

Degussa 

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Corporation

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8EHQ - 1197 - 13817s

VIA CERTIFIED MAIL R.R.R.

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October 30, 1997

8EHQ-96-13817

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OPPT Document Control Officer
East Tower, Room G-99 [7407]
U.S. Environmental Protection Agency
401 M Street S.W.
Washington, D.C. 20460
Attn: TSCA § 8(e) Coordinator

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Re: 8EHQ-96-13817
3-Chloro-2-hydroxypropyl-trimethylammonium chloride (CASRN 3327-22-8)

Dear Sir or Madam:

The enclosed information is intended to supplement the original TSCA § 8(e) notice pertaining to the substance, 3-Chloro-2-hydroxypropyl-trimethylammonium chloride or "QUAB" (CASRN 3327-22-8), that was submitted to EPA by Degussa Corporation (Degussa) on November 22, 1996. Since submitting the original notice, Degussa has received a copy of the "Results" section of the 2-year skin painting study on the product know commercially as "QUAB 188", which is an aqueous solution of 3-Chloro-2-hydroxypropyl-trimethylammonium chloride (65.79%) and water (32.36%). A copy of the Results section of the study is enclosed. The Results section does not contain any TSCA Business Confidential Information and should be handled accordingly.

The results of the study may be summarized as follows:

Purpose of Study

To determine the carcinogenic potential of QUAB 188 following repeated dermal applications of the product to the skin of mice (skin painting).

Study Design and Results

Carcinogenicity after repeated dermal application to mice (skin painting)

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Dosage/route/duration:

Groups of 50 NMRI-mice of each sex were treated twice weekly with 0.2 ml of 0, 0.018, and 0.18 ml of 3-Chloro-2-hydroxypropyl-trimethylammonium chloride dissolved in a 10% aqueous ethanol solution. This corresponds to doses of 0, 13.8, and 138 mg/animal applied to the clipped dorsal skin (2 cm²) of the animals. The study was terminated after 89 weeks for female animals and 105 weeks for male animals when the 25% survival limit was reached.

Results

Microscopic examination of the application site showed a minimal increase in hyperkeratosis and acanthosis probably reflecting a minimal irritation potential of the test substance after repeated application.

No tumors were observed at the site of application.

A dose related increase in the occurrence of bronchio-alveolar adenoma and/or carcinoma was noted in both sexes. The incidence of carcinoma did not reach formal significance, nor did the incidence of adenoma or hyperplasia. The overall tumor incidence (carcinoma and adenoma) was significantly increased in the high dose males. Additional statistical analysis showed some evidence of a dose related trend due to an increased tumor incidence in the high dose group. The incidence of these findings tended to be somewhat higher in the low dose group as well, contributing to the positive result in the trend test, but none of the group comparisons was statistically significant in any of the analyses.

A higher incidence of focal glandular hyperplasia of the stomach was observed in the high dose group of both sexes. The group comparison only showed significant differences for the high dose group females and for both sexes combined. This finding was mostly due to an increased incidence of minimal to slight hyperplasia.

No other treatment related changes were observed in the study.

Conclusion

In conclusion 3-Chloro-2-hydroxypropyl-trimethylammonium chloride caused minimal hyperkeratosis and acanthosis, but no local tumors at the site of application. However, an increased incidence of tumors and hyperplasia in the lungs of the animals seems to be a treatment related effect. The interpretation of these findings is difficult because the duration of the current study was considerably longer than the average study duration in published historic data of the same mouse strain and bronchio-alveolar hyperplasia and tumors are very common lesions in aging mice. Additionally, even if the slightly increased tumor incidence represents a real effect, its biological significance is unclear and may represent a promoting effect rather than a tumor inducing phenomenon.

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The slight increased incidence of glandular hyperplasia of the stomach seen in the high dose group may be due to unintended oral uptake of the test substance.

Degussa's Response to the Test Results

In response to the results of the study, Degussa has taken the following actions to minimize human exposure to QUAB 188 during manufacturing, processing, handling, etc. activities:

Degussa's Material Safety Data Sheet (MSDS) for QUAB 188 has been modified to reflect this information. The MSDS will also be revised to contain language that requires the use of respirators and dermal protection when the product is being handled and there is a potential for dermal and/or inhalation exposure.

