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Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, S. W.
Washington, D.C. 20460

ORIGINAL

Attn.: Section 8(e) Coordinator

Re: Substantial Risk Notification
Pursuant to TSCA Section 8(e)

Dear Sir:

We recently (May 6, 1994) submitted a Substantial Risk Notification for 1-Dodecanaminium, N-(3-chloro-2-hydroxypropyl)-N,N-dimethyl-chloride (which we identify by the trade names QUAB 342 and Lauryl QUAB) CASRN 41892-01-7.

In the submission, we mentioned that one of the attachments was a printout from the STN Chemical Abstracts Service Registry File. Apparently this was inadvertently not included, but it is attached now.

Sincerely,

John Lewinson

John Lewinson, Ph.D.
Manager, Product Regulatory Compliance

8EHQ-94-13022
SP001 C 05/31/94

89940000210

JL-94-198

cc: T. Grzymkowski
R. Marion, DCA
B. Santoro, DCRP

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65 Challenger Road Ridgefield Park NJ 07660 201-641-6100

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Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Lauryl-QUAB

Acute Toxicity
Testing the Acute Toxicity
after Single Oral Administration

R E P O R T
TRANSLATION

June 30, 1982

Summary

Lauryl-Quab was studied for acute toxicity after (single) oral administration in the rat.
The test substance, available as a liquid, was used as diluted aqueous solution (Aqua demin), or undiluted, with an increasing administration volume between 2.15 and 4.65 ml/kg.

The LD 50 values were for female rats 3.05 ml/kg (= 3066 mg/kg)
and for male rats 2.32 ml/kg (= 2332 mg/kg)

Intoxication was characterized by disturbances of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colourless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

ASTA WERKE 4800 Bielefeld 14
AKTIENGESELLSCHAFT
Toxikologisches Institut Degussa-Asta

Bericht Nr.: Ind.-TOX-79-81/82

DEGUSSA AG - US-IT - NR.
82 0086 DKT

BERICHT

über die toxikologische Prüfung von Lauryl-QUAB nach einmaliger oraler Gabe an der Ratte

Acute Oral Rat

30. Juni 1982

Versuchsbeginn	:	05.01.1982
Ende des tierexperimentellen Teiles	:	18.01.1982
Versuchsdurchführende Einrichtung	:	Toxikologisches Institut Degussa-Asta Artur-Ladebeck-Str. 128 - 152 D-4800 Bielefeld 14
Versuchsleiter	:	Dr. med. vet. H.-J. Zechel Fachtierarzt für Pharmakologie und Toxikologie
Pathologie und Laboratoriums-Diagnostik	:	--
Archivaufsicht	:	E. Fichtner (QS)
Berichtsdurchsicht	:	E. Fichtner (QS)

Zusammenfassung

Lauryl-QUAB wurde zur Bestimmung seiner akuten Toxizität oral an der Ratte verabfolgt. Das in flüssiger Form vorliegende Prüfprodukt kam in verdünnter wässriger Lösung (Aqua demin) sowie unverdünnt in steigendem Applikationsvolumen zwischen 2,15 und 4,65 ml/kg zur Anwendung.

Die erhobenen DL 50 - Werte betrugen für die weiblichen Tiere 3,05 ml/kg (≙ 3066 mg/kg) und für die männlichen Tiere 2,32 ml/kg (≙ 2332 mg/kg).

Im Vergiftungsbild standen Störungen des vegetativen Nervensystems und des Allgemeinbefindens (Zittern, Stelzengang, eingezogener sowie aufgeblähter Bauch, Speichelfluß, gesträubtes Fell, Durchfall, Abmagerung) im Vordergrund. Prämortal kam es teilweise zu Reflexausfall, Krämpfen, Mydriasis und Dyspnoe. Der Vergiftungsverlauf gestaltete sich akut bis protrahiert.

Makroskopisch-anatomisch wurden bei den verstorbenen Tieren Flüssigkeitsansammlungen, teils von klarem Aussehen, teils von roter oder gelber Farbe, in Magen und Darm festgestellt. Die Wandung dieser Organe wies Rötung auf. In einem Fall lag eine Perforation der Magenwand vor. Vereinzelt wurden Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Einleitung

Nachfolgend wird über die toxikologische Prüfung von Lauryl-QUAB berichtet.

Die Untersuchung erfolgte im Auftrage des Fachreferates für Industrielle Toxikologie der Degussa, Frankfurt/M. Sie wurde nach einmaliger oraler Gabe des Prüfproduktes an der Ratte durchgeführt.

Zweck der Studie war es, eine Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes vorzunehmen. Die erhobenen Befunde werden durch Angaben zur DL 50 ergänzt.

Lauryl-QUAB lag als farblose Flüssigkeit vor und kam tierexperimentell unverdünnt und verdünnt zur Anwendung.

Methodik

1. Versuchstiere

Spezies	:	Ratte
Stamm	:	Wistar (Bor: WISW)
Züchter	:	Winkelmann, Borchen
Geschlecht	:	♂ und ♀ Tiere
Gewicht	:	♂ 127 - 147 g; ♀ 127 - 141 g
Alter	:	♂ 57 - 62 Tg.; ♀ 57 - 58 Tg.
Eingewöhnungszeit vor Testbeginn	:	5 Tage
spezielle Angaben	:	siehe Versuchsdurchführung unter Pkt. 4

2. Versuchstierhaltung

Haltung	:	Makrolonkäfige Typ III
Tiere pro Käfig	:	1
Einstreu	:	Weichholzgranulat Typ 9 ALTROMIN®
Fütterung	:	Standardisiertes Versuchstierfutter ALTROMIN®
Tränkung	:	Wasser ad libitum
Raumtemperatur	:	$21^{o} \pm 2^{o}$ C
Relative Luftfeuchtigkeit	:	50 - 60 %
Raumbeleuchtung	:	12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. Dunkelheit
Raumhygiene	:	Labor- und Käfigreinigung mittels handel üblicher Antiseptika, Instrumente- und Geräte-Desinfektion mittels Autoklav und Heißluftsterilisator

3. Prüfsubstanz (spezielle Angaben)

Chemische Bezeichnung	:	Lauryl-QUAB
Chargen-Nr.	:	
Form und Aussehen	:	klare Flüssigkeit
Gehalt/Reinheit	:	27,87 %
Löslichkeit	:	Herstellg. verdünnter Lösungen in Aqua demi
Dichte	:	1,007 g/ml
pH-Wert	:	7,1

Verdünnungsfaktoren 1,47 und 2,1
Die entspr. Konzentrationen betrugen 685 mg/kg und 468 mg/kg b
Annahme einer Ausgangskonz. von 1007 mg/ml.

4. Versuchsdurchführung

Futterentzug	:	**16 Std. vor Versuchsbeginn**
Substanz-Applikation	:	oral mittels Schlundsonde
Dosenabstand	:	**in dezimalgeometrischer Folge**
Faktor	:	1,47 bzw. 2,15
Beobachtungszeit	:	14 Tage
Injektions-/ Applikationsvolumen	:	2,15 - 4,64 ml/kg; die Substanz kam verdünn und unverdünnt zur Anwendung
Tiere pro Dosis	:	5
Anzahl Dosierungs-gruppen	:	4 bei ♂ und 3 bei ♀
Anzahl Tiere zur Berechnung eines DL 50 - Wertes	:	20 ♂ und 15 ♀
Gesamtzahl der im Versuch verwendeten Tiere	:	35

5. Versuchszweck

Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes; [3]

Berechnung der DL 50 (Probitanalyse) [1,2,4,]

Ergebnisse

Lauryl-QUAB wurde zur Ermittlung seiner akuten Toxizität oral an der Ratte verabfolgt. Das Prüfprodukt lag in flüssiger Form vor und kam verdünnt mit Aqua demin und unverdünnt in steigenden Applikationsvolumina zwischen 2,15 und 4,64 ml/kg zur Anwendung. Bei der Berechnung der Dosen (mg/kg) wurde eine Dichte von 1,007 g/ml zugrunde gelegt. Der Dosenbereich erstreckte sich zwischen 1007 und 4672 mg/kg.

Das Vergiftungsbild war vorwiegend durch Symptome wie Zittern, Stelzengang, schleppender Gang, Speichelfluß, gesträubtes Fell, Durchfall und eingezogener bzw. aufgeblähter Bauch geprägt, was auf eine Störung des vegetativen Nervensystems hinweist.Darüber hinaus zeigten die männlichen Tiere während der 1. Beobachtungswoche nur eine mäßige Gewichtsentwicklung. Bei einigen Tieren war eine Zyanose zu beobachten. Prämortal kam es bei den weiblichen Tieren der höchsten Dosierungsgruppe zu vollständigem Reflexausfall, starken klonischen Krämpfen, Mydriasis und Dyspnoe.

Der Vergiftungsverlauf ist als akut bis protrahiert zu bezeichnen. Erste klinische Symptome traten 20 Minuten nach Substanzgabe auf und währten bei den Überlebenden maximal bis zu 1 Woche. Letalität stellte sich überwiegend während der ersten beiden Tage p. appl. ein, weitere Todesfälle traten noch in den folgenden 3 Tagen auf.

Bei der Sektion der verendeten Tiere kam es in Abhängigkeit von der verabfolgten Dosis zu einer zunehmenden Rötung von Magen- und Darmwand. Diese Organe wiesen, bedingt durch Flüssigkeitsansammlungen in ihrem Lumen (teils klar, teils von roter und gelber Farbe) eine pralle Füllung auf. Bei einem männlichen Tier wurde eine Perforation der Magenwand diagnostiziert. Dieser Befund steht nicht im Zusammenhang mit einem unphysiologisch hohen Applikationsvolumen; er muß als eine Folge der Substanzeinwirkung angesehen werden. In einzelnen Fällen wurden auch Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Die DL 50 - Werte sind in Tabelle 1a und 1b wiedergegeben.
Der zahlenmäßige Unterschied zwischen den DL 50 - Werten der weiblichen und männlichen Tiere ist statistisch nicht signifikant. Er trägt zufälligen Charakter.

In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

DL 50 - Werte von Lauryl-QUAB

Spezies : Ratte
Applikation : oral

Tabelle 1a

	DL 50 [ml/kg]
♀	2,32 (1,58 - 3,39)
♂	3,05 (2,25 - 4,13)

Tabelle 1b

	DL 50 [mg/kg]
♀	2372 (1593 - 3414)
♂	3066 (2261 - 4159)

Akute Toxizität DL 50 - Bestimmung
(Absterbedaten)

tanz/Präparat : Lauryl-QUAB
likationsart : oral

Spezies : Ratte
Stamm : Wistar (Bor: WI

Dosis mg/kg]	Geschlecht	Todes-rate		Überlebenszeit																			
				Std. nach Applikation						Tage nach Applikation													
		x/n	%	0,5	1	2	4	8	24	2	3	4	5	6	7	8	9	10	11	12	13	14
1473	♂	0/5	0																			
2165		1/5	20										1									
3182		2/5	40						1		1											
4672		5/5	100						2	3												
1007	♀	0/5	0																			
2165		2/5	40						2													
4672		5/5	100				3		1			1										

Literatur

1. BLISS, C.J.,The Statistics of Bioassay, Academic Press, New York, 1962

2. FINNEY, D.J., Probit Analysis, 2nd Ed., Cambridge Univ. Press, Cambridge 1952

3. HUNTER, W.J., Lingk, W. and Recht, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem. 62, 864 - 873, 1979

4. WEBER, E., Grundriß d. biol. Statistik, G. Fischer-Verlag, Stuttgart, 7. Auflage, 1972

Bielefeld, den 30.06.1982

Dr. med. vet. H.-J. Zechel
Fachtierarzt für Pharmakologie
und Toxikologie

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid
(Lauryl QUAB)
QUAB 342

Acute Toxicity after a Single Dermal Administration in Rabbits

R E P O R T
TRANSLATION

July 29, 1986

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

Summary:

Quab 342 was applied once * to the dorsal skin area of rabbits to determine its acute toxicity. The test substance, available as a colourless liquid, was used undiluted.

Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discolouration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart, and small intestine were oberserved.

The LD_{50} values were

probit analysis:
for male and female animals 2523 mg/kg (2.46 ml/kg)

graphical estimation:

for male animals	3072 mg/kg	(3.0 ml/kg)
for female animals	1946 mg/kg	(1.9 ml/kg)

* remark of the translater: (occlusive for 24 hours)

DEGUSSA AG - US-IT - NR.
86 0158 DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid (Lauryl QUAB) QUAB 342

Akute Toxizität

Prüfung der akuten Toxizität nach einmaliger dermaler Applikation am Kaninchen

B E R I C H T

Acute Dermal Rabbit

29. Juli 1986

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Der Bericht umfaßt 12 Seiten.

1. ALLGEMEINES

Auftraggeber	: DEGUSSA AG / ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Prüfeinrichtung	: ASTA-WERKE AG Chemische Fabrik Toxikologie Degussa-Asta Artur-Ladebeck-Str. 128-152 D-4800 Bielefeld 14
Prüfsubstanz	: QUAB 342
Art der Prüfung	: Prüfung der akuten Toxizität nach einmaliger dermaler Gabe am Kaninchen.
Prüfrichtlinie/-n	: OECD Guideline Nr. 402 (1)
Berichts-Nummer	: Ind.-TOX-508-85/86
Zeitplan	: Beginn: 23.10.84 Ende : 18.12.84
Prüfleiter	: Dr. rer. nat. W. Mayr
Durchführung der Studie	: R. Iselt
Berichtsdatum	: 29.07.1986

2. ZUSAMMENFASSUNG

QUAB 342 wurde zur Bestimmung seiner akuten Toxizität einmalig dermal auf die Rückenhaut des Kaninchens aufgetragen. Das Prüfprodukt lag als farblose Flüssigkeit vor und kam unverdünnt zur Anwendung.

Das Vergiftungsbild war gekennzeichnet durch Zittern, gesträubtes Fell und körperliche Schwäche. Das Vergiftungsbild ist als protrahiert zu bezeichnen. Todesfälle traten zwischen 2 und 7 Tagen p. appl. auf. Die Applikationsstellen zeigten Graufärbung, Rötung und Schorfbildung.

Bei der Autopsie waren nur bei den weiblichen Tieren unspezifische Veränderungen an Niere, Leber, Milz, Lunge, Herz und Dünndarm zu beobachten.

Die ermittelten LD 50-Werte betragen:

Probitanalyse:

männliche und weibliche Tiere 2523 mg/kg (2,46 ml/kg)

graphische Abschätzung:

männliche Tiere:	3072 mg/kg (3,0 ml/kg)
weibliche Tiere:	1946 mg/kg (1,9 ml/kg)

Bielefeld, den 29.07.1986

Prüfleiter:

Dr. rer. nat. W. Mayr

Institutsleiter:

7.8.86

Dr. med. vet. M. Herbst

3. EINLEITUNG

Nachfolgend wird über die toxikologische Prüfung von QUAB 342 nach einmaliger dermaler Gabe am Kaninchen berichtet.

Ziel der Studie war Art, Intensität und Dauer der klinischen Symptome sowie Todeszeitpunkte und Zahl der verstorbenen Tiere festzustellen (1 - 2). Die Beobachtungsdauer betrug 14 Tage. Aus den Mortalitätszahlen wurde die LD 50 ermittelt (3 - 5).

Das methodische Vorgehen basierte auf der OECD-Richtlinie Nr. 402 (1).

4. PRÜFSUBSTANZ

Prüfsubstanz /Handelsname	: QUAB 342
Chemische Bezeichnung /Synonym	: 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
CAS-Nr.	: ---
Chargen-Nr.	: 342 M 19
Form und Aussehen	: farblose Flüssigkeit
Gehalt/Reinheit	: ---
Löslichkeit	: ---
Dichte	: 1,024 g/ml
pH-Wert	: 1,5
Aufbewahrung und Stabilität	: Die Prüfsubstanz wurde bei Raumtemperatur aufbewahrt. Die Stabilität der Prüfsubstanz wurde vom Auftraggeber gewährleistet.

5. METHODIK

5.1 Prüfsystem

5.1.1 Versuchstier-Spezies	: Kaninchen
Stamm	: Weiße Russen (Albino)
Herkunft/Züchter	: Asta-Werke AG, D-4800 Bielefeld 14
Begründung der Versuchstierwahl	: Das Prüfsystem wurde aufgrund internationaler Empfehlungen gewählt. Danach ist die Spezies Kaninchen geeignet, potentielle toxische Wirkqualitäten von Prüfsubstanzen zu erfassen.

Alter der Tiere am Applikationstag	: männlich 9 - 12 Monate weiblich 9 - 10 Monate
Körpergewicht der Tiere am Applikationstag	: männlich 2,05 - 2,50 kg weiblich 2,40 - 2,75 kg
Eingewöhnungszeit	: Mindestens 5 Tage unter Prüfbedingungen vor Substanzapplikation

5.1.2 Versuchstierhaltung

Standort	: Toxikologie Degussa-Asta D-4800 Bielefeld 14, Gebäude B, Raum Nr. 214-5
Haltung	: Edelstahlkäfige Modell ASTA ASTA-WERKE AG, D-4800 Bielefeld 14
Tiere pro Käfig	: 1
Einstreu	: ohne, Drahtbodenkäfige
Futter	: Standardisiertes Versuchstierfutter ALTROMIN (R), Firma Altromin GMBH u. Co KG, 4937 Lage,
Trinkwasser	: Wasser ad libitum in Trinkwasserqualität der Stadtwerke Bielefeld, automatische Tränkung über Tränkventile
Raumtemperatur	: 22 +/- 2 Grad Celsius
Relative Luftfeuchtigkeit	: 55 +/- 15 %
Raumbeleuchtung	: 12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. natürlicher Hell- Dunkelrhythmus
Raumhygiene, Käfigreinigung	: Labor- und Käfigreinigung mittels handelsüblicher Antiseptika, Instrumente- und Gerätedesinfektion mittels Autoklav und Heißluftsterilisator.

5.1.3 Kennzeichnung der Tiere : mittels Ohrnummer (Tätowierung), Käfigbeschilderung mit gleicher Nummer

5.2 Durchführung der Prüfung

Futterentzug	: am Behandlungstage
Substanzapplikation	: dermal, zwischen der geschorenen Schulter- und Fersenbeinregion. Die Prüfsubstanz wurde mittels eines Patches unter Okklusiv-Bedingungen 24 Stunden lang mit der äußeren Haut in Kontakt gebracht. Nach Abnahme des Verbandes wurde die auf der Haut verbliebene Prüfsubstanz mit Wasser abgewaschen.
Begründung der Wahl der Applikationsart	: Die Prüfsubstanz wurde aufgrund der möglichen dermalen Exposition des Menschen in bezug auf seine Toxizität nach einmaliger oraler Applikation geprüft.
Häufigkeit der Applikation	: einmalige Gabe (24 Stunden-Patch)
Applikationsform	: unverdünntes Prüfprodukt
Konzentrationen	: ---
Applikationsvolumina	: m 0,909 - 4,22 ml/kg w 0,909 - 4,22 ml/kg
Dosenabstand	: in dezimalgeometrischer Reihenfolge
Faktor	: m 2,15 w 2,15
Anzahl der Dosisgruppen	: m 3 w 3
Tiere pro Dosisgruppe	: m 2 w 2
Gesamttierzahl	: 12
Beobachtungsdauer	: 14 Tage p. appl.

5.3 Beobachtungen

5.3.1 Verhalten und Allgemeinzustand der Tiere (klinische Vergiftungssymptome) : Beobachtung der Tiere am Applikationstag während 6-8 Stunden p. appl., danach täglich zweimal (vor- und nachmittags); an Samstagen, Sonn- und Feiertagen einmal.
Alle aufgetretenen Vergiftungserscheinungen sowie deren Beginn, Schwere und Dauer wurden protokolliert.

Mortalität : Todeszeitpunkt, Anzahl der verstorbenen Tiere pro Dosis

5.3.2 Pathologie : Alle gestorbenen bzw. am Ende der Beobachtunsperiode getöteten Tiere wurden obduziert und Äußeres, Körperöffnungen, Körperhöhlen (Brust- und Bauchhöhle) und deren Organe makroskopisch untersucht.

5.4 Bestimmung der LD_{50}-Werte : approximative Ermittlung, graphisch (6) sowie Probitanalyse (3 - 5)

6. ERGEBNISSE

Allgemeine Angaben

QUAB 342 wurde zur Ermittlung seiner akuten Toxizität dermal am Kaninchen verabfolgt. Die Prüfsubstanz lag als farblose Flüssigkeit vor und wurde unverdünnt appliziert. Unter Verwendung steigender Applikationsvolumina von 0,909 bis 4,22 ml/kg erstreckte sich der geprüfte Dosisbereich bei den männlichen und weiblichen Tieren zwischen 931 und 4321 mg/kg. Die Dosisangaben berücksichtigen eine Dichte von 1,024 g/ml.

Klinische Beobachtungen

Das Vergiftungsbild war gekennzeichnet durch mäßiges bis starkes Zittern, gesträubtes Fell sowie körperliche Schwäche.

Der Vergiftungsverlauf ist als protrahiert zu bezeichnen. Erste Vergiftungssymptome waren nach 1 bis 6 Tagen zu beobachten und dauerten bis zu 9 Tagen. Todesfälle traten zwischen 2 und 7 Tagen nach Substanzapplikation auf.

Nach Abnahme des Patches waren bei allen Tieren die behandelten Hautareale grau gefärbt und von lederartiger Beschaffenheit; zusätzlich waren Rötung und Schorfbildung zu beobachten.

Pathologie

Bei der Sektion war bei einem weiblichen Tier der hohen Dosisgruppe die Konsistenz der Milz erhöht und brüchig, die Leber marmoriert und die Lunge dunkel gefleckt. Geröteter Dünndarm, weißgefleckte Nieren weißes Endokard war bei einem weiblichen Tier der mittleren Dosisgruppe zu beobachten.

Statistik

Der LD 50-Wert wurde für beide Geschlechter graphisch nach Miller und Tainter (6) abgeschätzt und für beide Geschlechter zusammen mit der Probitanalyse ermittelt.

Die Werte sind in Tabelle 1 a und 1 b aufgeführt.
In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

Tabelle 1 Akute Toxizität - LD_{50}-Werte

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Tabelle 1a Graphische Abschätzung der LD 50 nach Miller und Tainter (6)
Vertrauensgrenze: 68,3 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche Tiere:	3,0 (0,355 - 25,322)	3072 (364 - 25930)
weibliche Tiere:	1,9 (0,303 - 11,925)	1946 (310 - 12211)

Tabelle 1b Rechnerische Bestimmung der LD 50 mittels Probitanalyse (3 - 5)
Vertrauensgrenze: 95 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche und weibliche Tiere:	2,46 (0,764 - 41,470)	2523 (782 - 42466)

Tabelle 2 Akute Toxizität - Absterbedaten

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Dosis (ml/kg)	Todesrate x/n	%	Überlebenszeit Std. n. Applikation 0,5	1	2	4	8	24	Tage nach Applikation 2	3	4	5	6	7	8	9	10	11	12	13	14
männliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50												1							
4,22	1/2	50									1										
weibliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50								1											
4,22	2/2	100							1												

8. LITERATUR

1. OECD-Guideline for Testing of Chemicals, Acute Dermal Toxicity, No. 402, OECD Paris (1981), ISBN-92-64-12221-4

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DEGUSSA AG - US-IT - NR.
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PROJECT 047665

Ames Test

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

WITH

QUAB 342

REPORT

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PREFACE

GENERAL

Sponsor:	DEGUSSA AG ZN Wolfang Rodenbacher Chaussee 4 D 6450 Hanau 1
Study Monitor:	Dr. O. Weiberg
Testing Facility:	R C C Research & Consulting Company AG CH - 4452 Itingen / Switzerland
RCC Project No.:	047665
Test Article:	QUAB 342
Title:	Salmonella/Mammalian-Microsome Mutagenicity Test with QUAB 342

SCHEDULE

Receipt of Test Article:	April 15, 1985
Start of Experiments:	May 28, 1985
Date of Completion:	June 17, 1985
Proposed Date of Final Report:	June 27, 1985/hac

PROJECT STAFF

Study Director: Dr N. Banduhn, Dipl Biol.

Date: July 3, 1985

Managing Director: Dr. K Sachsse

Date: July 3, 1985

Technical Coordinators: Mrs. M Maurer
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GUIDELINES

This study follows the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella Typhimurium, Reverse Mutation Assay", adopted May 26, 1983.

EEC Directive 84/449, Method No. B 14.

SUMMARY

This study was performed to investigate the potential gene mutagenic activity of QUAB 342 according to the plate incorporation test of Ames et al. (1) using the Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

The test was performed with and without liver microsomal activation. The test article was tested at the following concentrations: 1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate. Each concentration, including the controls, was tested in triplicate.

Toxic effects of the test article were observed in the toxicity experiments by a reduction of the relative survival rate.
Additionally, a reduction of the background lawn up to a clearing was observed. Reduced numbers of revertants were found in some strains at the highest concentration tested. The toxic effects were more prominent in the experiments without metabolic activation than in the experiments with metabolic activation.

Up to the highest investigated dose, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538 in both experiments, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. when tested without liver microsomal activation. In the first experiment, the revertant rate was increased but not doubled versus the control. In the second experiment, there was a dose-dependent increase with an exact doubling of the revertant rate at a concentration 46.4 mcg/pl.

CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay.

1 OBJECTIVES

1.1 PURPOSE

This in vitro experiment was performed to assess the potential gene mutagenic activity of QUAB 342.
The standard quality control principles of "Wegleitung der IKS (Interkantonale Kontrollstelle fuer Heilmittel) betreffend Gute Laborpraxis fuer nichtklinische Laborversuche" from April 28, 1980 (amended June 23, 1982), Berne/Switzerland were applied.

1.2 RATIONALE

The test procedure was according to Ames et al. (1)
The microbial assay is based on reverse mutation of Salmonella typhimurium from auxotrophism (histidine dependent) to prototrophism (histidine independent). When mutated Salmonella typhimurium are exposed to a mutagen, mutation to the histidine independent form takes place in a proportion of the bacterial population. These are readily detectable due to their ability to grow on a histidine deficient medium.

Exposure of strains of Salmonella typhimurium to a chemical mutagen may produce revertants which arise as a result of a base-pair substitution in the genetic material or as a frameshift mutation in which genetic material is either added, deleted or mis-coded.

Since many compounds do not exert their mutagenic activity until they have been metabolized by enzyme systems not available in the bacterial cell, the test article and the bacteria were incubated both in the absence and in the presence of a cofactor-supplemented post-mitochondrial fraction prepared from the livers of rats treated with enzyme-inducing agents Aroclor 1254.

To validate the test, reference mutagens were tested parallel to the test article.

2 MATERIALS AND METHODS

2.1 TEST ARTICLE

Identification	QUAB 342
Batch/Lot number	040485-342 MA 54
Chemical denomination	3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
Purity	40.01% (cf. Certificat of analysis, April 3, 85)
Physical appearance	colorless, clear fluid
Stability	not available from the sponsor
Storage	The test article was stored at room

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To avoid any light effects on the test compound, all experiments were performed under yellow light.

On the day of the experiment, the test article was dissolved in bidistilled water at the highest investigated dose. The other doses were dilutions from this stock solution with the solvent in third-log intervals.
The test article was investigated at the following concentrations:
1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate (mcg/pl.).

The highest concentration was limited by the cytotoxicity of the test article to bacteria. A preliminary test on strains TA 1537 and RTA of Salmonella typhimurium resulted in a strong toxic effect at a concentration of 500 mcg/pl. and higher.

2.2 CONTROLS

All control data were established on the same day as the experiment.

Negative Control:
Bidistilled water, solvent of the test article.

Positive Controls:
Reference mutagens not requiring microsomal drug-metabolizing enzyme.

Methyl methanesulfonate*	500 mcg/pl.	for TA 100
9-Aminoacridine*	40 mcg/pl.	for TA 1537
2-Nitrofluorene*	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538
N-Ethyl-N-Nitro-N-Nitrosoguanidine*	10 mcg/pl.	for TA 98
		TA 100
		TA 1535

Reference mutagens requiring microsomal drug-metabolizing enzyme

2-Aminoanthracene*	0.5 mcg/pl	for TA 98
		TA 100
		TA 1538
	1 mcg/pl	for TA 1535
	10 mcg/pl	for TA 1537
Benzo-(A)-pyrene**	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538

Aseptic Control:

An experiment with the S-9 mix was performed (cf. study procedure)

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* Aldrich, Chemie Brunschwig, 4000 Basel/Switzerland
** Fluka, 9470 Buchs/Switzerland

2 3 TEST SYSTEM

Bacterial test organisms:

Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538; obtained from B.N. Ames, University of California, Berkeley CA, 94720, USA, May 1981.

Characterization of strains:

The strains used are mutants derived from Salmonella typhimurium LT2 and have following genotype:

S.typhimurium TA 1535 his G46 rfa- uvrB-
S.typhimurium TA 1537 his C3076 rfa- uvrB-
S.typhimurium TA 1538 his D3052 rfa- uvrB-
S.typhimurium TA 98 his D3052 rfa- uvrB- R+
S.typhimurium TA 100 his G46 rfa- uvrB- R+

All the strains carry a mutation known as deep rough (rfa) which affects the cell membrane so that the normal lipopolysaccharide cell coat is defective These strains allow a greater permeability of the test agents across this membrane and into the cell.

In bacteria, almost all the primary damage caused to DNA by a mutagen is repaired by excision and recombination repair systems. Therefore, only a small percentage of the potential mutagenic damage is expressed. The tester strains used lack this excision repair system (uvrB), thus making them more sensitive to mutagens.

The strains TA 98 and TA 100 also carry an ampicillin resistance factor (R) which makes them particularly sensitive to mutagens.

The strains TA 1535 and TA 100 are sensitive to base-pair substitution mutagens; the strains TA 1537, TA 1538 and TA 98 are sensitive to frameshift mutagens.

The tester strains are checked at regular intervals for their genetic markers, according to published methods (1)

Storage

The strain cultures are stored in sterile 0 5 ml ampoules (0 45 ml bacterial culture and 0.05 ml Dimethylsulfoxide) at -70 degrees centrigrade

Pre-culture of strains:

The bacteria were grown in a shaking water bath for 16 hours overnight at 37 degrees centigrade in 2.5% Nutrient Broth No. 2*. After centrifugation, the bacteria were resuspended to a concentration of approximately 1 x 10 exp. 8 to 2 x 10 exp. 9 cells per milliliter in 0.16% Nutrient Broth and 0.5% sodium chloride. The concentration of germs was controlled photometrically and determined in an experimental test with Histidine-rich potassium chloride solution on selective agar plates (cf. study procedure).

2.4 LIVER MICROSOMAL FRACTION S-9 MIX

The S-9 mix was prepared on the day of the experiment

Specific pathogen-free male Wistar rats (180 g - 300 g, KFM-Ha, outbred**), received a single i.p. injection of Aroclor 1254*** dissolved in Oleum Arachidis (200 mg/ml) at a dosage of 500 milligrams per kilogram of body weight to induce liver microsomal enzyme activity five days before the experiment.

The rats were killed by cervical dislocation and exsanguinated on the fifth day post application following a 14-16 hours starvation period.

Livers were removed under aseptic conditions and homogenized with ice-cold 0.15 molar potassium chloride (5 g of liver to 15 g of potassium chloride). The homogenates were centrifuged at 9000 x g for 10 minutes at 0 to 2 degrees centrigrade. The supernatant fraction (S-9 fraction) was collected for the preparation of S-9 mix

Composition of 1 ml S-9 mix.

Na2HPO4****		100 micro moles
MgCL2	****	8 micro moles
KCl	****	33 micro moles
NADP	*****	4 micro moles
G-6-P	******	5 micro moles
S-9 Fraction		0.3 ml

* Oxoid; Fakola, 4000 Basel/Switzerland
** Kleintierfarm Madoerin AG, 4414 Fuellinsdorf/Switzerland
*** Analabs; Kontron, 8000 Zuerich/Switzerland
(Aroclor: polychlorinated biphenyl mixture)
**** ABS, 4000 Basel/Switzerland
***** Boehringer Mannheim, 6330 Cham/Switzerland
****** Fluka, 9470 Buchs/Switzerland

2.5 STUDY PROCEDURE

For each strain and dose level, including the controls, a minimum of three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates*:

100 microliters (mcl)	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 mcl	S-9 mix (for test with metabolic activation) or 0.15 M Na_2HPO_4, (for test without metabolic activation),
100 mcl	Bacteria suspension (cf. test system, pre-culture of the strains),
2 ml	Overlay agar consisting of 0.6 % Bacto agar** and 0.6 % NaCl supplemented with 10 % 0.5 mM L-Histidine*** and 0.5 mM Biotine*** solution.

The plates were incubated at 37 degrees centigrade in the dark for 3 days. The his+ revertant colonies were counted with an Artek 880 counter or counted by hand if precipitation of test article precluded automatic counting.

For the aseptic control experiment, 100 microliters of S-9 mix were added to 2 milliliters overlay agar and treated as mentioned above.

For each test, the germ concentration of the bacterial strains was determined by pouring a mixture of 100 microliters of a 2 x 10 exp.5 diluted bacteria suspension (overnight culture) with a Histidine-rich potassium chloride solution onto a selective agar plate. After three days of incubation at 37 degrees centrigrade, the colonies were counted and the counts multiplied with the dilution factor.

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* A Pourmatic (New Brunswick Scientific Comp.) was used to fill the plates with 22 ml of 1.5 % bacto agar in Vogel Bonner medium E with 2% Glucose.
** Chemie Brunschwig, 4000 Basel/Switzerland
*** Fluka, 9470 Buchs/Switzerland

2.6 TOXICITY TEST

To estimate the cytotoxicity of the test article, prototrophic bacteria (spontaneous revertants of TA 1537 = RTA) were added as an internal standard to the selective agar plates, together with TA 1537 (cf. study procedure). The difference in the number of colonies on plates with and without added prototrophic bacteria at each dose level of the test article was compared to the number of colonies obtained with the negative control. The ratio of the two values was expressed as relative survival rate with the negative control at a 100 % survival rate.

Additionally, the toxicity of the test article can be shown in the mutagenicity assay, by a reduction in the number of revertants or by a reduction or a clearing of the background lawn.

2.7 DATA RECORDING

The number of revertant colonies was recorded manually for each plate from the counter.
Individual plate counts and the mean number of revertant colonies per plate are presented in tabular form for the test article, positive and negative controls.

2.8 EVALUATION OF RESULTS

A material is identified as a mutagen in this test system if there is a reproducible demonstration of a dose effect relation with a 2-fold increase in the number of revertants over the controls in a minimum of one strain. With the strain TA 100, a 1.5-fold increase is the criterion for a positive result.

These criteria are generally in accordance with the international used standards for the evalutation of results obtained from the Ames Test (e.g. Ref. 1, 2, and 3).

3 RESULTS

In the current study, the following results have been compiled:

3.1 GERM CONCENTRATIONS

The concentrations of Salmonella typhimurium used in this assay were as follows:

	FIRST EXPERIMENT	SECOND EXPERIMENT
TA 98	= 1.2 x 10 exp.9 cells/ml	= 1.0 x 10 exp 9 cells/ml
TA 100	= 8.0 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1535	= 1.4 x 10 exp.9 cells/ml	= 9.9 x 10 exp 8 cells/ml
TA 1537	= 9.5 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1538	= 2.1 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments without metabolic activation,

TA 98	= 1.8 x 10 exp.9 cells/ml	= 9.4 x 10 exp 8 cells/ml
TA 100	= 6.8 x 10 exp.8 cells/ml	= 7.3 x 10 exp 8 cells/ml
TA 1535	= 1.1 x 10 exp.9 cells/ml	= 1.8 x 10 exp 9 cells/ml
TA 1537	= 1.4 x 10 exp.9 cells/ml	= 4.9 x 10 exp 8 cells/ml
TA 1538	= 1.8 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments with metabolic activation.

3.2 TOXICITY OF THE TEST ARTICLE

In the toxicity experiment with QUAB 342, a reduction of the relative survival rate was observed without metabolic activation starting at 46.4 mcg/pl and with metabolic activation at a concentration of 216 mcg/pl.
Additionally, a reduction up to a clearing of the background lawn was observed starting from 46.4 to 100 mcg/pl and reduced numbers of revertants were found in some strains at the highest concentrat (216 mcg/pl.) tested.

3.3 CONTROL EXPERIMENTS

Control plates with the solvent (negative control) showed numbers of spontaneous revertant colonies per plate which were within the normal range of those cited in the literature (1)

Control plates with reference mutagens (positive controls) showed a distinct increase of the revertant colonies with the tester strains. This confirms the reversion properties of each strain. The positive results of the mutagens 2-Aminoanthracene and Benzo(a)pyrene indicate that the metabolizing system was functioning.

3.4 TESTS WITH QUAB 342

In the described bacterial mutagenicity tests, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 at all dose levels tested when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. in both experiments when tested without liver microsomal activation. In the first experiment, the revertant rate was increased approximately 68 % versus the control. In the second experiment, the number of revertants increased starting at 10 mcg/pl. and reached an exact doubling versus the control at a concentration of 46.4 mcg/pl.

4 CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activiation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay

5 REFERENCES

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Distribution of Spontaneous Mutation Values in Ames Test with and without Metabolic Activation. In: Critical Evaluation of Mutagenicity Tests. Bass, R., Glocklin, V., Grosdanoff, P., Henschler, D., Kilbey B., Müller, D., Neubert, D., (eds.)
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6 ARCHIVING

R C C, CH - 4452 Itingen/Switzerland

Raw data, protocol, report, duplicate of report, and test article reference sample.

