EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Bishydroxyethyl Biscetyl Malonamide (Questamide H)

Adopted by the SCCP
during the 2nd plenary meeting of 7 December 2004

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1. BACKGROUND

Bishydroxyethyl biscetyl malonamide (Questamide H) (air and skin conditioning agent) is not regulated in an Annex to the Cosmetic Directive nor has it been evaluated by SCCP/SCCNFP before.

The SCCNP stated in its opinion of 25 September 2001 that substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic to reproduction, of category 3, and substances with similar potential, must not be intentionally added to cosmetic products unless it can be demonstrated that their levels do not pose a threat to the health of the consumer.

In April 2004, Enterprise Directorate-General was informed by industry that bishydroxyethyl biscetyl malonamide is notified in the United Kingdom under the Notification of New Substances Regulation 1993. As part of the assessment, the UK competent authority assigned an R62 ”possible risk of impaired fertility” classification. Therefore, bishydroxyethyl biscetyl malonamide has to be regarded as a substance with similar potential as substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic to reproduction, of category 3. This was recently confirmed, as the substance is now listed in the 29th adaptation to the technical progress of Directive 67/548/EEC (Dir 2004/73/EC) as a substance classified toxic to reproduction category 3.

The European Commission has received from industry a submission on bishydroxyethyl biscetyl malonamide which concludes that it is safe for continued use in cosmetic products under certain conditions of use.

2. TERMS OF REFERENCE

1. On the basis of provided data SCCP is asked to assess the risk to the consumer when Bishydroxyethyl biscetyl malonamide is used in cosmetic products.

2. And/or does the SCCP recommend any further restriction for its use as an ingredient in the cosmetic products?

3. OPINION

The present Opinion is primarily based on the industry submission on Questamide H and on the “National (Australia) Industrial Chemical Notification and Assessment Scheme”

Ref.: 1, 2

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Bishydroxyethyl biscetyl malonamide

3.1.1.2. Chemical names

N,N'-dihexadecyl-N,N'-bis(2-hydroxyethyl)propanediamide

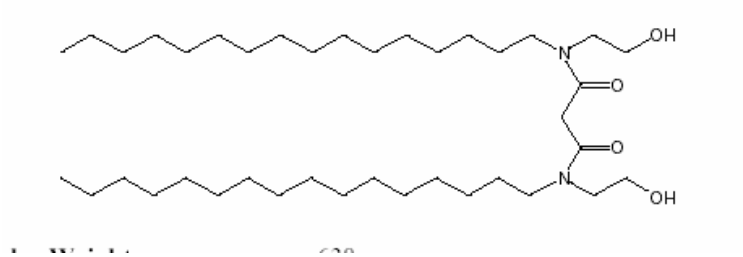
3.1.1.3. Trade names and abbreviations

Questamide H

3.1.1.4. CAS / EINECS number

CAS : 149591-38-8
EINECS : 422-560-9

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula : $C_{39}H_{78}N_2O_4$

3.1.2. Physical form

White/off-white power

3.1.3. Molecular weight

Molecular weight : 638

3.1.4. Purity, composition and substance codes

> 99%

3.1.5. Impurities / accompanying contaminants

Isomer of bishydroxyethyl biscetyl malonamide is present at less than 1%.

3.1.6.	Solubility

< 1 mg/l in water at 20 °C

3.1.7.	Partition coefficient (Log P_{ow})

no acceptable data

3.1.8.	Additional physical and chemical specifications

Organoleptic properties	:	white/off white waxy powder
Melting point	:	77°C (73 to 81°C)
Boiling point	:	/
Flash point	:	> 100°C
Vapour pressure	:	5.1 x 10^{-4} kPa at 25°C
Density	:	1.060 kg/m^3 at 20°C
Viscosity	:	/
pKa	:	/
Refractive index	:	1.490

General comments on analytical and physico-chemical characterisation

* A precise characterisation of Log P_{ow} is needed.
* No data on stability are provided.

3.2. Function and uses

Bishydroxyethyl biscetyl malonamide has been on the market and used in cosmetics since 1993. The projected usage in 2004 is 3,320 kg. It is used at concentration between 0.01 to 1.0% as a skin-conditioning agent in skin creams, shampoos, and conditioners.

The following fields of application are specified:
Hair care – shampoos and conditioners, leave-on masque, hair styling gels, balms, mousse, and spray.
Skin care – face cream, facial oil, makeup remover, eye gel, self-tanning, sun care lotion, and hand lotion.
Makeup – colour, cosmetics (foundation, powder, gloss, eye shadow, lipstick, mascara etc.), nail hardener, and nail polish.
Personal wash – bath salts, and soaps.

