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report no. 87/54

effects of skin contact with gasoline containing methanol

Prepared on behalf of CONCAWE's Health Management Group
by B.J. Simpson and A.R. Eyres

The Hague
February 1987

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CONTENTS

1. INTRODUCTION

The use of methanol as a blending component in gasoline raises two questions concerning potential health hazards from skin contact with liquid fuel:

1. Is gasoline containing methanol more irritant to the skin than conventional gasoline?

2. To what extent is methanol likely to be absorbed through the skin from gasoline containing methanol?

To provide information to assist in answering these questions and assessing the need to provide any additional precautionary handling advice for the protection of users, CONCAWE sponsored two limited studies which were carried out by Inveresk Research International (IRI), Musselburgh, Scotland. This report summarises the studies and findings and provides an interpretation of the results in relation to handling and use of gasoline containing methanol. Full details of the studies and results are contained in the IRI reports which are included as Appendices I and II.

2. SUMMARY OF STUDIES AND RESULTS

The objective of the first study was to compare the skin irritancy in rabbits of conventional unleaded gasoline with that of similar gasoline containing, firstly, 10 per cent methanol, designated as M10, and secondly, 3 per cent methanol and 2 per cent tertiarybutyl alcohol (TBA), designated as M3. The M3 blend was selected as being typical of marketed gasoline containing the oxygenate components methanol and TBA.

The second study was carried out to determine the extent to which methanol was absorbed through the rat skin after exposure to M3.

In the 24 hour occluded patch tests used in the irritancy study, the three types of gasoline tested all gave similar results. Primary irritation scores obtained were 0.79 for conventional unleaded gasoline, 0.67 for M10 and 0.63 for M3. All three materials would therefore be considered as slightly irritant to the rabbit skin but do not require classification and labelling as "irritant" according to the criteria of the EEC Dangerous Substances Directive.

In the skin absorption study which was carried out by the occluded application of M3 containing the C14 labelled isotope to the rat skin, evaporation was so rapid that less than 2 per cent of the applied gasoline could be recovered immediately after application. However, about 30 per cent of the small recovered dose was detected in the carcass at 1,24 and 72 hours after the exposure. It was concluded that more definitive evaluation of the extent of skin absorption of methanol from M3 would require a modified study design to keep liquid in contact with the skin for longer period. However, because such a modification would not be representative of the normal use situation, it was decided that further investigations of this type would not be useful.

3. INTERPRETATION OF RESULTS

The skin irritancy study has clearly demonstrated that gasoline containing methanol at levels up to 10 per cent is no more irritant than typical conventional unleaded gasoline. The results also confirm that gasoline is only slightly irritating to the skin under conditions of exposure which are likely to occur during normal handling and use, i.e. occasional splashes.

The skin absorption study has shown that small amounts of gasoline in contact with the skin evaporate rapidly. Therefore, in normal handling and use of gasoline, absorption through the skin of any methanol which may be present is likely to be at extremely low levels. Although it is concluded that there should be no increased hazards in normal handling and use of gasoline containing methanol, it is recognised that more prolonged skin contact may occur in some accident situations. Therefore, CONCAWE's previous advice, provided in July 1984 for participating companies to transmit to national poisons centres is repeated here:

"Due to changing refining patterns and requirements in Europe, some gasolines (petrol) now contain components such as methanol, tertiary butyl alcohol (TBA) and/or methyl tertiary butyl ether (MTBE), known generically as "oxygenates". Present evidence is that of these oxygenates, methanol, when added to gasoline, may be of toxicological significance.

These oxygenates typically are added in amounts of up to 3% by volume of methanol together with between 3 to 7% of TBA, or up to 10% TBA alone or up to 10% MTBE alone, although precise amounts and ratios will vary according to the origin of the gasoline and may, in occasional instances, exceed these values. With the exception of a very few special situations, where specifically adapted test fleets are running in restricted areas on "pure" methanol, the maximum methanol concentration in gasoline is unlikely to exceed 5%.

Therefore physicians who have to treat gasoline contact victims, or cases of suspected gasoline poisoning, should bear in mind that such casualties may have been exposed to gasoline containing methanol and should be examined for signs of methanol toxicity."

NOTE

The Inveresk study shows that only 0.6% of an applied dose of methanol (as M3) was absorbed. If it is assumed that absorption rates are similar for rat and human skin, the total doses of methanol likely to be absorbed would be:

18 mg from exposure to 100 ml of M3
90 mg from exposure to 500 ml of M3
180 mg from exposure to 1 litre of M3

These doses could be contrasted with 4800 mg methanol which is the lowest oral dose reported to be fatal in man (1). Therefore, it is considered that normal handling and use of gasoline containing methanol does not present any significant hazard of absorption of methanol through the skin.

Ref 1. Bennet, I.L., et al, (1953) Acute Methyl Alcohol Poisoning. A review based on experiences in an outbreak of 32 cases. Medicine 32, 431-463.

On the basis of these results, CONCAWE considers that the safe handling advice it has previously provided on fuels in Report No. 2/85 (Health aspects of petroleum fuels - general principles) and No. 85/51 (Health aspects of petroleum fuels - potential hazards and precautions for individual classes of fuels) will also protect users during normal handling and use of gasoline containing methanol. Briefly, this advice recommends avoiding unnecessary and in particular, repeated or prolonged skin contact, with the implementation of good standards of industrial and personal hygiene where more extended contact may be unavoidable.

