Probit function technical support document

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substance name	CAS number
Methacrylonitrile	**126-98-7**

This draft document describes the derivation of a probit function for application in a quantitative risk analysis (QRA).

This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM), and has been assigned the status "proposed". The document is open for discussion by the scientific expert panel on probit functions. Interested parties are invited to submit comments and suggestions concerning this document within 6 weeks after the issue date to the e-mail address mentioned above.

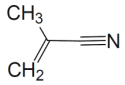
If the proposed probit function is approved by the expert panel on scientific grounds, the status of the document and probit function will be raised to "interim".

Subsequently the Ministry of Housing, Spatial Planning and the Environment (VROM) will perform a second tier evaluation to decide whether the probit function will be formally implemented. The decision on actual implementation will primarily be based on the results of a consequence analysis.

Detailed information on the procedures for derivation, evaluation and formalization of probit functions is available at http://www.rivm.nl/milieuportaal/bibliotheek/databases/probitrelaties.jsp.

Technical support document Methacrylonitrile

1. Substance identification



CAS-number:	126-98-7
IUPAC name:	methacrylonitrile
Synonyms:	
Molecular formula:	C_4H_5N
Molecular weight:	67.1 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	90°C (at 101.3 kPa)
Vapour pressure:	8.6 kPa (at 20°C)
Saturated vapour conc:	86000 ppm = 240 g/m^3 (at 20°C and 101.3 kPa)
Conversion factor:	1 mg/m^3 = 0.358 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.79 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	R: 11-23/24/25-43

2. Mechanism of action and toxicological effects following acute exposure[1]

Acute effects: Toxic effects of methacrylonitrile are mainly the result of metabolic cyanide formation and consistent with those produced after cyanide poisoning. The main target organs and tissues for inhalation exposure to methacrylonitrile are tissues with high oxygen demand such as the brain and heart. Cyanide inhibits the cellular respiration leading to increased oxygen tension and decreased unloading of oxyhaemoglobin. As oxidative metabolism slows down, the lack of energy results in central respiratory arrest and death.
The health endpoints are CNS dysfunction as well as irritation to the eyes, skin and upper respiratory tract. Dyspnoea, cyanosis, dizziness, hypoactivity, convulsions, coma, and pulmonary damage are symptoms after high exposure.
Long-term effects: Chronic exposure produces the same effects as acute exposure to cyanide.

3. Human toxicity data

No informative reports on health effects in humans following acute inhalation exposure were identified. Such reports are considered informative if both health effects as well as the exposure have been documented in sufficient detail.

4. Animal acute toxicity data

Animal lethal toxicity data considering acute exposure are described in Appendix 1. A total of 3 studies were identified -with 6 datasets for 4 species- with data on lethality following acute inhalation exposure. No datasets were assigned status A for deriving the human probit function, no datasets were assigned status B and 6 were assessed to be unfit (status C) for human probit function derivation.
During a litreature search the following technical support documents and databases have been consulted:

[1] AEGL interim TSD and AHLS provider manual.

1. AEGL interim TSD document (July 2007) and reference database for methacrylonitrile, covering references before 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, RTECS, with the following search terms:
   - Methcrylonitrile and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Sensory irritation
No studies were identified in which sensory irritation was studied.

5. Probit functions

It was not possible to derive a probit function for methacrylonitrile based on studies with A or B quality.

6. Evaluation

There is only one acute eligible inhalation toxicity study available for methacrylonitrile. This study doesn't meet the current standards regarding generation, delivery and analytical determination of test atmosphere nor post-mortem pathological evaluation of animals. There are three options for deriving a probit function for methacrylonitrile:

1. Disregard the Pozzani study, and not derive a probit function for methacrylonitrile.
2. Use the Pozzani study despite the considerable quality issues (cf AEGL).
3. Base the probit for methacrylonitrile on the existing probit for hydrogen cyanide.

To facilitate the discussion during the public review and by the scientific expert panel, probit functions have been developed following both approaches 2 and 3.