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	25		123		28		9		13	
	24		129		34		9		23	
	33	27	138	130	25	29	11	10	22	19
1.0 mcg	32		137		24		11		16	
	24		132		25		6		17	
	24	27	161	143	24	24	8	8	18	17
2.16 mcg	26		126		28		12		18	
	24		139		24		11		28	
	18	23	127	131	34	29	9	11	20	22
4.64 mcg	20		125		25		11		15	
	21		138		35		8		21	
	18	20	157	140	21	27	10	10	20	19
10.0 mcg	23		142		26		9		13	
	24		140		19		8		26	
	30	26	141	141	35	27	4	7	19	19
21.6 mcg	27		143		23		10		25	
	29		144		27		13		30	
	21	26	125	137	25	25	5	9	23	26
46.4 mcg	27		120		29		12		32	
	30		122		29		7		29	
	29	29	116	119	24	27	15	11	34	32
100.0 mcg	38		127		26		13		27	
	36		95		20		6		32	
	32	35	122	115	19	22	12	10	15	25
216.0 mcg	27		56		17		15		22	
	32		37		14		12		10	
	10	23	28	40	12	14	11	13	13	15
MMS 500 mcg	---		485		---		---		---	
	---		432		---		---		---	
	---	---	479	465	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		35		---	
	---		---		---		32		---	
	---	---	---	---	---	---	43	37	---	---
2-NF 5 mcg	493		486		---		44		830	
	509		443		---		47		882	
	500	501	367	432	---	---	54	48	886	866
ENNG 10 mcg	72		495		173		---		---	
	79		551		99		---		---	
	60	70	496	514	147	140	---	---	---	---

--- Not determined

SALMONE...A TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE, WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	41		125		16		35		35	
	37		95		19		48		34	
	36	38	124	115	20	18	37	40	38	36
1.0 mcg	46		115		17		30		24	
	43		122		18		40		39	
	47	45	110	116	16	17	37	36	36	33
2.16 mcg	39		93		26		35		40	
	39		109		23		35		31	
	55	44	127	110	20	23	36	35	31	34
4.64 mcg	44		134		21		41		25	
	40		123		26		30		40	
	47	44	113	123	22	23	30	34	34	33
10.0 mcg	31		122		35		36		35	
	40		125		29		32		40	
	47	39	136	128	22	29	29	32	27	34
21.6 mcg	46		129		18		40		29	
	46		109		21		43		39	
	49	47	99	112	15	18	35	39	28	32
46.4 mcg	47		118		19		31		33	
	43		107		12		32		43	
	40	43	117	114	22	18	36	33	34	37
100.0 mcg	45		113		18		36		34	
	36		119		15		29		37	
	55	45	136	123	19	17	14	26	39	37
216.0 mcg	41		72		25		47		44	
	58		58		10		26		33	
	53	51	61	64	21	19	33	35	38	38
2-AA 0.5 mcg	99		162		---		---		130	
	113		197		---		---		97	
	113	108	180	180	---	---	---	---	99	109
2-AA 1.0 mcg	---		---		89		---		---	
	---		---		81		---		---	
	---	---	---	---	65	78	---	---	---	---
2-AA 10.0 mcg	---		---		---		172		---	
	---		---		---		219		---	
	---	---	---	---	---	---	168	186	---	---
BaP 5 mcg	284		370		---		117		124	
	294		343		---		130		108	
	262	280	410	374	---	---	124	124	117	116

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	10	367	1.00
1.0 mcg	8	358	0.98
2.16 mcg	11	358	0.97
4.64 mcg	10	352	0.96
10.0 mcg	7	351	0.96
21.6 mcg	9	359	0.98
46.4 mcg	11	189	0.50
100.0 mcg	10	42	0.09
216.0 mcg	13	13	0.00

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	40	343	1.00
1.0 mcg	36	307	0.89
2.16 mcg	35	327	0.96
4.64 mcg	34	339	1.01
10.0 mcg	32	329	0.98
21.6 mcg	39	306	0.88
46.4 mcg	33	290	0.85
100.0 mcg	26	235	0.69
216.0 mcg	35	135	0.33

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	23		116		19		15		16	
	29		96		20		11		11	
	10	21	111	108	18	19	7	11	11	13
1.0 mcg	33		110		14		11		16	
	21		120		24		12		19	
	21	25	123	118	22	20	10	11	14	16
2.16 mcg	29		97		21		17		13	
	24		116		27		11		11	
	23	25	112	108	21	23	14	14	15	13
4.64 mcg	31		115		17		12		15	
	30		129		17		8		15	
	29	30	134	126	21	18	11	10	7	12
10 mcg	23		123		24		13		25	
	33		107		31		9		16	
	25	27	113	114	28	28	11	11	12	18
21.6 mcg	26		94		19		8		24	
	23		121		18		14		20	
	30	26	122	112	25	21	9	10	22	22
46.4 mcg	28		116		17		11		25	
	30		74		22		5		28	
	24	27	84	91	18	19	11	9	24	26
100 mcg	30		53		14		10		24	
	30		60		13		8		19	
	31	30	62	58	15	14	17	12	27	23
216 mcg	20		35		24		12		14	
	17		33		18		12		11	
	35	24	26	31	20	21	17	14	23	16
MMS 500 mcg	---		334		---		---		---	
	---		401		---		---		---	
	---	---	407	381	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		49		---	
	---		---		---		56		---	
	---	---	---	---	---	---	73	59	---	---
2-NF 5 mcg	470		383		---		57		859	
	461		357		---		53		864	
	476	469	393	378	---	---	48	53	764	829
ENNG 10 mcg	120		1103		1045		---		---	
	143		1145		681		---		---	
	133	132	885	1044	891	872	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	51		91		12		28		31	
	59		107		17		38		29	
	47	52	115	104	15	15	33	33	29	30
1.0 mcg	47		109		13		28		30	
	40		111		18		26		21	
	38	42	121	114	14	15	30	28	33	28
2.16 mcg	48		130		14		34		27	
	50		116		21		32		19	
	47	48	124	123	17	17	30	32	29	25
4.64 mcg	54		137		16		30		29	
	41		112		13		33		29	
	45	47	99	116	21	17	37	33	28	29
10 mcg	49		128		13		32		30	
	55		113		19		40		26	
	41	48	117	119	18	17	36	36	27	28
21.6 mcg	42		139		15		29		30	
	45		119		11		31		25	
	53	47	134	131	18	15	30	30	30	28
46.4 mcg	46		129		13		30		40	
	47		116		17		31		23	
	52	48	132	126	9	13	29	30	40	34
100 mcg	56		91		16		19		34	
	38		109		14		30		30	
	44	46	109	103	12	14	42	30	22	29
216 mcg	36		55		18		25		25	
	33		45		13		15		24	
	37	35	36	45	12	14	25	22	35	28
2-AA 0.5 mcg	73		---		---		---		99	
	76		172		---		---		---	
	---	75	172	172	---	---	---	---	72	86
2-AA 1.0 mcg	---		---		98		---		---	
	---		---		109		---		---	
	---	---	---	---	---	104	---	---	---	---
2-AA 10.0 mcg	---		---		---		136		---	
	---		---		---		146		---	
	---	---	---	---	---	---	150	144	---	---
BaP 5 mcg	135		257		---		---		84	
	124		---		---		95		81	
	138	132	270	264	---	---	94	95	---	83

--- Not determined

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	11	277	1.00
1.0 mcg	11	285	1.03
2.16 mcg	14	278	0.99
4.64 mcg	10	267	0.97
10 mcg	11	263	0.95
21.6 mcg	10	249	0.90
46.4 mcg	9	119	0.41
100 mcg	12	22	0.04
216 mcg	14	13	---

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	33	231	1.00
1.0 mcg	28	231	1.03
2.16 mcg	32	242	1.06
4.64 mcg	33	230	0.99
10 mcg	36	246	1.06
21.6 mcg	30	239	1.06
46.4 mcg	30	237	1.05
100 mcg	30	209	0.90
216 mcg	22	119	0.49

Study No.: 877858
Report

DEGUSSA AG - US-IT - NR.
92 0112 DGM

QUAB 342

Mouse Micronucleus Test
(Single Intraperitoneal Administration)

R E P O R T

July 15, 1992

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The report comprises 28 pages.

1. GENERAL INFORMATION

1.1 Study Specification

Sponsor	:	Degussa AG/ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Testing Facility	:	ASTA Medica AG Institute of Toxicology Kantstraße 2 D-4802 Halle-Künsebeck
Study Director, Veterinary Care, Pathology	:	Dr.med.vet. K. Krauser
Study Performance	:	Mrs. M. Beste, Mrs. A. Lagoudis
Bone Marrow Evaluation	:	Miss C. Barkey
Quality Assurance	:	K.E. Fichtner Representative: Dr. B. Wilker (formerly: Roos)
Test Material	:	QUAB 342
Objective	:	Testing the mutagenic properties (damage to the chromosomes and/or the mitotic apparatus)
Test Guidelines	:	OECD Guideline No. 474 (1) EEC Directive (84/449/EEC) (2)
Study Number	:	877858
Time Schedule	:	Protocol and 1 amendment Oct/04/1990 Day of Administration Oct/15/1990 Last Day of Bone Marrow Sampling Oct/18/1990 Date of Report Jul/15/1992
Quality Assurance	:	The study was performed according to the regulations for "Good Laboratory Practice" (3).
Archivation	:	The approved protocol, all raw data obtained in the course of the study, all bone marrow smears as well as a copy of the final report are kept in the archives of the Institute of Toxicology at least 30 years (starting with the report date). Afterwards the sponsor will decide on further use.

1.2 AUTHENTICATION

I, the undersigned, hereby declare that to the best of my knowledge the study was performed under my supervision in accordance with current Good Laboratory Practice (as laid down in the German Chemicals Act).

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

This report represents a true and accurate record of the results obtained.

Study Director : July 15, 1992
Dr.med.vet. K. Krauser

Report Review

Head of the
Institute of Toxicology : July 15, 1992
Dr.med.vet. W. Jahn

1.3 ASTA Medica AG
Corporate Quality Assurance
Section GLP/GCP

Quality Assurance Statement

The non-clinical laboratory study

QUAB 342
Mouse Micronucleus Test
(Single Intraperitoneal Administration)

performed at ASTA Medica AG, Bielefeld,

was inspected and audited for conformance to the principles of Good Laboratory Practice (GLP).

The dates of inspections and reports to study director and management are given below.

Phase of Study	Date of QA Inspection	Date of QA Report
Protocol Review	Oct. 15, 1990	
Experimental Period	Oct. 15, 1990 Oct. 16, 1990 Oct. 18, 1990	Oct. 19, 1990
(Evaluation)	Apr. 19, 1991	
(Statistics)	Jun. 24, 1991	Jun. 24. 1991
Final Report Audit	Jul. 15, 1992	Jul. 15, 1992

Date: July 29, 1992

Signed: K. E. Fichtner

2. SUMMARY

This in vivo micronucleus test in mice was performed to assess the potential mutagenic activity, induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus, according to SCHMID with the modifications of SALAMONE et al.

The experiment was performed with three groups, the negative and positive control groups, each containing 18 males and 18 females, and the test group, containing 21 males and 21 females.

Negative control group and test material group animals were given a single intraperitoneal administration of 10.0 ml/kg body weight. Positive control group animals received a single administration of 10.0 ml/kg body weight by oral gavage. No test material group animal died.

The negative control group received physiological saline solution (0.9%). The test material group animals received the maximum tolerated doses in respect of the micronucleus test, i.e. 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) of the test material QUAB 342 dissolved in aqua ad iniectabilia. The positive control group received 51.1 mg/kg body weight cyclophosphamide dissolved in physiological saline solution (0.9%).

QUAB 342 related toxic symptoms were registered in all test material group animals. The symptoms consisted in clonic convulsions, stilted gait, ruffled furs, ptosis, sunken sides, and hypokinesia.

Twenty-four (24), 48 and 72 hours after treatment, six mice per sex and control group and seven mice per sex of the test material group were sacrificed and bone marrow was removed from the femurs for examination.

For each sampling time five animals per sex and group were used for evaluation. One thousand polychromatic erythrocytes per animal were scored for micronuclei. The ratio of polychromatic to normochromatic erythrocytes was used to assess the toxicity of the test material by counting a total of 1000 erythrocytes.

The incidences of micronuclei were statistically analyzed by means of a POISSON test. Estimation and testing were performed at each sampling time for each treatment group and for each sex, as well as for both sexes combined.

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) no significant test material-related increase in micronucleated polychromatic erythrocytes was observed in both male and female animals, respectively males and females combined, when compared with corresponding negative control group animals at 24, 48, or 72 hours after administration. No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding control group animals, with the exception of one female at the 24-hour sampling time.

The positive control group animals, which received cyclophosphamide, exhibited a significant increase in the number of micronucleated polychromatic erythrocytes and thus validated the test system.

CONCLUSION

QUAB 342 at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) (single intraperitoneal administration) is non-mutagenic in the mouse micronucleus test under the described conditions.

3. INTRODUCTION

PURPOSE

This in-vivo experiment was performed to assess the potential mutagenic activity induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus.

RATIONALE

The test procedure was according to SCHMID (4) with the modifications of SALAMONE et al (5).

The micronucleus test is based on the principle that in many mammalian cells acentric chromatid and chromosome fragments induced by clastogenic agents are left behind in the anaphase stage of cell division. They are included in the daughter cells where they form micronuclei in the cytoplasm. These micronuclei persist in the erythrocyte after extrusion of the main nucleus and can be scored.

The object of the test is to detect an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated animals versus the controls. Differential staining allows micronuclei to be identified under the microscope (6).

To validate the test system, a reference mutagen is tested parallel to the test material.

4. M A T E R I A L S

4.1 TEST MATERIAL

Identification	: QUAB 342 (see Attachment 1)
Chemical Name/Synonym	: 3-Chloro-2-hydroxypropyl-N,N,N-dimethyldodecyl-ammoniumchloride
CAS No.	: 41892-01-7
Batch No.	: 90 / Ma - 03
Physical Appearance	: Slightly coloured solid
Content/Purity	: 85%
Solubility in H_2O	: appr. 40%
pH Value	: 3-6 (appr. 40% solution in water)
Storage	: The test material was stored in a refrigerator.
Stability	: The stability of the test material was guaranteed by the sponsor under the given storage conditions throughout the experimental period (until January 1991).
Additional Information	: See attachment 1 and under substance No. 89784 on file in ASTA Medica AG.
Formulation of the Test Material	: The test material was freshly dissolved in aqua ad iniectabilia (Ampuwa(R), batch No. PM 1032 E6, supplied by Fresenius AG, D-6380 Bad Homburg).

4.2 POSITIVE CONTROL MATERIAL

Identification	: Cyclophosphamide (see Attachment 2)
Chemical Designation	: 2-(Bis-(2-chloroethyl)-amino)-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide monohydrate
Batch/Lot Number	: 090029
Physical Appearance	: White crystalline powder
Purity	: 100.0%
pH Value	: 4.9 (2% aqueous solution)
Storage	: The positive control material was stored at room temperature.
Stability	: The stability of the positive control material was guaranteed for the test period (until Dec. 31, 1991)
Formulation of the Positive Control Material	: The positive control material was freshly dissolved in physiological saline solution (0.9%, batch No. AEW 09D, supplied by Fresenius AG, D-6380 Bad Homburg).

4.3 NEGATIVE CONTROL MATERIAL

Identification	: Physiological saline solution (0.9%) (Fresenius AG, D-6380 Bad Homburg).
Batch/Lot Number	: AEW 09 D

5. METHODS

5.1 TEST SYSTEM

5.1.1 Species : Mouse

Strain : BOR: NMRI, (SPF Han)

Breeder : Winkelmann, Versuchstierzucht GmbH & Co.KG., D-4799 Borchen

Justification for the Selection of the Test System : Mice are recommended for micronucleus tests as the international standard.

Initial Age at Test : 5 weeks

Initial Body Weight at Test : males : 29 - 35 g
females: 24 - 31 g

5.1.2 Husbandry

Housing : ASTA Medica AG, Institute of Toxicology, D-4800 Bielefeld 14
Building B, Room No. 214-1 (negative control) and Room No. 214-2 (positive control and test material group)

Caging : Macrolon cages, type II

Animals per Cage : 1

Bedding : Animal bedding-chips, Jelu-Werk, J. Ehrler Industriemehle, D-7092 Rosenberg/Württ.
According to information from the manufacturer contaminant analyses of the bedding are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Diet : Standard diet ad libitum, ssniff(R) M, "Special diet for Mice", supplied by SSNIFF Spezialdiäten GmbH, D-4770 Soest (composition of the diet see Attachment 3). According to information from the manufacturer contaminant analyses of the diet are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Water	: Water ad libitum in drinking water quality of the Stadtwerke Bielefeld (Municipal Works Bielefeld). Automatic watering system with drinking nipples. According to information from the Stadtwerke Bielefeld the water is investigated in appropriate intervals. Regarding these analyses, the contaminants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Room Temperature	: 22 ± 2 degrees centigrade
Relative Humidity	: 55% ± 15% Slight deviations from these values were present for a short period (approx. 4 hrs) the day before substance administration, because of a technical defect in the air-conditioning plant. These deviations are considered not having influenced the results of this study.
Room Lighting	: 6 a.m. - 6 p.m. CET artificial lighting 6 p.m. - 6 a.m. CET natural light/dark rhythm
Room Hygiene, Cage Cleaning	: The room was cleaned regularly with commercial antiseptics and the cages with the cage washing machine type HAMO-R-T-500D (supplied by Hamo AG, CH-Biel-Bienne, Data on the ingredients of the antiseptics used are on file in ASTA Medica AG.

5.1.3 Allocation and Identification of the Animals

Number of Animals	: 114 (57 males / 57 females)
Method of Randomization	: Animals were placed into the different groups using computer generated random numbers.
Identification of the Animals	: Different ear notching of the groups; cage labelling with study No., animal No., sex, material designation, sampling time and route of administration.
5.1.4 Acclimatization Time	: 5 days under test conditions with veterinary care.

5.2 EXPERIMENTAL DESIGN

5.2.1 Dose Selection : In an orientating acute toxicity study in mice after single intraperitoneal administration of 46.4 mg/kg (both sexes) and 31.6 mg/kg QUAB 342 (only males) severe symptoms of toxicity and death occurred, while at 21.5 mg/kg in males and 31.6 mg/kg in females clear symptoms of toxicity but no death were observed. Therefore 21.5 mg/kg (males) and 31.6 mg/kg (females) Quab 342 were considered to be the maximum tolerated doses.

5.2.2 Route of Administration : Intraperitoneal (test material, negative control material)
Oral by gavage (Nelaton catheter, red rubber, Charrière-No. 6, supplied by Willy Rüsch, D-7053 Rommelshausen/Stuttgart) (positive control material)

Justification for the Route of Administration : For methodical reasons and according to the test guidelines (1, 2) the intraperitoneal route of administration was chosen for negative control and test material group animals. For validation of the test system the positive control group animals were treated orally.

5.2.3 Administration Volume : 10.0 ml/kg body weight

5.2.4 Study Procedure : Three groups of mice, the negative and positive control groups containing each 18 males and 18 females and the test material group with 21 males and 21 females each received a single intraperitoneal administration or by oral gavage, respectively (diet withdrawal: 16 h before treatment).
Group 1, the negative control, received physiological saline solution (0.9%).
Group 2, the test material group, received 21.5 mg/kg body weight (males) and 31.6 mg/kg (females) of the test material. The test material was given as a freshly prepared solution in aqua ad iniectabilia.
Group 3, the positive control, received Cyclophosphamide (51.1 mg/kg b.w.) dissolved in physiological saline solution (0.9%).

The animals were observed for the first 4 to 6 hours after administration for the occurrence of toxicity symptoms; on days 2, 3, and 4 only once daily in the morning.

At 24-, 48- and 72-hour intervals after treatment, 6 mice per sex and control group and 7 mice per sex of the test material group were sacrificed to prepare bone marrow smears.

The groups were as follows:

Group	1 Negative Control	2 Test Material	3 Positive Control
Treatment (mg/kg)	Physiolocical saline solution (0.9%)	QUAB 342 M: 21.5 F: 31.6	Cyclophosphamide 51.1

Sampling time p. appl.		Animal Numbers		
24 h	M	1- 6	301-307	401-406
	F	19- 24	322, 323, 330, 337, 340	419-424
48 h	M	7- 12	308-314	407-412
	F	25- 30	324-329, 331	425-430
72 h	M	13- 18	315-321	413-418
	F	31- 36	332-336, 341, 342	431-436

Because of the strong toxic effects with risk of intercurrent death in severa female animals, the following changes were made in the test material group ani mals concerning planned and actual sampling times. The decision was based on th clinical symptoms.

Animal Numbers	Sampling Time p.appl. Planned	Sampling Time p.appl. Actual
324	24 h	48 h
325	"	"
326	"	"
327	"	"
328	"	"
330	48 h	24 h
332	"	72 h
333	"	"
334	"	"
335	"	"
337	72 h	24 h
338	"	"
339	"	"
340	"	"

5.2.5 Preparation of Bone Marrow for Evaluation : All mice were sacrificed by CO_2 overdose. Both femurs were removed from each mouse and the bone marrow cells flushed into a labelled centrifuge tube with approximately 1.5 ml of fetal calf serum (Art. No. 210463, Boehringer Mannheim, D-1800 Mannheim). The tubes were centrifuged at approx. 180 x g for 5 minutes (Eppendorf Zentrifuge 5415, Netheler & Hinz GmbH, D-2000 Hamburg 65), after which the supernatant serum was discarded and the bone marrow cells suspended upon a thin layer of serum. A small drop of the marrow serum suspension was smeared on a slide, which was identified by study number, animal number, species, sex, material (in the test material group), and date of preparation, and allowed to dry overnight.

At least two slides per animal were prepared. The following day, the smears were stained using the panoptic stain method developed by PAPPENHEIM (6).

5.2.6 Microscopical Evaluation of the Slides : For each sampling time the bone marrow smears from the first 5 animals per sex and group were used for evaluation. One slide per animal was examined. The remaining smears of each sex and group per interval were evaluated if macroscopical examination of the first smears revealed technical imperfections which precluded accurate microscopical analysis.

From each animal one thousand polychromatic erythrocytes (PCE) were scored under the microscope (magnification 650-1000 x, C. ZEISS, D-7082 Oberkochen) for the incidence of polychromatic erythrocytes with micronuclei.

The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was calculated, based on 1000 erythrocytes (PCE + NCE) scored per slide as a measure of the toxic efficacy of the test material.

5.3 DATA RECORDING : The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including test material group, negative and positive controls. The micronucleated cells per thousand polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes are given for each animal examined (Tables 1-3).

5.4 STATISTICAL METHODS : The frequencies of polychromatic erythrocytes with micronuclei of the test material group and the positive control group were compared with those of the negative control group at each sampling time.

A POISSON test (7) was applied (Tables 4 and 5). The data from each treatment group for each sex, as well as for both sexes combined, were compared with the respective negative control group data using a VAX 8200 computer (Digital Equipment Corp., D-8000 München 81). The values of the first 5 animals of each sex and sampling time were used for statistical evaluation.

5.5 CRITERIA OF ASSESSMENT : If a test material produced no statistically significant and reproducible positive response at any one of the test points compared to the negative control group, it is considered non-mutagenic in this system (significance level: 5%; $p \leq 0.05$).

5.6 Protocol Adherence : The study was conducted in accordance with the original protocol inclusive amendments with the following exceptions:

- Denotation of sponsor and testing facility (due to organizational changes) and change of address of the testing facility.
- Assignment of female test material group animals to sampling times (see page 14, point 5.2.4).
- The centrifuge tubes (see page 15, point 5.2.5) were centrifuged at approx. 180 x g.

6. RESULTS

The following results have been compiled:

6.1 CLINICAL EXAMINATION

Animals of the test material group exhibited clinical signs as slight to moderate clonic convulsions (21/21 males, 21/21 females), stilted gait (1/21 males, 13/21 females), ruffled furs (4/21 males, 11/21 females), ptosis (4/21 females), sunken sides (12/21 males, 16/21 females), and slight to moderate hypokinesia (3/21 females). No test material group animal died.

Negative and positive control group animals did not show any abnormal clinical signs.

6.2 EVALUATION OF BONE MARROW

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight in males and 31.6 mg/kg body weight in females no significant test material-related increase of micronucleated polychromatic erythrocytes was observed in either male or female animals at each of the three examination times, 24, 48, and 72 hours after administration, respectively. This was also true for the evaluation of the values from both sexes combined at 24 and 48 hours. For the 72-hour sampling time a p-value of 0.048 was found, when the incidence of micronucleated polychromatic erythrocytes was compared between the test material group and the negative control group for both sexes combined. This value at the border of a statisstical significance is due to the particularly low incidence of micronucleated polychromatic erythrocytes in negative control animals of the 72-hour sampling time (mean: 0.4‰), which is clearly lower than historical negative control values in the Institute of Toxicology of ASTA Medica AG and also than given in the literature (8). The number of micronucleated polychromatic erythrocytes in test material treated males of this sampling time was not increased, when compared to other negative control group animals. Therefore, the statistical p-value of 0.048 (rounded 0.05) is considered not to be of relevance for the estimation of the study results.

No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding negative control group animals, with exception of one female (No. 338) at the 24-hour sampling time.

The positive control group animals receiving cyclophosphamide exhibited a significant and clear increase in the number of micronucleated polychromatic erythrocytes and thus validated the test. The dose used for this reference mutagen had a toxic effect on bone marrow as shown by the reduced PCE/NCE ratio at 48 hours (males) and 72 hours (males and females), respectively, after administration.

7. CONCLUSION

Under the experimental conditions reported, Quab 342 induced no chromosome mutation in mice by damage to the chromosomes or the mitotic apparatus, at 24-, 48-, or 72-hour intervals after the animals had received a single intraperitoneal dose of 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females). Therefore, Quab 342 is considered non-mutagenic in the re-

8. REFERENCES

1. OECD: OECD Guidelines for Testing of Chemicals, No. 474, Micronucleus Test (1983)

2. EEC: Commission directive of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (84/449/EEC). Off. J., L251, 27, 137-139 (September 19, 1984), Part B: Methods for the determination of toxicity, B.12. Micronucleus Test

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9. T A B L E S 1-5

TABLE 1 Number of Micronuclei, Negative Control Group

TABLE 2 Number of Micronuclei, Test Material Group

TABLE 3 Number of Micronuclei, Positive Control Group

TABLE 4 Statistical Evaluation (Males / Females)

TABLE 5 Statistical Evaluation (Both Sexes Combined)

TABLE 1

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Negative control group: Physiological saline solution (0.9%), intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
1	3	2.05	7	1	1.40	13	0	2.55
2	0	1.71	8	2	2.04	14	1	4.05
3	1	1.93	9	0	2.09	15	1	2.70
4	2	2.45	10	0	1.20	16	0	2.80
5	2	1.89	11	0	1.75	17	0	3.41
Mean	1.6			0.6			0.4	
SD	1.14			0.89			0.55	
N	5			5			5	
Females								
19	1	1.77	25	1	2.13	31	0	2.41
20	1	1.97	26	2	1.86	32	0	3.33
21	2	1.67	27	1	1.72	33	1	3.47
22	2	1.67	28	2	1.57	34	1	3.13
23	1	1.96	29	2	1.42	35	1	1.78
Mean	1.4			1.6			0.6	
SD	0.55			0.55			0.55	
N	5			5			5	
Mean M+F	1.5			1.1			0.5	
SD	0.85			0.88			0.53	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 2

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Test material group: Males : 21.5 mg/kg body weight QUAB 342, intraperitoneal route
Females: 31.6

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
301	1	2.24	308	3	1.48	315	2	2.37
302	0	1.79	309	0	1.16	316	0	2.68
303	1	1.08	310	0	1.38	317	3	3.24
304	1	1.32	311	1	1.03	318	2	1.85
305	0	2.48	312	0	1.40	319	1	2.42
Mean	0.6			0.8			1.6	
SD	0.55			1.30			1.14	
N	5			5			5	
Females								
322	2	0.60	324	3	2.42	332	0	1.80
323	0	1.16	325	0	1.57	333	1	2.64
330	2	0.72	326	3	0.86	334	2	4.75
337	0	0.95	327	0	1.75	335	2	2.83
338	1	0.47	328	2	1.29	336	0	3.02
Mean	1.0			1.6			1.0	
SD	1.00			1.52			1.00	
N	5			5			5	
Mean								
M+F	0.8			1.2			1.3	
SD	0.79			1.40			1.06	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 3

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Positive control group: 51.1 mg/kg body weight cyclophosphamide, oral by gavage

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
401	24	2.24	407	13	0.37	413	8	0.46
402	43	2.21	408	17	0.62	414	8	0.33
403	19	2.40	409	22	0.32	415	8	0.16
404	48	1.70	410	21	0.47	416	4	0.19
405	20	2.19	411	22	0.37	417	7	0.30
Mean	30.8			19.0			7.0	
SD	13.66			3.94			1.73	
N	5			5			5	
Females								
419	27	1.82	425	5	1.16	431	3	0.94
420	26	0.90	426	3	0.91	432	4	1.31
421	35	1.02	427	4	0.75	433	5	0.34
422	27	0.98	428	7	1.19	434	2	0.98
423	23	1.05	429	10	0.80	435	5	0.48
Mean	27.6			5.8			3.8	
SD	4.45			2.77			1.30	
N	5			5			5	
Mean M+F	29.2			12.4			5.4	
SD	9.73			7.66			2.22	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

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TABLE 4

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test)

	24 h		48 h		72 h	
	m	f	m	f	m	f
No. of Micronuclei						
verum	3	5	4	8	8	5
neg. contr.	8	7	3	8	2	3
test statistic F	0.3333	0.6250	1.0000	0.8889	2.6667	1.2500
degrees of freedom f_1, f_2	18, 6	16, 10	8, 8	18, 16	6, 16	8, 10
p-value	0.967	0.806	0.500	0.598	0.055	0.363
No. of Micronuclei						
pos. contr.	154	138	95	29	35	19
neg. contr.	8	7	3	8	2	3
test statistic F	17.1111	17.2500	23.7500	3.2222	11.6667	4.7500
degrees of freedom f_1, f_2	18, 308	16, 276	8, 190	18, 58	6, 70	8, 38
p-value	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 5

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test) for Both Sexes Combined

	24 h	48 h	72 h
	m + f	m + f	m + f
No. of Micronuclei			
verum	8	12	13
neg. contr.	15	11	5
test statistic F	0.5000	1.0000	2.1667
degrees of freedom f_1, f_2	32, 16	24, 24	12, 26
p-value	0.953	0.500	0.048
No. of Micronuclei			
pos. contr.	292	124	54
neg. contr.	15	11	5
test statistic F	18.2500	10.3333	9.0000
degrees of freedom f_1, f_2	32, 584	24, 248	12, 108
p-value	0.000	0.000	0.000

10. A T T A C H M E N T S 1-3

ATTACHMENT 1. Test Material

ATTACHMENT 2. Positive Control Material

ATTACHMENT 3. Diet, Composition

Attachment 1

Degussa

Data on Test Substance No. Study No.

Supplier : DEGUSSA AG, ZN Wolfgang
Dept. IC-ATPS
P.O.Box 1345, D-6450 Hanau 1

Test Substance/
Trade Name/Identity: Quab 342 (Lauryl-Quab)

Synonym : 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid

CAS No. : 41892-01-7

Batch No./Date of
Production : Charge 90/ Ma - 03

Physical Appearance: slightly coloured solid

Purity (Method)
Known Impurities : Purity: 85 %
Diquaternary salt: 8,6 %
Glycol: 2,55 % Chlorhydrin: 84,8 %

Solubility : ca 40 % in water

Density : ca 1g/ml

pH-Value : 3-6 (ca 40 % solution in water)

Storage : 0 - 10 °C

Stability : Provided the a.m. storage conditions the test substance is stable until january'91 (no measurable loss of activity within 2 years)

Hu - Wolfg. 19.10.90
Place, Date

Signature

1.2	~~GC~~ 4.9	~~IR/LC~~ 05 2.0	~~Zyto~~ 3.0	~~Pirk~~ 3.5	Pyr	PGW

P 6899 1002 A.-Nr. 37 6 1

CYCLOPHOSPHAMID BP 88/USP XXI Charge: 090029 K.Nr.: 20/30

Menge: 112.05 kg/~~Lt~~/~~Stck~~. Abt.: PGW Ko.St.: 6127

3 Gebinde/~~Paletten~~ hergest.n.Vorschr.Nr. 15 v. Jan. 9

Herst.Datum: 07.09.90 WEGA – AMK X

Cyclophosphamid
K-Nr.: ~~20/0111 = 090025~~
~~20/1351 = 090026~~ 7.9.90
Cyclophosphamid roh

Verf.-Datum:

3 Etiketten SOLL K-Nr.: 200921K # 060526 IST

1. Beschreibung:	Weißes Krist. Pulver	entspr.: ja
2. Identifizierung:		
2.1. IR-Spektrum:	entspr. d. Stand. 50240	entspr.: ja
2. HPLC:	entspr. d. Vorschrift	entspr.: ja
2.3. Chlorid: Gesamtchlor	entspr. d. Vorschrift	entspr.: ja
3. Reinheitsprüfungen:		
3.1. Klarlöslichkeit:	n.m.a. 3.5 FTU	0,2 FTU
3.2. Reaktion:	pH = 4.0 bis 6.0	pH = 4,9
3.3. Chlorid:	n.m.a. 330 ppm	40 ppm
3.4. Schwermetalle:	n.m.a. 20 ppm	entspr.: ja
3.5. Wassergehalt:	6.0 bis 6.8 %	6,5 %
3.6. Schmelzpunkt:	49.5 bis 52.0 °C	50,5 °C
4. Wirkstoffgehalt:	98.0 bis 100.5 % wfr	100,0 %
5. Restlösemittel:		
Isopropanol:	n.m.a. 500 ppm	101 ppm
Diethylether:	n.m.a. 10 ppm	5.2 ppm
6. Sterilprüfung:	entspr. d. Vorschrift	entspr.: ja am: 19
7. Pyrogenprüfung:	entspr. d. Vorschrift	entspr.: ja am: 20.
WI Filtertest 17 g/	liegt vor	ja
WI Partikelzählung:	liegt vor	ja 12.9.90

PROBEMENGE : 2 x 100 ml Braunglasflasche dicht verschlossen
PROBEZIEHUNG : PGW
AM: 07.09.90

für toxikologische Untersuchungen bis 31.12.1991

FREIGABE DURCH QI AM : 25 SEP. 90

27.9.90

QUALITÄTSKONTROLLE freigegeben Jenapharm

10 JAN 1991

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Attachment 3

s s n i f f M
Special Diet for Mice

Nutrients (in % Diet)

Raw Protein	21.00%	Calcium	1.20%
Raw Fat	3.50%	Phosphorus	0.80%
Raw Fiber	4.50%	Sodium	0.30%
Raw Ash	6.50%	Magnesium	0.20%
		Potassium	1.00%

Amino Acids (in 1 kg Diet)

Lysine	1.40%
Methionine	0.45%
Cystine	0.30%
Glycine	1.00%
Leucine	1.70%
Isoleucine	1.00%
Arginine	1.40%
Phenylalanine	1.00%
Tryptophane	0.35%
Histidine	0.50%
Alanine	1.00%
Tyrosin	0.70%
Asparagine Acid	1.60%
Glutamine Acid	3.50%
Valine	1.00%

Vitamins (in 1 kg Diet)

A	25,000 I.E.
D_3	2,500 I.E.
E	75 mg
B_1	20 mg
B_2	40 mg
B_6	15 mg
B_{12}	80 mcg
Biotin	250 mcg
Pantothenic Acid	50 mg
Choline	2,000 mg
Folic Acid	2 mg
Nicotinic Acid	150 mg
K_3	5 mg

Trace Elements (in kg Diet)

Manganese	75 mg
Copper	12 mg
Zinc	80 mg
Iodine	1 mg
Ferrum	380 mg
Fluorine	10 mg

Aliphatic Acids

Data available on request

Triage of 8(e) Submissions

Date sent to triage: AUG 10 1991 NON-CAP CAP

Submission number: 13022-A TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.):

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

parts of this study are written in German

For Contractor Use Only

entire document: 0 1 2 **pages** 1,2,3 **pages** 1,2,3, tabs

Notes:

Contractor reviewer : DMC **Date:** 8/1

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0594 - 13022 SEQ. A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Degussa Corporation

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 05/06/94 OTS DATE: 05/10/94 CSRAD DATE: 06/16/94

CHEMICAL NAME: 1-Dodecanaminium, N-(3-chloro-2-hydroxypropyl)-N,N-dimethyl-chloride - QUAB 342

CAS# 41892-01-7 → Lauryl QUAB

"

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

Atox/neur

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES (CONTINUE)	YES (DROP/REFER)	RAT RBT	LOW Acute oral/dermal toxicity		
	NO (DROP)	NO (CONTINUE)	In vitro	MED Dermal Irritation		
	DETERMINE	REFER:	MUS	HIGH		

COMMENTS: Non-Cap

8(e)-13022A
MEDIUM

Dermal irritation (observed during the acute dermal toxicity test) is medium concern based on eschar formation and reddening at the application site. Acute oral toxicity in the rat is low concern based on LD_{50} values of 3066 mg/kg (♀) and 2322 mg/kg (♂). Clinical signs included tremor, abnormal gait, convulsions, and loss of reflexes. Gross changes of the GI tract were observed. Acute dermal toxicity in the rabbit is low concern based on a calculated LD_{50} of 2523 mg/kg. Clinical signs included tremors and reduced body strength. Gross changes of the kidneys, liver, spleen, lungs, heart and small intestines were observed.

Degussa

Degussa
Corporation

8EHQ-0594-13022

8e

Contains No CBI

ORIGINAL

May 6, 1994

(A)

8EHQ-94-13022
INIT 05/10/94

RECEIVED
OPPT CBIC
94 MAY 10 AM 8:35

Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, S. W.
Washington, D.C. 20460

88940000264

Attn.: Section 8(e) Coordinator

Re: Substantial Risk Notification
Pursuant to TSCA Section 8(e)

Dear Sir:

This Substantial Risk Notification is being submitted in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) by Degussa Corporation. In response to a bona fide intent to import, we were informed that the substance was listed on the confidential portion of the TSCA Inventory (EPA Control No. 528400314).

The chemical name is 1-Dodecanaminium, N-(3-chloro-2-hydroxypropyl)-N,N-dimethyl-chloride (which we identify by the trade names QUAB 342 and Lauryl QUAB) CASRN 41892-01-7.

We have just been made aware of some toxicity studies which can be considered to be reportable under TSCA Section 8(e) because of certain neurotoxic effects. Although we do not believe that the results reasonably support the conclusion that the substance presents a reasonable risk of injury to the health or the environment, based on EPA's guidelines of June 1991, we are submitting them to the TSCA Section 8(e) Office.

A summary of each study and a printout from the STN Chemical Abstracts Service Registry File follows; copies of the studies are attached. Because the oral and dermal toxicity reports are in German, we have provided English translations of the summaries.

Acute Oral Toxicity Study in Rats

Dosage/route/duration: The test material was administered by gavage diluted and undiluted to male and female rats at a volume between 2.15 and 4.65 ml/kg. Post-exposure observation was 14 days.