4. APPENDICES

APPENDIX I: Inveresk Research International Report No. 3361 - Gasoline with Added Methanol - Phases 1 and 2: Skin Irritation Tests in Rabbits.

APPENDIX II: Inveresk Research International Report No. 4101 - Percutaneous Absorption of Methanol from Gasoline containing 3% Methanol.

INVERESK RESEARCH INTERNATIONAL
Report No. 3361

CONFIDENTIAL

GASOLINE WITH ADDED METHANOL - PHASES 1 AND 2: SKIN IRRITATION TESTS IN RABBITS

IRI Project No. 233354

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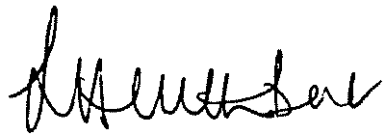
AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained."



J.A. Cuthbert, B.Sc.
Principal Investigator

Project No. 233354

Report No. 3361

QUALITY ASSURANCE AUTHENTICATION

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a pre-determined schedule.

This report has been audited by IRI Quality Assurance Personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

IRI Project No. 233354 Report No. 3361

Signed: Andrew Waddell (Quality Assurance Manager)

Date: 5th November 1985.

CONTENTS

PERSONNEL INVOLVED IN PROJECT 233354

Principal Investigator:	J.A. Cuthbert, B.Sc.
Project Leader:	S.M.A. Carr
Technical Assistance:	C. Barnes
Quality Assurance:	A.W. Waddell, B.Sc., Ph.D. S. Rae, B.Sc.

SUMMARY

A phased investigation into the skin irritancy potential of gasoline with added methanol under occlusion was carried out on rabbits.

Phase 1 on 3 rabbits resulted in only very slight erythema after various exposures to gasoline and M10, viz 4 h, 7 h and 24 h.

Phase 2 was a 24 h exposure on 6 rabbits and resulted in very similar irritant responses to each of the test materials. A primary irritation score for each of the materials was calculated as follows:

Gasoline	0.79
M10	0.67
M3	0.63

INTRODUCTION

Concawe requires information on the skin irritancy potential of gasoline with added methanol. Gasoline with 10% methanol is known as M10 and with 3% methanol is M3.

The investigation was carried out in a phased approach commencing with a rabbit skin irritation screening test in 3 rabbits. From the results of the screening test a confirmatory test on 6 rabbits was carried out.

The tests, which were performed at Elphinstone Research Centre, field station of Inveresk Research International Limited, were commenced on 2 April 1985 and completed on 15 April 1985.

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited for 5 years after issue of the final report.

TEST MATERIALS AND ANIMALS

Test Material

The following test materials were received at IRI on 19 March 1985:

Substance		Form	Gross Weights
Gasoline BPS (W85/38)		Liquid	4254.3
M3 BPS (W85/38)	95%		
MeoH	3%	Liquid	4369.4
TBA	2%		
M10 BPS (W85/38)	90%	Liquid	4509.3
MeoH	10%		

The test materials were stored in the dark under ambient conditions.

Animals

Rabbits were supplied by Cheshire Rabbit Farms Limited and arrived at Elphinstone Research Centre on 6 March 1985.

EXPERIMENTAL PROCEDURE

SKIN IRRITATION TESTS (Phases 1 and 2)

Test Materials

Gasoline (Phases 1 and 2)
M10 (Phases 1 and 2)
M3 (Phase 2)

Animals

Two male and 1 female (Phase 1) and 3 male and 3 female (Phase 2) New Zealand White rabbits, within the weight range 2.5-3.0 kg and approximately 11 weeks old, were used. They were fed on Special Diet Services Standard Rabbit Diet of satisfactory analysis and allowed food and water ad libitum. They were housed individually in cages with grid floors beneath which were peat moss filled trays. Mean environmental temperature was 16°C (extremes of 15°C-17°C) for Phase 1 and 16°C (extremes of 15°C-17°C) for Phase 2. Mean relative humidity was 60% (extremes of 54%-64%) for Phase 1 and 52% (extremes of 46%-54%) for Phase 2.

The rabbits were allowed an acclimatisation period of 27 days (Phase 1) and 36 days (Phase 2).

Method

Phase 1

Gasoline and M10 were tested on 3 rabbits. The hair was clipped from the back of each animal approximately 24 h before treatment.

Two patches of each material were applied to each of the rabbits. Approximately 0.5 ml of test material was applied under 2.5 cm x 2.5 cm patches of chromatography paper. The patches were covered with aluminium foil lined Blenderm and the whole trunk was bound with Sleek occlusive tape.

After 4 h, one patch of each material was removed from 2 rabbits (Nos. 1 and 2) and the skin reactions were scored. Since there was no excessive irritation noted the remaining patches were left.

After 7 h, one further patch of each material was removed from 2 rabbits (Nos. 1 and 3) and the skin reactions scored. Again, since there was no excessive irritation the remaining patches were left.

After 24 h the patches were removed from the remaining 2 rabbits (Nos. 2 and 3) and the skin reactions scored.

