death was attributed to circulatory collapse and postictal depression (Meyers, Autian, and Guess 1964).

An acute IP LD_{50} of >4000 mg/kg was reported for Acetyl Tributyl Citrate and the cause of death was the same. All deaths did not occur within the first hour after dosing, and a 72-hour observation period was required (Meyers, Autian, and Guess 1964). Behavioral studies on Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are summarized below.

Following the IP administration of graded doses of Acetyl Triethyl Citrate (in 3% acacia) to Swiss albino mice (number and weights not stated), doses slightly greater than 400 mg/kg induced a very rapid loss of righting reflex (without loss of consciousness). The animals usually regained their posture within 15 minutes. A marked increase in respiratory rate and frequent clonic convulsions also were observed (Meyers, Autian, and Guess 1964). Rapid loss of righting reflex (short duration) also was observed in Wistar rats at IP doses of Acetyl Triethyl Citrate slightly greater than 400 mg/kg. The number of animals as well as animal weights were not indicated (Meyers, Autian, and Guess 1964).

In the same study (same dosing procedure), Acetyl Tributyl Citrate in 3% acacia failed to induce rapid loss of the righting reflex in mice, but did cause an increase in respiratory rate. Frequently, the increase in respiratory rate was accompanied by clonic convulsions. Writhing was also observed during the first 10 minutes post injection. Similar effects were observed in Wistar rats dosed intraperitoneally with Acetyl Tributyl Citrate (Meyers, Autian, and Guess 1964).

Mortality was not observed in groups of mice or rats (males and females) dosed intraperitoneally (10,000 mg/kg body weight) with undiluted Acetyl Tributyl Citrate (Larionov and Cherkasova 1998).

Acute Intravenous Toxicity

The stimulatory effects of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate noted in the preceding acute IP toxicity studies were confirmed in experiments in which each chemical was administered intravenously to rabbits (number and weights not stated). Doses of 100 mg/kg caused marked increases in motor activity and respiration (Meyers, Autian, and Guess 1964).

Short-Term Oral Toxicity

The short-term oral toxicity of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate was evaluated using rats and cats in a series of experiments (Finklestein and Gold 1959). Study results are summarized below.

The short-term oral toxicity of Acetyl Triethyl Citrate was evaluated using groups of 21-day-old rats (males and females; ~8/group) with an average weight of approximately 85 g. It is important to note that an epidemic of pneumonia within the rat colony during the experiment resulted in a number of deaths in control and test groups, thereby reducing the number to ~5 rats per group. Three groups were fed 0.5%, 1.0%, and 2.0% Acetyl Triethyl Citrate in the diet, respectively, for 6 weeks. In the early part of the experiment, the three diets (0.5%, 1.0%, and 2.0%) represented an approximate daily consumption of 1, 2, and 4 g/kg, respectively. A fourth group was fed a control diet. None of the three test concentrations of Acetyl Triethyl Citrate had any effect on growth and the test substance did not induce toxicity. Ingestion of Acetyl Triethyl Citrate also had no effect on red blood cell count, white blood cell count, or differential blood count. Complete blood counts were taken at the beginning of the experiment and 4 and 8 weeks later (Finklestein and Gold 1959).

Acetyl Tributyl Citrate was fed to groups of four 21-day-old rats (males and females; strain not stated) for 6 weeks. Two groups were fed test substance concentrations of 5% and 10%, respectively, for 6 weeks. A third group served as the control. No deleterious effects on growth were noted following ingestion of the 5% diet. Feeding of the 10% diet resulted in growth reduction, which could have resulted from the diarrhea (Finklestein and Gold 1959). In a second feeding experiment using groups of four rats (same dietary concentrations of Acetyl Tributyl Citrate), complete blood counts were taken at the beginning of the experiment and 4 and 8 weeks later. At the end of the 8-week feeding period, the two groups of four animals were killed and gross and microscopic examination of internal organs was performed. Results indicated no conspicuous differences in red, white, and differential blood counts between test and control animals. No gross abnormalities were found in the thoracic or abdominal organs. At microscopic examination, no significant differences were found in the tissues between control and test animals (Finklestein and Gold 1959).

In another experiment, Acetyl Tributyl Citrate was administered via stomach tube to five rats (weights not stated) at doses ranging from 10 to 30 cc/kg. The animals were then observed for changes in appearance and behavior over a period of 21 days. Leakage of the test substance from the rectum was noted shortly (exact time not stated) after dosing. Initially, the animals appeared somewhat sluggish, but recovered promptly. No signs suggestive of systemic toxicity were noted during the observation period (Finklestein and Gold 1959).

Acetyl Triethyl Citrate (0.55 cc/kg) was administered to six cats (weights not stated) daily over a period of 2 months. Weakness, ataxia, and depression were noted after the fourth or fifth dose; however, all animals survived the 2-month treatment period. Clear differences in the following results between test and control animals were not demonstrated: weight, blood cell counts, hemoglobin, blood sugar, blood nitrogen, and electrocardiogram. Abnormalities of the thoracic or abdominal organs were not found at gross examination (Finklestein and Gold 1959).

In another experiment, Acetyl Tributyl Citrate was administered daily (5 cc/kg via stomach tube) to two cats (weights not stated) over a period of 2 months. Two additional cats served as controls. No changes in appearance or behavior were reported for treated animals; however, body weight was reduced by approximately 30%. The reduction in body weight could have been related to diarrhea. No effects on urine, blood chemistry, or blood cell counts were observed (Finklestein and Gold 1959).

Short-Term Dermal Toxicity

The application of undiluted Acetyl Tributyl Citrate to the skin of guinea pigs (number of animals and test procedure not stated) did not induce pathological reactions. At the end of the experiment, application of the test substance in fractions of 1/10 and 1/20 of the maximum 2,500 and 500 mg/kg dose and 1/10 and 1/20 of the threshold and permitted dose of 12,500 mg/kg did not produce significant effects. However, the periodic application of Acetyl Tributyl Citrate (250 and 500 mg/kg doses) during the experiment caused loss of body weight, a decrease in cerebral perfusion pressure, and an increase in the liver weight coefficient. Details concerning the study results were not included (Larionov and Cherkasova 1998).

Short-Term Intraperitoneal Toxicity

The short-term IP toxicity of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate (each in 3% acacia) was evaluated using groups of 20 Swiss albino mice (weights = 16–20 g). Acetyl Triethyl Citrate (230 mg/kg) and Acetyl Tributyl Citrate (900 mg/kg) were injected intraperitoneally daily for 14 days. The control group was dosed with 3% acacia according to the same procedure. At the end of the study, two mice from each group were killed and tissues prepared for microscopic examination. Both chemicals reduced body weight gain (significant within 95% confidence limits at day 7). Reduction of body weight gain was more pronounced after injection of Acetyl Tributyl Citrate. Compared to controls, no significant differences were observed in the following parameters in mice dosed with Acetyl Triethyl Citrate: erythrocyte counts, leucocyte counts, clotting times, and hemoglobin concentration. However, Acetyl Tributyl Citrate caused a significant (95% confidence) decrease in the erythrocyte count and hemoglobin concentration. Pathological changes were not observed in the liver, lungs, or kidneys (Meyers, Autian, and Guess 1964).