3.3. Toxicological Evaluation

3.3.1.	Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline	:	OECD Guideline 401 (EC Annex V Method B1).
Substance	:	Questamide H sample no S1999201, purity >99%.
Test formulation	:	200 mg/ml Questamide H in propylene glycol, freshly prepared.
Species/strain	:	Rat, Sprague-Dawley
Number/sex of animals:		5 males and 5 females, 6-8 weeks old, weight 134-184 g at dosing.
Observation period	:	14 days
Administration	:	Single gavage dose 2000 mg/kg in propylene glycol, constant volume 10 ml/kg
Clinical observations	:	Clinical signs recorded frequently on day of dosing, and once daily for 14 days following dosing. Rats were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.
Mortality	:	None
Morphological findings:		None
GLP	:	In compliance

Results
There was no mortality and no clinical signs were noted following a single oral dose of bishydroxyethyl biscetyl malonamide at a dose level of 2000 mg/kg. No abnormalities were detected at necropsy, and body weight gains were acceptable. LD_{50}: > 2 000 mg/kg

Ref.: 3

3.3.1.2. Acute dermal toxicity

Guideline	:	OECD TG 402
Substance	:	Questamide H sample no S199201, purity >99%.
Test formulation	:	Neat material as supplied was administered dermally.
Species/strain	:	Sprague-Dawley.
Number/sex of animals:		5 male and 5 female, 8-10 weeks old, weight range 203-271 g.
Observation period	:	14 days
Administration	:	2000 mg/kg was applied evenly to a gauze dressing (5cm x 5 cm) which was moistened with distilled water and applied to the shaved back of each rat. Approx. 25 cm^2 body surface (ca 10% total surface area) was in contact with test material. The trunk of the rat was then encircled with occlusive tape. Contact period 24 hours, then dressing removed and skin wiped with a water-dampened tissue.
Clinical observations	:	Clinical signs were recorded frequently on day of dosing, and once daily for 14 days following dosing. Animals were weighed immediately prior to dosing, 7 days after dosing and at sacrifice after 14 days. Skin reactions were assessed daily after patch removal for 14 days. No skin irritation observed at any time point.
Mortality	:	None
Morphological findings:		None
GLP	:	In compliance

LD_{50}: > 2 000 mg/kg

Results
There were no deaths, no clinical signs were noted and no evidence of irritation or other dermal changes were noted at the test sites. No abnormalities were detected at necropsy. LD50 >2000 mg/kg.

Ref.: 4

3.3.1.3. Acute inhalation toxicity

Not tested

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline	:	OECD TG404, EEC B4.
Substance	:	Questamide H sample no S1999201, purity > 99%
Test formulation	:	Neat material, 0.5 g moistened with water
Species/strain	:	Rabbit New Zealand White.
Number/sex of animals:		3 male, young adult.
Observation period	:	72 hours
Administration	:	The test material (0.5 g moistened with water) was applied to intact skin (from which hair had been clipped one day earlier) on each rabbit under a 2.5 cm x 2.5 cm patch of gauze. The patch was then covered with Micropore tape and the trunk was loosely bound with Elastoplast Elastic Bandage which remained in place for 4 hours. After 4 hours the patches were removed and the skin wiped with water-dampened tissues to remove surplus test material.
Clinical observations	:	Skin reactions were assessed 1, 24, 48 and 72 after patch removal
GLP	:	In compliance

Results
No erythematic or oedematous responses were noted. Draize scores were zero. Bishydroxyethyl biscetyl malonamide is non-irritant to rabbit skin.

Ref.: 5

3.3.2.2. Mucous membrane irritation

Guideline	:	OECD TG 405, EEC B5
Substance	:	Questamide H sample no S1999201, purity > 99%
Species/strain	:	Rabbit, New Zealand White
Number/sex of animals:		3 male, young adult
Observation period	:	6 days
Administration	:	100 mg test material was instilled into the right eye between lower eyelid and eyeball, the lids wee then held together for 1-2s
Clinical observations	:	Ocular reactions were assessed 1, 24, 48 and 72h after instillation
GLP	:	In compliance

Results
One animal was noted to paw at the treated eye for ca 1 min after dosing. This is indicative of a stinging response. No corneal opacity was noted in any of the treated eyes; however, a dulling of the normal lustre was reported over the 72h period following instillation. A slight iridial response, slight to moderate conjunctival redness and chemosis and a slight to severe discharge were noted up to 72h post instillation. The scores at 72h suggested a slight recovery and at 4 days a marked improvement was noted with only a slight conjunctival redness persisting. This response was still apparent at 5 days with a full recovery established at 6 days post instillation. Bishydroxyethyl biscetyl malonamide is moderately irritating to rabbit eyes. It requires Xi R36 "Irritating to eyes" classification in the EU.

Ref.: 6

3.3.3. Skin sensitisation

Guideline	:	OECD TG406, EEC B6 Magnusson/Kligman maximisation test
Substance	:	Questamide H sample no S1999201, purity >99%
Test formulation	:	5.0% a.i. Questamide H in 70% acetone/30% polyethylene glycol 400
Species/strain	:	Albino Dunkin/Hartley guinea pigs, 307-367 g.
Number of animals	:	20 animals (10 M, 10 F) in test group, and 10 animals (5 M, 5 F) in control
Induction phase		
Application	:	Injection – hair clipped from 2 cm x 4 cm area of skin in dorsal shoulder area, 3 pairs of injections made: 2 x 0.1 ml 50% Freunds complete Adjuvant (FCA) in 0.01% dodecylbenzene sulphonate/0.9% physiological saline 2 x 0.1 ml of 1% Questamide H as described above 2 x 0.1 ml test substance in 6% acetone/20% polyethylene glycol 400/0.01% dodecylbenzene sulphonate/0.9% physiological saline mixed 50:50 with FCA such that the final concentration of test materials injected was the same as that in 2nd injection set. Occluded patch application – 7 days later the same 4 cm x 2 cm area was clipped and shaved. A 2 cm x 4 cm filter paper patch, attached by double sided adhesive tape to a 4 cm x 6 cm piece of thin polythene, was saturated with 25.0% a.i. Bishydroxyethyl biscetyl malonamide in 70% Acetone/30% polyethylene glycol 400 and placed over the shaved side. The patch was held in place for 48 hours by adhesive plaster wrapped around the trunk behind the forelimbs.
Negative control	:	Ten control guinea pigs with comparable weights to the test animals were selected. They received four intradermal injections of 50% FCA in 0.01% dodecylbenzene sulphonate/0.9% physiological saline followed after one week by a 48 hour occluded patch of the test solvent over the injection sites. At challenge, five male and five female guinea pigs were challenged with test material in exactly the same way as the test animals.
Resting phase	:	14 days