Degussa believes that most, if not all, commercial processes involving the use of QUAB are closed systems and exposure to humans is minimized. Degussa's workers, customers, and other persons that may come into contact with QUAB are being notified of the study results and are being offered special advice and training on the safe handling of QUAB.

If you have any questions regarding the contents of this letter, please do not hesitate to contact me at (201) 807-3161.

Sincerely,



Jayne A. Pritchard
Regulatory Compliance Attorney

Encl.

DEGUSSA AG - USA - IT - NR.

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DGC

QUAB 188

Carcinogenicity Study after
Repeated Dermal Application to Mice
(Skin Painting)

Volume I of II

Report

No. 1 of 2 Originals

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RESULTS

Clinical Investigations

Clinical Symptoms

No treatment related clinical symptoms were observed during the study.

There were no significant differences between the treatment groups and the control group. The 25% survival limit in females was reached after 89 weeks of treatment. In males this limit was reached after 105 weeks of treatment.

- Point 9.1 - Summary tables of clinical signs
- Point 9.2 - Summary tables of nodules / masses
- Point 10.1 - Mortality Data
- Point 10.2 - Individual animal data of clinical signs
- Point 10.3 - Individual animal data of nodules and masses

Food Consumption

No treatment related changes in food consumption were observed during the study. However, occasionally statistical significant changes were noted. The high dose group males had slightly higher food consumption levels in weeks 3/4, 10/11, 25/26 and in week 97/98. During week 49/50 these animals had a slightly lower food consumption. Low dose group males had a slightly lower food consumption in week 65/66. The mean food consumption of male animals during the study was unaffected.

High dose group females had a marginally higher food consumption in weeks 2/3 to 9/10, 11/12 to 13/14, 65/66 to 73/74. Low dose group 2 females had a slightly increased food consumption in weeks 3/4, 11/12, and 81/82. The overall food consumption was marginally higher in group 2 females (3.27%) and slightly higher in group 3 females (6.56%).

These changes in both males and females are not considered to be treatment related but incidental since they were marginally (less than 10% overall) and are likely to reflect the biological variation present.

- Point 8 - Figures of mean food consumption
- Point 9.3 - Summary tables (mean values \pm St.Dev.) of food consumption
- Point 10.4 - Individual animal data of food consumption

Body Weights

No treatment related changes in body weights were observed during the study. However, occasionally statistical significant changes were noted. The body weight of group 2 males was slightly higher during weeks 11 to 21, 57 to 61 and in week 73. The body weight of group 2 and 3 females was slightly higher in week 65.

- Point 8 - Figures of mean body weights
- Point 9.4 - Summary tables (mean values \pm St.Dev.) of body weights
- Point 10.5 - Individual animal data of body weights

Reflex Testing, Control of Eyes, Hearing, and Teeth

Hearing, ophthalmology, and dental examinations did not reveal any treatment related changes. Individual animals in all dose groups showed signs compatible with the advanced age of these animals.

Clinical Pathology

Hematology

The hematology examinations revealed no treatment related changes. Individual animals in all dose groups showed abnormal values, most notably increased white blood cell count or granulocytosis, anaemia, thrombocytopenia and/or leucopenia. These changes are common in old mice and part of the expected background clinical pathology. Animals showing marked hematology changes indicative of leukemia were male animals 17, 26, 206, 219, and 241 as well as in the intercurrently killed animal No. 220. In females animals showing marked hematology changes indicative of leukemia are animals 55, 58, 66, 77, 85, 89, 171, 182, 192, 264, 273, 299.

Point 9.5 - Summary tables (mean values \pm St.Dev.) of hematology

Point 10.6 - Individual animal data of hematology, except for animals killed in extremis

Point 10.7 - Individual animal data of hematology / animals killed in extremis

Pathology

Organ Weights

Male animals did not show any treatment related changes. A marginal statistically lower absolute and relative weight of the left testis was noted in high dose group males. Female animals had in high dose group increased absolute and relative weights of the liver and adrenals. The absolute weight of the right kidney was also increased in high dose females.