The remaining 18 mice in each of the three groups were included in hexobarbital sleeping time experiments. No significant differences in sleeping time between test and control groups confirmed the assumption that doses of Acetyl Triethyl Citrate or Acetyl Tributyl Citrate over a period of 14 days did not impair liver function (Meyers, Autian, and Guess 1964).

Based on the blood effects induced by Acetyl Tributyl Citrate in this study, additional testing was done using albino rabbits. Acetyl Tributyl Citrate was injected into two rabbits (IP dose = 450 mg/kg) daily for 14 days and into two other rabbits (IP dose = 900 mg/kg) daily for 7 days. A decrease in red blood cell count (ranging from 0.5 to 2.5 million) and a corresponding decrease in hemoglobin concentration were noted in all animals. Bone marrow smears indicated no evidence of aplastic anemia (Meyers, Autian, and Guess 1964).

Subchronic Oral Toxicity

The subchronic oral toxicity of Acetyl Tributyl Citrate was evaluated using three groups of male and female rats (number of animals, weights, and strain not stated). The three groups were fed 100, 300, and 1000 mg/kg body weight, respectively, for 90 days. A fourth group served as the control. Isolated, statistically significant differences between test and control groups were noted. However, liver enlargement in rats (both sexes) of the 1000-mg/kg dose group and in male rats of the 300-mg/kg dose group was the only treatment-related change that was considered important. Liver enlargement was not accompanied by any evidence of hepatic damage (i.e., biochemical or histopathological changes), and was considered an adaptive response, rather than a treatment-related toxicological effect (CTFA 1998a). Furthermore, based on the results of an oral feeding study (CTFA 1998a) summarized in "Absorption, Metabolism, and Excretion," the liver enlargement was also considered a consequence of the increased metabolic load that resulted from the rapid absorption and metabolism of Acetyl Tributyl Citrate.

Chronic Oral Toxicity

Three groups of 1-month-old rats (Sherman strain, 20 rats/group) were fed diets containing 200, 2000, and 20,000 ppm Acetyl Tributyl Citrate, respectively, for 2 years. A fourth group of 40 rats was fed a control diet. Necropsy was performed on survivors of the 2-year study as well as animals that died spontaneously. Study results are summarized below (Soeler et al. 1998).

Compared to the control group, transient reduction of the growth rate was noted in all three test groups during weeks 5 to 15 of the study; however, the difference was not statistically significant. The difference in mortality between test and control groups also was not statistically significant. Twelve test animals and eight controls died spontaneously. Differences in behavior between test and control animals were not observed and the incidence of diarrhea in test animals was no greater than that noted for controls. At necropsy, inflammatory disease of the lungs was the most frequent finding. Pulmonary lesions ranged from bronchitis to severe suppurative and infectious necrotizing pneumonitis. Practically all rats (test and controls) had an appreciable amount of passive congestion of the viscera; however, it was assumed that these were agonal. The pathological findings between test and control groups were not statistically significant; the endocrine organs were free of abnormalities. Information on tumor incidence is included in "Carcinogenicity" later in the report. It was concluded that Acetyl Tributyl Citrate did not induce toxicity in the two-year study (Soeler et al. 1998).

Because transient depression of the growth rate was noted in all three test groups during weeks 5 to 15 of the preceding study, a second experiment was conducted using three additional groups of rats. Two groups of rats (10/group) were fed diets containing 200 and 2000 ppm Acetyl Tributyl Citrate, respectively, for 1 year, and 20 rats served as controls. Reduction of the growth rate was not observed in either of the two test groups. Additional study results are summarized below (Soeler et al. 1998).

In another experiment in the same study, two dogs (~6 months old) were fed a gelatin capsule containing Acetyl Tributyl Citrate

(140 mg) daily for 2 years. Venous blood was drawn periodically for hematological examination, and several urinalyses were also performed. The animals were killed at the end of the 2-year study and gross and microscopic examinations performed. No significant changes in the following hematological parameters were found during the study: hemoglobin, hematocrit, sedimentation rate, erythrocyte count, leucocyte count, and differential leucocyte count. Platelet counts were also normal. Platelet counts were not taken, but were studied using stained blood smears that were made for differential white blood cell count determinations. Urinalyses for protein and sugar were negative. At microscopic examination of the urine sediment, no abnormalities were found. Results of gross and microscopic examinations were unremarkable. It was concluded that Acetyl Tributyl Citrate did not induce toxicity in dogs in the 2-year study (Soeler et al. 1998).

In another chronic study, the oral toxicity of Acetyl Tributyl Citrate was evaluated using male and female mice and rats (numbers, strain, ages, and weights not stated). The induction of toxicity in animals (mice and rats) was achieved by introducing a milk solution of Acetyl Tributyl Citrate that was 1/10 and 1/20 of the acute reaction threshold (250 and 50 mg/kg, respectively). Group 1 animals received 250 mg/kg doses and group 2 animals received 50 mg/kg doses over a 1-year period. Group 3 animals served as controls. (During the ninth month of the study, the animals (rats and mice) were cross mated and a new generation of animals bred. The reproductive toxicity of Acetyl Tributyl Citrate was then evaluated. The results of this evaluation are summarized "Reproductive and Developmental Toxicity" later in this report.) The following dynamic factors of the animals were studied: behavior and body weight, CPP (cerebral perfusion pressure) and CDA (acronym not defined) activity, morphological composition of the blood, content of blood sulphydryl groups, catalytic activity, activity of cholinesterases, and blood peroxidases. At the conclusion of the experiment, secretory functions of the liver, and the weight coefficient of the organs and their pathomorphological changes were studied. Results of the 1-year chronic study are summarized below (Larionov and Cherkasova 1998).

Changes in the dynamic factors studied were reported for rats and mice in 250-mg/kg dose groups. However, toward the end of the study, practically none of the factors studied in test animals differed substantially from those of control animals. The researchers stated that these changes attest to the compensatory-adaptive reaction of rats to the effects of oral dosing with Acetyl Tributyl Citrate. Dosing with 50 mg/kg Acetyl Tributyl Citrate did not cause any changes in the parameters studied (Larionov and Cherkasova 1998).

Ocular Irritation

Acetyl Triethyl Citrate

The ocular irritation potential of Acetyl Triethyl Citrate was evaluated using three male albino rabbits. The test substance (0.1 ml) was instilled into the left conjunctival sac of each animal. Contralateral eyes served as controls. Reactions were scored at 20 miniutes and 3, 5, 24, 48, and 72 hours post instillation. Transient erythema (slight to moderate) of the palpebral conjunctivae was observed in two of the three rabbits. After 24 hours, the erythema was described as negligible (CTFA 1998b).