Challenge phase	:	Fourteen days after the application of the induction patch the guinea pigs were challenged on the clipped and shaved flank by an occluded patch.
Application	:	For each animal, an 8 mm diameter filter paper patch in an 11 mm aluminium patch test cup was saturated with 5.05 a.i. Questamide H in 70% acetone/30% polyethylene glycol 400 and the patch applied to the shaved flank. The patch was held in place for 24 hours by adhesive plaster wound around the trunk. The treatment sites were examined for evidence of sensitisation 24 and 48 hours after removal of the patches.
Observation of symptoms:		24 and 48 hours after removal.
GLP	:	In compliance

Results
There was no evidence of toxicity of the test substance as shown by the absence of significant differences in body weights between test and control guinea pigs. One animal was sacrificed as a lesion arising from neck induction was deteriorating to a point likely to cause undue pain and discomfort.

There was no evidence of sensitisation reactions in any of the nineteen test guinea pigs when challenged with bishydroxyethyl biscetyl malonamide. The control animals showed no response to bishydroxyethyl biscetyl malonamide. Under the conditions of this study, bishydroxyethyl biscetyl malonamide did not induce sensitisation in guinea pigs.

Ref.: 7

3.3.4. Dermal / percutaneous absorption

See Section 3.3.9.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Guideline	:	/
Substance	:	Questamide H sample no S1999201, purity >99%.
Species/strain	:	Sprague Dawley rats, 5-6 weeks old.
Number/sex of animals:		5 males and 5 females. Weight range 120.9 to 147.7 g.
Administration	:	Gavage in corn oil. 10.0 ml/kg b.w.
Dose/Study duration	:	0, 15, 150 or 1 000 mg/kg/day (control, low, mid or high doses, respectively) for 28 days
Clinical observations	:	The animals were observed up to 2 times per day for signs of ill health or reaction to treatment. Body weights were recorded at twice-weekly intervals. No deaths occurred during the study; nontreatment-related nasal and/or ocular discharge was observed in all groups; no treatment-related changes in body weight at week 4 or in body weight gain were observed during the study
Haematology	:	No statistically significant differences between control and treatment groups

Clinical chemistry	:	7% increase in magnesium levels in high dose males; 10% increase in aspartate transaminase, 68% increase in alanine transaminase, 32% increase in alkaline phosphatase levels in high dose males
GLP	:	In compliance

Histopathology:
Ovaries: Apoptosis in the *corpora lutea* was observed in 4 high dose rats and one mid dose rat; prominent vacuolated cells, possibly macrophages were present in the center of the *corpora lutea*.
Uterus: The rats with apoptosis in the *corpora lutea* also had pronounced dilatation of the uterine lumen; three of the high dose rats also had prominent keratinisation of the cervical epithelium one mid dose and one low dose rat were found to have luminal dilatation of the uterus, but this was less pronounced than the uterine changes identified as treatment-related. The incidence and degree of dilatation was considered to be within the normal physiological range, and therefore not due to treatment.
Mesenteric lymph nodes: Histiocytosis was present in the lymph nodes of high dose males and females; the medullary cords of all female rats and 3 male rats were affected as was the paracortex of 2 males and females. Histiocytosis was observed also in the sinuses of a number of rats from all treatment groups but the severity was increased in high dose animals.
Liver: Focal hepatocyte necrosis was observed in 3 female and one high dose male rat. An increase in the incidence of parenchymal mononuclear cells was noted in high dose females.
Spleen: A slight increase in extramedullary haemopoiesis was observed in high dose male and female rats. In view of this observation, a proportion of bone marrow smears from animals in this group and the control group were examined. No abnormalities were detected and smears from the two groups appeared similar; as the increase in splenic extramedullary haemopoiesis was marginal, within the normal background range and not supported by any alterations in haematology parameters or bone marrow smear morphology, it was judged not to be of any toxicological importance.

A variety of spontaneous changes were recorded in animals from all dose groups with no evidence of a treatment-related distribution. The findings were within the spectrum of spontaneous lesions commonly encountered in laboratory rats of this age and strain and were considered by the study authors to be unrelated to administration of the notified chemical.
Comments
The microscopic changes observed in the ovaries and uterus were judged to be consistent with, but more pronounced than the normal cyclic changes that occur during the pro-oestrus to oestrus stages of the rat oestrus cycle, apoptosis of luteal cells being a common feature of the ovary during oestrus. The incidence and severity of the genital tract changes increased with dose and were considered to be a direct toxic effect of bishydroxyethyl biscetyl malonamide or due to a hormonal imbalance resulting in an exaggeration of the normal changes that occur in the ovary in the course of the oestrus cycle.