Point 9.6 - Summary tables (mean values \pm St.Dev.) of organ weights and organ/body weight ratios

Point 10.8 - Individual animal data of organ weights and organ/body weight ratios

Gross Necropsy

Macroscopical examination revealed no treatment related effects indicative of neoplastic change in the animals. No macroscopic changes were noted at the application site.

The individual animal data are given in the Pathology Report (Volume II of II).

Histopathology

Microscopical examination revealed a higher dose related incidence of bronchiolo-alveolar hyperplasias and tumours in the lungs in both sexes. A slightly higher incidence and severity of minimal to mild focal hyperkeratosis was noted at the application site. The total tumour incidence was slightly increased in the high dose group, as was the incidence of low grade focal hyperplasia in the glandular mucosa of the stomach.

Application site

Microscopical examination of the application site revealed a slight dose related increase in the incidence of minimal to mild focal acanthosis and hyperkeratosis at the application site. This effect was marginal in the low dose group, but more pronounced in the high dose group. This probably reflects a minimal irritancy of the test material at the high dose. No other treatment related effects were noted. Only a few neoplastic and/or hyperplastic lesions were observed. Individual animals of all groups had metastasis/infiltration of malignant lymphoma. The striated muscle tissue adjacent to the application site of animal M143 was infiltrated by a carcinoma. One animal (M233) had a small well differentiated mast cell tumour in the dermis.

Another animal F58 had a benign basal cell tumour consisting of a hyperplasia of well differentiated basal cells with glandular pattern in the dermis. Animal M119 had a focal area with chronic infiltration and foamy macrophages in the dermis. The pathogenesis of the latter lesion is unknown. Individual animals of all groups also had mild to marked hyperplasia of

mammary tissue, which was occasionally and inconsistently present in variable amounts on the section of the application site. These neoplasms and hyperplastic lesions are not considered to be treatment related, but interpreted as part of the normal background lesions in this species.

Lungs

A dose related increase in the occurrence of bronchiolo-alveolar adenomas, and/or carcinomas was noted in both sexes. Bronchiolo-alveolar hyperplasias and tumours are very common lesions in aging mice. Macroscopically they are generally discrete pearly white nodules with a firm to rubbery consistency. Microscopically adenomas are well differentiated and circumscribed, whilst carcinomas have an increased cellular pleomorphism, atypia and/or mitosis and possibly local invasion or metastasis.

The following table gives an overview of the absolute incidence of these findings in the study (the table in the statistical analysis gives the incidence based on a per animal basis and is therefore not identical with the summary incidence table).

Incidental Analysis (Fisher Exact Test)	Organ/Finding/Animal	Dose Group		1		2		3	
		Sex		m	f	m	f	m	f
		No. Animals		50	50	50	50	50	50
Hyperplasia		4	0	5	2	4	3		
Benign Tumour		7	3	8	8	12	7		
Malignant Tumour		10	6	14	5	16	10		
Benign or Malignant Tumour		17	9	22	13	28*	17		

* $p < 0.05$

The incidence of carcinomas did not reach formal significance nor did the incidence of adenomas or hyperplasia. The overall tumour incidence (carcinomas and adenomas) was significantly increased in high dose group males. Additional statistical analyses showed some evidence of a positive dose related trend due to an increased incidence at 0.18 ml (high dose group). The incidence of these findings tended to be somewhat higher in the low dose group, contributing to the positive result ($p < 0.05$) in the trend test, but none of the group comparisons were statistically significant in any of the analyses. The pattern was the same for the combined incidence of tumour or hyperplasia, in which the high dose females had a significantly higher incidence in the group comparisons ($p < 0.05$) (see under section 11).

Stomach

A carcinoma of the glandular stomach was seen in one female (animal No. 185), with adenomas seen in a further three males (animal Nos. 106, 115, 129). Because all tumours were seen in the low dose group, there was a marginally significance between group variation in incidence. In the absence of a dose related trend for the tumour incidence, this can be considered a chance related spurious finding.