Acetyl Tributyl Citrate

The ocular irritation potential of Acetyl Tributyl Citrate was also evaluated according to the procedure in the preceding paragraph. Moderate erythema was observed in two of the three rabbits within 20 minutes post instillation. The erythema persisted to 3 hours post instillation and subsided in one of the rabbits after 5 hours. At 24 hours, the moderate erythema observed in one rabbit was slightly increased, whereas the other two rabbits were classified as negative for ocular irritation. All eyes were essentially negative at 48 and 72 hours post instillation (CTFA 1998b).

No inflammation was observed after a single drop of Acetyl Tributyl Citrate was instilled into the conjunctival sac of one rabbit (Larionov and Cherkasova 1998).

Acetyl Trihexyl Citrate

The ocular irritation potential of undiluted Acetyl Trihexyl Citrate was evaluated using six female albino rabbits. The test substance (0.1 ml) was instilled into the conjunctival sac of the left eye of each animal; eyes were not rinsed. Reactions were scored up to 7 days post instillation. Slight reddening of the palpebral conjunctivae was noted in four of six rabbits within $2^1/_3$ to $5^1/_2$ hours post instillation. In three of the rabbits with reactions, the slight reddening persisted through 24 hours, but had cleared by 48 hours. According to the investigators, a positive ocular irritation reaction was not observed in any of the rabbits tested. Furthermore, none of the following changes was observed: chemosis, discharge, iritis, or corneal opacity or denudation. Acetyl Triethyl Citrate was not an ocular irritant. All animals were alert and active throughout the study. Feed consumption and body weight gain were normal. At necropsy on day 7, no pathological changes were observed in any of the animals (CTFA 1982a).

Skin Irritation

Acetyl Triethyl Citrate

The skin irritation potential of Acetyl Triethyl Citrate was evaluated using three male albino rabbits. The test substance (1 cc/kg body weight) was inuncted onto intact abdominal skin that had been clipped free of hair. This procedure was repeated daily for 4 days. The animals were observed daily for up to 36 hours after the last application. Acetyl Triethyl Citrate did not induce skin irritation in any of the three rabbits tested (CTFA 1998b). In another experiment (same procedure), Acetyl Tributyl Citrate did not induce skin irritation in three additional rabbits (CTFA 1998b).

In another study, Acetyl Triethyl Citrate (1 cc/kg body weight) was inuncted onto intact abdominal skin of each of three male albino rabbits for a total of 18 applications (6 days/week). The animals were then observed over a period of 2 weeks. Body weights were normal throughout the study. Skin irritation was not observed in any of the animals (CTFA 1998b). In a second experiment (same procedure), Acetyl Tributyl Citrate did not induce skin irritation in three additional rabbits. Body weights also remained normal throughout the study (CTFA 1998b).

When the preceding experimental procedure was repeated using four male albino rabbits with abraded abdominal skin, neither Acetyl Triethyl Citrate nor Acetyl Tributyl Citrate induced skin irritation (CTFA 1998b).

Acetyl Trihexyl Citrate

Acetyl Trihexyl Citrate induced skin irritation in rabbits tested in the acute dermal toxicity study summarized is "Acute Dermal Toxicity." In the study, 10 albino rabbits were divided equally into two groups (intact and abraded test sites, respectively) and a single dose (2 g/kg) of undiluted test substance was applied topically to each animal. Sites were then covered with an occlusive dressing for 24 hours. No skin changes were apparent after removal of the occlusive dressing at 24 hours. However, by day 2, very slight erythema (score = 1) was noted in two animals (abraded sites). By day 3, the erythema had subsided completely in one animal, but persisted to the end of day 10 in the other. Apparently, the persistence of erythema to day 10 was not related to Acetyl Trihexyl Citrate application. Edema was not observed at abraded sites, and neither erythema nor edema was observed at intact test sites. Very slight to slight desquamation of the epidermis was observed in five rabbits (three abraded skin, two intact skin). Desquamation was first observed at abraded sites on day 5 or 6 and had cleared by day 10. At intact sites, desquamation was observed on day 6 or 7 only (CTFA 1982a).

In another experiment, the skin irritation potential of undiluted Acetyl Trihexyl Citrate was evaluated using six albino rabbits (three males, three females). Two occlusive patches, each containing 0.5 ml of test material, were applied to one abraded and one intact site per animal, respectively. The patches, secured with an occlusive dressing, remained in contact with the skin for 24 hours, and the animals were observed over a period of 7 days after patch removal. Erythema (very slight to barely perceptible, intact and abraded sites) was observed in one rabbit at 24, 48, and 72 hours post application and only at 48 hours in a second rabbit (intact and abraded site) and third rabbit (intact site only). Reactions had cleared by 96 hours in the first rabbit, and by 72 hours in the second and third rabbits. Edema was not observed in any of the three rabbits. Very slight to slight desquamation of the epidermis was observed at 6 to 7 of the 12 application sites (including 2 intact and 2 abraded sites in which erythema had not been observed previously). A primary irritation score of 0.17 was reported, indicating that Acetyl Trihexyl Citrate caused negligible skin irritation and was not a primary irritant. Throughout the study, all rabbits were alert and active and feed consumption and weight gain were normal. At necropsy at the end of the 7-day observation period, no pathological changes were found (CTFA 1982a).

Skin Irritation and Sensitization

Acetyl Triethyl Citrate and Acetyl Tributyl Citrate

The skin sensitization potential of Acetyl Triethyl Citrate was evaluated using 18 guinea pigs (10 test, 4 treated controls, and 4 untreated controls; strain not stated) according to a modification of the Magnusson and Kligman guinea pig maximization test. The six, 0.1-ml induction injections made in the shoulder region (2 × 4 cm) of each of the 10 test animals were defined as follows: two injections of 2.5% Acetyl Triethyl Citrate in 0.01% Dobs/Saline (0.01% dodecyl benzene sulfonate in physiological saline); two injections of 2.5% Acetyl Triethyl Citrate in 50% complete Freund's adjuvant (CFA); and two injections of 50% CFA in saline. At day 7 after the last injection, an induction patch (filter paper occluded with Blenderm and held in place with Poroplast) saturated with 100% Acetyl Triethyl Citrate was maintained in contact with the injection site for 48 hours. The challenge phase was initiated 14 days after application of the induction patch. An occlusive challenge patch containing 50% Acetyl Triethyl Citrate in absolute ethanol was applied to the flank. The challenge patch consisted of filter paper, saturated with the test solution, in a patch test cup. The cup was maintained in contact with the skin with Peroplast (wound around the trunk) for 24 hours. Reactions were scored at 24 and 48 hours post removal according to the following scale: 0 (no reaction) to +++ (intense erythema [deep pink] and edema). Reactions were considered positive only if they were + or greater and if skin irritation was not observed in control animals. The four treated controls consisted of four guinea pigs that were tested according to the preceding study protocol, with the exception that Acetyl Triethyl Citrate was omitted only from the intradermal and covered patch induction procedures. The untreated control group consisted of four previously untreated animals that were challenged with Acetyl Triethyl Citrate according to the procedure described earlier. Maximization test results are summarized below after the results for the preliminary dose range–finding study (Unilever Limited 1976).