RECEIVED
6/16/94

82pp

65 Challenger Road Ridgefield Park NJ 07660 201-641-6100

Results: Intoxication was characterized by disturbance of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colorless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

The LD_{50} value for female rats was 3066 mg/kg and for male rats 2332 mg/kg.

Acute Dermal Toxicity Study In Rabbits

Dosage/route/duration: The test material was administered undiluted to the dorsal skin of male and female rabbits at doses between 931 and 4321 mg/kg for 24 hours under occlusive conditions. The post-exposure observation was 14 days.

Results: Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discoloration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart and small intestine were observed.

The LD_{50} value for male and female rabbits was 2523 mg/kg.

In Vitro Ames Test

Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were tested with and without liver microsomal activation system (Arochlor induced rats) at concentrations between 1.0 to 216 micrograms per plate.

At a concentration of 46.4 microgram/plate a weak increase (< x 2) was observed in the test strain TA 1538 in the absence of S9-mix. At higher concentrations, the number of revertants were decreased. Cytotoxicity was observed at 46 microgram/plate and at higher concentrations.

In Vivo Micronucleus Test in Mice

21.5 mg/kg (males) or 31.6 mg/kg (females) of the test substance were administered intraperitoneal to mice. No significant increase in micronucleated polychromatic erythrocytes was observed in both male and female mice, respectively males and females combined, at 24, 48 or 72 hours after administration.

Sincerely,

John Lewinson

John Lewinson, Ph.D.
Manager, Product Regulatory Compliance

JL-94-178

cc: R. Marion, DCA
Dr. Pieter, DCRP
B. Santoro, DCRP

Contains No CBI

Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Lauryl-GUAR

Acute Toxicity
Testing the Acute Toxicity
after Single Oral Administration

REPORT
TRANSLATION

June 30, 1982

Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Summary

Lauryl-Quab was studied for acute toxicity after (single) oral administration in the rat.
The test substance, available as a liquid, was used as diluted aqueous solution (Aqua demin), or undiluted, with an increasing administration volume between 2.15 and 4.65 ml/kg.

The LD 50 values were for female rats 3.05 ml/kg (= 3066 mg/kg)
and for male rats 2.32 ml/kg (= 2332 mg/kg)

Intoxication was characterized by disturbances of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colourless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

ASTA WERKE 4800 Bielefeld 14
AKTIENGESELLSCHAFT
Toxikologisches Institut Degussa-Asta

Bericht Nr.: Ind.-TOX-79-81/82

DEGUSSA AG - US-IT - NR.
82 0086 DKT

BERICHT

über die toxikologische Prüfung von Lauryl-QUAB nach einmaliger oraler Gabe an der Ratte

Acute Oral Rat

30. Juni 1982

Versuchsbeginn	:	05.01.1982
Ende des tierexperimentellen Teiles	:	18.01.1982
Versuchsdurchführende Einrichtung	:	Toxikologisches Institut Degussa-Asta Artur-Ladebeck-Str. 128 - 152 D-4800 Bielefeld 14
Versuchsleiter	:	Dr. med. vet. H.-J. Zechel Fachtierarzt für Pharmakologie und Toxikologie
Pathologie und Laboratoriums-Diagnostik	:	--
Archivaufsicht	:	E. Fichtner (QS)
Berichtsdurchsicht	:	E. Fichtner (QS)

Zusammenfassung

Lauryl-QUAB wurde zur Bestimmung seiner akuten Toxizität oral an der Ratte verabfolgt. Das in flüssiger Form vorliegende Prüfprodukt kam in verdünnter wässriger Lösung (Aqua demin) sowie unverdünnt in steigendem Applikationsvolumen zwischen 2,15 und 4,65 ml/kg zur Anwendung.

Die erhobenen DL 50 - Werte betrugen für die weiblichen Tiere 3,05 ml/kg (≙ 3066 mg/kg) und für die männlichen Tiere 2,32 ml/kg (≙ 2332 mg/kg).

Im Vergiftungsbild standen Störungen des vegetativen Nervensystems und des Allgemeinbefindens (Zittern, Stelzengang, eingezogener sowie aufgeblähter Bauch, Speichelfluß, gesträubtes Fell, Durchfall, Abmagerung) im Vordergrund. Prämortal kam es teilweise zu Reflexausfall, Krämpfen, Mydriasis und Dyspnoe. Der Vergiftungsverlauf gestaltete sich akut bis protrahiert.

Makroskopisch-anatomisch wurden bei den verstorbenen Tieren Flüssigkeitsansammlungen, teils von klarem Aussehen, teils von roter oder gelber Farbe, in Magen und Darm festgestellt. Die Wandung dieser Organe wies Rötung auf. In einem Fall lag eine Perforation der Magenwand vor. Vereinzelt wurden Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Einleitung

Nachfolgend wird über die toxikologische Prüfung von Lauryl-QUAB berichtet.

Die Untersuchung erfolgte im Auftrage des Fachreferates für Industrielle Toxikologie der Degussa, Frankfurt/M. Sie wurde nach einmaliger oraler Gabe des Prüfproduktes an der Ratte durchgeführt.

Zweck der Studie war es, eine Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes vorzunehmen. Die erhobenen Befunde werden durch Angaben zur DL 50 ergänzt.

Lauryl-QUAB lag als farblose Flüssigkeit vor und kam tierexperimentell unverdünnt und verdünnt zur Anwendung.

Methodik

1. Versuchstiere

Spezies	:	Ratte
Stamm	:	Wistar (Bor: WISW)
Züchter	:	Winkelmann, Borchen
Geschlecht	:	♂ und ♀ Tiere
Gewicht	:	♂ 127 - 147 g; ♀ 127 - 141 g
Alter	:	♂ 57 - 62 Tg.; ♀ 57 - 58 Tg.
Eingewöhnungszeit vor Testbeginn	:	5 Tage
spezielle Angaben	:	siehe Versuchsdurchführung unter Pkt. 4

2. Versuchstierhaltung

Haltung	:	Makrolonkäfige Typ III
Tiere pro Käfig	:	1
Einstreu	:	Weichholzgranulat Typ 9 ALTROMIN®
Fütterung	:	Standardisiertes Versuchstierfutter ALTROMIN®
Tränkung	:	Wasser ad libitum
Raumtemperatur	:	$21^{o} \pm 2^{o}$ C
Relative Luftfeuchtigkeit	:	50 - 60 %
Raumbeleuchtung	:	12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. Dunkelheit
Raumhygiene	:	Labor- und Käfigreinigung mittels handel üblicher Antiseptika, Instrumente- und Geräte-Desinfektion mittels Autoklav und Heißluftsterilisator

3. Prüfsubstanz (spezielle Angaben)

Chemische Bezeichnung	:	Lauryl-QUAB
Chargen-Nr.	:	
Form und Aussehen	:	klare Flüssigkeit
Gehalt/Reinheit	:	27,87 %
Löslichkeit	:	Herstellg. verdünnter Lösungen in Aqua demir
Dichte	:	1,007 g/ml
pH-Wert	:	7,1

Verdünnungsfaktoren 1,47 und 2,15 Die entspr. Konzentrationen betrugen 685 mg/kg und 468 mg/kg be Annahme einer Ausgangskonz. von 1007 mg/ml.

4. Versuchsdurchführung

Futterentzug	:	16 Std. vor Versuchsbeginn
Substanz-Applikation	:	oral mittels Schlundsonde
Dosenabstand	:	in dezimalgeometrischer Folge
Faktor	:	1,47 bzw. 2,15
Beobachtungszeit	:	14 Tage
Injektions-/Applikationsvolumen	:	2,15 - 4,64 ml/kg; die Substanz kam verdünnt und unverdünnt zur Anwendung
Tiere pro Dosis	:	5
Anzahl Dosierungsgruppen	:	4 bei ♂ und 3 bei ♀
Anzahl Tiere zur Berechnung eines DL 50 - Wertes	:	20 ♂ und 15 ♀
Gesamtzahl der im Versuch verwendeten Tiere	:	35

5. Versuchszweck

Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes; [3]

Berechnung der DL 50 (Probitanalyse) [1,2,4,]

Ergebnisse

Lauryl-QUAB wurde zur Ermittlung seiner akuten Toxizität oral an der Ratte verabfolgt. Das Prüfprodukt lag in flüssiger Form vor und kam verdünnt mit Aqua demin und unverdünnt in steigenden Applikationsvolumina zwischen 2,15 und 4,64 ml/kg zur Anwendung. Bei der Berechnung der Dosen (mg/kg) wurde eine Dichte von 1,007 g/ml zugrunde gelegt. Der Dosenbereich erstreckte sich zwischen 1007 und 4672 mg/kg.

Das Vergiftungsbild war vorwiegend durch Symptome wie Zittern, Stelzengang, schleppender Gang, Speichelfluß, gesträubtes Fell, Durchfall und eingezogener bzw. aufgeblähter Bauch geprägt, was auf eine Störung des vegetativen Nervensystems hinweist. Darüber hinaus zeigten die männlichen Tiere während der 1. Beobachtungswoche nur eine mäßige Gewichtsentwicklung. Bei einigen Tieren war eine Zyanose zu beobachten. Prämortal kam es bei den weiblichen Tieren der höchsten Dosierungsgruppe zu vollständigem Reflexausfall, starken klonischen Krämpfen, Mydriasis und Dyspnoe.

Der Vergiftungsverlauf ist als akut bis protrahiert zu bezeichnen. Erste klinische Symptome traten 20 Minuten nach Substanzgabe auf und währten bei den Überlebenden maximal bis zu 1 Woche. Letalität stellte sich überwiegend während der ersten beiden Tage p. appl. ein, weitere Todesfälle traten noch in den folgenden 3 Tagen auf.

Bei der Sektion der verendeten Tiere kam es in Abhängigkeit von der verabfolgten Dosis zu einer zunehmenden Rötung von Magen- und Darmwand. Diese Organe wiesen, bedingt durch Flüssigkeitsansammlungen in ihrem Lumen (teils klar, teils von roter und gelber Farbe) eine pralle Füllung auf. Bei einem männlichen Tier wurde eine Perforation der Magenwand diagnostiziert. Dieser Befund steht nicht im Zusammenhang mit einem unphysiologisch hohen Applikationsvolumen; er muß als eine Folge der Substanzeinwirkung angesehen werden. In einzelnen Fällen wurden auch Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Die DL 50 - Werte sind in Tabelle 1a und 1b wiedergegeben.
Der zahlenmäßige Unterschied zwischen den DL 50 - Werten der weiblichen und männlichen Tiere ist statistisch nicht signifikant. Er trägt zufälligen Charakter.

In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

DL 50 - Werte von Lauryl-QUAB

Spezies : Ratte
Applikation : oral

Tabelle 1a

DL 50 [ml/kg]	
♀	2,32 (1,58 - 3,39)
♂	3,05 (2,25 - 4,13)

Tabelle 1b

DL 50 [mg/kg]	
♀	2372 (1593 - 3414)
♂	3066 (2261 - 4159)

Akute Toxizität DL 50 - Bestimmung
(Absterbedaten)

tanz/Präparat : Lauryl-QUAB
likationsart : oral

Spezies : Ratte
Stamm : Wistar (Bor: W.

Dosis mg/kg]	Geschlecht	Todes-rate		Überlebenszeit																		
				Std. nach Applikation						Tage nach Applikation												
		x/n	%	0,5	1	2	4	8	24	2	3	4	5	6	7	8	9	10	11	12	13	14
1473	♂	0/5	0																			
2165		1/5	20										1									
3182		2/5	40						1		1											
4672		5/5	100						2	3												
1007	♀	0/5	0																			
2165		2/5	40						2													
4672		5/5	100				3		1			1										

Literatur

1. BLISS, C.J.,The Statistics of Bioassay, Academic Press, New York, 1962

2. FINNEY, D.J., Probit Analysis, 2nd Ed., Cambridge Univ. Press, Cambridge 1952

3. HUNTER, W.J., Lingk, W. and Recht, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem. 62, 864 - 873, 1979

4. WEBER, E., Grundriß d. biol. Statistik, G. Fischer-Verlag, Stuttgart, 7. Auflage, 1972

Bielefeld, den 30.06.1982

Dr. med. vet. H.-J. Zechel
Fachtierarzt für Pharmakologie
und Toxikologie

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid
(Lauryl QUAB)
QUAB 342

Acute Toxicity after a Single Dermal Administration in Rabbits

R E P O R T
TRANSLATION

July 29, 1986

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

Summary:

Quab 342 was applied once * to the dorsal skin area of rabbits to determine its acute toxicity. The test substance, available as a colourless liquid, was used undiluted.

Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discolouration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart, and small intestine were oberserved.

The LD_{50} values were

probit analysis:
for male and female animals 2523 mg/kg (2.46 ml/kg)

graphical estimation:
for male animals 3072 mg/kg (3.0 ml/kg)
for female animals 1946 mg/kg (1.9 ml/kg)

* remark of the translater: (occlusive for 24 hours)

DEGUSSA AG - US-IT - NR.
86 0158 DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid (Lauryl QUAB) QUAB 342

Akute Toxizität

Prüfung der akuten Toxizität nach einmaliger dermaler Applikation am Kaninchen

B E R I C H T

Acute Dermal Rabbit

29. Juli 1986

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Der Bericht umfaßt 12 Seiten.

1. ALLGEMEINES

Auftraggeber	: DEGUSSA AG / ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Prüfeinrichtung	: ASTA-WERKE AG Chemische Fabrik Toxikologie Degussa-Asta Artur-Ladebeck-Str. 128-152 D-4800 Bielefeld 14
Prüfsubstanz	: QUAB 342
Art der Prüfung	: Prüfung der akuten Toxizität nach einmaliger dermaler Gabe am Kaninchen.
Prüfrichtlinie/-n	: OECD Guideline Nr. 402 (1)
Berichts-Nummer	: Ind.-TOX-508-85/86
Zeitplan	: Beginn: 23.10.84 Ende : 18.12.84
Prüfleiter	: Dr. rer. nat. W. Mayr
Durchführung der Studie	: R. Iselt
Berichtsdatum	: 29.07.1986

2. ZUSAMMENFASSUNG

QUAB 342 wurde zur Bestimmung seiner akuten Toxizität einmalig dermal auf die Rückenhaut des Kaninchens aufgetragen. Das Prüfprodukt lag als farblose Flüssigkeit vor und kam unverdünnt zur Anwendung.

Das Vergiftungsbild war gekennzeichnet durch Zittern, gesträubtes Fell und körperliche Schwäche. Das Vergiftungsbild ist als protrahiert zu bezeichnen. Todesfälle traten zwischen 2 und 7 Tagen p. appl. auf. Die Applikationsstellen zeigten Graufärbung, Rötung und Schorfbildung.

Bei der Autopsie waren nur bei den weiblichen Tieren unspezifische Veränderungen an Niere, Leber, Milz, Lunge, Herz und Dünndarm zu beobachten.

Die ermittelten LD 50-Werte betragen:

Probitanalyse:

männliche und weibliche Tiere 2523 mg/kg (2,46 ml/kg)

graphische Abschätzung:

männliche Tiere:	3072 mg/kg (3,0 ml/kg)
weibliche Tiere:	1946 mg/kg (1,9 ml/kg)

Bielefeld, den 29.07.1986

Prüfleiter:

Dr. rer. nat. W. Mayr

Institutsleiter:

7.8.86

Dr. med. vet. M. Herbst

3. EINLEITUNG

Nachfolgend wird über die toxikologische Prüfung von QUAB 342 nach einmaliger dermaler Gabe am Kaninchen berichtet.

Ziel der Studie war Art, Intensität und Dauer der klinischen Symptome sowie Todeszeitpunkte und Zahl der verstorbenen Tiere festzustellen (1 - 2). Die Beobachtungsdauer betrug 14 Tage. Aus den Mortalitätszahlen wurde die LD 50 ermittelt (3 - 5).

Das methodische Vorgehen basierte auf der OECD-Richtlinie Nr. 402 (1).

4. PRÜFSUBSTANZ

Prüfsubstanz /Handelsname	: QUAB 342
Chemische Bezeichnung /Synonym	: 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
CAS-Nr.	: ---
Chargen-Nr.	: 342 M 19
Form und Aussehen	: farblose Flüssigkeit
Gehalt/Reinheit	: ---
Löslichkeit	: ---
Dichte	: 1,024 g/ml
pH-Wert	: 1,5
Aufbewahrung und Stabilität	: Die Prüfsubstanz wurde bei Raumtemperatur aufbewahrt. Die Stabilität der Prüfsubstanz wurde vom Auftraggeber gewährleistet.

5. METHODIK

5.1 Prüfsystem

5.1.1 Versuchstier-Spezies	: Kaninchen
Stamm	: Weiße Russen (Albino)
Herkunft/Züchter	: Asta-Werke AG, D-4800 Bielefeld 14
Begründung der Versuchstierwahl	: Das Prüfsystem wurde aufgrund internationaler Empfehlungen gewählt. Danach ist die Spezies Kaninchen geeignet, potentielle toxische Wirkqualitäten von Prüfsubstanzen zu erfassen.

Alter der Tiere am Applikationstag	: männlich 9 - 12 Monate weiblich 9 - 10 Monate
Körpergewicht der Tiere am Applikationstag	: männlich 2,05 - 2,50 kg weiblich 2,40 - 2,75 kg
Eingewöhnungszeit	: Mindestens 5 Tage unter Prüfbedingungen vor Substanzapplikation

5.1.2 Versuchstierhaltung

Standort	: Toxikologie Degussa-Asta D-4800 Bielefeld 14, Gebäude B, Raum Nr. 214-5
Haltung	: Edelstahlkäfige Modell ASTA ASTA-WERKE AG, D-4800 Bielefeld 14
Tiere pro Käfig	: 1
Einstreu	: ohne, Drahtbodenkäfige
Futter	: Standardisiertes Versuchstierfutter ALTROMIN (R), Firma Altromin GMBH u. Co KG, 4937 Lage,
Trinkwasser	: Wasser ad libitum in Trinkwasserqualität der Stadtwerke Bielefeld, automatische Tränkung über Tränkventile
Raumtemperatur	: 22 +/- 2 Grad Celsius
Relative Luftfeuchtigkeit	: 55 +/- 15 %
Raumbeleuchtung	: 12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. natürlicher Hell- Dunkelrhythmus
Raumhygiene, Käfigreinigung	: Labor- und Käfigreinigung mittels handelsüblicher Antiseptika, Instrumente- und Gerätedesinfektion mittels Autoklav und Heißluftsterilisator.

5.1.3 Kennzeichnung der Tiere	: mittels Ohrnummer (Tätowierung), Käfigbeschilderung mit gleicher Nummer

5.2 Durchführung der Prüfung

Futterentzug	: am Behandlungstage
Substanzapplikation	: dermal, zwischen der geschorenen Schulter- und Fersenbeinregion Die Prüfsubstanz wurde mittels eines Patches unter Okklusiv-Bedingungen 24 Stunden lang mit der äußeren Haut in Kontakt gebracht. Nach Abnahme des Verbandes wurde die auf der Haut verbliebene Prüfsubstanz mit Wasser abgewaschen.
Begründung der Wahl der Applikationsart	: Die Prüfsubstanz wurde aufgrund der möglichen dermalen Exposition des Menschen in bezug auf seine Toxizität nach einmaliger oraler Applikation geprüft.
Häufigkeit der Applikation	: einmalige Gabe (24 Stunden-Patch)
Applikationsform	: unverdünntes Prüfprodukt
Konzentrationen	: ---
Applikationsvolumina	: m 0,909 - 4,22 ml/kg w 0,909 - 4,22 ml/kg
Dosenabstand	: in dezimalgeometrischer Reihenfolge
Faktor	: m 2,15 w 2,15
Anzahl der Dosisgruppen	: m 3 w 3
Tiere pro Dosisgruppe	: m 2 w 2
Gesamttierzahl	: 12
Beobachtungsdauer	: 14 Tage p. appl.

5.3 Beobachtungen

5.3.1 Verhalten und Allgemeinzustand der Tiere (klinische Vergiftungssymptome) : Beobachtung der Tiere am Applikationstag während 6-8 Stunden p. appl., danach täglich zweimal (vor- und nachmittags); an Samstagen, Sonn- und Feiertagen einmal.
Alle aufgetretenen Vergiftungserscheinungen sowie deren Beginn, Schwere und Dauer wurden protokolliert.

Mortalität : Todeszeitpunkt, Anzahl der verstorbenen Tiere pro Dosis

5.3.2 Pathologie : Alle gestorbenen bzw. am Ende der Beobachtunsperiode getöteten Tiere wurden obduziert und Äußeres, Körperöffnungen, Körperhöhlen (Brust- und Bauchhöhle) und deren Organe makroskopisch untersucht.

5.4 Bestimmung der LD_{50}-Werte : approximative Ermittlung, graphisch (6) sowie Probitanalyse (3 - 5)

6. ERGEBNISSE

Allgemeine Angaben

QUAB 342 wurde zur Ermittlung seiner akuten Toxizität dermal am Kaninchen verabfolgt. Die Prüfsubstanz lag als farblose Flüssigkeit vor und wurde unverdünnt appliziert. Unter Verwendung steigender Applikationsvolumina von 0,909 bis 4,22 ml/kg erstreckte sich der geprüfte Dosisbereich bei den männlichen und weiblichen Tieren zwischen 931 und 4321 mg/kg. Die Dosisangaben berücksichtigen eine Dichte von 1,024 g/ml.

Klinische Beobachtungen

Das Vergiftungsbild war gekennzeichnet durch mäßiges bis starkes Zittern, gesträubtes Fell sowie körperliche Schwäche.

Der Vergiftungsverlauf ist als protrahiert zu bezeichnen. Erste Vergiftungssymptome waren nach 1 bis 6 Tagen zu beobachten und dauerten bis zu 9 Tagen. Todesfälle traten zwischen 2 und 7 Tagen nach Substanzapplikation auf.

Nach Abnahme des Patches waren bei allen Tieren die behandelten Hautareale grau gefärbt und von lederartiger Beschaffenheit; zusätzlich waren Rötung und Schorfbildung zu beobachten.

Pathologie

Bei der Sektion war bei einem weiblichen Tier der hohen Dosisgruppe die Konsistenz der Milz erhöht und brüchig, die Leber marmoriert und die Lunge dunkel gefleckt. Geröteter Dünndarm, weißgefleckte Nieren weißes Endokard war bei einem weiblichen Tier der mittleren Dosisgruppe zu beobachten.

Statistik

Der LD 50-Wert wurde für beide Geschlechter graphisch nach Miller und Tainter (6) abgeschätzt und für beide Geschlechter zusammen mit der Probitanalyse ermittelt.

Die Werte sind in Tabelle 1 a und 1 b aufgeführt.
In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

Tabelle 1 Akute Toxizität - LD_{50}-Werte

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Tabelle 1a Graphische Abschätzung der LD 50 nach Miller und Tainter (6)
Vertrauensgrenze: 68,3 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche Tiere:	3,0 (0,355 - 25,322)	3072 (364 - 25930)
weibliche Tiere:	1,9 (0,303 - 11,925)	1946 (310 - 12211)

Tabelle 1b Rechnerische Bestimmung der LD 50 mittels Probitanalyse (3 - 5)
Vertrauensgrenze: 95 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche und weibliche Tiere:	2,46 (0,764 - 41,470)	2523 (782 - 42466)

Tabelle 2 Akute Toxizität - Absterbedaten

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Dosis (ml/kg)	Todes-rate x/n	%	Überlebenszeit Std. n. Applikation 0,5	1	2	4	8	24	Tage nach Applikation 2	3	4	5	6	7	8	9	10	11	12	13	14
männliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50												1							
4,22	1/2	50									1										
weibliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50								1											
4,22	2/2	100							1												

8. LITERATUR

1. OECD-Guideline for Testing of Chemicals, Acute Dermal Toxicity, No. 402, OECD Paris (1981), ISBN-92-64-12221-4

2. HUNTER, W. J., LINGK, W. and RECHT, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem 62, 864 - 873, 1979

3. BLISS, C. J., The Statistics of Bioassay, Academic Press, New York, 1962

4. FINNEY, D. J., Probit Analysis, 2nd Ed., Cambridge Univ. Press, Cambridge 1952

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6. MILLER, L.C. and TAINTER, M.L. Proc. Soc. Exp. Biol. Med. 57, 261 (1944)

RESEARCH & CONSULTING COMPANY AG

DEGUSSA AG - US-IT - NR.
85 0129 DKM

PROJECT 047665

Ames Test

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

WITH

QUAB 342

REPORT

P.O. Box 4452 Itingen/Switzerland
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PREFACE

GENERAL

Sponsor:	DEGUSSA AG ZN Wolfang Rodenbacher Chaussee 4 D 6450 Hanau 1
Study Monitor:	Dr. O. Weiberg
Testing Facility:	R C C Research & Consulting Company AG CH - 4452 Itingen / Switzerland
RCC Project No.:	047665
Test Article:	QUAB 342
Title:	Salmonella/Mammalian-Microsome Mutagenicity Test with QUAB 342

SCHEDULE

Receipt of Test Article:	April 15, 1985
Start of Experiments:	May 28, 1985
Date of Completion	June 17, 1985
Proposed Date of Final Report:	June 27, 1985/hac

PROJECT STAFF

Study Director: Dr. N. Banduhn, Dipl. Biol.

Date: July 3, 1985

Managing Director: Dr. K. Sachsse

Date: July 3, 1985

Technical Coordinators: Mrs. M. Maurer
Mrs. J. Gloor

GUIDELINES

This study follows the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella Typhimurium, Reverse Mutation Assay", adopted May 26, 1983.

EEC Directive 84/449, Method No. B 14.

SUMMARY

This study was performed to investigate the potential gene mutagenic activity of QUAB 342 according to the plate incorporation test of Ames et al. (1) using the Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

The test was performed with and without liver microsomal activation. The test article was tested at the following concentrations: 1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate. Each concentration, including the controls, was tested in triplicate.

Toxic effects of the test article were observed in the toxicity experiments by a reduction of the relative survival rate.
Additionally, a reduction of the background lawn up to a clearing was observed. Reduced numbers of revertants were found in some strains at the highest concentration tested. The toxic effects were more prominent in the experiments without metabolic activation than in the experiments with metabolic activation.

Up to the highest investigated dose, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538 in both experiments, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. when tested without liver microsomal activation. In the first experiment, the revertant rate was increased but not doubled versus the control. In the second experiment, there was a dose-dependent increase with an exact doubling of the revertant rate at a concentration 46.4 mcg/pl.

CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay.

1 OBJECTIVES

1.1 PURPOSE

This in vitro experiment was performed to assess the potential gene mutagenic activity of QUAB 342.
The standard quality control principles of "Wegleitung der IKS (Interkantonale Kontrollstelle fuer Heilmittel) betreffend Gute Laborpraxis fuer nichtklinische Laborversuche" from April 28, 1980 (amended June 23, 1982), Berne/Switzerland were applied.

1.2 RATIONALE

The test procedure was according to Ames et al. (1)
The microbial assay is based on reverse mutation of Salmonella typhimurium from auxotrophism (histidine dependent) to prototrophism (histidine independent). When mutated Salmonella typhimurium are exposed to a mutagen, mutation to the histidine independent form takes place in a proportion of the bacterial population. These are readily detectable due to their ability to grow on a histidine deficient medium.

Exposure of strains of Salmonella typhimurium to a chemical mutagen may produce revertants which arise as a result of a base-pair substitution in the genetic material or as a frameshift mutation in which genetic material is either added, deleted or mis-coded.

Since many compounds do not exert their mutagenic activity until they have been metabolized by enzyme systems not available in the bacterial cell, the test article and the bacteria were incubated both in the absence and in the presence of a cofactor-supplemented post-mitochondrial fraction prepared from the livers of rats treated with enzyme-inducing agents Aroclor 1254.

To validate the test, reference mutagens were tested parallel to the test article.

2 MATERIALS AND METHODS

2.1 TEST ARTICLE

Identification	QUAB 342
Batch/Lot number	040485-342 MA 54
Chemical denomination	3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
Purity	40.01% (cf. Certificat of analysis, April 3, 85)
Physical appearance	colorless, clear fluid
Stability	not available from the sponsor
Storage	The test article was stored at room

38

To avoid any light effects on the test compound, all experiments were performed under yellow light.

On the day of the experiment, the test article was dissolved in bidistilled water at the highest investigated dose. The other doses were dilutions from this stock solution with the solvent in third-log intervals.
The test article was investigated at the following concentrations:
1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate (mcg/pl.).

The highest concentration was limited by the cytotoxicity of the test article to bacteria. A preliminary test on strains TA 1537 and RTA of Salmonella typhimurium resulted in a strong toxic effect at a concentration of 500 mcg/pl. and higher

2.2 CONTROLS

All control data were established on the same day as the experiment.

Negative Control:
Bidistilled water, solvent of the test article.

Positive Controls:
Reference mutagens not requiring microsomal drug-metabolizing enzyme.

Methyl methanesulfonate*	500 mcg/pl.	for TA 100
9-Aminoacridine*	40 mcg/pl.	for TA 1537
2-Nitrofluorene*	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538
N-Ethyl-N-Nitro-N-Nitrosoguanidine*	10 mcg/pl.	for TA 98
		TA 100
		TA 1535

Reference mutagens requiring microsomal drug-metabolizing enzyme

2-Aminoanthracene*	0.5 mcg/pl	for TA 98
		TA 100
		TA 1538
	1 mcg/pl	for TA 1535
	10 mcg/pl	for TA 1537
Benzo-(A)-pyrene**	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538

Aseptic Control:

An experiment with the S-9 mix was performed (cf. study procedure)

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* Aldrich; Chemie Brunschwig, 4000 Basel/Switzerland
** Fluka, 9470 Buchs/Switzerland

2 3 TEST SYSTEM

Bacterial test organisms:

Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538; obtained from B.N. Ames, University of California, Berkeley CA, 94720, USA, May 1981.

Characterization of strains:

The strains used are mutants derived from Salmonella typhimurium LT2 and have following genotype:

S.typhimurium TA 1535 his G46 rfa- uvrB-
S.typhimurium TA 1537 his C3076 rfa- uvrB-
S.typhimurium TA 1538 his D3052 rfa- uvrB-
S.typhimurium TA 98 his D3052 rfa- uvrB- R+
S.typhimurium TA 100 his G46 rfa- uvrB- R+

All the strains carry a mutation known as deep rough (rfa) which affects the cell membrane so that the normal lipopolysaccharide cell coat is defective. These strains allow a greater permeability of the test agents across this membrane and into the cell.

In bacteria, almost all the primary damage caused to DNA by a mutagen is repaired by excision and recombination repair systems. Therefore, only a small percentage of the potential mutagenic damage is expressed. The tester strains used lack this excision repair system (uvrB), thus making them more sensitive to mutagens.

The strains TA 98 and TA 100 also carry an ampicillin resistance factor (R) which makes them particularly sensitive to mutagens.

The strains TA 1535 and TA 100 are sensitive to base-pair substitution mutagens; the strains TA 1537, TA 1538 and TA 98 are sensitive to frameshift mutagens.

The tester strains are checked at regular intervals for their genetic markers, according to published methods (1)

Storage:

The strain cultures are stored in sterile 0 5 ml ampoules (0 45 ml bacterial culture and 0.05 ml Dimethylsulfoxide) at -70 degrees centrigrade

Pre-culture of strains:

The bacteria were grown in a shaking water bath for 16 hours overnight at 37 degrees centigrade in 2.5% Nutrient Broth No. 2*. After centrifugation, the bacteria were resuspended to a concentration of approximately 1 x 10 exp. 8 to 2 x 10 exp. 9 cells per milliliter in 0.16% Nutrient Broth and 0.5% sodium chloride. The concentration of germs was controlled photometrically and determined in an experimental test with Histidine-rich potassium chloride solution on selective agar plates (cf. study procedure).

2.4 LIVER MICROSOMAL FRACTION S-9 MIX

The S-9 mix was prepared on the day of the experiment

Specific pathogen-free male Wistar rats (180 g - 300 g, KFM-Ha, outbred**), received a single i.p. injection of Aroclor 1254*** dissolved in Oleum Arachidis (200 mg/ml) at a dosage of 500 milligrams per kilogram of body weight to induce liver microsomal enzyme activity five days before the experiment.

The rats were killed by cervical dislocation and exsanguinated on the fifth day post application following a 14-16 hours starvation period.

Livers were removed under aseptic conditions and homogenized with ice-cold 0.15 molar potassium chloride (5 g of liver to 15 g of potassium chloride). The homogenates were centrifuged at 9000 x g for 10 minutes at 0 to 2 degrees centrigrade. The supernatant fraction (S-9 fraction) was collected for the preparation of S-9 mix

Composition of 1 ml S-9 mix.

Na2HPO4****	100	micro moles
MgCL2 ****	8	micro moles
KCl ****	33	micro moles
NADP *****	4	micro moles
G-6-P ******	5	micro moles
S-9 Fraction	0.3	ml

* Oxoid; Fakola, 4000 Basel/Switzerland
** Kleintierfarm Madoerin AG, 4414 Fuellinsdorf/Switzerland
*** Analabs; Kontron, 8000 Zuerich/Switzerland
(Aroclor: polychlorinated biphenyl mixture)
**** ABS, 4000 Basel/Switzerland
***** Boehringer Mannheim, 6330 Cham/Switzerland
****** Fluka, 9470 Buchs/Switzerland

2.5 STUDY PROCEDURE

For each strain and dose level, including the controls, a minimum of three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates*:

100 microliters (mcl)	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 mcl	S-9 mix (for test with metabolic activation) or 0.15 M Na_2HPO_4, (for test without metabolic activation),
100 mcl	Bacteria suspension (cf. test system, pre-culture of the strains),
2 ml	Overlay agar consisting of 0.6 % Bacto agar** and 0.6 % NaCl supplemented with 10 % 0.5 mM L-Histidine*** and 0.5 mM Biotine*** solution.

The plates were incubated at 37 degrees centigrade in the dark for 3 days. The his+ revertant colonies were counted with an Artek 880 counter or counted by hand if precipitation of test article precluded automatic counting.

For the aseptic control experiment, 100 microliters of S-9 mix were added to 2 milliliters overlay agar and treated as mentioned above.

For each test, the germ concentration of the bacterial strains was determined by pouring a mixture of 100 microliters of a 2 x 10 exp.5 diluted bacteria suspension (overnight culture) with a Histidine-rich potassium chloride solution onto a selective agar plate. After three days of incubation at 37 degrees centrigrade, the colonies were counted and the counts multiplied with the dilution factor.

---

* A Pourmatic (New Brunswick Scientific Comp.) was used to fill the plates with 22 ml of 1.5 % bacto agar in Vogel Bonner medium E with 2% Glucose.

** Chemie Brunschwig, 4000 Basel/Switzerland

*** Fluka, 9470 Buchs/Switzerland

2.6 TOXICITY TEST

To estimate the cytotoxicity of the test article, prototrophic bacteria (spontaneous revertants of TA 1537 = RTA) were added as an internal standard to the selective agar plates, together with TA 1537 (cf. study procedure). The difference in the number of colonies on plates with and without added prototrophic bacteria at each dose level of the test article was compared to the number of colonies obtained with the negative control. The ratio of the two values was expressed as relative survival rate with the negative control at a 100 % survival rate.

Additionally, the toxicity of the test article can be shown in the mutagenicity assay, by a reduction in the number of revertants or by a reduction or a clearing of the background lawn.

2.7 DATA RECORDING

The number of revertant colonies was recorded manually for each plate from the counter.
Individual plate counts and the mean number of revertant colonies per plate are presented in tabular form for the test article, positive and negative controls.

2.8 EVALUATION OF RESULTS

A material is identified as a mutagen in this test system if there is a reproducible demonstration of a dose effect relation with a 2-fold increase in the number of revertants over the controls in a minimum of one strain. With the strain TA 100, a 1.5-fold increase is the criterion for a positive result.

These criteria are generally in accordance with the international used standards for the evalutation of results obtained from the Ames Test (e.g. Ref. 1, 2, and 3).

3 RESULTS

In the current study, the following results have been compiled:

3.1 GERM CONCENTRATIONS

The concentrations of Salmonella typhimurium used in this assay were as follows:

FIRST EXPERIMENT	SECOND EXPERIMENT
TA 98 = 1.2 x 10 exp.9 cells/ml	= 1.0 x 10 exp 9 cells/ml
TA 100 = 8.0 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1535 = 1.4 x 10 exp.9 cells/ml	= 9.9 x 10 exp 8 cells/ml
TA 1537 = 9.5 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1538 = 2.1 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments without metabolic activation,

TA 98 = 1.8 x 10 exp.9 cells/ml	= 9.4 x 10 exp 8 cells/ml
TA 100 = 6.8 x 10 exp.8 cells/ml	= 7.3 x 10 exp 8 cells/ml
TA 1535 = 1.1 x 10 exp.9 cells/ml	= 1.8 x 10 exp 9 cells/ml
TA 1537 = 1.4 x 10 exp.9 cells/ml	= 4.9 x 10 exp 8 cells/ml
TA 1538 = 1.8 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments with metabolic activation.

3.2 TOXICITY OF THE TEST ARTICLE

In the toxicity experiment with QUAB 342, a reduction of the relative survival rate was observed without metabolic activation starting at 46.4 mcg/pl and with metabolic activation at a concentration of 216 mcg/pl.
Additionally, a reduction up to a clearing of the background lawn was observed starting from 46.4 to 100 mcg/pl and reduced numbers of revertants were found in some strains at the highest concentrati (216 mcg/pl.) tested.

3.3 CONTROL EXPERIMENTS

Control plates with the solvent (negative control) showed numbers of spontaneous revertant colonies per plate which were within the normal range of those cited in the literature (1)

Control plates with reference mutagens (positive controls) showed a distinct increase of the revertant colonies with the tester strains. This confirms the reversion properties of each strain. The positive results of the mutagens 2-Amino-anthracene and Benzo(a)pyrene indicate that the metabolizing system was functioning.

3.4 TESTS WITH QUAB 342

In the described bacterial mutagenicity tests, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 at all dose levels tested when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. in both experiments when tested without liver microsomal activation. In the first experiment, the revertant rate was increased approximately 68 % versus the control. In the second experiment, the number of revertants increased starting at 10 mcg/pl. and reached an exact doubling versus the control at a concentration of 46.4 mcg/pl.

4 CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activiation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay

5 REFERENCES

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6 ARCHIVING

R C C, CH - 4452 Itingen/Switzerland

Raw data, protocol, report, duplicate of report, and test article reference sample.