Calculation based on the methacrylonitrile study Pozzani *et al* (1968)

To derive the human probit function the weighted mean LC_{50} value of 969 mg/m^3 from Pozzani *et al* 1968 (C-quality study) have been used to derive a point of departure. The other available C-study (DuPont 1968) produced a higher 240-minute LC_{50} value of 1744 mg/m^3.

As point of departure for deriving the human probit function the 240 min LC_{50} value of 969 mg/m^3 for the rat from the Pozzani (C-quality) study was taken. The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	
RD_{50}	1	No RD_{50} data available
Nominal concentration	2	Well conducted analytically, unclear sampling location
Adequacy of database:	2	The study may have been conducted well, but the reported information does not allow to make a value judgement

The estimated human equivalent 240-minute LC_{50} value is 969 / 12 = **81 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2** was used. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.**.

The human probit function is then calculated on the human equivalent 240 min LC_{50} and using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1 \times \ln (81^2 \times 240)$ resulting in the a-value of **-9.263**.

$Pr = -9.26 + 1 \times \ln (C^2 \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically unacceptable basis. The probit function is based on 1 study in the rat with C quality, where 12 male and 12 female rats per concentration group were exposed to unknown concentrations for 240 minutes (total number of animals was not reported).

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 50 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 34 mg/m^3.

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
1% lethality, this probit	71	50
0.1% lethality, this probit	49	34
AEGL-3 (2007,interim)	88	69
ERPG-3		N/A
LBW (2007)		50

Comparing to equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are a little lower than the AEGL values, which use the same study for a point of departure. The AEGLs are calculated with an n-value of 3, the probits with an n-value of 2. The 2007 LBW was based on the absence of lethality after 3 hours exposure to 481 mg/m^3 in the Pozzani *et al* study.
These lethality estimates are not very consistent with the human data from Pozzani (1968) where a 10-minute exposure to 39 mg/m^3 produced odour detection in 7/7 tested human subjects, and some eye and nose irritation and tearing occurred. No severe health effects were reported.

Alternative calculation based on HCN equivalents.

Considering the weakness of the chemical specific database, an alternative approach may be to derive the probit function from HCN data. The cause of lethality seems to be related to cyanide formation. Even though hydrolysis is more rapid and complete than in e.g. acrylonitrile and oxalonitrile, a conservative probit is expected from an approach based on HCN equivalents.
The 240-minute rat LC_{50} of methacrylonitrile was calculated to be 969 mg/m^3 = 347 ppm. No 30-minute rat LC_{50} could be calculated from the data.
The 30-minute rat LC_{50} of HCN that was used as a point of departure for derivation of the HCN probit function was 190 mg/m^3 = 169 ppm (Lapin 1981).

One mole of methacrylonitrile can produce one mole of cyanide. Following this approach the POD for methacrylonitrile would be an estimated 30-minute rat LC_{50} of 169 ppm = 472 mg/m^3. The human equivalent LC_{50} was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	
RD_{50}	2	HCN RD_{50} value is just below the LC_{50} in the same study. Methacrylonitrile is reported to be irritating, and a precautionary assumption is made that rapid hydrolysis of the compound to cyanide contributes to this effect
Nominal concentration	1	Lapin HCN reported analytically determined concentrations
Adequacy of database:	1	The Lapin HCN study was a well conducted study

The estimated human equivalent 30-minute LC_{50} value is 472 / 6 = **79 mg/m^3**.
The experimentally determined n-value of HCN was **4.38** (Lapin 1981, HCN).
Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **0.457**.

The human probit function is then calculated on the human equivalent 30 min LC_{50} and using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 0.457 \times \ln (79^{4.38} \times 30)$ resulting in the a-value of **-5.283**.

$$Pr = -5.28 + 0.46 \times \ln (C^{4.38} \times t)$$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically weak basis. The probit function is based on an analogy with HCN which is only produced slowly by hydrolysis; the toxicity of the parent compound and of the concomitant production of cyanic acid cannot be taken into account.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 20 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 14 mg/m^3.