In the preceding study, the 2.5% test concentration for intradermal injection was selected based on the results of an intradermal injection, preliminary irritation test using four male guinea pigs. Test concentrations ranged from 0.05% to 1.25% in 0.01% Dobs/Saline. The lowest concentration tested (0.05%) induced faint, pink erythema in two animals and pale, pink erythema in the remaining two. Faint, pink erythema was observed in all four animals (edema in one animal) injected with the 1.0% concentration, and the 1.25% concentration was classified as a slight irritant (individual reactions not included). The patch test concentrations for induction (100% Acetyl Triethyl Citrate) and challenge (50% Acetyl Triethyl Citrate) phases were

selected based on the results of a covered patch, preliminary irritation test using four additional male guinea pigs. Reactions were scored at 24 and 48 hours after patch removal. At 24 hours, the 50% concentration induced scattered, mild (faint pink) erythema in one animal and the 75% concentration induced the same reaction in three animals. Reactions were not observed at 48 hours. Acetyl Triethyl Citrate (25%) did not cause irritation at 24 or 48 hours. Maximization test results are summarized below (Unilever Limited 1976).

At 24 and 48 hours post removal, challenge reactions of ++ or +++ to 50% Acetyl Triethyl Citrate predominated in 9 (5 males, 4 females) of the 10 guinea pigs tested. Only one animal had a + reaction (24-hour reading). No reaction was observed in the remaining male guinea pig at 24 or 48 hours. Reactions also were not observed in treated or untreated control groups. The results of a second challenge to evaluate the potential cross-reactivity of Acetyl Triethyl Citrate with Triethyl Citrate or Acetyl Tributyl Citrate indicated that Acetyl Triethyl Citrate cross-reacts with Triethyl Citrate, but that there is very little cross-reactivity of Acetyl Triethyl Citrate with Acetyl Tributyl Citrate. It is important to note that all 10 guinea pigs had sensitization reactions to Acetyl Triethyl Citrate at the second challenge and that, compared to the first challenge, the reactions were comparable in severity. Similar results were reported for nine guinea pigs tested with Triethyl Citrate according to the same procedure. Acetyl Triethyl Citrate and Triethyl Citrate were classified as strong sensitizers and were not considered suitable for use in products that are applied to the skin or come in contact with the skin during normal use (Unilever Limited 1976). The skin irritation/sensitization potential of Acetyl Tributyl Citrate was also tested according to the same test procedures. Study results are summarized below.

In the preliminary irritation test, the lowest test concentration of intradermally injected Acetyl Tributyl Citrate (0.05%) induced faint, pink or pale, pink edema in two guinea pigs and faint pink erythema with edema in the remaining two. Acetyl Tributyl Citrate (1%) induced pale, pink erythema with edema in three guinea pigs and faint, pink erythema with edema in one, and 1.25% Acetyl Tributyl Citrate was classified as not very irritating to the skin (individual reactions not included). In the covered patch preliminary irritation test, 100% Acetyl Tributyl Citrate induced barely perceptible erythema in one of four guinea pigs at 24 and 48 hours after patch removal. Reactions were not observed at lower test concentrations. Maximization test results for Acetyl Tributyl Citrate are summarized below (Unilever Limited 1976).

Acetyl Tributyl Citrate (50%) induced barely perceptible erythema at 24 and 48 hours after patch removal (one male guinea pig), barely perceptible erythema at 24 hours and scattered, mild erythema at 48 hours (one female guinea pig), and barely perceptible erythema at 48 hours (one female guinea pig). Similar reactions were observed following a second challenge. Reactions were not observed in treated or untreated control groups. The results of a third challenge to evaluate the potential cross-reactivity of Acetyl Tributyl Citrate with Acetyl Triethyl Citrate or Triethtyl Citrate indicated that Acetyl Tributyl Citrate did not cross-react with Acetyl Triethyl Citrate or Triethyl Citrate. Acetyl Tributyl Citrate was classified as a nonsensitizer (Unilever Limited 1976).

Neurotoxicity

Acetyl Triethyl Citrate and Acetyl Tributyl Citrate (both in 3% acacia, applied to sciatic nerve) induced complete, reversible sciatic nerve block during electrical stimulation of the sciatic nerve–anterior tibialis muscle in white rats. Complete blockage of the contralateral reflex was also demonstrated (Meyers, Autian, and Guess 1964). These experiments were conducted to identify effects on neuromuscular transmission, because neurological effects were reported in behavioral studies on both chemicals (see "Acute Intraperitoneal Toxicity").

Three drops of a 5% suspension of Acetyl Triethyl Citrate in a 3% gum acacia medium were instilled into the conjunctival sac of the eye of a rabbit. Corneal reflex action was temporarily abolished (local anesthetic effect). Similar results were reported following the instillation of Acetyl Tributyl Citrate according to the same procedure; however, duration of the blockage was longer (Meyers, Autian, and Guess 1964).

Cytotoxicity

The in vitro cytotoxicity of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate in HeLa cell cultures (human cancer cell line) was evaluated using the metabolic inhibition test, supplemented by microscopy of cells after 24 hours of incubation (the MIT-24 test system) (Ekwall 1980). Suspension cultures (in cups) containing the test substance were sealed with liquid paraffin and plates incubated for 7 days. After 24 hours, cell viability was determined by microscopy. Two endpoints of cytoinhibition (total and partial inhibition) were estimated after 24 hours, based on the absence or scarcity of spindle-shaped cells, and, after 7 days, based on the different degrees of basic pH change of the phenol red included in the cell medium. Cultures with 100% round cells were considered totally inhibited. Cultures with fewer fusiform cells, compared to normal reference cultures, were considered partially inhibited. Study results are summarized below.

Minimal inhibitory concentrations of Acetyl Triethyl Citrate were as follows: 4.9 mg/ml (for total inhibition at 24 hours), 2.2 mg/ml (for partial inhibition at 24 hours), and 4.9 mg/ml (for total and partial inhibition at 7 days). The following values for minimal inhibitory concentration were reported for Acetyl Tributyl Citrate: 13 mg/ml (for total inhibition at 24 hours), 3.8 mg/ml (for partial inhibition at 24 hours), and 5.7 mg/ml (for total and partial inhibition at 7 days). Acetyl Triethyl Citrate and Acetyl Tributyl Citrate induced little toxicity in HeLa cell cultures (Ekwall, Nordenstein, and Albanus 1982).

In another study, the in vitro cytotoxicity of Acetyl Tributyl Citrate (in ethyl alcohol) was evaluated using human KB cells (human epidermoid carcinoma cells), monkey Vero cells

(monkey kidney cell line), and dog MDCK cells (dog kidney cell line). Cultures were exposed to the test substance for 72 hours. The ID_{50} (50% inhibitory dose to growth of cells) served as the index of toxicity. Compared to controls, a dose-dependent decrease in growth was noted for all three cell types. ID_{50} values were as follows: 44.7 ± 2.99 μg/ml (KB cells), 39.9 ± 2.02 μg/ml (Vero cells), and 42.1 ± 2.02 μg/ml (dog MDCK cells) (Mochida, Gomyoda, and Fujita 1996).