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	25		123		28		9		13	
	24		129		34		9		22	
	33	27	138	130	25	29	11	10	22	19
1.0 mcg	32		137		24		11		16	
	24		132		25		6		17	
	24	27	161	143	24	24	8	8	18	17
2.16 mcg	26		126		28		12		18	
	24		139		24		11		28	
	18	23	127	131	34	29	9	11	20	22
4.64 mcg	20		125		25		11		15	
	21		138		35		8		21	
	18	20	157	140	21	27	10	10	20	19
10.0 mcg	23		142		26		9		13	
	24		140		19		8		26	
	30	26	141	141	35	27	4	7	19	19
21.6 mcg	27		143		23		10		25	
	29		144		27		13		30	
	21	26	125	137	25	25	5	9	23	26
46.4 mcg	27		120		29		12		32	
	30		122		29		7		29	
	29	29	116	119	24	27	15	11	34	32
100.0 mcg	38		127		26		13		27	
	36		95		20		6		32	
	32	35	122	115	19	22	12	10	15	25
216.0 mcg	27		56		17		15		22	
	32		37		14		12		10	
	10	23	28	40	12	14	11	13	13	15
MMS 500 mcg	---		485		---		---		---	
	---		432		---		---		---	
	---	---	479	465	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		35		---	
	---		---		---		32		---	
	---	---	---	---	---	---	43	37	---	---
2-NF 5 mcg	493		486		---		44		830	
	509		443		---		47		882	
	500	501	367	432	---	---	54	48	886	866
ENNG 10 mcg	72		425		173		---		---	
	79		551		99		---		---	
	60	70	496	514	147	140	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE, WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	41		125		16		35		35	
	37		95		19		48		34	
	36	38	124	115	20	18	37	40	38	36
1.0 mcg	46		115		17		30		24	
	43		122		18		40		39	
	47	45	110	116	16	17	37	36	36	33
2.16 mcg	39		93		26		35		40	
	39		109		23		35		31	
	55	44	127	110	20	23	36	35	31	34
4.64 mcg	44		134		21		41		25	
	40		123		26		30		40	
	47	44	113	123	22	23	30	34	34	33
10.0 mcg	31		122		35		36		35	
	40		125		29		32		40	
	47	39	136	128	22	29	29	32	27	34
21.6 mcg	46		129		18		40		29	
	46		109		21		43		39	
	49	47	99	112	15	18	35	39	28	32
46.4 mcg	47		118		19		31		33	
	43		107		12		32		43	
	40	43	117	114	22	18	36	33	34	37
100.0 mcg	45		113		18		36		34	
	36		119		15		29		37	
	55	45	136	123	19	17	14	26	39	37
216.0 mcg	41		72		25		47		44	
	58		58		10		26		33	
	53	51	61	64	21	19	33	35	38	38
2-AA 0.5 mcg	99		162		---		---		130	
	113		197		---		---		97	
	113	108	180	180	---	---	---	---	99	109
2-AA 1.0 mcg	---		---		89		---		---	
	---		---		81		---		---	
	---	---	---	---	65	78	---	---	---	---
2-AA 10.0 mcg	---		---		---		172		---	
	---		---		---		219		---	
	---	---	---	---	---	---	168	196	---	---
BaP 5 mcg	284		370		---		117		124	
	294		343		---		130		108	
	262	280	410	374	---	---	124	124	117	116

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	10	367	1.00
1.0 mcg	8	358	0.98
2.16 mcg	11	358	0.97
4.64 mcg	10	352	0.96
10.0 mcg	7	351	0.96
21.6 mcg	9	359	0.98
46.4 mcg	11	189	0.50
100.0 mcg	10	42	0.09
216.0 mcg	13	13	0.00

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	40	343	1.00
1.0 mcg	36	307	0.89
2.16 mcg	35	327	0.96
4.64 mcg	34	339	1.01
10.0 mcg	32	329	0.98
21.6 mcg	39	306	0.88
46.4 mcg	33	290	0.85
100.0 mcg	26	235	0.69
216.0 mcg	35	135	0.33

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	23		116		19		15		16	
	29		96		20		11		11	
	10	21	111	108	18	19	7	11	11	13
1.0 mcg	33		110		14		11		16	
	21		120		24		12		19	
	21	25	123	118	22	20	10	11	14	16
2.16 mcg	29		97		21		17		13	
	24		116		27		11		11	
	23	25	112	108	21	23	14	14	15	13
4.64 mcg	31		115		17		12		15	
	30		129		17		8		15	
	29	30	134	126	21	18	11	10	7	12
10 mcg	23		123		24		13		25	
	33		107		31		9		16	
	25	27	113	114	28	28	11	11	12	18
21.6 mcg	26		94		19		8		24	
	23		121		18		14		20	
	30	26	122	112	25	21	9	10	22	22
46.4 mcg	28		116		17		11		25	
	30		74		22		5		28	
	24	27	84	91	18	19	11	9	24	26
100 mcg	30		53		14		10		24	
	30		60		13		8		19	
	31	30	62	58	15	14	17	12	27	23
216 mcg	20		35		24		12		14	
	17		33		18		12		11	
	35	24	26	31	20	21	17	14	23	16
MMS 500 mcg	---		334		---		---		---	
	---		401		---		---		---	
	---	---	407	381	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		49		---	
	---		---		---		56		---	
	---	---	---	---	---	---	73	59	---	---
2-NF 5 mcg	470		383		---		57		859	
	461		357		---		53		864	
	476	469	393	378	---	---	48	53	764	829
ENNG 10 mcg	120		1103		1045		---		---	
	143		1145		681		---		---	
	133	132	885	1044	891	872	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	51		91		12		28		31	
	59		107		17		38		29	
	47	52	115	104	15	15	33	33	29	30
1.0 mcg	47		109		13		28		30	
	40		111		18		26		21	
	38	42	121	114	14	15	30	28	33	28
2.16 mcg	48		130		14		34		27	
	50		116		21		32		19	
	47	48	124	123	17	17	30	32	29	25
4.64 mcg	54		137		16		30		29	
	41		112		13		33		29	
	45	47	99	116	21	17	37	33	28	29
10 mcg	49		128		13		32		30	
	55		113		19		40		26	
	41	48	117	119	18	17	36	36	27	28
21.6 mcg	42		139		15		29		30	
	45		119		11		31		25	
	53	47	134	131	18	15	30	30	30	28
46.4 mcg	46		129		13		30		40	
	47		116		17		31		23	
	52	48	132	126	9	13	29	30	40	34
100 mcg	56		91		16		19		34	
	38		109		14		30		30	
	44	46	109	103	12	14	42	30	22	29
216 mcg	36		55		18		25		25	
	33		45		13		15		24	
	37	35	36	45	12	14	25	22	35	28
2-AA 0.5 mcg	73		---		---		---		99	
	76		172		---		---		---	
	---	75	172	172	---	---	---	---	72	86
2-AA 1.0 mcg	---		---		98		---		---	
	---		---		109		---		---	
	---	---	---	---	---	104	---	---	---	---
2-AA 10.0 mcg	---		---		---		136		---	
	---		---		---		146		---	
	---	---	---	---	---	---	150	144	---	---
BaP 5 mcg	135		257		---		---		84	
	124		---		---		95		81	
	138	132	270	264	---	---	94	95	---	83

--- Not determined

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	11	277	1.00
1.0 mcg	11	285	1.03
2.16 mcg	14	278	0.99
4.64 mcg	10	267	0.97
10 mcg	11	263	0.95
21.6 mcg	10	249	0.90
46.4 mcg	9	119	0.41
100 mcg	12	22	0.04
216 mcg	14	13	---

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	33	231	1.00
1.0 mcg	28	231	1.03
2.16 mcg	32	242	1.06
4.64 mcg	33	230	0.99
10 mcg	36	246	1.06
21.6 mcg	30	239	1.06
46.4 mcg	30	237	1.05
100 mcg	30	209	0.90
216 mcg	22	119	0.49

Study No.: 877858
Report

DEGUSSA AG - US-IT - NR.
92 0112 DGM

QUAB 342

Mouse Micronucleus Test
(Single Intraperitoneal Administration)

R E P O R T

July 15, 1992

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1. GENERAL INFORMATION

1.1 Study Specification

Sponsor	:	Degussa AG/ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Testing Facility	:	ASTA Medica AG Institute of Toxicology Kantstraße 2 D-4802 Halle-Künsebeck
Study Director, Veterinary Care, Pathology	:	Dr.med.vet. K. Krauser
Study Performance	:	Mrs. M. Beste, Mrs. A. Lagoudis
Bone Marrow Evaluation	:	Miss C. Barkey
Quality Assurance	:	K.E. Fichtner Representative: Dr. B. Wilker (formerly: Roos)
Test Material	:	QUAB 342
Objective	:	Testing the mutagenic properties (damage to the chromosomes and/or the mitotic apparatus)
Test Guidelines	:	OECD Guideline No. 474 (1) EEC Directive (84/449/EEC) (2)
Study Number	:	877858
Time Schedule	:	Protocol and 1 amendment Oct/04/1990 Day of Administration Oct/15/1990 Last Day of Bone Marrow Sampling Oct/18/1990 Date of Report Jul/15/1992
Quality Assurance	:	The study was performed according to the regulations for "Good Laboratory Practice" (3).
Archivation	:	The approved protocol, all raw data obtained in the course of the study, all bone marrow smears as well as a copy of the final report are kept in the archives of the Institute of Toxicology at least 30 years (starting with the report date). Afterwards the sponsor will decide on further use.

1.2 AUTHENTICATION

I, the undersigned. hereby declare that to the best of my knowledge the study was performed under my supervision in accordance with current Good Laboratory Practice (as laid down in the German Chemicals Act).

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

This report represents a true and accurate record of the results obtained.

Study Director : July 15/1992 K. Krauser
Dr.med.vet. K. Krauser

Report Review

Head of the
Institute of Toxicology : July 15, 1992
Dr.med.vet. W. Jahn

1.3 ASTA Medica AG
Corporate Quality Assurance
Section GLP/GCP

Quality Assurance Statement

The non-clinical laboratory study

QUAB 342
Mouse Micronucleus Test
(Single Intraperitoneal Administration)

performed at ASTA Medica AG, Bielefeld,

was inspected and audited for conformance to the principles of Good Laboratory Practice (GLP).

The dates of inspections and reports to study director and management are given below.

Phase of Study	Date of QA Inspection	Date of QA Report
Protocol Review	Oct. 15, 1990	
Experimental Period	Oct. 15, 1990 Oct. 16, 1990 Oct. 18, 1990	Oct. 19, 1990
(Evaluation)	Apr. 19, 1991	
(Statistics)	Jun. 24, 1991	Jun. 24. 1991
Final Report Audit	Jul. 15, 1992	Jul. 15, 1992

Date: July 29, 1992

Signed: K. E. Fichtner

2. SUMMARY

This in vivo micronucleus test in mice was performed to assess the potential mutagenic activity, induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus, according to SCHMID with the modifications of SALAMONE et al.

The experiment was performed with three groups, the negative and positive control groups, each containing 18 males and 18 females, and the test group, containing 21 males and 21 females.

Negative control group and test material group animals were given a single intraperitoneal administration of 10.0 ml/kg body weight. Positive control group animals received a single administration of 10.0 ml/kg body weight by oral gavage. No test material group animal died.

The negative control group received physiological saline solution (0.9%). The test material group animals received the maximum tolerated doses in respect of the micronucleus test, i.e. 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) of the test material QUAB 342 dissolved in aqua ad iniectabilia. The positive control group received 51.1 mg/kg body weight cyclophosphamide dissolved in physiological saline solution (0.9%).

QUAB 342 related toxic symptoms were registered in all test material group animals. The symptoms consisted in clonic convulsions, stilted gait, ruffled furs, ptosis, sunken sides, and hypokinesia.

Twenty-four (24), 48 and 72 hours after treatment, six mice per sex and control group and seven mice per sex of the test material group were sacrificed and bone marrow was removed from the femurs for examination.

For each sampling time five animals per sex and group were used for evaluation. One thousand polychromatic erythrocytes per animal were scored for micronuclei. The ratio of polychromatic to normochromatic erythrocytes was used to assess the toxicity of the test material by counting a total of 1000 erythrocytes.

The incidences of micronuclei were statistically analyzed by means of a POISSON test. Estimation and testing were performed at each sampling time for each treatment group and for each sex, as well as for both sexes combined.

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) no significant test material-related increase in micronucleated polychromatic erythrocytes was observed in both male and female animals, respectively males and females combined, when compared with corresponding negative control group animals at 24, 48, or 72 hours after administration. No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding control group animals, with the exception of one female at the 24-hour sampling time.

The positive control group animals, which received cyclophosphamide, exhibited a significant increase in the number of micronucleated polychromatic erythrocytes and thus validated the test system.

CONCLUSION

QUAB 342 at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) (single intraperitoneal administration) is non-mutagenic in the mouse micronucleus test under the described conditions.

3. INTRODUCTION

PURPOSE

This in-vivo experiment was performed to assess the potential mutagenic activity induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus.

RATIONALE

The test procedure was according to SCHMID (4) with the modifications of SALAMONE et al (5).

The micronucleus test is based on the principle that in many mammalian cells acentric chromatid and chromosome fragments induced by clastogenic agents are left behind in the anaphase stage of cell division. They are included in the daughter cells where they form micronuclei in the cytoplasm. These micronuclei persist in the erythrocyte after extrusion of the main nucleus and can be scored.

The object of the test is to detect an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated animals versus the controls. Differential staining allows micronuclei to be identified under the microscope (6).

To validate the test system, a reference mutagen is tested parallel to the test material.

4. MATERIALS

4.1 TEST MATERIAL

Identification	: QUAB 342 (see Attachment 1)
Chemical Name/Synonym	: 3-Chloro-2-hydroxypropyl-N,N,N-dimethyldodecyl-ammoniumchloride
CAS No.	: 41892-01-7
Batch No.	: 90 / Ma - 03
Physical Appearance	: Slightly coloured solid
Content/Purity	: 85%
Solubility in H_2O	: appr. 40%
pH Value	: 3-6 (appr. 40% solution in water)
Storage	: The test material was stored in a refrigerator.
Stability	: The stability of the test material was guaranteed by the sponsor under the given storage conditions throughout the experimental period (until January 1991).
Additional Information	: See attachment 1 and under substance No. 89784 on file in ASTA Medica AG.
Formulation of the Test Material	: The test material was freshly dissolved in aqua ad iniectabilia (Ampuwa(R), batch No. PM 1032 E6, supplied by Fresenius AG, D-6380 Bad Homburg).

4.2 POSITIVE CONTROL MATERIAL

Identification	: Cyclophosphamide (see Attachment 2)
Chemical Designation	: 2-(Bis-(2-chloroethyl)-amino)-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide monohydrate
Batch/Lot Number	: 090029
Physical Appearance	: White crystalline powder
Purity	: 100.0%
pH Value	: 4.9 (2% aqueous solution)
Storage	: The positive control material was stored at room temperature.
Stability	: The stability of the positive control material was guaranteed for the test period (until Dec. 31, 1991)
Formulation of the Positive Control Material	: The positive control material was freshly dissolved in physiological saline solution (0.9%, batch No. AEW 09D, supplied by Fresenius AG, D-6380 Bad Homburg).

4.3 NEGATIVE CONTROL MATERIAL

Identification	: Physiological saline solution (0.9%) (Fresenius AG, D-6380 Bad Homburg).
Batch/Lot Number	: AEW 09 D

5. METHODS

5.1 TEST SYSTEM

5.1.1 Species	: Mouse
Strain	: BOR: NMRI, (SPF Han)
Breeder	: Winkelmann, Versuchstierzucht GmbH & Co.KG., D-4799 Borchen
Justification for the Selection of the Test System	: Mice are recommended for micronucleus tests as the international standard.
Initial Age at Test	: 5 weeks
Initial Body Weight at Test	: males : 29 - 35 g females: 24 - 31 g

5.1.2 Husbandry

Housing	: ASTA Medica AG, Institute of Toxicology, D-4800 Bielefeld 14 Building B, Room No. 214-1 (negative control) and Room No. 214-2 (positive control and test material group)
Caging	: Macrolon cages, type II
Animals per Cage	: 1
Bedding	: Animal bedding-chips, Jelu-Werk, J. Ehrler Industriemehle, D-7092 Rosenberg/Württ. According to information from the manufacturer contaminant analyses of the bedding are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Diet	: Standard diet ad libitum, ssniff(R) M, "Special diet for Mice", supplied by SSNIFF Spezialdiäten GmbH, D-4770 Soest (composition of the diet see Attachment 3). According to information from the manufacturer contaminant analyses of the diet are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Water	: Water ad libitum in drinking water quality of the Stadtwerke Bielefeld (Municipal Works Bielefeld). Automatic watering system with drinking nipples. According to information from the Stadtwerke Bielefeld the water is investigated in appropriate intervals. Regarding these analyses, the contaminants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Room Temperature	: 22 ± 2 degrees centigrade
Relative Humidity	: 55% ± 15% Slight deviations from these values were present for a short period (approx. 4 hrs) the day before substance administration, because of a technical defect in the air-conditioning plant. These deviations are considered not having influenced the results of this study.
Room Lighting	: 6 a.m. - 6 p.m. CET artificial lighting 6 p.m. - 6 a.m. CET natural light/dark rhythm
Room Hygiene, Cage Cleaning	: The room was cleaned regularly with commercial antiseptics and the cages with the cage washing machine type HAMO-R-T-500D (supplied by Hamo AG, CH-Biel-Bienne, Data on the ingredients of the antiseptics used are on file in ASTA Medica AG.

5.1.3 Allocation and Identification of the Animals

Number of Animals	: 114 (57 males / 57 females)
Method of Randomization	: Animals were placed into the different groups using computer generated random numbers.
Identification of the Animals	: Different ear notching of the groups; cage labelling with study No., animal No., sex, material designation, sampling time and route of administration.
5.1.4 Acclimatization Time	: 5 days under test conditions with veterinary care.

5.2 EXPERIMENTAL DESIGN

5.2.1 Dose Selection : In an orientating acute toxicity study in mice after single intraperitoneal administration of 46.4 mg/kg (both sexes) and 31.6 mg/kg QUAB 342 (only males) severe symptoms of toxicity and death occurred, while at 21.5 mg/kg in males and 31.6 mg/kg in females clear symptoms of toxicity but no death were observed. Therefore 21.5 mg/kg (males) and 31.6 mg/kg (females) Quab 342 were considered to be the maximum tolerated doses.

5.2.2 Route of Administration : Intraperitoneal (test material, negative control material)
Oral by gavage (Nelaton catheter, red rubber, Charrière-No. 6, supplied by Willy Rüsch, D-7053 Rommelshausen/Stuttgart) (positive control material)

Justification for the Route of Administration : For methodical reasons and according to the test guidelines (1, 2) the intraperitoneal route of administration was chosen for negative control and test material group animals. For validation of the test system the positive control group animals were treated orally.

5.2.3 Administration Volume : 10.0 ml/kg body weight

5.2.4 Study Procedure : Three groups of mice, the negative and positive control groups containing each 18 males and 18 females and the test material group with 21 males and 21 females each received a single intraperitoneal administration or by oral gavage, respectively (diet withdrawal: 16 h before treatment).
Group 1, the negative control, received physiological saline solution (0.9%).
Group 2, the test material group, received 21.5 mg/kg body weight (males) and 31.6 mg/kg (females) of the test material. The test material was given as a freshly prepared solution in aqua ad iniectabilia.
Group 3, the positive control, received Cyclophosphamide (51.1 mg/kg b.w.) dissolved in physiological saline solution (0.9%).

The animals were observed for the first 4 to 6 hours after administration for the occurrence of toxicity symptoms; on days 2, 3, and 4 only once daily in the morning.

At 24-, 48- and 72-hour intervals after treatment, 6 mice per sex and control group and 7 mice per sex of the test material group were sacrificed to prepare bone marrow smears.

The groups were as follows:

Group	1 Negative Control	2 Test Material	3 Positive Control
Treatment (mg/kg)	Physiolocical saline solution (0.9%)	QUAB 342 M: 21.5 F: 31.6	Cyclophosphamide 51.1

Sampling time p. appl.		Animal Numbers		
24 h	M	1- 6	301-307	401-406
	F	19- 24	322, 323, 330, 337, 340	419-424
48 h	M	7- 12	308-314	407-412
	F	25- 30	324-329, 331	425-430
72 h	M	13- 18	315-321	413-418
	F	31- 36	332-336, 341, 342	431-436

Because of the strong toxic effects with risk of intercurrent death in severa female animals, the following changes were made in the test material group ani mals concerning planned and actual sampling times. The decision was based on th clinical symptoms.

Animal Numbers	Sampling Time p.appl. Planned	Sampling Time p.appl. Actual
324	24 h	48 h
325	"	"
326	"	"
327	"	"
328	"	"
330	48 h	24 h
332	"	72 h
333	"	"
334	"	"
335	"	"
337	72 h	24 h
338	"	"
339	"	"
340	"	"

5.2.5 Preparation of Bone Marrow for Evaluation : All mice were sacrificed by CO_2 overdose. Both femurs were removed from each mouse and the bone marrow cells flushed into a labelled centrifuge tube with approximately 1.5 ml of fetal calf serum (Art. No. 210463, Boehringer Mannheim, D-1800 Mannheim). The tubes were centrifuged at approx. 180 x g for 5 minutes (Eppendorf Zentrifuge 5415, Netheler & Hinz GmbH, D-2000 Hamburg 65), after which the supernatant serum was discarded and the bone marrow cells suspended upon a thin layer of serum. A small drop of the marrow serum suspension was smeared on a slide, which was identified by study number, animal number, species, sex, material (in the test material group), and date of preparation, and allowed to dry overnight.

At least two slides per animal were prepared. The following day, the smears were stained using the panoptic stain method developed by PAPPENHEIM (6).

5.2.6 Microscopical Evaluation of the Slides : For each sampling time the bone marrow smears from the first 5 animals per sex and group were used for evaluation. One slide per animal was examined. The remaining smears of each sex and group per interval were evaluated if macroscopical examination of the first smears revealed technical imperfections which precluded accurate microscopical analysis.

From each animal one thousand polychromatic erythrocytes (PCE) were scored under the microscope (magnification 650-1000 x, C. ZEISS, D-7082 Oberkochen) for the incidence of polychromatic erythrocytes with micronuclei.

The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was calculated, based on 1000 erythrocytes (PCE + NCE) scored per slide, as a measure of the toxic efficacy of the test material.

5.3 DATA RECORDING : The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including test material group, negative and positive controls. The micronucleated cells per thousand polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes are given for each animal examined (Tables 1-3).

5.4 STATISTICAL METHODS : The frequencies of polychromatic erythrocytes with micronuclei of the test material group and the positive control group were compared with those of the negative control group at each sampling time.

A POISSON test (7) was applied (Tables 4 and 5). The data from each treatment group for each sex, as well as for both sexes combined, were compared with the respective negative control group data using a VAX 8200 computer (Digital Equipment Corp., D-8000 München 81). The values of the first 5 animals of each sex and sampling time were used for statistical evaluation.

5.5 CRITERIA OF ASSESSMENT : If a test material produced no statistically significant and reproducible positive response at any one of the test points compared to the negative control group, it is considered non-mutagenic in this system (significance level: 5%; $p \leq 0.05$).

5.6 Protocol Adherence : The study was conducted in accordance with the original protocol inclusive amendments with the following exceptions:

- Denotation of sponsor and testing facility (due to organizational changes) and change of address of the testing facility.
- Assignment of female test material group animals to sampling times (see page 14, point 5.2.4).
- The centrifuge tubes (see page 15, point 5.2.5) were centrifuged at approx. 180 x g.

6. R E S U L T S

The following results have been compiled:

6.1 CLINICAL EXAMINATION

Animals of the test material group exhibited clinical signs as slight to moderate clonic convulsions (21/21 males, 21/21 females), stilted gait (1/21 males, 13/21 females), ruffled furs (4/21 males, 11/21 females), ptosis (4/21 females), sunken sides (12/21 males, 16/21 females), and slight to moderate hypokinesia (3/21 females). No test material group animal died.

Negative and positive control group animals did not show any abnormal clinical signs.

6.2 EVALUATION OF BONE MARROW

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight in males and 31.6 mg/kg body weight in females no significant test material-related increase of micronucleated polychromatic erythrocytes was observed in either male or female animals at each of the three examination times, 24, 48, and 72 hours after administration, respectively. This was also true for the evaluation of the values from both sexes combined at 24 and 48 hours. For the 72-hour sampling time a p-value of 0.048 was found, when the incidence of micronucleated polychromatic erythrocytes was compared between the test material group and the negative control group for both sexes combined. This value at the border of a statisstical significance is due to the particularly low incidence of micronucleated polychromatic erythrocytes in negative control animals of the 72-hour sampling time (mean: 0.4‰), which is clearly lower than historical negative control values in the Institute of Toxicology of ASTA Medica AG and also than given in the literature (8). The number of micronucleated polychromatic erythrocytes in test material treated males of this sampling time was not increased, when compared to other negative control group animals. Therefore, the statistical p-value of 0.048 (rounded 0.05) is considered not to be of relevance for the estimation of the study results.

No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding negative control group animals, with exception of one female (No. 338) at the 24-hour sampling time.

The positive control group animals receiving cyclophosphamide exhibited a significant and clear increase in the number of micronucleated polychromatic erythrocytes and thus validated the test. The dose used for this reference mutagen had a toxic effect on bone marrow as shown by the reduced PCE/NCE ratio at 48 hours (males) and 72 hours (males and females), respectively, after administration.

7. C O N C L U S I O N

Under the experimental conditions reported, Quab 342 induced no chromosome mutation in mice by damage to the chromosomes or the mitotic apparatus, at 24-, 48-, or 72-hour intervals after the animals had received a single intraperitoneal dose of 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females). Therefore, Quab 342 is considered non-mutagenic in the re-

8. REFERENCES

1. OECD: OECD Guidelines for Testing of Chemicals, No. 474, Micronucleus Test (1983)

2. EEC: Commission directive of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (84/449/EEC). Off. J., L251, 27, 137-139 (September 19, 1984), Part B: Methods for the determination of toxicity, B.12. Micronucleus Test

3. Law on protection from dangerous substances (German Chemicals Act), August 1, 1990, Amendment 1: Principles of Good Laboratory Practice (GLP), amended at: "Dritte Verordnung zur Änderung der Gefahrstoffverordnung" dated June 5, 1991, published in Bundesgesetzblatt, Volume 1991, Part 1, No. 35, June 14, 1991.

4. SCHMID W (1975): The micronucleus test. Mutation Res. 31, 9-15

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9. T A B L E S 1-5

TABLE 1 Number of Micronuclei, Negative Control Group

TABLE 2 Number of Micronuclei, Test Material Group

TABLE 3 Number of Micronuclei, Positive Control Group

TABLE 4 Statistical Evaluation (Males / Females)

TABLE 5 Statistical Evaluation (Both Sexes Combined)

TABLE 1

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Negative control group: Physiological saline solution (0.9%), intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
1	3	2.05	7	1	1.40	13	0	2.55
2	0	1.71	8	2	2.04	14	1	4.05
3	1	1.93	9	0	2.09	15	1	2.70
4	2	2.45	10	0	1.20	16	0	2.80
5	2	1.89	11	0	1.75	17	0	3.41
Mean	1.6			0.6			0.4	
SD	1.14			0.89			0.55	
N	5			5			5	
Females								
19	1	1.77	25	1	2.13	31	0	2.41
20	1	1.97	26	2	1.86	32	0	3.33
21	2	1.67	27	1	1.72	33	1	3.47
22	2	1.67	28	2	1.57	34	1	3.13
23	1	1.96	29	2	1.42	35	1	1.78
Mean	1.4			1.6			0.6	
SD	0.55			0.55			0.55	
N	5			5			5	
Mean M+F	1.5			1.1			0.5	
SD	0.85			0.88			0.53	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 2

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Test material group: Males : 21.5 / Females: 31.6 mg/kg body weight QUAB 342, intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
301	1	2.24	308	3	1.48	315	2	2.37
302	0	1.79	309	0	1.16	316	0	2.68
303	1	1.08	310	0	1.38	317	3	3.24
304	1	1.32	311	1	1.03	318	2	1.85
305	0	2.48	312	0	1.40	319	1	2.42
Mean	0.6			0.8			1.6	
SD	0.55			1.30			1.14	
N	5			5			5	
Females								
322	2	0.60	324	3	2.42	332	0	1.80
323	0	1.16	325	0	1.57	333	1	2.64
330	2	0.72	326	3	0.86	334	2	4.75
337	0	0.95	327	0	1.75	335	2	2.83
338	1	0.47	328	2	1.29	336	0	3.02
Mean	1.0			1.6			1.0	
SD	1.00			1.52			1.00	
N	5			5			5	
Mean								
M+F	0.8			1.2			1.3	
SD	0.79			1.40			1.06	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 3

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Positive control group: 51.1 mg/kg body weight cyclophosphamide, oral by gavage

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
401	24	2.24	407	13	0.37	413	8	0.46
402	43	2.21	408	17	0.62	414	8	0.33
403	19	2.40	409	22	0.32	415	8	0.16
404	48	1.70	410	21	0.47	416	4	0.19
405	20	2.19	411	22	0.37	417	7	0.30
Mean	30.8			19.0			7.0	
SD	13.66			3.94			1.73	
N	5			5			5	
Females								
419	27	1.82	425	5	1.16	431	3	0.94
420	26	0.90	426	3	0.91	432	4	1.31
421	35	1.02	427	4	0.75	433	5	0.34
422	27	0.98	428	7	1.19	434	2	0.98
423	23	1.05	429	10	0.80	435	5	0.48
Mean	27.6			5.8			3.8	
SD	4.45			2.77			1.30	
N	5			5			5	
Mean M+F	29.2			12.4			5.4	
SD	9.73			7.66			2.22	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 4

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test)

	24 h		48 h		72 h	
	m	f	m	f	m	f
No. of Micronuclei						
verum	3	5	4	8	8	5
neg. contr.	8	7	3	8	2	3
test statistic F	0.3333	0.6250	1.0000	0.8889	2.6667	1.2500
degrees of freedom f_1, f_2	18, 6	16, 10	8, 8	18, 16	6, 16	8, 10
p-value	0.967	0.806	0.500	0.598	0.055	0.363
No. of Micronuclei						
pos. contr.	154	138	95	29	35	19
neg. contr.	8	7	3	8	2	3
test statistic F	17.1111	17.2500	23.7500	3.2222	11.6667	4.7500
degrees of freedom f_1, f_2	18, 308	16, 276	8, 190	18, 58	6, 70	8, 38
p-value	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 5

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test) for Both Sexes Combined

	24 h	48 h	72 h
	m + f	m + f	m + f
No. of Micronuclei			
verum	8	12	13
neg. contr.	15	11	5
test statistic F	0.5000	1.0000	2.1667
degrees of freedom f_1, f_2	32, 16	24, 24	12, 26
p-value	0.953	0.500	0.048
No. of Micronuclei			
pos. contr.	292	124	54
neg. contr.	15	11	5
test statistic F	18.2500	10.3333	9.0000
degrees of freedom f_1, f_2	32, 584	24, 248	12, 108
p-value	0.000	0.000	0.000

10. A T T A C H M E N T S 1-3

ATTACHMENT 1. Test Material

ATTACHMENT 2. Positive Control Material

ATTACHMENT 3. Diet, Composition

Attachment 1

Degussa

Data on Test Substance No. Study No.

Supplier : DEGUSSA AG, ZN Wolfgang
Dept. IC-ATPS
P.O.Box 1345, D-6450 Hanau 1

Test Substance/
Trade Name/Identity: Quab 342 (Lauryl-Quab)

Synonym : 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid

CAS No. : 41892-01-7

Batch No./Date of
Production : Charge 90/ Ma - 03

Physical Appearance: slightly coloured solid

Purity (Method)
Known Impurities : Purity: 85 %
Diquaternary salt: 8,6 %
Glycol: 2,55 % Chlorhydrin: 84,8 %

Solubility : ca 40 % in water

Density : ca 1g/ml

pH-Value : 3-6 (ca 40 % solution in water)

Storage : 0 - 10 °C

Stability : Provided the a.m. storage conditions the test substance is stable until january'91 (no measurable loss of activity within 2 years)

Hu - Wolfgang, 19.10.90
Place, Date

Signature

IR/KC 0.5 2.0 | Lyto 3.0 | Part 3.5 | Pyr | PGW

P 6899 1002

A.-Nr. 3761

CYCLOPHOSPHAMID BP 88/USP XXI

Charge: 090029 K.Nr.: 201301

Menge: 102.05 kg/~~l~~/~~Stck.~~

Abt.: PGW Ko.St.: 6127

3 Gebinde/~~Paletten~~

hergest.n.Vorschr.Nr. 15 v. Jan.90

Herst.Datum: 07.09.90

WEGA — AMK X

Cyclophosphamid

K-Nr.: ~~200011k#070025~~ ~~201130k#080026~~ 07.9.90

Verf.-Datum:

Cyclophosphamid roh

3 Etiketten

SOLL K-Nr.: 200921k#060526 IST

1. Beschreibung:	Weißes Krist. Pulver	entspr.: ja
2. Identifizierung:		
2.1. IR-Spektrum:	entspr. d. Stand. 50240	entspr.: ja
2. HPLC:	entspr. d. Vorschrift	entspr.: ja
2.3. Chlorid: Kochsalzchlor	entspr. d. Vorschrift	entspr.: ja
3. Reinheitsprüfungen:		
3.1. Klarlöslichkeit:	n.m.a. 3.5 FTU	0,2 FTU
3.2. Reaktion:	pH = 4.0 bis 6.0	pH = 4,9
3.3. Chlorid:	n.m.a. 350 ppm	40 ppm
3.4. Schwermetalle:	n.m.a. 20 ppm	entspr.: ja
3.5. Wassergehalt:	6.0 bis 6.8 %	6,5 %
3.6. Schmelzpunkt:	49.5 bis 53.0 °C	50,5 °C
4. Wirkstoffgehalt:	98.0 bis 100.5 % wfr	100,0 %
5. Restlösemittel:		
Isopropanol:	n.m.a. 500 ppm	101 ppm
Diethylether:	n.m.a. 10 ppm	5.2 ppm
6. Sterilprüfung:	entspr. d. Vorschrift	entspr.: ja am: 19
7. Pyrogenprüfung:	entspr. d. Vorschrift	entspr.: ja am: 20.
WI Filtertest 17 g/	liegt vor	ja
WI Partikelzählung:	liegt vor	ja 12.9.90

PROBEMENGE : 2 x 100 ml Braunglasflasche dicht verschlossen
PROBEZIEHUNG : PGW
AM: 07.09.90

für toxikologische Untersuchungen bis 31.12.1991

20 SEP. 90

FREIGABE DURCH QI AM :

27.9.90

QUALITÄTSKONTROLLE freigegeben

- 81 -

Attachment 3

s s n i f f M
Special Diet for Mice

Nutrients (in % Diet)

Raw Protein	21.00%
Raw Fat	3.50%
Raw Fiber	4.50%
Raw Ash	6.50%

Calcium	1.20%
Phosphorus	0.80%
Sodium	0.30%
Magnesium	0.20%
Potassium	1.00%

Amino Acids (in 1 kg Diet)

Lysine	1.40%
Methionine	0.45%
Cystine	0.30%
Glycine	1.00%
Leucine	1.70%
Isoleucine	1.00%
Arginine	1.40%
Phenylalanine	1.00%
Tryptophane	0.35%
Histidine	0.50%
Alanine	1.00%
Tyrosin	0.70%
Asparagine Acid	1.60%
Glutamine Acid	3.50%
Valine	1.00%

Vitamins (in 1 kg Diet)

A	25,000 I.E.
D_3	2,500 I.E.
E	75 mg
B_1	20 mg
B_2	40 mg
B_6	15 mg
B_{12}	80 mcg
Biotin	250 mcg
Pantothenic Acid	50 mg
Choline	2,000 mg
Folic Acid	2 mg
Nicotinic Acid	150 mg
K_3	5 mg

Trace Elements (in kg Diet)

Manganese	75 mg
Copper	12 mg
Zinc	80 mg
Iodine	1 mg
Ferrum	380 mg
Fluorine	10 mg

Aliphatic Acids

Data available on request

Contains No CBI

Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Lauryl-QUAB

Acute Toxicity
Testing the Acute Toxicity
after Single Oral Administration

R E P O R T
TRANSLATION

June 30, 1982

Summary

Lauryl-Quab was studied for acute toxicity after (single) oral administration in the rat.
The test substance, available as a liquid, was used as diluted aqueous solution (Aqua demin), or undiluted, with an increasing administration volume between 2.15 and 4.65 ml/kg.

The LD 50 values were for female rats 3.05 ml/kg (= 3066 mg/kg)
and for male rats 2.32 ml/kg (= 2332 mg/kg)

Intoxication was characterized by disturbances of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colourless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

ASTA WERKE 4800 Bielefeld 14
AKTIENGESELLSCHAFT
Toxikologisches Institut Degussa-Asta

Bericht Nr.: Ind.-TOX-79-81/82

DEGUSSA AG - US-IT - NR.
82 0086 DKT

BERICHT

über die toxikologische Prüfung von Lauryl-QUAB nach einmaliger oraler Gabe an der Ratte

Acute Oral Rat

30. Juni 1982

Versuchsbeginn	:	05.01.1982
Ende des tierexperimentellen Teiles	:	18.01.1982
Versuchsdurchführende Einrichtung	:	Toxikologisches Institut Degussa-Asta Artur-Ladebeck-Str. 128 - 152 D-4800 Bielefeld 14
Versuchsleiter	:	Dr. med. vet. H.-J. Zechel Fachtierarzt für Pharmakologie und Toxikologie
Pathologie und Laboratoriums-Diagnostik	:	--
Archivaufsicht	:	E. Fichtner (QS)
Berichtsdurchsicht	:	E. Fichtner (QS)

Zusammenfassung

Lauryl-QUAB wurde zur Bestimmung seiner akuten Toxizität oral an der Ratte verabfolgt. Das in flüssiger Form vorliegende Prüfprodukt kam in verdünnter wässriger Lösung (Aqua demin) sowie unverdünnt in steigendem Applikationsvolumen zwischen 2,15 und 4,65 ml/kg zur Anwendung.

Die erhobenen DL 50 - Werte betrugen für die weiblichen Tiere 3,05 ml/kg (≙ 3066 mg/kg) und für die männlichen Tiere 2,32 ml/kg (≙ 2332 mg/kg).