The glandular mucosa had a higher incidence of focal glandular hyperplasia in the high dose group of both sexes resulting in significant dose related trend ($p < 0.05$). The group comparison only showed significant differences in high dose group females and for the sexes combined. This finding was predominantly due to an increased incidence of minimal or slight hyperplasia, rather than an increase in high grade lesions in the high dose group animals.

All other findings did not show any clear treatment related changes. These tumours occurring in this study are discussed in the following section in the organ order of the tabulated tables.

Liver

Hepatocellular carcinomas were seen in 22 males, with hepatocellular adenomas seen in a further 22 males and in one female, and focal hepatocellular hyperplasia seen in a further 4 males and one female. The incidence of hepatocellular carcinoma was lowest in the high dose group (0.18 ml present in 6/49 animals examined, with 7/50 in the low dose group and 9/50 in the control group). Neither the trend nor the pairwise difference from the control group was significant in any analyses. The incidence of hemangiosarcomas seen in three males, one in each dose group (animal Nos. 15, 112, 216), was unrelated to treatment.

Urinary bladder

Transitional cell carcinomas were seen in five males (animal Nos. 3, 9, 102, 126, 235), with further transitional cell papillomas in animal Nos. 126 and 242. Focal hyperplasia was noted in a further 7 males and one female. The incidence of these findings was unrelated to treatment.

Nasal cavity

Focal hyperplasia was noted in a few animals and was unrelated to treatment. Primary nasal cavity tumours were not observed in this study. Because of the presence of part of the oral cavity on this section and the proximity of this tissue, a few primary oral cavity lesions were noted in this section and registered as such. Three male animals had an odontoma (animal Nos. 38, 125, 149), the incidence of which was not treatment related. One female control (animal No. 83) had an incidental carcinoma, probably derived from the oral cavity in this tissue. One male control animal (No. 23) had a malignant schwannoma in this location.

Pancreas

Islet cell adenomas were seen in 17 males and two females, with islet cell hyperplasia seen in a further 90 males and 89 females. The incidence of these findings was not treatment related. One female high dose group animal (No. 291) had a malignant granular cell tumour variant with consisting of large epithelioid to fusiform cells with an abundant granular eosinophilic cytoplasm. This finding is considered to be an incidental finding.

Mesenteric lymph node

A hemangiosarcoma was noted in one male (animal No. 241), with hemangiomas seen in a further 5 males and 9 females. The incidence was unrelated to treatment.

Hematopoietic/lymphoreticular system

The occurrence of any systemic tumours such as malignant lymphoma, myeloid leukemia or histiocytic sarcoma was recorded under this artificial site as primary tumour. The organs in which tumour cells were detected were recorded as invaded site and the degree of infiltration graded. One histiocytic sarcoma was localized in the skin only and therefore not recorded as systemic tumour (animal No. 136). This tumour was included in the overall analysis of histiocytic sarcomas. One high dose group female (animal No. 273) had a myeloid leukemia. The incidence of all of these tumours was unrelated to treatment.

Intestines

The large intestine had a small carcinoma in situ in one female control animal (No. 92). One control and one mid dose group animal had focal hyperplasia in the large intestine. One high dose group male (animal No. 204) had a hemangiosarcoma in the small intestine. All these findings were incidental and unrelated to treatment.

Testes

Leydig cell carcinomas were seen in four males with Leydig cell adenomas seen in a further 8 males and Leydig cell hyperplasia in a further 91 males. Incidence of tumours was unrelated to treatment. The analysis of the combined incidence of tumours or hyperplasia showed a marginally significant ($p < 0.05$) reduced incidence in the low dose group (0.018 ml). This finding, in the absence of a dose related trend, was considered to be a chance finding.

Seminal vesicles

A carcinoma was seen in a low dose group male (animal No. 132) with an adenoma in another animal (No. 124) of this dose group. Focal hyperplasia was seen in a further 9 males. The incidence of these findings was unrelated to treatment.

Malignant granular cell tumours were seen in three males, with benign granular cell tumours present in a further two males of the control or low dose group. Incidence was not significantly related to treatment.