Cardiovascular Effects

The intravenous administration of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate to cats and rabbits induced a dose-related decrease in blood pressure. Both chemicals caused complete loss of blood pressure when administered at toxic doses. The results of experiments conducted on the isolated rabbit heart indicated that this effect on blood pressure was due to cardiac inhibition (Meyers, Autian, and Guess 1964). These experiments were conducted to investigate the suspicion of cardiovascular collapse in acute intraperitoneal toxicity experiments summarized earlier (see "Acute Intraperitoneal Toxicity").

Effects on Smooth Muscle

Acetyl Tributyl Citrate had a significant, characteristic effect on the isolated guinea pig ileum, characterized by rapid contractions and relaxations. The magnitude of the spasms induced was concentration dependent at test concentrations ranging from 1 to 13.3 μg/ml. Test concentrations were added to the tissue bath for 5 minutes at 8-minute intervals. Contractions were not reduced when Acetyl Tributyl Citrate (8 μg/ml) remained in the tissue bath for 30 minutes. The addition of tetrodotoxin, selective blocker of nerve-mediated responses, had no effect on the induction of spasms; however, contractions elicited by transmural electrical stimulation of the ileum were abolished in the presence of tetrodotoxin. Thus, the muscle spasms probably resulted from a direct effect of Acetyl Tributyl Citrate on smooth muscle, and were not induced via a neuronal mechanism (Hollingsworth 1975).

Acetyl Tributyl Citrate did not induce spasms in longitudinal smooth strips of human small intestine and colon at test concentrations of 1 to 13.3 μg/ml (Hollingsworth 1975).

GENOTOXICITY

Acetyl Tributyl Citrate

The mutagenicity of Acetyl Tributyl Citrate was evaluated using the Ames test (Ames, McCann, and Yamasaki 1975) and the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538. Acetyl Tributyl Citrate (29.71 mg/3 ml dimethylsulfoxide) solutions containing 9, 50, 99, and 495 μg were tested on all strains without metabolic activation. Nitrofluorene served as the positive control. If the number of induced revertants, compared to the spontaneous reversion rate of a particular strain, was less than twofold, then the response was classified as negative. Acetyl Tributyl Citrate was not mutagenic in any of the strains tested with or without metabolic activation. The positive control was mutagenic (Heath and Reilly 1981).

In another study, the mutagenicity of Acetyl Tributyl Citrate (in DMSO) was evaluated using the same *Salmonella typhimurium* strains indicated in the preceding study, TA98, TA 100, TA 1535, TA 1537, and TA 1538. Acetyl Tributyl Citrate was tested in the standard plate test at concentrations of 333 to 10,000 μg/plate with and without metabolic activation. Two different metabolic activation assays (rat liver S-9, hamster liver S-9) were used per strain tested. Acetyl Tributyl Citrate was not mutagenic to any strain tested with or without metabolic activation (Chemical Carcinogenesis Research Information System [CCRIS] 1998).

The mutagenicity of Acetyl Tributyl Citrate was evaluated using the L5178Y (TK+/TK−) mouse lymphoma suspension/plate assay. Acetyl Tributyl Citrate, in DMSO, was tested at concentrations of 10 to 230 μg/ml (without metabolic activation) and 200 to 480 μg/ml (with metabolic activation). The test substance was not mutagenic with or without metabolic activation (CCRIS 1998).

Acetyl Trihexyl Citrate

The mutagenicity of Acetyl Trihexyl Citrate was evaluated in the Ames test using *S. typhimurium* strains TA1537, TA98, and TA100. Acetyl Trihexyl Citrate was tested (without metabolic activation) at concentrations ranging from 0.02 to 10 mg/plate. The average number of colonies per plate in test cultures was compared with the average number of spontaneous revertant colonies per control plate. A positive response was defined as a threefold increase over the control value. Sodium nitrite, 9-aminoacridine, and 2-nitrofluorene served as positive controls. Acetyl Trihexyl Citrate did not induce significant mutagenic activity in any of the three bacterial strains tested without metabolic activation. The positive controls were mutagenic (CTFA 1982b).

The mutagenicity of Acetyl Trihexyl Citrate (without metabolic activation) was also evaluated in the Ames test using the following *S. typhimurium* strains: TA 1535, TA 1537, TA 98, and TA100. Assays were conducted using compound control, metabolic activation, and metabolic activation control plates. Mouse and rat liver S-9 fractions were used in two separate experiments, respectively. Metabolic activation plates were identical to metabolic activation control plates, except for the inclusion of NADP. Acetyl Trihexyl Citrate was tested at concentrations ranging from 0.02 to 2 mg/plate. A positive response in the presence of metabolic activation was defined as a dose-related, reproducible threefold increase in the average number of revertant colonies on activation plates, compared to their respective activation control plates. Positive controls were as follows: sodium nitrite, 9-aminoacridine, 2-nitrofluorene, nitrofurantoin, and 2-anthramine. Acetyl Trihexyl Citrate was not mutagenic to any of the four bacterial strains tested with metabolic activation

(rat or mouse liver S-9 fractions). The positive controls were mutagenic (CTFA 1982b).

The presence of mutagenic excretory products resulting from the oral administration of Acetyl Trihexyl Citrate was evaluated. The test substance was administered orally to two sets of groups of 14 male HaM/ICR mice (weights = 31–42 g) in doses of 50, 500, and 1000 mg/kg. The animals were maintained in metabolic cages for 18 hours post dosing. Positive controls were as follows: sodium nitrate, 9-aminoacridine, 2-nitrofluorene, and nitrofurantoin. The mutagenicity of pooled urine samples from each group was then assayed using the following *S. typhimurium* strains: TA1535, TA1537, TA98, and TA100. Compared to controls, Acetyl Trihexyl Citrate did not cause significant increases in the number of revertant colonies per plate. The positive controls were mutagenic (CTFA 1982b).

The in vitro mutagenicity of Acetyl Trihexyl Citrate was tested in the L5178Y/TK gene mutation assay. Three experimental trials were included. Acetyl Trihexyl Citrate was tested at concentrations ranging from 6 to 45 μg/ml (with and without metabolic activation) using suspensions of L5178Y/TK+/− mouse lymphoma cells with and without metabolic activation. Test and control cell cultures were plated with and without trifluorothymidine and incubated for 7 to 12 days. Mutation to the TK−/− or thymidine kinase–deficient state confers stable resistance to trifluorothymidine (irreversible inhibitor of thymidylate synthetase when phosphorylated). In the first experimental trial (without metabolic activation), Acetyl Trihexyl Citrate was not mutagenic. No conclusion on mutagenicity in the second trial (with metabolic activation) was reached due to the lack of sufficient toxicity. In the third trial (with metabolic activation), Acetyl Triethyl Citrate was not mutagenic. The positive controls (ethyl methanesulfonate and 3-methylcholanthrene) were mutagenic (CTFA 1982b).