Estimated level	**30 min (mg/m^3)**	**60 min (mg/m^3)**
1% lethality, this probit	24	20
0.1% lethality, this probit	16	14
AEGL-3 (2007,interim)	88	69
ERPG-3		N/A
LBW (2007)		50

Comparing to equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are much lower than the AEGL values, which use the Pozzani *et al* study for a point of departure. The 2007 LBW was based on the absence of lethality after 3 hours exposure to 481 mg/m^3 in the Pozzani *et al* study.

These lethality estimates appear to conflict with the human data from Pozzani (1968) where a 10-minute exposure to 39 mg/m^3 produced odour detection in 7/7 tested human subjects, and some eye and nose irritation and tearing occurred. No severe health effects were reported.

Appendix 1 Animal experimental research

This section will be updated once the final version on the HCN TSD is available

Study ID: A.1

Author, year: Lapin, 1981

Substance: ***Hydrogen cyanide***

Species, strain, sex: Rat, Crl:CD, male

Number/sex/concentration group: 6 (restrained), 10 (freely moving)

Age and weight: weights 250 ± 25 gram, age not specified.

Observation period: at least 7 days

Criteria	*Comment*
Study carried out according to GLP	*No declaration of GLP was given.*
Study carried out according to guideline(s)	*No*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Restrained: Head only. Rats were restrained in whole body holders inside the chamber (175 l) used (in some cases simultaneously) for unrestrained animals. By using a switch a hinged box was swung down to start an exposure (see figure below).* *Unrestrained: whole body*
Pressure distribution.	*Not specified*
Homogeneity of test atmosphere at breathing zone of animals	*Test atmosphere was generated by dilution of bottled gas and passed into the chamber by flow-through.*
Number of air changes per hour	*Unknown*
Actual concentration measurement	*Continuous measurements with infrared spectrophotometry. This method was validated by gas chromatography. No details were presented.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*N/A*
Assessment of Reliability	***A*** *Study data were suitable to derive a probit function. Multiple concentration levels and durations were tested, resulting in a good concentration response relation with mortality of 0-100% (5 minute data were excluded from analysis).*

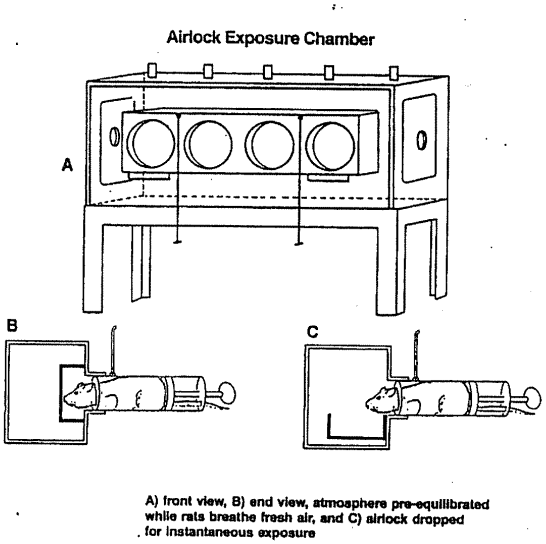


Figure showing how the restrained animals were exposed. Figures A and B show respectively a closed and opened hinged box.