The hepatocyte necrosis noted in three high dose females and one high dose male rat comprised only a few discrete foci with no predilection for any particular region of the liver lobule and was considered to be unlike xenobiotic-induced cytotoxic hepatocyte necrosis which generally has a more specific distribution within the liver lobule and is usually more widespread. The effect on the liver of female rats was more severe than in males and an increase in parenchymal mononuclear cells was noted also in the majority of high dose female rats. There was, however,

no increase in liver weight and the plasma activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were only significantly increased in male rats. It was concluded that the pattern of hepatocyte necrosis seen in this study, which is occasionally noted in untreated control rats and has been attributed to pathological organisms arriving from the intestines via the portal blood supply, represented a treatment-related exacerbation of a spontaneous change rather than a direct toxic effect (although no such lesions were seen in control animals).

Accumulation of histiocytes in the mesenteric lymph nodes is a common response to the oral administration of xenobiotics. There was no evidence of any degenerative effect associated with these histiocytes. The enlarged mesenteric lymph nodes noted at necropsy in one high dose male animal were considered to be consistent with this microscopic finding.

Genital tract and liver changes similar to those seen after treatment with bishydroxyethyl biscetyl malonamide can occur in control animals. It was considered to be possible that a chance imbalanced incidence of these background findings resulted in an apparently treatment-related distribution, a problem exacerbated by the small group size and known to occur in tissues such as the genital tract, which undergo cyclic changes.

Results
The NOEL for the notified chemical was judged to be 15 mg/kg/day with target organs being the ovary, uterus, liver and mesenteric lymph node; effects at 150 mg/kg/day were limited to the ovary and uterus.

Ref.: 8

3.3.5.2.	Sub-chronic (90 days) oral / dermal / inhalation toxicity

No data submitted.

3.3.5.3.	Chronic (> 12 months) toxicity

No data submitted

3.3.6.	Mutagenicity / Genotoxicity

***Salmonella typhimurium* Reverse Mutation Assay**

Guideline	:	/
Substance	:	Questamide H sample no S1999201, purity > 99%
Test formulation	:	The solvent used was 95% ethanol
Strains	:	TA 1535, TA 1537, TA 98, TA 100
Concentration range	:	5, 50, 500, 5 000 µg/plate
Metabolic activation	:	Liver fraction from Aroclor 1254-induced rats (S9 fraction). The tests were performed in the presence of 0%, 10%, and 30% S9 fraction in the first test and 0% and 10% S9 in the second test.
GLP	:	In compliance

Comments

Bishydroxyethyl biscetyl malonamide was toxic at the top dose as judged by lack of effect on microcolony formation; precipitation above 500 µg/plate was predicted to obscure scoring of mutants and some precipitation was seen at this dose; positive control mutagens, which established the sensitivity of the assay were: 2-aminoanthracene: +S9; 2-nitrofluorene: TA 98 –S9; sodium azide: TA 1535 and TA 100 –S9; 9-aminoacridine: TA 1537 –S9

Results
Bishydroxyethyl biscetyl malonamide was not mutagenic at doses up to 500 µg/plate in bacteria in either the absence or presence of metabolic activation provided by Aroclor 1254-induced SD rat liver S9 fraction.

Ref.: 9

***In vitro* Cytogenetic Study in Human Lymphocytes**

Guideline	:	/
Substance	:	Questamide H sample no S1999201, purity > 99%
Test formulation	:	The solvent used was 95% ethanol
Cells	:	Phytohaemagglutinin-stimulated human peripheral lymphocytes from female donors
Doses	:	62.5, 125, 250, and 500 µg/mL for approximately 19 or 43 hours in the absence of metabolic activation provided Aroclor 1254-induced rat liver S9 fraction (S9) and in its presence for 3 hours.
GLP	:	In compliance

Chromosome preparation
The cells were treated with bishydroxyethyl biscetyl malonamide both in the absence and presence of S9mix. Cultures were treated with test or control compounds 48 h after initiation. Negative solvent control cultures and positive controls were included to monitor the performance of the assays. Cultures were harvested 18 –20 h after start of the treatment. The study was performed at two occasions and on the second occasion additional cultures were harvested after a further 24 h of culture.

Comments
The dose level was chosen to reduce the mitotic index to 40 – 80% of controls; negative controls were within the expected limits; the positive control substances were ethylmethane sulphonate (500 µg/mL) or cyclophosphamide (12.5 µg/mL) and demonstrated the sensitivity of the assay

Results
Under the conditions of the study, Questamide H showed no evidence of clastogenicity in human lymphocytes.

Ref.: 10

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

Preliminary screening studies of developmental toxicity and teratogenicity

Study 1

Guideline	:	/
Substance	:	Questamide H sample no S1999201, purity > 99%
Test formulation	:	Suspension in corn oil.
Species/strain	:	Spraque-Dawley, 7.5 to 8.5 weeks old at start of study and 11.5 to 12.5 weeks old at pairing. The body range of the females at the start of the study was 168.4 – 196.5 g, and at pairing was 187.5 – 231.2g.
Number/sex of animals:		20 test females; 20 control females, 20 untreated males for mating
Administration	:	Gavage
Dose/Study duration	:	0, 1 000 mg/kg/day for 28 days followed by necropsy of half of the females on day 29 and mating of the other half and continued dosing until gestation day 19; on day 20 the females were killed and examined for maternal, foetal and reproductive parameters
Clinical observations	:	No decedents and no treatment-related clinical signs; body weight and body weight gain were not affected by treatment; no effect on oestrus cycle; pregnancy rate was 90% in the treated group and 100% in the control group
GLP	:	In compliance

Reproduction and foetal parameters: Uteri of the treated group were normal and there was no treatment-related effect on deaths as a percentage of implantations, percentage of late foetal deaths, number of *corpora lutea*, implantations or live foetuses, numbers of live male and female foetuses and sex ratio, pre- and post-implantation losses and foetal weight.
Macroscopic findings: For rats killed after 28 days of dosing, one treated rat had enlarged mesenteric lymph nodes and another rat had a few pale foci on the liver for rats killed on gestation day 20 there were no treatment-related findings abdominal fat deposition was similar in treated and control rats.