Skin reactions were scored according to the system detailed in the Appendix at 1 h, 24 h, 48 h and 72 h after patch removal.

Phase 2

Four test sites on each of 6 rabbits were used. The test materials were gasoline, M10, M3 and a blank patch. Each patch was applied in the same manner as for Phase 1 and left in contact for 24 h. The skin was assessed 1 h, 24 h, 48 h and 72 h after patch removal.

RESULTS

Phase 1 Details are given in Tables 1-3.

After a 4 h exposure, both gasoline and M10 treated sites showed very slight erythema at 1 h only. Skin was normal at 24 h, 48 h and 72 h.

After a 7 h exposure, sites treated with both test materials showed very slight erythema at 1 h and 24 h only, thereafter skin was normal.

After a 24 h exposure, sites treated with both test materials showed very slight erythema at 1 h and 24 h only. Skin was normal at 48 h and 72 h.

Phase 2 Details are given in Tables 4-8.

Since no excessive irritation was noted in Phase 1, Phase 2 was carried out. Very slight-well defined erythema was noted at gasoline, M10 and M3 treated sites at 1 h and 24 h with mild oedema at gasoline and M10 sites at 1 h only.

At 48 h, 3/6 treated sites showed only very slight erythema and by 72 h all treated sites were normal.

No irritation was noted at the sites treated with a blank patch.

DISCUSSION AND CONCLUSIONS

The results of the Phase 1 test gave confidence to proceed to Phase 2. Skin responses seen in Phase 2 were very similar for gasoline, M10 and M3. Gasoline gave a slightly higher primary irritation score of 0.79 with M10 and M3 giving scores of 0.67 and 0.63 respectively. All irritant responses were transient in nature and all treated sites had returned to normal by 72 h.

TABLE 1

Phase 1: Skin Irritation Test (4 h exposure)
Reaction Scores

Time*	Rabbit No./ Sex	Patch Arrangement		Skin Score: Erythema and Eschar		Oedema	
1 h	1♂	A	B	1	1	0	0
		A	B	-	-	-	-
	2♀	A	B	1	1	0	0
		A	B	-	-	-	-
24 h	1♂	A	B	0	0	0	0
		A	B	-	-	-	-
	2♀	A	B	0	0	0	0
		A	B	-	-	-	-
48 h	1♂	A	B	0	0	0	0
		A	B	-	-	-	-
	2♀	A	B	0	0	0	0
		A	B	-	-	-	-
72 h	1♂	A	B	0	0	0	0
		A	B	-	-	-	-
	2♀	A	B	0	0	0	0
		A	B	-	-	-	-

A = Gasoline
B = M10
- = Not scored at this observation
* = Assessment time (h) after patch removal

TABLE 2

Phase 1: Skin Irritation Test (7 h exposure)
Reaction Scores

Time*	Rabbit No./ Sex	Patch Arrangement		Skin Score: Erythema and Eschar		Skin Score: Oedema	
1 h	1♂	A	B	-	-	-	-
		A	B	1	1	0	0
	3♂	A	B	1	1	0	0
		A	B	-	-	-	-
24 h	1♂	A	B	-	-	-	-
		A	B	1	1	0	0
	3♂	A	B	1	1	0	0
		A	B	-	-	-	-
48 h	1♂	A	B	-	-	-	-
		A	B	0	0	0	0
	3♂	A	B	0	0	0	0
		A	B	-	-	-	-
72 h	1♂	A	B	-	-	-	-
		A	B	0	0	0	0
	3♂	A	B	0	0	0	0
		A	B	-	-	-	-

A = Gasoline
B = M10
- = Not scored at this observation
* = Assessment time (h) after patch removal

TABLE 3

Phase 1: Skin Irritation Test (24 h exposure)
Reaction Scores

Time*	Rabbit No./ Sex	Patch Arrangement		Skin Score: Erythema and Eschar		Oedema	
1 h	2♀	A	B	-	-	-	-
		A	B	1	1	0	0
	3♂	A	B	-	-	-	-
		A	B	1	1	0	0
24 h	2♀	A	B	-	-	-	-
		A	B	1	1	0	0
	3♂	A	B	-	-	-	-
		A	B	1	1	0	0
48 h	2♀	A	B	-	-	-	-
		A	B	0	0	0	0
	3♂	A	B	-	-	-	-
		A	B	0	0	0	0
72 h	2♀	A	B	-	-	-	-
		A	B	0	0	0	0
	3♂	A	B	-	-	-	-
		A	B	0	0	0	0

A = Gasoline
B = M10
- = Not scored at this observation
* = Assessment time (h) after patch removal

