File No: NA/456

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Spirambrene

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Spirambrene

1. APPLICANT

Givaudan-Roure Pty Ltd of 96 South Creek Road DEE WHY 2099 has submitted a limited notification statement in support of their application for an assessment certificate for Spirambrene.

2. IDENTITY OF THE CHEMICAL

Marketing Name:	Spirambrene
Molecular Weight:	238.37

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	colourless to pale yellow liquid
Boiling Point:	252°C
Density:	D_4^{20} = approximately 0.987
Vapour Pressure:	0.39 Pa at 20°C
Water Solubility:	47 mg/L at 25°C
Partition Co-efficient (n-octanol/water):	log P_{ow} = approximately 4.9
Hydrolysis as a Function of pH:	$T_{1/2}$ at pH 4.0 < 24 hours at 25°C (100% degradation at 50°C in 3 hours) $T_{1/2}$ at pH 7.0 and 25°C estimated as between 200 hours and 8 700 hours $T_{1/2}$ at pH 9.0 and 25°C estimated as between 500 hours and 8 700 hours
Adsorption/Desorption:	not determined
Dissociation Constant:	not determined

Flash Point: 130°C (closed cup)

Autoignition Temperature: 245°C

Surface Activity: 55 $mN.m^{-1}$ at 19-19.5°C

Henry's Law Constant: 2 $Pa.m^3.mol^{-1}$

Comments on Physico-Chemical Properties

Tests were performed according to OECD test guidelines at facilities complying with OECD Principles of Good Laboratory Practice.

No information was provided on the adsorption/desorption properties of the chemical. Given the chemical's high partition coefficient it is anticipated that it will strongly adsorb to soils.

The notified chemical contains no dissociable hydrogens or basic functionalities.

The notified chemical is expected to be slightly surface active. By definition, a chemical has surface activity when the surface tension is less than 60 $mN.m^{-1}$ (1).

4. PURITY OF THE CHEMICAL

Degree of Purity: minimum 90%

Toxic or Hazardous Impurities: none

5. USE, VOLUME AND FORMULATION

The notified chemical is a fragrance ingredient for use in alcoholic perfumery, cosmetics, toileteries, household products, soaps, detergents and industrial perfumery. It will be imported as a component (maximum 3%) of a fragrance package at a rate of less than 1 tonne per year for the first five years.

6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported in aluminium or steel containers varying in size from 1 to 200 kg. Exposure of transport or warehouse workers is only likely in the rare event of an accident or leaking packaging.

When received by the cosmetic compounder, the contents of the containers are removed by decanting or pumping to a mixing vessel. Mixing is accomplished in various ways depending on the end product but local exhaust ventilation is normally

employed. The notifier states that all efforts are employed to minimise fragrance loss by spillage or heat and that these controls serve to minimise worker exposure. Following mixing, the product is automatically packaged and transported to the retailer.

The duration of worker exposure is estimated by the notifier at several minutes per batch.

7. PUBLIC EXPOSURE

Since the notified chemical will be used in cosmetics, toiletries and household detergents, widespread public exposure could occur by the dermal and/or inhalational routes. It is estimated that the maximum amount of chemical applied per day would be 10 mg from alcoholic perfumes or a cosmetic preparation. Exposure from rinse-off toiletry products or detergents should be less than that from cosmetic products.

8. ENVIRONMENTAL EXPOSURE

Release

If any reformulation of the notified chemical into end use products takes place locally it will result in a maximum of 2% wastage (a maximum of 20 kg) of the chemical based on overseas experience. This would be released to the sewer from the washing of equipment.

Given the use pattern indicated by the notifier the majority of the notified chemical will be released to waste water either directly (e.g. soaps and detergents) or as the result of washing (alcoholic perfumery, cosmetics and toiletry). The use of products containing the chemical would be widespread but diffuse in small quantities. In the Environmental Risk Assessment Report prepared for notification in the United Kingdom supplied by the notifier it is assumed that all the notified chemical will be discharged to waste water.

Assuming 1% remains in containers, 10 kg would be released to the environment through disposal in landfill Australia wide.