Cytogenetic assays (in vivo and in vitro) were also used to evaluate the genotoxicity of Acetyl Trihexyl Citrate. In in vivo experiments, groups of five CD-1 mice (6 to 8 weeks old; weights = 30–40 g) received single oral doses of Acetyl Trihexyl Citrate (1000 mg/kg) in distilled water and were killed at 6, 12, or 24 hours post dosing. An additional five mice received daily doses of 500 mg/kg/day for 5 days and were killed 6 hours after administration of the last dose. All animals were dosed with colchicine 2 hours before they were killed. Bone marrow cellular suspensions were prepared, and 50 metaphase figures per mouse were examined for chromosome damage. The results of in vivo experiments indicated no evidence of test substance–induced chromosome breakage over that observed in control cellular suspensions (CTFA 1982b).

In in vitro experiments from the preceding study, cultured human lymphocytes from normal subjects were used to determine the potential of Acetyl Trihexyl Citrate to induce chromosome damage. The test substance was added to cultures at concentrations of 10, 100, and 1000 μg/ml of culture medium, respectively. Fifty metaphase figures from each culture were examined for structural aberrations. Additionally, the frequency of mitosis in each culture was determined using 100-cell samples. At test concentrations of 1000 μg/ml and below, no statistically significant differences in chromosome breakage were found between test cultures and concurrent or historical controls. Furthermore, the observed variation in mitotic frequency was within the range normally reported for the test system that was used (CTFA 1982b).

CARCINOGENICITY

In a 2-year, chronic oral toxicity study, three groups of 1-month-old rats (Sherman strain, 20 rats/group) were fed diets containing 200, 2000, and 20,000 ppm Acetyl Tributyl Citrate, respectively, for 2 years. A fourth group of 40 rats was fed a control diet. Necropsy was performed on survivors of the 2-year study as well as animals that died spontaneously. Lymphomas were observed in 3 of the 60 rats fed Acetyl Tributyl Citrate, whereas, 4 of the 40 control rats had lymphomas (Soeler et al. 1998).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Test results from a 2-year chronic oral toxicity study by Larionov and Cherkasova (1998) (see "Chronic Oral Toxicity") that relate to the reproductive and developmental toxicity of Acetyl Tributyl Citrate are summarized in this section. Group 1 mice and rats received oral doses of 250 mg/kg and group 2 mice and rats received oral doses of 50 mg/kg. During the ninth month of the 1-year study, animals of each group were cross-mated and a new generation of animals bred. The reproductive toxicity of Acetyl Tributyl Citrate was then evaluated. Acetyl Tributyl Citrate did not cause any significant effects on male gonads in mice or rats of either treatment group. Additional study results are summarized below (Larionov and Cherkasova 1998).

In group 1 rats and mice (250 mg/kg oral doses), the spermatogenesis index was equal to 2.92 (range = 2.83–3.01). A spermatogenic index of 3.38 (range = 2.38–4.38) was reported for control animals. Study results also indicated a difference in desquamatosed spermatogenic epithelium between group 1 animals (24.6; range = 22.2–26.6) and controls (11.25; range = 7.74–14.56). These values were based on measurements per 100 tubules (Larionov and Cherkasova 1998).

Early and late embryonic death served as indicators of embryotoxicity. These indicators were determined by calculating the numbers of yellow bodies, places of implantation, and the number of normal, resorptive, and deformed tissues. The length of newborns and the size and weight of the placenta were also determined. Doses of 250 mg/kg Acetyl Tributyl Citrate caused increases in body weight, size of progeny, and weight of the placenta. Experimental progeny weighed 1.4 g (range = 1.3–1.5 g) and were 24 mm (range = 23.2–24.8 mm) in length. Control progeny weighed 0.89 g (range = 0.80–0.98 g) and were 18 mm (range = 17.5–18.5 mm) in length (Larionov and Cherkasova 1998).

Differences in the fertility rate and the number of animals born per pregnant female (rats and mice) were not noted when test (mice and rats, both treatment groups) and control groups were compared. Additionally, no difference in physiological development of the progeny was noted between test and control groups of mice and rats (both treatment groups). For mice and rats dosed orally with Acetyl Tributyl Citrate, it was concluded that the test substance did not cause significant effects on male sexual cells, did not induce embryotoxicity, and did not affect the growth and development of the progeny (Larionov and Cherkasova 1998).

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

The skin irritation potential of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate was evaluated using 59 men and women (age range = 21–60 years), all of whom had no history of diabetes, psoriasis, or active dermatoses. Two of the original 61 subjects withdrew from the study for personal reasons. Occlusive patches (one per test substance) moistened with 0.4 ml of the test solution were applied to the upper arms of each subject on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. Each patch was removed at 24 hours post application. Induction reactions were scored prior to patch applications (second through ninth visits) and at the time of the tenth visit. Duplicate challenge applications of each test material were made after a 2-week nontreatment period. For each test substance, one challenge patch was applied to the original test site, and, another, to an adjacent site. Challenge reactions were scored at 48 and 96 hours post application. Both Acetyl Triethyl Citrate and Acetyl Tributyl Citrate were nonirritating to the skin, and reactions suggestive of contact sensitization were not observed during the study (Hill Top Research 1978).

SUMMARY

The safety of the following aliphatic esters in cosmetics is reviewed in this report: Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate. The available information on methods of production indicates that Acetyl Triethyl Citrate is produced via the reaction of citric acid triethyl ester with acetic anhydride and that Acetyl Tributyl Citrate is produced via the reaction of tri-n-butyl citrate with acetic anhydride. Collectively, the purity of Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, and Acetyl Trihexyl Citrate ranges from 97% to 99%, with impurities (such as heavy metals and volatiles) accounting for a minor proportion of the composition of each. In an impurities analysis of deuterated [$^2H_4$] Acetyl Tributyl Citrate, small amounts of tributyl citrate and tributylpropene 1,2,3-tricarboxylate were detected. It is important to note that, in this analysis, the production process yielded [$^2H_4$] Acetyl Tributyl Citrate that was only 79% pure.

The four ingredients included in this review all function as plasticizers in cosmetics. Acetyl Trihexyl Citrate and Acetyl Trioctyl Citrate also function as skin-conditioning agents—emollients. Frequency of use data submitted to FDA in 1998 indicated use of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate in 9 and 27 cosmetic products (mostly nail products), respectively. No uses were reported for the remaining two ingredients. Concentration of use data submitted to the CTFA in 1999 indicate that Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are used in nail products at concentrations up to 7% and that Acetyl Tributyl Citrate is used in eye area products at concentrations up to 3%.

The following metabolites were identified after oral administration of a single dose of Acetyl Tributyl Citrate to male rats: acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate, and acetyl dibutyl citrate. At 48 hours post dosing, approximately 99% of the administered dose had been excreted either in the urine, feces, or expired air. In an in vitro study, the hydrolysis of Acetyl Trihexyl Citrate to hexanol and acetyl diethyl citrate was demonstrated. Citric acid has been identified as the theoretical hydrolysis product of Acetyl Tributyl Citrate.

Percutaneous absorption data on the ingredients included in this review were not identified in the published literature.