Rats killed following 28 days of dosing.

Ovaries: Vacuolated cells were present in the *corpora lutea* of 6/10 treated rats; granulomata were present in the *corpora lutea* of 4/10 treated rats; in total, 9/10 rats showed a histological change in the ovary.
Liver: Granulomata in the liver of a single treated rat corresponding with the pale foci noted macroscopically in this animal; for this reason the tissue was processed as an abnormality.
Mesenteric lymph node: Marked histiocytosis in the mesenteric lymph node of a single treated rat, corresponding with the enlargement noted macroscopically.
Incidental findings: There was a higher incidence of luminal dilatation of the uterus in treated, but this was consistent with the stage of the oestrous cycle that they were in and is not considered likely to be related to treatment; treated rats were in a variety of different stages of the oestrous cycle, such as would normally be expected in rats of this age.

Rats killed on day 20 of gestation

Ovaries: There were vacuolated cells in the *corpora lutea* and granulomata in the ovaries of the majority of treated rats.

Liver: There were granulomata in the livers of two treated rats (these livers were processed due to the presence of macroscopic abnormalities). All other findings were of a nature and incidence commonly found in rats of this age and strain, and were considered of no toxicological importance; there were no histological findings in tissues from the pair of rats that did not breed successfully to suggest any infertility.
Foetal observations:
External observations at dissection, narrow and flattened heads were seen in 15/113 foetuses from 7/9 litters treated with bishydroxyethyl biscetyl malonamide. 27/113 treated foetuses were observed to have narrow heads and 13/113 flattened heads; this finding was not seen in the 125 control foetuses. The incidence was significantly higher in the treated foetuses and occurred throughout the range of litter sizes and range of foetal weights, it was not associated with either large or small litters, or heavy or lighter foetuses. The appearance of narrow/flattened heads was different from that of domed heads, often associated with smaller foetuses, seen in both control and treated foetuses.

There was no significant difference in fixed weights.

Visceral observations: A range of findings was observed on visceral examination of the fixed fetuses. No significant differences in the incidence of these findings were observed.

Skeletal examination there were no noticeable differences between the treatment and control groups with respect to the number of morphological changes or in the degree ossification and in particular no structural deviations in the skull bones that might underlie the finding of narrow/flattened heads at necropsy.
Examination of thick slices of heads. Heads of a proportion of affected foetuses plus unaffected foetuses from the same litters and also control foetuses were sectioned and assessed. Examination of intact heads or heads after freehand serial slicing did not reveal any differences between treated or control foetuses or any structural abnormalities.

Comments
There was no indication of a treatment-related effect on hormone levels, particularly follicle stimulating hormone, a marker of ovarian toxicity. The only treatment-related finding in the foetus was the observation of slightly narrowed/flattened heads in some treated foetuses at caesarean section. Other external and visceral findings did not appear to show a treatment related incidence and were judged to be spontaneous. On examination of skeletons and thick sections of heads no structural abnormalities or morphological changes in soft tissue or cranial bones were found. The view of the pathologists was that the narrow and flattened appearance of the head was not a structural defect, but may have been due to compression of the foetus in the uterus, a physiological/ pharmacological effect. It was considered to be a slight effect, because there were no other signs of uterine compression, such as wavy ribs or limb flexures in the foetuses.

There was no indication that treatment had adverse effects on the oestrous cycle, mating, caused maternal toxicity, embryolethality or structural abnormalities in fetuses. While slight changes in head shape were observed at caesarean section, the no effect level for this could not be determined. These changes were considered not to be a structural defect.

Administration of bishydroxyethyl biscetyl malonamide elicited microscopic changes in the ovaries, liver and mesenteric lymph node as noted in a previous 28-day oral toxicity study (see section 3.3.5.1.).

Vacuolated cells in the *corpora lutea* were judged to represent macrophages. Granulomata were present in the *corpora lutea* of rats at 28 days; in rats killed on day 20 of pregnancy granulomata were still present and better defined, but their location was less clearly evident. Use of a reticulin stain showed that the granulomata were walled off by reticulin fibres in a circular pattern, suggesting that they were inside old *corpora lutea.* Control rats killed on day 20 of gestation generally had only large *corpora lutea* of pregnancy present in their ovaries. The corresponding treated rats, however, also had these smaller granulomatous structures present, suggesting persistence of older generations of *corpora lutea.*

If the incidence of microscopic changes in the 28-day dose group is compared with that in the rats killed on gestation day 20 (which had received at least 48 days dosing), effects on the ovary appear more frequently in the latter. Granuloma(ta) in the ovary and limited numbers of livers examined were observed in this study but not in the previous 28-day oral toxicity study (see section 3.3.5.1).