TABLE 4

Phase 2: Skin Irritation Test (24 h exposure)
Reaction Scores

Time*	Rabbit No./ Sex	Patch Arrangement		Skin Score: Erythema and Eschar		Skin Score: Oedema	
1 h	4♂	A	B	2	2	0	0
		C	D	1	0	0	0
	5♀	A	B	2	1	1	0
		C	D	2	0	1	0
	6♂	A	B	1	1	0	0
		C	D	1	0	0	0
	7♀	A	B	1	0	0	0
		C	D	1	0	0	0
	8♂	A	B	1	0	0	0
		C	D	0	0	0	0
	9♀	A	B	1	1	0	0
		C	D	1	0	0	0
24 h	4♂	A	B	2	2	0	0
		C	D	1	0	0	0
	5♀	A	B	2	2	0	0
		C	D	2	0	0	0
	6♂	A	B	1	1	0	0
		C	D	1	0	0	0
	7♀	A	B	0	0	0	0
		C	D	0	0	0	0
	8♂	A	B	1	1	0	0
		C	D	1	0	0	0
	9♀	A	B	1	1	0	0
		C	D	1	0	0	0

A = Gasoline
B = M3
C = M10
D = Blank
*= Assessment time (h) after patch removal

TABLE 4 (continued)

Time*	Rabbit No./ Sex	Patch Arrangement		Skin Score: Erythema and Eschar		Skin Score: Oedema	
48 h	4♂	A	B	1	1	0	0
		C	D	1	0	0	0
	5♀	A	B	1	1	0	0
		C	D	1	0	0	0
	6♂	A	B	0	0	0	0
		C	D	0	0	0	0
	7♀	A	B	0	0	0	0
		C	D	0	0	0	0
	8♂	A	B	0	0	0	0
		C	D	0	0	0	0
	9♀	A	B	1	1	0	0
		C	D	1	0	0	0
72 h	4♂	A	B	0	0	0	0
		C	D	0	0	0	0
	5♀	A	B	0	0	0	0
		C	D	0	0	0	0
	6♂	A	B	0	0	0	0
		C	D	0	0	0	0
	7♀	A	B	0	0	0	0
		C	D	0	0	0	0
	8♂	A	B	0	0	0	0
		C	D	0	0	0	0
	9♀	A	B	0	0	0	0
		C	D	0	0	0	0

A = Gasoline
B = M3
C = M10
D = Blank
• = Assessment time (h) after patch removal

TABLE 5

Phase 2 (Gasoline)
Mean Scores and Primary Irritation Scores

	Assessment Time (h) After Patch Removal	Reaction Score
Erythema and Eschar Formation		
	1 h	1.33
	24 h	1.17
	48 h	0.50
	72 h	0.00
Sub total		3.00
Oedema Formation		
	1 h	0.17
	24 h	0.00
	48 h	0.00
	72 h	0.00
Sub total		0.17
Total		3.17
Primary Irritation Score		0.79

The reaction score is the average value of results from the 6 test animals. The primary irritation score is obtained in the following way: values for erythema and eschar formation at 1 h, 24 h, 48 h and 72 h (4 values) are added to the values for oedema at 1 h, 24 h, 48 h and 72 h (4 values) and the figure is divided by 4.

TABLE 6

Phase 2 (M10)
Mean Scores and Primary Irritation Scores

	Assessment Time (h) After Patch Removal	Reaction Score
Erythema and Escher Formation		
	1 h	1.00
	24 h	1.00
	48 h	0.50
	72 h	0.00
Sub total		2.50
Oedema Formation		
	1 h	0.17
	24 h	0.00
	48 h	0.00
	72 h	0.00
Sub total		0.17
Total		2.67
Primary Irritation Score		0.67

The reaction score is the average value of results from the 6 test animals. The primary irritation score is obtained in the following way: values for erythema and eschar formation at 1 h, 24 h, 48 h and 72 h (4 values) are added to the values for oedema at 1 h, 24 h, 48 h and 72 h (4 values) and the figure is divided by 4.

TABLE 7

Phase 2 (M3)
Mean Scores and Primary Irritation Scores

	Assessment Time (h) After Patch Removal	Reaction Score
Erythema and Eschar Formation		
	1 h	0.83
	24 h	1.17
	48 h	0.50
	72 h	0.00
Sub total		2.50
Oedema Formation		
	1 h	0.00
	24 h	0.00
	48 h	0.00
	72 h	0.00
Sub total		0.00
Total		2.50
Primary Irritation Score		0.63

The reaction score is the average value of results from the 6 test animals. The primary irritation score is obtained in the following way: values for erythema and eschar formation at 1 h, 24 h, 48 h and 72 h (4 values) are added to the values for oedema at 1 h, 24 h, 48 h and 72 h (4 values) and the figure is divided by 4.

TABLE 8

Phase 2 (Blank Patch)
Mean Scores and Primary Irritation Scores

	Assessment Time (h) After Patch Removal	Reaction Score
Erythema and Eschar Formation		
	1 h	0.00
	24 h	0.00
	48 h	0.00
	72 h	0.00
Sub total		0.00
Oedema Formation		
	1 h	0.00
	24 h	0.00
	48 h	0.00
	72 h	0.00
Sub total		0.00
Total		0.00
Primary Irritation Score		0.00

The reaction score is the average value of results from the 6 test animals. The primary irritation score is obtained in the following way: values for erythema and eschar formation at 1 h, 24 h, 48 h and 72 h (4 values) are added to the values for oedema at 1 h, 24 h, 48 h and 72 h (4 values) and the figure is divided by 4.