The acute oral LD_{50} for Acetyl Triethyl Citrate in cats and rats was approximately 7 cc/kg (doses administered = 5–15 cc/kg). In another acute oral toxicity study, Acetyl Triethyl Citrate caused progressive lowering of blood pressure (resulting in shock) and a progressive decrease in the heart rate, but no effect on neuromuscular transmission.

In mice or rats, the acute oral LD_{50} for Acetyl Tributyl Citrate was greater than 25 g/kg. In another experiment, single oral doses of up to 30 cc/kg did not cause death in rats. The oral dosing of cats with single doses of Acetyl Tributyl Citrate (30 to 50 cc/kg) did not cause systemic toxicity or any hematological effects.

The acute dermal LD_{50} for Acetyl Trihexyl Citrate in albino rabbits was >2 g/kg. None of the animals died and there was no evidence of systemic toxicity. In a parenteral toxicity study using albino mice, the acute IP LD_{50} for Acetyl Triethyl Citrate and Acetyl Tributyl Citrate (both in 3% acacia) were 1150 mg/kg and >4000 mg/kg, respectively. Both ingredients (in 3% acacia) caused an increase in respiratory rate and clonic convulsions. Increased respiration was also noted in rabbits, following acute intravenous exposure to Acetyl Triethyl Citrate (100 mg/kg) and Acetyl Tributyl Citrate (100 mg/kg).

Acetyl Triethyl Citrate did not induce toxicity or have any effect on growth in groups of rats fed dietary concentrations of up to 2.0% for 6 weeks. Additionally, no effect on red or white blood cell count was noted. Similar results were reported for groups of rats fed 5% and 10% Acetyl Tributyl Citrate for 6 weeks. At necropsy and microscopic examination of internal organs, no test substance–related toxic effects were observed. In another experiment, oral doses of Acetyl Tributyl Citrate (10 to 30 cc/kg) did not induce systemic toxicity in rats.

None of the six cats dosed orally with Acetyl Triethyl Citrate daily (5 cc/day) for 2 months died. No differences in body weight, hematological test results, or electrocardiograms were found between test and control groups. Following oral administration of Acetyl Tributyl Citrate to two cats for 2 months (5 cc/kg/day), no differences in hematological test results or urine samples were observed between test and control groups. The reduction in body weight was attributed to diarrhea.

The application of pure Acetyl Tributyl Citrate to the skin of guinea pigs did not result in any pathological reactions. However, the periodic introduction of Acetyl Tributyl Citrate at doses of 250 and 500 mg/kg during the experiment caused what was described as "authenticated deviation in the increase of body weight, a decrease in cerebral perfusion pressure, and an increase in the weight coefficient of the liver."

The short-term (14 days) IP toxicity of Acetyl Triethyl Citrate (230 mg/kg/day) and Acetyl Tributyl Citrate (900 mg/kg/day) in 3% acacia was evaluated using groups of 20 Swiss albino mice. Compared to controls, Acetyl Triethyl Citrate did not cause any significant hematological effects. Acetyl Tributyl Citrate caused a significant decrease in the erythrocyte count and hemoglobin concentration. Microscopic changes were not observed in the liver, lungs, or kidneys. A decrease in erythrocyte count and hemoglobin concentration was also noted after IP administration of Acetyl Tributyl Citrate to four albino rabbits for 14 days. Two rabbits received 450 mg/kg/day and the remaining two rabbits received daily doses of 900 mg/kg daily. However, based on study of bone marrow smears, no indication of aplastic anemia was found.

Liver enlargement was noted in rats dosed with Acetyl Tributyl Citrate in a subchronic (90 days) oral toxicity study. Neither biochemical nor histopathological changes indicative of liver damage were observed. Thus, liver enlargement was considered an adaptive change, rather than an Acetyl Tributyl Citrate–induced toxicological effect. It is important to note that, based on the results of a metabolism study (oral feeding study) of Acetyl Tributyl Citrate, the liver enlargement observed in the subchronic study was considered a consequence of the increased metabolic load that resulted from the rapid absorption and metabolism of Acetyl Tributyl Citrate.

In a 2-year chronic oral toxicity study, inflammatory disease of the lungs (not statistically significant) was the most common finding in groups of rats fed diets containing up to 20,000 ppm Acetyl Tributyl Citrate. Effects on the endocrine system were not evident. In the same study, no significant hematological effects were observed in dogs fed a gelatin capsule containing Acetyl Tributyl Citrate (140 mg) daily for 2 years. Urinalyses for protein and sugar were negative and findings of both gross and microscopic examination were unremarkable.

In another chronic oral toxicity study, groups of mice and rats received 50 or 250 mg/kg doses of Acetyl Tributyl Citrate over a 1-year period. Compared to controls, substantial changes in certain dynamic factors evaluated (e.g., body weight, cerebral perfusion pressure, and hematological parameters) were noted early in the study in 250-mg/kg dose groups. However, toward the end of the study, practically none of the differences between test and control animals were considered substantial. Dosing with 50 mg/kg did not result in remarkable changes in any of the dynamic factors studied.

Acetyl Triethyl Citrate induced slight to moderate erythema in the eyes of two of three albino rabbits; reactions had cleared by 24 hours post instillation. Moderate erythema was observed in two of three rabbits tested with Acetyl Tributyl Citrate. Reactions persisted through 24 hours post instillation and were essentially negative at 48 and 72 hours post instillation. Acetyl Trihexyl Citrate induced slight erythema in the eyes of four of six albino rabbits; reactions had cleared by 48 hours post instillation.

Acetyl Triethyl Citrate did not induce skin irritation when inuncted (1 cc/kg body weight) onto intact abdominal skin of six rabbits daily for 4 days. Similar results were reported for two groups of three rabbits that received applications of Acetyl Triethyl Citrate and Acetyl Tributyl Citrate, respectively, and four albino rabbits that received applications of either test substance to abraded abdominal skin (same procedure) six days per week for 3 weeks.

Very slight erythema, but no edema, was observed in two of five albino rabbits 24 hours after a single dose of Acetyl Trihexyl Citrate (2 g/kg) was applied topically to abraded skin. Topical application was followed by application of an occlusive dressing for 24 hours. Very slight to slight desquamation was also noted in three of the five rabbits with abraded skin. Two of five rabbits (second group) tested with Acetyl Trihexyl Citrate at intact sites according to the same procedure had very slight to slight desquamation, but no erythema or edema. Similarly, Acetyl Trihexyl Citrate induced negligible skin irritation (not a primary irritant) in another study. The test substance (0.5 ml) was applied to an intact and abraded site on each of six albino rabbits, and sites were then covered with an occlusive dressing for 24 hours.

In the guinea pig maximization test, Acetyl Triethyl Citrate was classified as a strong sensitizer, whereas Acetyl Tributyl Citrate was classified as a nonsensitizer.