Results (restrained animals)

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	363	5	6	0
Rat	386	5	6	1
Rat	447	5	6	3
Rat	468	5	6	3
Rat	478	5	6	5
Rat	605	5	6	6
Rat	81	15	6	0
Rat	158	15	6	3
Rat	168	15	6	1
Rat	222	15	6	4
Rat	264	15	6	6
Rat	325	15	6	6
Rat	402	15	6	6
Rat	38	30	6	0
Rat	60	30	6	2
Rat	110	30	6	3
Rat	121	30	6	4
Rat	143	30	6	4
Rat	185	30	6	6
Rat	319	30	6	6
Rat	47	60	6	0
Rat	61	60	6	2
Rat	81	60	6	4
Rat	116	60	6	6

198 **Results (freely moving animals)**

Species	Concentration (mg/m^3)	Exposure duration (min)	Exposed	Responded
Rat	307	5	10	0
Rat	368	5	10	1
Rat	382	5	10	2
Rat	396	5	10	5
Rat	495	5	10	9
Rat	554	5	10	10
Rat	570	5	10	10
Rat	124	15	10	0
Rat	197	15	10	2
Rat	211	15	10	4
Rat	229	15	10	7
Rat	258	15	10	7
Rat	282	15	10	10
Rat	318	15	10	10
Rat	453	15	10	10
Rat	144	30	10	0
Rat	167	30	10	0
Rat	180	30	10	4
Rat	206	30	10	8
Rat	249	30	10	9
Rat	344	30	10	10
Rat	85	60	10	0
Rat	120	60	10	1
Rat	173	60	10	7
Rat	206	60	10	9
Rat	249	60	10	10

199
200

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2006) as

$Pr = a + b \times \ln(C) + c \times \ln(t)$

with C for concentration in mg/m^3 and t for time in minutes.

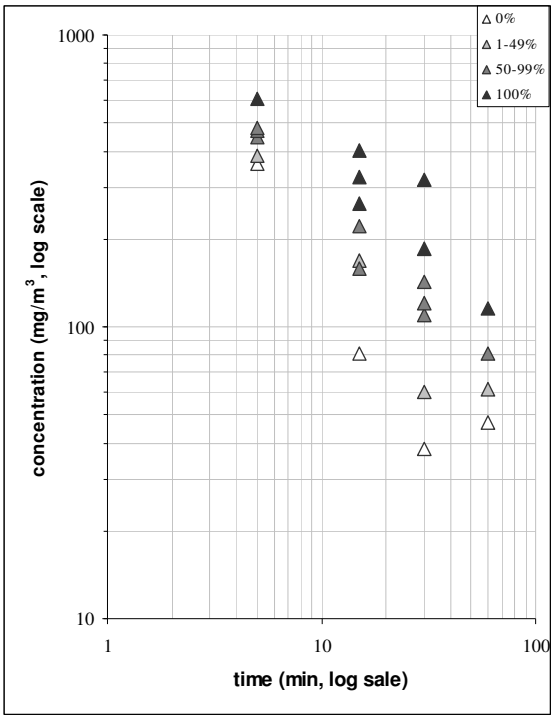
As only male rats were used in the study, sex could not be included as covariate in the probit function. The method of exposing the animals was taken up as covariate in the analysis of the data combined. It appeared that the results for the restrained animals and freely moving animals were significantly different. This conclusion was based on the comparison of the ln(likelihood) of the fitted data. The ln(likelihood) for the data combined with method as covariate was -52.16, whereas the ln(likelihood)s of the separated data were both approximately -17. As a general rule, a model is considered a better fit when the additional use of parameters (in this case by fitting the data separately) lead to a significant increase in the ln(likelihood). Since the ln(likelihood)s indicate that analysing the data separately is significantly better (-52 versus -34), it is not appropriate to combine the data.

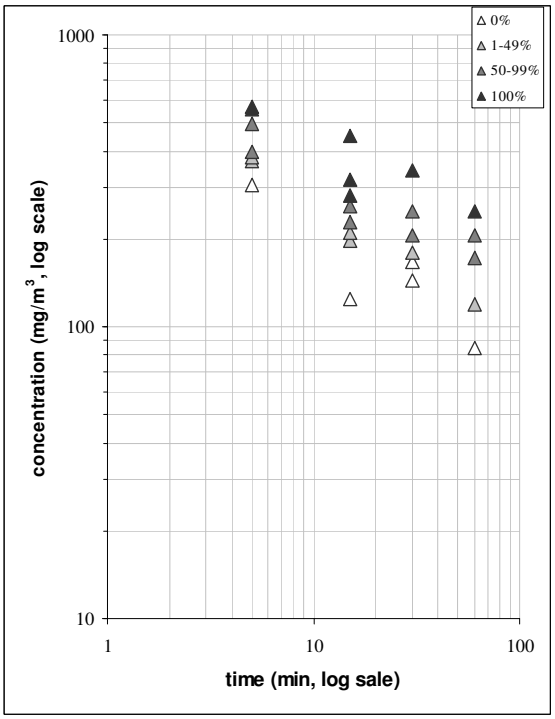
All analyses excluded the five minute data.