APPENDIX

FDA: Acute Dermal Irritation Test in Rabbits
Grades for Skin Lesions

Erythema and Eschar Formation	Grade
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (area raised approximately 1 mm)	3
Severe oedema (raised by more than 1 mm and extending beyond the area of exposure)	4

The reaction score is the mean value for the 6 animals

APPENDIX II

INVERESK RESEARCH INTERNATIONAL
Report No. 4101

CONFIDENTIAL

PERCUTANEOUS ABSORPTION OF METHANOL FROM GASOLINE CONTAINING 3% METHANOL

IRI Project No. 131140

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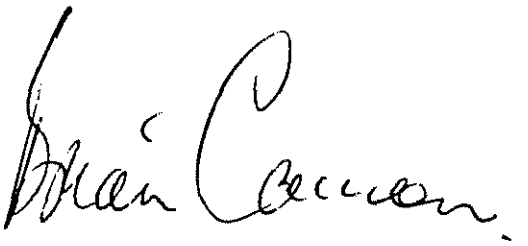
February 1986

AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my direction, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."



B.D. Cameron, B.Sc., C.Biol.,
M.I.Biol.
Principal Investigator

Project No. 131140

Report No. 4101

CONTENTS

PERSONNEL INVOLVED IN PROJECT 131140

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Project Leader:	J.P. Dunsire, C.Biol., M.I.Biol.
Technical Assistance:	A. Cochrane, O.N.C.

SUMMARY

1. Gasoline containing 3% [^{14}C]-methanol was applied to rat skin and the treated area occluded with a dressing.

2. Very little of the administered [^{14}C]-methanol remained in contact with the skin for any length of time - less than 2% of the dose was recovered at 0 h post dose.

3. Of the very small amount of radioactivity recovered about 30% was absorbed into the animal body.

INTRODUCTION

This study was designed to investigate the percutaneous absorption in the rat of methanol from gasoline containing 3% methanol. To facilitate the measurement of methanol, the ^{14}C-analogue was used. A copy of IRI Protocol No. 131140 is provided in Appendix 1.

The studies were carried out at the following location:

Inveresk Gate, Musselburgh EH21 7UB, Scotland

Starting date: 16 May 1985
Completion date: 12 July 1985

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited for 5 years after issue of the final report.

EXPERIMENTAL PROCEDURES

Materials

[^{14}C]-methanol (5 mCi, 369 μCi.mg^{-1}) was obtained from Amersham International p.l.c. (Buckinghamshire, England) in gaseous form. A copy of the Amersham analysis sheet is provided in Appendix 2.

Gasoline containing 3% methanol was supplied by the Sponsor.

All other material were supplied by IRI. Chemicals used were of analytical grade or equivalent.

Animals

Male Olac pigmented rats of body weight 185-216 g were used. The animals were weighed accurately predose and were uniquely identified by tail marking. Food (SQC Rat and Mouse Maintenance Diet No. 1, BP Nutrition (U.K.) Limited) and water was available at all times.

Twenty four hours prior to dosing the back of each rat was shaved with animal clippers, care being taken not to abrade the skin, and a clearly defined circular area (12.5 cm^2) was marked out with indelible ink.

Dose Preparation

The [^{14}C]-methanol (ca 13.5 mg in gaseous form) was supplied in a double compartment borosilicate glass ampoule. The lower compartment containing the [^{14}C]-methanol vapour was under vacuum and was separated from the upper compartment by a glass seal. The upper compartment was filled with gasoline and the glass seal broken by means of a metal plunger, allowing the gasoline to flood the lower compartment. The gasoline/[^{14}C]-methanol mixture was then transferred to a 25 ml volumetric flask and made up to volume with gasoline.

Analysis of 6 x 2 µl aliquots by liquid scintillation counting showed the dose mixture to contain 3.32 mCi, i.e. 9 mg of [^{14}C]-methanol. The dose mixture thus contained a total of 759 mg methanol at a calculated specific activity of 4.37 µCi.mg^{-1}. It was intended to confirm the radiochemical purity and specific activity of the prepared dose mixture, but all development work was halted before this could be achieved, on instruction of the Sponsor.

Radioactive doses of 250 µl were administered using a 250 µl Hamilton syringe. Doses administered were calculated by measuring the amount of radioactivity associated with 'mock doses' taken at the time of dosing. Individual animal doses are provided in Appendix 3.

Animal Experimentation

Prior to initiating the topical administration dosimetry study, a pilot study was carried out to determine the amount of [^{14}C]-methanol and/or [^{14}C]-carbon dioxide produced by an animal dosed topically with the prepared dose mixture. Two rats each received a 250 µl topical dose of the prepared gasoline/methanol mixture and the dose area was immediately occluded with a Sleek^{R} and aluminium foil dressing. The rats were placed in Jencon's all-glass metabolism cages specially designed for the collection of expired air. Results of this study (Appendix 4) showed that less than 1% of the administered dose was recovered in expired air over a 17 h period. At this point in time it was not known that virtually all of the [^{14}C]-methanol evaporated from the dose site before occlusion.