Fate

The majority of the notified chemical (up to 990 kg) will be released to waste water as a result of the reformulation into or use of the products containing the chemical. The high partition coefficient of the chemical indicates that it is likely to adsorb strongly to sewerage sludge which will be landfilled or incinerated. The notifier has estimated that approximately 85% of the notified chemical will adsorb to sewerage sludge using the "Simple Treat" model (2), with 14.5% remaining in water and only 0.6% released to air.

Any incineration of the notified chemical will produce oxides of carbon and water.

The substance was examined for biodegradation potential using OECD Test Guideline 301C (modified MITI Test (I)) for ready biodegradability and OECD Test Guideline 302C (modified MITI Test (II)) for inherent biodegradability. The substance exhibited no degradation in either test, indicating that it is not readily or inherently biodegradable under the conditions of the tests. It was also found that the substance was not inhibitory to bacteria under these conditions.

The chemical structure, molecular weight, resistance to biodegradation, partition coefficient, water solubility and non-ionisable nature would suggest significant potential for bioaccumulation (3). However, this potential will be mediated by the low amount likely to be introduced and the low level but widespread exposure to the aquatic compartment of the chemical.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Spirambrene

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	$LD_{50} > 5\ 100\ mg.kg^{-1}$	(4)
acute dermal toxicity	rat	$LD_{50} > 2\ 000\ mg.kg^{-1}$	(5)
skin irritation	rabbit	slight to moderate irritant	(6)
eye irritation	rabbit	slight irritant	(7)
skin sensitisation	guinea pig	non-sensitiser	(8)

9.1.1 Oral Toxicity (4)

Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	gavage
Clinical observations:	various behavioural modifications for up to 3 days following intubation, including abormal position, slowing of motor activity, abnormal response to stimuli, halting breath, piloerection and some inhibition of the clinging reflex
Mortality:	none
Morphological findings:	two gastric ulcers in one female rat

Test method:	according to OECD Guidlines (9)
LD_{50}:	> 5 100 $mg.kg^{-1}$
Result:	the notified chemical was of low acute oral toxicity in rats

9.1.2 Dermal Toxicity (5)

Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	pure substance applied under occlusive dressing for 24 hours
Clinical observations:	none
Mortality:	none
Morphological findings:	none
Test method:	internal protocol no. 108 G 93
LD_{50}:	> 2 000 $mg.kg^{-1}$
Result:	the notified chemical was of low acute dermal toxicity in rats

9.1.3 Skin Irritation (6)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3 males
Observation period:	14 days
Method of administration:	0.5 g of technical grade Spirambrene was applied under occlusive dressing for 4 hours

Draize scores (10):

Time after decontamination

Animal	*1 h*	*1d*	*2d*	*3d*	*4d*	*5d*	*6d*	*7d*	*9d*	*11d*	*12d*	*14d*
Erythema												
1	1[a]	2	2	2	2	2[b]	1[b]	1[bc]	1[e]	0[e]	0[e]	0[e]
2	1	1	1	2	2	2[bc]	1[bc]	1[bc]	1[ce]	0[e]	0[e]	0[e]
3	1	2	2	2	2	2	2	2	1	0[e]	0[e]	0[e]
Oedema	very slight oedema in animal no. 1 at 3-6 days after decontamination											

[a] see Attachment 1 for Draize scales h = hours, d = days after dosing [b] dosed area dry and cracked [c] scabbing evident a dose site [e] desquamation present at dose site

Test method: according to OECD Guidelines (9)

Result: the notified chemical was a slight to moderate skin irritant in rabbits

9.1.4 Eye Irritation (7)

Species/strain: rabbit/New Zealand White

Number/sex of animals: 3 males

Observation period: 4 days

Method of administration: 0.1 mL of technical grade Spirambrene was instilled into the conjunctival sac of the right eye of each animal

Draize scores (10) of unirrigated eyes:

	Time after instillation														
Animal	***1 hour***			***1 day***			***2 days***			***3 days***			***4 days***		
Cornea	no corneal effects seen														
Iris	slight iridal effect seen in rabbit #3 at 1 hour post-instillation														
Conjunctiva	***r[a]***	***c[b]***	***d[c]***	***r[a]***	***c[b]***	***d[c]***	***r[a]***	***c[b]***	***d[c]***	***r[a]***	***c[b]***	***d[c]***	***r[a]***	***c[b]***	***d[c]***
1	1[1]	1	0	1	0	0	0	0	0	1	0	0	0	0	0
2	1	1	sl*	1	0	0	0	0	0	0	0	0			
3	1	1	sl*	1	0	0	0	0	0	0	0	0			