Probit function	*Species*	*a*	*b*	*c*	*n-value*
restrained	*Rat*	*-14.4*	*2.80*	*1.87*	*1.50 (1.05-1.94)*
Freely moving	*Rat*	*-33.5*	*6.39*	*1.46*	*4.38 (2.87-5.88)*

Duration (minutes)	*LC_{50} (mg/m^3) 95%-C.I.* ***Male (restrained)***	*LC_{50} (mg/m^3) 95%-C.I.* ***Male (freely moving)***
10	*224 (168-283)*	*244 (224-266)*
30	*108 (93.7-120)*	*190 (181-199)*
60	*67.6 (55.1-82.3)*	*162 (149-176)*

A graphical overview of the data is presented below. Each concentration-time combination represents one point in the plot.





Top: data of restrained animals, bottom: data of freely moving animals.

Study ID: C.1

Author, year: Pozzani et al, 1968

Substance: Methacrylonitrile

Species, strain, sex: Male and female Harlan/Wistar rats and male albino guinea pigs, albino rabbits and A/J strain mice.

Number/sex/concentration group: 6 / concentration level

Age and weight: not specified

Observation period: 14 days

Criteria	*Comment*
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to guideline(s)	*OECD guideline 403 did not exist at the time*
Stability of test compound in test atmosphere	*No information provided*
Use of vehicle (other than air)	*No information provided*
Whole body / nose-only (incl. head/nose-only) exposure	*No information provide, probably whole-body*
Pressure distribution.	*No information provided*
Homogeneity of test atmosphere at breathing zone of animals	*No information provided*
Number of air changes per hour	*No information provided*
Actual concentration measurement	*A gas chromatographic procedure was described for the analysis of actual concentration measurements. The frequency and location of samples was not described.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*No aerosol formation and aerosol exposure monitoring reported.*
Assessment of Reliability	***C*** *No information provided to assess the reliability of this study*

Pozzani et al. (1968) exposed groups of 6 male and 6 female Harlan-Wistar rats, 6 male albino guinea pigs, 4 male rabbits and 6 male mice to methacrylonitrile for 4 hours. The study did not report the actual concentrations tested, but stated that the 'concentrations were varied by a factor of two'. The study only reported the LC_{50} values and the 'range' (which may be a confidence interval). In male and female rats 2 groups were tested; judging from the animals' weight this concerned groups of younger and older animals but the authors do not discuss this any further.

In addition to the experiments reported below, Pozzani *et al* also tested survival after brief exposure to 'essentially saturated vapour' and 3-7 hour exposures of 3 dogs. The results of these experiments will not be reported here.

Results

Species and sex	Weight range (gram)	LC_{50} and 'range' (mg/m^3)	Exposure duration (min)	Symptoms observed from exposure to the lowest tested concentration
Rat F	213-317	1954 (594 – 6495)	240	loss of consciousness within 3 hr at 491 mg/m^3, but no deaths
Rat F	95-172	1384 (698 – 2771)	240	loss of consciousness within 3 hr at 491 mg/m^3, but no deaths
Rat M	344-510	915 (581 – 1440)	240	loss of consciousness within 3 hr at 491 mg/m^3, but no deaths
Rat M	123-207	915 (645 – 1658)	240	loss of consciousness within 3 hr at 491 mg/m^3, but no deaths
Guinea pig M	585-1035	246 (173 – 346)	240	147 mg/m^3 caused no symptoms
Rabbit M	2360-4290	103 (64 – 159)	240	55 mg/m^3 caused no symptoms
Mouse M	25-33	100 (70 – 120)	240	55 mg/m^3 caused no symptoms

Probit function

The data did not allow to calculate a probit function and associated LC-values. The reported 'range' appears to be substantially larger in the female rats than in the male rats. In addition, for the female rats there is a tendency towards a slightly higher LC_{50} value for older animals.