Im Vergiftungsbild standen Störungen des vegetativen Nervensystems und des Allgemeinbefindens (Zittern, Stelzengang, eingezogener sowie aufgeblähter Bauch, Speichelfluß, gesträubtes Fell, Durchfall, Abmagerung) im Vordergrund. Prämortal kam es teilweise zu Reflexausfall, Krämpfen, Mydriasis und Dyspnoe. Der Vergiftungsverlauf gestaltete sich akut bis protrahiert.

Makroskopisch-anatomisch wurden bei den verstorbenen Tieren Flüssigkeitsansammlungen, teils von klarem Aussehen, teils von roter oder gelber Farbe, in Magen und Darm festgestellt. Die Wandung dieser Organe wies Rötung auf. In einem Fall lag eine Perforation der Magenwand vor. Vereinzelt wurden Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Einleitung

Nachfolgend wird über die toxikologische Prüfung von Lauryl-QUAB berichtet.

Die Untersuchung erfolgte im Auftrage des Fachreferates für Industrielle Toxikologie der Degussa, Frankfurt/M. Sie wurde nach einmaliger oraler Gabe des Prüfproduktes an der Ratte durchgeführt.

Zweck der Studie war es, eine Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes vorzunehmen. Die erhobenen Befunde werden durch Angaben zur DL 50 ergänzt.

Lauryl-QUAB lag als farblose Flüssigkeit vor und kam tierexperimentell unverdünnt und verdünnt zur Anwendung.

Methodik

1. Versuchstiere

Spezies	:	Ratte
Stamm	:	Wistar (Bor: WISW)
Züchter	:	Winkelmann, Borchen
Geschlecht	:	♂ und ♀ Tiere
Gewicht	:	♂ 127 - 147 g; ♀ 127 - 141 g
Alter	:	♂ 57 - 62 Tg.; ♀ 57 - 58 Tg.
Eingewöhnungszeit vor Testbeginn	:	5 Tage
spezielle Angaben	:	siehe Versuchsdurchführung unter Pkt. 4

2. Versuchstierhaltung

Haltung	:	Makrolonkäfige Typ III
Tiere pro Käfig	:	1
Einstreu	:	Weichholzgranulat Typ 9 ALTROMIN®
Fütterung	:	Standardisiertes Versuchstierfutter ALTROMIN®
Tränkung	:	Wasser ad libitum
Raumtemperatur	:	$21^{o} \pm 2^{o}$ C
Relative Luftfeuchtigkeit	:	50 - 60 %
Raumbeleuchtung	:	12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. Dunkelheit
Raumhygiene	:	Labor- und Käfigreinigung mittels handel üblicher Antiseptika, Instrumente- und Geräte-Desinfektion mittels Autoklav und Heißluftsterilisator

3. Prüfsubstanz (spezielle Angaben)

Chemische Bezeichnung	:	Lauryl-QUAB
Chargen-Nr.	:	
Form und Aussehen	:	klare Flüssigkeit
Gehalt/Reinheit	:	27,87 %
Löslichkeit	:	Herstellg. verdünnter Lösungen in Aqua demi
Dichte	:	1,007 g/ml
pH-Wert	:	7,1

Verdünnungsfaktoren 1,47 und 2,1
Die entspr. Konzentrationen betrugen 685 mg/kg und 468 mg/kg b
Annahme einer Ausgangskonz. von 1007 mg/ml.

4. Versuchsdurchführung

Futterentzug	:	**16 Std. vor Versuchsbeginn**
Substanz-Applikation	:	oral mittels Schlundsonde
Dosenabstand	:	**in dezimalgeometrischer Folge**
Faktor	:	1,47 bzw. 2,15
Beobachtungszeit	:	14 Tage
Injektions-/ Applikationsvolumen	:	2,15 - 4,64 ml/kg; die Substanz kam verdünn und unverdünnt zur Anwendung
Tiere pro Dosis	:	5
Anzahl Dosierungs-gruppen	:	4 bei ♂ und 3 bei ♀
Anzahl Tiere zur Berechnung eines DL 50 - Wertes	:	20 ♂ und 15 ♀
Gesamtzahl der im Versuch verwendeten Tiere	:	35

5. Versuchszweck

Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes; [3]

Berechnung der DL 50 (Probitanalyse) [1,2,4,]

Ergebnisse

Lauryl-QUAB wurde zur Ermittlung seiner akuten Toxizität oral an der Ratte verabfolgt. Das Prüfprodukt lag in flüssiger Form vor und kam verdünnt mit Aqua demin und unverdünnt in steigenden Applikationsvolumina zwischen 2,15 und 4,64 ml/kg zur Anwendung. Bei der Berechnung der Dosen (mg/kg) wurde eine Dichte von 1,007 g/ml zugrunde gelegt. Der Dosenbereich erstreckte sich zwischen 1007 und 4672 mg/kg.

Das Vergiftungsbild war vorwiegend durch Symptome wie Zittern, Stelzengang, schleppender Gang, Speichelfluß, gesträubtes Fell, Durchfall und eingezogener bzw. aufgeblähter Bauch geprägt, was auf eine Störung des vegetativen Nervensystems hinweist.Darüber hinaus zeigten die männlichen Tiere während der 1. Beobachtungswoche nur eine mäßige Gewichtsentwicklung. Bei einigen Tieren war eine Zyanose zu beobachten. Prämortal kam es bei den weiblichen Tieren der höchsten Dosierungsgruppe zu vollständigem Reflexausfall, starken klonischen Krämpfen, Mydriasis und Dyspnoe.

Der Vergiftungsverlauf ist als akut bis protrahiert zu bezeichnen. Erste klinische Symptome traten 20 Minuten nach Substanzgabe auf und währten bei den Überlebenden maximal bis zu 1 Woche. Letalität stellte sich überwiegend während der ersten beiden Tage p. appl. ein, weitere Todesfälle traten noch in den folgenden 3 Tagen auf.

Bei der Sektion der verendeten Tiere kam es in Abhängigkeit von der verabfolgten Dosis zu einer zunehmenden Rötung von Magen- und Darmwand. Diese Organe wiesen, bedingt durch Flüssigkeitsansammlungen in ihrem Lumen (teils klar, teils von roter und gelber Farbe) eine pralle Füllung auf. Bei einem männlichen Tier wurde eine Perforation der Magenwand diagnostiziert. Dieser Befund steht nicht im Zusammenhang mit einem unphysiologisch hohen Applikationsvolumen; er muß als eine Folge der Substanzeinwirkung angesehen werden. In einzelnen Fällen wurden auch Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Die DL 50 - Werte sind in Tabelle 1a und 1b wiedergegeben.
Der zahlenmäßige Unterschied zwischen den DL 50 - Werten der weiblichen und männlichen Tiere ist statistisch nicht signifikant. Er trägt zufälligen Charakter.

In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

DL 50 - Werte von Lauryl-QUAB

Spezies : Ratte
Applikation : oral

Tabelle 1a

	DL 50 [ml/kg]
♀	2,32 (1,58 - 3,39)
♂	3,05 (2,25 - 4,13)

Tabelle 1b

	DL 50 [mg/kg]
♀	2372 (1593 - 3414)
♂	3066 (2261 - 4159)

Akute Toxizität DL 50 - Bestimmung
(Absterbedaten)

tanz/Präparat : Lauryl-QUAB
likationsart : oral

Spezies : Ratte
Stamm : Wistar (Bor: WI

Dosis mg/kg]	Geschlecht	Todes-rate		Überlebenszeit																			
				Std. nach Applikation						Tage nach Applikation													
		x/n	%	0,5	1	2	4	8	24	2	3	4	5	6	7	8	9	10	11	12	13	14
1473	♂	0/5	0																			
2165		1/5	20										1									
3182		2/5	40						1		1											
4672		5/5	100						2	3												
1007	♀	0/5	0																			
2165		2/5	40						2													
4672		5/5	100				3		1			1										

Literatur

1. BLISS, C.J.,The Statistics of Bioassay, Academic Press, New York, 1962

2. FINNEY, D.J., Probit Analysis, 2nd Ed., Cambridge Univ. Press, Cambridge 1952

3. HUNTER, W.J., Lingk, W. and Recht, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem. 62, 864 - 873, 1979

4. WEBER, E., Grundriß d. biol. Statistik, G. Fischer-Verlag, Stuttgart, 7. Auflage, 1972

Bielefeld, den 30.06.1982

Dr. med. vet. H.-J. Zechel
Fachtierarzt für Pharmakologie
und Toxikologie

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid
(Lauryl QUAB)
QUAB 342

Acute Toxicity after a Single Dermal Administration in Rabbits

R E P O R T
TRANSLATION

July 29, 1986

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

Summary:

Quab 342 was applied once * to the dorsal skin area of rabbits to determine its acute toxicity. The test substance, available as a colourless liquid, was used undiluted.

Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discolouration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart, and small intestine were oberserved.

The LD_{50} values were

probit analysis:
for male and female animals 2523 mg/kg (2.46 ml/kg)

graphical estimation:

for male animals	3072 mg/kg	(3.0 ml/kg)
for female animals	1946 mg/kg	(1.9 ml/kg)

* remark of the translater: (occlusive for 24 hours)

DEGUSSA AG - US-IT - NR.
86 0158 DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid (Lauryl QUAB) QUAB 342

Akute Toxizität

Prüfung der akuten Toxizität nach einmaliger dermaler Applikation am Kaninchen

B E R I C H T

Acute Dermal Rabbit

29. Juli 1986

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Der Bericht umfaßt 12 Seiten.

1. ALLGEMEINES

Auftraggeber	: DEGUSSA AG / ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Prüfeinrichtung	: ASTA-WERKE AG Chemische Fabrik Toxikologie Degussa-Asta Artur-Ladebeck-Str. 128-152 D-4800 Bielefeld 14
Prüfsubstanz	: QUAB 342
Art der Prüfung	: Prüfung der akuten Toxizität nach einmaliger dermaler Gabe am Kaninchen.
Prüfrichtlinie/-n	: OECD Guideline Nr. 402 (1)
Berichts-Nummer	: Ind.-TOX-508-85/86
Zeitplan	: Beginn: 23.10.84 Ende : 18.12.84
Prüfleiter	: Dr. rer. nat. W. Mayr
Durchführung der Studie	: R. Iselt
Berichtsdatum	: 29.07.1986

2. ZUSAMMENFASSUNG

QUAB 342 wurde zur Bestimmung seiner akuten Toxizität einmalig dermal auf die Rückenhaut des Kaninchens aufgetragen. Das Prüfprodukt lag als farblose Flüssigkeit vor und kam unverdünnt zur Anwendung.

Das Vergiftungsbild war gekennzeichnet durch Zittern, gesträubtes Fell und körperliche Schwäche. Das Vergiftungsbild ist als protrahiert zu bezeichnen. Todesfälle traten zwischen 2 und 7 Tagen p. appl. auf. Die Applikationsstellen zeigten Graufärbung, Rötung und Schorfbildung.

Bei der Autopsie waren nur bei den weiblichen Tieren unspezifische Veränderungen an Niere, Leber, Milz, Lunge, Herz und Dünndarm zu beobachten.

Die ermittelten LD 50-Werte betragen:

Probitanalyse:

männliche und weibliche Tiere 2523 mg/kg (2,46 ml/kg)

graphische Abschätzung:

männliche Tiere:	3072 mg/kg (3,0 ml/kg)
weibliche Tiere:	1946 mg/kg (1,9 ml/kg)

Bielefeld, den 29.07.1986

Prüfleiter:

Dr. rer. nat. W. Mayr

Institutsleiter:

7.8.86

Dr. med. vet. M. Herbst

3. EINLEITUNG

Nachfolgend wird über die toxikologische Prüfung von QUAB 342 nach einmaliger dermaler Gabe am Kaninchen berichtet.

Ziel der Studie war Art, Intensität und Dauer der klinischen Symptome sowie Todeszeitpunkte und Zahl der verstorbenen Tiere festzustellen (1 - 2). Die Beobachtungsdauer betrug 14 Tage. Aus den Mortalitätszahlen wurde die LD 50 ermittelt (3 - 5).

Das methodische Vorgehen basierte auf der OECD-Richtlinie Nr. 402 (1).

4. PRÜFSUBSTANZ

Prüfsubstanz /Handelsname	: QUAB 342
Chemische Bezeichnung /Synonym	: 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
CAS-Nr.	: ---
Chargen-Nr.	: 342 M 19
Form und Aussehen	: farblose Flüssigkeit
Gehalt/Reinheit	: ---
Löslichkeit	: ---
Dichte	: 1,024 g/ml
pH-Wert	: 1,5
Aufbewahrung und Stabilität	: Die Prüfsubstanz wurde bei Raumtemperatur aufbewahrt. Die Stabilität der Prüfsubstanz wurde vom Auftraggeber gewährleistet.

5. METHODIK

5.1 Prüfsystem

5.1.1 Versuchstier-Spezies	: Kaninchen
Stamm	: Weiße Russen (Albino)
Herkunft/Züchter	: Asta-Werke AG, D-4800 Bielefeld 14
Begründung der Versuchstierwahl	: Das Prüfsystem wurde aufgrund internationaler Empfehlungen gewählt. Danach ist die Spezies Kaninchen geeignet, potentielle toxische Wirkqualitäten von Prüfsubstanzen zu erfassen.

Alter der Tiere am Applikationstag	: männlich 9 - 12 Monate weiblich 9 - 10 Monate
Körpergewicht der Tiere am Applikationstag	: männlich 2,05 - 2,50 kg weiblich 2,40 - 2,75 kg
Eingewöhnungszeit	: Mindestens 5 Tage unter Prüfbedingungen vor Substanzapplikation

5.1.2 Versuchstierhaltung

Standort	: Toxikologie Degussa-Asta D-4800 Bielefeld 14, Gebäude B, Raum Nr. 214-5
Haltung	: Edelstahlkäfige Modell ASTA ASTA-WERKE AG, D-4800 Bielefeld 14
Tiere pro Käfig	: 1
Einstreu	: ohne, Drahtbodenkäfige
Futter	: Standardisiertes Versuchstierfutter ALTROMIN (R), Firma Altromin GMBH u. Co KG, 4937 Lage,
Trinkwasser	: Wasser ad libitum in Trinkwasserqualität der Stadtwerke Bielefeld, automatische Tränkung über Tränkventile
Raumtemperatur	: 22 +/- 2 Grad Celsius
Relative Luftfeuchtigkeit	: 55 +/- 15 %
Raumbeleuchtung	: 12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. natürlicher Hell- Dunkelrhythmus
Raumhygiene, Käfigreinigung	: Labor- und Käfigreinigung mittels handelsüblicher Antiseptika, Instrumente- und Gerätedesinfektion mittels Autoklav und Heißluftsterilisator.

5.1.3 Kennzeichnung der Tiere : mittels Ohrnummer (Tätowierung), Käfigbeschilderung mit gleicher Nummer

5.2 Durchführung der Prüfung

Futterentzug	: am Behandlungstage
Substanzapplikation	: dermal, zwischen der geschorenen Schulter- und Fersenbeinregion. Die Prüfsubstanz wurde mittels eines Patches unter Okklusiv-Bedingungen 24 Stunden lang mit der äußeren Haut in Kontakt gebracht. Nach Abnahme des Verbandes wurde die auf der Haut verbliebene Prüfsubstanz mit Wasser abgewaschen.
Begründung der Wahl der Applikationsart	: Die Prüfsubstanz wurde aufgrund der möglichen dermalen Exposition des Menschen in bezug auf seine Toxizität nach einmaliger oraler Applikation geprüft.
Häufigkeit der Applikation	: einmalige Gabe (24 Stunden-Patch)
Applikationsform	: unverdünntes Prüfprodukt
Konzentrationen	: ---
Applikationsvolumina	: m 0,909 - 4,22 ml/kg w 0,909 - 4,22 ml/kg
Dosenabstand	: in dezimalgeometrischer Reihenfolge
Faktor	: m 2,15 w 2,15
Anzahl der Dosisgruppen	: m 3 w 3
Tiere pro Dosisgruppe	: m 2 w 2
Gesamttierzahl	: 12
Beobachtungsdauer	: 14 Tage p. appl.

5.3 Beobachtungen

5.3.1 Verhalten und Allgemeinzustand der Tiere (klinische Vergiftungssymptome) : Beobachtung der Tiere am Applikationstag während 6-8 Stunden p. appl., danach täglich zweimal (vor- und nachmittags); an Samstagen, Sonn- und Feiertagen einmal.
Alle aufgetretenen Vergiftungserscheinungen sowie deren Beginn, Schwere und Dauer wurden protokolliert.

Mortalität : Todeszeitpunkt, Anzahl der verstorbenen Tiere pro Dosis

5.3.2 Pathologie : Alle gestorbenen bzw. am Ende der Beobachtunsperiode getöteten Tiere wurden obduziert und Äußeres, Körperöffnungen, Körperhöhlen (Brust- und Bauchhöhle) und deren Organe makroskopisch untersucht.

5.4 Bestimmung der LD_{50}-Werte : approximative Ermittlung, graphisch (6) sowie Probitanalyse (3 - 5)

6. ERGEBNISSE

Allgemeine Angaben

QUAB 342 wurde zur Ermittlung seiner akuten Toxizität dermal am Kaninchen verabfolgt. Die Prüfsubstanz lag als farblose Flüssigkeit vor und wurde unverdünnt appliziert. Unter Verwendung steigender Applikationsvolumina von 0,909 bis 4,22 ml/kg erstreckte sich der geprüfte Dosisbereich bei den männlichen und weiblichen Tieren zwischen 931 und 4321 mg/kg. Die Dosisangaben berücksichtigen eine Dichte von 1,024 g/ml.

Klinische Beobachtungen

Das Vergiftungsbild war gekennzeichnet durch mäßiges bis starkes Zittern, gesträubtes Fell sowie körperliche Schwäche.

Der Vergiftungsverlauf ist als protrahiert zu bezeichnen. Erste Vergiftungssymptome waren nach 1 bis 6 Tagen zu beobachten und dauerten bis zu 9 Tagen. Todesfälle traten zwischen 2 und 7 Tagen nach Substanzapplikation auf.

Nach Abnahme des Patches waren bei allen Tieren die behandelten Hautareale grau gefärbt und von lederartiger Beschaffenheit; zusätzlich waren Rötung und Schorfbildung zu beobachten.

Pathologie

Bei der Sektion war bei einem weiblichen Tier der hohen Dosisgruppe die Konsistenz der Milz erhöht und brüchig, die Leber marmoriert und die Lunge dunkel gefleckt. Geröteter Dünndarm, weißgefleckte Nieren weißes Endokard war bei einem weiblichen Tier der mittleren Dosisgruppe zu beobachten.

Statistik

Der LD 50-Wert wurde für beide Geschlechter graphisch nach Miller und Tainter (6) abgeschätzt und für beide Geschlechter zusammen mit der Probitanalyse ermittelt.

Die Werte sind in Tabelle 1 a und 1 b aufgeführt.
In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

Tabelle 1 Akute Toxizität - LD_{50}-Werte

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Tabelle 1a Graphische Abschätzung der LD 50 nach Miller und Tainter (6)
Vertrauensgrenze: 68,3 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche Tiere:	3,0 (0,355 - 25,322)	3072 (364 - 25930)
weibliche Tiere:	1,9 (0,303 - 11,925)	1946 (310 - 12211)

Tabelle 1b Rechnerische Bestimmung der LD 50 mittels Probitanalyse (3 - 5)
Vertrauensgrenze: 95 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche und weibliche Tiere:	2,46 (0,764 - 41,470)	2523 (782 - 42466)

Tabelle 2 Akute Toxizität - Absterbedaten

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Dosis (ml/kg)	Todesrate x/n	%	Überlebenszeit Std. n. Applikation 0,5	1	2	4	8	24	Tage nach Applikation 2	3	4	5	6	7	8	9	10	11	12	13	14
männliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50												1							
4,22	1/2	50									1										
weibliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50								1											
4,22	2/2	100							1												

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RESEARCH & CONSULTING COMPANY AG

DEGUSSA AG - US-IT - NR.
85 0129 DKM

PROJECT 047665

Ames Test

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

WITH

QUAB 342

REPORT

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PREFACE

GENERAL

Sponsor:	DEGUSSA AG ZN Wolfang Rodenbacher Chaussee 4 D 6450 Hanau 1
Study Monitor:	Dr. O. Weiberg
Testing Facility:	R C C Research & Consulting Company AG CH - 4452 Itingen / Switzerland
RCC Project No.:	047665
Test Article:	QUAB 342
Title:	Salmonella/Mammalian-Microsome Mutagenicity Test with QUAB 342

SCHEDULE

Receipt of Test Article:	April 15, 1985
Start of Experiments:	May 28, 1985
Date of Completion:	June 17, 1985
Proposed Date of Final Report:	June 27, 1985/hac

PROJECT STAFF

Study Director: Dr N. Banduhn, Dipl Biol.

Date: July 3, 1985

Managing Director: Dr. K Sachsse

Date: July 3, 1985

Technical Coordinators: Mrs. M Maurer
Mrs J. Gloor

GUIDELINES

This study follows the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella Typhimurium, Reverse Mutation Assay", adopted May 26, 1983.

EEC Directive 84/449, Method No. B 14.

SUMMARY

This study was performed to investigate the potential gene mutagenic activity of QUAB 342 according to the plate incorporation test of Ames et al. (1) using the Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

The test was performed with and without liver microsomal activation. The test article was tested at the following concentrations: 1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate. Each concentration, including the controls, was tested in triplicate.

Toxic effects of the test article were observed in the toxicity experiments by a reduction of the relative survival rate.
Additionally, a reduction of the background lawn up to a clearing was observed. Reduced numbers of revertants were found in some strains at the highest concentration tested. The toxic effects were more prominent in the experiments without metabolic activation than in the experiments with metabolic activation.

Up to the highest investigated dose, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538 in both experiments, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. when tested without liver microsomal activation. In the first experiment, the revertant rate was increased but not doubled versus the control. In the second experiment, there was a dose-dependent increase with an exact doubling of the revertant rate at a concentration 46.4 mcg/pl.

CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay.

1 OBJECTIVES

1.1 PURPOSE

This in vitro experiment was performed to assess the potential gene mutagenic activity of QUAB 342.
The standard quality control principles of "Wegleitung der IKS (Interkantonale Kontrollstelle fuer Heilmittel) betreffend Gute Laborpraxis fuer nichtklinische Laborversuche" from April 28, 1980 (amended June 23, 1982), Berne/Switzerland were applied.

1.2 RATIONALE

The test procedure was according to Ames et al. (1)
The microbial assay is based on reverse mutation of Salmonella typhimurium from auxotrophism (histidine dependent) to prototrophism (histidine independent). When mutated Salmonella typhimurium are exposed to a mutagen, mutation to the histidine independent form takes place in a proportion of the bacterial population. These are readily detectable due to their ability to grow on a histidine deficient medium.

Exposure of strains of Salmonella typhimurium to a chemical mutagen may produce revertants which arise as a result of a base-pair substitution in the genetic material or as a frameshift mutation in which genetic material is either added, deleted or mis-coded.

Since many compounds do not exert their mutagenic activity until they have been metabolized by enzyme systems not available in the bacterial cell, the test article and the bacteria were incubated both in the absence and in the presence of a cofactor-supplemented post-mitochondrial fraction prepared from the livers of rats treated with enzyme-inducing agents Aroclor 1254.

To validate the test, reference mutagens were tested parallel to the test article.

2 MATERIALS AND METHODS

2.1 TEST ARTICLE

Identification	QUAB 342
Batch/Lot number	040485-342 MA 54
Chemical denomination	3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
Purity	40.01% (cf. Certificat of analysis, April 3, 85)
Physical appearance	colorless, clear fluid
Stability	not available from the sponsor
Storage	The test article was stored at room

38

To avoid any light effects on the test compound, all experiments were performed under yellow light.

On the day of the experiment, the test article was dissolved in bidistilled water at the highest investigated dose. The other doses were dilutions from this stock solution with the solvent in third-log intervals.
The test article was investigated at the following concentrations:
1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate (mcg/pl.).

The highest concentration was limited by the cytotoxicity of the test article to bacteria. A preliminary test on strains TA 1537 and RTA of Salmonella typhimurium resulted in a strong toxic effect at a concentration of 500 mcg/pl. and higher.

2.2 CONTROLS

All control data were established on the same day as the experiment.

Negative Control:
Bidistilled water, solvent of the test article.

Positive Controls:
Reference mutagens not requiring microsomal drug-metabolizing enzyme.

Methyl methanesulfonate*	500 mcg/pl.	for TA 100
9-Aminoacridine*	40 mcg/pl.	for TA 1537
2-Nitrofluorene*	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538
N-Ethyl-N-Nitro-N-Nitrosoguanidine*	10 mcg/pl.	for TA 98
		TA 100
		TA 1535

Reference mutagens requiring microsomal drug-metabolizing enzyme

2-Aminoanthracene*	0.5 mcg/pl	for TA 98
		TA 100
		TA 1538
	1 mcg/pl	for TA 1535
	10 mcg/pl	for TA 1537
Benzo-(A)-pyrene**	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538

Aseptic Control:

An experiment with the S-9 mix was performed (cf. study procedure)

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* Aldrich, Chemie Brunschwig, 4000 Basel/Switzerland
** Fluka, 9470 Buchs/Switzerland

2 3 TEST SYSTEM

Bacterial test organisms:

Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538; obtained from B.N. Ames, University of California, Berkeley CA, 94720, USA, May 1981.

Characterization of strains:

The strains used are mutants derived from Salmonella typhimurium LT2 and have following genotype:

S.typhimurium TA 1535 his G46 rfa- uvrB-
S.typhimurium TA 1537 his C3076 rfa- uvrB-
S.typhimurium TA 1538 his D3052 rfa- uvrB-
S.typhimurium TA 98 his D3052 rfa- uvrB- R+
S.typhimurium TA 100 his G46 rfa- uvrB- R+

All the strains carry a mutation known as deep rough (rfa) which affects the cell membrane so that the normal lipopolysaccharide cell coat is defective These strains allow a greater permeability of the test agents across this membrane and into the cell.

In bacteria, almost all the primary damage caused to DNA by a mutagen is repaired by excision and recombination repair systems. Therefore, only a small percentage of the potential mutagenic damage is expressed. The tester strains used lack this excision repair system (uvrB), thus making them more sensitive to mutagens.

The strains TA 98 and TA 100 also carry an ampicillin resistance factor (R) which makes them particularly sensitive to mutagens.

The strains TA 1535 and TA 100 are sensitive to base-pair substitution mutagens; the strains TA 1537, TA 1538 and TA 98 are sensitive to frameshift mutagens.

The tester strains are checked at regular intervals for their genetic markers, according to published methods (1)

Storage

The strain cultures are stored in sterile 0 5 ml ampoules (0 45 ml bacterial culture and 0.05 ml Dimethylsulfoxide) at -70 degrees centrigrade

Pre-culture of strains:

The bacteria were grown in a shaking water bath for 16 hours overnight at 37 degrees centigrade in 2.5% Nutrient Broth No. 2*. After centrifugation, the bacteria were resuspended to a concentration of approximately 1 x 10 exp. 8 to 2 x 10 exp. 9 cells per milliliter in 0.16% Nutrient Broth and 0.5% sodium chloride. The concentration of germs was controlled photometrically and determined in an experimental test with Histidine-rich potassium chloride solution on selective agar plates (cf. study procedure).

2.4 LIVER MICROSOMAL FRACTION S-9 MIX

The S-9 mix was prepared on the day of the experiment

Specific pathogen-free male Wistar rats (180 g - 300 g, KFM-Ha, outbred**), received a single i.p. injection of Aroclor 1254*** dissolved in Oleum Arachidis (200 mg/ml) at a dosage of 500 milligrams per kilogram of body weight to induce liver microsomal enzyme activity five days before the experiment.

The rats were killed by cervical dislocation and exsanguinated on the fifth day post application following a 14-16 hours starvation period.

Livers were removed under aseptic conditions and homogenized with ice-cold 0.15 molar potassium chloride (5 g of liver to 15 g of potassium chloride). The homogenates were centrifuged at 9000 x g for 10 minutes at 0 to 2 degrees centrigrade. The supernatant fraction (S-9 fraction) was collected for the preparation of S-9 mix

Composition of 1 ml S-9 mix.

Na2HPO4****		100 micro moles
MgCL2	****	8 micro moles
KCl	****	33 micro moles
NADP	*****	4 micro moles
G-6-P	******	5 micro moles
S-9 Fraction		0.3 ml

* Oxoid; Fakola, 4000 Basel/Switzerland
** Kleintierfarm Madoerin AG, 4414 Fuellinsdorf/Switzerland
*** Analabs; Kontron, 8000 Zuerich/Switzerland
(Aroclor: polychlorinated biphenyl mixture)
**** ABS, 4000 Basel/Switzerland
***** Boehringer Mannheim, 6330 Cham/Switzerland
****** Fluka, 9470 Buchs/Switzerland

2.5 STUDY PROCEDURE

For each strain and dose level, including the controls, a minimum of three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates*:

100 microliters (mcl)	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 mcl	S-9 mix (for test with metabolic activation) or 0.15 M Na_2HPO_4, (for test without metabolic activation),
100 mcl	Bacteria suspension (cf. test system, pre-culture of the strains),
2 ml	Overlay agar consisting of 0.6 % Bacto agar** and 0.6 % NaCl supplemented with 10 % 0.5 mM L-Histidine*** and 0.5 mM Biotine*** solution.

The plates were incubated at 37 degrees centigrade in the dark for 3 days. The his+ revertant colonies were counted with an Artek 880 counter or counted by hand if precipitation of test article precluded automatic counting.

For the aseptic control experiment, 100 microliters of S-9 mix were added to 2 milliliters overlay agar and treated as mentioned above.

For each test, the germ concentration of the bacterial strains was determined by pouring a mixture of 100 microliters of a 2 x 10 exp.5 diluted bacteria suspension (overnight culture) with a Histidine-rich potassium chloride solution onto a selective agar plate. After three days of incubation at 37 degrees centrigrade, the colonies were counted and the counts multiplied with the dilution factor.

---

* A Pourmatic (New Brunswick Scientific Comp.) was used to fill the plates with 22 ml of 1.5 % bacto agar in Vogel Bonner medium E with 2% Glucose.
** Chemie Brunschwig, 4000 Basel/Switzerland
*** Fluka, 9470 Buchs/Switzerland

2.6 TOXICITY TEST

To estimate the cytotoxicity of the test article, prototrophic bacteria (spontaneous revertants of TA 1537 = RTA) were added as an internal standard to the selective agar plates, together with TA 1537 (cf. study procedure). The difference in the number of colonies on plates with and without added prototrophic bacteria at each dose level of the test article was compared to the number of colonies obtained with the negative control. The ratio of the two values was expressed as relative survival rate with the negative control at a 100 % survival rate.

Additionally, the toxicity of the test article can be shown in the mutagenicity assay, by a reduction in the number of revertants or by a reduction or a clearing of the background lawn.

2.7 DATA RECORDING

The number of revertant colonies was recorded manually for each plate from the counter.
Individual plate counts and the mean number of revertant colonies per plate are presented in tabular form for the test article, positive and negative controls.

2.8 EVALUATION OF RESULTS

A material is identified as a mutagen in this test system if there is a reproducible demonstration of a dose effect relation with a 2-fold increase in the number of revertants over the controls in a minimum of one strain. With the strain TA 100, a 1.5-fold increase is the criterion for a positive result.

These criteria are generally in accordance with the international used standards for the evalutation of results obtained from the Ames Test (e.g. Ref. 1, 2, and 3).

3 RESULTS

In the current study, the following results have been compiled:

3.1 GERM CONCENTRATIONS

The concentrations of Salmonella typhimurium used in this assay were as follows:

	FIRST EXPERIMENT	SECOND EXPERIMENT
TA 98	= 1.2 x 10 exp.9 cells/ml	= 1.0 x 10 exp 9 cells/ml
TA 100	= 8.0 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1535	= 1.4 x 10 exp.9 cells/ml	= 9.9 x 10 exp 8 cells/ml
TA 1537	= 9.5 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1538	= 2.1 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments without metabolic activation,

TA 98	= 1.8 x 10 exp.9 cells/ml	= 9.4 x 10 exp 8 cells/ml
TA 100	= 6.8 x 10 exp.8 cells/ml	= 7.3 x 10 exp 8 cells/ml
TA 1535	= 1.1 x 10 exp.9 cells/ml	= 1.8 x 10 exp 9 cells/ml
TA 1537	= 1.4 x 10 exp.9 cells/ml	= 4.9 x 10 exp 8 cells/ml
TA 1538	= 1.8 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments with metabolic activation.

3.2 TOXICITY OF THE TEST ARTICLE

In the toxicity experiment with QUAB 342, a reduction of the relative survival rate was observed without metabolic activation starting at 46.4 mcg/pl and with metabolic activation at a concentration of 216 mcg/pl.
Additionally, a reduction up to a clearing of the background lawn was observed starting from 46.4 to 100 mcg/pl and reduced numbers of revertants were found in some strains at the highest concentrat (216 mcg/pl.) tested.

3.3 CONTROL EXPERIMENTS

Control plates with the solvent (negative control) showed numbers of spontaneous revertant colonies per plate which were within the normal range of those cited in the literature (1)

Control plates with reference mutagens (positive controls) showed a distinct increase of the revertant colonies with the tester strains. This confirms the reversion properties of each strain. The positive results of the mutagens 2-Aminoanthracene and Benzo(a)pyrene indicate that the metabolizing system was functioning.

3.4 TESTS WITH QUAB 342

In the described bacterial mutagenicity tests, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 at all dose levels tested when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. in both experiments when tested without liver microsomal activation. In the first experiment, the revertant rate was increased approximately 68 % versus the control. In the second experiment, the number of revertants increased starting at 10 mcg/pl. and reached an exact doubling versus the control at a concentration of 46.4 mcg/pl.

4 CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activiation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay

5 REFERENCES

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Distribution of Spontaneous Mutation Values in Ames Test with and without Metabolic Activation. In: Critical Evaluation of Mutagenicity Tests. Bass, R., Glocklin, V., Grosdanoff, P., Henschler, D., Kilbey B., Müller, D., Neubert, D., (eds.)
BGA Schriften 3/84, MMV Medizin Verlag München, 1984, 465-475.

6 ARCHIVING

R C C, CH - 4452 Itingen/Switzerland

Raw data, protocol, report, duplicate of report, and test article reference sample.

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	25		123		28		9		13	
	24		129		34		9		23	
	33	27	138	130	25	29	11	10	22	19
1.0 mcg	32		137		24		11		16	
	24		132		25		6		17	
	24	27	161	143	24	24	8	8	18	17
2.16 mcg	26		126		28		12		18	
	24		139		24		11		28	
	18	23	127	131	34	29	9	11	20	22
4.64 mcg	20		125		25		11		15	
	21		138		35		8		21	
	18	20	157	140	21	27	10	10	20	19
10.0 mcg	23		142		26		9		13	
	24		140		19		8		26	
	30	26	141	141	35	27	4	7	19	19
21.6 mcg	27		143		23		10		25	
	29		144		27		13		30	
	21	26	125	137	25	25	5	9	23	26
46.4 mcg	27		120		29		12		32	
	30		122		29		7		29	
	29	29	116	119	24	27	15	11	34	32
100.0 mcg	38		127		26		13		27	
	36		95		20		6		32	
	32	35	122	115	19	22	12	10	15	25
216.0 mcg	27		56		17		15		22	
	32		37		14		12		10	
	10	23	28	40	12	14	11	13	13	15
MMS 500 mcg	---		485		---		---		---	
	---		432		---		---		---	
	---	---	479	465	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		35		---	
	---		---		---		32		---	
	---	---	---	---	---	---	43	37	---	---
2-NF 5 mcg	493		486		---		44		830	
	509		443		---		47		882	
	500	501	367	432	---	---	54	48	886	866
ENNG 10 mcg	72		495		173		---		---	
	79		551		99		---		---	
	60	70	496	514	147	140	---	---	---	---

--- Not determined

SALMONE...A TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE, WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	41		125		16		35		35	
	37		95		19		48		34	
	36	38	124	115	20	18	37	40	38	36
1.0 mcg	46		115		17		30		24	
	43		122		18		40		39	
	47	45	110	116	16	17	37	36	36	33
2.16 mcg	39		93		26		35		40	
	39		109		23		35		31	
	55	44	127	110	20	23	36	35	31	34
4.64 mcg	44		134		21		41		25	
	40		123		26		30		40	
	47	44	113	123	22	23	30	34	34	33
10.0 mcg	31		122		35		36		35	
	40		125		29		32		40	
	47	39	136	128	22	29	29	32	27	34
21.6 mcg	46		129		18		40		29	
	46		109		21		43		39	
	49	47	99	112	15	18	35	39	28	32
46.4 mcg	47		118		19		31		33	
	43		107		12		32		43	
	40	43	117	114	22	18	36	33	34	37
100.0 mcg	45		113		18		36		34	
	36		119		15		29		37	
	55	45	136	123	19	17	14	26	39	37
216.0 mcg	41		72		25		47		44	
	58		58		10		26		33	
	53	51	61	64	21	19	33	35	38	38
2-AA 0.5 mcg	99		162		---		---		130	
	113		197		---		---		97	
	113	108	180	180	---	---	---	---	99	109
2-AA 1.0 mcg	---		---		89		---		---	
	---		---		81		---		---	
	---	---	---	---	65	78	---	---	---	---
2-AA 10.0 mcg	---		---		---		172		---	
	---		---		---		219		---	
	---	---	---	---	---	---	168	186	---	---
BaP 5 mcg	284		370		---		117		124	
	294		343		---		130		108	
	262	280	410	374	---	---	124	124	117	116

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	10	367	1.00
1.0 mcg	8	358	0.98
2.16 mcg	11	358	0.97
4.64 mcg	10	352	0.96
10.0 mcg	7	351	0.96
21.6 mcg	9	359	0.98
46.4 mcg	11	189	0.50
100.0 mcg	10	42	0.09
216.0 mcg	13	13	0.00

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	40	343	1.00
1.0 mcg	36	307	0.89
2.16 mcg	35	327	0.96
4.64 mcg	34	339	1.01
10.0 mcg	32	329	0.98
21.6 mcg	39	306	0.88
46.4 mcg	33	290	0.85
100.0 mcg	26	235	0.69
216.0 mcg	35	135	0.33

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	23		116		19		15		16	
	29		96		20		11		11	
	10	21	111	108	18	19	7	11	11	13
1.0 mcg	33		110		14		11		16	
	21		120		24		12		19	
	21	25	123	118	22	20	10	11	14	16
2.16 mcg	29		97		21		17		13	
	24		116		27		11		11	
	23	25	112	108	21	23	14	14	15	13
4.64 mcg	31		115		17		12		15	
	30		129		17		8		15	
	29	30	134	126	21	18	11	10	7	12
10 mcg	23		123		24		13		25	
	33		107		31		9		16	
	25	27	113	114	28	28	11	11	12	18
21.6 mcg	26		94		19		8		24	
	23		121		18		14		20	
	30	26	122	112	25	21	9	10	22	22
46.4 mcg	28		116		17		11		25	
	30		74		22		5		28	
	24	27	84	91	18	19	11	9	24	26
100 mcg	30		53		14		10		24	
	30		60		13		8		19	
	31	30	62	58	15	14	17	12	27	23
216 mcg	20		35		24		12		14	
	17		33		18		12		11	
	35	24	26	31	20	21	17	14	23	16
MMS 500 mcg	---		334		---		---		---	
	---		401		---		---		---	
	---	---	407	381	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		49		---	
	---		---		---		56		---	
	---	---	---	---	---	---	73	59	---	---
2-NF 5 mcg	470		383		---		57		859	
	461		357		---		53		864	
	476	469	393	378	---	---	48	53	764	829
ENNG 10 mcg	120		1103		1045		---		---	
	143		1145		681		---		---	
	133	132	885	1044	891	872	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	51		91		12		28		31	
	59		107		17		38		29	
	47	52	115	104	15	15	33	33	29	30
1.0 mcg	47		109		13		28		30	
	40		111		18		26		21	
	38	42	121	114	14	15	30	28	33	28
2.16 mcg	48		130		14		34		27	
	50		116		21		32		19	
	47	48	124	123	17	17	30	32	29	25
4.64 mcg	54		137		16		30		29	
	41		112		13		33		29	
	45	47	99	116	21	17	37	33	28	29
10 mcg	49		128		13		32		30	
	55		113		19		40		26	
	41	48	117	119	18	17	36	36	27	28
21.6 mcg	42		139		15		29		30	
	45		119		11		31		25	
	53	47	134	131	18	15	30	30	30	28
46.4 mcg	46		129		13		30		40	
	47		116		17		31		23	
	52	48	132	126	9	13	29	30	40	34
100 mcg	56		91		16		19		34	
	38		109		14		30		30	
	44	46	109	103	12	14	42	30	22	29
216 mcg	36		55		18		25		25	
	33		45		13		15		24	
	37	35	36	45	12	14	25	22	35	28
2-AA 0.5 mcg	73		---		---		---		99	
	76		172		---		---		---	
	---	75	172	172	---	---	---	---	72	86
2-AA 1.0 mcg	---		---		98		---		---	
	---		---		109		---		---	
	---	---	---	---	---	104	---	---	---	---
2-AA 10.0 mcg	---		---		---		136		---	
	---		---		---		146		---	
	---	---	---	---	---	---	150	144	---	---
BaP 5 mcg	135		257		---		---		84	
	124		---		---		95		81	
	138	132	270	264	---	---	94	95	---	83

--- Not determined

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	11	277	1.00
1.0 mcg	11	285	1.03
2.16 mcg	14	278	0.99
4.64 mcg	10	267	0.97
10 mcg	11	263	0.95
21.6 mcg	10	249	0.90
46.4 mcg	9	119	0.41
100 mcg	12	22	0.04
216 mcg	14	13	---

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	33	231	1.00
1.0 mcg	28	231	1.03
2.16 mcg	32	242	1.06
4.64 mcg	33	230	0.99
10 mcg	36	246	1.06
21.6 mcg	30	239	1.06
46.4 mcg	30	237	1.05
100 mcg	30	209	0.90
216 mcg	22	119	0.49

Study No.: 877858
Report

DEGUSSA AG - US-IT - NR.
92 0112 DGM

QUAB 342

Mouse Micronucleus Test
(Single Intraperitoneal Administration)

R E P O R T

July 15, 1992

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The report comprises 28 pages.