[1] see Attachment 1 for Draize scales
[a] redness [b] chemosis [c] discharge * slight discharge

Test method: according to OECD Guidelines (9)

Result: the notified chemical was a slight eye irritant in rabbits

9.1.5 Skin Sensitisation (8)

Species/strain: guinea pig/Himalayan White spotted

Number of animals: 15/sex

Induction procedure: three pairs of intradermal injections (0.1 mL/site):

- Freund's Complete Adjuvant (FCA) plus ethanol (1:1)
- 1% technical grade Spirambrene in ethanol
- 1% technical grade Spirambrene in FCA plus ethanol (1:1)

one week after the injections, the same scapular area was treated with undiluted technical grade Spirambrene under occlusive dressing for 48 hours

Challenge procedure: two weeks after the epidermal induction, undiluted technical grade Spirambrene was applied to the flanks under occlusive dressing for 24 hours; a second challenge was performed 2 weeks later

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
100%	0/20**	0/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response

Test method: according to OECD Guidelines (9)

Result: the notified chemical was not a skin sensitiser in guinea pigs

9.1.6 Repeat Insult Patch Test (11)

Species/strain: human subjects

Number/sex: 2 males, 53 females (2 subjects withdrew prior to study termination)

Method of administration: nine applications of 2% test article under occlusive dressing adjacent to the spinal mid-line for 3 weeks each Monday, Wednesday and Friday during the induction phase; after a

	2 week rest period, a challenge patch was applied to a previously untreated site, which was scored 24 and 72 hours after application
Test method:	not specified
Result:	two subjects exhibited transient, barely perceptible responses (one at induction exposure no. 4, the other at 24 hours after challenge); responses considered to be non-specific under the conditions of the study, neither irritation nor sensitisation was observed in humans exposed to 2% technical grade Spirambrene

9.2 Repeated Dose Toxicity (12)

Species/strain:	rat/Wistar
Number/sex of animals:	6 rats/sex/group
Method of administration:	gavage; vehicle: 0.5% sodium carboxymethyl cellulose, 0.9% NaCl
Dose/Study duration:	0, 100 $mg.kg^{-1}.d^{-1}$ (low dose), 320 $mg.kg^{-1}.d^{-1}$ (mid dose), 1 000 $mg.kg^{-1}.d^{-1}$ (high dose) for 28 days
Clinical observations:	a body weight retardation of 12.5% was observed among male rats in the high dose group related to a 6.1% reduction in feed intake
Clinical chemistry/Haematology	the concentration of plasma triglycerides was increased among female rats in the high dose group (2.2-fold on day 9 and 2.8-fold on day 24); a number of haematological and biochemical parameters exhibited statistically significant differences from control values, generally in the high dose group, however, these were considered by the study authors not to be biologically meaningful or indicative of a treatment effect; urinalysis results indicated a slightly increased total protein concentration among males and females in the high dose group

Macroscopic findings:	liver weight was increased by 34% among males of the mid dose group; liver weights were increased among males (by 37%) and females (by 39%) of the high dose group; no other macroscopic findings were reported
Histopathology:	no microscopic findings distinguished the animals of the high dose group from control animals
Test method:	according to OECD Guidelines (9)
Result:	the target organ for the notified chemical was the liver with the lowest dose at which an effect was seen being 320 $mg.kg^{-1}.d^{-1}$

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (13)

Strains:	TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration range:	0.5 - 50 $\mu g.plate^{-1}$
Test method:	according to OECD Guidelines (9)
Result:	the toxicity of the test article limited the maximum concentration to 50 $\mu g.plate^{-1}$, at which concentration no increase in mutant frequency was observed in any strain either in the presence or absence of metablic activation provided by rat liver S9 fraction;

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (14)