Ovary

Tubulostromal carcinomas were seen in two females, with tubulostromal adenomas seen in a further 16 females and tubulostromal hyperplasia and tubular stromal hyperplasia present in a further 95 females. The incidence of these findings was unrelated to treatment.

Malignant sex cord stromal tumours (granulosa cell) were seen in two females (animal Nos. 52 and 260), with benign sex cord stromal tumours (granulosa, sertoli, or luteoma) seen in a further 19 females. Incidence was unrelated to treatment. The incidence of hemangiomas seen in five females was unrelated to treatment.

Uterus

One high dose group female had a uterine carcinoma (animal No. 285). A few animals in all dose groups had uterine polyps or granular cell tumours. The incidence of these tumours or hyperplasias was unrelated to treatment. One animal in the control and each dose group had a hemangioma. The incidence of which was also unrelated to treatment. Individual animals from the high dose group had a leiomyoma, leiomyosarcoma, and malignant schwanoma.

Skin

Malignant tumours of hair follicles were seen in three females, with atypical sebaceous gland hyperplasia in a further 2 females. Incidence was unrelated to treatment.

Mammary gland

Adenocarcinomas were seen in 18 females, with adenomas seen in a further two females. Incidence was unrelated to treatment. Mammary gland hyperplasia was seen in four males and 100 females. A slight positive trend in incidence was not statistically significant ($0.05 < p < 0.1$).

Pituitary gland

A carcinoma of the pars distalis was seen in one male (animal No. 108), with adenomas of the pars distalis seen in a further 3 males and 17 females and focal hyperplasia of the pars distalis seen in a further 5 males and 15 females. Incidence of these findings was unrelated to treatment. One animal had an adenoma of the pars intermedia.

Thyroid gland

One male (animal No. 242) and one female (animal No. 270) had an adenoma in the thyroid gland.

Parathyroid gland

Three animals had adenomas of the parathyroids (animal Nos. 133, 184, 226), the incidence of which was unrelated to treatment.

Adrenals

Adrenal cortex: Adenocarcinomas were seen in 8 males, with adenomas seen in a further 5 males and 3 females, and focal hyperplasia seen in a further 19 males and 7 females. Incidence was unrelated to treatment. Type A adenomas were seen in four males and 5 females, with type A hyperplasia seen in a further 121 males and 139 females. Incidence was unrelated to treatment.

Type B adenomas were seen in 67 males and 3 females, with type B hyperplasia seen in a further 39 males and 10 females. Incidence was not significantly related to treatment, although low dose group animals showed a slightly lower incidence.

Adrenal medulla: Malignant medullary tumours were seen in 7 males and in one female, with benign medullary tumours seen in a further 8 males and two females and focal medullary hyperplasia seen in a further 19 males and 7 females. Incidence was unrelated to treatment.

Harderian gland

Carcinomas were seen in 9 males and 2 females, with adenomas seen in a further 19 males and 15 females, and focal hyperplasia seen in a further two males and two females. Carcinomas in males were seen in 1/50, 4/50, and 4/50 animals of the control, low, and high dose groups respectively. Two control females also had a harderian gland tumour. Adenomas were seen in 6/50 males and 4/50 females in the control and low dose groups and 10/50 males and 8/50 females in the high dose group. There was no significant relationship between treatment and incidence of carcinomas, but when results for the sexes were combined there was a marginally significant ($p < 0.05$) positive trend in overall tumour incidence. A similar trend was not significant ($0.05 < p < 0.1$) when the combined incidence of tumours and hyperplasia was analysed. Comparisons for the individual sexes were also not significant. This is a common lesion in mice with quite a variable incidence. The findings were considered to be incidental, as the individual sex analysis did not show any treatment related effect.

Bone

One mid dose group female (animal No. 156) had an osteosarcoma. This finding was unrelated to treatment.

Body cavities

One control (animal No. 2) and one high dose group animal (No. 206) had a malignant mesothelioma, the incidence of which is unrelated to treatment.