Results
It was concluded that bishydroxyethyl biscetyl malonamide administered to rats by gavage for 28 days, or for an additional period up to day 20 of gestation, produced some morphological changes in the ovary, liver and mesenteric lymph node, but had no effect of fertility, hormone levels or the oestrus cycle and was not maternotoxic. Narrow and flattened heads were seen in some treated foetuses (13%, and occurred in 7/9 litters) on day 20. No structural abnormalities were observed. A more complete assessment would, however, be needed before it is possible to conclude that bishydroxyethyl biscetyl malonamide has no adverse reproductive effects. The effects on offspring and on treated males have not yet been assessed. A continuous breeding protocol would be a suitable way of conducting any further investigations into the reproductive effects of bishydroxyethyl biscetyl malonamide.

Ref.: 11

Study 2

Guideline	:	/
Method	:	Investigation of the effect of Questamide H on embryonic and foetal development of the rat when administered during the period of organogenesis (days 6 to 17 of pregnancy.
Substance	:	Questamide H batch 51433688029, purity > 99%
Test formulation	:	Suspension in corn oil.
Species/strain	:	Female, timed-mated Spraque-Dawley rats.
Number/sex of animals:		10 test females/dose; 10 control females
Dose/Study duration	:	0, 15, 150 or 1 000 mg/kg/day by gavage dosed once daily from gestation day 6 to 17 inclusive.
Clinical observations	:	No premature deaths or treatment-related clinical signs; no effect of treatment on maternal bodyweights or food consumption.
GLP	:	In compliance

Reproduction and foetal parameters: All females in the control and mid dose groups and 9/10 females in the other groups became pregnant. No effect of treatment was observed on the numbers of *corpora lutea*, implantations or live foetuses or pre- or post-implantation losses. Although pre-implantation losses in the high dose group were higher than in the other groups, they were considered to be unrelated to treatment because implantation generally occurs before

day 6 of pregnancy (the first day of dosing). The foetal sex ratio was unaffected by treatment as were mean foetal and placental weights. There were 1, 0, 1 and 0 foetuses with major abnormalities observed in the control, low, mid and high dose groups, respectively. For the foetus in the control group, the abnormalities observed were the absence of one or more thoracic centra and thoracic neural arch and major fusion of the ribs. For the foetus in the mid dose group, the abnormality observed was an interrupted aortic arch. Due to the low incidence of major foetal abnormalities, the fact that no major abnormalities were observed in the high dose group and that one of these foetuses was in the control group, these abnormalities were considered not to be related to treatment. There was no effect of treatment on the overall incidences of minor foetal abnormalities or variations. The only minor skeletal abnormality observed was incomplete ossification of the sacral neural arches. However, there was no statistical difference between the treatment groups and the control. In the mid and high dose groups, the observation of one skeletal variation - non-ossification of the hyoid bone of the skull, was statistically significantly increased in comparison with the control group. However, although the highest incidences of these findings were at 1000 mg/kg/day, both were within background range and in the absence of further increased incidences of any similar findings, were considered not to be related to treatment. It should be noted that these findings are considered to be transitory and would be expected to disappear once the pups were born and were weaning. Other variations observed that were increased in the treated groups included increased renal pelvic cavitation, dilation of the ureter, incomplete ossification of the interparietals and occipital bones of the skull, vestigial 14th ribs and bilobed thoracic centra. However, all of these findings were within background range and considered not to be related to treatment.

Results
Bishydroxyethyl biscetyl malonamide elicited no maternal or foetal developmental toxicity following oral (gavage) administration to pregnant rats during organogenesis at dose levels up to 1000 mg/kg/day.

Ref.: 12

3.3.9. Toxicokinetics

([2-14C] Malonyl)Questamide was prepared and used on the experiments below.

Toxicokinetics in the Rat after Gavage, Intravenous and Topical Administration

Bishydroxyethyl biscetyl malonamide labelled with ^{14}C was administered (by gavage) to six male and six female rats at a dose of 6 mg/kg. Over 96 hours, virtually the entire administered label was present in the faeces with 0.3% present in urine. It was estimated that 2 – 3% was absorbed from the gastrointestinal tract. Excretion of label in the faeces was very high within the first 24 hours (> 96%). After 96 hours approximately 2% of the label was present in the carcass. No $^{14}CO_2$ was detected in expired air.

The absorbed label accumulated in a number of organs and tissues, particularly the liver, spleen, adrenals and ovaries (females). Lower levels of accumulation were observed in the lungs and bone marrow. Clearance of the label was very slow. There was little metabolism of the notified chemical but degradation was observed after contact with rat faeces in aqueous slurry and dry forms. Three unidentified metabolites were present at low concentrations.

For intravenous administration, 6 male rats were dosed via the tail vein with bishydroxyethyl biscetyl malonamide labelled with ^{14}C in PEG 400. After 1, 2, 4, 8 and 24 hours blood, liver,

spleen, kidney, brain and adrenals were assayed for ^{14}C. Following intravenous injection of Bishydroxyethyl biscetyl malonamide at 2.6 mg/kg, 2.6% of the dose was excreted within 24 hours equally distributed in faeces and urine. There was no expiration of $^{14}CO_2$. The administered ^{14}C was rapidly cleared from the blood and deposited into the liver, adrenals, spleen and kidneys. Within 1 hour of dosing the liver contained 53% of the dose and over the following 24 hours the level increased to 89%. The spleen contained 6% to 10% of the dose but a slightly higher concentration per gram of tissue than the liver. The spleen ^{14}C level increased slightly over the first 4 hours before falling back to the 1hour level by 24 hours. The brain, adrenals and kidneys accounted for very low levels of ^{14}C and the carcass contained 13% of the dose at 24 hours. The label was removed very slowly from the tissues analysed. Whole body autoradiography showed that the bone marrow was also a target organ. Approximately 8% of the ^{14}C in the liver was a possible metabolite.