Species/strain:	mouse/OF 1
Number and sex of animals:	5/sex/group
Doses:	5 mL/kg
Method of administration:	sub-cutaneous
Test method:	according to OECD Guidelines (9)
Result:	no treatment-related increase in the frequency of micronucleated polychromatic erythrocytes was observed at either 24, 48 or 72 hours

post-treatment

9.4 Overall Assessment of Toxicological Data

The notified chemical was of low acute oral toxicity (LD_{50} > 5 100 $mg.kg^{-1}$) and low acute dermal toxicity (LD_{50} > 2 000 $mg.kg^{-1}$) in rats. It was a slight to moderate skin irritant and a slight eye irritant in rabbits and was not a skin sensitiser in guinea pigs. In a human repeat insult patch test with 2% of the technical grade material neither irritation nor sensitisation was observed.

The target organ identified in a 28-day repeat dose oral study in rats was the liver with the lowest dose at which effects were noted being 320 $mg.kg^{-1}day^{-1}$. No toxic effects were observed at 100 $mg.kg^{-1}day^{-1}$.

The notified chemical was not mutagenic in *S. typhimurium* at a maximum dose of 50 μg/plate (used because of toxicity at higher doses) and was not clastogenic in a mouse bone marrow micronucleus assay.

The notified chemical would not be classified as hazardous according to Worksafe Australia's *Approved Criteria for Classifying Hazardous Substances* (Approved Criteria) (15).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Although no ecotoxicological data has to be provided for chemicals imported at rates less than 1 000 kg per annum according to the Act, the company did provide data. The tests were carried out to OECD Test Methods (9).

Species	***Test***	***Results***	***Ref.***
Rainbow Trout *Salmo gairdneri*	Acute Toxicity (OECD Method 203)	LC_{50} = 7.5 $mg.L^{-1}$ NOEC = 1.8 $mg.L^{-1}$	(16)
Daphnia magna	Acute Immobilisation (OECD Method 202, Part 1)	11.7 mg/L < EC_{50} < 22.1 $mg.L^{-1}$	(17)
Algae *Selenastrum capricornutum*	Growth Inhibition (OECD Method 201)	NOEC = 2.8 $mg.L^{-1}$ (72 hour) E_bC_{50} = 23 $mg.L^{-1}$ (72 hour) E_rC_{50} = 38 $mg.L^{-1}$ (0-72 hour)	(18)
Aerobic Waste Water Bacteria	Respiration Inhibition (OECD Method 209)	EC_{50} > 3 200 $mg.L^{-1}$ (3 hour)	(19)

The test results indicate the chemical is moderately toxic to fish, and slightly toxic to *Daphnia* and algae. The activated sludge respiration inhibition test indicated that Spirambrene does not inhibit the respiration of microorganisms.

In the Acute Fish Toxicity test (OECD Method 203) all fish died when the concentration of the test substance was 10 $mg.L^{-1}$. Additionally, several sub-lethal

effects were noted including increased pigmentation, loss of equilibrium, swimming at the surface and lethargy. Increased pigmentation was observed in 60% of the test animals at a test concentration of 3.2 $mg.L^{-1}$. This increased to 100% at a concentration of 5.6 $mg.L^{-1}$. The other sub-lethal effects were noted at concentrations greater than 5.6 $mg.L^{-1}$.

In the Acute *Daphnia* Toxicity test (OECD Guideline 202, Part 1) the effect of Spirambrene was investigated at a range of concentrations of the active ingredient (1.44, 2.76, 5.65, 11.7 and 22.1 $mg.L^{-1}$). The data from the two highest concentrations were used to calculate an EC_{50} (17.9 $mg.L^{-1}$) reported by the notifier. The lower of these two concentrations resulted in no *Daphnia* immobility, while in the higher concentration 85% of the *Daphnids* were immobilised. Hence, these data cannot be used to calculate the EC50 for *Daphnia* and the true EC_{50} value most likely lies between the two extremes (i.e. 11.7 $mg.L^{-1} < EC_{50} < 22.1$ $mg.L^{-1}$). Additionally, in the two highest concentrations 1 to 2 *Daphnids* were trapped at the surface of the test medium.