Dosimetry Study

Eight male Olac pigmented rats each received a topical dose of 250 µl of the prepared gasoline/methanol mixture. The dose was quickly applied to the prepared area which was immediately occluded with a Sleek^{R} and aluminium foil dressing. The animals were housed

individually in polycarbonate and stainless steel cages with raised wire-mesh floors to prevent coprophagy. One rat was sacrificed by N_2 inhalation at each of the following times post dose: 0, 0.25, 0.5, 1, 2, 8, 24 and 72 h. Immediately following sacrifice the animals were quickly skinned and the skin and dressings immediately placed in closed containers of methanol. Radioactivity was separately measured in the skin, skin dressings and residual carcass.

On examination of results from this dosimetry study, the Sponsor instructed that all further development work should be cancelled. This decision was due to the observation that [^{14}C]-methanol only remained in contact with the skin for a very short period of time.

Quantitation of Radioactivity

Radioactivity was analysed using a liquid scintillation counter (Philips, Holland) with automatic quench correction by external standard-channels ratio. Each individual sample was analysed for 5 min. Wherever possible, samples were measured in duplicate. Vials were allowed to heat and light stabilise overnight prior to analysis. Prior to calculation of each result, a background count rate was determined and subtracted from each sample count rate.

A limit of reliable determination of 30 dpm above background has been instituted in these laboratories. If results have arisen from data below the limit of reliable determination, the fact is so noted.

Samples for combustion (ca 0.4 g) were weighed into Combustacones^R (Packard Instrument Company Limited) and combusted using a Model 306 Tricarb automatic sample oxidiser (Packard). The resultant $^{14}CO_2$ was absorbed in Carbosorb^R (Packard) and mixed automatically with Permafluor^R scintillator (Packard). Comhustion efficiency and carry over were checked routinely throughout each production run. Combustion efficiency was shown to be greater than 96% throughout the experimental period.

Liquid Samples

Aliquots of dose solution and mock dose solutions were made up to 1 ml with distilled water if necessary and mixed with Unisolve^{R} (Koch-Light) scintillator (10 ml).

Carcasses

Residual carcasses were finely minced then homogenised using a Waring Commercial Blendor^{R}. The homogenates were stabilised by addition of carboxymethylcellulose and representative samples (ca 0.4 g) were taken for combustion.

Skins

The whole skin was treated as 'treated skin', and was plunged into 200 ml methanol in a closed container immediately after sacrifice. The skin was washed once more with a further 250 ml methanol, and 2 x 1 ml aliquots of each wash were mixed with 10 ml 'Unisolve' for scintillation counting.

Skin residues were then finely chopped and homogenised and aliquots (ca 0.4 g) taken for combustion.

Skin Dressings

Skin dressings were plunged into 250 ml methanol in a closed container immediately after sacrifice. The dressings were washed once more with a further 250 ml methanol, and 2 x 1 ml aliquots of each wash were mixed with 10 ml 'Unisolve' for scintillation counting.

RESULTS AND DISCUSSION

Results of the topical administration dosimetry study described in this report are provided in Table 1. It appears from these results that very little of the administered [^{14}C]-methanol remains in contact with the skin for any appreciable length of time - less than 2% of the dose was recovered at 0 h post dose and this amount decreased with time until at 72 h post dose only 0.37% was recovered. However, of the small amount of dose actually recovered a significant portion had been absorbed - after 1 h about 30% of the radioactivity recovered was detected in the carcass and this ratio was maintained over the 72 h period.

Although every precaution had been taken to eliminate the possiblility of evaporation of the [^{14}C]-methanol these precautions appeared to be insufficient and virtually all the methanol had evaporated even in the 0 h control animal.

A novel design of study will be required to establish the possibility of absorbtion of methanol possibly from an occlusive glass container glued and stitched to the rats back. These results would only be of empirical interest since the artificial conditions do not simulate the inuse situation.

After examination of these results, the Sponsor instructed IRI to cease all further development work and to cancel the remaining phases of this study.

TABLE 1

Recovery of Total Radioactivity Following Topical Application of Gasoline Containing 3% [^{14}C]-Methanol to Male Pigmented Rats

Results expressed as % dose administered recovered

	Animal Number and Time of Sacrifice							
	1♂ (0 h)	2♂ (0.25 h)	3♂ (0.5 h)	4♂ (1 h)	5♂ (2 h)	6♂ (8 h)	7♂ (24 h)	8♂ (72 h)
Skin	1.14	0.86	0.58	0.51	0.25	0.33	0.25	0.15
Skin Dressing	0.38	0.44	0.36	0.34	0.30	0.17	0.09	0.10
Carcass	0.05*	0.09*	0.27	0.35	0.23	0.24	0.15	0.12
Total	1.57	1.39	1.21	1.20	0.78	0.74	0.49	0.37

* = Results obtained from data <30 dpm above background

APPENDIX 1

Copy of IRI Protocol No. 131140

Concawe
Babylon-Kantoren A
Koningin Julianaplein 30-9
2595 AA Den Haag
Netherlands

PROTOCOL TITLE:

Percutaneous Absorption of Methanol from Gasoline Containing 3% Methanol

IRI PROJECT NO: 131140

TEST MATERIAL: ^{14}C-Methanol

IRI PROTOCOL CODE: Final

PRINCIPAL INVESTIGATOR: J McDougall, BSc PhD

PROJECTED TIMINGS: Begin: December 1984

Draft Report: February 1985

PRINCIPAL INVESTIGATOR: _____ DATE: _____

PROTOCOL ACCEPTED BY: _____ DATE: _____

(No of pages : 5)

MATERIALS

1. IRI will purchase high specific activity $^{14}CH_3OH$ (1mCi mg^{-1}, 5 mCi) from Amersham International PLC.

2. Concawe will supply IRI with gasoline containing 3% methanol. IRI will 'spike' the mixture with ^{14}C methanol. IRI will establish the specific activity and radiochemical purity of the prepared mixture.