The reported LC_{50} for both sexes did not differ by more than a factor 2. This does not support that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analyzed to derive the animal probit function. The overall LC_{50} value was calculated as a weighted mean of all reported rat LC_{50} values. For this calculation it was necessary to assume that the reported 'range' was a confidence interval; this assumption was supported by the fact the reported values did not fit well in a series of concentrations that increased by a factor of two. The unweighted geometric mean of the four reported LC_{50} values was 1227 mg/m^3.

Duration (minutes)	*LC_{50} (mg/m^3)* ***Combined***
240	969 (no cfd-i calculated)

Human subjects

Pozzani *et al* also exposed human subjects to determine the sensory response to inhalation exposure. A 10-minute exposure to 39 mg/m^3 produced odour detection in 7/7 tested subjects, some eye and nose irritation and tearing. No severe health effects were reported.

Study ID: C studies

A group of 4 mature male rats (strain not specified) were exposed to a 'concentrated atmosphere' of methacrylonitrile (Younger Labs, 1969). The vapours were produced by passing a stream of air through 106.0 grams of methacrylonitrile contained in a 350 ml erlenmeyer flask. Vapours from the flask passed into a one litre bottle to remove droplets. The vapour then passed into the 35-litre metal chamber. Air flow through the chamber was 4 litres/minute and the average chamber temperature was 23°C and average humidity was 58%. All animals died within 25 minutes after the start of exposure. Laboured breathing, pawing at the face and nose, cyanosis, and collapse were observed during exposure. At autopsy, lung and liver hyperemia, dilated coronary arteries, and aortic aneurysms were observed.
Description adapted from AEGL interim TSD 07/2007.

Groups of ten young adult male ChR-CD rats were exposed to methacrylonitrile (only highest concentration of 1744 mg/m^3 was reported) for 4 hours and observed for up to 14 days (DuPont, 1968). The test sample was uniformly metered by a syringe drive into a stainless steel T-tube whose internal temperature was above the boiling point of the methacrylonitrile. A metered stream of air passing through the T-tube carried the vapours to the exposure chamber where the atmosphere was analyzed every half-hour by gas chromatography. Irregular respiration, hyperaemia followed by pale ears, unresponsiveness, tremors, convulsions, and face pawing and lacrimation (at 1744 mg/m^3 only) were observed during exposure. Mild initial erratic weight loss followed by normal weight gain was observed after the exposure period. An LC_{50} of 1228 (1061-1423) mg/m^3 was calculated. No other experimental details were reported.

Appendix 2 Reference list

DuPont. Initial Submission: Acute Inhalation Toxicity in Rats with Acrylonitrile (Uninhibited), Methacrylonitrile (Inhibited), and Acetonitrile with Cover Letter Dated 101592. OTS0571605. Haskell Lab Report 226-68, October 1968.

Lapin, C.A.: Inhalation toxicity of common combustion gases. E.I. du Pont de Nemours and Company. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No. 238-81 (1981).

NAC/AEGL. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Interim TSD for Methacrylonitrile. Washington, US EPA, July 2007.

Pozzani, U.C., Kinkead, E.R., and King, J.M. The mammalian toxicity of methacrylonitrile. Am. Ind. Hyg. Assoc. J. 1968;29:202-210.

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Younger Labs. Initial Submission: Toxicological Investigation of Methacrylonitrile with Cover Letter Dated 072392. OTS0570516. 1969.