1. GENERAL INFORMATION

1.1 Study Specification

Sponsor	:	Degussa AG/ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Testing Facility	:	ASTA Medica AG Institute of Toxicology Kantstraße 2 D-4802 Halle-Künsebeck
Study Director, Veterinary Care, Pathology	:	Dr.med.vet. K. Krauser
Study Performance	:	Mrs. M. Beste, Mrs. A. Lagoudis
Bone Marrow Evaluation	:	Miss C. Barkey
Quality Assurance	:	K.E. Fichtner Representative: Dr. B. Wilker (formerly: Roos)
Test Material	:	QUAB 342
Objective	:	Testing the mutagenic properties (damage to the chromosomes and/or the mitotic apparatus)
Test Guidelines	:	OECD Guideline No. 474 (1) EEC Directive (84/449/EEC) (2)
Study Number	:	877858
Time Schedule	:	Protocol and 1 amendment Oct/04/1990 Day of Administration Oct/15/1990 Last Day of Bone Marrow Sampling Oct/18/1990 Date of Report Jul/15/1992
Quality Assurance	:	The study was performed according to the regulations for "Good Laboratory Practice" (3).
Archivation	:	The approved protocol, all raw data obtained in the course of the study, all bone marrow smears as well as a copy of the final report are kept in the archives of the Institute of Toxicology at least 30 years (starting with the report date). Afterwards the sponsor will decide on further use.

1.2 AUTHENTICATION

I, the undersigned, hereby declare that to the best of my knowledge the study was performed under my supervision in accordance with current Good Laboratory Practice (as laid down in the German Chemicals Act).

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

This report represents a true and accurate record of the results obtained.

Study Director : July 15, 1992
Dr.med.vet. K. Krauser

Report Review

Head of the
Institute of Toxicology : July 15, 1992
Dr.med.vet. W. Jahn

1.3 ASTA Medica AG
Corporate Quality Assurance
Section GLP/GCP

Quality Assurance Statement

The non-clinical laboratory study

QUAB 342
Mouse Micronucleus Test
(Single Intraperitoneal Administration)

performed at ASTA Medica AG, Bielefeld,

was inspected and audited for conformance to the principles of Good Laboratory Practice (GLP).

The dates of inspections and reports to study director and management are given below.

Phase of Study	Date of QA Inspection	Date of QA Report
Protocol Review	Oct. 15, 1990	
Experimental Period	Oct. 15, 1990 Oct. 16, 1990 Oct. 18, 1990	Oct. 19, 1990
(Evaluation)	Apr. 19, 1991	
(Statistics)	Jun. 24, 1991	Jun. 24. 1991
Final Report Audit	Jul. 15, 1992	Jul. 15, 1992

Date: July 29, 1992

Signed: K. E. Fichtner

2. SUMMARY

This in vivo micronucleus test in mice was performed to assess the potential mutagenic activity, induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus, according to SCHMID with the modifications of SALAMONE et al.

The experiment was performed with three groups, the negative and positive control groups, each containing 18 males and 18 females, and the test group, containing 21 males and 21 females.

Negative control group and test material group animals were given a single intraperitoneal administration of 10.0 ml/kg body weight. Positive control group animals received a single administration of 10.0 ml/kg body weight by oral gavage. No test material group animal died.

The negative control group received physiological saline solution (0.9%). The test material group animals received the maximum tolerated doses in respect of the micronucleus test, i.e. 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) of the test material QUAB 342 dissolved in aqua ad iniectabilia. The positive control group received 51.1 mg/kg body weight cyclophosphamide dissolved in physiological saline solution (0.9%).

QUAB 342 related toxic symptoms were registered in all test material group animals. The symptoms consisted in clonic convulsions, stilted gait, ruffled furs, ptosis, sunken sides, and hypokinesia.

Twenty-four (24), 48 and 72 hours after treatment, six mice per sex and control group and seven mice per sex of the test material group were sacrificed and bone marrow was removed from the femurs for examination.

For each sampling time five animals per sex and group were used for evaluation. One thousand polychromatic erythrocytes per animal were scored for micronuclei. The ratio of polychromatic to normochromatic erythrocytes was used to assess the toxicity of the test material by counting a total of 1000 erythrocytes.

The incidences of micronuclei were statistically analyzed by means of a POISSON test. Estimation and testing were performed at each sampling time for each treatment group and for each sex, as well as for both sexes combined.

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) no significant test material-related increase in micronucleated polychromatic erythrocytes was observed in both male and female animals, respectively males and females combined, when compared with corresponding negative control group animals at 24, 48, or 72 hours after administration. No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding control group animals, with the exception of one female at the 24-hour sampling time.

The positive control group animals, which received cyclophosphamide, exhibited a significant increase in the number of micronucleated polychromatic erythrocytes and thus validated the test system.

CONCLUSION

QUAB 342 at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) (single intraperitoneal administration) is non-mutagenic in the mouse micronucleus test under the described conditions.

3. INTRODUCTION

PURPOSE

This in-vivo experiment was performed to assess the potential mutagenic activity induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus.

RATIONALE

The test procedure was according to SCHMID (4) with the modifications of SALAMONE et al (5).

The micronucleus test is based on the principle that in many mammalian cells acentric chromatid and chromosome fragments induced by clastogenic agents are left behind in the anaphase stage of cell division. They are included in the daughter cells where they form micronuclei in the cytoplasm. These micronuclei persist in the erythrocyte after extrusion of the main nucleus and can be scored.

The object of the test is to detect an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated animals versus the controls. Differential staining allows micronuclei to be identified under the microscope (6).

To validate the test system, a reference mutagen is tested parallel to the test material.

4. M A T E R I A L S

4.1 TEST MATERIAL

Identification	: QUAB 342 (see Attachment 1)
Chemical Name/Synonym	: 3-Chloro-2-hydroxypropyl-N,N,N-dimethyldodecyl-ammoniumchloride
CAS No.	: 41892-01-7
Batch No.	: 90 / Ma - 03
Physical Appearance	: Slightly coloured solid
Content/Purity	: 85%
Solubility in H_2O	: appr. 40%
pH Value	: 3-6 (appr. 40% solution in water)
Storage	: The test material was stored in a refrigerator.
Stability	: The stability of the test material was guaranteed by the sponsor under the given storage conditions throughout the experimental period (until January 1991).
Additional Information	: See attachment 1 and under substance No. 89784 on file in ASTA Medica AG.
Formulation of the Test Material	: The test material was freshly dissolved in aqua ad iniectabilia (Ampuwa(R), batch No. PM 1032 E6, supplied by Fresenius AG, D-6380 Bad Homburg).

4.2 POSITIVE CONTROL MATERIAL

Identification	: Cyclophosphamide (see Attachment 2)
Chemical Designation	: 2-(Bis-(2-chloroethyl)-amino)-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide monohydrate
Batch/Lot Number	: 090029
Physical Appearance	: White crystalline powder
Purity	: 100.0%
pH Value	: 4.9 (2% aqueous solution)
Storage	: The positive control material was stored at room temperature.
Stability	: The stability of the positive control material was guaranteed for the test period (until Dec. 31, 1991)
Formulation of the Positive Control Material	: The positive control material was freshly dissolved in physiological saline solution (0.9%, batch No. AEW 09D, supplied by Fresenius AG, D-6380 Bad Homburg).

4.3 NEGATIVE CONTROL MATERIAL

Identification	: Physiological saline solution (0.9%) (Fresenius AG, D-6380 Bad Homburg).
Batch/Lot Number	: AEW 09 D

5. METHODS

5.1 TEST SYSTEM

5.1.1 Species : Mouse

Strain : BOR: NMRI, (SPF Han)

Breeder : Winkelmann, Versuchstierzucht GmbH & Co.KG., D-4799 Borchen

Justification for the Selection of the Test System : Mice are recommended for micronucleus tests as the international standard.

Initial Age at Test : 5 weeks

Initial Body Weight at Test : males : 29 - 35 g
females: 24 - 31 g

5.1.2 Husbandry

Housing : ASTA Medica AG, Institute of Toxicology, D-4800 Bielefeld 14
Building B, Room No. 214-1 (negative control) and Room No. 214-2 (positive control and test material group)

Caging : Macrolon cages, type II

Animals per Cage : 1

Bedding : Animal bedding-chips, Jelu-Werk, J. Ehrler Industriemehle, D-7092 Rosenberg/Württ.
According to information from the manufacturer contaminant analyses of the bedding are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Diet : Standard diet ad libitum, ssniff(R) M, "Special diet for Mice", supplied by SSNIFF Spezialdiäten GmbH, D-4770 Soest (composition of the diet see Attachment 3). According to information from the manufacturer contaminant analyses of the diet are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Water	: Water ad libitum in drinking water quality of the Stadtwerke Bielefeld (Municipal Works Bielefeld). Automatic watering system with drinking nipples. According to information from the Stadtwerke Bielefeld the water is investigated in appropriate intervals. Regarding these analyses, the contaminants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Room Temperature	: 22 ± 2 degrees centigrade
Relative Humidity	: 55% ± 15% Slight deviations from these values were present for a short period (approx. 4 hrs) the day before substance administration, because of a technical defect in the air-conditioning plant. These deviations are considered not having influenced the results of this study.
Room Lighting	: 6 a.m. - 6 p.m. CET artificial lighting 6 p.m. - 6 a.m. CET natural light/dark rhythm
Room Hygiene, Cage Cleaning	: The room was cleaned regularly with commercial antiseptics and the cages with the cage washing machine type HAMO-R-T-500D (supplied by Hamo AG, CH-Biel-Bienne, Data on the ingredients of the antiseptics used are on file in ASTA Medica AG.

5.1.3 Allocation and Identification of the Animals

Number of Animals	: 114 (57 males / 57 females)
Method of Randomization	: Animals were placed into the different groups using computer generated random numbers.
Identification of the Animals	: Different ear notching of the groups; cage labelling with study No., animal No., sex, material designation, sampling time and route of administration.
5.1.4 Acclimatization Time	: 5 days under test conditions with veterinary care.

5.2 EXPERIMENTAL DESIGN

5.2.1 Dose Selection : In an orientating acute toxicity study in mice after single intraperitoneal administration of 46.4 mg/kg (both sexes) and 31.6 mg/kg QUAB 342 (only males) severe symptoms of toxicity and death occurred, while at 21.5 mg/kg in males and 31.6 mg/kg in females clear symptoms of toxicity but no death were observed. Therefore 21.5 mg/kg (males) and 31.6 mg/kg (females) Quab 342 were considered to be the maximum tolerated doses.

5.2.2 Route of Administration : Intraperitoneal (test material, negative control material)
Oral by gavage (Nelaton catheter, red rubber, Charrière-No. 6, supplied by Willy Rüsch, D-7053 Rommelshausen/Stuttgart) (positive control material)

Justification for the Route of Administration : For methodical reasons and according to the test guidelines (1, 2) the intraperitoneal route of administration was chosen for negative control and test material group animals. For validation of the test system the positive control group animals were treated orally.

5.2.3 Administration Volume : 10.0 ml/kg body weight

5.2.4 Study Procedure : Three groups of mice, the negative and positive control groups containing each 18 males and 18 females and the test material group with 21 males and 21 females each received a single intraperitoneal administration or by oral gavage, respectively (diet withdrawal: 16 h before treatment).
Group 1, the negative control, received physiological saline solution (0.9%).
Group 2, the test material group, received 21.5 mg/kg body weight (males) and 31.6 mg/kg (females) of the test material. The test material was given as a freshly prepared solution in aqua ad iniectabilia.
Group 3, the positive control, received Cyclophosphamide (51.1 mg/kg b.w.) dissolved in physiological saline solution (0.9%).

The animals were observed for the first 4 to 6 hours after administration for the occurrence of toxicity symptoms; on days 2, 3, and 4 only once daily in the morning.

At 24-, 48- and 72-hour intervals after treatment, 6 mice per sex and control group and 7 mice per sex of the test material group were sacrificed to prepare bone marrow smears.

The groups were as follows:

Group	1 Negative Control	2 Test Material	3 Positive Control
Treatment (mg/kg)	Physiolocical saline solution (0.9%)	QUAB 342 M: 21.5 F: 31.6	Cyclophosphamide 51.1

Sampling time p. appl.		Animal Numbers		
24 h	M	1- 6	301-307	401-406
	F	19- 24	322, 323, 330, 337, 340	419-424
48 h	M	7- 12	308-314	407-412
	F	25- 30	324-329, 331	425-430
72 h	M	13- 18	315-321	413-418
	F	31- 36	332-336, 341, 342	431-436

Because of the strong toxic effects with risk of intercurrent death in severa female animals, the following changes were made in the test material group ani mals concerning planned and actual sampling times. The decision was based on th clinical symptoms.

Animal Numbers	Sampling Time p.appl. Planned	Sampling Time p.appl. Actual
324	24 h	48 h
325	"	"
326	"	"
327	"	"
328	"	"
330	48 h	24 h
332	"	72 h
333	"	"
334	"	"
335	"	"
337	72 h	24 h
338	"	"
339	"	"
340	"	"

5.2.5 Preparation of Bone Marrow for Evaluation : All mice were sacrificed by CO_2 overdose. Both femurs were removed from each mouse and the bone marrow cells flushed into a labelled centrifuge tube with approximately 1.5 ml of fetal calf serum (Art. No. 210463, Boehringer Mannheim, D-1800 Mannheim). The tubes were centrifuged at approx. 180 x g for 5 minutes (Eppendorf Zentrifuge 5415, Netheler & Hinz GmbH, D-2000 Hamburg 65), after which the supernatant serum was discarded and the bone marrow cells suspended upon a thin layer of serum. A small drop of the marrow serum suspension was smeared on a slide, which was identified by study number, animal number, species, sex, material (in the test material group), and date of preparation, and allowed to dry overnight.

At least two slides per animal were prepared. The following day, the smears were stained using the panoptic stain method developed by PAPPENHEIM (6).

5.2.6 Microscopical Evaluation of the Slides : For each sampling time the bone marrow smears from the first 5 animals per sex and group were used for evaluation. One slide per animal was examined. The remaining smears of each sex and group per interval were evaluated if macroscopical examination of the first smears revealed technical imperfections which precluded accurate microscopical analysis.

From each animal one thousand polychromatic erythrocytes (PCE) were scored under the microscope (magnification 650-1000 x, C. ZEISS, D-7082 Oberkochen) for the incidence of polychromatic erythrocytes with micronuclei.

The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was calculated, based on 1000 erythrocytes (PCE + NCE) scored per slide as a measure of the toxic efficacy of the test material.

5.3 DATA RECORDING : The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including test material group, negative and positive controls. The micronucleated cells per thousand polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes are given for each animal examined (Tables 1-3).

5.4 STATISTICAL METHODS : The frequencies of polychromatic erythrocytes with micronuclei of the test material group and the positive control group were compared with those of the negative control group at each sampling time.

A POISSON test (7) was applied (Tables 4 and 5). The data from each treatment group for each sex, as well as for both sexes combined, were compared with the respective negative control group data using a VAX 8200 computer (Digital Equipment Corp., D-8000 München 81). The values of the first 5 animals of each sex and sampling time were used for statistical evaluation.

5.5 CRITERIA OF ASSESSMENT : If a test material produced no statistically significant and reproducible positive response at any one of the test points compared to the negative control group, it is considered non-mutagenic in this system (significance level: 5%; $p \leq 0.05$).

5.6 Protocol Adherence : The study was conducted in accordance with the original protocol inclusive amendments with the following exceptions:

- Denotation of sponsor and testing facility (due to organizational changes) and change of address of the testing facility.
- Assignment of female test material group animals to sampling times (see page 14, point 5.2.4).
- The centrifuge tubes (see page 15, point 5.2.5) were centrifuged at approx. 180 x g.

6. RESULTS

The following results have been compiled:

6.1 CLINICAL EXAMINATION

Animals of the test material group exhibited clinical signs as slight to moderate clonic convulsions (21/21 males, 21/21 females), stilted gait (1/21 males, 13/21 females), ruffled furs (4/21 males, 11/21 females), ptosis (4/21 females), sunken sides (12/21 males, 16/21 females), and slight to moderate hypokinesia (3/21 females). No test material group animal died.

Negative and positive control group animals did not show any abnormal clinical signs.

6.2 EVALUATION OF BONE MARROW

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight in males and 31.6 mg/kg body weight in females no significant test material-related increase of micronucleated polychromatic erythrocytes was observed in either male or female animals at each of the three examination times, 24, 48, and 72 hours after administration, respectively. This was also true for the evaluation of the values from both sexes combined at 24 and 48 hours. For the 72-hour sampling time a p-value of 0.048 was found, when the incidence of micronucleated polychromatic erythrocytes was compared between the test material group and the negative control group for both sexes combined. This value at the border of a statisstical significance is due to the particularly low incidence of micronucleated polychromatic erythrocytes in negative control animals of the 72-hour sampling time (mean: 0.4‰), which is clearly lower than historical negative control values in the Institute of Toxicology of ASTA Medica AG and also than given in the literature (8). The number of micronucleated polychromatic erythrocytes in test material treated males of this sampling time was not increased, when compared to other negative control group animals. Therefore, the statistical p-value of 0.048 (rounded 0.05) is considered not to be of relevance for the estimation of the study results.

No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding negative control group animals, with exception of one female (No. 338) at the 24-hour sampling time.

The positive control group animals receiving cyclophosphamide exhibited a significant and clear increase in the number of micronucleated polychromatic erythrocytes and thus validated the test. The dose used for this reference mutagen had a toxic effect on bone marrow as shown by the reduced PCE/NCE ratio at 48 hours (males) and 72 hours (males and females), respectively, after administration.

7. CONCLUSION

Under the experimental conditions reported, Quab 342 induced no chromosome mutation in mice by damage to the chromosomes or the mitotic apparatus, at 24-, 48-, or 72-hour intervals after the animals had received a single intraperitoneal dose of 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females). Therefore, Quab 342 is considered non-mutagenic in the re-

8. REFERENCES

1. OECD: OECD Guidelines for Testing of Chemicals, No. 474, Micronucleus Test (1983)

2. EEC: Commission directive of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (84/449/EEC). Off. J., L251, 27, 137-139 (September 19, 1984), Part B: Methods for the determination of toxicity, B.12. Micronucleus Test

3. Law on protection from dangerous substances (German Chemicals Act), August 1, 1990, Amendment 1: Principles of Good Laboratory Practice (GLP), amended at: "Dritte Verordnung zur Änderung der Gefahrstoffverordnung" dated June 5, 1991, published in Bundesgesetzblatt, Volume 1991, Part 1, No. 35, June 14, 1991.

4. SCHMID W (1975): The micronucleus test. Mutation Res. 31, 9-15

5. SALAMONE M, HEDDLE J, STUART E, KATZ M (1980): Toward an improved micronucleus test, Studies on 3 model agents, mitomycin C, cyclophosphamide, and dimethylbenzanthracene. Mutation Res. 74, 347-356

6. QUEISSER W (1978) Das Knochenmark. Georg Thieme Verlag, Stuttgart

7. STANGE K (1970) Angewandte Statistik I. Springer, Berlin

8. MAVOURNIN KH, BLAKEY DH, CIMINO MC, SALAMONE MF, HEDDLE JA (1990): The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 239, 29-80

9. T A B L E S 1-5

TABLE 1 Number of Micronuclei, Negative Control Group

TABLE 2 Number of Micronuclei, Test Material Group

TABLE 3 Number of Micronuclei, Positive Control Group

TABLE 4 Statistical Evaluation (Males / Females)

TABLE 5 Statistical Evaluation (Both Sexes Combined)

TABLE 1

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Negative control group: Physiological saline solution (0.9%), intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
1	3	2.05	7	1	1.40	13	0	2.55
2	0	1.71	8	2	2.04	14	1	4.05
3	1	1.93	9	0	2.09	15	1	2.70
4	2	2.45	10	0	1.20	16	0	2.80
5	2	1.89	11	0	1.75	17	0	3.41
Mean	1.6			0.6			0.4	
SD	1.14			0.89			0.55	
N	5			5			5	
Females								
19	1	1.77	25	1	2.13	31	0	2.41
20	1	1.97	26	2	1.86	32	0	3.33
21	2	1.67	27	1	1.72	33	1	3.47
22	2	1.67	28	2	1.57	34	1	3.13
23	1	1.96	29	2	1.42	35	1	1.78
Mean	1.4			1.6			0.6	
SD	0.55			0.55			0.55	
N	5			5			5	
Mean M+F	1.5			1.1			0.5	
SD	0.85			0.88			0.53	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 2

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Test material group: Males : 21.5 mg/kg body weight QUAB 342, intraperitoneal route
Females: 31.6

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
301	1	2.24	308	3	1.48	315	2	2.37
302	0	1.79	309	0	1.16	316	0	2.68
303	1	1.08	310	0	1.38	317	3	3.24
304	1	1.32	311	1	1.03	318	2	1.85
305	0	2.48	312	0	1.40	319	1	2.42
Mean	0.6			0.8			1.6	
SD	0.55			1.30			1.14	
N	5			5			5	
Females								
322	2	0.60	324	3	2.42	332	0	1.80
323	0	1.16	325	0	1.57	333	1	2.64
330	2	0.72	326	3	0.86	334	2	4.75
337	0	0.95	327	0	1.75	335	2	2.83
338	1	0.47	328	2	1.29	336	0	3.02
Mean	1.0			1.6			1.0	
SD	1.00			1.52			1.00	
N	5			5			5	
Mean								
M+F	0.8			1.2			1.3	
SD	0.79			1.40			1.06	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 3

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Positive control group: 51.1 mg/kg body weight cyclophosphamide, oral by gavage

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
401	24	2.24	407	13	0.37	413	8	0.46
402	43	2.21	408	17	0.62	414	8	0.33
403	19	2.40	409	22	0.32	415	8	0.16
404	48	1.70	410	21	0.47	416	4	0.19
405	20	2.19	411	22	0.37	417	7	0.30
Mean	30.8			19.0			7.0	
SD	13.66			3.94			1.73	
N	5			5			5	
Females								
419	27	1.82	425	5	1.16	431	3	0.94
420	26	0.90	426	3	0.91	432	4	1.31
421	35	1.02	427	4	0.75	433	5	0.34
422	27	0.98	428	7	1.19	434	2	0.98
423	23	1.05	429	10	0.80	435	5	0.48
Mean	27.6			5.8			3.8	
SD	4.45			2.77			1.30	
N	5			5			5	
Mean M+F	29.2			12.4			5.4	
SD	9.73			7.66			2.22	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

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TABLE 4

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test)

	24 h		48 h		72 h	
	m	f	m	f	m	f
No. of Micronuclei						
verum	3	5	4	8	8	5
neg. contr.	8	7	3	8	2	3
test statistic F	0.3333	0.6250	1.0000	0.8889	2.6667	1.2500
degrees of freedom f_1, f_2	18, 6	16, 10	8, 8	18, 16	6, 16	8, 10
p-value	0.967	0.806	0.500	0.598	0.055	0.363
No. of Micronuclei						
pos. contr.	154	138	95	29	35	19
neg. contr.	8	7	3	8	2	3
test statistic F	17.1111	17.2500	23.7500	3.2222	11.6667	4.7500
degrees of freedom f_1, f_2	18, 308	16, 276	8, 190	18, 58	6, 70	8, 38
p-value	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 5

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test) for Both Sexes Combined

	24 h	48 h	72 h
	m + f	m + f	m + f
No. of Micronuclei			
verum	8	12	13
neg. contr.	15	11	5
test statistic F	0.5000	1.0000	2.1667
degrees of freedom f_1, f_2	32, 16	24, 24	12, 26
p-value	0.953	0.500	0.048
No. of Micronuclei			
pos. contr.	292	124	54
neg. contr.	15	11	5
test statistic F	18.2500	10.3333	9.0000
degrees of freedom f_1, f_2	32, 584	24, 248	12, 108
p-value	0.000	0.000	0.000

10. A T T A C H M E N T S 1-3

ATTACHMENT 1. Test Material

ATTACHMENT 2. Positive Control Material

ATTACHMENT 3. Diet, Composition

Attachment 1

Degussa

Data on Test Substance No. Study No.

Supplier : DEGUSSA AG, ZN Wolfgang
Dept. IC-ATPS
P.O.Box 1345, D-6450 Hanau 1

Test Substance/
Trade Name/Identity: Quab 342 (Lauryl-Quab)

Synonym : 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid

CAS No. : 41892-01-7

Batch No./Date of
Production : Charge 90/ Ma - 03

Physical Appearance: slightly coloured solid

Purity (Method)
Known Impurities : Purity: 85 %
Diquaternary salt: 8,6 %
Glycol: 2,55 % Chlorhydrin: 84,8 %

Solubility : ca 40 % in water

Density : ca 1g/ml

pH-Value : 3-6 (ca 40 % solution in water)

Storage : 0 - 10 °C

Stability : Provided the a.m. storage conditions the test substance is stable until january'91 (no measurable loss of activity within 2 years)

Hu - Wolfg. 19.10.90
Place, Date

Signature

1.2	~~GC~~ 4.9	~~IR/LC~~ 05 2.0	~~Zyto~~ 3.0	~~Pirk~~ 3.5	Pyr	PGW

P 6899 1002 A.-Nr. 37 6 1

CYCLOPHOSPHAMID BP 88/USP XXI Charge: 090029 K.Nr.: 20/30

Menge: 112.05 kg/~~Lt~~/~~Stck~~. Abt.: PGW Ko.St.: 6127

3 Gebinde/~~Paletten~~ hergest.n.Vorschr.Nr. 15 v. Jan. 9

Herst.Datum: 07.09.90 WEGA – AMK X

Cyclophosphamid
K-Nr.: ~~20/0111 = 090025~~
~~20/1351 = 090026~~ 7.9.90
Cyclophosphamid roh

Verf.-Datum:

3 Etiketten SOLL K-Nr.: 200921K # 060526 IST

1. Beschreibung:	Weißes Krist. Pulver	entspr.: ja
2. Identifizierung:		
2.1. IR-Spektrum:	entspr. d. Stand. 50240	entspr.: ja
2. HPLC:	entspr. d. Vorschrift	entspr.: ja
2.3. Chlorid: Gesamtchlor	entspr. d. Vorschrift	entspr.: ja
3. Reinheitsprüfungen:		
3.1. Klarlöslichkeit:	n.m.a. 3.5 FTU	0,2 FTU
3.2. Reaktion:	pH = 4.0 bis 6.0	pH = 4,9
3.3. Chlorid:	n.m.a. 330 ppm	40 ppm
3.4. Schwermetalle:	n.m.a. 20 ppm	entspr.: ja
3.5. Wassergehalt:	6.0 bis 6.8 %	6,5 %
3.6. Schmelzpunkt:	49.5 bis 52.0 °C	50,5 °C
4. Wirkstoffgehalt:	98.0 bis 100.5 % wfr	100,0 %
5. Restlösemittel:		
Isopropanol:	n.m.a. 500 ppm	101 ppm
Diethylether:	n.m.a. 10 ppm	5.2 ppm
6. Sterilprüfung:	entspr. d. Vorschrift	entspr.: ja am: 19
7. Pyrogenprüfung:	entspr. d. Vorschrift	entspr.: ja am: 20.
WI Filtertest 17 g/	liegt vor	ja
WI Partikelzählung:	liegt vor	ja 12.9.90

PROBEMENGE : 2 x 100 ml Braunglasflasche dicht verschlossen
PROBEZIEHUNG : PGW
AM: 07.09.90

für toxikologische Untersuchungen bis 31.12.1991

FREIGABE DURCH QI AM : 25 SEP. 90

27.9.90

QUALITÄTSKONTROLLE freigegeben Jenapharm

10 JAN 1991

81

Attachment 3

s s n i f f M
Special Diet for Mice

Nutrients (in % Diet)

Raw Protein	21.00%	Calcium	1.20%
Raw Fat	3.50%	Phosphorus	0.80%
Raw Fiber	4.50%	Sodium	0.30%
Raw Ash	6.50%	Magnesium	0.20%
		Potassium	1.00%

Amino Acids (in 1 kg Diet)

Lysine	1.40%
Methionine	0.45%
Cystine	0.30%
Glycine	1.00%
Leucine	1.70%
Isoleucine	1.00%
Arginine	1.40%
Phenylalanine	1.00%
Tryptophane	0.35%
Histidine	0.50%
Alanine	1.00%
Tyrosin	0.70%
Asparagine Acid	1.60%
Glutamine Acid	3.50%
Valine	1.00%

Vitamins (in 1 kg Diet)

A	25,000 I.E.
D_3	2,500 I.E.
E	75 mg
B_1	20 mg
B_2	40 mg
B_6	15 mg
B_{12}	80 mcg
Biotin	250 mcg
Pantothenic Acid	50 mg
Choline	2,000 mg
Folic Acid	2 mg
Nicotinic Acid	150 mg
K_3	5 mg

Trace Elements (in kg Diet)

Manganese	75 mg
Copper	12 mg
Zinc	80 mg
Iodine	1 mg
Ferrum	380 mg
Fluorine	10 mg

Aliphatic Acids

Data available on request

Triage of 8(e) Submissions

Date sent to triage: AUG 10 1991 NON-CAP CAP

Submission number: 13022-A TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.):

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

parts of this study are written in German

For Contractor Use Only

entire document: 0 1 2 **pages** 1,2,3 **pages** 1,2,3, tabs

Notes:

Contractor reviewer : DMC **Date:** 8/1

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0594 - 13022 SEQ. A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Degussa Corporation

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 05/06/94 OTS DATE: 05/10/94 CSRAD DATE: 06/16/94

CHEMICAL NAME: 1-Dodecanaminium, N-(3-chloro-2-hydroxypropyl)-N,N-dimethyl-chloride - QUAB 342

CAS# 41892-01-7 → Lauryl QUAB

"

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

Atox/neur

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES (CONTINUE)	YES (DROP/REFER)	RAT RBT	LOW Acute oral/dermal toxicity		
	NO (DROP)	NO (CONTINUE)	In vitro	MED Dermal Irritation		
	DETERMINE	REFER:	MUS	HIGH		

COMMENTS: Non-Cap

8(e)-13022A
MEDIUM

Dermal irritation (observed during the acute dermal toxicity test) is medium concern based on eschar formation and reddening at the application site. Acute oral toxicity in the rat is low concern based on LD_{50} values of 3066 mg/kg (♀) and 2322 mg/kg (♂). Clinical signs included tremor, abnormal gait, convulsions, and loss of reflexes. Gross changes of the GI tract were observed. Acute dermal toxicity in the rabbit is low concern based on a calculated LD_{50} of 2523 mg/kg. Clinical signs included tremors and reduced body strength. Gross changes of the kidneys, liver, spleen, lungs, heart and small intestines were observed.

Degussa

Degussa
Corporation

8EHQ-0594-13022

8e

Contains No CBI

ORIGINAL

May 6, 1994

(A)

8EHQ-94-13022
INIT 05/10/94

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94 MAY 10 AM 8:35

Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, S. W.
Washington, D.C. 20460

88940000264

Attn.: Section 8(e) Coordinator

Re: Substantial Risk Notification
Pursuant to TSCA Section 8(e)

Dear Sir:

This Substantial Risk Notification is being submitted in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) by Degussa Corporation. In response to a bona fide intent to import, we were informed that the substance was listed on the confidential portion of the TSCA Inventory (EPA Control No. 528400314).

The chemical name is 1-Dodecanaminium, N-(3-chloro-2-hydroxypropyl)-N,N-dimethyl-chloride (which we identify by the trade names QUAB 342 and Lauryl QUAB) CASRN 41892-01-7.

We have just been made aware of some toxicity studies which can be considered to be reportable under TSCA Section 8(e) because of certain neurotoxic effects. Although we do not believe that the results reasonably support the conclusion that the substance presents a reasonable risk of injury to the health or the environment, based on EPA's guidelines of June 1991, we are submitting them to the TSCA Section 8(e) Office.

A summary of each study and a printout from the STN Chemical Abstracts Service Registry File follows; copies of the studies are attached. Because the oral and dermal toxicity reports are in German, we have provided English translations of the summaries.

Acute Oral Toxicity Study in Rats

Dosage/route/duration: The test material was administered by gavage diluted and undiluted to male and female rats at a volume between 2.15 and 4.65 ml/kg. Post-exposure observation was 14 days.

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6/16/94

82pp

65 Challenger Road Ridgefield Park NJ 07660 201-641-6100

Results: Intoxication was characterized by disturbance of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colorless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

The LD_{50} value for female rats was 3066 mg/kg and for male rats 2332 mg/kg.

Acute Dermal Toxicity Study In Rabbits

Dosage/route/duration: The test material was administered undiluted to the dorsal skin of male and female rabbits at doses between 931 and 4321 mg/kg for 24 hours under occlusive conditions. The post-exposure observation was 14 days.

Results: Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discoloration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart and small intestine were observed.

The LD_{50} value for male and female rabbits was 2523 mg/kg.

In Vitro Ames Test

Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were tested with and without liver microsomal activation system (Arochlor induced rats) at concentrations between 1.0 to 216 micrograms per plate.

At a concentration of 46.4 microgram/plate a weak increase (< x 2) was observed in the test strain TA 1538 in the absence of S9-mix. At higher concentrations, the number of revertants were decreased. Cytotoxicity was observed at 46 microgram/plate and at higher concentrations.

In Vivo Micronucleus Test in Mice

21.5 mg/kg (males) or 31.6 mg/kg (females) of the test substance were administered intraperitoneal to mice. No significant increase in micronucleated polychromatic erythrocytes was observed in both male and female mice, respectively males and females combined, at 24, 48 or 72 hours after administration.

Sincerely,

John Lewinson

John Lewinson, Ph.D.
Manager, Product Regulatory Compliance

JL-94-178

cc: R. Marion, DCA
Dr. Pieter, DCRP
B. Santoro, DCRP

Contains No CBI

Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Lauryl-GUAR

Acute Toxicity
Testing the Acute Toxicity
after Single Oral Administration

REPORT
TRANSLATION

June 30, 1982

Project No.:
Ind.-TOX-79-81/82
US-IT-No.: 82-0086-DKT

Summary

Lauryl-Quab was studied for acute toxicity after (single) oral administration in the rat.
The test substance, available as a liquid, was used as diluted aqueous solution (Aqua demin), or undiluted, with an increasing administration volume between 2.15 and 4.65 ml/kg.

The LD 50 values were for female rats 3.05 ml/kg (= 3066 mg/kg)
and for male rats 2.32 ml/kg (= 2332 mg/kg)

Intoxication was characterized by disturbances of the autonomic nervous system and the general condition (tremor, stilted gait, sunken sides or abdominal distension, salivation, ruffled fur, diarrhea, loss of body weight). Ante mortem loss of reflexes, convulsions, mydriasis and dyspnoea occurred in some individuals. The course of intoxication was acute to prolonged.