3. All other materials will be supplied by IRI.

PREPARATION OF ^{14}C METHANOL INTO DOSING SOLUTIONS

1. Topical Application

For topical application, a minimum quantity of ^{14}C methanol will be added to gasoline containing methanol such that the concentration of methanol will be minimally affected. Thus 1mg ^{14}C methanol (1mCi) will be added to 5ml gasoline containing 3% methanol.

Each animal will receive 250ul gasoline as a topical dose. This is equivalent to ca 6mg methanol and ca 0.05mg ^{14}C methanol.

2. Intravenous Administration

For intravenous administration, the dose level will be adjusted according to the proportion absorbed following topical application. Thus the dose level may be 0.05 - 6mg. For each animal, 50ug ^{14}C methanol (50 uCi) will be prepared, diluted with up to 6mg of methanol, and administered in solution in 250ul water for injection.

ANIMAL STUDIES

Olac pigmented rats (18 male) of body weight ca 200 - 250g will be used. The animals will be individually housed and allowed free access to food and water at all times.

A. Topical Application (13 male rats)

Each rat will be shaved with animal clippers and a clearly defined circular area (12.5cm^2) marked out. 250ul of the prepared gasoline solution containing 3% ^{14}C methanol will be placed onto the prepared area and immediately covered with aluminium foil and sleek waterproof dressing.

Following topical application the animals will be placed in single polycarbonate cages in a well ventilated room

1. Dosimetry Study (8 rats)

The rats will be killed at 0, 0.25, 0.5, 1, 2, 8, 24, 72 h post dose.

Following sacrifice the animals will be quickly skinned and the skin and dressings immediately added to a closed cylinder of methanol. Radioactivity will be separately measured in the skin, skin dressings and the residual carcass.

2. Plasma Level Study (5 rats)

Following dose application, blood samples will be taken from the tail veins at the following times post dose for measurement of total radioactivity in plasma. 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 48, 72 h.

B. Intravenous Administration (5 male rats)

The dose level given (mg kg^{-1}) will be adjusted by using the experience gained above to give plasma levels of methanol in the same order of magnitude as those observed in A2 above. The radioactive dose will be ca 50 uCi. 250ul of water for injection containing the relevant proportion of methanol wil be administered into the cephalic vein. Blood samples will be taken from the tail vein at the following times post dose for the measurement of radioactivity in plasma.

0.1, 0.25, 0.5, 1.0, 2, 3, 4, 5, 6, 8, 24, 48 and 72 h.

QUANTITATION OF RADIOACTIVITY

Samples of plasma will be measured for radioactivity by direct liquid scintillation counting. Carcasses will be minced, homogenised, and samples oxidised to $^{14}CO_2$ and absorbed in a CO_2 absorbant scintillation system.

Skins and dressings will be extracted several times in methanol and radioactivity measured in the extracts by direct liquid scintillation counting. The residues of skins will be minced and processed as for carcasses.

PRESENTATION OF DATA

Data will be presented in the form of tables and figures. The areas under plasma profiles will be calculated and be used as a measure of the extent of absorption of ^{14}C methanol from the gasoline formulation. The data will be compiled in a draft report which will include introduction, methods, results and discussion together with a copy of this protocol. Concawe will be invited to comment on the draft report before issuing a final report. The final report will contain a quality assurance authentication.

QUALITY ASSURANCE

During the conduct of this study, periodic inspections will be made by the IRI Quality Assurance Unit.

STANDARD OPERATING PROCEDURES

These studies will be performed according to Standard Operating Procedures as they relate to metabolic studies. These operating procedures have been published (Standard Operating Procedures, Vol.4, Analytical Chemistry and Metabolic Studies, 1981, MTP Press)

GOOD LABORATORY PRACTICE

The experiments will be carried out in such a way as to comply with our understanding of the following :

1. The Good Laboratory Practice Regulations for Non-clinical Studies laid down by the United States Department of Health, Education and Welfare, Food and Drug Administration. published in the Federal Register on 22 December 1978, Vo. 43, No. 247. pp 59986-60025.

2. The IRI Code of Good Laboratory Practice, Document CGP/791 issued in March 1979.

DATA STORAGE

All original data and this protocol, all chromatographic hard copy and print out and all documents relating to the conduct of the practical phases of this study will be stored in the archives of IRI for a period of 5 years.