Acetyl Triethyl Citrate and Acetyl Tributyl Citrate (both in 3% acacia) induced complete, reversible sciatic nerve block during electrical stimulation of the sciatic nerve–anterior tibialis muscle in white rats. Complete blockage of the contralateral reflex was also demonstrated. Acetyl Triethyl Citrate or Acetyl Tributyl Citrate in 3% acacia also temporarily abolished corneal reflex action (local anesthetic effect), following instillation into the conjunctival sac of the eye of one rabbit.

A low level of cytotoxicity was induced by Acetyl Triethyl Citrate and Acetyl Tributyl Citrate in HeLa cell cultures (human cancer cell line).

Intravenous administration of Acetyl Triethyl Citrate or Acetyl Tributyl Citrate to cats and rabbits caused a dose-related decrease in blood pressure. Toxic doses resulted in the complete loss of blood pressure.

Acetyl Tributyl Citrate induced concentration-dependent (1 to 13.3 μg/ml) muscular spasms in the isolated guinea pig ileum. However, this effect was not noted when sections of human small intestine and colon were exposed to the same test concentrations.

Ames test results for Acetyl Tributyl Citrate in DMSO were negative (with or without metabolic activation) in the following *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538. Acetyl Tributyl Citrate also was not mutagenic with or without metabolic activation when tested in the L5178Y/TK+/− mouse lymphoma suspension/plate assay.

Acetyl Trihexyl Citrate had negative Ames test results in the following *S. typhimurium* strains (without metabolic activation): TA98, TA100, TA1535, and TA1537. It also was not mutagenic in the L5178Y/TK+/− mouse lymphoma assay with or without metabolic activation.

In an in vivo cytogenetic assay, Acetyl Trihexyl Citrate did not induce chromosome breakage in cellular suspensions from CD-1 mice. The results of an in vitro cytogenetic assay on Acetyl Trihexyl Citrate using cultured human lymphocytes indicated no statistically significant differences in chromosome breakage between test and concurrent or historical controls.

Groups of 20, 1-month-old Sherman rats were fed Acetyl Tributyl Citrate at concentrations up to 20 ppm in the diet for 2 years. Lymphomas were observed in three of the 60 test animals. However, similar neoplasms were observed in 4 of the 40 control rats.

Results concerning the reproductive and developmental toxicity of Acetyl Tributyl Citrate were included in a 2-year chronic oral toxicity study. In this study, groups of male and female mice and rats receiving oral doses of 50 or 250 mg/kg Acetyl Tributyl Citrate were mated during the ninth month. No significant, test substance–related effects on male gonads were noted in rats or mice. However, an authenticated difference in desquamatosed spermatogenic epithelium between 250-mg/kg dose groups (mice and rats) and controls was observed. It was also determined that both doses of Acetyl Tributyl Citrate did not induce embryotoxicity or affect the growth or development of the progeny.

Neither a test solution of Acetyl Triethyl Citrate nor Acetyl Tributyl Citrate induced skin irritation or sensitization in 59 men and women tested in a repeated insult patch test (occlusive patches).

DISCUSSION

Acetyl Triethyl Citrate was classified as a strong sensitizer in the guinea pig maximization test, whereas Acetyl Tributyl Citrate was classified as a nonsensitizer. Both ingredients were tested at a concentration of 50% during the challenge phase. After reviewing these data and considering that Acetyl Triethyl Citrate and Acetyl Tributyl Citrate are used mostly in nail products at concentrations up to 7%, the Panel concluded that is not likely that either of the esters in this safety assessment, as used in cosmetics, poses a risk for sensitization.

The Cosmetic Ingredient Review (CIR) Expert Panel was aware of the absence of toxicity data on Acetyl Trioctyl Citrate. However, it was determined that because of structural similarities between the aliphatic esters included in this review, the available toxicity data on Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, and Acetyl Trihexyl Citrate are sufficient for evaluating the safety of Acetyl Triocyl Citrate in cosmetics. Furthermore, after considering these data along with current use concentration data (on Acetyl Triethyl Citrate and Acetyl Tributyl Citrate) provided by the cosmetics industry, the Panel determined that the available data do not warrant any restrictions on the use of these ingredients in rinse-off or leave-on cosmetic products. The Panel recognized that there are no reported uses of Acetyl Trihexyl Citrate or Acetyl Trioctyl Citrate in cosmetics; were these ingredients to be used in the future, their concentration of use is expected to be similar to that reported for Acetyl Triethyl Citrate and Acetyl Tributyl Citrate.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Acetyl Triethyl Citrate, Acetyl Tributyl Citrate, Acetyl Trihexyl Citrate, and Acetyl Trioctyl Citrate are safe as used in cosmetics.

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2019 FDA VCRP Data

Acetyl Triethyl Citrate

05B - Hair Spray (aerosol fixatives)	10
05F - Shampoos (non-coloring)	1
08A - Basecoats and Undercoats	2
08E - Nail Polish and Enamel	9
Total	**22**

Acetyl Tributyl Citrate

03B - Eyeliner	1
03F - Mascara	2
03G - Other Eye Makeup Preparations	2
05B - Hair Spray (aerosol fixatives)	1
05I - Other Hair Preparations	2
07I - Other Makeup Preparations	2
08A - Basecoats and Undercoats	44
08C - Nail Creams and Lotions	6
08D - Nail Extenders	1
08E - Nail Polish and Enamel	343
08F - Nail Polish and Enamel Removers	1
08G - Other Manicuring Preparations	33
Total	**438**

Acetyl Triethylhexyl Citrate - No FDA Data

Acetyl Trihexyl Citrate - No FDA Data

1998 FDA VCRP Data

Acetyl Triethyl Citrate

05I - Other Hair Preparations	1
08A - Basecoats and Undercoats	1
08E - Nail Polish and Enamel	6
08G - Other Manicuring Preparations	1
Total	**9**

Acetyl Tributyl Citrate

03B - Eyeliner	2
03G - Other Eye Makeup Preparations	1
05I - Other Hair Preparations	1
08A - Basecoats and Undercoats	4
08E - Nail Polish and Enamel	15
08F - Nail Polish and Enamel Removers	1
08G - Other Manicuring Preparations	3
Total	**27**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: January 8, 2019

SUBJECT: Concentration of Use Information: Acetyl Trialkyl Citrates

Concentration of Use – Acetyl Trialkyl Citrates*

Acetyl Tributyl Citrate
Acetyl Triethyl Citrate
Acetyl Triethylhexyl Citrate
Acetyl Trihexyl Citrate

Ingredient	Product Category	Maximum Concentration of Use
Acetyl Tributyl Citrate	Eye shadows (3C)	7.5%
Acetyl Tributyl Citrate	Perfumes (4B)	0.0015%
Acetyl Tributyl Citrate	Hair sprays Aerosol (5B)	0.09%
Acetyl Tributyl Citrate	Foundations (7C)	5.8%
Acetyl Tributyl Citrate	Basecoats and undercoats (manicuring preparations) (8A)	6.6-7%
Acetyl Tributyl Citrate	Nail polish and enamel (8E)	7-7.9%
Acetyl Tributyl Citrate	Other manicuring preparations (8G)	6-8.9%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2018
Table prepared January 7, 2019