At necropsy of the deceased animals, stomach and intestine contained a colourless, red or yellow liquid. The mucosa of these organs was reddened. In one animal the stomach was perforated. In some cases gathering of liquid was detected in abdominal and thoracal cavity.

ASTA WERKE 4800 Bielefeld 14
AKTIENGESELLSCHAFT
Toxikologisches Institut Degussa-Asta

Bericht Nr.: Ind.-TOX-79-81/82

DEGUSSA AG - US-IT - NR.
82 0086 DKT

BERICHT

über die toxikologische Prüfung von Lauryl-QUAB nach einmaliger oraler Gabe an der Ratte

Acute Oral Rat

30. Juni 1982

Versuchsbeginn	:	05.01.1982
Ende des tierexperimentellen Teiles	:	18.01.1982
Versuchsdurchführende Einrichtung	:	Toxikologisches Institut Degussa-Asta Artur-Ladebeck-Str. 128 - 152 D-4800 Bielefeld 14
Versuchsleiter	:	Dr. med. vet. H.-J. Zechel Fachtierarzt für Pharmakologie und Toxikologie
Pathologie und Laboratoriums-Diagnostik	:	--
Archivaufsicht	:	E. Fichtner (QS)
Berichtsdurchsicht	:	E. Fichtner (QS)

Zusammenfassung

Lauryl-QUAB wurde zur Bestimmung seiner akuten Toxizität oral an der Ratte verabfolgt. Das in flüssiger Form vorliegende Prüfprodukt kam in verdünnter wässriger Lösung (Aqua demin) sowie unverdünnt in steigendem Applikationsvolumen zwischen 2,15 und 4,65 ml/kg zur Anwendung.

Die erhobenen DL 50 - Werte betrugen für die weiblichen Tiere 3,05 ml/kg (≙ 3066 mg/kg) und für die männlichen Tiere 2,32 ml/kg (≙ 2332 mg/kg).

Im Vergiftungsbild standen Störungen des vegetativen Nervensystems und des Allgemeinbefindens (Zittern, Stelzengang, eingezogener sowie aufgeblähter Bauch, Speichelfluß, gesträubtes Fell, Durchfall, Abmagerung) im Vordergrund. Prämortal kam es teilweise zu Reflexausfall, Krämpfen, Mydriasis und Dyspnoe. Der Vergiftungsverlauf gestaltete sich akut bis protrahiert.

Makroskopisch-anatomisch wurden bei den verstorbenen Tieren Flüssigkeitsansammlungen, teils von klarem Aussehen, teils von roter oder gelber Farbe, in Magen und Darm festgestellt. Die Wandung dieser Organe wies Rötung auf. In einem Fall lag eine Perforation der Magenwand vor. Vereinzelt wurden Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Einleitung

Nachfolgend wird über die toxikologische Prüfung von Lauryl-QUAB berichtet.

Die Untersuchung erfolgte im Auftrage des Fachreferates für Industrielle Toxikologie der Degussa, Frankfurt/M. Sie wurde nach einmaliger oraler Gabe des Prüfproduktes an der Ratte durchgeführt.

Zweck der Studie war es, eine Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes vorzunehmen. Die erhobenen Befunde werden durch Angaben zur DL 50 ergänzt.

Lauryl-QUAB lag als farblose Flüssigkeit vor und kam tierexperimentell unverdünnt und verdünnt zur Anwendung.

Methodik

1. Versuchstiere

Spezies	:	Ratte
Stamm	:	Wistar (Bor: WISW)
Züchter	:	Winkelmann, Borchen
Geschlecht	:	♂ und ♀ Tiere
Gewicht	:	♂ 127 - 147 g; ♀ 127 - 141 g
Alter	:	♂ 57 - 62 Tg.; ♀ 57 - 58 Tg.
Eingewöhnungszeit vor Testbeginn	:	5 Tage
spezielle Angaben	:	siehe Versuchsdurchführung unter Pkt. 4

2. Versuchstierhaltung

Haltung	:	Makrolonkäfige Typ III
Tiere pro Käfig	:	1
Einstreu	:	Weichholzgranulat Typ 9 ALTROMIN®
Fütterung	:	Standardisiertes Versuchstierfutter ALTROMIN®
Tränkung	:	Wasser ad libitum
Raumtemperatur	:	$21^{o} \pm 2^{o}$ C
Relative Luftfeuchtigkeit	:	50 - 60 %
Raumbeleuchtung	:	12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. Dunkelheit
Raumhygiene	:	Labor- und Käfigreinigung mittels handel üblicher Antiseptika, Instrumente- und Geräte-Desinfektion mittels Autoklav und Heißluftsterilisator

3. Prüfsubstanz (spezielle Angaben)

Chemische Bezeichnung	:	Lauryl-QUAB
Chargen-Nr.	:	
Form und Aussehen	:	klare Flüssigkeit
Gehalt/Reinheit	:	27,87 %
Löslichkeit	:	Herstellg. verdünnter Lösungen in Aqua demir
Dichte	:	1,007 g/ml
pH-Wert	:	7,1

Verdünnungsfaktoren 1,47 und 2,15 Die entspr. Konzentrationen betrugen 685 mg/kg und 468 mg/kg be Annahme einer Ausgangskonz. von 1007 mg/ml.

4. Versuchsdurchführung

Futterentzug	:	16 Std. vor Versuchsbeginn
Substanz-Applikation	:	oral mittels Schlundsonde
Dosenabstand	:	in dezimalgeometrischer Folge
Faktor	:	1,47 bzw. 2,15
Beobachtungszeit	:	14 Tage
Injektions-/Applikationsvolumen	:	2,15 - 4,64 ml/kg; die Substanz kam verdünnt und unverdünnt zur Anwendung
Tiere pro Dosis	:	5
Anzahl Dosierungsgruppen	:	4 bei ♂ und 3 bei ♀
Anzahl Tiere zur Berechnung eines DL 50 - Wertes	:	20 ♂ und 15 ♀
Gesamtzahl der im Versuch verwendeten Tiere	:	35

5. Versuchszweck

Bestimmung des Vergiftungsbildes, des Vergiftungsverlaufes und des Todeszeitpunktes; [3]

Berechnung der DL 50 (Probitanalyse) [1,2,4,]

Ergebnisse

Lauryl-QUAB wurde zur Ermittlung seiner akuten Toxizität oral an der Ratte verabfolgt. Das Prüfprodukt lag in flüssiger Form vor und kam verdünnt mit Aqua demin und unverdünnt in steigenden Applikationsvolumina zwischen 2,15 und 4,64 ml/kg zur Anwendung. Bei der Berechnung der Dosen (mg/kg) wurde eine Dichte von 1,007 g/ml zugrunde gelegt. Der Dosenbereich erstreckte sich zwischen 1007 und 4672 mg/kg.

Das Vergiftungsbild war vorwiegend durch Symptome wie Zittern, Stelzengang, schleppender Gang, Speichelfluß, gesträubtes Fell, Durchfall und eingezogener bzw. aufgeblähter Bauch geprägt, was auf eine Störung des vegetativen Nervensystems hinweist. Darüber hinaus zeigten die männlichen Tiere während der 1. Beobachtungswoche nur eine mäßige Gewichtsentwicklung. Bei einigen Tieren war eine Zyanose zu beobachten. Prämortal kam es bei den weiblichen Tieren der höchsten Dosierungsgruppe zu vollständigem Reflexausfall, starken klonischen Krämpfen, Mydriasis und Dyspnoe.

Der Vergiftungsverlauf ist als akut bis protrahiert zu bezeichnen. Erste klinische Symptome traten 20 Minuten nach Substanzgabe auf und währten bei den Überlebenden maximal bis zu 1 Woche. Letalität stellte sich überwiegend während der ersten beiden Tage p. appl. ein, weitere Todesfälle traten noch in den folgenden 3 Tagen auf.

Bei der Sektion der verendeten Tiere kam es in Abhängigkeit von der verabfolgten Dosis zu einer zunehmenden Rötung von Magen- und Darmwand. Diese Organe wiesen, bedingt durch Flüssigkeitsansammlungen in ihrem Lumen (teils klar, teils von roter und gelber Farbe) eine pralle Füllung auf. Bei einem männlichen Tier wurde eine Perforation der Magenwand diagnostiziert. Dieser Befund steht nicht im Zusammenhang mit einem unphysiologisch hohen Applikationsvolumen; er muß als eine Folge der Substanzeinwirkung angesehen werden. In einzelnen Fällen wurden auch Flüssigkeitsansammlungen in Bauchhöhle und Thorax beobachtet.

Die DL 50 - Werte sind in Tabelle 1a und 1b wiedergegeben.
Der zahlenmäßige Unterschied zwischen den DL 50 - Werten der weiblichen und männlichen Tiere ist statistisch nicht signifikant. Er trägt zufälligen Charakter.

In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

DL 50 - Werte von Lauryl-QUAB

Spezies : Ratte
Applikation : oral

Tabelle 1a

DL 50 [ml/kg]	
♀	2,32 (1,58 - 3,39)
♂	3,05 (2,25 - 4,13)

Tabelle 1b

DL 50 [mg/kg]	
♀	2372 (1593 - 3414)
♂	3066 (2261 - 4159)

Akute Toxizität DL 50 - Bestimmung
(Absterbedaten)

tanz/Präparat : Lauryl-QUAB
likationsart : oral

Spezies : Ratte
Stamm : Wistar (Bor: W.

Dosis mg/kg]	Geschlecht	Todes-rate		Überlebenszeit																		
				Std. nach Applikation						Tage nach Applikation												
		x/n	%	0,5	1	2	4	8	24	2	3	4	5	6	7	8	9	10	11	12	13	14
1473	♂	0/5	0																			
2165		1/5	20										1									
3182		2/5	40						1		1											
4672		5/5	100						2	3												
1007	♀	0/5	0																			
2165		2/5	40						2													
4672		5/5	100				3		1			1										

Literatur

1. BLISS, C.J.,The Statistics of Bioassay, Academic Press, New York, 1962

2. FINNEY, D.J., Probit Analysis, 2nd Ed., Cambridge Univ. Press, Cambridge 1952

3. HUNTER, W.J., Lingk, W. and Recht, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem. 62, 864 - 873, 1979

4. WEBER, E., Grundriß d. biol. Statistik, G. Fischer-Verlag, Stuttgart, 7. Auflage, 1972

Bielefeld, den 30.06.1982

Dr. med. vet. H.-J. Zechel
Fachtierarzt für Pharmakologie
und Toxikologie

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid
(Lauryl QUAB)
QUAB 342

Acute Toxicity after a Single Dermal Administration in Rabbits

R E P O R T
TRANSLATION

July 29, 1986

Project No.:
Ind.-TOX-508-85/86
US-IT-No.: 86-0158-DKT

Summary:

Quab 342 was applied once * to the dorsal skin area of rabbits to determine its acute toxicity. The test substance, available as a colourless liquid, was used undiluted.

Intoxication was characterized by tremor, ruffled fur, and reduction of body strength. Intoxication was prolonged. Deaths occurred between 2 and 7 days after application. At the application site grey discolouration, reddening, and eschar formation occurred.

At necropsy unspecific changes in kidneys, liver, spleen, lungs, heart, and small intestine were oberserved.

The LD_{50} values were

probit analysis:
for male and female animals 2523 mg/kg (2.46 ml/kg)

graphical estimation:
for male animals 3072 mg/kg (3.0 ml/kg)
for female animals 1946 mg/kg (1.9 ml/kg)

* remark of the translater: (occlusive for 24 hours)

DEGUSSA AG - US-IT - NR.
86 0158 DKT

3-Chlor-2-hydroxypropyl-N,N,N-dimethyldodecylammoniumchlorid (Lauryl QUAB) QUAB 342

Akute Toxizität

Prüfung der akuten Toxizität nach einmaliger dermaler Applikation am Kaninchen

B E R I C H T

Acute Dermal Rabbit

29. Juli 1986

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Der Bericht umfaßt 12 Seiten.

1. ALLGEMEINES

Auftraggeber	: DEGUSSA AG / ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Prüfeinrichtung	: ASTA-WERKE AG Chemische Fabrik Toxikologie Degussa-Asta Artur-Ladebeck-Str. 128-152 D-4800 Bielefeld 14
Prüfsubstanz	: QUAB 342
Art der Prüfung	: Prüfung der akuten Toxizität nach einmaliger dermaler Gabe am Kaninchen.
Prüfrichtlinie/-n	: OECD Guideline Nr. 402 (1)
Berichts-Nummer	: Ind.-TOX-508-85/86
Zeitplan	: Beginn: 23.10.84 Ende : 18.12.84
Prüfleiter	: Dr. rer. nat. W. Mayr
Durchführung der Studie	: R. Iselt
Berichtsdatum	: 29.07.1986

2. ZUSAMMENFASSUNG

QUAB 342 wurde zur Bestimmung seiner akuten Toxizität einmalig dermal auf die Rückenhaut des Kaninchens aufgetragen. Das Prüfprodukt lag als farblose Flüssigkeit vor und kam unverdünnt zur Anwendung.

Das Vergiftungsbild war gekennzeichnet durch Zittern, gesträubtes Fell und körperliche Schwäche. Das Vergiftungsbild ist als protrahiert zu bezeichnen. Todesfälle traten zwischen 2 und 7 Tagen p. appl. auf. Die Applikationsstellen zeigten Graufärbung, Rötung und Schorfbildung.

Bei der Autopsie waren nur bei den weiblichen Tieren unspezifische Veränderungen an Niere, Leber, Milz, Lunge, Herz und Dünndarm zu beobachten.

Die ermittelten LD 50-Werte betragen:

Probitanalyse:

männliche und weibliche Tiere 2523 mg/kg (2,46 ml/kg)

graphische Abschätzung:

männliche Tiere:	3072 mg/kg (3,0 ml/kg)
weibliche Tiere:	1946 mg/kg (1,9 ml/kg)

Bielefeld, den 29.07.1986

Prüfleiter:

Dr. rer. nat. W. Mayr

Institutsleiter:

7.8.86

Dr. med. vet. M. Herbst

3. EINLEITUNG

Nachfolgend wird über die toxikologische Prüfung von QUAB 342 nach einmaliger dermaler Gabe am Kaninchen berichtet.

Ziel der Studie war Art, Intensität und Dauer der klinischen Symptome sowie Todeszeitpunkte und Zahl der verstorbenen Tiere festzustellen (1 - 2). Die Beobachtungsdauer betrug 14 Tage. Aus den Mortalitätszahlen wurde die LD 50 ermittelt (3 - 5).

Das methodische Vorgehen basierte auf der OECD-Richtlinie Nr. 402 (1).

4. PRÜFSUBSTANZ

Prüfsubstanz /Handelsname	: QUAB 342
Chemische Bezeichnung /Synonym	: 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
CAS-Nr.	: ---
Chargen-Nr.	: 342 M 19
Form und Aussehen	: farblose Flüssigkeit
Gehalt/Reinheit	: ---
Löslichkeit	: ---
Dichte	: 1,024 g/ml
pH-Wert	: 1,5
Aufbewahrung und Stabilität	: Die Prüfsubstanz wurde bei Raumtemperatur aufbewahrt. Die Stabilität der Prüfsubstanz wurde vom Auftraggeber gewährleistet.

5. METHODIK

5.1 Prüfsystem

5.1.1 Versuchstier-Spezies	: Kaninchen
Stamm	: Weiße Russen (Albino)
Herkunft/Züchter	: Asta-Werke AG, D-4800 Bielefeld 14
Begründung der Versuchstierwahl	: Das Prüfsystem wurde aufgrund internationaler Empfehlungen gewählt. Danach ist die Spezies Kaninchen geeignet, potentielle toxische Wirkqualitäten von Prüfsubstanzen zu erfassen.

Alter der Tiere am Applikationstag	: männlich 9 - 12 Monate weiblich 9 - 10 Monate
Körpergewicht der Tiere am Applikationstag	: männlich 2,05 - 2,50 kg weiblich 2,40 - 2,75 kg
Eingewöhnungszeit	: Mindestens 5 Tage unter Prüfbedingungen vor Substanzapplikation

5.1.2 Versuchstierhaltung

Standort	: Toxikologie Degussa-Asta D-4800 Bielefeld 14, Gebäude B, Raum Nr. 214-5
Haltung	: Edelstahlkäfige Modell ASTA ASTA-WERKE AG, D-4800 Bielefeld 14
Tiere pro Käfig	: 1
Einstreu	: ohne, Drahtbodenkäfige
Futter	: Standardisiertes Versuchstierfutter ALTROMIN (R), Firma Altromin GMBH u. Co KG, 4937 Lage,
Trinkwasser	: Wasser ad libitum in Trinkwasserqualität der Stadtwerke Bielefeld, automatische Tränkung über Tränkventile
Raumtemperatur	: 22 +/- 2 Grad Celsius
Relative Luftfeuchtigkeit	: 55 +/- 15 %
Raumbeleuchtung	: 12 Std. Leuchtstoffröhren-Beleuchtung 12 Std. natürlicher Hell- Dunkelrhythmus
Raumhygiene, Käfigreinigung	: Labor- und Käfigreinigung mittels handelsüblicher Antiseptika, Instrumente- und Gerätedesinfektion mittels Autoklav und Heißluftsterilisator.

5.1.3 Kennzeichnung der Tiere	: mittels Ohrnummer (Tätowierung), Käfigbeschilderung mit gleicher Nummer

5.2 Durchführung der Prüfung

Futterentzug	: am Behandlungstage
Substanzapplikation	: dermal, zwischen der geschorenen Schulter- und Fersenbeinregion Die Prüfsubstanz wurde mittels eines Patches unter Okklusiv-Bedingungen 24 Stunden lang mit der äußeren Haut in Kontakt gebracht. Nach Abnahme des Verbandes wurde die auf der Haut verbliebene Prüfsubstanz mit Wasser abgewaschen.
Begründung der Wahl der Applikationsart	: Die Prüfsubstanz wurde aufgrund der möglichen dermalen Exposition des Menschen in bezug auf seine Toxizität nach einmaliger oraler Applikation geprüft.
Häufigkeit der Applikation	: einmalige Gabe (24 Stunden-Patch)
Applikationsform	: unverdünntes Prüfprodukt
Konzentrationen	: ---
Applikationsvolumina	: m 0,909 - 4,22 ml/kg w 0,909 - 4,22 ml/kg
Dosenabstand	: in dezimalgeometrischer Reihenfolge
Faktor	: m 2,15 w 2,15
Anzahl der Dosisgruppen	: m 3 w 3
Tiere pro Dosisgruppe	: m 2 w 2
Gesamttierzahl	: 12
Beobachtungsdauer	: 14 Tage p. appl.

5.3 Beobachtungen

5.3.1 Verhalten und Allgemeinzustand der Tiere (klinische Vergiftungssymptome) : Beobachtung der Tiere am Applikationstag während 6-8 Stunden p. appl., danach täglich zweimal (vor- und nachmittags); an Samstagen, Sonn- und Feiertagen einmal.
Alle aufgetretenen Vergiftungserscheinungen sowie deren Beginn, Schwere und Dauer wurden protokolliert.

Mortalität : Todeszeitpunkt, Anzahl der verstorbenen Tiere pro Dosis

5.3.2 Pathologie : Alle gestorbenen bzw. am Ende der Beobachtunsperiode getöteten Tiere wurden obduziert und Äußeres, Körperöffnungen, Körperhöhlen (Brust- und Bauchhöhle) und deren Organe makroskopisch untersucht.

5.4 Bestimmung der LD_{50}-Werte : approximative Ermittlung, graphisch (6) sowie Probitanalyse (3 - 5)

6. ERGEBNISSE

Allgemeine Angaben

QUAB 342 wurde zur Ermittlung seiner akuten Toxizität dermal am Kaninchen verabfolgt. Die Prüfsubstanz lag als farblose Flüssigkeit vor und wurde unverdünnt appliziert. Unter Verwendung steigender Applikationsvolumina von 0,909 bis 4,22 ml/kg erstreckte sich der geprüfte Dosisbereich bei den männlichen und weiblichen Tieren zwischen 931 und 4321 mg/kg. Die Dosisangaben berücksichtigen eine Dichte von 1,024 g/ml.

Klinische Beobachtungen

Das Vergiftungsbild war gekennzeichnet durch mäßiges bis starkes Zittern, gesträubtes Fell sowie körperliche Schwäche.

Der Vergiftungsverlauf ist als protrahiert zu bezeichnen. Erste Vergiftungssymptome waren nach 1 bis 6 Tagen zu beobachten und dauerten bis zu 9 Tagen. Todesfälle traten zwischen 2 und 7 Tagen nach Substanzapplikation auf.

Nach Abnahme des Patches waren bei allen Tieren die behandelten Hautareale grau gefärbt und von lederartiger Beschaffenheit; zusätzlich waren Rötung und Schorfbildung zu beobachten.

Pathologie

Bei der Sektion war bei einem weiblichen Tier der hohen Dosisgruppe die Konsistenz der Milz erhöht und brüchig, die Leber marmoriert und die Lunge dunkel gefleckt. Geröteter Dünndarm, weißgefleckte Nieren weißes Endokard war bei einem weiblichen Tier der mittleren Dosisgruppe zu beobachten.

Statistik

Der LD 50-Wert wurde für beide Geschlechter graphisch nach Miller und Tainter (6) abgeschätzt und für beide Geschlechter zusammen mit der Probitanalyse ermittelt.

Die Werte sind in Tabelle 1 a und 1 b aufgeführt.
In Tabelle 2 finden sich Angaben zur prozentualen Todesrate und zur Überlebenszeit.

Tabelle 1 Akute Toxizität - LD_{50}-Werte

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Tabelle 1a Graphische Abschätzung der LD 50 nach Miller und Tainter (6)
Vertrauensgrenze: 68,3 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche Tiere:	3,0 (0,355 - 25,322)	3072 (364 - 25930)
weibliche Tiere:	1,9 (0,303 - 11,925)	1946 (310 - 12211)

Tabelle 1b Rechnerische Bestimmung der LD 50 mittels Probitanalyse (3 - 5)
Vertrauensgrenze: 95 %

	LD 50 (ml/kg)	LD 50 (mg/kg)
männliche und weibliche Tiere:	2,46 (0,764 - 41,470)	2523 (782 - 42466)

Tabelle 2 Akute Toxizität - Absterbedaten

Prüfsubstanz : QUAB 342

Spezies : Kaninchen Applikationsart: dermal

Dosis (ml/kg)	Todes-rate x/n	%	Überlebenszeit Std. n. Applikation 0,5	1	2	4	8	24	Tage nach Applikation 2	3	4	5	6	7	8	9	10	11	12	13	14
männliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50												1							
4,22	1/2	50									1										
weibliche Tiere																					
0,909	0/2	0																			
2,0	1/2	50								1											
4,22	2/2	100							1												

8. LITERATUR

1. OECD-Guideline for Testing of Chemicals, Acute Dermal Toxicity, No. 402, OECD Paris (1981), ISBN-92-64-12221-4

2. HUNTER, W. J., LINGK, W. and RECHT, P., Intercomparison Study on the Determination of Single Administration Toxicity in Rats, Commission of the European Communities, Health and Safety Directorate, J. Assoc. Off. Anal. Chem 62, 864 - 873, 1979

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RESEARCH & CONSULTING COMPANY AG

DEGUSSA AG - US-IT - NR.
85 0129 DKM

PROJECT 047665

Ames Test

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

WITH

QUAB 342

REPORT

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PREFACE

GENERAL

Sponsor:	DEGUSSA AG ZN Wolfang Rodenbacher Chaussee 4 D 6450 Hanau 1
Study Monitor:	Dr. O. Weiberg
Testing Facility:	R C C Research & Consulting Company AG CH - 4452 Itingen / Switzerland
RCC Project No.:	047665
Test Article:	QUAB 342
Title:	Salmonella/Mammalian-Microsome Mutagenicity Test with QUAB 342

SCHEDULE

Receipt of Test Article:	April 15, 1985
Start of Experiments:	May 28, 1985
Date of Completion	June 17, 1985
Proposed Date of Final Report:	June 27, 1985/hac

PROJECT STAFF

Study Director: Dr. N. Banduhn, Dipl. Biol.

Date: July 3, 1985

Managing Director: Dr. K. Sachsse

Date: July 3, 1985

Technical Coordinators: Mrs. M. Maurer
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GUIDELINES

This study follows the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella Typhimurium, Reverse Mutation Assay", adopted May 26, 1983.

EEC Directive 84/449, Method No. B 14.

SUMMARY

This study was performed to investigate the potential gene mutagenic activity of QUAB 342 according to the plate incorporation test of Ames et al. (1) using the Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

The test was performed with and without liver microsomal activation. The test article was tested at the following concentrations: 1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate. Each concentration, including the controls, was tested in triplicate.

Toxic effects of the test article were observed in the toxicity experiments by a reduction of the relative survival rate.
Additionally, a reduction of the background lawn up to a clearing was observed. Reduced numbers of revertants were found in some strains at the highest concentration tested. The toxic effects were more prominent in the experiments without metabolic activation than in the experiments with metabolic activation.

Up to the highest investigated dose, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538 in both experiments, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. when tested without liver microsomal activation. In the first experiment, the revertant rate was increased but not doubled versus the control. In the second experiment, there was a dose-dependent increase with an exact doubling of the revertant rate at a concentration 46.4 mcg/pl.

CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay.

1 OBJECTIVES

1.1 PURPOSE

This in vitro experiment was performed to assess the potential gene mutagenic activity of QUAB 342.
The standard quality control principles of "Wegleitung der IKS (Interkantonale Kontrollstelle fuer Heilmittel) betreffend Gute Laborpraxis fuer nichtklinische Laborversuche" from April 28, 1980 (amended June 23, 1982), Berne/Switzerland were applied.

1.2 RATIONALE

The test procedure was according to Ames et al. (1)
The microbial assay is based on reverse mutation of Salmonella typhimurium from auxotrophism (histidine dependent) to prototrophism (histidine independent). When mutated Salmonella typhimurium are exposed to a mutagen, mutation to the histidine independent form takes place in a proportion of the bacterial population. These are readily detectable due to their ability to grow on a histidine deficient medium.

Exposure of strains of Salmonella typhimurium to a chemical mutagen may produce revertants which arise as a result of a base-pair substitution in the genetic material or as a frameshift mutation in which genetic material is either added, deleted or mis-coded.

Since many compounds do not exert their mutagenic activity until they have been metabolized by enzyme systems not available in the bacterial cell, the test article and the bacteria were incubated both in the absence and in the presence of a cofactor-supplemented post-mitochondrial fraction prepared from the livers of rats treated with enzyme-inducing agents Aroclor 1254.

To validate the test, reference mutagens were tested parallel to the test article.

2 MATERIALS AND METHODS

2.1 TEST ARTICLE

Identification	QUAB 342
Batch/Lot number	040485-342 MA 54
Chemical denomination	3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid
Purity	40.01% (cf. Certificat of analysis, April 3, 85)
Physical appearance	colorless, clear fluid
Stability	not available from the sponsor
Storage	The test article was stored at room

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To avoid any light effects on the test compound, all experiments were performed under yellow light.

On the day of the experiment, the test article was dissolved in bidistilled water at the highest investigated dose. The other doses were dilutions from this stock solution with the solvent in third-log intervals.
The test article was investigated at the following concentrations:
1.0, 2.16, 4.64, 10, 21.6, 46.4, 100 and 216 micrograms per plate (mcg/pl.).

The highest concentration was limited by the cytotoxicity of the test article to bacteria. A preliminary test on strains TA 1537 and RTA of Salmonella typhimurium resulted in a strong toxic effect at a concentration of 500 mcg/pl. and higher

2.2 CONTROLS

All control data were established on the same day as the experiment.

Negative Control:
Bidistilled water, solvent of the test article.

Positive Controls:
Reference mutagens not requiring microsomal drug-metabolizing enzyme.

Methyl methanesulfonate*	500 mcg/pl.	for TA 100
9-Aminoacridine*	40 mcg/pl.	for TA 1537
2-Nitrofluorene*	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538
N-Ethyl-N-Nitro-N-Nitrosoguanidine*	10 mcg/pl.	for TA 98
		TA 100
		TA 1535

Reference mutagens requiring microsomal drug-metabolizing enzyme

2-Aminoanthracene*	0.5 mcg/pl	for TA 98
		TA 100
		TA 1538
	1 mcg/pl	for TA 1535
	10 mcg/pl	for TA 1537
Benzo-(A)-pyrene**	5 mcg/pl.	for TA 98
		TA 100
		TA 1537
		TA 1538

Aseptic Control:

An experiment with the S-9 mix was performed (cf. study procedure)

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* Aldrich; Chemie Brunschwig, 4000 Basel/Switzerland
** Fluka, 9470 Buchs/Switzerland

2 3 TEST SYSTEM

Bacterial test organisms:

Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538; obtained from B.N. Ames, University of California, Berkeley CA, 94720, USA, May 1981.

Characterization of strains:

The strains used are mutants derived from Salmonella typhimurium LT2 and have following genotype:

S.typhimurium TA 1535 his G46 rfa- uvrB-
S.typhimurium TA 1537 his C3076 rfa- uvrB-
S.typhimurium TA 1538 his D3052 rfa- uvrB-
S.typhimurium TA 98 his D3052 rfa- uvrB- R+
S.typhimurium TA 100 his G46 rfa- uvrB- R+

All the strains carry a mutation known as deep rough (rfa) which affects the cell membrane so that the normal lipopolysaccharide cell coat is defective. These strains allow a greater permeability of the test agents across this membrane and into the cell.

In bacteria, almost all the primary damage caused to DNA by a mutagen is repaired by excision and recombination repair systems. Therefore, only a small percentage of the potential mutagenic damage is expressed. The tester strains used lack this excision repair system (uvrB), thus making them more sensitive to mutagens.

The strains TA 98 and TA 100 also carry an ampicillin resistance factor (R) which makes them particularly sensitive to mutagens.

The strains TA 1535 and TA 100 are sensitive to base-pair substitution mutagens; the strains TA 1537, TA 1538 and TA 98 are sensitive to frameshift mutagens.

The tester strains are checked at regular intervals for their genetic markers, according to published methods (1)

Storage:

The strain cultures are stored in sterile 0 5 ml ampoules (0 45 ml bacterial culture and 0.05 ml Dimethylsulfoxide) at -70 degrees centrigrade

Pre-culture of strains:

The bacteria were grown in a shaking water bath for 16 hours overnight at 37 degrees centigrade in 2.5% Nutrient Broth No. 2*. After centrifugation, the bacteria were resuspended to a concentration of approximately 1 x 10 exp. 8 to 2 x 10 exp. 9 cells per milliliter in 0.16% Nutrient Broth and 0.5% sodium chloride. The concentration of germs was controlled photometrically and determined in an experimental test with Histidine-rich potassium chloride solution on selective agar plates (cf. study procedure).

2.4 LIVER MICROSOMAL FRACTION S-9 MIX

The S-9 mix was prepared on the day of the experiment

Specific pathogen-free male Wistar rats (180 g - 300 g, KFM-Ha, outbred**), received a single i.p. injection of Aroclor 1254*** dissolved in Oleum Arachidis (200 mg/ml) at a dosage of 500 milligrams per kilogram of body weight to induce liver microsomal enzyme activity five days before the experiment.

The rats were killed by cervical dislocation and exsanguinated on the fifth day post application following a 14-16 hours starvation period.

Livers were removed under aseptic conditions and homogenized with ice-cold 0.15 molar potassium chloride (5 g of liver to 15 g of potassium chloride). The homogenates were centrifuged at 9000 x g for 10 minutes at 0 to 2 degrees centrigrade. The supernatant fraction (S-9 fraction) was collected for the preparation of S-9 mix

Composition of 1 ml S-9 mix.

Na2HPO4****	100	micro moles
MgCL2 ****	8	micro moles
KCl ****	33	micro moles
NADP *****	4	micro moles
G-6-P ******	5	micro moles
S-9 Fraction	0.3	ml

* Oxoid; Fakola, 4000 Basel/Switzerland
** Kleintierfarm Madoerin AG, 4414 Fuellinsdorf/Switzerland
*** Analabs; Kontron, 8000 Zuerich/Switzerland
(Aroclor: polychlorinated biphenyl mixture)
**** ABS, 4000 Basel/Switzerland
***** Boehringer Mannheim, 6330 Cham/Switzerland
****** Fluka, 9470 Buchs/Switzerland

2.5 STUDY PROCEDURE

For each strain and dose level, including the controls, a minimum of three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates*:

100 microliters (mcl)	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 mcl	S-9 mix (for test with metabolic activation) or 0.15 M Na_2HPO_4, (for test without metabolic activation),
100 mcl	Bacteria suspension (cf. test system, pre-culture of the strains),
2 ml	Overlay agar consisting of 0.6 % Bacto agar** and 0.6 % NaCl supplemented with 10 % 0.5 mM L-Histidine*** and 0.5 mM Biotine*** solution.

The plates were incubated at 37 degrees centigrade in the dark for 3 days. The his+ revertant colonies were counted with an Artek 880 counter or counted by hand if precipitation of test article precluded automatic counting.

For the aseptic control experiment, 100 microliters of S-9 mix were added to 2 milliliters overlay agar and treated as mentioned above.

For each test, the germ concentration of the bacterial strains was determined by pouring a mixture of 100 microliters of a 2 x 10 exp.5 diluted bacteria suspension (overnight culture) with a Histidine-rich potassium chloride solution onto a selective agar plate. After three days of incubation at 37 degrees centrigrade, the colonies were counted and the counts multiplied with the dilution factor.

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* A Pourmatic (New Brunswick Scientific Comp.) was used to fill the plates with 22 ml of 1.5 % bacto agar in Vogel Bonner medium E with 2% Glucose.

** Chemie Brunschwig, 4000 Basel/Switzerland

*** Fluka, 9470 Buchs/Switzerland

2.6 TOXICITY TEST

To estimate the cytotoxicity of the test article, prototrophic bacteria (spontaneous revertants of TA 1537 = RTA) were added as an internal standard to the selective agar plates, together with TA 1537 (cf. study procedure). The difference in the number of colonies on plates with and without added prototrophic bacteria at each dose level of the test article was compared to the number of colonies obtained with the negative control. The ratio of the two values was expressed as relative survival rate with the negative control at a 100 % survival rate.

Additionally, the toxicity of the test article can be shown in the mutagenicity assay, by a reduction in the number of revertants or by a reduction or a clearing of the background lawn.

2.7 DATA RECORDING

The number of revertant colonies was recorded manually for each plate from the counter.
Individual plate counts and the mean number of revertant colonies per plate are presented in tabular form for the test article, positive and negative controls.

2.8 EVALUATION OF RESULTS

A material is identified as a mutagen in this test system if there is a reproducible demonstration of a dose effect relation with a 2-fold increase in the number of revertants over the controls in a minimum of one strain. With the strain TA 100, a 1.5-fold increase is the criterion for a positive result.

These criteria are generally in accordance with the international used standards for the evalutation of results obtained from the Ames Test (e.g. Ref. 1, 2, and 3).

3 RESULTS

In the current study, the following results have been compiled:

3.1 GERM CONCENTRATIONS

The concentrations of Salmonella typhimurium used in this assay were as follows:

FIRST EXPERIMENT	SECOND EXPERIMENT
TA 98 = 1.2 x 10 exp.9 cells/ml	= 1.0 x 10 exp 9 cells/ml
TA 100 = 8.0 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1535 = 1.4 x 10 exp.9 cells/ml	= 9.9 x 10 exp 8 cells/ml
TA 1537 = 9.5 x 10 exp.8 cells/ml	= 5.8 x 10 exp 8 cells/ml
TA 1538 = 2.1 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments without metabolic activation,

TA 98 = 1.8 x 10 exp.9 cells/ml	= 9.4 x 10 exp 8 cells/ml
TA 100 = 6.8 x 10 exp.8 cells/ml	= 7.3 x 10 exp 8 cells/ml
TA 1535 = 1.1 x 10 exp.9 cells/ml	= 1.8 x 10 exp 9 cells/ml
TA 1537 = 1.4 x 10 exp.9 cells/ml	= 4.9 x 10 exp 8 cells/ml
TA 1538 = 1.8 x 10 exp.9 cells/ml	= 1.3 x 10 exp 9 cells/ml

in the experiments with metabolic activation.

3.2 TOXICITY OF THE TEST ARTICLE

In the toxicity experiment with QUAB 342, a reduction of the relative survival rate was observed without metabolic activation starting at 46.4 mcg/pl and with metabolic activation at a concentration of 216 mcg/pl.
Additionally, a reduction up to a clearing of the background lawn was observed starting from 46.4 to 100 mcg/pl and reduced numbers of revertants were found in some strains at the highest concentrati (216 mcg/pl.) tested.

3.3 CONTROL EXPERIMENTS

Control plates with the solvent (negative control) showed numbers of spontaneous revertant colonies per plate which were within the normal range of those cited in the literature (1)

Control plates with reference mutagens (positive controls) showed a distinct increase of the revertant colonies with the tester strains. This confirms the reversion properties of each strain. The positive results of the mutagens 2-Amino-anthracene and Benzo(a)pyrene indicate that the metabolizing system was functioning.

3.4 TESTS WITH QUAB 342

In the described bacterial mutagenicity tests, no relevant increase of the revertant colony numbers was obtained in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 at all dose levels tested when compared with the corresponding controls. The presence of liver microsomal activation did not influence these findings.

In the strain TA 1538, the number of revertant colonies was increased at a concentration of 46.4 mcg/pl. in both experiments when tested without liver microsomal activation. In the first experiment, the revertant rate was increased approximately 68 % versus the control. In the second experiment, the number of revertants increased starting at 10 mcg/pl. and reached an exact doubling versus the control at a concentration of 46.4 mcg/pl.

4 CONCLUSION

A weak, but reproducible increase of revertant colony numbers was found in the strain TA 1538 without liver microsomal activiation in two independent experiments. In the first experiment, the number of revertants was not doubled, but in both experiments the increase of revertants was found to be strain specific, detected in the same concentration range and in the absence of liver microsomal activation.

For this, it is concluded that during the described mutagenicity test and under the experimental conditions reported, the test article induced point mutations by frameshifts in the genome of the strain TA 1538.