For study of toxicokinetics after topical application, ^{14}C-labelled bishydroxyethyl biscetyl malonamide in acetone was applied to the skin (9.6 cm^2 with 0.1 ml of a 9.6 mg/ml solution) of 4 male rats and occluded for 48 hours. Approximately 0.2% (0.24 $\mu g/cm^2$) of the applied dose was absorbed through the skin and 0.1% retained in the carcass. Between 7% and 8% of the dose was retained at the site of application and the remainder of the dose was rinsed off. The results suggest considerable penetration into the skin but poor removal via the peripheral blood.

In a second study of toxicokinetics after topical application, ^{14}C-labelled bishydroxyethyl biscetyl malonamide in acetone was applied to the skin of 2 female rats followed by 24 hours of occlusion. Approximately 0.01% of the dose was absorbed through the skin and traces levels were found in the urine, faeces and carcass. Blood levels were below the detection limit. Bishydroxyethyl biscetyl malonamide was mainly localised in the *stratum corneum* and around the hairs and upper regions of the hair follicles. Very little label was present in the dermis.

Ref.: 13

Toxicokinetics in the Rat after Multiple Gavage Administration

Bishydroxyethyl biscetyl malonamide labelled with ^{14}C was administered (by gavage) to female rats at a dose of 1000 mg/kg/day on 3 consecutive days. At time points up to 36 days, animals were killed and selected known target organs were assayed for ^{14}C. ^{14}C levels in urine and faeces were also monitored up to day 36. The distribution of ^{14}C was examined by whole body autoradiography after a single dose and after the three consecutive doses. Administration of the test material proved very difficult due to high viscosity and only 3 of a planned 8 daily doses were administered. Abnormal bodyweight loss was observed in all rats on the 3 days of dosing and for the first 48 hours of the recovery period. High individual variability of fate and disposition of the test substance observed on days 1 and 2 was judged to be the result of high abnormal stress levels. Faecal production during the 3 days after the first dose was much lower than normal in most rats, and judged to be due to a combination of reduced diet intake, treatment related stress and the viscous nature of the preparation. The urine ^{14}C level peaked 48 hours after the final gavage treatment at 1854 dpm/mL of urine and the faecal ^{14}C level peaked 24 hours after the final gavage treatment at 17.7 x 10^6 dpm/g of dry faeces. After these points the level of ^{14}C in both excreta declined rapidly. Labelled bishydroxyethyl biscetyl malonamide was rapidly taken up by various tissues in small amounts and slowly cleared. Half-lives of clearance from the ovary was between 9 and 16 days, from the liver between 15 and 27 days and from the blood between 19.5 and 25 days. Maximum levels were reached after two gavage treatments for the adrenals (25520 dpm/g) and blood (796 dpm/g) and on day 5 for the ovary (27531 dpm/g) and liver (10241 dpm/g).

Ref.: 14

Toxicokinetics in the Rat after Topical Application

This study was conducted in two parts. In the first part groups of four female rats were treated topically with bishydroxyethyl biscetyl malonamide labelled with ^{14}C under occlusive dressing for 48 hours at doses of 0, 0.07, 0.48 or 2.6 mg/cm^2 over an area of 9.6 cm^2 of skin. The chemical vehicle was Petroleum Jelly (vaseline). Approximately 0.02, 0.1 and 0.03% of the applied dose was absorbed, respectively, in the low, mid and high dose groups. The amount remaining within treated skin was 26, 19 and 33% for the low, mid and high dose groups. Unabsorbed material ranged from 63 – 73%. No label could be detected in the brain, liver, ovaries, uterus, kidneys, lungs, femurs, small intestine or adrenals. It was concluded that only very low absorption of the notified chemical occurred after topical application. The ultimate fate of skin residues or unabsorbed chemical at times greater than 48 hours is not known. In the second part of the study, groups of twenty female rats were treated topically with 0.1 g of bishydroxyethyl biscetyl malonamide labelled with ^{14}C in either a lotion formulation or in Petroleum Jelly (vaseline) under occlusive dressing for 24 hours. After 1, 7, 14 or 28 days rats were killed and the treated site, heart blood, brain, liver, ovaries, uterus, kidneys, lungs, femurs, small intestine (with contents) and adrenals were removed for ^{14}C assay. During the 24 hours of occlusion, the overall level of percutaneous absorption of ^{14}C was 0.1% of the applied dose for both treatment groups. Between 48 and 90% was removed by skin wiping, and approximately 6% (lotion base) and 4% (Petroleum Jelly) remained in the treatment site. During the six days after removal of the device protecting the treatment site, the level of ^{14}C in faeces increased to a peak of 0.5% of the dose on day 4 indicating that further absorption from skin residues occurred after 24 hours. This is supported by measurements in the skin, showing a sharp decline between day 1 and day 7. Urine levels remained fairly constant at approximately 0.02% during the same six days. On days 7, 14 and 28 the overall level of ^{14}C in the tissues remained constant at approximately 0.01% of the dose in the liver and small intestine (with contents) whereas the level in the other organs was below the detection limit. Blood levels of ^{14}C remained very low throughout the course of the experiment. It was concluded that the notified chemical applied to rat skin in either a lotion base or in vaseline was poorly absorbed across the skin.