STAFF INVOLVED

B.D. Cameron, BSc, C.Biol, MIBiol.	Study Co-ordinator
J. McDougall, BSc, PhD.	Principal Investigator
C. Young, BSc.	Project Leader

APPENDIX 2

Copy of Amersham Data Sheet

Amersham International plc
White Lion Road Amersham
Buckinghamshire England HP7 9LL

Radiochemical batch analysis

Caution: The product is prepared for laboratory use only and not warranted for use in humans or for clinical diagnosis

[^{14}C]METHANOL
Code CFA.7 C3

TECHNICAL DATA

Specific activity :	11.8 mCi/mmol,	437 MBq/mmol
	369 μCi/mg,	13.7 MBq/mg

Molecular weight : 32

Radiochemical purity

by gas-solid radiochromatography on Chromosorb 102 : 99%

by gas-solid radiochromatography on Carbowax 1540 (water content) : 2.1%

Analysed 26th October 1984

STABILITY AND STORAGE RECOMMENDATIONS

To minimise decomposition stocks of [^{14}C]methanol should be stored at +2°C. Under these conditions decomposition should not exceed 2% per annum.

PACKAGING

[^{14}C]Methanol is supplied as a vapour or liquid in borosilicate glass break-seal ampoules.

PREPARATION

[^{14}C]Methanol is prepared by the catalytic reduction of [^{14}C]carbon dioxide essentially as described by the method of OTT, D.C., WHALEY, T.W., BENZIGER, T., and ROHWER, R.K., J Labelled Compounds, 10, 315, 1974. The [^{14}C]methanol is purified by gas-liquid chromatography.

CH_3OH

HINTS FOR OPENING BREAK-SEAL AMPOULES

This product is supplied in borosilicate glass break-seal ampoules, type P1, which are sealed under vacuum.

For most convenient handling, these ampoules can be attached to a vacuum manifold system, and the contents are transferred to a receiving vessel following breakage of the seal by a previously inserted 'magnetic hammer' (see Method A below). If the ampoule contents are intended to be diluted with a liquid 'carrier' solvent or solution at room temperature, it is not always necessary to use a vacuum transfer system and a simpler method (Method B below) may be employed as an alternative. Method A is recommended in preference to Method B where loss of expensive materials during transfer must be minimised.

Method A - Transfer Under Vacuum

(i) Carefully introduce a 'magnetic hammer' (a glass-covered, soft, iron cylinder 5 mm diameter, 20 mm long) into the neck of the ampoule so that it rests on the glass hook of the seal.

(ii) Attach the ampoule to the vacuum line via the ground glass joint.

(iii) Open the stopcocks to remove air and moisture from the system and the top part of the ampoule. Close them after a good vacuum is obtained and test the system for leaks.

(iv) Cool the receiver by placing a Dewar flask, containing a refrigerant liquid, around it.

(v) Use a magnet to raise the 'hammer' about 5-10 cm above the break-seal, and then remove the magnet so that the 'hammer' falls and breaks the glass seal.

(vi) Open the stopcocks above the receiver and above the ampoule to let the ampoule contents distil rapidly into the receiver. For efficient transfer of the material, it is most important to maintain good vacuum conditions and no air must leak into the system. Transfer of material may be facilitated by warming the ampoule (for example, with a hot air blower). Some low boiling gases, such as methane or carbon monoxide, require the use of a Toepler (or similar) pump for complete transfer.

(vii) Close the stopcock above the receiver.

Method B - Dilution or Dissolution of Ampoule Contents

(i) Carefully introduce a glass-covered 'magnetic hammer' (5 mm diameter) so that it rests on the hook of the break-seal and then support the ampoule vertically in a clamp.

(ii) Place the solvent (or solution) in the space above the break-seal and close the tube by means of a ground-glass cap. Note that there must be enough solvent to cover the top of the hook, and that not all the solvent will pass into the ampoule.

(iii) Using a magnet as in Method A, raise the 'hammer' and let it fall and break the seal. The solution passes quickly into the ampoule which is normally under reduced pressure. Allow the contents to equilibrate for at least 15 minutes, by which time the mixture should be homogeneous.

(iv) Make a scratch mark around the ampoule just below the level of the break-seal using a glass cutting knife or sharp file. Hold a heated (red hot) glass rod against the scratch mark until a crack develops along the mark. The top of the ampoule can then be easily snapped off and the contents removed.

NOTE: Care is needed in this final stage if flammable solvents are involved.

APPENDIX 3

Individual Animal Dose Summary

Animal Number	Body Weight (g)	Dose Received ([^{14}C]-Methanol)			
		uCi	mg	mg.cm^{-2}	mg.kg^{-1}
1	205	27.05	6.19	0.50	30.19
2	207	27.05	6.19	0.50	29.90
3	216	27.05	6.19	0.50	28.66
4	203	27.05	6.19	0.50	30.49
5	204	27.05	6.19	0.50	30.34
6	193	27.05	6.19	0.50	32.07
7	185	27.05	6.19	0.50	33.46
8	185	27.05	6.19	0.50	33.46

APPENDIX 4

Results of Pilot Study
Animals in Closed Metabolism Cages

	Rat A	Rat B
Dose Administered (dpm)	73,606,221	73,606,221
% Dose Recovered 0-1 h†	0.29	0.15
% Dose Recovered 0-2 h†	0.46	0.29
% Dose Recovered 0-17 h†	0.90	0.72

† = Expired air passed through 3 traps in series containing the following:

Trap 1 100 ml water
Trap 2 100 ml NaOH (10% solution)
Trap 3 100 ml methanol