Therefore, this sample of QUAB 342 is considered to exhibit a weak mutagenic activity in this Salmonella typhimurium reverse mutation assay

5 REFERENCES

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Distribution of Spontaneous Mutation Values in Ames Test with and without Metabolic Activation. In: Critical Evaluation of Mutagenicity Tests. Bass, R., Glocklin, V., Grosdanoff, P., Henschler, D., Kilbey B., Müller, D., Neubert, D., (eds.)
BGA Schriften 3/84, MMV Medizin Verlag München, 1984, 465-475.

6 ARCHIVING

R C C, CH - 4452 Itingen/Switzerland

Raw data, protocol, report, duplicate of report, and test article reference sample.

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	25		123		28		9		13	
	24		129		34		9		22	
	33	27	138	130	25	29	11	10	22	19
1.0 mcg	32		137		24		11		16	
	24		132		25		6		17	
	24	27	161	143	24	24	8	8	18	17
2.16 mcg	26		126		28		12		18	
	24		139		24		11		28	
	18	23	127	131	34	29	9	11	20	22
4.64 mcg	20		125		25		11		15	
	21		138		35		8		21	
	18	20	157	140	21	27	10	10	20	19
10.0 mcg	23		142		26		9		13	
	24		140		19		8		26	
	30	26	141	141	35	27	4	7	19	19
21.6 mcg	27		143		23		10		25	
	29		144		27		13		30	
	21	26	125	137	25	25	5	9	23	26
46.4 mcg	27		120		29		12		32	
	30		122		29		7		29	
	29	29	116	119	24	27	15	11	34	32
100.0 mcg	38		127		26		13		27	
	36		95		20		6		32	
	32	35	122	115	19	22	12	10	15	25
216.0 mcg	27		56		17		15		22	
	32		37		14		12		10	
	10	23	28	40	12	14	11	13	13	15
MMS 500 mcg	---		485		---		---		---	
	---		432		---		---		---	
	---	---	479	465	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		35		---	
	---		---		---		32		---	
	---	---	---	---	---	---	43	37	---	---
2-NF 5 mcg	493		486		---		44		830	
	509		443		---		47		882	
	500	501	367	432	---	---	54	48	886	866
ENNG 10 mcg	72		425		173		---		---	
	79		551		99		---		---	
	60	70	496	514	147	140	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE, WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	41		125		16		35		35	
	37		95		19		48		34	
	36	38	124	115	20	18	37	40	38	36
1.0 mcg	46		115		17		30		24	
	43		122		18		40		39	
	47	45	110	116	16	17	37	36	36	33
2.16 mcg	39		93		26		35		40	
	39		109		23		35		31	
	55	44	127	110	20	23	36	35	31	34
4.64 mcg	44		134		21		41		25	
	40		123		26		30		40	
	47	44	113	123	22	23	30	34	34	33
10.0 mcg	31		122		35		36		35	
	40		125		29		32		40	
	47	39	136	128	22	29	29	32	27	34
21.6 mcg	46		129		18		40		29	
	46		109		21		43		39	
	49	47	99	112	15	18	35	39	28	32
46.4 mcg	47		118		19		31		33	
	43		107		12		32		43	
	40	43	117	114	22	18	36	33	34	37
100.0 mcg	45		113		18		36		34	
	36		119		15		29		37	
	55	45	136	123	19	17	14	26	39	37
216.0 mcg	41		72		25		47		44	
	58		58		10		26		33	
	53	51	61	64	21	19	33	35	38	38
2-AA 0.5 mcg	99		162		---		---		130	
	113		197		---		---		97	
	113	108	180	180	---	---	---	---	99	109
2-AA 1.0 mcg	---		---		89		---		---	
	---		---		81		---		---	
	---	---	---	---	65	78	---	---	---	---
2-AA 10.0 mcg	---		---		---		172		---	
	---		---		---		219		---	
	---	---	---	---	---	---	168	196	---	---
BaP 5 mcg	284		370		---		117		124	
	294		343		---		130		108	
	262	280	410	374	---	---	124	124	117	116

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	10	367	1.00
1.0 mcg	8	358	0.98
2.16 mcg	11	358	0.97
4.64 mcg	10	352	0.96
10.0 mcg	7	351	0.96
21.6 mcg	9	359	0.98
46.4 mcg	11	189	0.50
100.0 mcg	10	42	0.09
216.0 mcg	13	13	0.00

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	40	343	1.00
1.0 mcg	36	307	0.89
2.16 mcg	35	327	0.96
4.64 mcg	34	339	1.01
10.0 mcg	32	329	0.98
21.6 mcg	39	306	0.88
46.4 mcg	33	290	0.85
100.0 mcg	26	235	0.69
216.0 mcg	35	135	0.33

SALMONELLA TEST WITHOUT S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	23		116		19		15		16	
	29		96		20		11		11	
	10	21	111	108	18	19	7	11	11	13
1.0 mcg	33		110		14		11		16	
	21		120		24		12		19	
	21	25	123	118	22	20	10	11	14	16
2.16 mcg	29		97		21		17		13	
	24		116		27		11		11	
	23	25	112	108	21	23	14	14	15	13
4.64 mcg	31		115		17		12		15	
	30		129		17		8		15	
	29	30	134	126	21	18	11	10	7	12
10 mcg	23		123		24		13		25	
	33		107		31		9		16	
	25	27	113	114	28	28	11	11	12	18
21.6 mcg	26		94		19		8		24	
	23		121		18		14		20	
	30	26	122	112	25	21	9	10	22	22
46.4 mcg	28		116		17		11		25	
	30		74		22		5		28	
	24	27	84	91	18	19	11	9	24	26
100 mcg	30		53		14		10		24	
	30		60		13		8		19	
	31	30	62	58	15	14	17	12	27	23
216 mcg	20		35		24		12		14	
	17		33		18		12		11	
	35	24	26	31	20	21	17	14	23	16
MMS 500 mcg	---		334		---		---		---	
	---		401		---		---		---	
	---	---	407	381	---	---	---	---	---	---
9-AMA 40 mcg	---		---		---		49		---	
	---		---		---		56		---	
	---	---	---	---	---	---	73	59	---	---
2-NF 5 mcg	470		383		---		57		859	
	461		357		---		53		864	
	476	469	393	378	---	---	48	53	764	829
ENNG 10 mcg	120		1103		1045		---		---	
	143		1145		681		---		---	
	133	132	885	1044	891	872	---	---	---	---

--- Not determined

SALMONELLA TEST WITH S-9 MIX

NUMBER OF REVERTANT COLONIES PER PLATE WITH GROUP MEANS

DOSE/PLATE	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
0 mcg control	51		91		12		28		31	
	59		107		17		38		29	
	47	52	115	104	15	15	33	33	29	30
1.0 mcg	47		109		13		28		30	
	40		111		18		26		21	
	38	42	121	114	14	15	30	28	33	28
2.16 mcg	48		130		14		34		27	
	50		116		21		32		19	
	47	48	124	123	17	17	30	32	29	25
4.64 mcg	54		137		16		30		29	
	41		112		13		33		29	
	45	47	99	116	21	17	37	33	28	29
10 mcg	49		128		13		32		30	
	55		113		19		40		26	
	41	48	117	119	18	17	36	36	27	28
21.6 mcg	42		139		15		29		30	
	45		119		11		31		25	
	53	47	134	131	18	15	30	30	30	28
46.4 mcg	46		129		13		30		40	
	47		116		17		31		23	
	52	48	132	126	9	13	29	30	40	34
100 mcg	56		91		16		19		34	
	38		109		14		30		30	
	44	46	109	103	12	14	42	30	22	29
216 mcg	36		55		18		25		25	
	33		45		13		15		24	
	37	35	36	45	12	14	25	22	35	28
2-AA 0.5 mcg	73		---		---		---		99	
	76		172		---		---		---	
	---	75	172	172	---	---	---	---	72	86
2-AA 1.0 mcg	---		---		98		---		---	
	---		---		109		---		---	
	---	---	---	---	---	104	---	---	---	---
2-AA 10.0 mcg	---		---		---		136		---	
	---		---		---		146		---	
	---	---	---	---	---	---	150	144	---	---
BaP 5 mcg	135		257		---		---		84	
	124		---		---		95		81	
	138	132	270	264	---	---	94	95	---	83

--- Not determined

TOXICITY TEST WITHOUT S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	11	277	1.00
1.0 mcg	11	285	1.03
2.16 mcg	14	278	0.99
4.64 mcg	10	267	0.97
10 mcg	11	263	0.95
21.6 mcg	10	249	0.90
46.4 mcg	9	119	0.41
100 mcg	12	22	0.04
216 mcg	14	13	---

TOXICITY TEST WITH S-9 MIX

DOSE	MEAN NUMBERS OF REVERTANT COLONIES		RELATIVE SURVIVAL RATE
	TA 1537	TA 1537 + RTA	
0 mcg control	33	231	1.00
1.0 mcg	28	231	1.03
2.16 mcg	32	242	1.06
4.64 mcg	33	230	0.99
10 mcg	36	246	1.06
21.6 mcg	30	239	1.06
46.4 mcg	30	237	1.05
100 mcg	30	209	0.90
216 mcg	22	119	0.49

Study No.: 877858
Report

DEGUSSA AG - US-IT - NR.
92 0112 DGM

QUAB 342

Mouse Micronucleus Test
(Single Intraperitoneal Administration)

R E P O R T

July 15, 1992

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The report comprises 28 pages.

1. GENERAL INFORMATION

1.1 Study Specification

Sponsor	:	Degussa AG/ZN Wolfgang Industrielle Toxikologie (US-IT) Postfach 13 45 D-6450 Hanau 1
Testing Facility	:	ASTA Medica AG Institute of Toxicology Kantstraße 2 D-4802 Halle-Künsebeck
Study Director, Veterinary Care, Pathology	:	Dr.med.vet. K. Krauser
Study Performance	:	Mrs. M. Beste, Mrs. A. Lagoudis
Bone Marrow Evaluation	:	Miss C. Barkey
Quality Assurance	:	K.E. Fichtner Representative: Dr. B. Wilker (formerly: Roos)
Test Material	:	QUAB 342
Objective	:	Testing the mutagenic properties (damage to the chromosomes and/or the mitotic apparatus)
Test Guidelines	:	OECD Guideline No. 474 (1) EEC Directive (84/449/EEC) (2)
Study Number	:	877858
Time Schedule	:	Protocol and 1 amendment Oct/04/1990 Day of Administration Oct/15/1990 Last Day of Bone Marrow Sampling Oct/18/1990 Date of Report Jul/15/1992
Quality Assurance	:	The study was performed according to the regulations for "Good Laboratory Practice" (3).
Archivation	:	The approved protocol, all raw data obtained in the course of the study, all bone marrow smears as well as a copy of the final report are kept in the archives of the Institute of Toxicology at least 30 years (starting with the report date). Afterwards the sponsor will decide on further use.

1.2 AUTHENTICATION

I, the undersigned. hereby declare that to the best of my knowledge the study was performed under my supervision in accordance with current Good Laboratory Practice (as laid down in the German Chemicals Act).

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

This report represents a true and accurate record of the results obtained.

Study Director : July 15/1992 K. Krauser
Dr.med.vet. K. Krauser

Report Review

Head of the
Institute of Toxicology : July 15, 1992
Dr.med.vet. W. Jahn

1.3 ASTA Medica AG
Corporate Quality Assurance
Section GLP/GCP

Quality Assurance Statement

The non-clinical laboratory study

QUAB 342
Mouse Micronucleus Test
(Single Intraperitoneal Administration)

performed at ASTA Medica AG, Bielefeld,

was inspected and audited for conformance to the principles of Good Laboratory Practice (GLP).

The dates of inspections and reports to study director and management are given below.

Phase of Study	Date of QA Inspection	Date of QA Report
Protocol Review	Oct. 15, 1990	
Experimental Period	Oct. 15, 1990 Oct. 16, 1990 Oct. 18, 1990	Oct. 19, 1990
(Evaluation)	Apr. 19, 1991	
(Statistics)	Jun. 24, 1991	Jun. 24. 1991
Final Report Audit	Jul. 15, 1992	Jul. 15, 1992

Date: July 29, 1992

Signed: K. E. Fichtner

2. SUMMARY

This in vivo micronucleus test in mice was performed to assess the potential mutagenic activity, induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus, according to SCHMID with the modifications of SALAMONE et al.

The experiment was performed with three groups, the negative and positive control groups, each containing 18 males and 18 females, and the test group, containing 21 males and 21 females.

Negative control group and test material group animals were given a single intraperitoneal administration of 10.0 ml/kg body weight. Positive control group animals received a single administration of 10.0 ml/kg body weight by oral gavage. No test material group animal died.

The negative control group received physiological saline solution (0.9%). The test material group animals received the maximum tolerated doses in respect of the micronucleus test, i.e. 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) of the test material QUAB 342 dissolved in aqua ad iniectabilia. The positive control group received 51.1 mg/kg body weight cyclophosphamide dissolved in physiological saline solution (0.9%).

QUAB 342 related toxic symptoms were registered in all test material group animals. The symptoms consisted in clonic convulsions, stilted gait, ruffled furs, ptosis, sunken sides, and hypokinesia.

Twenty-four (24), 48 and 72 hours after treatment, six mice per sex and control group and seven mice per sex of the test material group were sacrificed and bone marrow was removed from the femurs for examination.

For each sampling time five animals per sex and group were used for evaluation. One thousand polychromatic erythrocytes per animal were scored for micronuclei. The ratio of polychromatic to normochromatic erythrocytes was used to assess the toxicity of the test material by counting a total of 1000 erythrocytes.

The incidences of micronuclei were statistically analyzed by means of a POISSON test. Estimation and testing were performed at each sampling time for each treatment group and for each sex, as well as for both sexes combined.

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) no significant test material-related increase in micronucleated polychromatic erythrocytes was observed in both male and female animals, respectively males and females combined, when compared with corresponding negative control group animals at 24, 48, or 72 hours after administration. No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding control group animals, with the exception of one female at the 24-hour sampling time.

The positive control group animals, which received cyclophosphamide, exhibited a significant increase in the number of micronucleated polychromatic erythrocytes and thus validated the test system.

CONCLUSION

QUAB 342 at 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females) (single intraperitoneal administration) is non-mutagenic in the mouse micronucleus test under the described conditions.

3. INTRODUCTION

PURPOSE

This in-vivo experiment was performed to assess the potential mutagenic activity induced by QUAB 342 through damage to the chromosomes or to the mitotic apparatus.

RATIONALE

The test procedure was according to SCHMID (4) with the modifications of SALAMONE et al (5).

The micronucleus test is based on the principle that in many mammalian cells acentric chromatid and chromosome fragments induced by clastogenic agents are left behind in the anaphase stage of cell division. They are included in the daughter cells where they form micronuclei in the cytoplasm. These micronuclei persist in the erythrocyte after extrusion of the main nucleus and can be scored.

The object of the test is to detect an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of treated animals versus the controls. Differential staining allows micronuclei to be identified under the microscope (6).

To validate the test system, a reference mutagen is tested parallel to the test material.

4. MATERIALS

4.1 TEST MATERIAL

Identification	: QUAB 342 (see Attachment 1)
Chemical Name/Synonym	: 3-Chloro-2-hydroxypropyl-N,N,N-dimethyldodecyl-ammoniumchloride
CAS No.	: 41892-01-7
Batch No.	: 90 / Ma - 03
Physical Appearance	: Slightly coloured solid
Content/Purity	: 85%
Solubility in H_2O	: appr. 40%
pH Value	: 3-6 (appr. 40% solution in water)
Storage	: The test material was stored in a refrigerator.
Stability	: The stability of the test material was guaranteed by the sponsor under the given storage conditions throughout the experimental period (until January 1991).
Additional Information	: See attachment 1 and under substance No. 89784 on file in ASTA Medica AG.
Formulation of the Test Material	: The test material was freshly dissolved in aqua ad iniectabilia (Ampuwa(R), batch No. PM 1032 E6, supplied by Fresenius AG, D-6380 Bad Homburg).

4.2 POSITIVE CONTROL MATERIAL

Identification	: Cyclophosphamide (see Attachment 2)
Chemical Designation	: 2-(Bis-(2-chloroethyl)-amino)-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide monohydrate
Batch/Lot Number	: 090029
Physical Appearance	: White crystalline powder
Purity	: 100.0%
pH Value	: 4.9 (2% aqueous solution)
Storage	: The positive control material was stored at room temperature.
Stability	: The stability of the positive control material was guaranteed for the test period (until Dec. 31, 1991)
Formulation of the Positive Control Material	: The positive control material was freshly dissolved in physiological saline solution (0.9%, batch No. AEW 09D, supplied by Fresenius AG, D-6380 Bad Homburg).

4.3 NEGATIVE CONTROL MATERIAL

Identification	: Physiological saline solution (0.9%) (Fresenius AG, D-6380 Bad Homburg).
Batch/Lot Number	: AEW 09 D

5. METHODS

5.1 TEST SYSTEM

5.1.1 Species	: Mouse
Strain	: BOR: NMRI, (SPF Han)
Breeder	: Winkelmann, Versuchstierzucht GmbH & Co.KG., D-4799 Borchen
Justification for the Selection of the Test System	: Mice are recommended for micronucleus tests as the international standard.
Initial Age at Test	: 5 weeks
Initial Body Weight at Test	: males : 29 - 35 g females: 24 - 31 g

5.1.2 Husbandry

Housing	: ASTA Medica AG, Institute of Toxicology, D-4800 Bielefeld 14 Building B, Room No. 214-1 (negative control) and Room No. 214-2 (positive control and test material group)
Caging	: Macrolon cages, type II
Animals per Cage	: 1
Bedding	: Animal bedding-chips, Jelu-Werk, J. Ehrler Industriemehle, D-7092 Rosenberg/Württ. According to information from the manufacturer contaminant analyses of the bedding are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Diet	: Standard diet ad libitum, ssniff(R) M, "Special diet for Mice", supplied by SSNIFF Spezialdiäten GmbH, D-4770 Soest (composition of the diet see Attachment 3). According to information from the manufacturer contaminant analyses of the diet are performed in appropriate intervals. The pollutants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.

Water	: Water ad libitum in drinking water quality of the Stadtwerke Bielefeld (Municipal Works Bielefeld). Automatic watering system with drinking nipples. According to information from the Stadtwerke Bielefeld the water is investigated in appropriate intervals. Regarding these analyses, the contaminants are toxicologically insignificant in the quantities detected for the experiment performed. Certificates of analysis are on file in ASTA Medica AG.
Room Temperature	: 22 ± 2 degrees centigrade
Relative Humidity	: 55% ± 15% Slight deviations from these values were present for a short period (approx. 4 hrs) the day before substance administration, because of a technical defect in the air-conditioning plant. These deviations are considered not having influenced the results of this study.
Room Lighting	: 6 a.m. - 6 p.m. CET artificial lighting 6 p.m. - 6 a.m. CET natural light/dark rhythm
Room Hygiene, Cage Cleaning	: The room was cleaned regularly with commercial antiseptics and the cages with the cage washing machine type HAMO-R-T-500D (supplied by Hamo AG, CH-Biel-Bienne, Data on the ingredients of the antiseptics used are on file in ASTA Medica AG.

5.1.3 Allocation and Identification of the Animals

Number of Animals	: 114 (57 males / 57 females)
Method of Randomization	: Animals were placed into the different groups using computer generated random numbers.
Identification of the Animals	: Different ear notching of the groups; cage labelling with study No., animal No., sex, material designation, sampling time and route of administration.
5.1.4 Acclimatization Time	: 5 days under test conditions with veterinary care.

5.2 EXPERIMENTAL DESIGN

5.2.1 Dose Selection : In an orientating acute toxicity study in mice after single intraperitoneal administration of 46.4 mg/kg (both sexes) and 31.6 mg/kg QUAB 342 (only males) severe symptoms of toxicity and death occurred, while at 21.5 mg/kg in males and 31.6 mg/kg in females clear symptoms of toxicity but no death were observed. Therefore 21.5 mg/kg (males) and 31.6 mg/kg (females) Quab 342 were considered to be the maximum tolerated doses.

5.2.2 Route of Administration : Intraperitoneal (test material, negative control material)
Oral by gavage (Nelaton catheter, red rubber, Charrière-No. 6, supplied by Willy Rüsch, D-7053 Rommelshausen/Stuttgart) (positive control material)

Justification for the Route of Administration : For methodical reasons and according to the test guidelines (1, 2) the intraperitoneal route of administration was chosen for negative control and test material group animals. For validation of the test system the positive control group animals were treated orally.

5.2.3 Administration Volume : 10.0 ml/kg body weight

5.2.4 Study Procedure : Three groups of mice, the negative and positive control groups containing each 18 males and 18 females and the test material group with 21 males and 21 females each received a single intraperitoneal administration or by oral gavage, respectively (diet withdrawal: 16 h before treatment).
Group 1, the negative control, received physiological saline solution (0.9%).
Group 2, the test material group, received 21.5 mg/kg body weight (males) and 31.6 mg/kg (females) of the test material. The test material was given as a freshly prepared solution in aqua ad iniectabilia.
Group 3, the positive control, received Cyclophosphamide (51.1 mg/kg b.w.) dissolved in physiological saline solution (0.9%).

The animals were observed for the first 4 to 6 hours after administration for the occurrence of toxicity symptoms; on days 2, 3, and 4 only once daily in the morning.

At 24-, 48- and 72-hour intervals after treatment, 6 mice per sex and control group and 7 mice per sex of the test material group were sacrificed to prepare bone marrow smears.

The groups were as follows:

Group	1 Negative Control	2 Test Material	3 Positive Control
Treatment (mg/kg)	Physiolocical saline solution (0.9%)	QUAB 342 M: 21.5 F: 31.6	Cyclophosphamide 51.1

Sampling time p. appl.		Animal Numbers		
24 h	M	1- 6	301-307	401-406
	F	19- 24	322, 323, 330, 337, 340	419-424
48 h	M	7- 12	308-314	407-412
	F	25- 30	324-329, 331	425-430
72 h	M	13- 18	315-321	413-418
	F	31- 36	332-336, 341, 342	431-436

Because of the strong toxic effects with risk of intercurrent death in severa female animals, the following changes were made in the test material group ani mals concerning planned and actual sampling times. The decision was based on th clinical symptoms.

Animal Numbers	Sampling Time p.appl. Planned	Sampling Time p.appl. Actual
324	24 h	48 h
325	"	"
326	"	"
327	"	"
328	"	"
330	48 h	24 h
332	"	72 h
333	"	"
334	"	"
335	"	"
337	72 h	24 h
338	"	"
339	"	"
340	"	"

5.2.5 Preparation of Bone Marrow for Evaluation : All mice were sacrificed by CO_2 overdose. Both femurs were removed from each mouse and the bone marrow cells flushed into a labelled centrifuge tube with approximately 1.5 ml of fetal calf serum (Art. No. 210463, Boehringer Mannheim, D-1800 Mannheim). The tubes were centrifuged at approx. 180 x g for 5 minutes (Eppendorf Zentrifuge 5415, Netheler & Hinz GmbH, D-2000 Hamburg 65), after which the supernatant serum was discarded and the bone marrow cells suspended upon a thin layer of serum. A small drop of the marrow serum suspension was smeared on a slide, which was identified by study number, animal number, species, sex, material (in the test material group), and date of preparation, and allowed to dry overnight.

At least two slides per animal were prepared. The following day, the smears were stained using the panoptic stain method developed by PAPPENHEIM (6).

5.2.6 Microscopical Evaluation of the Slides : For each sampling time the bone marrow smears from the first 5 animals per sex and group were used for evaluation. One slide per animal was examined. The remaining smears of each sex and group per interval were evaluated if macroscopical examination of the first smears revealed technical imperfections which precluded accurate microscopical analysis.

From each animal one thousand polychromatic erythrocytes (PCE) were scored under the microscope (magnification 650-1000 x, C. ZEISS, D-7082 Oberkochen) for the incidence of polychromatic erythrocytes with micronuclei.

The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was calculated, based on 1000 erythrocytes (PCE + NCE) scored per slide, as a measure of the toxic efficacy of the test material.

5.3 DATA RECORDING : The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including test material group, negative and positive controls. The micronucleated cells per thousand polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes are given for each animal examined (Tables 1-3).

5.4 STATISTICAL METHODS : The frequencies of polychromatic erythrocytes with micronuclei of the test material group and the positive control group were compared with those of the negative control group at each sampling time.

A POISSON test (7) was applied (Tables 4 and 5). The data from each treatment group for each sex, as well as for both sexes combined, were compared with the respective negative control group data using a VAX 8200 computer (Digital Equipment Corp., D-8000 München 81). The values of the first 5 animals of each sex and sampling time were used for statistical evaluation.

5.5 CRITERIA OF ASSESSMENT : If a test material produced no statistically significant and reproducible positive response at any one of the test points compared to the negative control group, it is considered non-mutagenic in this system (significance level: 5%; $p \leq 0.05$).

5.6 Protocol Adherence : The study was conducted in accordance with the original protocol inclusive amendments with the following exceptions:

- Denotation of sponsor and testing facility (due to organizational changes) and change of address of the testing facility.
- Assignment of female test material group animals to sampling times (see page 14, point 5.2.4).
- The centrifuge tubes (see page 15, point 5.2.5) were centrifuged at approx. 180 x g.

6. R E S U L T S

The following results have been compiled:

6.1 CLINICAL EXAMINATION

Animals of the test material group exhibited clinical signs as slight to moderate clonic convulsions (21/21 males, 21/21 females), stilted gait (1/21 males, 13/21 females), ruffled furs (4/21 males, 11/21 females), ptosis (4/21 females), sunken sides (12/21 males, 16/21 females), and slight to moderate hypokinesia (3/21 females). No test material group animal died.

Negative and positive control group animals did not show any abnormal clinical signs.

6.2 EVALUATION OF BONE MARROW

After a single intraperitoneal administration of the test material at 21.5 mg/kg body weight in males and 31.6 mg/kg body weight in females no significant test material-related increase of micronucleated polychromatic erythrocytes was observed in either male or female animals at each of the three examination times, 24, 48, and 72 hours after administration, respectively. This was also true for the evaluation of the values from both sexes combined at 24 and 48 hours. For the 72-hour sampling time a p-value of 0.048 was found, when the incidence of micronucleated polychromatic erythrocytes was compared between the test material group and the negative control group for both sexes combined. This value at the border of a statisstical significance is due to the particularly low incidence of micronucleated polychromatic erythrocytes in negative control animals of the 72-hour sampling time (mean: 0.4‰), which is clearly lower than historical negative control values in the Institute of Toxicology of ASTA Medica AG and also than given in the literature (8). The number of micronucleated polychromatic erythrocytes in test material treated males of this sampling time was not increased, when compared to other negative control group animals. Therefore, the statistical p-value of 0.048 (rounded 0.05) is considered not to be of relevance for the estimation of the study results.

No clear reduction in the PCE/NCE ratio was present in test material group animals when compared with the corresponding negative control group animals, with exception of one female (No. 338) at the 24-hour sampling time.

The positive control group animals receiving cyclophosphamide exhibited a significant and clear increase in the number of micronucleated polychromatic erythrocytes and thus validated the test. The dose used for this reference mutagen had a toxic effect on bone marrow as shown by the reduced PCE/NCE ratio at 48 hours (males) and 72 hours (males and females), respectively, after administration.

7. C O N C L U S I O N

Under the experimental conditions reported, Quab 342 induced no chromosome mutation in mice by damage to the chromosomes or the mitotic apparatus, at 24-, 48-, or 72-hour intervals after the animals had received a single intraperitoneal dose of 21.5 mg/kg body weight (males) and 31.6 mg/kg body weight (females). Therefore, Quab 342 is considered non-mutagenic in the re-

8. REFERENCES

1. OECD: OECD Guidelines for Testing of Chemicals, No. 474, Micronucleus Test (1983)

2. EEC: Commission directive of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (84/449/EEC). Off. J., L251, 27, 137-139 (September 19, 1984), Part B: Methods for the determination of toxicity, B.12. Micronucleus Test

3. Law on protection from dangerous substances (German Chemicals Act), August 1, 1990, Amendment 1: Principles of Good Laboratory Practice (GLP), amended at: "Dritte Verordnung zur Änderung der Gefahrstoffverordnung" dated June 5, 1991, published in Bundesgesetzblatt, Volume 1991, Part 1, No. 35, June 14, 1991.

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9. T A B L E S 1-5

TABLE 1 Number of Micronuclei, Negative Control Group

TABLE 2 Number of Micronuclei, Test Material Group

TABLE 3 Number of Micronuclei, Positive Control Group

TABLE 4 Statistical Evaluation (Males / Females)

TABLE 5 Statistical Evaluation (Both Sexes Combined)

TABLE 1

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Negative control group: Physiological saline solution (0.9%), intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
1	3	2.05	7	1	1.40	13	0	2.55
2	0	1.71	8	2	2.04	14	1	4.05
3	1	1.93	9	0	2.09	15	1	2.70
4	2	2.45	10	0	1.20	16	0	2.80
5	2	1.89	11	0	1.75	17	0	3.41
Mean	1.6			0.6			0.4	
SD	1.14			0.89			0.55	
N	5			5			5	
Females								
19	1	1.77	25	1	2.13	31	0	2.41
20	1	1.97	26	2	1.86	32	0	3.33
21	2	1.67	27	1	1.72	33	1	3.47
22	2	1.67	28	2	1.57	34	1	3.13
23	1	1.96	29	2	1.42	35	1	1.78
Mean	1.4			1.6			0.6	
SD	0.55			0.55			0.55	
N	5			5			5	
Mean M+F	1.5			1.1			0.5	
SD	0.85			0.88			0.53	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 2

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Test material group: Males : 21.5 / Females: 31.6 mg/kg body weight QUAB 342, intraperitoneal route

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
301	1	2.24	308	3	1.48	315	2	2.37
302	0	1.79	309	0	1.16	316	0	2.68
303	1	1.08	310	0	1.38	317	3	3.24
304	1	1.32	311	1	1.03	318	2	1.85
305	0	2.48	312	0	1.40	319	1	2.42
Mean	0.6			0.8			1.6	
SD	0.55			1.30			1.14	
N	5			5			5	
Females								
322	2	0.60	324	3	2.42	332	0	1.80
323	0	1.16	325	0	1.57	333	1	2.64
330	2	0.72	326	3	0.86	334	2	4.75
337	0	0.95	327	0	1.75	335	2	2.83
338	1	0.47	328	2	1.29	336	0	3.02
Mean	1.0			1.6			1.0	
SD	1.00			1.52			1.00	
N	5			5			5	
Mean								
M+F	0.8			1.2			1.3	
SD	0.79			1.40			1.06	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 3

MOUSE MICRONUCLEUS TEST, QUAB 342

Micronuclei scored in one thousand polychromatic erythrocytes with ratio PCE/NCE

Positive control group: 51.1 mg/kg body weight cyclophosphamide, oral by gavage

24 hrs post-appl.			48 hrs post-appl.			72 hrs post-appl.		
Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE	Animal No.	MN	Ratio PCE/NCE
Males								
401	24	2.24	407	13	0.37	413	8	0.46
402	43	2.21	408	17	0.62	414	8	0.33
403	19	2.40	409	22	0.32	415	8	0.16
404	48	1.70	410	21	0.47	416	4	0.19
405	20	2.19	411	22	0.37	417	7	0.30
Mean	30.8			19.0			7.0	
SD	13.66			3.94			1.73	
N	5			5			5	
Females								
419	27	1.82	425	5	1.16	431	3	0.94
420	26	0.90	426	3	0.91	432	4	1.31
421	35	1.02	427	4	0.75	433	5	0.34
422	27	0.98	428	7	1.19	434	2	0.98
423	23	1.05	429	10	0.80	435	5	0.48
Mean	27.6			5.8			3.8	
SD	4.45			2.77			1.30	
N	5			5			5	
Mean M+F	29.2			12.4			5.4	
SD	9.73			7.66			2.22	
N	10			10			10	

MN = micronuclei; PCE = polychromatic erythrocytes;
NCE = normochromatic erythrocytes

TABLE 4

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test)

	24 h		48 h		72 h	
	m	f	m	f	m	f
No. of Micronuclei						
verum	3	5	4	8	8	5
neg. contr.	8	7	3	8	2	3
test statistic F	0.3333	0.6250	1.0000	0.8889	2.6667	1.2500
degrees of freedom f_1, f_2	18, 6	16, 10	8, 8	18, 16	6, 16	8, 10
p-value	0.967	0.806	0.500	0.598	0.055	0.363
No. of Micronuclei						
pos. contr.	154	138	95	29	35	19
neg. contr.	8	7	3	8	2	3
test statistic F	17.1111	17.2500	23.7500	3.2222	11.6667	4.7500
degrees of freedom f_1, f_2	18, 308	16, 276	8, 190	18, 58	6, 70	8, 38
p-value	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 5

MOUSE MICRONUCLEUS TEST, QUAB 342

Statistical Evaluation of the Data (POISSON Test) for Both Sexes Combined

	24 h	48 h	72 h
	m + f	m + f	m + f
No. of Micronuclei			
verum	8	12	13
neg. contr.	15	11	5
test statistic F	0.5000	1.0000	2.1667
degrees of freedom f_1, f_2	32, 16	24, 24	12, 26
p-value	0.953	0.500	0.048
No. of Micronuclei			
pos. contr.	292	124	54
neg. contr.	15	11	5
test statistic F	18.2500	10.3333	9.0000
degrees of freedom f_1, f_2	32, 584	24, 248	12, 108
p-value	0.000	0.000	0.000

10. A T T A C H M E N T S 1-3

ATTACHMENT 1. Test Material

ATTACHMENT 2. Positive Control Material

ATTACHMENT 3. Diet, Composition

Attachment 1

Degussa

Data on Test Substance No. Study No.

Supplier : DEGUSSA AG, ZN Wolfgang
Dept. IC-ATPS
P.O.Box 1345, D-6450 Hanau 1

Test Substance/
Trade Name/Identity: Quab 342 (Lauryl-Quab)

Synonym : 3-Chlor-2-hydroxypropyl-N,N,N-dimethyl-dodecylammoniumchlorid

CAS No. : 41892-01-7

Batch No./Date of
Production : Charge 90/ Ma - 03

Physical Appearance: slightly coloured solid

Purity (Method)
Known Impurities : Purity: 85 %
Diquaternary salt: 8,6 %
Glycol: 2,55 % Chlorhydrin: 84,8 %

Solubility : ca 40 % in water

Density : ca 1g/ml

pH-Value : 3-6 (ca 40 % solution in water)

Storage : 0 - 10 °C

Stability : Provided the a.m. storage conditions the test substance is stable until january'91 (no measurable loss of activity within 2 years)

Hu - Wolfgang, 19.10.90
Place, Date

Signature

IR/KC 0.5 2.0 | Lyto 3.0 | Part 3.5 | Pyr | PGW

P 6899 1002

A.-Nr. 3761

CYCLOPHOSPHAMID BP 88/USP XXI

Charge: 090029 K.Nr.: 201301

Menge: 102.05 kg/~~l~~/~~Stck.~~

Abt.: PGW Ko.St.: 6127

3 Gebinde/~~Paletten~~

hergest.n.Vorschr.Nr. 15 v. Jan.90

Herst.Datum: 07.09.90

WEGA — AMK X

Cyclophosphamid

K-Nr.: ~~200011k#070025~~ ~~201130k#080026~~ 07.9.90

Verf.-Datum:

Cyclophosphamid roh

3 Etiketten

SOLL K-Nr.: 200921k#060526 IST

1. Beschreibung:	Weißes Krist. Pulver	entspr.: ja
2. Identifizierung:		
2.1. IR-Spektrum:	entspr. d. Stand. 50240	entspr.: ja
2. HPLC:	entspr. d. Vorschrift	entspr.: ja
2.3. Chlorid: Kochsalzchlor	entspr. d. Vorschrift	entspr.: ja
3. Reinheitsprüfungen:		
3.1. Klarlöslichkeit:	n.m.a. 3.5 FTU	0,2 FTU
3.2. Reaktion:	pH = 4.0 bis 6.0	pH = 4,9
3.3. Chlorid:	n.m.a. 350 ppm	40 ppm
3.4. Schwermetalle:	n.m.a. 20 ppm	entspr.: ja
3.5. Wassergehalt:	6.0 bis 6.8 %	6,5 %
3.6. Schmelzpunkt:	49.5 bis 53.0 °C	50,5 °C
4. Wirkstoffgehalt:	98.0 bis 100.5 % wfr	100,0 %
5. Restlösemittel:		
Isopropanol:	n.m.a. 500 ppm	101 ppm
Diethylether:	n.m.a. 10 ppm	5.2 ppm
6. Sterilprüfung:	entspr. d. Vorschrift	entspr.: ja am: 19
7. Pyrogenprüfung:	entspr. d. Vorschrift	entspr.: ja am: 20.
WI Filtertest 17 g/	liegt vor	ja
WI Partikelzählung:	liegt vor	ja 12.9.90

PROBEMENGE : 2 x 100 ml Braunglasflasche dicht verschlossen
PROBEZIEHUNG : PGW
AM: 07.09.90

für toxikologische Untersuchungen bis 31.12.1991

20 SEP. 90

FREIGABE DURCH QI AM :

27.9.90

QUALITÄTSKONTROLLE freigegeben

- 81 -

Attachment 3

s s n i f f M
Special Diet for Mice

Nutrients (in % Diet)

Raw Protein	21.00%
Raw Fat	3.50%
Raw Fiber	4.50%
Raw Ash	6.50%

Calcium	1.20%
Phosphorus	0.80%
Sodium	0.30%
Magnesium	0.20%
Potassium	1.00%

Amino Acids (in 1 kg Diet)

Lysine	1.40%
Methionine	0.45%
Cystine	0.30%
Glycine	1.00%
Leucine	1.70%
Isoleucine	1.00%
Arginine	1.40%
Phenylalanine	1.00%
Tryptophane	0.35%
Histidine	0.50%
Alanine	1.00%
Tyrosin	0.70%
Asparagine Acid	1.60%
Glutamine Acid	3.50%
Valine	1.00%

Vitamins (in 1 kg Diet)

A	25,000 I.E.
D_3	2,500 I.E.
E	75 mg
B_1	20 mg
B_2	40 mg
B_6	15 mg
B_{12}	80 mcg
Biotin	250 mcg
Pantothenic Acid	50 mg
Choline	2,000 mg
Folic Acid	2 mg
Nicotinic Acid	150 mg
K_3	5 mg

Trace Elements (in kg Diet)

Manganese	75 mg
Copper	12 mg
Zinc	80 mg
Iodine	1 mg
Ferrum	380 mg
Fluorine	10 mg

Aliphatic Acids

Data available on request