Ref.: 15

Skin Deposition

The levels of deposition of bishydroxyethyl biscetyl malonamide onto pig skin were determined after topical application in a rinse-off toiletry product (shower gel) at a concentration of 0.5%. ^{14}C-labelled bishydroxyethyl biscetyl malonamide was applied to isolated whole pig skin at 31 μg/cm^2 for 5 minutes. Following topical application most, but variable amounts (69 – 85%) was removed with distilled water. Soap solutions (8% soap – shower gel, Unilever No. 2 soap base or Dove soap base) were able to remove between 3.6 and 6.2% and between 1.9 and 4.1% remained on the skin. It was concluded that low levels of bishydroxyethyl biscetyl malonamide would be deposited onto skin from rinse-off skin cleansing systems and the depot formed is fairly resistant to rinsing with water or surfactants.

Ref.: 16

Skin Absorption

The *in vitro* percutaneous absorption of bishydroxyethyl biscetyl malonamide was measured using Franz flowthrough cells and keratomed pig, human and rat skin and heat separated pig and human skin. The heat separation technique resulted in considerable skin damage. Therefore results from keratomed skin are given. Bishydroxyethyl biscetyl malonamide was shown to penetrate skin poorly. The greatest penetration was 1.2% with rat skin, suggested to be related to the higher number of hair follicles. Bishydroxyethyl biscetyl malonamide penetrated pig skin to a level of 0.06%. When an aqueous ethanol receptor solution was used with human skin, penetration was 0.04% of the applied dose. When a receptor solution containing bovine serum albumin was used with human skin (a situation closer to the *in vivo* situation), penetration was 0.004%.

Ref.: 17

3.3.10.	Photo-induced toxicity

It is stated that bishydroxyethyl biscetyl malonamide did not absorb in the visible region and had negligible absorbance of near ultraviolet radiation, hence further photobiological evaluation was considered unnecessary.

3.3.10.1.	Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2.	Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11.	Human data

No data submitted

3.3.12.	Special investigations

No data submitted

3.3.13.	Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14.	Discussion

Bishydroxyethyl biscetyl malonamide was of low oral toxicity in rats (LD_{50} > 2000 mg/kg) and low dermal toxicity in rats (LD_{50} > 2000 mg/kg). It was not a skin irritant in rabbits and not a

skin sensitiser in guinea pigs. In an eye irritation study in rabbits, bishydroxyethyl biscetyl malonamide was moderately irritating, with iris effects observed in all animals and the substance is labeled “Irritating to eyes” in EU.

A 28-day oral (gavage) repeated dose study with bishydroxyethyl biscetyl malonamide revealed that target organs were the ovary, uterus, liver and mesenteric lymph node. The NOAEL was 15 mg/kg/day, based on fluid distension of the uterus and microscopic changes in the ovaries and uterus at the 150 and 1000 mg/kg/d. It was estimated that 2 – 3% was absorbed from the gastrointestinal tract.

Bishydroxyethyl biscetyl malonamide was shown to be absorbed slowly across human, rat and pig skin. In human skin, penetration was shown to be a maximum of 0.04% of the absorbed dose. Following deposition of bishydroxyethyl biscetyl malonamide on skin in a rinse-off cosmetic product, low levels remained but were resistant to removal. There is some evidence that skin residues continue to be absorbed.
In the gavage studies the major route of elimination was in the faeces. After intravenous administration, most of the chemical accumulated in the liver and spleen. In general the target organs were those identified in the toxicokinetic studies as those involved in uptake of bishydroxyethyl biscetyl malonamide.

In a preliminary screening study of developmental toxicity and teratogenicity, bishydroxyethyl biscetyl malonamide was administered to dams for 28 days prior to and during organogenesis, at 1000 mg/kg/day. Morphological changes to the ovary, liver and mesenteric lymph nodes were observed, however, no significant effects on fertility, hormone levels or the oestrous cycle were apparent. In the treated foetuses, a significantly higher incidence of narrow and flattened heads was observed at dissection on day 20 of gestation, which could not be explained. In a developmental toxicity screening study, bishydroxyethyl biscetyl malonamide was not maternotoxic or teratogenic in pregnant rats when administered during organogenesis at dose levels up to 1000 mg/kg/day. In the repeated dose study, the principal target organs were the ovaries and uterus, with morphological changes to the ovaries also observed in a preliminary developmental and teratogenicity study. Bishydroxyethyl biscetyl malonamide is classified as toxic to reproduction category 3 with the risk phrase “Possible risk of impaired fertility”. The substance may have a weak oestrogen activity due to the effect on uterus/ovary. The high incidence of narrow and flattened heads in the preliminary study indicates some cause for concern regarding human developmental toxicity. A more complete assessment would, however, be needed before it is possible to conclude that bishydroxyethyl biscetyl malonamide has no adverse reproductive effects. The effects on offspring and on treated males have not yet been assessed. A continuous breeding protocol would be a suitable way of conducting any further investigations into the reproductive effects of bishydroxyethyl biscetyl malonamide.

Bishydroxyethyl biscetyl malonamide was not an *in vitro* mutagen as judged by negative results in studies of mutagenesis in bacteria and chromosomal aberrations in human peripheral lymphocytes.

4. CONCLUSION

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required:

* a sub-chronic (90-day) oral toxicity study;

* a full developmental toxicity study;

* a two-generation reproduction toxicity study, with emphasis on hormonal effects in addition to the usual end-points;

* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

The requested information must be submitted by 31 July 2006.

5. MINORITY OPINION

Not applicable

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