Overall tumour incidence

One hundred and four males and 102 females had a malignant tumour of any site, with a further 38 males and 18 females having a benign, but not a malignant tumour. Overall tumour incidence was not significantly treatment related. Also the analysis of the incidence of the presence of different tumours at different sites did not show any treatment related effect. Defined this way, there were 35 males and 33 females which had malignant tumours of at least two sites and a further 72 males and 51 females which had tumours of at least two sites, no more than one of which was malignant.

In all these analyses, however, the incidence is greatest in the high group (0.18 ml). The trend was only significant ($p < 0.05$), in the analysis of malignant tumours of multiple sites for the sexes combined. This is considered to be an artificial effect, due to the higher incidence in pulmonary tumours and incidental tumours in this dose group. There was a higher frequency of tumours with a very low incidence (1-2) in the analysis of the overall tumour incidence. These tumours are listed in table 33 of the overall tumour incidence. These tumours are very varied and do not represent a single target tissue type. This finding is considered to be an artefact, in which several unrelated tumour types are pooled, even though this finding has a high statistical significance ($p < 0.01$).

Non neoplastic findings

A target organ with treatment related toxic changes was not observed in this study. All other findings are considered to be part of the normal background findings in this species.

The summary tables and individual animal data are given in the Pathology Report (Volume II of II).

Discussion

The results of this study revealed that QUAB 188 in a dermal carcinogenicity study in NMRI mice did not have any local tumourigenic potential. A probable treatment related increase in the incidence of bronchiolo-alveolar tumours and hyperplasia was observed for both sexes. The group comparison was only significant for the high dose group and not for the low dose. The glandular stomach also showed an increased incidence of glandular hyperplasia in the high dose group. All other analysis showed only marginally significant trends or pairwise differences from the control, which were interpreted as due to chance.

The absence of a local dermal carcinogenic potential of QUAB 188 is in contrast to the results obtained in a similar study with the corresponding epoxid (2,3-epoxypropyl-N,N,N-trimethylammonium chloride, QUAB 151). The latter showed a significant incidence of skin tumours, but also the occurrence of mamma and lung tumours was reported. However, kind and incidence of the lung tumours were not described (DFG/MAK 1991).

The significance of the increase in bronchiolo-alveolar tumour and/or hyperplasia in the study with QUAB 188 is equivocal. In interpreting the results of the statistical analysis of tumour incidence data there is a possibility of false positive relationships, this is particularly the case when the p-value is in the range of 0.05 to 0.01. In classifying findings as definite or probably treatment related effects and those which can be dismissed as chance, various criteria has to be taken into account such as the level of significance, the existence of a dose related trend, the consistency of results over two sexes and the consistency of results in analysis of malignant tumour, of tumour or hyperplasia and by severity of hyperplasia. For interpretation the incidence of tumours in the historical data is also important. Although there are no direct in house historical data available for this strain, historical data from another company using the same strain and breeder are available and have been published (5a, 5b, 5c). Bearing these considerations in mind, there is only one endpoint in the tumour data, bronchiolo-alveolar tumours and hyperplasia, for which there is considerable certainty that a possible effect of treatment occurred. Incidences in the high dose group (0.18 ml) exceeded that in controls in both sexes and in each analysis. The incidence was intermediate in the low dose group at 0.018 ml, but this did not represent a statistical significant excess. The incidence of these tumours in the high dose group is also above the historical data. However, the duration of this study was considerably longer than the average study duration in the historical data. The longer duration of the current study will lead to a tendency to have a higher background incidence of both common tumours and rare tumours.

If the increased incidence of bronchio-alveolar tumours and hyperplasia is really treatment related, it can be assumed that the substance was bioavailable. Apart from the dermal exposure route an oral exposure cannot be excluded, due to licking of the treated skin site. The slightly increased incidence of glandular hyperplasia in the stomach might be indicative for an oral exposure, however may well be incidental, as this is a common lesion in aged mice. The absence of plasma levels and exposure data precludes an assessment.

Even if the slightly increased incidence of lung tumours in this study should present a real effect, its biological significance remains unclear and it may represent a promoting effect rather than a tumour inducing phenomenon.

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