No unusual observations were made during either the Algal Growth Inhibition test and the Activated Sludge Respiration Inhibition test.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

As a fragrance ingredient for use in alcoholic perfumery, cosmetics, toiletries, household products, soaps, detergents and industrial perfumery the use of the chemical will be widespread across Australia. Release of the notified chemical to the environment will occur as a result of formulation and use of the end use products in which it is incorporated. It is anticipated that most of the chemical will be released into waste water as a result of the use pattern of the end use products.

As a worst case, an environmental concentration of 1 ppb is predicted if all of the imported chemical remains suspended in sewage waters (assuming: 990 kg maximum annual use, an Australian population of 18 million and a daily per capita waste water discharge of 150 L). This is three orders of magnitude lower than the most sensitive aquatic toxicity result (LC_{50} 7.5 $mg.L^{-1}$ for rainbow trout). Further, most (~85%) is expected to adsorb to sewerage sludge which will be landfilled or incinerated. In landfill the substance is not expected to be mobile or degrade due to its high partition coefficient, moderately low water solubility and lack of biodegradability. The overall environmental hazard of the chemical can be rated as low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical would not be classfied as hazardous according to the Approved Criteria in relation to acute or sub-chronic toxicity, skin or eye irritancy, skin sensitisation or genotoxicity. However, there may be potential for slight to moderate skin irritancy in some individuals and some potential for liver toxicity following long term exposure to high concentrations.

The maximum concentration of the notified chemical to be imported is 3%. Thus exposure to transport or warehouse workers will be low even in the rare event of an accident or leaking packaging.

Exposure of workers involved in incorporating the imported fragrance package into various products is expected to be low. This is because the concentration of notified chemical in the fragrance package is low and engineering controls are used to minimise fragrance loss. The point of maximum worker exposure is likely to be when the fragrance package is decanted or pumped into the initial mixing vessel and the duration of these operations is expected to be short.

Given the low likely worker exposure coupled with low hazard, the risk of adverse health effects resulting from import, storage, use and disposal is expected to be minimal.

The highest public exposure is expected to be from cosmetic use and this level is calculated at 10 mg per day. Assuming a bodyweight of 40 kg, in the case of a young adolescent, this would represent a dose of 0.25 $mg.kg^{-1}.d^{-1}$, or four hundredths of the oral NOEL in the 28-day rat study. In practice the safety factor would probably be much higher, given that absorption through the skin is likely to be less extensive than via the oral route. Therefore, the risk of adverse public health effects from use of the notified chemical is expected to be minimal.

13. RECOMMENDATIONS

To minimise occupational exposure to the notified chemical the following guidelines and precautions should be observed:

- Spillage of the notified chemical should be avoided, spillages should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the Material Safety Data Sheet (MSDS) should be easily accessible to employees.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (20).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

16. REFERENCES

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7. Jackson, D. & Ogilvie, S.W. 1993, *Spirambrene Acute Eye Irritation Test in Rabbits*, Project no., 553821, Inveresk Research International, Tranent, Scotland.

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15. National Occupational Health and Safety Commission 1994, *Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1994)]*, Australian Government Publishing Service, Canberra.

16. Douglas, M.T. & Sewell, I.G. 1988, *Acute Toxicity of LRG 1579 to Rainbow Trout (Salmo gairdneri)*, Project no., ROU 1(b)/88311, Huntingdon Research Centre Ltd, Cambridgeshire, England.

17. Grützner, I. 1995, *48-Hour Acute Toxicity of Spirambrene to Daphnia magna ((OECD Method 102) Immobilization Test)*, Project no., 386212, RCC Umweltchemie AG, Iningen/BL, Switzerland.

18. Bell, G. 1994, *48-Hour Acute Toxicity of Spirambrene to Daphnia magna ((OECD Method 102) Immobilization Test)*, Project no., GVD 1/941010, Huntingdon Research Centre Ltd, Cambridgeshire, England.

19. Rudio, J. 1993, *Activated Sludge Respiration Inhibition Test on Spirambrene according to (OECD Method 102) Guideline No. 209*, Project no., 93-E23, Vernier/Geneva, Switzerland.

20. National Occupational Health and Safety Commission 1994, *National Code of Practice for the Preparation of Material Safety Data Sheets [NOHSC:2011(1994)]*, Australian Government Publishing Service, Canberra.

Attachment 1

The Draize Scale for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe