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Toxicological Review of Ammonia Noncancer Inhalation

(CASRN 7664-41-7)

Supplemental Information

June 2016

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ABBREVIATIONS

AEGL	Acute Exposure Guideline Level
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	body mass index
BrDU	bromodeoxyuridine
BUN	blood urea nitrogen
CAC	cumulative ammonia concentration
CI	confidence interval
COPD	chronic obstructive pulmonary disease
DAP	diammonium phosphate
EU	endotoxin unit
FDA	Food and Drug Administration
FEF	forced expiratory flow
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GABA	gamma-aminobutyric acid
HERO	Health and Environmental Research Online
IgE	immunoglobulin E
IgG	immunoglobulin G
IRIS	Integrated Risk Information System
LC_{50}	50% lethal concentration
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MMEF	mean midexpiratory flow
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRL	minimal risk level
NH_3	ammonia
NH_4^+	ammonium ion
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAS	periodic acid-Schiff
PEF	peak expiratory flow
PEFR	peak expiratory flow rate
PEL	Permissible Exposure Limit
RD_{50}	50% response dose
REL	Recommended Exposure Limit
SD	standard deviation
SIFT-MS	selected ion flow tube mass spectrometry
TWA	time-weighted average
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Toxicity values and other health-related regulatory limits for ammonia that have been developed by other national and international health agencies are summarized in Table A-1.

Table A-1. Assessments by other national and international health agency assessments for ammonia

Organization	Toxicity value
Agency for Toxic Substances and Disease Registry (ATSDR, 2004)	Chronic inhalation MRL = 0.1 ppm (0.07 mg/m^3) ***Basis***: Lack of significant alterations in lung function in chronically exposed workers (Holness et al., 1989) and a composite UF of 30 (10 for human variability and a modifying factor of 3 for the lack of reproductive and developmental studies).
National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NRC, 2008)	**AEGL-1** (nondisabling) = 30 ppm (21 mg/m^3) for exposures ranging from 10 mins to 8 hrs to protect against mild irritation ***Basis***: mild irritation in human subjects (MacEwen et al., 1970) **AEGL-2** (disabling) = 220 ppm (154 mg/m^3) for a 10-min exposure to 110 ppm (77 mg/m^3) for an 8-hr exposure ***Basis***: irritation (eyes and throat; urge to cough) in human subjects (Verberk, 1977) **AEGL-3** (lethal) = 2,700 ppm (1,888 mg/m^3) for a 10-min exposure to 390 ppm (273 mg/m^3) for an 8-hr exposure ***Basis***: lethality in the mouse (Kapeghian et al., 1982; MacEwen and Vernot, 1972)
National Institute for Occupational Safety and Health (NIOSH, 2015) REL established in 1992	REL = 25 ppm (18 mg/m^3)[a] TWA for up to a 10-hr workday and a 40-hr work week ***Basis***: To project against respiratory and eye irritation. References cited in support of the REL included review documents for the years up to 1992; no specific reference served as the basis for the REL.
Occupational Safety and Health Administration (OSHA, 2006) PEL established in early 1970s	PEL for general industry = 50 ppm (35 mg/m^3) TWA for an 8-hr workday ***Basis***: The 1968 ACGIH TLV was promulgated as the OSHA PEL soon after adoption of the Occupational Safety and Health Act in 1970. The ACGIH TLV from 1968 was intended to protect against irritation of ammonia in humans; no specific reference served as the basis for the 1968 TLV.
Food and Drug Admistration (FDA, 2011a, b)	Ammonium hydroxide: direct food substance affirmed as generally recognized as safe (21 CFR 184.1139); substance generally recognized as safe when used in accordance with good manufacturing or feeding practices (21 CFR 582.1139).

[a]NIOSH used slightly different ppm to mg/m^3 conversion factors.
TWA = time weighted average; UF = uncertainty factor

ATSDR MRL = minimal risk level. An MRL is an estimate of daily human exposure to a hazardous substance that

Table A-1. Assessments by other national and international health agency assessments for ammonia

Organization	Toxicity value

is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure (http://www.atsdr.cdc.gov/mrls/index.asp; accessed 2/26/2016).

AEGL = acute exposure guideline level. AEGLs are used by emergency planners and responders as guidance in dealing with rare, usually accidental, releases of chemicals into the air and are calculated for exposure periods of 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours. At concentrations above specificied levels, the general population could experience the following: Level 1: notable discomfort, irritation, or certain asymptomatic non-sensory effects; Level 2: ireversible or other serious, long-lasting adverse health effects or an impaired ability to escape; and Level 3: life-threatening health effects or death (http://www.epa.gov/aegl/about-acute-exposure-guideline-levels-aegls; accessed 2/26/2016).

NIOSH REL = recommended exposure limit. An REL is an occupational exposure limit recommended by NIOSH to OSHA for adoption as a permissible exposure limit. The REL is a level that NIOSH believes would be protective of worker safety and health over a working lifetime if used in combination with engineering and work practice controls, exposure and medical monitoring, posting and labeling of hazards, worker training and personal protective equipment (http://www.cdc.gov/niosh/npg/pgintrod.html; accessed 2/26/2016).

OSHA PEL = permissible exposure limit. PELs are legally enforceable occupational standards (https://www.osha.gov/dsg/annotated-pels/; accessed 2/26/2016).

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APPENDIX B. ADDITIONAL DETAILS OF LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

Table B-1. Literature search strings for computerized databases

Database	Query strings	Hits
PubMed		
Period: March 2013–September 2015 Search date: 9/11/2015	((("Ammonia"[MeSH Terms] OR "ammonium hydroxide" [Supplementary Concept]) AND (("ammonia/adverse effects"[MeSH Terms] OR "ammonia/antagonists and inhibitors"[MeSH Terms] OR "ammonia/blood"[MeSH Terms] OR "ammonia/cerebrospinal fluid"[MeSH Terms] OR "ammonia/pharmacokinetics"[MeSH Terms] OR "ammonia/poisoning"[MeSH Terms] OR "ammonia/toxicity"[MeSH Terms] OR "ammonia/urine"[MeSH Terms]) OR ("hydroxides/adverse effects"[MeSH Terms] OR "hydroxides/antagonists and inhibitors"[MeSH Terms] OR "hydroxides/blood"[MeSH Terms] OR "hydroxides/cerebrospinal fluid"[MeSH Terms] OR "hydroxides/pharmacokinetics"[MeSH Terms] OR "hydroxides/poisoning"[MeSH Terms] OR "hydroxides/toxicity"[MeSH Terms] OR "hydroxides/urine"[MeSH Terms]) OR (("ammonia/metabolism"[MeSH Terms] OR "hydroxides/metabolism"[MeSH Terms]) AND (animals[MeSH Terms] OR humans[MeSH Terms])) OR (ci[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR risk[MeSH Terms] OR cancer[sb]) OR ((ammonia[majr] OR "ammonium hydroxide"[Supplementary Concept]) AND (dose-response relationship, drug[MeSH Terms] OR pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR mammals[MeSH Terms]))) OR ((Ammonia [Title] OR "Ammonium hydroxide"[Title] OR "Spirit of hartshorn"[Title] OR Aquammonia[Title]) NOT medline[sb])) OR ((inhal* OR (air OR breath OR exhal* OR respiration) OR (biological markers[MeSH Terms] AND (air OR breath OR exhal* OR respiration)) OR ("air pollutants"[MeSH Terms] AND (breath OR exhal*)) OR breath OR (analysis[Subheading] AND breath) OR (respiration[MeSH Terms] OR breath tests[MeSH Terms] OR exhalation[MeSH Terms])) AND (7664-41-7[rn] OR 1336-21-6[rn]))) AND (2013/03/01:3000[crdat] OR 2013/03/01:3000[mhda] OR 2013/03/01:3000[edat])	1,473
Period: March 2012–March 2013 Search date: 3/13/2013	("2012/03/26"[Date - Publication] : "3000"[Date - Publication]) AND (("Ammonia"[MeSH Terms] OR "ammonium hydroxide" [Supplementary Concept]) AND (("ammonia/adverse effects"[MeSH Terms] OR "ammonia/antagonists and inhibitors"[MeSH Terms] OR "ammonia/blood"[MeSH Terms] OR "ammonia/cerebrospinal fluid"[MeSH Terms] OR "ammonia/pharmacokinetics"[MeSH Terms] OR "ammonia/poisoning"[MeSH Terms] OR "ammonia/toxicity"[MeSH Terms] OR "ammonia/urine"[MeSH Terms]) OR ("hydroxides/adverse effects"[MeSH Terms] OR "hydroxides/antagonists and inhibitors"[MeSH Terms] OR "hydroxides/blood"[MeSH Terms] OR "hydroxides/cerebrospinal	410

Database	Query strings	Hits
	fluid"[MeSH Terms] OR "hydroxides/pharmacokinetics"[MeSH Terms] OR "hydroxides/poisoning"[MeSH Terms] OR "hydroxides/toxicity"[MeSH Terms] OR "hydroxides/urine"[MeSH Terms]) OR (("ammonia/metabolism"[MeSH Terms] OR "hydroxides/metabolism"[MeSH Terms]) AND (animals[MeSH Terms] OR humans[MeSH Terms])) OR (ci[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR risk[MeSH Terms] OR cancer[sb]) OR ((ammonia[majr] OR "ammonium hydroxide"[Supplementary Concept]) AND (dose-response relationship, drug[MeSH Terms] OR pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR mammals[MeSH Terms]))) OR ((Ammonia [Title] OR "Ammonium hydroxide"[Title] OR “Spirit of hartshorn"[Title] OR Aquammonia[Title]) NOT medline[sb]))	
	("2012/03/26"[Date - Publication] : "3000"[Date - Publication]) AND ((inhal* OR (air OR breath OR exhal* OR respiration) OR (biological markers[MeSH Terms] AND (air OR breath OR exhal* OR respiration)) OR ("air pollutants"[MeSH Terms] AND (breath OR exhal*)) OR breath OR (analysis[Subheading] AND breath) OR (respiration[MeSH Terms] OR breath tests[MeSH Terms] OR exhalation[MeSH Terms])) AND (7664-41-7[rn] OR 1336-21-6[rn]))	50
Period: March 2012–March 2013 Search date: 9/10/2015[a]	(((((((("Ammonia"[MeSH Terms] OR "ammonium hydroxide" [Supplementary Concept]) AND (("ammonia/adverse effects"[MeSH Terms] OR "ammonia/antagonists and inhibitors"[MeSH Terms] OR "ammonia/blood"[MeSH Terms] OR "ammonia/cerebrospinal fluid"[MeSH Terms] OR "ammonia/pharmacokinetics"[MeSH Terms] OR "ammonia/poisoning"[MeSH Terms] OR "ammonia/toxicity"[MeSH Terms] OR "ammonia/urine"[MeSH Terms]) OR ("hydroxides/adverse effects"[MeSH Terms] OR "hydroxides/antagonists and inhibitors"[MeSH Terms] OR "hydroxides/blood"[MeSH Terms] OR "hydroxides/cerebrospinal fluid"[MeSH Terms] OR "hydroxides/pharmacokinetics"[MeSH Terms] OR "hydroxides/poisoning"[MeSH Terms] OR "hydroxides/toxicity"[MeSH Terms] OR "hydroxides/urine"[MeSH Terms]) OR (("ammonia/metabolism"[MeSH Terms] OR "hydroxides/metabolism"[MeSH Terms]) AND (animals[MeSH Terms] OR humans[MeSH Terms])) OR (ci[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR risk[MeSH Terms] OR cancer[sb]) OR ((ammonia[majr] OR "ammonium hydroxide"[Supplementary Concept]) AND (dose-response relationship, drug[MeSH Terms] OR pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR mammals[MeSH Terms]))) OR ((Ammonia [Title] OR "Ammonium hydroxide"[Title] OR “Spirit of hartshorn"[Title] OR Aquammonia[Title]) NOT medline[sb]))) OR (((inhal* OR (air OR breath OR exhal* OR respiration) OR (biological markers[MeSH Terms] AND (air OR breath OR exhal* OR respiration)) OR ("air pollutants"[MeSH Terms] AND (breath OR exhal*)) OR breath OR (analysis[Subheading] AND breath) OR (respiration[MeSH Terms] OR breath tests[MeSH Terms] OR exhalation[MeSH Terms])) AND (7664-41-7[rn] OR 1336-21-6[rn])))))))) AND ((2012/03/26:2013/03/13[crdat] OR 2012/03/26:2013/03/13[mhda] OR 2012/03/26:2013/03/13[edat]) NOT (2012/03/26:2013/03/13[dp]))	159

Database	Query strings	Hits
No date limit Search date: 3/26/2012	(("Ammonia"[MeSH Terms] OR "ammonium hydroxide" [Supplementary Concept]) AND (("ammonia/adverse effects"[MeSH Terms] OR "ammonia/antagonists and inhibitors"[MeSH Terms] OR "ammonia/blood"[MeSH Terms] OR "ammonia/cerebrospinal fluid"[MeSH Terms] OR "ammonia/pharmacokinetics"[MeSH Terms] OR "ammonia/poisoning"[MeSH Terms] OR "ammonia/toxicity"[MeSH Terms] OR "ammonia/urine"[MeSH Terms]) OR ("hydroxides/adverse effects"[MeSH Terms] OR "hydroxides/antagonists and inhibitors"[MeSH Terms] OR "hydroxides/blood"[MeSH Terms] OR "hydroxides/cerebrospinal fluid"[MeSH Terms] OR "hydroxides/pharmacokinetics"[MeSH Terms] OR "hydroxides/poisoning"[MeSH Terms] OR "hydroxides/toxicity"[MeSH Terms] OR "hydroxides/urine"[MeSH Terms]) OR (("ammonia/metabolism"[MeSH Terms] OR "hydroxides/metabolism"[MeSH Terms]) AND (animals[MeSH Terms] OR humans[MeSH Terms])) OR (ci[Subheading] OR "environmental exposure"[MeSH Terms] OR "endocrine system"[MeSH Terms] OR "hormones, hormone substitutes, and hormone antagonists"[MeSH Terms] OR risk[MeSH Terms] OR cancer[sb]) OR ((ammonia[majr] OR "ammonium hydroxide"[Supplementary Concept]) AND (dose-response relationship, drug[MeSH Terms] OR pharmacokinetics[MeSH Terms] OR metabolism[MeSH Terms]) AND (humans[MeSH Terms] OR mammals[MeSH Terms]))) OR ((Ammonia [Title] OR "Ammonium hydroxide"[Title] OR “Spirit of hartshorn"[Title] OR Aquammonia[Title]) NOT medline[sb])	13,012
	Additional Search on Exhaled Breath (inhal* OR (air OR breath OR exhal* OR respiration) OR (biological markers[MeSH Terms] AND (air OR breath OR exhal* OR respiration)) OR ("air pollutants"[MeSH Terms] AND (breath OR exhal*)) OR breath OR (analysis[Subheading] AND breath) OR (respiration[MeSH Terms] OR breath tests[MeSH Terms] OR exhalation[MeSH Terms])) AND (7664-41-7[rn] OR 1336-21-6[rn])	1,600
ToxLine		
Period: March 2012 – September 2015 Search date: 9/14/2015[b]	@or+(piscesqcorrection+"ammonia"+"ammonium hydroxide"+"Spirit of hartshorn"+"aquammonia"+@term+@rn+7664-41-7+@term+@rn+1336-21-6)+@AND+@range+yr+2012+2015+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	33
Period: March 2012– March 2013 Search date: 3/13/2013	@AND+@OR+("7664-41-7"+"1336-21-6"+@TERM+@rn+7664-41-7+@TERM+@rn+1336-21-6)+@na+ammon*+@RANGE+yr+2012+2013	100
No date limit Search date: 3/26/2012	Searched via NLM (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE): Limited to ammon* in title. This covered all synonyms listed to both ammonia and ammonium hydroxide with the exception of “spirit of hartshorn” which found no results when limited to the title	2,417
TSCATS		
TSCATS2: recent notices	7664-41-7	0 TSCATS2

Database	Query strings	Hits
Search date: 9/15/2015	1336-21-6 EPA receipt date: 01/01/2012-08/31/2015	0 recent notices
TSCATS, TSCATS2, TSCA: recent notices No date limit Search date: 3/26/2012	7664-41-7 1336-21-6	50 TSCATS1 7 TSCATS2 1 recent notice
Web of Science		
Period: March 2012–September 2015 Search date: 9/10/2015	(TS="ammonia" OR TS="ammonium hydroxide" OR TS="Spirit of hartshorn" OR TS="aquammonia") AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology" OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*) OR (TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="WORKERS" OR TS="HUMAN" OR TS=patient* OR TS=mother OR TS=fetal OR TS=fetus OR TS=citizens OR TS=milk OR TS=formula)) OR TI=toxic*) Indexes=SCI-EXPANDED, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC Timespan=2012-2015	3,691
	(TS="ammonia" OR TS="ammonium hydroxide" OR TS="Spirit of hartshorn" OR TS="aquammonia") AND (TS=breath OR TS=exhale* OR TS="expired air")	125

Database	Query strings	Hits
	Indexes=SCI-EXPANDED, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC Timespan=2012-2015	
Toxcenter		
No date limit Search date: 3/27/2012	((7664-41-7 OR 1336-21-6) not (patent/dt OR tscats/fs)) and (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon?) AND (((biosis/fs AND py>1999 AND (hominidae/ct,st,it OR human/ct,st,it OR humans/ct,st,it OR mammals/ct,st,it OR mammal/ct,st,it OR mammalia/ct,st,it)) OR ipa/fs OR (caplus/fs AND 4-?/cc) OR ammonia/ti OR "ammonium hydroxide"/ti OR "spirit of hartshorn"/ti OR aquammonia/ti) Dupicates were removed; Biosis subfile results were date limited to avoid extensive overlap with Toxline	2,591
	Additional Search on Exhaled Breath (7664-41-7 OR 1336-21-6) AND (breath OR exhale? OR "expired air")	81
HERO		
SQL statement run on 3/14/13	select r.reference_id from tbl_reference r where r.sdelete = 'No' and (lower (r.title) like '%ammonia%' or lower (r.title) like '%ammonium hydroxide%' or lower (r.abstract) like '%ammonia%' or lower (r.abstract) like '%ammonium hydroxide%') and r.year > 2011 and r.reference_id not in (select reference_id from tbl_reference_project where project_id = 36);	115
Search date: 3/27/2012	ammonia OR ammonium hydroxide	5,295
Combined Reference Set	duplicates eliminated electronically	~28,000

[a]This query expands the 2013 PubMed search from items published from March 2012 to March 2013 to all items added to PubMed during that timeframe.
[b]This query expands the 2013 Toxline search to include synonym searches for items not also appearing in the PubMed database.

1

Table B-2. Processes used to augment the search of core computerized databases for ammonia

System used	Key reference or source
Manual search of citations from health assessment documents	ATSDR (2004). Toxicological profile for ammonia [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=11&tid=2
	NRC (2008). Acute exposure guideline levels for selected airborne chemicals: Volume 6. Washington, DC: The National Academies Press. http://www.nap.edu/catalog.php?record_id=12018
	ACGIH (2001). Ammonia [TLV/BEI]. In Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH.
	NIOSH (2010). NIOSH pocket guide to chemical hazards: Ammonia. http://www.cdc.gov/niosh/npg/npgd0028.html
	OSHA. (2006). Table Z-1: Limits for air contaminants, 29 CFR § 1910.1000 http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992
	FDA (2011a) Direct food substances affirmed as generally recognized as safe (GRAS): Ammonium hydroxide, 21 CFR § 184.1139. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1139
	FDA (2011b) Substances generally recognized as safe: General purpose food additives: Ammonium hydroxide, 21 CFR § 582.1139. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=582.1139
Manual search of citations from key studies in cleaning and hospital worker literature	Dumas, O; Donnay, C; Heederik, DJ; Héry, M; Choudat, D; Kauffmann, F; Le Moual, N. (2012). Occupational exposure to cleaning products and asthma in hospital workers. Occup Environ Med 69: 883-889. http://dx.doi.org/10.1136/oemed-2012-100826 (Dumas et al., 2012)
	Zock, JP; Vizcaya, D; Le Moual, N. (2010). Update on asthma and cleaners [Review]. Curr Opin Allergy Clin Immunol 10: 114-120. http://dx.doi.org/10.1097/ACI.0b013e32833733fe (Zock et al., 2010)
	Mirabelli, MC; Zock, J-P; Plana, E; Maria Anto, J; Benke, G; Blanc, PD; Dahlman-Hoglund, A; Jarvis, DL; Kromhout, H; lillienberg, L; Norback, D; Olivieri, M; Radon, K; Sunyer, J; Toren, K; van Sprundel, M; Villani, S; Kogevinas, M. (2007). Occupational risk factors for asthma among nurses and related healthcare professionals in an international study. Occup Environ Med 64: 474-479. (Mirabelli et al., 2007)
Web of Science forward search (performed in 2013 and updated in 2015)	Kennedy, SM; Le Moual, N; Choudat, D; Kauffmann, F. (2000). Development of an asthma specific job exposure matrix and its application in the epidemiological study of genetics and environment in asthma (EGEA). Occup Environ Med 57: 635-641. (Kennedy et al., 2000)

System used	Key reference or source
Search of Online Chemical Assessment-Related Websites	Combination of CASRN and synonyms searched on the following websites:
Period: No limit– March 2012; updated 2012–August 2015 Search date: 8/10/2015	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA (Office of Environmental Health Hazard Assessment) (http://www.oehha.ca.gov/risk.html) eChemPortal (includes: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, US EPA IRIS, US EPA SRS) (http://www.echemportal.org/echemportal/participant/page.action?pageID=9) EPA Acute Exposure Guideline Levels (http://www.epa.gov/aegl/access-acute-exposure-guideline-levels-aegls-values#chemicals) EPA – IRIS Assessments (http://cfpub.epa.gov/ncea/iris2/atoz.cfm) EPA NSCEP (http://www.epa.gov/nscep) EPA Science Inventory (http://cfpub.epa.gov/si/) Federal Docket (www.regulations.gov) Health Canada First Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php) Health Canada Second Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php) IARC (http://monographs.iarc.fr/htdig/search.html) IPCS INCHEM (http://www.inchem.org/) NAS via NAP (http://www.nap.edu/) NCI (http://www.cancer.gov) NCTR (http://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/default.htm) National Institute for Environmental Health Sciences (NIEHS) (http://www.niehs.nih.gov/) NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) NTP – RoC, status, results, and management reports (http://ntpsearch.niehs.nih.gov/query.html) WHO assessments – CICADS, EHC (http://www.who.int/ipcs/assessment/en/)
Period: No limit–August 2015 Search date: 8/10/2015	ACGIH (http://www.acgih.org/home.htm) AICS (http://www.nicnas.gov.au/regulation-and-compliance/aics/aics-search-page) AIHA: WEELs (https://www.aiha.org/get-involved/AIHAGuidelineFoundation/WEELs/Documents/2011WEELValues.pdf); ERPGs (https://www.aiha.org/get-involved/AIHAGuidelineFoundation/EmergencyResponsePlanningGuidelines/Documents/2014%20ERPG%20Values.pdf) CalEPA Drinking Water Notification Levels (http://www.swrcb.ca.gov/drinking_water/certlic/drinkingwater/NotificationLevels.shtml) CalEPA Office of Environmental Health Hazard Assessment: OEHHA Toxicity Criteria Database (http://www.oehha.ca.gov/tcdb/index.asp); Biomonitoring California-Priority Chemicals (http://www.oehha.ca.gov/multimedia/biomon/pdf/PriorityChemsCurrent.pdf); Biomonitoring California-Designated Chemicals

System used	Key reference or source
	(http://www.oehha.ca.gov/multimedia/biomon/pdf/DesignatedChemCurrent.pdf); Cal/Ecotox Database (http://www.oehha.ca.gov/scripts/cal_ecotox/CHEMLIST.ASP); OEHHA Fact Sheets (http://www.oehha.ca.gov/public_info/facts/index.html); Non-cancer health effects Table-RELs (http://www.oehha.ca.gov/air/allrels.html); Cancer Potency Factors-Appendix A and AppendixB (http://www.oehha.ca.gov/air/hot_spots/tsd052909.html) CHRIP (http://www.safe.nite.go.jp/english/db.html) CPSC (http://www.cpsc.gov) ECHA Chem (http://echa.europa.eu/) Environment Canada – Search entire site (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) EPA HPVIS (http://java.epa.gov/chemview) – Limit output selection to High Production Volume Information System; EPA OPP Pesticide Chemical Search (http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1) FDA (http://www.fda.gov/) Health Canada: Toxic Substances Managed Under CEPA (http://www.ec.gc.ca/toxiques-toxics/Default.asp?lang=En&n=98E80CC6-1); Final Assessments (http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=09F567A7-B1EE-1FEE-73DB-8AE6C1EB7658); Draft Assessments (http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=6892C255-5597-C162-95FC-4B905320F8C9); Health Canada Drinking Water Documents (http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/index-eng.php#tech_doc) NICNAS - PEC only covered by eChemPortal (http://www.nicnas.gov.au/chemical-information) NIOSH (http://www.cdc.gov/niosh/topics/) NRC – AEGLs via NAP (http://www.nap.edu/) OECD HPV (http://webnet.oecd.org/hpv/ui/Search.aspx) OSHA (http://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html) RTECS (http://ccinfoweb.ccohs.ca/rtecs/search.html)

Table B-3. Disposition of studies from the cleaning and hospital worker literature

Studies selected for full text review	Review of full text or abstract?	Disposition based on inclusion/exclusion criteria in Table LS-1
References identified by manual backward search of seminal studies identified in March 2013 and forward search of Kennedy et al. (2000) performed in 2013		
Kogevinas, M; Zock, JP; Jarvis, D; Kromhout, H; Lillienberg, L; Plana, E; Radon, K; Toren, K; Alliksoo, A; Benke, G; Blanc, PD; Dahlman-Hoglund, A; D'Errico, A; Hery, M; Kennedy, S; Kunzli, N; Leynaert, B; Mirabelli, MC; Muniozguren, N; Norback, D; Olivieri, M; Payo, F; Villani, S; van Sprundel, M; Urrutia, I; Wieslander, G; Sunyer, J; Anto, JM. (2007). Exposure to	Full-text	Exclude No ammonia-specific data

Studies selected for full text review	Review of full text or abstract?	Disposition based on inclusion/exclusion criteria in Table LS-1
substances in the workplace and new-onset asthma: an international prospective population-based study (ECRHS-II). Lancet 370: 336-341.		
Mirabelli, MC; Zock, JP; Plana, E; Anto, JM; Benke, G; Blanc, PD; Dahlman-Hoglund, A; Jarvis, DL; Kromhout, H; Lillienberg, L; Norback, D; Olivieri, M; Radon, K; Sunyer, J; Toren, K; van Sprundel, M; Villani, S; Kogevinas, M. (2007). Occupational risk factors for asthma among nurses and related healthcare professionals in an international study. Occup Environ Med 64: 474-479.	Full-text	Exclude No ammonia-specific data
Zock, JP; Plana, E; Jarvis, D; Anto, JM; Kromhout, H; Kennedy, SM; Kunzli, N; Villani, S; Olivieri, M; Toren, K; Radon, K; Sunyer, J; Dahlman-Hoglund, A; Norback, D; Kogevinas, M. (2007). The use of household cleaning sprays and adult asthma: an international longitudinal study. Am J Respir Crit Care Med 176: 735-741.	Full-text	**Include**
Zock, JP; Plana, E; Anto, JM; Benke, G; Blanc, PD; Carosso, A; Dahlman-Hoglund, A; Heinrich, J; Jarvis, D; Kromhout, H; Lillienberg, L; Mirabelli, MC; Norback, D; Olivieri, M; Ponzio, M; Radon, K; Soon, A; van Sprundel, M; Sunyer, J; Svanes, C; Toren, K; Verlato, G; Villani, S; Kogevinas, M. (2009). Domestic use of hypochlorite bleach, atopic sensitization, and respiratory symptoms in adults. J Allergy Clin Immunol 124: 731-738 e731.	Abstract	Exclude No ammonia-specific data
Orriols, R; Costa, R; Albanell, M; Alberti, C; Castejon, J; Monso, E; Panades, R; Rubira, N; Zock, JP. (2006). Reported occupational respiratory diseases in Catalonia. Occup Environ Med 63: 255-260.	Abstract	Exclude No ammonia-specific data
Cherry, N; Beach, J; Burstyn, I; Fan, X; Guo, N; Kapur, N. (2009). Data linkage to estimate the extent and distribution of occupational disease: new onset adult asthma in Alberta, Canada. Am J Ind Med 52: 831-840.	Full-text	Exclude No ammonia-specific data
Mazurek, JM; Filios, M; Willis, R; Rosenman, KD; Reilly, MJ; McGreevy, K; Schill, DP; Valiante, D; Pechter, E; Davis, L; Flattery, J; Harrison, R. (2008). Work-related asthma in the educational services industry: California, Massachusetts, Michigan, and New Jersey, 1993-2000. Am J Ind Med 51: 47-59.	Abstract	Exclude No ammonia-specific data
Obadia, M; Liss, GM; Lou, W; Purdham, J; Tarlo, SM. (2009). Relationships between asthma and work exposures among non-domestic cleaners in Ontario. Am J Ind Med 52: 716-723.	Abstract	Exclude No ammonia-specific data
Lynde, CB; Obadia, M; Liss, GM; Ribeiro, M; Holness, DL; Tarlo, SM. (2009). Cutaneous and respiratory symptoms among professional cleaners. Occup Med (Lond) 59: 249-254.	Abstract	Exclude No ammonia-specific data
Massin, N; Hecht, G; Ambroise, D; Hery, M; Toamain, JP; Hubert, G; Dorotte, M; Bianchi, B. (2007). Respiratory symptoms and bronchial responsiveness among cleaning and disinfecting workers in the food industry. Occup Environ Med 64: 75-81.	Full-text	Exclude Quarternary ammonia
de Fatima Macaira, E; Algranti, E; Medina Coeli Mendonca, E; Antonio Bussacos, M. (2007). Rhinitis and asthma symptoms in non-domestic cleaners from the Sao Paulo metropolitan area, Brazil. Occup Environ Med 64: 446-453.	Full-text	Exclude Ammonium
Medina-Ramon, M; Zock, JP; Kogevinas, M; Sunyer, J; Basagana, X; Schwartz, J; Burge, PS; Moore, V; Anto, JM. (2006). Short-term respiratory	Full-text	**Include**

Studies selected for full text review	Review of full text or abstract?	Disposition based on inclusion/exclusion criteria in Table LS-1
effects of cleaning exposures in female domestic cleaners. Eur Respir J 27: 1196-1203.		
Bernstein, JA; Brandt, D; Rezvani, M; Abbott, C; Levin, L. (2009). Evaluation of cleaning activities on respiratory symptoms in asthmatic female homemakers. Ann Allergy Asthma Immunol 102: 41-46.	Full-text	Exclude No ammonia-specific data
Delclos, GL; Gimeno, D; Arif, AA; Burau, KD; Carson, A; Lusk, C; Stock, T; Symanski, E; Whitehead, LW; Zock, JP; Benavides, FG; Anto, JM. (2007). Occupational risk factors and asthma among health care professionals. Am J Respir Crit Care Med 175: 667-675.	Full-text	Exclude No ammonia-specific data
Arif, AA; Delclos, GL; Serra, C. (2009). Occupational exposures and asthma among nursing professionals. Occup Environ Med 66: 274-278.	Full-text	Exclude No ammonia-specific data
Liss, GM; Buyantseva, L; Luce, CE; Ribeiro, M; Manno, M; Tarlo, SM. (2011). Work-related asthma in health care in Ontario. Am J Ind Med 54: 278-284.	Abstract	Exclude No ammonia-specific data
Pechter, E; Davis, LK; Tumpowsky, C; Flattery, J; Harrison, R; Reinisch, F; Reilly, MJ; Rosenman, KD; Schill, DP; Valiante, D; Filios, M. (2005). Work-related asthma among health care workers: surveillance data from California, Massachusetts, Michigan, and New Jersey, 1993-1997. Am J Ind Med 47: 265-275.	Full-text	Exclude No ammonia-specific data
Arif, AA; Delclos, GL. (2012). Association between cleaning-related chemicals and work-related asthma and asthma symptoms among healthcare professionals. Occup Environ Med 69: 35-40.	Full-text	**Include**
Vizcaya, D; Mirabelli, MC; Anto, JM; Orriols, R; Burgos, F; Arjona, L; Zock, JP. (2011). A workforce-based study of occupational exposures and asthma symptoms in cleaning workers. Occup Environ Med 68: 914-919.	Full-text	**Include**
Quirce, S; Barranco, P. (2010). Cleaning agents and asthma. J Investig Allergol Clin Immunol 20: 542-550.	Full-text	Exclude Review article (references checked; no new refereces identified)
Chan-Yeung, M; Malo, JL. (1994). Aetiological agents in occupational asthma. Eur Respir J 7: 346-371.	Full-text	Exclude Review article
Medina-Ramon, M; Zock, JP; Kogevinas, M; Sunyer, J; Torralba, Y; Borrell, A; Burgos, F; Anto, JM. (2005). Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: a nested case-control study. Occup Environ Med 62: 598-606.	Full-text	**Include**
Le Moual, N; Varraso, R; Siroux, V; Dumas, O; Nadif, R; Pin, I; Zock, JP; Kauffmann, F. (2012). Domestic use of cleaning sprays and asthma activity in females. Eur Respir J 40: 1381-1389.	Full-text	Exclude No ammonia-specific data (Ammonia analyzed as part of "Factor 3", with decalcifers, acids, stain removers, and sprays for carpets, rugs and curtains)

Studies selected for full text review	Review of full text or abstract?	Disposition based on inclusion/exclusion criteria in Table LS-1
Ghosh, RE; Cullinan, P; Fishwick, D; Hoyle, J; Warburton, CJ; Strachan, DP; Butland, BK; Jarvis, D. (2013). Asthma and occupation in the 1958 birth cohort. Thorax 68: 365-371.	Full-text	Exclude No ammonia-specific data
Lemiere, C; Begin, D; Camus, M; Forget, A; Boulet, LP; Gerin, M. (2012). Occupational risk factors associated with work-exacerbated asthma in Quebec. Occup Environ Med 69: 901-907.	Full-text	**Include**
References identified in September 2015 update of forward search of Kennedy et al. (2000)		
Beach, J; Burstyn, I; Cherry, N. (2012). Estimating the extent and distribution of new-onset adult asthma in British Columbia using frequentist and Bayesian approaches. The Annals of occupational hygiene 56: 719-727. http://dx.doi.org/10.1093/annhyg/mes004	Full-text	Exclude No ammonia-specific data
Casas, L; Nemery, B. (2014). Irritants and asthma. The European respiratory journal 44: 562-564. http://dx.doi.org/10.1183/09031936.00090014	Full-text	Exclude Editorial
Christensen, BH; Thulstrup, A; Hougaard, KS; Skadhauge, LR; Hansen, KS; Schlunssen, V. (2013). Occupational exposure during pregnancy and the risk of hay fever in 7-year-old children. Clinical Respiratory Journal 7: 183-188. http://dx.doi.org/10.1111/j.1752-699X.2012.00300.x	Full-text	Exclude No ammonia-specific data
Christensen, BH; Thulstrup, An; Hougaard, KS; Skadhauge, LR; Hansen, KS; Frydenberg, M; Schlunssen, V. (2013). Maternal occupational exposure to asthmogens during pregnancy and risk of asthma in 7-year-old children: a cohort study. BMJ Open 3. http://dx.doi.org/10.1136/bmjopen-2012-002401	Full-text	Exclude No ammonia-specific data
Dumas, O; Laurent, E; Bousquet, J; Metspalu, A; Milani, L; Kauffmann, F; Le Moual, N. (2014). Occupational irritants and asthma: an Estonian cross-sectional study of 34 000 adults. The European respiratory journal 44: 647-656. http://dx.doi.org/10.1183/09031936.00172213	Full-text	Exclude No ammonia-specific data
Dumas, O; Le Moual, N; Siroux, V; Heederik, D; Garcia-Aymerich, J; Varraso, R; Kauffmann, F; Basagana, X. (2013). Work related asthma. A causal analysis controlling the healthy worker effect. Occupational and environmental medicine 70: 603-610. http://dx.doi.org/10.1136/oemed-2013-101362	Full-text	Exclude No ammonia-specific data
Dumas, O; Siroux, V; Luu, F; Nadif, R; Zock, Ja; Kauffmann, F; Le Moual, N. (2014). Cleaning and Asthma Characteristics in Women. Am J Ind Med 57: 303-311. http://dx.doi.org/10.1002/ajim.22244	Full-text	Exclude No ammonia-specific data
Henneberger, PK; Liang, X; Lillienberg, L; Dahlman-Hoglund, A; Toren, K; Andersson, E. (2015). Occupational exposures associated with severe exacerbation of asthma. Int J Tuberc Lung Dis 19: 244-250. http://dx.doi.org/10.5588/ijtld.14.0132	Full-text	Exclude No ammonia-specific data
Jeebhay, MF; Ngajilo, D; Le Moual, N. (2014). Risk factors for nonwork-related adult- onset asthma and occupational asthma: a comparative review. Curr Opin Allergy Clin Immunol 14: 84-94. http://dx.doi.org/10.1097/ACI.0000000000000042	Full-text	Exclude Review article (references checked; no new refereces identified)
Kellberger, J; Peters-Weist, AS; Reinrich, S; Pfeiffer, S; Vogelberg, C; Roller, D; Genuneit, Jo; Weinmayr, G; von Mutius, E; Heumann, C; Nowak, D;	Full-text	Exclude

Studies selected for full text review	Review of full text or abstract?	Disposition based on inclusion/exclusion criteria in Table LS-1
Radon, K. (2014). Predictors of work-related sensitisation, allergic rhinitis and asthma in early work life. The European respiratory journal 44: 657-665. http://dx.doi.org/10.1183/09031936.00153013		No ammonia-specific data
Koehoorn, M; Tamburic, L; McLeod, CB; Demers, PA; Lynd, L; Kennedy, SM. (2013). Population-based surveillance of asthma among workers in British Columbia, Canada. 33: 88-94.	Full-text	Exclude No ammonia-specific data
Le Moual, N; Carsin, AE; Siroux, V; Radon, K; Norback, Da; Toren, K; Olivieri, M; Urrutia, I; Cazzoletti, L; Jacquemin, B; Benke, G; Kromhout, H; Mirabelli, MC; Mehta, AJ; Schluenssen, V; Sigsgaard, T; Blanc, PD; Kogevinas, M; Anto, JM; Zock, J. (2014). Occupational exposures and uncontrolled adult-onset asthma in the European Community Respiratory Health Survey II. The European respiratory journal 43: 374-386. http://dx.doi.org/10.1183/09031936.00034913	Full-text	Exclude No ammonia-specific data
Lillienberg, L; Andersson, Ev; Janson, C; Dahlman-Hoglund, A; Forsberg, B; Holm, M; Gislason, T; Joegi, R; Omenaas, E; Schlunssen, V; Sigsgaard, T; Svanes, C; Toren, K. (2013). Occupational Exposure and New-onset Asthma in a Population-based Study in Northern Europe (RHINE). The Annals of occupational hygiene 57: 482-492. http://dx.doi.org/10.1093/annhyg/mes083	Full-text	Exclude No ammonia-specific data
Lillienberg, L; Dahlman-Höglund, A; Schiöler, L; Torén, K; Andersson, E. (2014). Exposures and asthma outcomes using two different job exposure matrices in a general population study in northern Europe. The Annals of occupational hygiene 58: 469-481. http://dx.doi.org/10.1093/annhyg/meu002	Full-text	Exclude No ammonia-specific data
Lindstrom, I; Suojalehto, H; Pallasaho, P; Luukkonen, R; Karjalainen, J; Lauerma, A; Karjalainen, A. (2013). Middle-Aged Men With Asthma Since Youth The Impact of Work on Asthma. J Occup Environ Med 55: 917-923. http://dx.doi.org/10.1097/JOM.0b013e31828dc9c9	Full-text	Exclude No ammonia-specific data
Mirabelli, MC; London, SJ; Charles, LE; Pompeii, LA; Wagenknecht, LE. (2012). Occupation and three-year incidence of respiratory symptoms and lung function decline: the ARIC Study. Respir Res 13. http://dx.doi.org/10.1186/1465-9921-13-24	Full-text	Exclude No ammonia-specific data
Thilsing, T; Rasmussen, J; Lange, B; Kjeldsen, AD; Al-Kalemji, A; Baelum, J. (2012). Chronic rhinosinusitis and occupational risk factors among 20- to 75-year-old Danes-A GA(2) LEN-based study. Am J Ind Med 55: 1037-1043. http://dx.doi.org/10.1002/ajim.22074	Full-text	Exclude No ammonia-specific data

Table B-4. Electronic screening inclusion terms (and fragments) for ammonia

(gastrointestinal OR gastro-intestinal OR digestive tract OR stomach* OR (gastric AND (mucosa* OR cancer* OR tumor* OR tumour* OR neoplas*)) OR (ammoni*[title] AND intestin*[title or keyword]) OR genotox* OR (genetic* AND toxic*) OR ames assay* OR ames test* OR aneuploid* OR chromosom*[title] OR clastogen* OR cytogen* OR dominant lethal OR genetic*[title] OR genotox* OR hyperploid* OR micronucle* OR mitotic* OR mutagen*[title] OR mutat*[title] OR recessive lethal OR sister chromatid OR ((kidney* OR renal) AND (toxic* OR poisoning OR adverse OR congestion OR calcif*)) OR nephrotox* OR ((spleen* OR splenic) AND (toxic* OR poisoning OR adverse OR congestion OR enlarged)) OR absorption OR distribution OR metabolism[title or

keywords] OR excret* OR PBPK OR toxicokinetic* OR pharmacokin* OR exhal* OR breath OR (expired AND air) OR (respiratory AND (irritation OR symptom* or disease* OR adverse OR chemically induced)) OR lung* OR (pulmonary AND (irritation* OR function*)) OR FVC OR Forced vital capacity OR Forced expiratory volume OR FEV OR FEV1 OR inflammation OR congest* OR edema* OR hemorrhag* OR discharge* OR phlegm* OR cough* OR wheez* OR dyspnea OR bronchitis OR pneumonitis OR asthma* OR nose OR nasal OR throat OR trachea* OR bronchial OR airway* OR (chest AND tightness) OR epithelium* OR epithelia* OR immune OR immun*[title] OR antibod* OR antigen* OR autoimmun* OR cytokine* OR granulocyte* OR interferon OR interleukin* OR leukocyte* OR lymph* OR lymphocyt* OR monocyt* OR immunosupress* OR immunotox* OR (immun* AND toxic*) OR hypersensitivity OR ((dermal OR skin) AND lesion*) OR erythema* OR host resistance OR ((bacterial OR bacteria) AND coloniz*) OR T cell* OR T-Lymphocyte* OR thymocyte* OR ((liver* OR hepatic) AND (function* OR congest* OR toxic* OR poisoning OR adverse)) OR hepatotox* OR fatty liver OR clinical chemistry OR adrenal OR ((heart* OR cardiac) AND (toxic* OR adverse OR poisoning)) OR cardiotox* OR myocardium OR myocardial OR lacrimation OR ocular OR (eye* AND discharge*) OR opacity OR blood pH OR neurotransmitter* OR (amino acid* AND brain) OR reproduct*[title] OR reproductive OR developmental[title or keywords] OR terato* OR (ammoni*[title] AND (abort* OR cleft* OR embryo* OR fertilit* OR fetal OR fetus* OR foetal OR foetus* OR gestation* OR infertilit* OR malform* OR neonat* OR newborn* OR ova OR ovaries OR ovary OR ovum OR perinatal OR placenta* OR postnatal OR pregnan* OR prenatal OR sperm OR sterilit* OR zygote*)) NOT (hyperammon* OR ammonemia OR ammonaemia OR hepatic coma OR liver failure OR (reye AND syndrome) OR ((hepatic OR liver OR portosystemic OR portal-systemic) AND (encephalopathy OR cirrhosis)) OR fish OR fishes OR carp OR catfish OR crayfish OR jellyfish OR daphnia OR shrimp OR frog OR frogs OR amphibians OR bivalve OR bivalves OR clam OR crustacea OR crustaceans)

Table B-5. Disposition of epidemiology studies identified in September 2015 literature search update of core databases

Epidemiology study	Review of full text or abstract?	Disposition
Folletti, I; Zock, JP; Moscato, G; Siracusa, A (2014). Asthma and rhinitis in cleaning workers: a systematic review of epidemiological studies. Journal of Asthma 2014; 51 (1): 18-28.	Full-text	Exclude Review article
Siracusa, A; De Blay, F; Folletti, I; Moscato, G; Olivieri, M; Quirce, S; Raulf-Heimsoth, M; Sastre, J; Tarlo, SM; Walusiak-Skorupa, J; Zock, JP (2013). Asthma and exposure to cleaning products – a European Academy of Allergy and Clinical Immunology task force consensus statement. European Journal of Allergy and Clinical Immunology 2013; 68: 1532-1545.	Full-text	Exclude Review article
Casas, L; Zock, JP; Torrent, M; Garcia-Esteban; Gracia-Lavedan, E; Hyvarinen, A; Sunyer, J (2013). Use of household cleaning products, exhaled nitric oxide and lung function in children. Eur Respir J 2013; 42: 1415-1418.	Full-text	**Include**
Hovland, KH; Skogstad, M; Bakke, B; Skare, O; Skyberg, K (2013). Longitudinal lung function decline among workers in a nitrate fertilizer production plant. International Journal of Occupational and Environmental Health 19 (2): 119-126.	Full-text	Exclude Extremely low ammonia concentrations (maximum concentration of 0.1 mg/m^3) and mandatory respiratory protection. Not expected to be

Epidemiology study	Review of full text or abstract?	Disposition
		informative for evaluating relationships between ammonia exposure and health effects
Hovland, KH; Skogstad, M; Bakke, B; Skare, O; Skyberg, K (2014). Longitudinal decline in pulmonary diffusing capacity among nitrate fertilizer workers. Occupational Medicine 64: 181-187.	Full-text	Exclude No ammonia concentrations reported
Loftus, C; Yost, M; Sampson, P; Torres, E; Arias, G; Vasquez, VB; Hartin, K; Armstrong, J; Tchong-French, M; Vedal, S; Bhatti, P; Karr, C (2015). Ambient Ammonia Exposures in an Agricultural Community and Pediatric Asthma Morbidity. Epidemiology 26 (6): 794-801.	Full-text	**Include**
Nemer, M; Sikkeland, LIB; Kasem, M; Kristensen, P; Nijem, K; Bjertness, E; Skare, O; Bakke, B; Kongerud, J; Skogstad, M (2015). Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study. Occup Environ Med 72: 428-434.	Full-text	**Include**
Ulvestad, B; Lund, MB; Bakke, B; Thomassen, Y; Ellingsen, DG (2014). Short-term lung function decline in tunnel construction workers. Occup Environ Med 72: 108-113.	Full-text	Exclude No analysis of ammonia-specific exposures; confounding of respiratory effects by silica exposure

Table B-6. Evaluation of epidemiology studies summarized in Table 1-2 (industrial settings/respiratory measures)

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
Respiratory symptoms						
Nemer et al. (2015)	Palestine; laboratory at Hebron University; cross-sectional study of female hairdressers in 13 hair salons from 10/2012–03/2013 Exposed: n = 33 nonsmoking female hairdressers (age 19–50 yrs; mean 38 yrs); selected from a cohort of 200 hairdressers studied previously (every sixth participant from a list sorted by salon name was invited to participate) Controls: n = 35; nonsmoking female students from Hebron University (n = 27) and staff (n = 8); age of all controls 18–49 yrs, mean 24 yrs; recruited through advertisements	Ammonia air concentrations measured in 13 salons using an electrochemical sensor instrument (direct reading device) affixed to one hairdresser in each salon; sample duration 45–305 mins; concentration range 0–202 mg/m^3; duration variation due to the variation in the number of customers serviced Limited specificity for measuring ammonia compared to other gases	Modified version of a standardized respiratory questionnaire from the American Thoracic Society, including questions on respiratory symptoms (chest tightness, shortness of breath, coughing, wheezing, phlegm production during the past 12 months and doctors' diagnosed asthma)	Other hair salon exposures known to cause irritation, inflammation or other respiratory effects (such as persulfates) were not measured Factors potentially predicting ammonia exposure, including size of salon, number of hairdressers at work and number of customers, tasks being done (coloring, bleaching, cutting, spraying), were evaluated No adjustment for smoking since all participants were non-smokers	Statistical software was used to calculate arithmetic means and standard deviations for exposure data and outcome variables	Device used for exposure measurements had limited specificity for measuring ammonia relative to other gases (potential false positives from other gases); potential selection bias in control group due to differences in recruitment (self-selected based on interest in the study) or workload; small sample size and only a single measurement of ammonia at each salon (which may not have been representative of salon exposures)
Rahman et al. (2007)	Bangladesh, urea fertilizer factory; cross sectional study Exposed: n = 88 (24 ammonia plant workers and 64 urea plant workers) Controls: n = 25 Exposed: production operators in ammonia (low exposure; 24 out of 63 workers participated)[a] and urea (high exposure, 64 out of 77 workers participated)[b] plants, 5–9 out of 15–19 per shift selected. Excluded if planned to have less than a four-hour work day. Mean	Personal airborne levels of ammonia exposure by two direct-reading methods: Dräger diffusion tube and Dräger PAC III monitoring instrument[c]; 1 worker per day per measure. Correlation between methods; r = 0.80, but higher absolute values (by four to fivefold) using Dräger diffusion tubes[c] Concentrations based on	Respiratory symptoms (5 point scale for severity over last shift), based on Optimal Symptom Score Questionnaire)	Nitrogen dioxide (measured by Drager tubes) was below detection limit in all areas (urea plant, ammonia plant and administration area); other workplace exposures not assessed. Exposure analysis adjusted for current smoking and duration	Fisher's exact test; repeated excluding 33 current smokers or workers with history of previous respiratory disease	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect Differences in exposure measurement methods (Dräger diffusion tube and Dräger PAC III monitoring instrument) considered limitation for quantitation of exposure-response

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	age ~40 yrs, mean duration ~18 yrs; never smoked ~52%. Controls: from administration building, 4–7 per day over 5 days selected. Mean age ~43 yrs, mean duration ~16 yrs; never smoked ~72%.	PAC III monitoring: Low-exposure group (ammonia plant): 6.9 ppm (4.9 mg/m^3) High-exposure group (urea plant): 26.1 ppm (18.5 mg/m^3)				relationship but not a limitation for hazard identification due to uncertainty in the absolute value, but not the relative ranking, of exposure
Ballal et al. (1998)	Saudi Arabia; two urea fertilizer factories; cross sectional study; all males Exposed: n = 161 Factory A: n = 84 Factory B: n = 77 Controls: n = 355 Exposed: 20% of workers selected (systematic sample representing different workplaces using payroll lists); 100% participation rate. Mean age 30 yrs, mean duration 51.8 months; never smoked ~59%. Controls: administrative staff from other companies in the area (same sampling system as exposed); participation rate 100%. Mean age 34 yrs, mean duration 73 months; never smoked ~49%.	Area monitors (3 sets in each work section taken at least 3 months apart, mean 16 measures per set); spectrophotometric absorption measure. Computed geometric mean concentration per section and cumulative ammonia concentration (a function of both exposure intensity and duration of service) assigned to each worker.	Prevalence of respiratory symptoms and conditions based on the British Medical Research Council questionnaire	Authors stated no other pollutants in workplace. Stratified or adjusted for smoking	Contingency tables (stratified by smoking); logistic regression of exposure measures, adjusted for duration, smoking (yes, no)	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect
Holness et al. (1989)	Canada, sodium carbonate (soda ash) production plant; cross sectional study Exposed: n = 58 Controls: n = 31 Exposed: 52 of 64 available production workers (82%) and 6 maintenance workers; all males. Mean age 39 yrs, mean duration 14.4 yrs, nonsmokers ~29%. Controls from stores and office workers in the plant; excluded if previous ammonia exposure. Participation rate not reported. Mean age 43 yrs, mean duration	Airborne levels of ammonia (mean = 6.5 mg/m^3 for exposed; mean = 0.2 mg/m^3 for controls) using NIOSH-recommended protocol for personal sampling and analysis (measured over one work-shift per person, mean 8.4 hours)	Prevalence of self-reported symptoms and conditions obtained through questionnaire based on American Thoracic Society questionnaire	Adjusted for smoking (pack-yrs); other workplace exposures not assessed, but study authors note high level of control of exposures in the plant	Comparison between groups by logistic regression. Also analyzed by three categories of exposure.	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect Relatively small sample size—potential of not being able to detect a difference between controls and exposed when one might exist Low exposure concentrations—potential

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	12.2 yrs; nonsmokers ~39%. Indication of self-selection of exposed out of workplace based on atopy (lower prevalence of hay fever).					that an effect level may not have been reached
Lung function						
Nemer et al. (2015)	Palestine; laboratory at Hebron University; cross-sectional study of female hairdressers in 13 hair salons from 10/2012–03/2013 Exposed: n = 33 nonsmoking female hairdressers (age 19–50 yrs; mean 38 yrs); selected from a cohort of 200 hairdressers studied previously (every sixth participant from a list sorted by salon name was invited to participate) Controls: n = 35; nonsmoking female students from Hebron University (n = 27) and staff (n = 8); age of all controls 18–49 yrs, mean 24 yrs; recruited through advertisements	Ammonia air concentrations measured in 13 salons using an electrochemical sensor instrument (direct reading device) affixed to one hairdresser in each salon; sample duration 45–305 mins; concentration range 0–202 mg/m^3; duration variation due to the variation in the number of customers serviced Limited specificity for measuring ammonia compared to other gases	Lung function test performed according to American Thoracic Society/European Respiratory Standards guidelines using a PC spirometer; data adjusted for age, height, and body mass index	Other hair salon exposures known to cause irritation, inflammation or other respiratory effects (such as persulfates) were not measured Factors potentially predicting ammonia exposure, including size of salon, number of hairdressers at work and number of customers, tasks being done (coloring, bleaching, cutting, spraying), were evaluated No adjustment for smoking since all participants were non-smokers	Linear regression used to assess the relationship between ammonia exposure and lung function	Device used for exposure measurements had limited specificity for measuring ammonia relative to other gases (potential false positives from other gases); potential selection bias in control group due to differences in recruitment (self-selected based on interest in the study) or workload; small sample size and only a single measurement of ammonia at each salon (which may not have been representative of salon exposures)
Rahman et al. (2007)	Bangladesh, urea fertilizer factory; cross sectional study Exposed: n = 88 (24 ammonia plant workers and 64 urea plant workers); production operators in ammonia (low exposure; 24 out of 63 workers participated)[a] and urea (high exposure, 64 out of 77 workers participated)[b] plants, 5–9 out of 15–19 per shift selected. Excluded if planned to have less than a four-hour work day. Mean age ~40 yrs, mean duration ~18	Personal airborne levels of ammonia exposure by two direct-reading methods: Dräger diffusion tube and Dräger PAC III monitoring instrument[c]; 1 worker per day per measure. Correlation between methods; r = 0.80, but higher absolute values (by four- to fivefold) using Dräger diffusion tubes.[c] Concentrations based on	Spirometry by standard protocol, beginning and end of shift	Nitrogen dioxide (measured by Dräger tubes) was below detection limit in all areas (urea plant, ammonia plant, and administration area); other workplace exposures not assessed. Exposure analysis adjusted for current smoking and duration	Paired t-tests compared cross shift differences in lung function within and between plants; analyses repeated excluding workers with previous respiratory diseases. Multiple linear regression analyzed exposure level and change in lung function for n = 23 with both concurrent measure	Study population and design: "healthy" workers; long duration–potential for lack of complete ascertainment of effect Differences in exposure measurement methods (Dräger diffusion tube and Dräger PAC III monitoring instrument) considered limitation for quantitation of exposure-response

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	yrs; never smoked ~52%	PAC III monitoring: Low-exposure group (ammonia plant): 6.9 ppm (4.9 mg/m^3) High-exposure group (urea plant): 26.1 ppm (18.5 mg/m^3)				relationship but not a limitation for hazard identification due to uncertainty in the absolute value, but not the relative ranking, of exposure
Ali et al. (2001)	Saudi Arabia; urea fertilizer factory; cross sectional study (appears to be same as Factory A in Ballal et al. (1998) Exposed: n = 73 Controls: n = 348 Exposed: 20% of workers selected (systematic sample representing different workplaces using payroll lists); 95% participation rate. Mean age 30 yrs, mean duration 51.8 months; nonsmokers ~49%. Controls: administrative staff from 4 industrial groups (same sampling system as exposed); participation rate 98%. Mean age 34 yrs; nonsmokers ~42%	Ammonia concentration in air determined by sampling pump with a flow rate of 1 L/min for 4 hours for each measurement and spectrophotometry (i.e., by absorption techniques and comparison to a standard). Computed cumulative ammonia concentration (a function of both exposure level and duration of service) assigned to each worker, dichotomized to high and low at 50 mg/m^3-yrs	Spirometry by standard protocol, morning measurement, 3 or more replicates	Stratified by smoking status	T-tests and Chi-square tests for comparisons between groups and by exposure level among exposed	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect
Bhat and Ramaswamy (1993)	Mangalore; fertilizer chemical plant; cross sectional study Exposed: n = 91 Controls: n = 68 Exposed: 30 urea plant workers, 30 DAP plant workers, and 31 ammonia plant workers; sex of workers not reported; age, sex, height, weight, and duration of exposure were recorded but not reported; duration of exposure dichotomized into two groups (up to 10 yrs and more than 10 yrs); smokers excluded. Controls: people having comparable body surface area chosen from the same socio-	No measurement of exposure made	Spirometry by standard protocol, 3 replicates with highest reading retained for calculation	All smokers excluded from study; other workplace exposures not assessed	Paired t-test for comparisons between exposed and controls	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	economic status and sex; smokers excluded; no other information provided on participant selection.					
Holness et al. (1989)	Canada, sodium carbonate (soda ash) production plant; cross sectional study Exposed: n=58 Controls: n=31 Exposed: 52 of 64 available production workers (82%) and 6 maintenance workers; all males, mean age 39 yrs, mean duration 14.4 yrs; nonsmokers ~29%. Controls from stores and office workers in the plant; excluded if previous ammonia exposure. Participation rate not reported. Mean age 43 yrs, mean duration 12.2 yrs; nonsmokers ~39%. Indication of self-selection of exposed out of workplace based on atopy (lower prevalence of hay fever)	Airborne levels of ammonia (mean = 6.5 mg/ m^3 for exposed; mean = 0.2 mg/m^3 for controls) using NIOSH-recommended protocol for personal sampling and analysis (measured over one work-shift per person, mean 8.4 hours)	Spirometry by standard protocol, beginning and end of shift, 3–6 replicates, each worker measured on two test days	Adjusted for smoking (pack-yrs); other workplace exposures not assessed	Baseline lung function compared between groups using linear regression, adjusting for age, height, and pack-yrs (linear regression). Unpaired t-tests compared change in lung function over workshift between groups. Percent predicted lung function at baseline and change in lung function also analyzed by three categories of exposure	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect Relatively small sample size—potential of not being able to detect a difference between controls and exposed when one might exist Low exposure concentrations—potential that an effect level may not have been reached
Sputum, exhaled NO (eNO) and blood parameters						
Nemer et al. (2015)	Palestine; laboratory at Hebron University; cross-sectional study of female hairdressers in 13 hair salons from 10/2012–03/2013 Exposed: n = 33 nonsmoking female hairdressers (age 19–50 yrs; mean 38 yrs); selected from a cohort of 200 hairdressers studied previously (every sixth participant from a list sorted by salon name was invited to participate) Controls: n = 35; nonsmoking female students from Hebron University (n = 27) and staff (n = 8); age of all controls 18–49 yrs, mean	Ammonia air concentrations measured in 13 salons using an electrochemical sensor instrument (direct reading device) affixed to one hairdresser in each salon; sample duration 45–305 mins; concentration range 0–202 mg/m^3; duration variation due to the variation in the number of customers serviced Limited specificity for measuring ammonia	Sputum collected; total cell count and cell viability; differentiate cell counts Exhaled NO (eNO) measured using the NIOX MINO device (flow rate 50 mL/s), in accordance with manufacturer's protocol and American Thoracic Society recommendations;	Other hair salon exposures known to cause irritation, inflammation or other respiratory effects (such as persulfates) were not measured Factors potentially predicting ammonia exposure, including size of salon, number of hairdressers at work and number of customers, tasks being done (coloring, bleaching, cutting, spraying), were evaluated	Median regression used to compare inflammatory cell levels in the sputum, eNO levels, and blood parameters between hairdressers and control group	Device used for exposure measurements had limited specificity for measuring ammonia relative to other gases (potential false positives from other gases); potential selection bias in control group due to differences in recruitment (self-selected based on interest in the study) or workload; small sample size and only a single measurement of ammonia at each salon (which may not have been

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	24 yrs; recruited through advertisements	compared to other gases	eNO data adjusted for height and age Blood samples analyzed for a complete blood count; blood parameters adjusted for body mass index and age	No adjustment for smoking since all participants were non-smokers		representative of salon exposures)

[a]Ammonia plant workers checked temperature, pressure, and concentration of ammonia and checked the pumps, prepared solutions, and checked the revolutions per minute of various motors. These are considered the low-exposure group.

[b]Urea plant workers purged solution and washed pipelines, operated various pumps, and washed and cleaned the cooling fluidized bed in the production area. These are considered the high-exposure group.

[c]Based on communication with technical support at Dräger Safety Inc. (Bacom and Yanosky, 2010), the U.S. Environmental Protection Agency (U.S. EPA) considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, more confidence is attributed to the PAC III air measurements of ammonia for the Rahman et al. (2007) study.

Table B-7. Evaluation of epidemiology studies summarized in Table 1-3 (use in cleaning/disinfection settings)

Reference	Study setting/ participant selection	Exposure measure	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
Casas et al. (2013)	Menorca, Spain. Population based cross sectional birth cohort study; recruitment during pregnancy; 432 infants were enrolled; 295 individuals completed the 10-year follow up visit and the cleaning products questionnaire and performed the FeNO and/or lung function test 35% of recruited population were excluded because information on use of cleaning products and/or respiratory tests not available 46 individuals reported use of ammonia	Interviewer-led questionnaire on the frequency of use of 10 different cleaning products (bleach, ammonia, polishes or waxes, acids, solvents, furniture sprays, glass cleaning sprays, degreasing sprays, air freshening sprays and air freshening plug-ins). The means of the reported days of use per week (never = 0, <1 day per week = 0.5, 1–3 days per week = 2 and 4–7 days per week = 5.5) for each product were summed providing a score ranging from 0 (no exposure) to 55 (exposed to all 10 products used 4–7 days per week).	Questionnaires on wheezing, asthma, treatment and allergies were administered by mother from birth to age 10; at age 10–13 FeNO and lung function tests were carried out	Models adjusted for sex, age, maternal education, parental smoking indoors, asthma medication, season of respiratory test measurement, and height and weight (lung function measurement only) Measurements of indoor volatile organic compounds or home inspections were not performed	Multivariate linear regression models were developed for FeNO, FVC and FEV_1 to predict log-transformed FeNO concentration and non-transformed levels of FVC and FEV_1	Sample size was relatively small (n=46 for ammonia use) and may have limited power; exposure to cleaning products was assessed by parental report; over-reporting the use of cleaning products or changes in behavior related to their use was possible
Dumas et al. (2012)	France. Nested case-control study of adult asthma cases recruited from pulmonary clinics in 1991–1995; follow-up in 2003–2007. Drawn from the Epidemiological study on the Genetics and Environment in Asthma (EGEA) study (included first degree relatives of cases and population control group). Study base = 1,355: included if had occupation data, excluded if asthma at baseline or and missing data on smoking. Selected if ever worked in hospital (exposure group) and referent group Hospital workers: 179 (43 men, 136 women) Referent group: 545 (212 men, 333 women)	Exposure to specific agents based on three methods (ever exposed, based on all jobs held at least 3 months): • Self-report: two job exposure questionnaire modules for health care workers (including frequency of use of specific products) [possible underestimate of exposure] • Expert assessment – hospital workers (probability, frequency, intensity; 18 products) • Asthma-specific job exposure matrix (22 agents) with expert review	Asthma attack, respiratory symptoms or asthma treatment in the last 12 months (based on standardized questionnaire)	Adjusted for age and smoking status. Additional adjustment for body mass index tested. Association with ammonia stronger than that seen with bleach (OR 1.87 and 0.93, respectively, for ammonia and bleach)	Products analyzed if 5 or more exposed cases. Analyses stratified by sex (small sample size for men so focused on women). Familial dependence in data accounted for by generalized estimating equations	

Table B-7. Evaluation of epidemiology studies summarized in Table 1-3 (use in cleaning/disinfection settings)

Reference	Study setting/ participant selection	Exposure measure	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	Smoking history and age similar for women; smoking history similar for men (but mean age approximately 5 yrs higher in hospital workers) Possible "healthy worker" bias, with underestimation of associations from movement out of jobs or avoidance of specific jobs by affected individuals	Control group: "Never exposed to cleaning/disinfecting products" based on each of the methods described above, plus expert review of additional (broader) information from main occupation questionnaire				
Arif and Delclos (2012)	United States (Texas). Survey of 3,650 licensed health care professionals (physicians, nurses, respiratory therapists, occupational therapists). Response rate 66% (3,650 out of 5,600)	For longest job held: frequency of use of specific products (never/once a month, at least once a week, more than once a day, every day) (for 2,049 of the 3,650, current/most recent job was longest held job) For all jobs: ever been in contact with list of 28 products at least once a month for a period of 6 months or longer (ammonia part of general cleaning factor in factor analysis)	• Four outcomes, based on structured questionnaire • Work Related Asthma Symptoms (WRAS): wheezing/whistling at work or shortness of breath at works that gets better away from work or worse at work • Work Related Asthma (WRA): same as above and physician-diagnosed asthma (n = 74) • Work exacerbated asthma (WEA): onset before began work (n = 41) • Occupational asthma (OA): onset after began work (n = 33)	Adjusted for age, sex, race/ethnicity, body mass index, seniority, atopy and smoking status.	Multinomial logistic regression with four asthma outcome categories: WRAS, WEA, OA and none. Oversampling nurses and physicians was accounted for with post-stratification weights	Limited exposure assessment (i.e., "ever exposed")
Lemiere et al. (2012)	Quebec. Case-control study. Workers with work-related asthma (WRA) seen at two tertiary care centers; WRA based on specific inhalation challenges (SIC); reversible airflow limitation or airway hyper-responsiveness (provocative concentration of methacholine)	Structured interview about last/current job (including job title, tasks, machines, materials), work environment, protective equipment. This information used in conjunction with other material (e.g., technical and material safety data sheets,	• Diagnoses made based on reference tests • Occupational asthma (OA) if specific inhalation challenge test was positive (n = 67); • Work exacerbated asthma (WEA) if specific	Assessed confounding effects of age, smoking, occupational exposure to heat, cold, humidity, dryness and physical strain; not included in final models because none acted as	Logistic regression	

Table B-7. Evaluation of epidemiology studies summarized in Table 1-3 (use in cleaning/disinfection settings)

Reference	Study setting/ participant selection	Exposure measure	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	inducing a 20% fall in FEV_1 equal or lower than 8 mg/ml. Controls: Non-work related asthma (NWRA) seen at same clinics but symptoms did not worsen at work. Total n = 153 (33 controls, 120 work related asthma)	occupational hygiene literature, data bases and web sites) for expert review and classification of exposure to 41 specific agents, blinded to case status. Semiquantitative estimate (low=1, medium=2, high=3) for intensity, frequency, and confidence.	inhalation test was negative but symptoms worsened at work (n = 53)	confounders of exposures under study		
Vizcaya et al. (2011)	Barcelona, Spain Survey of 1,018 cleaning services to find companies willing to participate; 286 (28%) not eligible (no longer in business); 37 agreed to participate (n workers ranged from 6 to >1,000). 4,993 questionnaires distributed by company representatives to employees; 950 (19%) completed; 33 excluded because of missing data. Total n = 917. Two companies completed non-responder survey (sex, age, nationality, job position); no major differences with responders. Selection bias unlikely	Standardized questionnaire about cleaning tasks and products used in the last year Reference group = never cleaners AND current cleaners who had not used bleach, degreasers, multi-purpose cleaners, glass cleaners, perfumed products, air fresheners, mop products, hydrochloric acid, ammonia, polishes or waxes, solvents, or carpet cleaners in the last year	• Current asthma based on structured questionnaire (in past 12 months, woken by an attack of shortness of breath, had an attack of asthma or currently taking any asthma medications (including inhalers, aerosols or tablets) • Asthma score: Sum of "yes" answers to five questions on asthma symptoms in last 12 months (wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest, attack of shortness of breath after exercise, woken by attack of shortness of breath)	Adjusted for age, country of birth (Spanish vs non-Spanish), sex, and smoking status	Asthma: logistic regression Asthma score: Negative binomial regression (to account for over-dispersion in the data)	Exposure assessment limited (use in past year; no frequency data)
Zock et al. (2007)	Europe (22 sites in 10 countries). Longitudinal study. Random population sample, ages 20–44 yrs (the European Community Respiratory Health Survey), 9-yr follow-up period. Excluded 764 individuals with asthma at baseline. Analysis limited to	At follow-up, standardized interview about use of 15 cleaning products in the home (frequency never, <1 day/week, 1 to 3 days/week, 4 to 7 days/week)	• Incident (since baseline survey) current asthma, defined by asthma attack or nocturnal shortness of breath in the past 12 months or current use of medication for asthma	Adjusted for sex, age, smoking, employment in a cleaning job during follow-up, and study center; heterogeneity by center also assessed. Correlations among	Incident asthma and wheeze: log-binomial regression Incident physician diagnosed asthma: Cox proportional	Referent group included some exposure (to the product, and to other products); could underestimate risk; although it is an incident study, the exposure

Table B-7. Evaluation of epidemiology studies summarized in Table 1-3 (use in cleaning/disinfection settings)

Reference	Study setting/ participant selection	Exposure measure	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
	individuals reporting doing the cleaning or washing in their home (n = 3,503).	Reference group: did not use the product or used <1 day/week	• Incident physician-diagnosed asthma, defined as above with confirmation by a physician and information on age or date of first attack • Incident (since baseline survey) current wheeze, defined as wheezing or whistling in the chest in last 12 months when not having a cold.	products generally weak (Spearman rho < 0.3)	hazards regression, with date on onset defined as reported date of first attack. Referent category = used product never or <1 day/week	information was collected at follow-up so may not reflect pre-disease patterns (if practices changed because of symptoms) or could be influenced by knowledge of outcome
Medina-Ramón et al. (2006)	Cornellà, Spain. Two-week diary and pulmonary function study, 2001–2002. Female domestic cleaners aged 31–66 yrs with a history of obstructive lung disease, recruited from participants in a nested case–control based on population survey from 2000–2001 (see Medina-Ramón et al. (2005), below). Selected if reported current asthma symptoms or chronic bronchitis in 2000–2001 survey (standard definitions). Excluded if illiterate or unable to complete diary (n = 57). 80 met eligibility criteria; 51 (64%) completed diary. Participants and non-participants similar except for higher prevalence of bronchial hyperresponsiveness and shorter duration of domestic cleaning employment among responders	2-week diary recorded daily use of cleaning products and cleaning tasks (checklist of cleaning exposures, number of hours cleaning in each house).	• Respiratory symptoms based on 2-week daily diary (7 symptoms, 5 point intensity scale); summed score for upper respiratory symptoms (blocked nose, throat irritation, watery eyes) and lower respiratory symptoms (chest tightness, wheezing, shortness of breath and cough). • PEF measured with mini-Wright peak flow meter (with training and written instructions); measured morning, lunchtime, night (3 measurements each; highest recorded). • Occupational asthma based on analysis of PEF patterns by occupational asthma system (OASYS)	Adjusted for respiratory infection, use of maintenance medication and age; daily number of cigarettes smoked, yrs of employment in domestic cleaning and/or weekly working hours in domestic cleaning also assessed and included as necessary	Respiratory symptom scores dichotomized as > and <2 for use in logistic regression. PEF analysis based on night time and the next morning values; linear regression	Pulmonary function measured by participant; validation of method not reported. Potential for knowledge of exposure to affect reporting of symptoms

Table B-7. Evaluation of epidemiology studies summarized in Table 1-3 (use in cleaning/disinfection settings)

Reference	Study setting/ participant selection	Exposure measure	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding potential major limitations
Medina-Ramón et al. (2005)	Cornellà, Spain. Nested case-control study in 2001–2002 of 650 cleaning workers drawn from population-based survey in 2000–2001, 4,521 women ages 30–65 yrs. Cases: 160 identified, 117 still employed in domestic cleaning, 87 (74%) agreed to participate, 40 met final case definition Controls: 386 identified, 281 still employed in domestic cleaning, 194 (69%) agreed to participate, 155 met final control definition	Job-specific questionnaire for cleaning workers, frequency of use of 22 specific products (times per week, month, or yr); summed across each home and personal home and divided into two groups (cut-point = 12 times per yr). Also assessed accidental exposures (e.g., spills) Measurements taken in 10 cleaning sessions to obtain data on exposure to chlorine and ammonia during specific tasks and with specific products (ammonia used in kitchen cleaning; median 0.6–6.4 ppm; peaks >50 ppm)	Case based on asthma and/or bronchitis at both assessments. Asthma = asthma attack or being woken by attack or shortness of breath in past 12 months. Chronic bronchitis = regular cough or regular bringing up phlegm for at least 3 months each year. Controls: no history of respiratory symptoms in preceding year and no asthma at either assessment	Correlations among tasks/products reported to be generally weak (but specific values for ammonia and other products not reported). Multivariate model adjusted for age tertile and smoking status (but results for ammonia in this model only reported as "not statistically significant"—no information on effect estimate/variability)	Logistic regression	Results of adjusted model not reported in detail, but confounding unlikely major factor if correlations weak

Table B-8. Evaluation of epidemiology study summarized in Table 1-6 (industrial setting/serum chemistry measures)

Reference	Study setting/ participant selection	Exposure parameters	Outcome measured	Consideration of confounding	Statistical analysis	Comments regarding major limitations
Abdel Hamid and El-Gazzar (1996)	Egypt, urea fertilizer production plant; cross sectional study. Exposed: n = 30 Controls: n = 30 Exposed: workers selected randomly (process not described). Mean age 36 yrs, mean duration 12 yrs. Controls from administrative departments with no known history of ammonia exposure; matched to exposed by age, educational status, and socioeconomic status. Mean age 35 yrs	No direct measurement of ammonia exposure; blood urea was used as a surrogate measure (ammonia is detoxified mainly through the formation of urea in the liver) Mean (± SD) mg/dl ($p < 0.01$) Exposed: 31.9 (± 7.6) Controls: 20.3 (± 5.1) The reliability of blood urea and correlation with ammonia exposure not reported	Fasting blood sample for AST, ALT (measures of liver function), hemoglobin, catalase enzyme activity as mediator of cell membrane permeability and serum monoamine oxidase enzyme activity as mediator of effects on nervous system	No information on exposure to other contaminants; no information on smoking status	Type of statistical test not reported (EPA assumes to be t-test); data presented as group means ± SD, with *p*-value.	Study population and design: "healthy" workers; long duration—potential for lack of complete ascertainment of effect Lack of information on smoking, and alcohol use—potential for possible confounding for liver function measures; uncertain effect on enzyme measures

ALT = alanine aminotransferase; AST = asparate aminotransferase; SD = standard deviation

APPENDIX C. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ANALYSIS

C.1. TOXICOKINETICS

C.1.1. Absorption

Inhalation Exposure

A study in volunteers[1] indicated that ammonia is almost completely retained in the nasal mucosa (83–92%) during short-term acute exposure (i.e., up to 120 seconds) over a wide exposure range (40–354 mg/m^3) (Landahl and Herrmann, 1950). Longer-term acute exposure (10–27 minutes) to 354 mg/m^3 ammonia resulted in lower retention (4–30%), with expired breath concentrations of 247–283 mg/m^3 observed by the end of the exposure period (Silverman et al., 1949), suggesting saturation of absorption into the nasal mucosa. Nasal and pharyngeal irritation, but not tracheal irritation, suggests that ammonia is retained in the upper respiratory tract. Unchanged levels of blood urea nitrogen (BUN), nonprotein nitrogen, urinary urea, and urinary ammonia following these acute exposures are evidence of low absorption into the blood.

Data in rabbits and dogs provide supporting evidence for high-percentage nasal retention, resulting in a lower fraction of the inhaled dose reaching the lower respiratory tract (Egle, 1973; Dalhamn, 1963; Boyd et al., 1944). Continuous exposure of rats to ammonia at concentrations up to 23 mg/m^3 for 24 hours did not result in statistically significant increases in blood ammonia levels, whereas exposures to 219–818 mg/m^3 resulted in significantly increased blood concentrations of ammonia within 8 hours of exposure initiation, indicating a potential for systemic absorption of inhaled ammonia (Schaerdel et al., 1983).

Gastrointestinal Contributions to Systemic Ammonia

Ammonia as NH_4^+ is endogenously produced in the human intestines through the use of amino acids as an energy source (glutamine deamination) (Taylor and Curthoys, 2004; Mcfarlane Anderson et al., 1976) and by bacterial degradation of nitrogenous compounds from ingested food (Romero-Gómez et al., 2009). About 99% of the ammonia produced in the intestines is systemically absorbed. Evidence suggests that fractional absorption of ammonia increases as the lumen pH increases, and that transport occurs at lower pH levels (absorption has been detected at a pH as low as 5) (Castell and Moore, 1971; Mossberg and Ross, 1967). NH_4^+ absorbed from the gastrointestinal tract travels via the hepatic portal vein directly to the liver where, in healthy individuals, most of it is converted to urea and glutamine.

C.1.2. Distribution

The range of mean ammonia concentrations in humans as a result of endogenous production was reported as 0.1–0.6 µg/mL in arterial blood and 0.2–1.7 µg/mL in venous blood (Huizenga et al., 1994). More recent sources provide values for the normal range of blood ammonia of 0.1–0.8 µg/ml (venous blood) and 0.15–0.45 µg/ml.[2] Given its importance in amino acid metabolism, the urea cycle, and acid-base balance, ammonia is homeostatically regulated to remain at low concentrations in the blood. At normal physiological blood pH, 98.3% of total ammonia is present as NH_4^+, and 1.7% as NH_3 (Weiner and Verlander, 2013).[3]

Ammonia is present in fetal circulation. In vivo studies in several animal species and in vitro studies of human placenta suggest that ammonia is produced within the uteroplacenta and released into the fetal and maternal circulations (Bell et al., 1989; Johnson et al., 1986; Hauguel et al., 1983; Meschia et al., 1980; Remesar et al., 1980; Holzman et al., 1979; Holzman et al., 1977; Rubaltelli and Formentin, 1968; Luschinsky, 1951). Jóźwik et al. (2005) reported that ammonia levels in human fetal blood (specifically umbilical arterial and venous blood) at birth were 1.0–1.4 µg/mL, compared to 0.5 µg/mL in the mothers' venous blood. DeSanto et al. (1993) similarly collected human umbilical arterial and venous blood at delivery and found that umbilical arterial ammonia concentrations (0.51–5.9 µg/mL) from 15–17 caesarian section deliveries, intended to better represent in utero values were significantly higher than venous concentrations (0.43–5.13 µg/mL). There was no correlation between umbilical ammonia levels and gestational age (range of 25–43 weeks of gestation; vaginal and cesarean section deliveries). In sheep, the uteroplacental tissue is the main site of ammonia production, with outputs of ammonia into both the uterine and umbilical circulations (Jóźwik et al., 1999). In late-gestation pregnant sheep that were catheterized to allow measurement of ammonia exposure to the fetus, concentrations of ammonia in umbilical arterial and venous blood and uterine arterial and venous blood ranged from approximately 0.39 to 0.60 µg/mL (Jóźwik et al., 2005; Jóźwik et al., 1999).

Ammonia is present in human breast milk as one of the sources of nonprotein nitrogen (Atkinson et al., 1980).

Little information on the distribution of inhaled ammonia was found in the available literature. Information on the distribution of endogenously produced ammonia suggests that any ammonia absorbed through inhalation would be distributed to all body compartments via the blood, where it would be used in protein synthesis as a buffer, reduced to normal concentrations by urinary excretion, or converted by the liver to glutamine and urea (Takagaki et al., 1961). Rats inhaling 212 mg/m^3 ammonia 6 hours/day for 15 days exhibited increased blood ammonia (200%) and brain glutamine (28%) levels at 5 days of exposure, but not at 10 or 15 days (Manninen et al., 1988), demonstrating transient distribution of ammonia to the brain.

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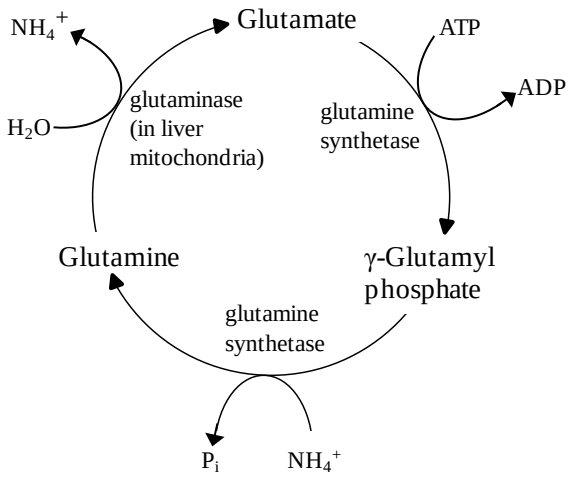
[2]University of Rochester Medical Center, Health Encyclopedia: Ammonia. https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=167&ContentID=ammonia, accessed 1/19/2016, and U.S. National Library of Medicine. Medline Plus. Ammonia blood test. https://www.nlm.nih.gov/medlineplus/ency/article/003506.htm, accessed 1/19/2016.

[3]The relative amounts of NH_4^+ and NH_3 are determined by pH. For every 0.3 pH unit change, the amount of NH_3 changes in parallel by 100% (i.e., at pH 7.70, total ammonia present as NH_3 is 3.4%, and at pH 7.10, 0.85%). The amount of NH_4^+ changes in the opposite direction by an equivalent absolute amount (decreases 1.7% to 96.7% at pH 7.70, and increases 0.85% to 99.15% at pH 7.10) (Weiner and Verlander, 2013).

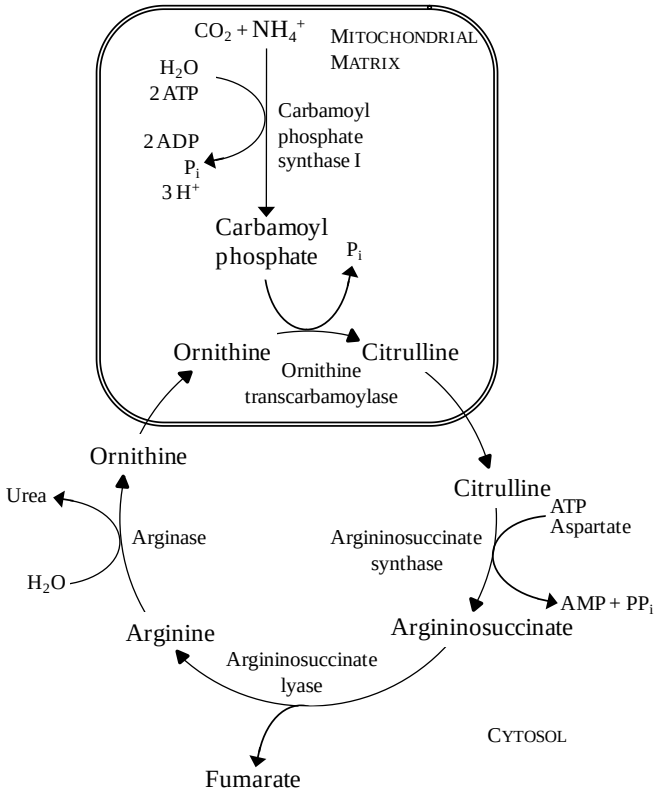
C.1.3. Metabolism/Endogenous Production of Ammonia

Ammonia is produced endogenously by catabolism of amino acids by glutamate dehydrogenase or glutaminase primarily in the liver, renal cortex and intestines, but also in the brain and heart (Souba, 1987). In skeletal muscle, ammonia may be produced by metabolism of amino acids or adenosine monophosphate via adenylate deaminase. Ammonia is metabolized to glutamine via glutamine synthetase in the glutamine cycle (Figure C-1), or incorporated into urea as part of the urea cycle as observed in the hepatic mitochondria and cytosol (Figure C-2) (Nelson and Cox, 2008) before entering the systemic circulation. Van de Poll et al. (2008) reported that the liver removes an amount of ammonia from circulation equal to the amount added by the intestines at metabolic steady state, indicating that the gut does not contribute significantly to systemic ammonia release. However, when hepatic function is disrupted (see Section 1.3.2, Susceptible Populations and Lifestages), intestine-derived ammonia may reach the systemic circulation (Van de Poll et al., 2008; Romero-Gómez et al., 2004).



Adapted from: Nelson and Cox (2008).

Figure C-1. Glutamine cycle.



Adapted from: Nelson and Cox (2008).

Figure C-2. The urea cycle showing the compartmentalization of its steps within liver cells.

Ammonia generated in the renal proximal tubule cells can be eliminated via the kidneys (Weiner and Verlander, 2013; Kim, 2009). While renal elimination via the kidney is a major contributor to ammonia homeostasis, the kidneys are themselves a source of ammonia. Renal ammonia is derived from the utilization of glutamate as an energy source by the renal proximal tubule cells and in the maintenance of the acid-base balance (Weiner and Verlander, 2013; Kim, 2009). The fact that the sum of urinary ammonia and renal vein ammonia substantially exceeds renal arterial ammonia delivery (Weiner and Verlander, 2011) indicates that that the kidney adds ammonia to the body. This is demonstrated in studies of patients with renal artery stenosis where the concentrations of ammonia in the renal vein are slightly higher than those in systemic circulation (Olde-Damink et al., 2002).

Ammonia can also be produced in the gastrointestinal tract. The enzymatic activity of glutaminase, which produces ammonia, is high in the gastrointestinal tract. Such enzymatic activity in the small intestines is approximately fourfold that found in the large intestine mucosa (James et al., 1998). While bacterial content of the gut may contribute to circulating levels of ammonia, results from studies with germ-free animals suggest that hyperammonemia can be produced without bacterial involvement (Nance and Kline, 1971; Warren and Newton, 1959).

In addition to the production of endogenous ammonia from the liver, kidneys and intestine, exercising skeletal muscle liberates ammonia by deamination of adenosine monophosphate. Ammonia

produced from the skeletal muscle is also effectively incorporated into glutamine in these cells before entering the circulation (Huizenga et al., 1996). Under conditions of prolonged exercise, skeletal muscle may derive as much as 10% of its energy from amino acid metabolism (Graham and MacLean, 1992).

Given its important metabolic role, ammonia exists in a homeostatically regulated equilibrium in the body. In particular, free ammonia has been shown to be homeostatically regulated to remain at low concentrations, with 95–98% of body burden existing in the blood (at physiological pH) as NH_4^+ (da Fonseca-Wollheim, 1995; Souba, 1987). Two studies in rats (Manninen et al., 1988; Schaerdel et al., 1983) provide evidence that exposure to environmental ammonia at concentrations ≤18 mg/m^3 do not measurably alter blood ammonia concentrations. Schaerdel et al. (1983) exposed rats to ammonia for 24 hours at concentrations of 11, 23, 219, or 818 mg/m^3. Exposure to 11 and 23 mg/m^3 ammonia did not statistically significantly increase blood ammonia concentrations after 24 hours; the difference between pre- and postexposure concentrations ranged from −0.58 to 0.006 millimoles/L whole blood. Concentrations ≥219 mg/m^3 caused an exposure-released increase in blood ammonia, but blood ammonia levels at 12- and 24-hour sampling periods were lower than at 8 hours (increase at 8 hours of 0.192–0.244 millimoles/L compared to pre-exposures levels), suggesting compensation by increasing ammonia metabolism. Any changes in blood gas (pO_2, pCO_2, pH) and liver microsomal activity (ethylmorphine-N-demethylase, cytochrome P450) were small and not associated with environmental ammonia concentrations, suggesting no measureable effect of short-term environmental ammonia exposure on the parameters measured in this study. In rats inhaling 18 mg/m^3 ammonia 6 hours/day for 5, 10, or 15 days (Manninen et al., 1988), blood ammonia levels (0.021–0.057 millimoles/L) were not statistically significantly different from controls (0.032–0.043 millimoles/L). Rats inhaling 212 mg/m^3 exhibited statistically significantly increased levels of blood ammonia (threefold) at 5 days of exposure, but not at 10 or 15 days. Brain ammonia levels did not differ from controls at either exposure concentration. Blood glutamine (at 212 mg/m^3) and brain glutamine (at 18 and 212 mg/m^3) on day 5 was increased over control, but were no longer elevated on days 10 and 15. The return of blood ammonia and blood and brain glutamine levels to control levels within days is consistent with metabolic adaptation, and these data suggest that animals have the capacity to handle high concentrations of inhaled ammonia.

Various disease states can affect the rate of glutamine uptake and catabolism and thereby affect the blood and tissue levels of ammonia. Acute renal failure can result in increased renal glutamine consumption and ammonia production with a decreased capability of eliminating urea in the urine (Souba, 1987). Abnormally elevated levels of breath ammonia (and corresponding increases in plasma urea) are indicative of end-stage renal failure (Davies et al., 1997). Both acute (e.g., fulminant hepatitis) and chronic (e.g., end-stage liver failure; hepatic cirrhosis) liver disease may result in decreased ureagenesis and increased levels of ammonia in blood (hyperammonemia), leading to increased uptake into the brain and the onset of hepatic encephalopathy. The increased metabolic alkalosis associated with hepatic encephalopathy may result in a shift in the NH_4^+/NH_3 ratio in the direction of ammonia, which may pass through the blood-brain barrier more effectively than ammonium (Katayama, 2004). In patients with liver cirrhosis and acute clinical hepatic encephalopathy, the mean net metabolic flux of [^{13}N]-ammonia from the blood into the brain was three- to fivefold higher in patients with cirrhosis than healthy controls; cerebral trapping of ammonia was primarily attributable to increased blood ammonia (Keiding et al., 2010; Keiding

et al., 2006). Sørensen et al. (2009) demonstrated greater unidirectional clearance of ammonia from the blood to brain cells than metabolic clearance of ammonia from the blood both in healthy controls and in cirrhotic patients with and without hepatic encephalopathy.

C.1.4. Elimination

Ammonia is excreted by the kidneys as urea. Elimination of ammonia in the kidney involves specific proteins mediating transport of NH_3 and NH_4^+. For example, in the proximal tubule the apical Na^+/H^+ exchanger, NHE-3, preferentially secretes NH_4^+. The Rhesus glycoproteins, Rh B glycoprotein (Rhbg) and Rh C glycoprotein (Rhcg), are ammonia transporters in the distal tubule and collecting duct (Weiner and Verlander, 2011; Bishop et al., 2010; Lee et al., 2010; Lee et al., 2009; Han et al., 2006; Handlogten et al., 2005). Angiotensin II is one of the factors that modulates ammonia release from renal proximal tubule cells (Nagami and Warech, 1992; Chobanian and Julin, 1991). Diseases and conditions that increase angiotensin II may thus increase production and decrease elimination of ammonia (Agroyannis et al., 1998).

Ammonia is also eliminated through the skin through sweat production or possibly due to direct diffusion of systemic plasma NH_4^+ (Schmidt et al., 2013).

Additionally, ammonia is eliminated in the expired air of all humans (Manolis, 1983). Exhalation serves as a clearance mechanism. Several investigators specifically measured ammonia in breath exhaled from the nose (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977) (Solga et al., 2013). Smith et al. (2008) reported median ammonia concentrations of 0.059–0.078 mg/m^3 in exhaled breath from the nose of three healthy volunteers (with samples collected daily over a 4-week period); these concentrations were similar to or slightly higher than the mean laboratory air level of ammonia reported in this study of 0.056 mg/m^3. In another study of 20 health volunteers, the mean ammonia concentration in exhaled breath from the nose was 0.032 mg/m^3 (range: 0.0092–0.1 mg/m^3) (Schmidt et al., 2013). Larson et al. (1977) reported that the median concentration of ammonia collected from air samples exhaled from the nose ranged from 0.013 to 0.046 mg/m^3. One sample collected from the trachea via a tube inserted through the nose of one subject was 0.029 mg/m^3—a concentration within the range of that found in breath exhaled through the nose (Larson et al., 1977). Solga et al. (2013) reported 0.682 mg/m^3 ammonia in expired breath of a single subject during "mouth-closed breathing."

Higher and more variable ammonia concentrations are reported in breath exhaled from the mouth or oral cavity than in breath exhaled from the nose. In studies that reported ammonia in breath samples from the mouth or oral cavity, ammonia concentrations were commonly found in the range of 0.085–2.1 mg/m^3 (Schmidt et al., 2013; Solga et al., 2013; Smith et al., 2008; Španěl et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977), and strongly correlated with saliva pH (Schmidt et al., 2013). These higher concentrations are largely attributed to the production of ammonia by bacterial degradation of food protein in the oral cavity or gastrointestinal tract (Turner et al., 2006; Smith et al., 1999; Vollmuth and Schlesinger, 1984). This source of ammonia in breath was demonstrated by Smith et al. (1999), who observed elevated ammonia concentrations in the expired air of six healthy volunteers following the ingestion of a protein-rich meal.

Other factors that can affect ammonia levels in breath exhaled from the mouth or oral cavity include diet, oral hygiene, age, living conditions, and disease state. Norwood et al. (1992) reported decreases in baseline ammonia levels (0.085–0.905 mg/m^3) in exhaled breath following tooth brushing (<50% depletion), a distilled water oral rinse (<50% depletion), and an acid oral rinse (80–90% depletion). Solga et al. (2013) similarly reported decreased ammonia in the expired breath of a single subject following rinses with water, hydrogen peroxide, and Coca Cola, and an increase with Mylanta, which has a basic pH. These findings are consistent with ammonia generation in the oral cavity by bacterial and/or enzymatic activity. Several investigators have reported that ammonia in breath from the mouth and oral cavity increases with age (Španěl et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003), with ammonia concentrations increasing on average about 0.1 mg/m^3 for each 10 years of life (Španěl et al., 2007a). Turner et al. (2006) reported that the age of the individual accounts for about 25% of the variation observed in mean breath ammonia levels, and the remaining 75% is due to factors other than age. Certain disease states can also influence ammonia levels in exhaled breath. Ammonia is greatly elevated in the breath of patients in renal failure (Španěl et al., 2007a; Davies et al., 1997). These studies are further described in Table C-1.

Because ammonia measured in samples of breath exhaled from the mouth or oral cavity can be generated in the oral cavity and may thus be substantially influenced by diet and other factors, ammonia levels measured in mouth or oral cavity breath samples do not likely reflect systemic (blood) levels of ammonia. Ammonia concentrations in breath exhaled from the nose appear to better represent levels at the alveolar interface of the lung and are thought to be more relevant to understanding systemic levels of ammonia (Schmidt et al., 2013; Smith et al., 2008). That said, the amount of ammonia that equilibrates between the endogenous lung metabolic pool and alveolar air is likely to be small even under hyperammonemic conditions. In a study that measured the amount of label in exhaled air of anesthetized rats administered an intravenous dose of [^{13}N]ammonia (Cooper and Freed, 2005), trace amounts of label could be detected in the expired breath over a five minute period, whereas approximately 30% of the administered dose passed through the lungs within seconds, with most of the blood-derived ammonia in the rat lung incorporated into glutamine.

In evaluating measures of ammonia in expired air, it is important to recognize that ammonia in ambient air is the source of some of the ammonia in exhaled breath. Studies of ammonia in exhaled breath (see Table C-1) were conducted in environments with measureable levels of ambient (exogenous) ammonia rather than in ammonia-free environments, and it has been established that concentrations of certain trace compounds in exhaled breath are correlated with their ambient concentrations (Španěl et al., 2013). Španěl et al. (2013) determined that the concentration of ammonia in inhaled breath could account for approximately 70% of the ammonia in exhaled breath. It is likely that ammonia concentrations in exhaled breath, and particularly from the nose, would be lower if the inspired air were free of ammonia.

Ammonia has also been detected in the expired air of animals. Whittaker et al. (2009) observed a significant association between ambient ammonia concentrations and increases in exhaled ammonia in stabled horses. Analysis of endogenous ammonia levels in the expired air of rats showed concentrations of 0.007–0.250 mg/m^3 (mean = 0.06 mg/m^3) (Barrow and Steinhagen, 1980). Larson et al. (1980) reported

ammonia concentrations measured in the larynx of dogs exposed to sulfuric acid ranging between 0.02 and 0.16 mg/m^3 following mouth breathing and between 0.04 and 0.16 mg/m^3 following nose breathing.

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Breath samples from the nose and trachea					
Single test subject (no information on age, health status)	Subject exhaled into breath sampler for at least 10 seconds while maintaining constant exhalation flow rate of 50 mL/s (maintained via orifice in breath sampler). Ammonia in exhaled breath measured for open and closed mouth breathing and with a water rinse (closed breathing only). 11 breath collections over 10 consecutive work days.	Mean pre-rinse baseline concentration of breath ammonia: Mouth closed: 0.682 (+/- 0.315) mg/m^3 Post-rinse (water) concentration of breath ammonia: Mouth closed: 0.119 (+/- 0.062) mg/m^3	Continuous wave (CW) distributed feedback quantum cascade laser (DFB-QCL) based sensor coupled to breath sampling device measuring both mouth pressure and real-time concentration of carbon dioxide	Rinsing the mouth with water significantly lowered the amount of breath ammonia exhaled.	Solga et al. (2013)
20 healthy volunteers (13 males and 7 females aged 22–61 yrs)	Subjects fasted overnight and refrained from exercise in the morning before sampling; samples collected between 8 and 11 AM; end-tidal breath samples collected from the nose; subjects breathed continuously into the sampling piece for 3–5 min to obtain stable sample; samples also collected after an acidic mouth wash	Concentrations in exhaled breath from the nose (mg/m^3): Range = 0.0092–0.10 Mean = 0.032 (95% CI: 0.021–0.042) Median = 0.024 Concentrations following acidic mouth wash (mg/m^3): Range = 0.011–0.027 Mean = 0.016 (95% CI: 0.014–0.018) Median = 0.015	Commercial cavity ring-down spectrometer	Ammonia concentrations in outdoor air were down to 0.0004 g/m^3, in indoor air were 0.002–0.004 mg/m^3, and in indoor air in the presence of humans were 0.006–0.007 mg/m^3	Schmidt et al. (2013)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Three healthy male volunteers (>30 yrs of age)	Ammonia levels measured in nose-exhaled breath of test subjects each morning about 2 hrs after eating a regular breakfast; samples collected daily over a 4-wk period	Volunteer A = 0.0728 ± 0.000848 mg/m^3 Volunteer B = 0.0777 ± 0.000919 mg/m^3 Volunteer C = 0.0587 ± 0.000848 mg/m^3 (median ammonia levels estimated as geometric mean ± geometric SD)	SIFT-MS analysis	Mean ambient air level of ammonia was 0.056 ± 0.0071 mg/m^3 The authors indicated that ammonia measured in mouth-exhaled breath may be generated in the oral cavity and suggested that concentrations in nose-exhaled breath may better represent systemic conditions (such as metabolic disease)	Smith et al. (2008)
Sixteen healthy subjects (9 males aged 25–63 yrs and 7 females aged 23–41 yrs); subgroups tested were all male	Breath samples collected during quiet nose breathing, and direct sampling during a deep inspiration followed by breath-holding with the glottis closed	Ammonia concentrations ranged from 0.013 to 0.046 mg/m^3 during nose breathing (median 0.025 mg/m^3) (five male subjects), and 0.029 mg/m^3 from an air sample collected from the trachea (collected from a tube inserted into one male subject's nose and into the trachea)	Chemiluminescence		Larson et al. (1977)
Breath samples from the mouth and oral cavity					
Single test subject (no information on age, health status)	Subject exhaled into breath sampler for at least 10 seconds while maintaining constant exhalation flow rate of 50 mL/s (maintained via orifice in breath sampler). Ammonia in exhaled breath measured for open and closed mouth breathing and with different rinses (water, hydrogen peroxide, Mylanta, Coca Cola). 11 breath collections over 10 consecutive work days.	Mean pre-rinse baseline concentration of breath ammonia: Mouth open: 0.719 (+/- 0.291) mg/m^3 Post-rinse (water) concentration of breath ammonia: Mouth open: 0.121 (+/- 0.057) mg/m^3	Continuous wave (CW) distributed feedback quantum cascade laser (DFB-QCL) based sensor coupled to breath sampling device measuring both mouth pressure and real-time concentration of carbon dioxide	Rinsing the mouth with water and the two acidic rinses significantly lowered the amount of breath ammonia exhaled (by ~ 50–75%). The basic rinse, Mylanta, significantly increased breath ammonia (by ~40%). In trails with different rinses, the study subject breathed without direction as to the mode of breathing; EPA assumed that this included mouth breathing.	Solga et al. (2013)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
20 healthy volunteers (13 males and 7 females aged 22–61 yrs)	Subjects fasted overnight and refrained from exercise in the morning before sampling; samples collected between 8 and 11 AM; end-tidal breath samples collected from the mouth; subjects breathed continuously into the sampling piece for 3–5 min to obtain stable sample; samples also collected after an acidic mouth wash	Concentrations in exhaled breath from the mouth (mg/m^3): Range = 0.28–1.5 Mean = 0.55 (95% CI: 0.42–0.68) Median = 0.49 Concentrations following acidic mouth wash (mg/m^3): Range = 0.010–0.027 Mean = 0.015 (95% CI: 0.014–0.018) Median = 0.015	Commercial cavity ring-down spectrometer	Ammonia concentrations in outdoor air were down to 0.0004 mg/m^3, in indoor air were 0.002–0.004 mg/m^3, and in indoor air were 0.006–0.007 mg/m^3	Schmidt et al. (2013)
Three healthy male volunteers (>30 yrs of age)	Ammonia levels measured in mouth-exhaled breath and in the closed mouth cavity of test subjects each morning about 2 hrs after eating a regular breakfast; samples collected daily over a 4-wk period	Via mouth: Volunteer A = 0.769 ± 0.000919 mg/m^3 Volunteer B = 0.626 ± 0.000919 mg/m^3 Volunteer C = 0.604 ± 0.000919 mg/m^3 Via oral cavity: Volunteer A = 1.04 ± 0.000990 mg/m^3 Volunteer B = 1.52 ± 0.00106 mg/m^3 Volunteer C = 1.31 ± 0.000919 mg/m^3 (median ammonia levels estimated as geometric mean ± geometric SD)	SIFT-MS analysis	Mean ambient air level of ammonia was 0.056 ± 0.0071 mg/m^3 The authors indicated that ammonia measured in mouth-exhaled breath may be generated in the oral cavity and suggested that concentrations in nose-exhaled breath may better represent systemic conditions (such as metabolic disease)	Smith et al. (2008)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Four healthy children (two males and two females, 4–6 yrs old) Thirteen senior volunteers (11 males and 2 females, 60–83 yrs old); four had type-2 diabetes mellitus with onset at ages between 50 and 70 yrs, and controlled by diet All subjects had their regular breakfast without any specific restrictions	Breath samples collected in morning at least 1 hr after breakfast and at least 1 hr prior to lunch; each volunteer performed two exhalation/inhalation cycles (both about 5–10 sec in duration)	Children = range 0.157–0.454 mg/m^3 Seniors = 0.224–1.48 mg/m^3	SIFT-MS analysis	Ammonia breath levels significantly increased with age Some seniors reported diabetes Measured ammonia level in breath reported for each subject	Španěl et al. (2007a)
Twenty-six secondary school students (10 males and 16 females, 17–18 yrs old and one 19-yr-old)	Three sequential breath exhalations collected over 5 min following the students listening to a 1-hr presentation (at least 1 hr following breakfast and before lunch); alveolar portion measured (identified using humidity)	Median values reported for: 17-yr-olds = 0.165 mg/m^3 18-yr-olds = 0.245 mg/m^3	SIFT-MS analysis	Significant differences in ammonia levels in exhaled breath between 17- and 18-yr-olds ($p < 10^{-8}$) were reported (statistical test not reported)	Španěl et al. (2007b)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Thirty healthy volunteers (19 males and 11 females, 24–59 yrs, 28 Caucasian, 1 African, and 1 mixed race); volunteers were instructed to maintain their normal daily routines and to not rinse out their mouths prior to providing a breath sample	Breath samples collected in the morning prior to lunch at approximately weekly intervals for about 6 mo; some volunteers provided samples more frequently than others; 480 samples collected and analyzed for ammonia	Geometric mean and geometric SD = 0.589 ± 0.00114 mg/m^3 Median = 0.595 mg/m^3 Range = 0.175–2.08 mg/m^3	SIFT-MS analysis	Ammonia breath levels were shown to increase with age Background levels in the testing laboratory were typically around 0.28 mg/m^3	Turner et al. (2006)
Five subjects (two females, three males; age range 27–65 yrs)	Breath samples collected between 8 and 9 AM in three sequential breath exhalations on multiple days (12–30 d) over the course of a month	Ammonia concentrations were 0.298–1.69 mg/m^3	SIFT-MS analysis	Differences in ammonia breath levels between individuals were significant ($p < 0.001$; ANOVA test)	Diskin et al. (2003)
Six normal nonsmoking male volunteers (24–61 yrs old), fasted for 12 hrs prior to testing	Baseline breath sample obtained; breath samples collected 20, 40, and 60 min and 5 hrs following the ingestion of a liquid protein-calorie meal	Premeal levels were 0.2–0.4 mg/m^3; Postmeal levels at 30 min were 0.1 mg/m^3 increasing to maximum values at 5 hrs of 0.4–1.3 mg/m^3	SIFT-MS analysis	A biphasic response in breath ammonia concentration was observed after eating	(Smith et al., 1999)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Fourteen healthy, nonsmoking subjects (age range 21–54 yrs) performed one or more of the following hygiene maneuvers: (1) acidic oral rinse (pH 2.5) (2) tooth brushing followed by acidic oral rinse (3) tooth brushing followed by distilled water rinse (4) distilled water rinse	Subjects fasted for 8 hrs prior to baseline measurement, refrained from oral hygiene after their most recent meal, refrained from heavy exercise for 12 hrs, and had no liquid intake for several hours; initial breath ammonia was measured between 8 and 10 AM, then subjects performed one or more of the hygiene measures listed (at 30-min intervals for a total 90-min period; samples collected over 5 min)	Baseline levels varied from 0.085 to 0.905 mg/m^3	Nitrogen oxide analyzer with an ammonia conversion channel (similar to chemi-luminescence)	An 80–90% depletion of volatile ammonia emissions was seen within 10 min of acid rinsing; <50% depletion of ammonia was seen following tooth brushing or distilled water rinse; gaseous ammonia levels increased after all rinse procedures over time	Norwood et al. (1992)
Sixteen healthy subjects (nine males aged 25–63 yrs and seven females aged 23–41 yrs); subgroups tested were all male	Breath samples collected during quiet mouth breathing	Ammonia concentrations ranged from 0.029 to 0.52 mg/m^3 during mouth breathing (median of 0.17 mg/m^3)	Chemiluminescence	The oral cavity appears to be a source of breath ammonia; no attempt was made to control the diet of subjects or standardize the interval between the last meal and the measurement	Larson et al. (1977)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Breath samples: source (nose/mouth/oral cavity) not specified					
Sixteen healthy, nonsmoking subjects (4 females and 12 males, 29 ± 7 yrs); no significant differences in mean age, height, weight, BMI, or time since last oral intake; 10 subjects tested in each experiment	Experiment 1: single whole-breath samples collected from each subject (same samples immediately reanalyzed within <10 sec to assess instrument specific variability) Experiment 2: three repeat breath samples collected from each subject (to evaluate intra-subject differences); this experiment evaluated differences based on standardization of expiratory pressure and flow Experiment 3: two mixed breath samples and two bag alveolar breath samples collected in short succession from each subject	Experiment 1: 0.843 ± 0.0601 mg/m^3 (median ± measurement error) Experiment 2: Nonstandardized = 0.712 ± 0.130 mg/m^3 (median ± SD) Standardized = 1.01 ± 0.113 mg/m^3 (median ± SD) Experiment 3: Mixed = 0.860 ± 0.585 mg/m^3 (median ± SD) Alveolar = 0.920 ± 0.559 mg/m^3 (median ± SD)	SIFT-MS analysis This study established that SIFT-MS analysis is reliable and repeatable	Relatively small number of healthy subjects used Did not address the breath of those with disease Intra- and inter-day repeatability were not investigated	Boshier et al. (2010)

Table C-1. Ammonia levels in exhaled breath of volunteers

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Eight healthy subjects (average age 39.8 ± 9.6 yrs)	Subjects fasted for 6 hrs prior to samples being collected; subjects breathed normally into collection device for 5 min	Mean breath ammonia = 0.35 ± 0.17 mg/m^3	Fiber optic sensor	This study measured ammonia levels in healthy volunteers compared to *Helicobacter pylori* positive individuals (five subjects) (data not shown); the experiment also included a challenge with a 300 mg urea capsule to evaluate the urease activity of healthy versus infected individuals (data not shown); the authors concluded that breath ammonia measurement may be feasible as a diagnostic test for *H. pylori*	Kearney et al. (2002)
Three groups of children were used as test subjects: (1) 68 asthmatic children residing in a National Park in the mountains (mean age 10 yrs, 48 boys, 20 girls) (2) 52 asthmatic children in an urban area (mean age 9 yrs, 35 boys, 17 girls) (3) 20 healthy children from the same urban area as a control group (mean age 10 yrs, 12 boys, 8 girls)	Subjects performed a 5-sec breath-hold and exhaled slowly into collection device	Asthmatic children from National Park = 0.0040 ± 0.0033 mg/m^3 Asthmatic urban children: Mean NH_3 = 0.0101 ± 0.00721 mg/m^3 Urban children control group: Mean NH_3 = 0.0105 ± 0.00728 mg/m^3	Chemiluminescence	Both groups of asthmatic children had some subjects on glucocorticoids, often combined with histamine antagonists and/or b2 agonists, while others were left untreated; ammonia concentrations in exhaled breath appeared to be correlated with exposure to urban air	Giroux et al. (2002)

ANOVA = analysis of variance; BMI = body mass index; CI = confidence interval; SD = standard deviation; SIFT-MS = selected ion flow tube mass spectrometry

Physiologically Based Pharmacokinetic Models

No physiologically based pharmacokinetic models have been developed for ammonia. An expanded one-compartment toxicokinetic model in rats was developed by Diack and Bois (2005), which used physiological values to represent first-order uptake and elimination of inhaled ammonia (and other chemicals). The model is not useful for dose-response assessment of ammonia because: (1) it cannot specify time-dependent amounts or concentrations of ammonia in specific target tissues, (2) it has not been verified against experimental data for ammonia, glutamate, or urea levels in tissues, and (3) it does not support extrapolation of internal doses of ammonia between animals and humans.

C.2. HUMAN STUDIES

More detailed summaries are provided of epidemiology studies of workers in industrial exposure settings that examined respiratory parameters; information from these studies was used as the basis for the RfC.

C.2.1. Occupational Studies in Industrial Worker Populations

Holness et al. (1989)

Holness et al. (1989) conducted a cross-sectional study of workers in a soda ash (sodium carbonate) plant[4] who had chronic, low-level exposure to ammonia. The cohort consisted of 58 workers and 31 controls from stores and office areas of the plant. All workers were males (average age 43 years), and the average exposure duration for the exposed workers at the plant was 12 years. The mean time-weighted average (TWA) ammonia exposure of the exposed group based on personal sampling over one work shift (mean sample collection time 8.4 hours) was 9.2 ppm (6.5 mg/m^3) compared to 0.3 ppm (0.2 mg/m^3) for the control group. The average concentrations of ammonia to which workers were exposed were determined using the procedure recommended by the National Institute for Occupational Safety and Health (NIOSH), which involves the collection of air samples on sulfuric acid-treated silica gel adsorption tubes (NIOSH, 1979).

No statistically significant differences were observed in age, height, years worked, percentage of smokers, or pack-years smoked for exposed versus control workers. Exposed workers weighed approximately 8% ($p < 0.05$) more than control workers. Information regarding past occupational exposures, working conditions, and medical and smoking history, as well as respiratory symptoms and eye and skin complaints was obtained by means of a questionnaire that was based on an American Thoracic Society questionnaire (Ferris, 1978). Each participant's sense of smell was evaluated at the beginning and end of the work week using several concentrations of pyridine (0.4, 0.66, or 10 ppm). Lung function tests were conducted at the beginning and end of the work shift on the first and last days of their work week (four tests administered). Differences in reported symptoms and lung function between groups were evaluated using the actual exposure

[4]At this plant, ammonia, carbon dioxide, and water were the reactants used to form ammonium bicarbonate, which in turn was reacted with salt to produce sodium bicarbonate and subsequently processed to form sodium carbonate. Ammonia and carbon dioxide were recovered in the process and reused.

values with age, height, and pack-years smoked as covariates in linear regression analysis. Exposed workers were grouped into three exposure categories (high = >12.5 ppm [>8.8 mg/m^3], medium = 6.25–12.5 ppm [4.4–8.8 mg/m^3], and low = <6.25 ppm [<4.4 mg/m^3]) for analysis of symptom reporting and lung function data.

Endpoints evaluated in the study included sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, skin problems, and lung function parameters (forced vital capacity [FVC], forced expiratory volume in 1 second [FEV_1], FEV_1/FVC, forced expiratory flow [FEF_{50}], and FEF_{75}). No statistical differences in the prevalence of respiratory irritation or eye irritation were evident between the exposed and control groups (Table C-2).

There was a statistically significant increase ($p < 0.05$) in the prevalence of skin problems in workers in the lowest exposure category (<4.4 mg/m^3) compared to controls; however, the prevalence was not increased among workers in the two higher exposure groups. Workers also reported that exposure at the plant had aggravated specific symptoms including coughing, wheezing, nasal complaints, eye irritation, throat discomfort, and skin problems. Odor detection threshold and baseline lung functions were similar in the exposed and control groups. No changes in lung function were demonstrated over either work shift (days 1 or 2) or over the work week in the exposed group compared with controls. No relationship was demonstrated between chronic ammonia exposure and baseline lung function changes either in terms of the level or duration of exposure. Study investigators noted that this finding was limited by the lack of adequate exposure data collected over time, precluding development of a meaningful index accounting for both level and length of exposure. Based on the lack of exposure-related differences in subjective symptomatology, sense of smell, and measures of lung function, EPA identified the high-exposure category (≥8.8 mg/m^3) as the no-observed-adverse-effect level (NOAEL). A lowest-observed-adverse-effect level (LOAEL) was not identified for this study.

Table C-2. Symptoms and lung function results of workers exposed to different levels of TWA ammonia concentrations

Parameter	Ammonia concentration			
	Control 0.2 mg/m³	Exposed <4.4 mg/m³	Exposed 4.4–8.8 mg/m³	Exposed >8.8 mg/m³
Symptom				
Cough	3/31 (10)[a]	6/34 (18)	1/12 (8)	2/12 (17)
Sputum	5/31 (16)	9/34 (26)	3/12 (25)	1/12 (8)
Wheeze	3/31 (10)	5/34 (15)	1/12 (8)	0/12 (0)
Chest tightness	2/31 (6)	2/34 (6)	0/12 (0)	0/12 (0)
Shortness of breath	4/31 (13)	3/34 (9)	1/12 (8)	0/12 (0)
Nasal complaints	6/31 (19)	4/34 (12)	2/12 (17)	0/12 (0)
Eye irritation	6/31 (19)	2/34 (6)	2/12 (17)	1/12 (8)
Throat irritation	1/31 (3)	2/34 (6)	1/12 (8)	1/12 (8)
Skin problems	2/31 (6)	10/34* (29)	1/12 (8)	1/12 (8)
Lung function (% predicted)				
FVC	98.6	96.7	96.9	96.8
FEV_1	95.1	93.7	93.9	95.3
FEF_{50}	108.4	106.9	106.2	111.2
FEF_{75}	65.2	71.0	67.8	78.8

[a]Number affected/number examined. The percentage of workers reporting symptoms is indicated in parentheses.

*Significantly different from controls, $p < 0.05$, by Fisher's exact test performed for this review.

Source: Holness et al. (1989).

Ballal et al. (1998)

Ballal et al. (1998) conducted a cross-sectional study of male workers at two urea fertilizer factories in Saudi Arabia[5]. The cohort consisted of 161 exposed subjects (84 from factory A and 77 from factory B) and 355 unexposed controls. Workers in factory A were exposed to air ammonia levels of 2–130 mg/m³, and workers in factory B were exposed to levels of 0.02–7 mg/m³. Mean duration of employment was 51.8 months for exposed workers and 73.1 months for controls. Exposure levels were estimated by analyzing a total of 97 air samples collected over 8-hour shifts close to the employee's work site. The prevalence of respiratory symptoms and diseases was determined by administration of a questionnaire. The authors stated that there were no other chemical pollutants in the workplace that might have affected the respiratory system. Smoking habits were similar for exposed workers and controls.

In factory A, the relative risks for respiratory symptoms (cough, phlegm, wheezing, dyspnea) were elevated in smokers, whereas in factory B, all relative risks were nonsignificant. The

[5]The process of fertilizer production involved synthesis of ammonia from natural gas, followed by reaction of the ammonia and carbon dioxide to form ammonium carbamide, which was then converted to urea.

prevalence rate of hemoptysis (coughing up blood) was higher in factory A (RR = 4.1, 95% CI 1.63–10.28) than factory B (RR = 0.47, 95% CI 0.06–3.66), although chest roentgenograms showed no specific pulmonary changes. Stratifying the workers by ammonia exposure levels (above or below the American Conference of Governmental Industrial Hygienists [ACGIH] threshold limit value [TLV] of 18 mg/m^3) showed that those exposed to ammonia concentrations higher than the TLV had 2.2- to 4-fold higher relative risks for cough, phlegm, wheezing, dyspnea, and asthma than workers exposed to levels below the TLV (Table C-3). The relative risk for wheezing was also elevated among those exposed to ammonia levels at or below the TLV. Distribution of symptoms by cumulative ammonia concentration (CAC, mg/m^3-years) also showed 2- to 4.8-fold higher relative risk for all of the above symptoms among those with higher CAC (Table C-3). Results of the logistic regression analysis showed that ammonia concentration was significantly related to cough, phlegm, wheezing with and without shortness of breath, and asthma (Table C-4).

Table C-3. The prevalence of respiratory symptoms and disease in urea fertilizer workers exposed to ammonia

Respiratory symptom/disease	Relative risk (95% CI)			
	Exposure category		CAC[a] (mg/m^3-yrs)	
	≤ACGIH TLV (18 mg/m^3) (n = 138)	>ACGIH TLV (18 mg/m^3) (n = 17)	≤50 (n = 130)	>50 (n = 30)
Cough	0.86 (0.48–1.52)	3.48 (1.84–6.57)	0.72 (0.38–1.35)	2.82 (1.58–5.03)
Wheezing	2.26 (1.32–3.88)	5.01 (2.38–10.57)	1.86 (1.04–3.32)	5.24 (2.85–9.52)
Phlegm	0.79 (0.43–1.47)	3.75 (1.97–7.11)	0.63 (0.31–1.26)	3.03 (1.69–5.45)
Dyspnea	1.13 (0.62–2.04)	4.57 (2.37–8.81)	1.19 (0.66–2.17)	2.59 (1.25–5.36)
Chronic bronchitis	1.43 (0.49–4.19)	2.32 (0.31–17.28)	0.61 (0.13–2.77)	5.32 (1.72–16.08)
Bronchial asthma	1.15 (0.62–2.15)	4.32 (2.08–8.98)	1.22 (0.66–2.28)	2.44 (1.10–5.43)
Chronic bronchitis and bronchial asthma	2.57 (0.53–12.59)	6.96 (0.76–63.47)	1.82 (0.31–10.77)	8.38 (1.37–45.4)

[a] = one missing value

Source: Ballal et al. (1998).

Table C-4. Logistic regression analysis of the relationship between ammonia concentration and respiratory symptoms or disease in exposed urea fertilizer workers

Respiratory symptom/disease	OR (95% CI)
Cough	1.32 (1.08–1.62)*
Phlegm	1.36 (1.10–1.67)*
Shortness of breath with wheezing	1.26 (1.04–1.54)*
Wheezing alone	1.55 (1.17–2.06)*
Dyspnea on effort	0.83 (0.68–1.02)
Diagnosis of asthma	1.33 (1.07–1.65)*

*$p \leq 0.05$.

OR = odds ratio

Source: Ballal et al. (1998).

Ali et al. (2001)

Results from limited spirometry testing of workers from factory A were reported in a followup study (Ali et al., 2001). The lung function indices measured in 73 ammonia workers and 348 control workers included FEV_1 and FVC. Prediction equations for these indices were developed for several nationalities (Saudis, Arabs, Indians, and other Asians), and corrected values were expressed as the percentage of the predicted value for age and height. Workers with cumulative exposure >50 mg/m^3-years had significantly lower FEV_1% predicted (7.4% decrease, $p < 0.006$) and FVC% predicted (5.4% decrease, $p \leq 0.030$) than workers with cumulative exposure ≤50 mg/m^3-years. A comparison between symptomatic and asymptomatic exposed workers showed that FEV_1% predicted and FEV_1/FVC% were significantly lower among symptomatic workers (9.2% decrease in FEV_1% predicted, $p < 0.001$, and 4.6% decrease in FEV_1/FVC%, $p < 0.02$).[6]

Rahman et al. (2007)

[6] Table 3 of Ali et al. (2001) provided a comparison of pulmonary function indices for exposed workers and controls. FVC% predicted was statistically significantly higher than the control group; FEV_1% predicted and FEV_1/FVC% were not. Based on comparison of values for exposed workers in Tables 3, 4, and 5 of the paper, EPA concluded that the value for FVC% in the exposed group (Table 3) was likely an error. FVC% predicted for all exposed workers (n = 73) in Table 3 was 105.65. Tables 4 and 5 provided values for FVC% predicted for exposed workers subdivided two different ways: (1) exposed workers with cumulative exposures ≤50 mg/m^3-years (105.64; n = 45) and >50 mg/m^3-year (100.22; n = 28) (Table 4), and (2) exposed workers that were symptomatic (102.23; n = 33) and asymptomatic (104.58) (n = 40) (Table 5). The values for the exposed workers when subdivided (either by cumulative exposure or by presence/absence of symptoms) should bracket the FVC% predicted value for all exposed workers (105.65). This was not the case in either instance. Two of the authors of the study were contacted (email from Dr. H.O. Ahmed to S. Rieth, U.S. EPA, on May 14, 2013; email from Dr. S.G. Ballal to S. Rieth, U.S. EPA, on May 9, 2013); both reported that the original data were no longer available. Given concerns about the pulmonary function values in Table 3, only evidence from Tables 4 and 5 of the Ali et al. (2001) study were considered in this assessment.

Rahman et al. (2007) conducted a cross-sectional study of workers at a urea fertilizer factory in Bangladesh that consisted of an ammonia plant and a urea plant. The exposed group consisted of 24 participants of the 63 operators in the ammonia plant and 64 participants of the 77 operators in the urea plant; 25 individuals from the administration building served as a control group. Mean duration of employment exceeded 16 years in all groups. Personal ammonia exposures were measured by two different methods (Dräger PAC III and Dräger tube) in five to nine exposed workers per day for 10 morning shifts in the urea plant (for a total of 64 workers) and in five to nine exposed workers per day for 4 morning shifts from the ammonia plant (for a total of 24 workers). Four to seven volunteer workers per day were selected from the administration building as controls, for a total of 25 workers over a 5-day period. Questionnaires were administered to inquire about demographics, past chronic respiratory disease, past and present occupational history, smoking status, respiratory symptoms (cough, chest tightness, runny nose, stuffy nose, and sneezing), and use of protective devices. Lung function tests (FVC, FEV_1, and peak expiratory flow rate [PEFR]) were administered preshift and postshift (8-hour shifts) to the 88 exposed workers after exclusion of workers who had planned to have less than a 4-hour working day; lung function was not tested in the control group. Personal ammonia exposure and lung function were measured on the same shift for 28 exposed workers. Linear multiple regression was used to analyze the relationship between workplace and the percentage cross-shift change in FEV_1 ($\Delta FEV_1\%$) while adjusting for current smoking.

Mean exposure levels at the ammonia plant determined by the Dräger tube and Dräger PAC III methods were 25.0 and 6.9 ppm (17.7 and 4.9 mg/m^3), respectively; the corresponding means in the urea plant were 124.6 and 26.1 ppm (88.1 and 18.5 mg/m^3) (Rahman et al., 2007). Although the Dräger tube measurements indicated ammonia levels about 4–5 times higher than levels measured with the PAC III instrument, there was a significant correlation between the ammonia concentrations measured by the two methods (p = 0.001). No ammonia was detected in the control area using the Dräger tube (concentrations less than the measuring range of 2.5–200 ppm [1.8–141 mg/m^3]). The study authors observed that their measurements indicated only relative differences in exposures between workers and production areas, and that the validity of the exposure measures could not be evaluated based on their results. Based on an evaluation of the two monitoring methods and communication with technical support at Dräger Safety Inc. (Bacom and Yanosky, 2010), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, the PAC III air measurements were considered the more reliable measurement of exposure to ammonia for the Rahman et al. (2007) study.

The prevalence of respiratory irritation and decreased lung function was higher in the urea plant than in the ammonia plant or in the administration building. Comparison between the urea plant and the administration building showed that cough and chest tightness were statistically higher in the former; a similar comparison of the ammonia plant and the administration building showed no statistical difference in symptom prevalence between the two groups (Table C-5). Preshift measurement of FVC, FEV_1, and PEFR did not differ between urea plant and ammonia plant workers. Significant cross-shift reductions in FVC and FEV_1 were reported in the urea plant (2 and

3%, respectively, $p \leq 0.05$), but not in the ammonia plant. When controlled for current smoking, a significant decrease in ΔFEV_1% was observed in the urea plant ($p \leq 0.05$). Among 23 workers with concurrent measurements of ammonia and lung function on the same shift, ammonia exposure and years working in the factory were correlated with a cross-shift decline in FEV_1. EPA identified a NOAEL of 4.9 mg/m^3 and a LOAEL of 18.5 mg/m^3 in the Rahman et al. (2007) study based on increased prevalence of respiratory symptoms and a decrease in lung function.

Table C-5. Prevalence of respiratory symptoms and cross-shift changes in lung function among workers exposed to ammonia in a urea fertilizer factory

Parameter	Ammonia plant (4.9 mg/m^3)[a]	Urea plant (18.5 mg/m^3)[a]	Administration building (concentration not determined)[b]
Respiratory symptoms			
Cough	4/24 (17%)[c]	18/64 (28%)*	2/25 (8%)
Chest tightness	4/24 (17%)	21/64 (33%)*	2/25 (8%)
Stuffy nose	3/24 (12%)	10/64 (16%)	1/25 (4%)
Runny nose	1/24 (4%)	10/64 (16%)	1/25 (4%)
Sneeze	0/24 (0%)	14/64 (22%)	2/25 (8%)
Lung function parameters (cross-shift percentage change)[d,e]			
FVC	0.2 ± 9.3 (Pre-shift: 3.308; Post-shift: 3.332)	-2.3 ± 8.8 (Pre-shift: 3.362; Post-shift: 3.258)	No data
FEV_1	3.4 ± 13.3 (Pre-shift: 2.627; Post-shift: 2.705)	-1.4 ± 8.9 (Pre-shift: 2.701; Post-shift: 2.646)	No data
PEFR	2.9 ± 11.1 (Pre-shift: 8.081; Post-shift: 8.313)	-1.0 ± 16.2 (Pre-shift: 7.805; Post-shift: 7.810)	No data

[a]Mean ammonia concentrations measured by the Dräger PAC III method.

[b]Concentrations in the administration building were rejected by study authors due to relatively large drift in the zero levels.

[c]Values are presented as incidence (prevalence expressed as a percentage).

[d]Calculated as ([postshift - preshift]/preshift) × 100.

[e]Values are presented as mean ± standard deviation (SD).

*Statistically significant ($p \leq 0.05$) by Fisher's exact test, comparing exposed workers to administrators.

Source: Rahman et al. (2007).

Bhat and Ramaswamy (1993)

A cross-sectional study of workers exposed to fertilizer chemicals in a plant in Mangalore, India (Bhat and Ramaswamy, 1993) showed significant reduction in lung function parameters (PEFR/min and FEV_1) compared to a control group. The exposed group consisted of 91 workers who underwent lung function testing, and included 30 urea plant workers, 30 diammonium

phosphate (DAP) plant workers, and 31 ammonia plant workers. The controls were a group of 68 people having comparable body surface area and were chosen from the same socioeconomic status and sex. All smokers were excluded from the study to avoid the effect of smoking on lung function. Other workplace exposures were not assessed. The duration of exposure was dichotomized into two groups (≤10 and >10 years), but no exposure measurements were made.

Lung function parameters (FVC, FEV_1, and PEFR/minute) were measured by a standard spirometry protocol for all workers in the study, and the highest of three replicates were retained for calculation. A comparison of FVC, FEV_1, and PEFR/minute was made between controls and fertilizer workers as a whole and also between controls and urea workers, DAP workers, and ammonia workers individually. The ammonia plant workers showed a significant decrease in FEV_1 ($p < 0.05$) and PERF/minute ($p < 0.001$) when compared to controls, but no significant decrease in FVC (Table C-6). PEFR/minute, a measure of airflow in the bronchi, was reduced in all plant workers (urea, DAP, and ammonia), indicating that these fertilizer chemicals affected the larger airways. The reduction of FEV_1, a measure of the amount of air that can be exhaled in 1 second, in ammonia plant workers suggested that ammonia can enter into the smaller bronchioles and cause bronchospasm. NOAEL and LOAEL values were not identified by the authors of this study or by EPA due to the lack of exposure concentration measurements in this study.

Table C-6. Comparison of lung function parameters in ammonia plant workers with controls

Parameter	Controls (n = 68) (mean ± standard error)	Ammonia Plant (n = 31) (mean ± standard error)
FVC	3.43 ± 0.21	3.19 ± 0.07
FEV_1	2.84 ± 0.10	2.52 ± 0.1*
PEFR/min	383.3 ± 7.6	314 ± 19.9**

*Significantly different from controls ($p < 0.05$); paired t-test.
**Significantly different from controls ($p < 0.001$); paired t-test.

Source: Bhat and Ramaswamy (1993).

C.2.2. Studies of Populations in Agricultural Settings (Livestock Farmers/Populations in Close Proximity to Animal Feeding Operations)

Several studies have investigated respiratory health and other outcomes related to ammonia exposure in agricultural settings. Some of these studies have also demonstrated respiratory effects associated with exposure to other air constituents (e.g., respirable dust, endotoxin). Ammonia exposure was associated with a decrease in lung function measures in six of the eight studies (Loftus et al., 2015; Monsó et al., 2004; Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Zejda et al., 1994; Heederik et al., 1990) examining this outcome (Table C-7). Six of these studies addressed confounding in some way; four of these studies controlled for co-exposures (e.g., endotoxin, dust, disinfectants) (Reynolds et al.,

1996; Donham et al., 1995; Preller et al., 1995) (Melbostad and Eduard, 2001), one study noted only weak correlations (i.e., Spearman r < 0.20) between ammonia and dust or endotoxin (Donham et al., 2000), and one study observed associations with ammonia but not with endotoxin or dust measures (Heederik et al., 1990). Two studies did not address confounding (Monsó et al., 2004; Zejda et al., 1994), and one study noted a lack of analysis for other potential confounders (Loftus et al., 2015).

The studies that controlled for co-exposures (e.g., endotoxin, dust, disinfectants) (Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995) (Melbostad and Eduard, 2001), noted only weak correlations (i.e., Spearman r < 0.20) between ammonia and dust or endotoxin (Donham et al., 2000), or observed associations with ammonia but not with endotoxin or dust measures (Heederik et al., 1990), are the studies EPA considered to be methodologically strongest (see Literature Search Strategy | Study Selection and Evaluation section). In summary, this set of studies provides relatively consistent evidence of an association between ammonia exposure and reduced lung function in studies of populations in agricultural settings, accounting for endotoxin and dust.

Some of these studies in agricultural settings also included analyses of respiratory outcomes in relation to exposure, based on ammonia measurements. The studies analyzing prevalence of respiratory symptoms (including cough, phlegm, wheezing, chest tightness, and eye, nasal, and throat irritation) in relation to ammonia provide generally negative results (Melbostad and Eduard, 2001; Preller et al., 1995; Zejda et al., 1994). Two other studies reported an increased prevalence of respiratory symptoms in pig farmers (Choudat et al., 1994; Crook et al., 1991). The authors of these studies measured air ammonia, but did not include a direct analysis of respiratory symptoms in relation to ammonia (Table C-8). One study found no relationship between reported asthma symptoms or medication use for asthma and ammonia exposure (Loftus et al., 2015).

Table C-7. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings with direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Lung function	
Monsó et al. (2004) 105 never-smoking farmers (84 males, 21 females) working inside animal confinement buildings; sampled from the European Farmers' Study; mean age 45 yrs **Exposure:** Area samples (confinement building, morning) Median ammonia: 10 ppm (7 mg/m^3) total dust: 5.6 mg/m^3 total endotoxin: 687.1 units/m^3 **Outcome:** Lung function (standard spirometry, before and after shift; chronic obstructive pulmonary disease (COPD) defined as FEV_1 <70 (n = 18; 17%).	COPD, Odds ratio (95% CI), by quartile of ammonia (1st and 2nd groups = referent) ppm: 0 to 10; >10–17; >17–60 OR: 1.0; 0.73; 1.32 (95%CI): (referent); (0.17, 3.20); (0.34, 5.12) Adjusted for age, gender, types of farming Monsó et al. (2004)

Table C-7. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings with direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Donham et al. (2000) (United States, Iowa) 257 poultry workers (30% women, 70% men); 150 controls (42% women, 58% men; postal workers and electronics plant) **Exposure:** Personal samples (workshift) Mean ammonia 18.4 ppm (13 mg/m^3) total dust 6.5 mg/m^3 respirable dust 0.63 mg/m^3 total endotoxin 1,589 EU/m^3 (0.16 µg/m^3) respirable endotoxin 58.9 EU/m^3 (0.006 µg/m^3) **Outcome:** Lung function (standard spirometry, before and after work shift)	OR (95% CI) for 3% or greater cross-shift decline in FEV_1, by quartile of ammonia ppm OR (95%CI) >0 to ≤5 1.88 (0.68, 5.14) 5 to ≤12 1.93 (0.72, 5.17) 12 to ≤25 4.25 (1.60, 11.2) >25 2.45 (0.88, 6.85) Adjusted for age, years worked in poultry industry, gender, smoking status, education. In linear regression, ammonia was statistically significant predictor of 5% decline in FEF_{25-75} (p = 0.045; Beta not reported) Correlations between ammonia and other exposures relatively weak (Spearman r < 0.20).
Reynolds et al. (1996) (United States, Iowa) 151 men ≥18 yrs of age employed at swine farms and spent time in swine confinement buildings (mean years of employment = 12.4); a farm comparison group (nonconfinement production) was included (number not given). Follow-up study of Donham et al. (1995). **Exposure:** Personal samples (workshift) Geometric Mean (Time 2) ammonia 5.15 ppm (4 mg/m^3) total dust 3.45 mg/m^3 respirable dust 0.26 mg/m^3 total endotoxin 176.12 EU/m^3 respirable endotoxin 11.86 EU/m^3 Ammonia levels similar at time 1 (5.65 ppm), but total dust and respirable dust higher at time 1 than time 2 **Outcome:** Lung function (standard spirometry, before and after work shift at two times, two years apart (same season)	Correlation between cross-shift decline in FEV_1 and ammonia: Spearman r = 0.18 (p < 0.05); strongest for 0-6 and 10-13 yrs duration Predictive model relating ammonia to cross-shift change in FEV_1 developed at baseline was corroborated by Time 2 data; dust and endotoxin did not add to the significance of ammonia as predictor
Donham et al. (1995) (United States, Iowa) 201 men ≥18 yrs of age employed at swine farms and spent time in swine confinement buildings (mean years of employment = 9.6); a farm comparison group (nonconfinement production) was included (number not given) **Exposure:** Personal samples Geometric Mean ammonia 5.64 ppm (4 mg/m^3) total dust 4.53 mg/m^3 respirable dust 0.23 mg/m^3 total endotoxin 202.35 EU/m^3 respirable endotoxin 16.59 EU/m^3 **Outcome:** Lung function (standard spirometry, before shift and then after a minimum of 2 hrs of exposure)	Ammonia was significant predictor of cross-shift decline in lung function (included with age, duration, smoking, total dust, respirable dust, and total endotoxin in the models, as well as interaction terms) Positive correlations were associated with changes in lung function and exposure to total dust, respirable dust, respirable endotoxin, and ammonia; dust was related to all lung function measures; ammonia results more variable across measures and duration strata—strongest for 7–9 yrs duration); exposure to ammonia concentrations of ≥7.5 ppm (5 mg/m^3) were predictive of a ≥3% decrease in FEV_1

Table C-7. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings with direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Heederik et al. (1990) (Netherlands) 27 pig farmers (mean age of 29 yrs; 43% current smokers) **Exposure:** Area samples, used in conjunction with duration of specific tasks to calculate an individual exposure measure ammonia — Mean 5.6 mg/m^3 total dust — 1.57 mg/m^3 total endotoxin — 24 ng/m^3 **Outcome:** Lung function (standard spirometry, before and after work shift, taken on Monday, Tuesday, and Friday)	Change (ml) in cross-shift lung function per 5 mg/m^3 increase in ammonia FVC: -3 (35) FEV_1: -112 (38), (< 0.05) MMEF: -330 (131), (< 0.05) PEF: -170 (335) MEF_{75}: -505 (300), (< 0.05) MEF_{50}: -404 (215), (< 0.05) MEF_{25}: -70 (179) Results from Tuesday measures presented; other days reported to be similar patterns but not as strong No association between dust or endotoxins with the lung function variables
Lung function and respiratory symptoms	
Loftus et al. (2015) (USA) Animal feeding operations; health and environmental data collected from AFARE (Aggravating Factors of Asthma in a Rural Environment) project n = 59 asthmatic children enrolled (inclusion criteria: school-age, no serious illness other than asthma); n = 51 (exposed) participated in the study (86.4% participation rate) **Exposure**: 14 ammonia monitoring devices located outside the home of a subset of the participants throughout study area 24-hour ammonia concentrations ranged from 0.0002–0.238 mg/m^3; median ammonia concentration measured at each site ranged from 0.0029–0.0727 mg/m^3; annual average ammonia concentrations in study region was 0.019 mg/m^3 **Outcome:** Lung function (FEV_1) measurements, twice daily by child given instructions for proper use according to American Thoracic Society guidelines; Asthma symptoms (nighttime waking, shortness of breath, limitation of activities, wheezing and morning asthma symptom) and medication use (frequency of use of short-acting bronchodilator)	Associations between **FEV_1%** and estimated ammonia concentrations measured at the nearest neighbor monitors Point estimates and 95% CI of FEV_1%[a] Entire cohort (n = 51) / Subjects within 1 km (n = 23) One day lag: -3.8% (0.2, 7.3) / -6.0% (0.4, 12.5)[b] Two day lag: -3.0% (0.5, 5.8) / -6.3% (2.3, 10.0) [a] Point estimates and 95% CI represent changes associated with an IQR increase in 24-hour average ammonia (25 µg/m^3); FEV1% indicates forced expiratory volume in 1 second as percent of predicted; IQR, interquartile range [b] This value was estimated from Figure 3 in Loftus et al. (2015) Odds of specific asthma symptoms associated with estimated weekly ammonia **Symptom or Medication Use OR (95% CI)[a]** Limitation of activities: 1.1 (0.79, 1.4) Wheezing: 0.99 (0.77, 1.3) Nighttime waking: 0.92 (0.76, 1.3) Shortness of breath: 1.1 (0.86, 1.3) Symptoms worse in morning: 0.88 (0.75, 1.0) Use of short-acting "relief" medication: 0.97 (0.82, 1.2) [a]OR is the odds ratio for report of any symptom/medication use in week prior associated with an IQR increase in weekly ammonia (18 µg/m^3). IQR indicates interquartile increase; OR, odds ratio.

Table C-7. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings with direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Preller et al. (1995) (Netherlands) 194 swine farmers (94 with chronic respiratory symptoms, 100 without symptoms); 106 with complete data for lung function analysis. **Exposure:** Personal samples (two workshifts; winter and summer) Mean ammonia 2 mg/m^3 total dust 2.7 mg/m^3 total endotoxin 112 ng/m^3 Long-term average exposure derived based on measured values and model based on farm characteristics and tasks **Outcome:** Lung function (standard spirometry, single measure); standardized questionnaire for respiratory symptoms	Association between ammonia and lung function (n = 106) Beta (SE) (*p*-value) FVC (l) -0.05 (0.13) (0.36) FEV_1 (l) -0.27 (0.13) (0.022) MMEF (l/s) -0.68 (0.23) (0002) PEF (l/s) -0.77 (0.43) (0.039) Adjusted for age, height, smoking, endotoxin, disinfection variables Stronger patterns seen in symptomatic group (n = 55). No association with respiratory symptoms (chronic cough, chronic phlegm, wheezing, shortness of breath, chest tightness)
Zejda et al. (1994) 54 male swine producers (mean age = 36.3 yrs; mean duration of employment = 10.7 yrs) **Exposure:** Area samples Mean ammonia 11.3 ppm (8 mg/m^3) total dust 2.93 mg/m^3 respirable dust 0.13 mg/m^3 total endotoxin 11,332 units/m^3 Exposure measures categorized into tertiles (cut-points 10.2 and 12.7 ppm) for some analyses. **Outcome:** Lung function (standard spirometry, single measure); respiratory symptoms-based on standardized questionnaire (cough, phlegm, chest wheeze, chest tightness)	Correlation coefficients (Spearman r) with ammonia with hr/day interaction FVC (% predicted) 0.18 -0.13 FEV_1 (% predicted) 0.18 -0.16 FEV_1/FVC 0.00 -0.06 FEF (% predicted) 0.08 -0.09 Adjusted for age, height, and smoking Some symptoms associated with ammonia exposure—hours/day interaction but it is difficult to distinguish these effects from the other exposures and interactions in the analyses (particularly endotoxin)Zejda et al. (1994)
Respiratory symptoms (without lung function measures)	
Melbostad and Eduard (2001) Survey of 8,482 farmers and spouses; exposure study conducted in 102 farmers **Exposure:** personal samples Range ammonia 0 to 8.2 ppm (0–6 mg/m^3) total dust 0.4–5.1 mg/m^3 total endotoxin 500–28000 EU/m^3 fungal spores 0.02–2.0 10^6/m^3 bacteria 0.2–48 10^6/m^3 **Outcome:** Respiratory symptoms (standard questionnaire); eye, nose, and throat irritation, cough, chest tightness, and wheezing	Negative correlation (r = -0.64) with total symptom prevalence

EU = endotoxin unit (10 EU/ng)

1

1

Table C-8. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings without direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Lung function and respiratory symptoms	
Crook et al. (1991) (Scotland) 29 swine farmers (from 12 farms); 48 electronic workers (controls for IgE/IgG serum analysis only) **Exposure:** Area samples of 20 pig houses were monitored for dust and ammonia concentrations over a working shift every 4 weeks over a 24 week period lasting from July to December; aerobiological analysis was conducted once in 6 pig houses Mean ammonia[a] 1.50–13.23 ppm (1–9 mg/m^3) total dust[a,b] 1.66–21.04 mg/m^3 airborne microorganisms 10^5–10^7 CFU/m^3 airborne endotoxin 1.9–28.5 ng/m^3 [a]Mean concentrations were higher in winter due to decreased ventilation [b]Mean concentrations were higher in pig houses using restricted feeding systems **Outcome:** Lung function (FEV_1, FVC); respiratory symptoms (standard questionnaire); serum measurements of IgG and IgE antibodies specific to pig skin, pig urine, and pig feed components	Impaired lung function (decreased FEV_1 and FVC) was observed in 3/29 swine farmers (quantitative values not reported) Respiratory symptoms Incidence nasal/eye irritation 20/29 cough 15/29 wheeze/chest tightness 13/29 any respiratory complaint 23/29 The study authors suggested that the presence of IgE in some farmers with wheeze (and absence in asymptomatic farmers) may indicate the involvement of an allergic response in these farmers, rather than a respiratory response to ammonia exposure

Table C-8. Evidence pertaining to respiratory effects in populations exposed to ammonia in agricultural settings without direct analysis of the relationship between ammonia exposure and measured outcomes

Study design and reference	Results
Choudat et al. (1994) (France) 102 male swine farmers who worked at least half-time in a swine confinement building (mean age 39.7 yrs; mean duration of employment of 15.7 yrs); 51 male dairy farmers (mean age 40.1 yrs; mean duration of employment of 20.3 yrs); and 81 male dairy industry workers (referents; mean age 38.5 yrs; mean duration of employment of 15.7 yrs) **Exposure:** Area samples in 28 swine confinement buildings from 6 farms ammonia: 48 samples; Mean 8.5 mg/m^3 total dust: 21 samples; 2.41 mg/m^3 inspirable particles: 28 samples; 1.82 mg/m^3 respirable fraction: 24 samples; 0.17 mg/m^3 carbon dioxide: 28 samples; 1,000–5,000 ppm (1,800–9,000 mg/m^3) Personal samples in swine farmers (n=4) ammonia: Mean 3.23 mg/m^3 inspirable particles: Mean 3.63 mg/m^3 Airborne exposure levels were not measured in dairy farm or industry buildings; study authors noted that dairy workers do not work in confinement buildings. The percentage of smokers in the pig and dairy farm groups (28.4 and 27.4%, respectively) was significantly lower than in the referent group (44.4%). **Outcome:** Lung function tests (FEV_1, FVC, PF) before and after bronchial responsiveness (methacholine challenge); respiratory symptoms (standard questionnaire)	No significant differences in baseline lung function observed between groups Prevalence (%) of bronchial hyperreactivity to methacholine responders (≥10% decrease in FEV_1): Swine farmers 17.9*; Dairy farmers 35.6***; Dairy industry 6.7 responders (≥15% decrease in FEV_1): Swine farmers 6.3; Dairy farmers 17.8*; Dairy industry 4.0 Prevalence (%) of respiratory symptoms (in general) morning cough: Swine farmers 13.3*; Dairy farmers 10.4; Dairy industry 3.8 diurnal cough: Swine farmers 13.3**; Dairy farmers 6.2; Dairy industry 1.3 fits of coughing: Swine farmers 24.0**; Dairy farmers 22.9*; Dairy industry 9.0 morning phlegm: Swine farmers 10.2; Dairy farmers 16.7; Dairy industry 7.7 chest tightness: Swine farmers 3.1; Dairy farmers 10.4*; Dairy industry 1.3 sneezing: Swine farmers 29.6; Dairy farmers 25.2; Dairy industry 19.2 Prevalence (%) of respiratory symptoms at work Fits of coughing: Swine farmers 24.5***; Dairy farmers 8.3; Dairy industry 5.1 Sneezing: Swine farmers 21.4*; Dairy farmers 10.4; Dairy industry 9.0 No significant differences in the prevalence of wheezing, shortness of breath, or rhinitis (in general or at work) between pig or dairy farmers and dairy industry workers *p < 0.05; **p < 0.01; ***p < 0.001 (compared with dairy industry referents)

FEV_1 = forced expiratory volume during 1 second; FVC = forced vital capacity; PF = peak flow rate

C.2.3. Controlled Human Inhalation Exposure Studies

Controlled exposure studies conducted with volunteers to evaluate irritation effects and changes in lung function following acute inhalation exposure to ammonia are summarized in Table C-9.

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results
Lung function	
Sigurdarson et al. (2004)[b] (United States, Iowa) 6 healthy volunteers (2 males, 4 females; 25–45 yrs old) and 8 volunteers with mild asthma (4 males, 4 females; 18–52 yrs old) **Exposure:** Volunteers were exposed to ammonia, grain dust, or ammonia+dust for 30-min sessions in an exposure hood with 1 wk between different exposure scenario sessions; a nose-clip was used to ensure mouth breathing Exposure levels: ammonia 16–20 ppm (11–14 mg/m^3) total dust 4 mg/m^3 respirable fraction 1 mg/m^3 endotoxin content 4 µg/m^3 **Outcome**: Lung function (FEV_1, DLCO, exhaled NO) before and after exposure, post-exposure bronchial responsiveness (methacholine challenge)	*Ammonia-only* No significant changes in lung function in healthy or asthmatic subjects *Ammonia + dust or Dust-only* Significantly decreased FEV_1 and increased bronchial hyperreactivity were observed in asthmatic subjects post-exposure. No significant changes were observed in DLCO or exhaled NO in asthmatic subjects. No significant changes in lung function were observed in healthy subjects.
Cormier et al. (2000)[b] (Canada) Eight healthy male volunteers (23–28 yrs old) **Exposure:** Volunteers were exposed for 4 hrs to ambient air in eight swine confinement buildings with 1 wk between different site exposures Area samples in eight confinement buildings: ammonia: Mean 20.7 ppm (14.6 mg/m^3); Range 2.80–38.55 ppm (1.98–27.25 mg/m^3) total dust: Mean 3.54 mg/m^3; Range 2.20–5.62 mg/m^3 bacteria: Mean 4.25×10^5 CFU/m^3; Range 1.67×10^5–9.29×10^5 CFU/m^3 endotoxin: Mean 404 EU/m^3; Range 215–596 EU/m^3 mold: Mean 883 CFU/m^3; Range 138–1805 CFU/m^3 **Outcome:** Lung function (FEV_1, FVC) before and after exposure; post-exposure bronchial responsiveness (methacholine challenge); nasal lavage levels of white blood cells and IL-8	FEV_1 and FVC values were significantly decreased after exposure in each of the eight swine confinement buildings, but values were not significantly correlated with any airborne exposures Pearson's correlation coefficients (*p*-values) between airborne exposures and changes in lung function ammonia: ΔFEV_1 -0.29 (0.49); ΔFVC -0.22 (0.60) total dust: ΔFEV_1 0.07 (0.87); ΔFVC -0.24 (0.57) bacteria: ΔFEV_1 0.36 (0.38); ΔFVC 0.40 (0.32) endotoxin: ΔFEV_1 -0.01 (0.99); ΔFVC -0.07 (0.87) mold: ΔFEV_1 0.30 (0.47); ΔFVC 0.19 (0.66) Bronchial responsiveness was increased in 3/64 measurements; this increase was significant only in swine confinement building 2 (lowest ammonia concentration, second highest mold concentration; all other airborne values were mid-range) Nasal lavage levels of total white blood cells, neutrophils, and IL-8 were significantly or near-significantly ($p = 0.06$) increased after exposure in each of the eight swine confinement buildings (data presented graphically); the only significant correlation between airborne exposure and nasal lavage endpoints was a significant positive correlation between endotoxin level and IL-8 (correlation coefficient = 0.72; *p*-value = 0.05)

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results
Lung function and irritation effects	
Petrova et al. (2008)[b] (United States, Pennsylvania) 25 healthy volunteers (mean age 29.7 yrs) and 15 mild/moderate persistent asthmatic volunteers (mean age 29.1 yrs) **Exposure:** Volunteers were exposed to 20 dilution steps of ammonia (2–500 ppm [1–354 mg/m^3]) via a nasal cannula and/or a specially fitted set of googles for up to 1.5 hrs; two separate sessions were conducted, separated by at least 48 hrs **Outcome**: Lung function (FEV_1) before, during, and after exposure; subjective reporting of odor intensity, annoyance, and irritation threshold (with or without velopharyngeal [VP] closure manipulation to isolate the throat from the nasal passages by raising the soft palate)	No significant changes in lung function were observed for healthy or asthmatic subjects during or after exposure Reported irritation thresholds in ppm (mg/m^3) Exposure — Asthmatic — Healthy nasal — 167 (116) — 179 (125) ocular — 133 (93) — 127 (88) combined (VP open) — 94 (65) — 87 (61) combined (VP closed) — 77 (54) — 102 (71) Odor intensity, irritation, and annoyance scores during combined ocular and nasal exposure were not significantly different between healthy volunteers and asthmatics at personal threshold concentrations or 2 dilution steps above threshold.
Sundblad et al. (2004)[b] (Sweden) 12 healthy volunteers (7 females, 5 males; mean age 25 yrs) **Exposure:** Volunteers were exposed to each of the following concentrations in randomized order during three separate exposures in inhalation chamber: 0, 5, and 25 ppm (0, 4, and 18 mg/m^3). Exposure duration was 3 hrs, in which 50% of the time was spent resting and 50% exercising on a stationary bike (alternating every 30 min); exposures were separated by at least 1 week. **Outcome**: Lung function (VC, TLC, FEV_1, PEF, exhaled NO) and questionnaire for irritation and respiratory effects (0–100 mm visual analogue scale) before, during, and 7 hrs after exposure; post-exposure bronchial responsiveness (methacholine challenge); determination of total cell and cytokine (IL-6, IL-8) concentration in nasal lavage fluid	No significant changes in lung functions or bronchial responsiveness were observed for healthy or asthmatic subjects during or after exposure Change in symptom rating during exposure compared with pre-exposure rating Exposure in ppm (mg/m^3) 0 — 5 (4) — 25 (18) eye irritation — -0.5 — 3.6* — 14.8* nose irritation — -4.7 — 3.4 — 15.3* throat/airway irritation — -2.9 — 1.2 — 14.2* breathing difficulty — -1.2 — 2.3 — 12.2* solvent smell — 0.2 — 38.1* — 61.8* *$p < 0.05$ No significant changes in total cell concentration or IL-8 concentration were observed in nasal lavage fluid. IL-6 in lavage fluid was below the level of detection

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results
Cole et al. (1977)[c] (United Kingdom) 18 healthy servicemen volunteers (mean age 24.1 yrs) **Exposure:** Exposure to ammonia for half-day sessions during exercise on a cycle ergometer; exercise sessions in ambient air the day before and the day after acted as controls (two separate studies were conducted) Ammonia concentrations in exposure chamber samples: Study 1, morning session — Mean 71 mg/m^3 Study 1, afternoon session — 144 mg/m^3 Study 2, morning session — 106 mg/m^3 Study 2, afternoon session — 235 mg/m^3 **Outcomes:** Lung function endpoints during exercise: exercise cardiac frequency at 45 mmol O_2/min (fC_{45}); ventilation minute volume at 45 mmol O_2/min (VE_{45}); exercise tidal volume at ventilation volume of 30 L/min (Vt_{30}); and mean respiratory frequency at a ventilation volume of 30 L/min (fR_{30}); irritation effects (subjective reporting)	Percent change in lung function during exercise + ammonia exposure, compared with exercise + ambient air Exposure in mg/m^3 0 / 71 / 106 / 144 / 235 VE_{45}: – / -4 / -8* / -10* / -6* Vt_{30}: – / 2 / 3* / -9* / -8* fR_{30}: – / -2 / -3* / 10* / 8* *$p < 0.05$ Subjective complaints during ammonia exposure included “a prickling sensation in the nose and slight dryness of the mouth,” but incidence data for these self-reported symptoms were not reported

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results
Ferguson et al. (1977)[c] (United States, New Jersey) 5 male and 1 female volunteers (24–46 yrs old); no previous occupational exposure to ammonia **Exposure:** Volunteers were exposed to 25, 50, or 100 ppm (18, 35, and 71 mg/m^3) ammonia for 2–6 hr/d, 1 d/wk over 6 wks; occasional brief exposure to 150–200 ppm (106–141 mg/m^3) were reported. Note: exposure durations were inconsistent across exposure levels Exposure in ppm (mg/m^3)† 25 (18) / 50 (35) / 100 (71) Group A (n = 2): Wks 1, 4 (2 hr/d) / Wks 2, 5 (4 hr/d) / Wks 3, 6 (6 hr/d) Group B (n =2): NA / Wks 1–6 (6 hr/d) / NA Group C (n=2): Wk 3 (2 hr/d), Wk 4 (6 hr/d) / Wk 2, 5 (4 hr/d) / Wk 1 (6 hr/d), Wk 6 (2 hr/d †For the 25- and 50-ppm levels, locations were occupational work sites with reportedly stable ammonia levels; the 100 ppm location was a temporary exposure chamber. However, no monitoring data for ammonia concentrations were presented **Outcome:** Lung function (FEV_1, FVC) tests were conducted by subjects irritation effects were evaluated by a physician looking at eyes and mucosa of the nose and throat before, during, and after exposure. Note: The study authors did not indicate whether or not pre-exposure lung function measurements were performed	Study authors report that lung function was not impaired with exposure at any concentration Incidence of physician-reported eye, nose or throat irritations (per total number of observations) Exposure in ppm (mg/m^3) 0 (pre-exposure) / 25 (18) / 50 (35) / 100 (71) incidence (%): 4/45 (9%) / 2/78 (3%) / 22/198 (11%) / 11/84 (13%) Volunteers did not make any subjective complaints at concentrations ≤100 ppm. All subjects reported mild eye, nose, and throat irritation after brief exposures ≥150 ppm
Verberk (1977)[c] (Netherlands) 16 volunteers; 8 were considered experts (knew effects of ammonia from literature; 7 males, 1 female; 29–53 yrs), 8 were non-science students and considered non-experts (not familiar with effects of ammonia; 6 males, 2 females; 18–30 yrs) **Exposure:** Volunteers were exposed to 50, 80, 110, and 140 ppm ammonia (35, 57, 78, and 99 mg/m^3) for 2 hrs in an exposure chamber at 1-wk intervals **Outcome:** Lung function (VC, FEV_1, FIV_1) before and after exposure; irritation effects during exposure (subjective reporting on scale of 1–5); post-exposure bronchial responsiveness (histamine challenge)	VC, FEV_1, or FIV_1 were within 10% of pre-exposure values in all subjects Incidence of subjects reporting at least one symptom (smell, irritation of eyes, nose, throat, and/or urge to cough) with score ≥3/5 (= nuisance) after 30 min exposure Exposure in ppm (mg/m^3) 50 (35) / 80 (56) / 110 (77) / 140 (98) expert: 2/8 / 2/8 / 5/8 / 7/8 non-expert: 5/8 / 7/8 / 7/8 / 8/8 All non-experts found 140 ppm “unbearable” and left the exposure chamber within 2 hrs; all experts tolerated 140 ppm for the full 4-hr exposure

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results
Silverman et al. (1949)[c] (United States, Massachusetts) 7 adult male volunteers **Exposure:** Volunteers were exposed to 500 ppm (354 mg/m^3) for 15–30 min via nose and mouth breathing mask **Outcome:** Lung function (respiratory rate, minute volume) before, during and after exposure, irritation effects (subjective reporting)	Respiratory rate and minute volume were increased by 50–250% during exposure, compared with pre-exposure values; elevated minute volumes during exposure showed cyclic variation (~25% decrease from peak values every 4–7 min) Subjective complaints included excessive lacrimation (2/7), nasal irritation (5/7), and nasal and throat irritation lasting up to 24 hrs after exposure (2/7)
Irritation effects (without lung function measures)	
Smeets et al. (2007)[b] (Netherlands) 24 healthy female volunteers (mean age 29.9 yrs) **Exposure:** Volunteers were exposed to a concentration series (maximum of 2 sec per concentration, 30–60-sec break between exposures) via static or dynamic nasal exposure through fitted nosepieces (separate airstreams to each nostril); each subject was exposed twice by each method over a 2-wk period Exposure range ppm (mg/m^3) Static: 1.23×10^{-6}–341.95 (0.87×10^{-6}–241.76) Dynamic: 0.10–615.38 (0.07–435.07) **Outcome**: Odor and irritation threshold	Reported thresholds (geometric mean) in ppm (mg/m^3) Static / Dynamic odor: 2.56 (1.81) / 2.62 (1.85) irritation: 31.69 (22.4) / 60.92 (43.07) Values determined via static or dynamic methods were not statistically significantly different
Ihrig et al. (2006)[b] (Germany) 10 healthy male volunteers exposed to ammonia regularly at the workplace (mean age 33 yrs) and 33 healthy male volunteers unfamiliar with the smell of ammonia (naïve; mean age 29 yrs) **Exposure:** Volunteers were exposed to 0, 10, 20, and 50 ppm (0, 7, 14, and 35 mg/m^3) for 4 hrs/d (D 1, 2, 3, and 5 of study, respectively) in an exposure chamber; on D 4, volunteers were exposed to 20 ppm for 4 hrs with 2 peak 30-min exposures at 40 ppm (14 + 28 mg/m^3) **Outcome:** Irritation effects during exposure (standardized questionnaire with rating scale of 1–5); data shown graphically	Mean intensity ratings for irritation in naïve volunteers (but not in workers) significantly increased with increasing exposure level during exposure; mean intensity ratings were <2 in all groups ("somewhat irritative") The increased ratings were driven primarily by olfactory symptom; at 50 ppm, naïve volunteers had an average rating of between 3 ("rather much") and 4 ("considerably" irritative), compared with a rating of ~2 in workers
Douglas and Coe (1987)[c] (England) Unspecified number of volunteer subjects **Exposure:** Volunteers were exposed to a concentration series via tight fitting goggles (up to 15 sec) or mouthpiece (10 inhaled breaths while wearing nose clip); one concentration was tested per day; concentrations used and duration of experiment were not specified **Outcome:** Ocular and pulmonary irritation threshold	Reported thresholds in ppm (mg/m^3) lachrymatory: 55 (39) bronchoconstriction: 85 (60)

Table C-9. Evidence pertaining to irritation effects and changes in lung function in controlled human exposure studies[a]

Study design and reference	Results		
MacEwen et al. (1970)[b] (United States, Ohio) 6 male volunteers (mean age 31 yrs) **Exposure:** Volunteers were exposed to 30 and 50 ppm (21 or 35 mg/m^3) for 10 min in a head-only inhalation chamber **Outcome:** Self-reported ocular and nasal irritation, odor intensity	Mean rating	Exposure in ppm (mg/m^3)	
		30 (21)	50 (35)
	eye/nasal irritation (scale 0–4)	0.4	1.5
	odor intensity (scale 0–5)	3.6	4.0

[a]Kalandarov et al. (1984), a study of the effect of ammonia on the adrenocortical system in 20 volunteer subjects, was eliminated from further consideration because of concerns regarding ethical conduct of the study, including the absence of information on ethical procedures followed and statement of informed consent by volunteers, and lack of clarity about the reported exposures (reported as 17–37 days in a sealed chamber).

[b]Investigators reported the use of ethical standards involving informed consent by volunteers and/or study approval by an Institutional Review Board or other ethics committee.

[c]This controlled-exposure study did not provide information on the human subjects research ethics procedures undertaken in the study; however, there is no evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

CFU = colony forming unit; DLCO = diffusion capacity of the lung for carbon monoxide; EU = endotoxin unit; *f*C$_{45}$ = exercise cardiac frequency at 45 mmol O2/minute; FEV$_1$ = forced expiratory volume during 1 second; FIV$_1$ = forced inspiratory volume during 1 second; *f*R$_{30}$ = mean respiratory frequency at a ventilation volume of 30 L/minute; IL-6 = interleukin-6; IL-8 = interleukin-8; NO = nitric oxide; PEF = peak expiratory flow; TLC = total lung capacity; TV = tidal volume; VC = vital capacity; VE$_{45}$ = ventilation minute volume at 45 mmol 02 /minute; Vt$_{30}$ = exercise tidal volume at ventilation volume of 30L/minute

Twelve healthy volunteers exposed to 4 and 18 mg/m^3 ammonia on three different occasions for 1.5 hours in an exposure chamber while exercising on a stationary bike reported discomfort in the eyes and odor detection at 4 mg/m^3 (Sundblad et al., 2004). Eye irritation was also shown to increase in a concentration-dependent manner in 16 volunteers exposed to ammonia for 2 hours in an exposure chamber at concentrations of 50, 80, 110 and 40 ppm (35, 57, 78, and 99 mg/m^3); ammonia concentrations of 99 mg/m^3 caused severe and intolerable irritation (Verberk, 1977). The lachrymatory threshold was determined to be 39 mg/m^3 in volunteers exposed to ammonia gas inside tight-fitting goggles for an acute duration of up to 15 seconds (Douglas and Coe, 1987). In contrast, exposures to up to 35 mg/m^3 ammonia gas did not produce severe lacrimation in seven volunteers after 10 minutes in an exposure chamber, although increased eye erythema was reported (MacEwen et al., 1970). Exposure to 354 mg/m^3 of ammonia gas for 30 minutes through a masked nose and throat inhalation apparatus resulted in two of seven volunteers reporting lacrimation and two of seven reporting nose and throat irritation that lasted up to 24 hours after exposure (Silverman et al., 1949).

Petrova et al. (2008) investigated irritation threshold differences between 25 healthy volunteers and 15 mild-to-moderate persistent asthmatic volunteers exposed to ammonia via the

eyes and nose at concentrations of 1–354 mg/m^3 for durations lasting up to 2.5 hours. Irritation threshold, odor intensity, and annoyance were not significantly different between the two groups. The nasal and eye irritation thresholds were reported to be 91 and 124 mg/m^3, respectively. Smeets et al. (2007) investigated odor and irritation thresholds for ammonia vapor in 24 healthy female volunteers at concentrations of 0.02–435 mg/m^3. This study found a mean odor detection threshold of 2 mg/m^3 and a mean irritation threshold of 22 or 43 mg/m^3, depending on the olfactometry methodology followed (static versus dynamic, respectively). Irritation thresholds may be higher in people who have had prior experience with ammonia exposure (Ihrig et al., 2006). Thirty male volunteers who had not experienced the smell of ammonia and 10 male volunteers who had regular workplace exposure to ammonia were exposed to ammonia vapors at concentrations of 0, 7, 14, and 35 mg/m^3 on 5 consecutive days (4 hours/day) in an exposure chamber; an additional group was exposed to 14 mg/m^3 plus two peak exposures to 28 mg/m^3 for 30 minutes. Volunteers in the group familiar with the smell of ammonia reported fewer symptoms than the nonhabituated group, but at a concentration of 14 mg/m^3, there were no differences in perceived symptoms between the groups. However, the perceived intensity of symptoms was concentration-dependent in both groups, but was only significant in the group of volunteers not familiar with ammonia exposure (Ihrig et al., 2006). Ferguson et al. (1977) reported habituation to eye, nose, and throat irritation in six male and female volunteers after 2–3 weeks of exposure to ammonia concentrations of 18, 35, and 71 mg/m^3 during a 6-week study (6 hours/day, 1 time/week). Continuous exposure to even the highest concentration tested became easily tolerated with no general health effects occurring after acclimation.

Several studies evaluated lung functions following acute inhalation exposure to ammonia. Volunteers exposed to ammonia (lung only) through a mouthpiece for 10 inhaled breaths of gas experienced bronchioconstriction at a concentration of 60 mg/m^3 (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in seven volunteers exposed to ammonia at concentrations of 21, 35, and 64 mg/m^3 for 10 minutes in an exposure chamber (MacEwen et al., 1970). Similarly, 12 healthy volunteers exposed to ammonia on three separate occasions to 4 and 18 mg/m^3 for 1.5 hours in an exposure chamber while exercising on a stationary bike did not have changes in bronchial responsiveness, upper airway inflammation, exhaled nitric oxide levels, or lung function as measured by vital capacity and FEV_1 (Sundblad et al., 2004). In another study, 18 healthy servicemen volunteers were placed in an exposure chamber for 3 consecutive half-day sessions. Exposure to ammonia at concentrations of 50–344 mg/m^3 occurred on the second session, with sessions 1 and 3 acting as controls (Cole et al., 1977). The no-effect concentration was determined to be 71 mg/m^3. Exercise tidal volume was increased at 106 mg/m^3, but then decreased at higher concentrations in a concentration-dependent manner (Cole et al., 1977). Decreased FEV_1 and FVC were reported in eight healthy male volunteers exposed to a mean airborne ammonia concentration of 15 mg/m^3 in swine confinement buildings for 4 hours at 1-week intervals; however, swine confinement buildings also include confounding exposures to dust, bacteria, endotoxin, and molds, thereby making measurement of effects due to ammonia uncertain in this study (Cormier et al., 2000).

Differences in lung function between healthy and asthmatic volunteers exposed to ammonia were evaluated in several studies. There were no changes in lung function as measured by FEV_1 in 25 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers after ocular and nasal exposure to 1–354 mg/m^3 ammonia at durations lasting up to 2.5 hours (Petrova et al., 2008). In another study, six healthy volunteers and eight mildly asthmatic volunteers were exposed to 11–14 mg/m^3 ammonia, ammonia and dust, and dust alone for 30-minute sessions, with 1 week between sessions (Sigurdarson et al., 2004). There were no significant changes in lung function as measured by FEV_1 in the healthy volunteers for any exposure. A decrease in FEV_1 was reported in asthmatics exposed to dust and ammonia, but not to ammonia alone; similarly, increased bronchial hyperreactivity was reported in asthmatics after exposure to dust and ammonia, but not to ammonia alone. Exposure to dust alone caused similar effects, suggesting that dust was responsible for decreased lung function (Sigurdarson et al., 2004).

In summary, controlled human exposure studies demonstrate that eye irritation can occur following acute exposure to ammonia at concentrations as low as 4 mg/m^3. Irritation thresholds may be higher in people who have had prior experience with ammonia exposure, and habituation to eye, nose, and throat irritation occurs over time. Lung function was not affected in workers acutely exposed to ammonia concentrations as high as 71 mg/m^3. Studies comparing the lung function of asthmatics and healthy volunteers exposed to ammonia do not suggest that asthmatics are more sensitive to the lung effects of ammonia.

C.2.4. Case Reports of Human Exposure to Ammonia

Inhalation is the most frequently reported route of exposure and cause of morbidity and fatality, and often occurs in conjunction with dermal and ocular exposures. Acute effects from inhalation have been reported to range from mild to severe, with mild symptoms consisting of nasal and throat irritation, sometimes with perceived tightness in the throat (Price and Watts, 2008; Prudhomme et al., 1998; Weiser and Mackenroth, 1989; Yang et al., 1987; O'Kane, 1983; Ward et al., 1983; Caplin, 1941). Moderate effects are described as moderate to severe pharyngitis; tachycardia; frothy, often blood-stained sputum; moderate dyspnea; rapid, shallow breathing; cyanosis; some vomiting; transient bronchospasm; edema and some evidence of burns to the lips and oral mucosa; and localized to general rhonchi in the lungs (Weiser and Mackenroth, 1989; Yang et al., 1987; O'Kane, 1983; Ward et al., 1983; Couturier et al., 1971; Caplin, 1941). Severe effects include second- and third-degree burns to the nasal passages, soft palate, posterior pharyngeal wall, and larynx; upper airway obstruction; loss of consciousness; bronchospasm, dyspnea; persistent, productive cough; bilateral diffuse rales and rhonchi; production of large amounts of mucous; pulmonary edema; marked hypoxemia; local necrosis of the lung; deterioration of the whole lung; and fatality. Delayed effects of acute exposure to high concentrations of ammonia include bronchiectasis; bronchitis; bronchospasm/asthma; dyspnea upon exertion and chronic productive cough; bronchiolitis; severe pulmonary insufficiency; and chronic obstructive pulmonary disease (Ortiz-Pujols et al., 2014; Lalić et al., 2009; Leduc et al., 1992; Bernstein and

Bernstein, 1989; Flury et al., 1983; Ward et al., 1983; Stroud, 1981; Close et al., 1980; Taplin et al., 1976; Walton, 1973; Kass et al., 1972; Slot, 1938).

Respiratory effects were also observed following chronic occupational exposure to ammonia. After 18 months and 1 year on the job, respectively, two men developed cough, chest tightness, and wheezing, typically after 2–6 hours from the beginning of each work day, but not on weekends or holidays. In another case, progressive deterioration of the clinical condition of a 68-year-old male was documented for 4 years, and development of diffuse interstitial and severe restrictive lung disease was reported following long-term repetitive occupational exposure to ammonia at or above the odor recognition level of 3–50 ppm (Brautbar et al., 2003). Lee et al. (1993) reported a case of a 39-year-old man who developed occupational asthma 5 months after beginning a job requiring the polishing of silverware. The room in which he worked was poorly ventilated. The product used contained ammonia and isopropyl alcohol and the measured ammonia concentration in the breathing zone when using this product was found to be 6–11 mg/m^3.

Acute dermal exposure to anhydrous (liquid) ammonia and ammonia vapor has resulted in caustic burns of varying degrees to the skin and eyes. There are numerous reports of exposures from direct contact with anhydrous ammonia in which first-, second-, and third-degree burns occurred over as much as 50% of the total body surface (Lalić et al., 2009; Pirjavec et al., 2009; Arwood et al., 1985). Frostbite injury has also been reported in conjunction with exposure to sudden decompression of liquefied ammonia, which is typically stored at -33°F (George et al., 2000; Sotiropoulos et al., 1998; Arwood et al., 1985). However, direct contact is not a prerequisite for burn injury. Several reports have indicated that burns to the skin occurred with exposure to ammonia gas or vapor. Kass et al. (1972) reported one woman with chemical burns to her abdomen, left knee, and forearm and another with burns to the feet when exposed to anhydrous ammonia gas released from a derailed train in the vicinity. Several victims at or near the scene of an overturned truck that had been carrying 8,000 gallons of anhydrous ammonia were reported as having second- and third-degree burns over exposed portions of the body (Burns et al., 1985; Close et al., 1980; Hatton et al., 1979). In a case involving a refrigeration leak in a poorly ventilated room, workers located in an adjacent room reported a "burning skin" sensation (de la Hoz et al., 1996), while in another case involving the sudden release of ammonia from a pressure valve in a refrigeration unit, one victim received burns to the leg and genitalia (O'Kane, 1983).

In addition to the skin, the eyes are particularly vulnerable to ammonia burns due to the highly water-soluble nature of the chemical and the ready dissociation of ammonium hydroxide to release hydroxyl ions. When ammonia or ammonia in solution has been splashed or sprayed into the face (accidently or intentionally), immediate effects include temporary blindness, blepharospasm, conjunctivitis, corneal burns, ulceration, edema, chemosis, and loss of corneal epithelium (George et al., 2000; Helmers et al., 1971; Highman, 1969; McGuiness, 1969; Levy et al., 1964; Abramovicz, 1925). The long-term effects included photophobia, progressive loss of sensation, formation of bilateral corneal opacities and cataracts, recurrent corneal ulcerations, nonreactive pupil, and gradual loss of vision (Yang et al., 1987; Kass et al., 1972; Helmers et al.,

1971; Highman, 1969; Osmond and Tallents, 1968; Levy et al., 1964; Abramovicz, 1925). White et al. (2007) reported a case with acute bilateral corneal injury that developed into bilateral uveitis with stromal vascularization and stromal haze and scarring, and pigmented keratic precipitates that resulted in legal blindness. An increase in intraocular pressure, resembling acute-angle closure glaucoma, was reported by Highman (1969) following ammonia intentionally sprayed into the eyes during robbery attempts.

C.3. ANIMAL STUDIES INVOLVING INHALATI0N EXPOSURE

Anderson et al. (1964)

Anderson et al. (1964) exposed a group of 10 guinea pigs (strain not given) and 10 Swiss albino mice of both sexes continuously to 20 ppm (14 mg/m^3) ammonia vapors for up to 6 weeks (anhydrous ammonia, purity not reported). Controls (number not specified) were maintained under identical conditions except for the exposure to ammonia. An additional group of six guinea pigs was exposed to 50 ppm (35 mg/m^3) for 6 weeks. The animals were observed daily for abnormal signs or lesions. At termination, the mice and guinea pigs were sacrificed (two per group at 1, 2, 3, 4, and 6 weeks of exposure), and selected tissues (lungs, trachea, turbinates, liver, and spleen) were examined for gross and microscopic pathological changes. No significant effects were observed in animals exposed for up to 4 weeks, but exposure to 14 mg/m^3 for 6 weeks caused darkening, edema, congestion, and hemorrhage in the lung. Exposure of guinea pigs to 35 mg/m^3 ammonia for 6 weeks caused grossly enlarged and congested spleens, congested livers and lungs, and pulmonary edema.

Coon et al. (1970)

Coon et al. (1970) exposed groups of male and female Sprague-Dawley and Long-Evans rats, male and female Princeton-derived guinea pigs, male New Zealand rabbits, male squirrel monkeys, and purebred male beagle dogs to 0, 155, or 770 mg/m^3 ammonia for 8 hours/day, 5 days/week for 6 weeks (anhydrous ammonia, >99% pure). The investigators stated that a typical loaded chamber contained 15 rats, 15 guinea pigs, 3 rabbits, 3 monkeys, and 2 dogs. Blood samples were taken before and after the exposures for determination of hemoglobin concentration, packed erythrocyte volume, and total leukocyte counts. Animals were routinely checked for clinical signs of toxicity. At termination, sections of the heart, lung, liver, kidney, and spleen were processed for microscopic examination in approximately half of the surviving rats and guinea pigs and all of the surviving dogs and monkeys. Sections of the brain, spinal cord, and adrenals from dogs and monkeys were also retained, as were sections of the thyroid from the dogs. The nasal passages were not examined in this study.

Exposure to 155 mg/m^3 ammonia did not result in any deaths or adverse clinical signs of toxicity in any of the animals. Hematological values were within normal limits for the laboratory and there were no significant gross alterations in the organs examined. Microscopic examination showed evidence of focal pneumonitis in the lung of one of three monkeys. Exposure to 770 mg/m^3 caused initial mild to moderate lacrimation and dyspnea in rabbits and dogs. However, these

clinical signs disappeared by the second week of exposure. No significant alterations were observed in hematology tests or upon gross or microscopic examinations at the highest dose. However, consistent nonspecific inflammatory changes (not further described) that were more extensive than in control animals (incidence not reported) were observed in the lungs from rats and guinea pigs in the high-dose group.

Coon et al. (1970) also exposed rats (15–51/group) continuously to ammonia (anhydrous ammonia, >99% pure) at 0, 40, 127, 262, 455, or 470 mg/m^3 for 90–114 days. Fifteen guinea pigs, three rabbits, two dogs, and three monkeys were also exposed continuously under similar conditions to ammonia at either 40 or 470 mg/m^3. No significant effects were reported in any animals exposed to 40 mg/m^3 ammonia. Exposure of rats to 262 mg/m^3 ammonia caused nasal discharge in 25%; nonspecific circulatory and degenerative changes in the lungs and kidneys were also demonstrated (not further described, incidence not reported), which the authors stated were difficult to relate to ammonia inhalation. A frank effect level at 455 mg/m^3 was observed due to high mortality in the rats (50/51). Thirty-two of 51 rats died by day 25 of exposure; no histopathological examinations were conducted in these rats. Exposure to 470 mg/m^3 caused death in 13/15 rats and 4/15 guinea pigs and marked eye irritation in dogs and rabbits. Dogs experienced heavy lacrimation and nasal discharge, and corneal opacity was noted in rabbits. Hematological values did not differ significantly from controls in animals exposed to 470 mg/m^3 ammonia. Histopathological evaluation of animals exposed to 470 mg/m^3 consistently showed focal or diffuse interstitial pneumonitis in all animals and alterations in the kidneys (calcification and proliferation of tubular epithelium), heart (myocardial fibrosis), and liver (fatty change) in several animals of each species (incidence not reported). The study authors did not determine a NOAEL or LOAEL concentration from this study. EPA identified a NOAEL of 262 mg/m^3 and a LOAEL of 455 mg/m^3 based on nonspecific inflammatory changes in the lungs and kidneys in rats exposed to ammonia for 90 days.

Stombaugh et al. (1969)

Stombaugh et al. (1969) exposed groups of Duroc pigs (9/group) to measured concentrations of 12, 61, 103, or 145 ppm ammonia (8, 43, 73, or 103 mg/m^3) continuously for 5 weeks (anhydrous ammonia, purity not reported). Endpoints evaluated included clinical signs, food consumption (measured 3 times/week), weight gain (measured weekly), and gross and microscopic examination of the respiratory tract at termination. A control group was not included. In general, exposure to ammonia reduced food consumption and body weight gain, but because a control group was not used, it could not be determined whether this reduction was statistically significant. Food efficiency (food consumed/kg body weight gain) was not affected. Exposure to ≥73 mg/m^3 ammonia appeared to cause excessive nasal, lacrimal, and mouth secretions and increased the frequency of cough (incidence data for these effects were not reported). Examination of the respiratory tract did not reveal any significant exposure-related alterations. The study authors did not identify a NOAEL or LOAEL concentration from this study.

Doig and Willoughby (1971)

Doig and Willoughby (1971) exposed groups of six specific-pathogen-free derived Yorkshire Landrace pigs to 0 or 100 ppm ammonia (0 or 71 mg/m^3) continuously for up to 6 weeks. The mean concentration of ammonia in the control chamber was 8 ppm (6 mg/m^3). Additional groups of pigs were exposed to similar levels of ammonia as well as to 0.3 mg/ft^3 of ground corn dust to simulate conditions on commercial farms. Pigs were monitored daily for clinical signs and changes in behavior. Initial and terminal body weights were measured to determine body weight gain during the exposure period. Blood samples were collected prior to the start of each experiment and at study termination for hematology (packed cell volume, white blood cell, differential leukocyte percentage, and total serum lactate dehydrogenase). Two pigs (one exposed and one control) were necropsied at weekly intervals, and tracheal swabs for bacterial and fungal culture were taken. Histological examination was conducted on tissue samples from the lung, trachea, and bronchial lymph nodes.

During the first week of exposure, exposed pigs exhibited slight signs of conjunctival irritation including photophobia and excessive lacrimation. These irritation effects were not apparent beyond the first week. Measured air concentrations in the exposure chambers increased to more than 150 ppm (106 mg/m^3) on two occasions. Doig and Willoughby (1971) reported that, at this concentration, the signs of conjunctival irritation were more pronounced in all pigs. No adverse effects on body weight gain were apparent. Hematological parameters and gross pathology were comparable between exposed and control pigs. Histopathology revealed epithelial thickening in the trachea of exposed pigs and a corresponding decrease in the numbers of goblet cells (see Table C-10). Tracheal thickening was characterized by thinning and irregularity of the ciliated brush border and an increased number of cell layers. Changes in bronchi and bronchioles, characterized as lymphocytic cuffing, were comparable between exposed and control pigs. Similarly, intraalveolar hemorrhage and lobular atelectasis were common findings in both exposed and control pigs. Pigs exposed to both ammonia and dust exhibited similar reactions as those pigs exposed only to ammonia, although initial signs of conjunctival irritation were more severe in these pigs, and these pigs demonstrated lesions in the nasal epithelium similar to those observed in the tracheal epithelium of pigs exposed only to ammonia.

Table C-10. Summary of histological changes observed in pigs exposed to ammonia for 6 weeks

Duration of exposure (wks)	Thickness of tracheal epithelium (µm)		Number of tracheal goblet cells (per 500 µm)	
	Control	71 mg/m^3 NH_3	Control	71 mg/m^3 NH_3
1	15.7	21.0	13.6	24.0
2	20.4	29.3	22.7	10.3
3	20.4	36.6	18.9	7.3
4	21.8	36.2	18.3	10.7
5	19.3	33.2	20.2	10.0
6	18.9	41.6	20.0	1.3
Mean ± SD	19.4 ± 2.1	32.9 ± 7.2	18.9 ± 3.0	10.6 ± 7.5

Source: Doig and Willoughby (1971).

Doig and Willoughby (1971) concluded that ammonia exposure at 71 mg/m^3 may be detrimental to young pigs. The authors suggested that although the structural damage to the upper respiratory epithelium was slight, such changes may cause severe functional impairment. The study authors did not identify a NOAEL or LOAEL concentration from this study. EPA identified a LOAEL of 71 mg/m^3 based on damage to the upper respiratory epithelium. A NOAEL could not be identified from this single-concentration study.

Broderson et al. (1976)

Broderson et al. (1976) exposed groups of Sherman rats (5/sex/dose) continuously to 10 or 150 ppm ammonia (7 or 106 mg/m^3, respectively) for 75 days (anhydrous ammonia, purity not reported). The 7 mg/m^3 exposure level represented the background ammonia concentration resulting from cage bedding that was changed 3 times/week. The 106 mg/m^3 concentration resulted from cage bedding that was replaced occasionally, but never completely changed. F344 rats (6/sex/group) were exposed to ammonia in an inhalation chamber at concentrations of 0 or 250 ppm (177 mg/m^3) continuously for 35 days. Rats were sacrificed at the end of the exposure period, and tissues were prepared for histopathological examination of nasal passages, middle ear, trachea, lungs, liver, kidneys, adrenal, pancreas, testicle, mediastinal lymph nodes, and spleen.

Histopathological changes were observed in the nasal passage of rats exposed to 106 mg/m^3 for 75 days (from bedding) or 177 mg/m^3 for 35 days (inhalation chamber). Nasal lesions were most extensive in the anterior portions of the nose compared with posterior sections of the nasal cavity. The respiratory and olfactory mucosa was similarly affected with a three- to fourfold increase in the thickness of the epithelium. Pyknotic nuclei and eosinophilic cytoplasm were observed in epithelial cells located along the basement membrane. Epithelial cell hyperplasia and formation of glandular crypts were observed, and neutrophils were located in the epithelial layer, the lumina of submucosal glands, and the nasal passages. Dilation of small blood vessels and edema were observed in the submucosa of affected areas. Collagen replacement of submucosal

glands and the presence of lymphocytes and neutrophils were also observed. No histopathological alterations were seen in control rats (7 mg/m^3 from bedding or 0 mg/m^3 from the inhalation chamber). Broderson et al. (1976) did not identify a NOAEL or LOAEL from this study. EPA identified a NOAEL of 7 mg/m^3 and a LOAEL of 106 mg/m^3 based on nasal lesions in rats exposed to ammonia (from bedding) for 75 days.

Gaafar et al. (1992)

Gaafar et al. (1992) exposed 50 adult male white albino mice under unspecified conditions to ammonia vapor derived from a 12% ammonia solution (air concentrations were not reported) for 15 minutes/day, 6 days/week for up to 8 weeks. Twenty-five additional mice served as controls. Starting the fourth week, 10 exposed and 5 control mice were sacrificed weekly. Following sacrifice, the nasal mucosa was removed and examined histologically. Frozen sections of the nasal mucosa were subjected to histochemical analysis (succinic dehydrogenase, nonspecific estrase, acid phosphatase, and alkaline phosphatase [ALP]). Histological examination revealed a progression of changes in the nasal mucosa of exposed rats from the formation of crypts and irregular cell arrangements at 4 and 5 weeks; epithelial hyperplasia, patches of squamous metaplasia, and loss of cilia at 6 weeks; and dysplasia in the nasal epithelium at 7 weeks. Similar changes were exaggerated in the nasal mucosa of rats sacrificed at 8 weeks. Neoplastic changes included a carcinoma in situ in the nostril of one rat sacrificed at 7 weeks, and an invasive adenocarcinoma in one rat sacrificed at 8 weeks. Histochemical results revealed changes in succinic dehydrogenase, acid phosphatase, and ALP in exposed mice compared to controls (magnitude of change not reported), especially in areas of the epithelium characterized by dysplasia. Succinic dehydrogenase and acid phosphatase changes were largest in the superficial layer of the epithelium, although the acid phosphatase reaction was stronger in the basal and intermediate layers in areas of squamous metaplasia. The presence of ALP was greatest in the goblet cells from the basal part of the epithelium and basement membrane.

In summary, Gaafar et al. (1992) observed that ammonia exposure induces histological changes in the nasal mucosa of male mice that increase in severity over longer exposure periods. Corresponding abnormalities in histochemistry suggest altered cell metabolism and energy production, cell injury, cell proliferation, and possible chronic inflammation and neoplastic transformation. The study authors did not determine a NOAEL or LOAEL concentration from this study. EPA did not identify a NOAEL or LOAEL because air concentrations were not reported in the study.

Done et al. (2005)

Done et al. (2005) continuously exposed groups of 24 weaned pigs of several breeds in an experimental facility to atmospheric ammonia at 0, 0.6, 10, 18.8, or 37 ppm (0, 0.4, 7, 13.3, or 26 mg/m^3) and 1.2, 2.7, 5.1, or 9.9 mg/m^3 inhalable dust for 5 weeks (16 treatment combinations). The concentrations of ammonia and dust used were representative of those found commercially. A split-plot design was used in which one dust concentration was allocated to a "batch" (which involved five lots of 24 pigs each) and the four ammonia concentrations were allocated to the four lots within that batch. The fifth lot served as a control. Each batch was replicated.

$$2 \times [4 \text{ dust concentrations} \times 4 \text{ ammonia concentrations} + 4 \text{ controls}] = 40 \text{ lots total}$$

In total, 960 pigs (460 males and 500 females) were used in the study; 560 pigs were given postmortem examinations. Blood was collected from 15 sows before the start of the experiment and tested for porcine reproductive and respiratory syndrome virus and swine influenza. Five sentinel pigs were sacrificed at the start of each batch, and lung, nasal cavity, and trachea, together with material from any lesions, were examined postmortem and subjected to bacteriological examination.

Postmortem examination involved examination of the pigs' external surfaces for condition and abnormalities, examination of the abdomen for peritonitis and lymph node size, internal gross examination of the stomach for abnormalities, and gross examination of the nasal turbinates, thorax, larynx, trachea, tracheobronchial lymph nodes, and lung. Pigs were monitored for clinical signs (daily), growth rate, feed consumption, and feed conversion efficiency (frequency of observations not specified). After 37 days of exposure, eight pigs from each lot were sacrificed. Swabs of the nasal cavity and trachea were taken immediately after death for microbiological analysis, and the pigs were grossly examined postmortem. On day 42, the remaining pigs were removed from the exposure facility and transferred to a naturally ventilated building for a recovery period of 2 weeks. Six pigs from each lot were assessed for evidence of recovery and the remaining 10 pigs were sacrificed and examined postmortem.

The pigs in this study demonstrated signs of respiratory infection and disease common to young pigs raised on a commercial farm (Done et al., 2005). The different concentrations of ammonia and dust did not have a significant effect on the pathological findings in pigs or on the incidence of pathogens. In summary, exposure to ammonia and inhalable dust at concentrations commonly found at pig farms was not associated with increase in the incidence of respiratory or other disease. The study authors did not identify a NOAEL or LOAEL concentration from this study. EPA identified a NOAEL of 26 mg/m^3, based on the lack of respiratory or other disease following exposure to ammonia in the presence of respirable dust.

Weatherby (1952)

Weatherby (1952) exposed a group of 12 guinea pigs (strain not reported) to a target concentration of 170 ppm (120 mg/m^3) 6 hours/day, 5 days/week for up to 18 weeks (anhydrous

ammonia, purity not reported). The actual concentration measured in the exposure chamber varied between 140 ppm (99 mg/m^3) and 200 ppm (141 mg/m^3). A control group of six guinea pigs was exposed to room air. All animals were weighed weekly. Interim sacrifices were conducted at intervals of 6 weeks (four exposed and two control guinea pigs), and the heart, lungs, liver, stomach and small intestine, spleen, kidneys, and adrenal glands were removed for microscopic examination; the upper respiratory tract was not examined.

No exposure-related effects were observed in guinea pigs sacrificed after 6 or 12 weeks of exposure. However, guinea pigs exposed to ammonia for 18 weeks showed considerable congestion of the spleen, liver, and kidneys, and early degenerative changes in the adrenal gland. The most severe changes occurred in the spleen and the least severe changes occurred in the liver. The spleen of exposed guinea pigs contained a large amount of hemosiderin, and kidney tubules showed cloudy swelling with precipitated albumin in the lumens and some urinary casts (cylindrical structures indicative of disease). The incidence of histopathological lesions was not reported. EPA identified the ammonia concentration of 120 mg/m^3 to be a LOAEL based on congestion of the spleen, liver, and kidneys and early degenerative changes in the adrenal gland. A NOAEL could not be identified in this single-concentration study.

Curtis et al. (1975)

Curtis et al. (1975) exposed groups of crossbred pigs (4–8/group) to 0, 50, or 75 ppm ammonia (0, 35, or 53 mg/m^3) continuously for up to 109 days (anhydrous ammonia, >99.9% pure). Endpoints evaluated included clinical signs and body weight gain. At termination, all pigs were subjected to a complete gross examination and representative tissues from the respiratory tract, the eye and its associated structures, and the visceral organs (not specified) were taken for subsequent microscopic examination. Weight gain was not significantly affected by exposure to ammonia, and the results of the evaluations of tissues and organs were unremarkable. The turbinates, trachea, and lungs of all pigs were classified as normal. The study authors did not identify a NOAEL or LOAEL from this study. EPA identified a NOAEL of 53 mg/m^3 based on the absence of effects occurring in pigs exposed to ammonia; a LOAEL was not identified from this study.

C.3.3. Reproductive/Developmental Studies

Diekman et al. (1993)

Diekman et al. (1993) reared 80 crossbred gilts (young female pigs) in a conventional grower from 2 to 4.5 months of age; pigs were exposed naturally during that time to *Mycoplasma hyopneumoniae* and *Pasteurella multocida*, which causes pneumonia and atrophic rhinitis, respectively. At 4.5 months of age, the pigs were transferred to environmentally regulated rooms where they were exposed continuously to a mean concentration of ammonia of 7 ppm (range, 4–12 ppm) (5 mg/m^3; range, 3–8.5 mg/m^3) or 35 ppm (range, 26–45 ppm) (25 mg/m^3; range, 18–32 mg/m^3) for 6 weeks (Diekman et al., 1993). A control group was not included in this study. The low concentration of ammonia was obtained by the flushing of manure pits weekly and the higher

concentration of ammonia was maintained by adding anhydrous ammonia (purity not reported) to manure pits that were not flushed. After 6 weeks of exposure, 20 gilts from each group were sacrificed, and sections of the lungs and snout were examined for gross lesions. In addition, the ovaries, uterus, and adrenal glands were weighed. The remaining 20 gilts/group were mated with mature boars and continued being exposed to ammonia until gestation day 30, at which time they were sacrificed. Fetuses were examined for viability, weight, and length, and the number of corpora lutea were counted.

Gilts exposed to 25 mg/m^3 ammonia gained less weight than gilts exposed to 5 mg/m^3 during the first 2 weeks of exposure (7% decrease, $p < 0.01$), but growth rate recovered thereafter. Mean scores for lesions in the lungs and snout were not statistically different between the two exposure groups, and there were no differences in the weight of the ovaries, uterus, and adrenals. Age at puberty did not differ significantly between the two groups, but gilts exposed to 25 mg/m^3 ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m^3. In gilts that were mated, conception rates were similar between the two groups (94.1 versus 100% in low versus high exposure, respectively). At sacrifice on day 30 of gestation, body weights were not significantly different between the two groups. In addition, there were no significant differences between the two groups regarding percentage of lung tissue with lesions and mean snout grade. Number of corpora lutea, number of live fetuses, and weight and length of the fetuses on day 30 of gestation were not significantly different between treatment groups. Diekman et al. (1993) did not identify NOAEL or LOAEL concentrations for maternal or fetal effects in this study. EPA did not identify NOAEL or LOAEL values from this study due to the absence of a no ammonia control group and due to confounding exposures to bacterial and mycoplasm pathogens.

C.3.4. Acute and Short-term Inhalation Toxicity Studies

Table C-11 provides information on animal studies of acute and short-term inhalation exposure to ammonia.

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Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Rats					
Female Porton rats (16/group)	0 or 141	Continuous exposure for 4, 8, or 12 d	Histology of the trachea	4 d: transitional-stratified appearance of the epithelium 8 d: gross change with disappearance of cilia and stratification on luminal surface 12 d: increased epithelial thickness	Gamble and Clough (1976)
Male OFA rats (27/group)	0 or 354	Continuous exposure for 1–8 wks	Body weight, organ weights, airway structure, cell population, alveolar macrophages	No deaths occurred; decreased food consumption and body weight gain; increased lung and kidney weights; at 3 wks, nasal irritation and upper respiratory tract inflammation, but no effect on lower airways; slight decrease in alveolar macrophages; no histopathological effects seen at 8 wks, suggesting adaptation to exposure	Richard et al. (1978a)
Male and female Wistar rats (5/sex/group)	9,898–37,825; no mention of control group	10, 20, 40, or 60 min	Clinical signs, pathology, LC_{50}	Eye irritation, eye and nasal discharge, dyspnea; hemorrhagic lungs on necropsy; 10-min LC_{50} = 28,492 mg/m^3 20-min LC_{50} = 20,217 mg/m^3 40-min LC_{50} = 14,352 mg/m^3 60-min LC_{50} = 11,736 mg/m^3	Appelman et al. (1982)
Male Crl:COBS CD Sprague-Dawley rats (8/group)	11, 23, 219, and 818; arterial blood collected prior to exposure served as control	24 hrs	Clinical signs, histology, blood pH, blood gas measurement	No clinical signs of toxicity, no histologic differences in tracheal or lung sections, no change in blood pH or pCO_2, minor changes in pO_2	Schaerdel et al. (1983)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male Crl:COBS CD Sprague-Dawley rats (14/group)	3, 17, 31, 117, and 505; arterial blood collected prior to exposure served as control	3 and 7 d	Hepatic cytochrome P450 content and ethylmorphine-N-demethylase activity	No dose-related change in P450 content or enzyme activity	Schaerdel et al. (1983)
Male Long-Evans rats (4/group)	70 and 212; results were compared to "control", but it was not clear if the authors were referring to historical or concurrent controls	6 hrs	Clinical signs, behavioral observation	Decreased running, decreased activity	Tepper et al. (1985)
Female Wistar rats (5/group)	0, 18, or 212	6 hrs/d for 5, 10, or 15 d	Blood ammonia, urea, glutamine, and pH; brain ammonia, glutamine; histopathology of lungs, heart, liver, and kidneys (light and electron microscopy)	Brain and blood glutamine increased; slight acidosis (i.e., decreased blood pH) at 212 mg/m^3; lung hemorrhage observed in some exposed rats	Manninen et al. (1988)
Female Wistar rats (5/group)	0, 18, or 212	6 hrs/d for 5 d	Plasma and brain ammonia and amino acid analysis	Increase in brain and plasma glutamine concentrations; increased brain/plasma ratio of threonine	Manninen and Savolainen (1989)
Female albino rats (8/group)	0, 848–1,068	3 hrs	Mortality, respiratory movement, and O_2 consumption	No deaths reported; inhibition of external respiration and decreased O_2 consumption	Rejniuk et al. (2007)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male Sprague-Dawley rats (number/group not given)	Air concentration not given; ammonia vapor added to inspiratory line of ventilator; controls exposed to same volume of room air	20 sec	Activity of upper thoracic spinal neurons	Lower airway irritation, activation of vagal pulmonary afferents and upper thoracic spinal neurons receiving pulmonary sympathetic input	(Qin et al. (2007a), 2007b))
Male rats (10/group)	0, 848–1,068 at the beginning and end of the exposure period	3 hrs	Oxygen consumption	Decreased O_2 consumption	Rejniuk et al. (2008)
Male Wistar rats (4/group)	0, 92–1,243; the preexposure period was used as the control for each animal	45 min	Airway reflexes by the changes in respiratory patterns elicited by ammonia in either dry, steam-humidified, or aqueous aerosol-containing atmospheres	Ammonia-induced upper respiratory tract sensory irritation is not affected to any appreciable extent by wet atmospheres (with or without aerosol) up to 1,243 mg/m^3	Li and Pauluhn (2010)
Mice					
Mice (20/group, species, sex not specified)	6,080–7,070; no controls	10 min	LC_{50}	LC_{50} = 7,056 mg/m^3	Silver and McGrath (1948)
Male Swiss albino mice (4/group)	5,050–20,199; no controls	30–120 min	LC_{50}	LC_{50} (30 min) = 15,151 mg/m^3	Hilado et al. (1977)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Albino mice (sex not specified; 6/dose)	Air concentration not measured; results were compared to "control", but it was not clear if the authors were referring to historical or concurrent controls	Continuously for 2 or 5 d	Regional brain metabolism (cerebral cortex, cerebellum, brainstem); MAO, enzymes of glutamate and gamma-aminobutyric acid (GABA) metabolism, and (Na^+-K^+)-ATPase; amino acid levels in the brain	Altered activities of MAO, glutamate decarboxylase, ALT, GABA-transaminase, and (Na^+-K^+)-ATPase; increased alanine and decreased glutamate	(Sadasivudu et al. (1979); Sadasivudu and Radha Krishna Murthy (1978))
Male Swiss-Webster mice (4/group)	Concentrations not given; baseline levels established prior to exposure	10 min	Reflex decrease in respiratory rate was used as an index of sensory irritation; RD_{50} = the concentration associated with a 50% decrease in the respiratory rate	RD_{50} = 214 mg/m^3	Kane et al. (1979)
Male albino ICR mice (12/dose)	0–3,436	1 hr (14-d followup)	Clinical signs, body weight, organ weight, histopathology, LC_{50}	Eye and nose irritation, dyspnea, ataxia, seizures, coma, and death; decreased body weight and increased liver to body weight ratio in mice surviving to 14 d; effects in the lung included focal pneumonitis, atelectasis, and intralveolar hemorrhage; liver effects included hepatocellular swelling and necrosis, vascular congestion; LC_{50} = 2,990 mg/m^3	Kapeghian et al. (1982)
Male Swiss-Webster mice (16–24/group)	0 or 216	6 hrs/d for 5 d	Respiratory tract histopathology	Lesions in the nasal respiratory epithelium (moderate inflammation, minimal necrosis, exfoliation, erosion, or ulceration); no lesions in trachea or lungs	Buckley et al. (1984)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male albino ICR mice (12/dose)	0, 954, 3,097, or 3,323	4 hrs	Hexobarbital sleeping time, microsomal protein content, liver microsomal enzyme activity	Increased hexobarbital sleeping time (3,097 mg/m^3), increased microsomal protein content and aminopyrene-N-deethylase and aniline hydroxylase activities (3,323 mg/m^3)	Kapeghian et al. (1985)
Male albino ICR mice (12/dose)	0, 81, or 233	4 hrs/d for 4 d	Microsomal protein content, liver microsomal enzyme activity	No dose-dependent effects on microsomal enzymes	Kapeghian et al. (1985)
Male Swiss mice (6/dose)	71 and 212; data collected during the 2 d separating each ammonia exposure served as the control baseline	6 hrs	Clinical signs, behavioral observation	Decreased running, decreased activity	Tepper et al. (1985)
Mice (sex not specified; 4/group)	3, 21, 40, or 78, lowest measured concentration was the nominal control group	2 d	Responses to atmospheric ammonia in an environmental preference chamber with four chambers of different concentrations of ammonia	No distinguishable preference for, or aversion to, different ammonia concentrations	Green et al. (2008)
Male OF1 mice (4/group)	0, 92–1,243; the preexposure period was used as the control for each animal	45 min	Airway reflexes by the changes in respiratory patterns elicited by ammonia in either dry, steam-humidified, or aqueous aerosol containing atmospheres	Ammonia-induced upper respiratory tract sensory irritation is not affected to any appreciable extent by wet atmospheres (with or without aerosol) up to 1,243 mg/m^3	Li and Pauluhn (2010)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Rabbits					
Female New Zealand White rabbits (7–9/dose)	0, 35, or 71	2.5–3.0 hrs	Lung function	Decreased respiratory rate at both concentrations	Mayan and Merilan (1972)
Rabbits (species, sex, number/dose not specified)	0, 707–14,140	15–180 min	Lung function, death	Bradycardia at 1,768 mg/m^3; arterial pressure variations and blood gas modifications (acidosis indicated by decreased pH and increased pCO_2) at 3,535 mg/m^3; death occurred at 4,242 mg/m^3	Richard et al. (1978b)
New Zealand White rabbits (sex not specified; 16 total; 8/dose)	Peak concentrations: 24,745–27,573; concurrent controls tested	4 min	Lung function, heart rate, blood pressure, blood gases	Lung injury was evident after 2–3 min (decreased pO_2 increased airway pressure)	Sjöblom et al. (1999)
Cats					
Mixed breed stray cats (sex not specified; 5/group)	0 or 707	10 min	Lung function, lung histopathology on 1, 7, 21, and 35 d postexposure	Lung function deficits were correlated with lung histopathology; acute effects were followed by chronic respiratory dysfunction (secondary bronchitis, bronchiolitis, and bronchopneumonia)	Dodd and Gross (1980)
Pigs					
Young pigs (sex not specified; 2/group)	0, 35, 71, or 106	Continuous exposure for 4 wks	Clinical signs, food consumption, body weight, gross necropsy, organ weight, histopathology	Lethargy and histopathological alterations in the tracheal and nasal epithelium were observed at 71 and 106 mg/m^3; decreased body weight occurred at all concentrations (7–19% decrease from control)	Drummond et al. (1980)
Male and female Belgian Landrace pigs (4/group)	0, 18, 35, or 71	6 d	Clinical signs, body weight, lung function	Lethargy and decreased body weight gain (all concentrations); no effect on lung microvascular hemodynamics and permeability	Gustin et al. (1994)

Table C-11. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Belgian Landrace pigs (sex not specified; 4/group)	0, 18, 35, or 71	6 d	Clinical signs, body weight, neutrophil count, and albumin in nasal lavage fluid	Nasal irritation (increased neutrophils in nasal lavage fluid) and decreased body weight gain at all concentrations	Urbain et al. (1994)
Landrace-Yorkshire pigs (sex not specified; 4/group)	0 or 42	15 min/d for 8 wks	Thromboxane A2 (TXA2), leukotriene C4 (LTC4), and prostaglandin (PGI2) production	Significant increases in TXA2 and LTC4, no significant effect on PGI2 production	Chaung et al. (2008)
Hybrid gilts (White synthetic Pietrain, white Duroc, Landrace, Large White) (14 pigs/group)	<4 (control) or 14	15 wks	Salivary cortisol, adrenal morphometry, body weight, food conversion efficiency, general health scores, play behavior; reaction to light and noise intensity tested concurrently	Decreased salivary cortisol, larger adrenal cortices, less play behavior, no measurable impact on productivity or physiological parameters	O'Connor et al. (2010)

C.4. ESTIMATING THE MEAN EXPOSURE CONCENTRATION IN THE HIGH-EXPOSURE GROUP

To estimate the mean exposure concentration in the high-exposure group, the exposure concentration was assumed to follow the lognormal distribution. This assumption is reasonable given the typically skewed nature of chemical exposures. The frequency distribution provided in Holness et al. (1989) was used to estimate the parameters (log-scale mean and standard deviation) of the lognormal distribution that best fit the data. This frequency distribution is provided in Table C-12.

Table C-12. Frequency distribution of ammonia exposure from Holness et al. (1989)

Exposure group	Interval of exposures (mg/m^3)	Interval of exposures (ppm)	Number of exposed workers
Low	0–4.4	0–6.25	34
Medium	4.4–8.8	6.25–12.5	12
High[a]	8.8–17.7	12.5–25	9
	>17.7	>25	3

[a]EPA divided the high-exposure group into two subgroups based on the statement in Holness, "Three workers were exposed to TWA concentrations of ammonia in excess of 25 ppm, the current exposure guideline."

The lognormal parameter estimates were obtained by applying the maximum likelihood method to this frequency distribution. Using the estimated distribution defined by these parameter estimates, the estimated mean exposure in the high-exposure group and 95% lower confidence bound on this mean were calculated:

mean exposure estimate = 17.9 mg/m^3
95% lower confidence bound = 13.6 mg/m^3

Using Pearson's chi-square goodness-of-fit test, the fit of the estimated lognormal distribution to the frequency distribution was determined to be plausible (p-value = 0.49). Details on the estimation methods and goodness-of-fit test are provided in the remainder of this section. All calculations were done in R, version 3.1.2; for the code used, please see U.S. EPA (2016).

Documentation of the estimation of the mean ammonia concentration in high-exposure group from Holness et al. (1989)

Assuming the data are lognormal, the log-scale mean μ and standard deviation σ were estimated using the frequency distribution in Table C-12 and the mean exposure subsequently estimated. For ease of calculation, the distribution was parametrized using $a = -\mu/\sigma$ and $b = 1/\sigma$, and the likelihood function was written in terms of a and b. Generalizing the data grouping into four intervals with non-random interval limits, assume t_1, t_2, t_3, and t_4 represent the number

of workers in the low and medium exposure groups and the two high (8.8-17.7 mg/m^3 and above 17.7 mg/m^3) exposure groups, respectively. The log-likelihood function of a and b is given by

$$\mathcal{L}(a, b; \boldsymbol{t}) = \sum_{i=1}^{4} t_i \log\left[\Phi(a + b \cdot \log x_i) - \Phi(a + b \cdot \log x_{i-1})\right],$$

where $x_0 = 0, x_4 = \infty$, and Φ is the CDF of the standard normal distribution. The log-likelihood was maximized by finding the roots of its first derivatives with respect to a and b, using the function 'nleqslv' in R ('nleqslv' package). The resulting parameter estimates were $\hat{a} = -1.23, \hat{b} = 0.970$, and thus the log-scale mean and standard deviation of the estimated lognormal distribution were $\hat{\mu} = -\hat{a}/\hat{b} = 1.27, \hat{\sigma} = 1/\hat{b} = 1.03$. Using these parameter estimates, the mean of the high exposure group is calculated from the following formula (from p. 241 of Johnson et al. (1994), with r = 1 to represent the 1st moment).

$$\hat{\mathrm{E}}(Y|Y > 8.8) = \exp\left(\hat{\mu} + \frac{\hat{\sigma}^2}{2}\right)\frac{1 - \Phi(U_0 - \hat{\sigma})}{1 - \Phi(U_0)} = 17.9 \text{ mg/m}^3,$$

where $U_0 = \frac{\log(8.8) - \hat{\mu}}{\hat{\sigma}}$.

To test the adequacy of the estimated lognormal distribution as a model of the frequency data from Holness et al. (1989), a Pearson's chi-square goodness-of-fit test was conducted. Here, the observed frequencies were set equal to the interval frequencies listed in Table C-12, and the expected frequencies were calculated under the lognormal assumption with log-scale mean $\hat{\mu}$ and standard deviation $\hat{\sigma}$. The observed and expected frequencies are listed in Table C-13.

Table C-13. Observed and expected frequencies of ammonia exposure from Holness et al. (1989)

Exposure group	Interval of exposures (mg/m^3)	Observed frequencies	Expected frequencies
Low	0–4.4	34	33.8
Medium	4.4–8.8	12	13.2
High[a]	8.8–17.7	9	7.5
	>17.7	3	3.5

[a]EPA divided the high-exposure group into two subgroups based on the statement in Holness, "Three workers were exposed to TWA concentrations of ammonia in excess of 25 ppm, the current exposure guideline."

The results of the test were

$$\chi_1^2 = 0.467, p\text{-value} = 0.49,$$

where the degrees of freedom of the test statistic were equal to (number of intervals – number of estimated parameters estimated – 1) = 1. Because the p-value of the test was above 0.05, the

lognormal fit was determined not to be inadequate for this dataset. It should be noted that because of the low degrees of freedom, the power of this test is very low.

Figure C-3 presents a histogram of the data in Table C-12 with the superimposed estimated lognormal density.

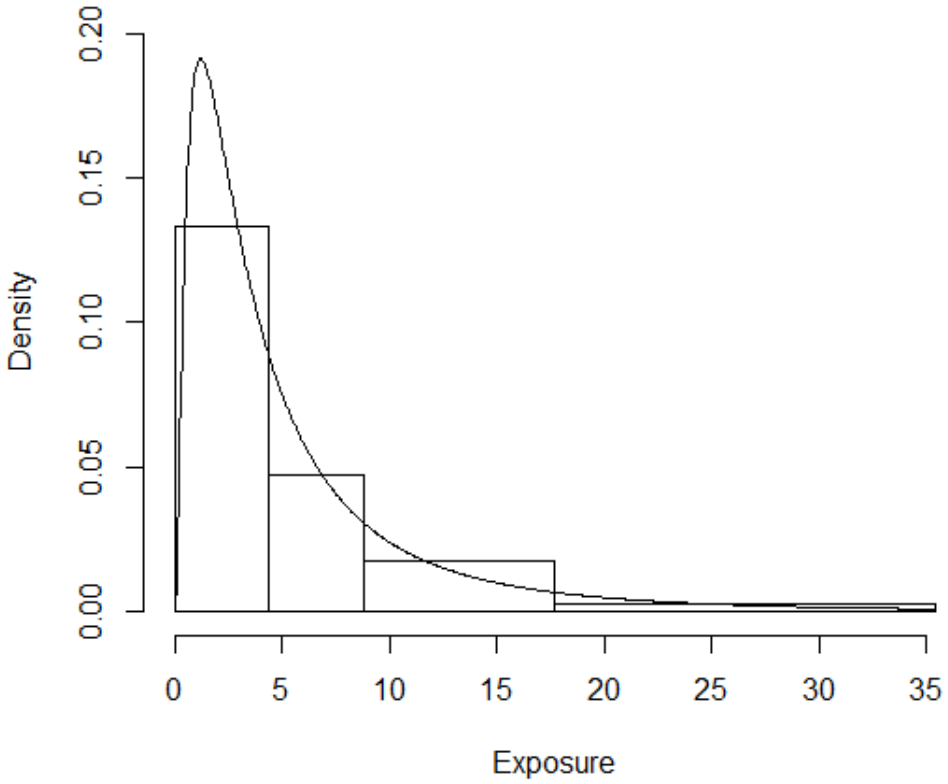

Figure C-3. Histogram of Holness.dose

To obtain a 95% lower confidence bound on the mean of the high exposure group, 10,000 bootstrap samples were randomly selected from the lognormal distribution with log-scale mean and standard deviation equal to $(\hat{\mu}, \hat{\sigma}) = (1.27, 1.03)$ from the original sample. The estimated mean exposure in the high-exposure group was calculated for each bootstrap sample using the same method as for the original sample. Specifically, each bootstrap sample was grouped into the four exposure intervals listed in Table C-12, the MLEs of the log-scale mean and standard deviation were calculated based on the group frequencies, and the estimated mean exposure in the high exposure group was calculated based on these MLEs using the mean formula presented above. The 95% lower confidence bound was set equal to the 5th percentile of the 10,000 high-exposure group mean estimates:

95% lower confidence bound = 13.6 mg/m^3.

As expected, a histogram of these means revealed high skewness, with 28 means ranging from 50 to 181 mg/m^3 and the remaining means less than 50 mg/ m^3. Figure C-4 is a histogram of the estimated mean exposures from the bootstrap samples. To alleviate the bunching of data points on the low end, the 28 means that exceeded 50 mg/m^3 were omitted from the histogram.

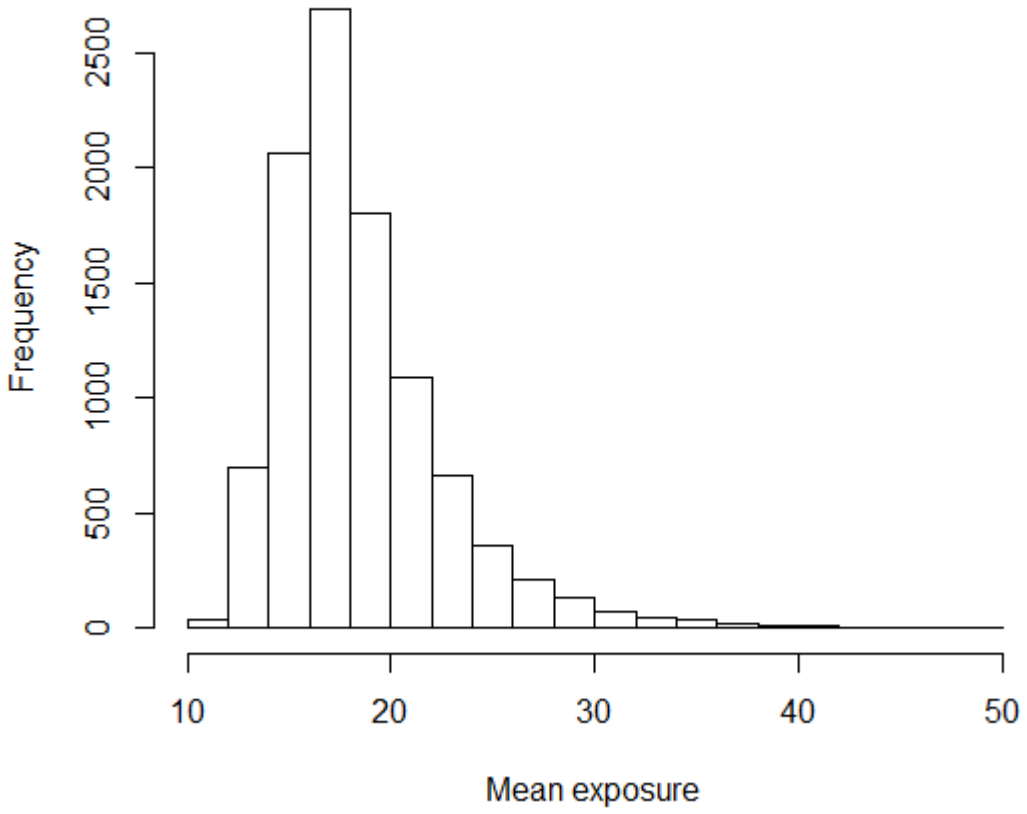

Figure C-4. Histogram of mean exposures in high-exposure group

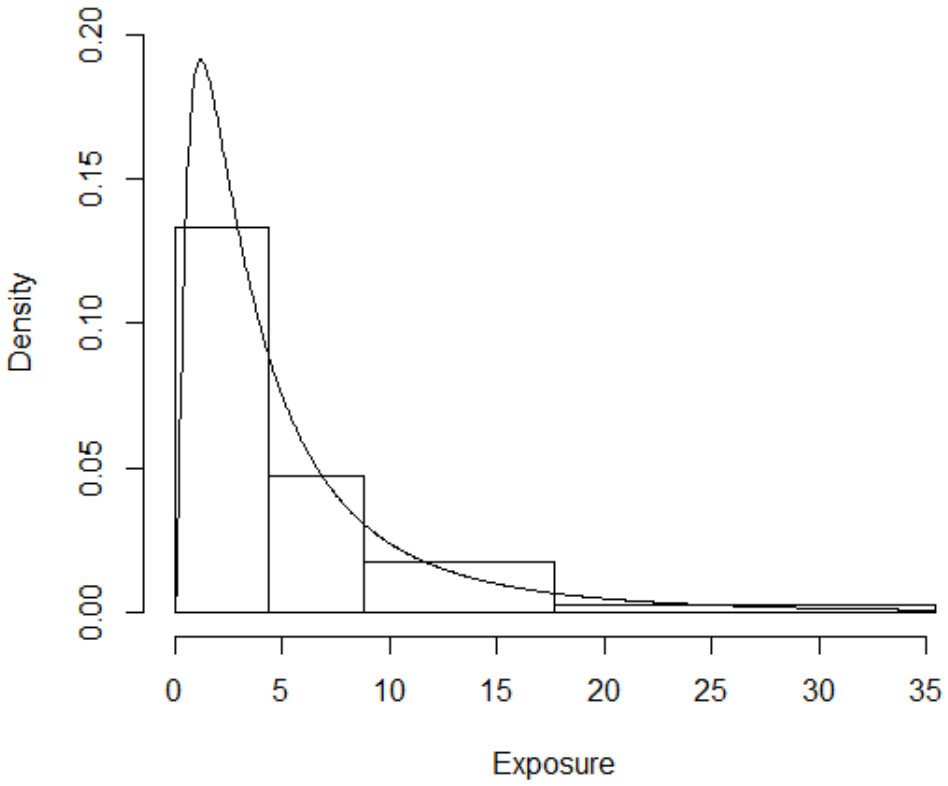

Figure C-3. Histogram of Holness.dose

To obtain a 95% lower confidence bound on the mean of the high exposure group, 10,000 bootstrap samples were randomly selected from the lognormal distribution with log-scale mean and standard deviation equal to $(\hat{\mu}, \hat{\sigma}) = (1.27, 1.03)$ from the original sample. The estimated mean exposure in the high-exposure group was calculated for each bootstrap sample using the same method as for the original sample. Specifically, each bootstrap sample was grouped into the four exposure intervals listed in Table C-12, the MLEs of the log-scale mean and standard deviation were calculated based on the group frequencies, and the estimated mean exposure in the high exposure group was calculated based on these MLEs using the mean formula presented above. The 95% lower confidence bound was set equal to the 5th percentile of the 10,000 high-exposure group mean estimates:

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As expected, a histogram of these means revealed high skewness, with 28 means ranging from 50 to 181 mg/m^3 and the remaining means less than 50 mg/ m^3. Figure C-4 is a histogram of the estimated mean exposures from the bootstrap samples. To alleviate the bunching of data points on the low end, the 28 means that exceeded 50 mg/m^3 were omitted from the histogram.

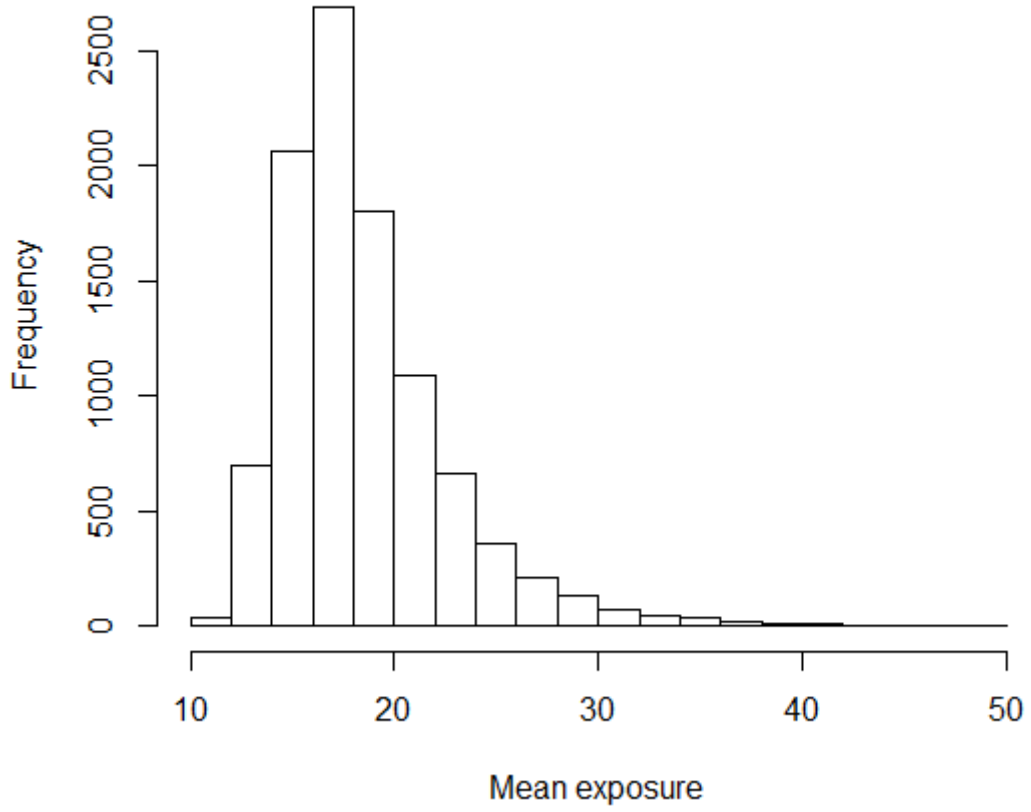

Figure C-4. Histogram of mean exposures in high-exposure group

APPENDIX D. SUMMARY OF SAB PEER REVIEW COMMENTS AND EPA's DISPOSITION

The draft *Toxicological Review of Ammonia,* dated August 2013, underwent a formal external peer review in accordance with EPA guidance on peer review (U.S. EPA, 2006). This peer review was conducted by the Chemical Assessment Advisory Committee (CAAC) Augmented for the IRIS Ammonia Assessment (CAAC Ammonia panel) of EPA's Science Advisory Board (SAB). An external peer review workshop was held on July 14–16, 2014. Public teleconferences of the CAAC Ammonia panel were held on December 17 and 19, 2014, to discuss the Panel's draft review report. The SAB held a public teleconference on June 8, 2015 to conduct a quality review of the draft peer review report. The final report of the SAB was released in August 2015 (U.S. EPA, 2015).

The SAB was tasked with providing feedback in response to charge questions related to the hazard identification and dose-response assessment of ammonia, as well as EPA's implementation of recommendations of the National Research Council (NRC) for improving the development of IRIS assessments. A summary of the SAB's major recommendations, and EPA's responses to these recommendations, follows and is organized by charge question. In addition, the SAB offered editorial suggestions to improve the clarity of specific portions of the text; changes in response to these editorial suggestions were incorporated in the Toxicological Review as appropriate and are not included below in the summary of major SAB recommendations.

The SAB generally commended EPA for progress in implementing NRC's recommendations and the new document structure for IRIS toxicological reviews. The SAB concurred with the selection of the study used to derive the inhalation RfC, with respiratory effects as the critical effect, and with the application of uncertainty factors (UFs), but offered recommendations related to the identification of the point of departure (POD) for the RfC. Changes to the POD resulted in an increase in the RfC from 0.3 to 0.5 mg/m^3 (see Charge Questions E2 and E3). In response to SAB recommendations on the evaluation of the toxicity of ingested ammonia, the scope of this assessment was revised to contain evaluation of the toxicity of inhaled ammonia only. An evaluation of ammonia's oral toxicity will be conducted as a separate assessment in order to expand the evaluation to include a systematic review of the ammonium salts literature (see Charge Question D1).

Charge Question 1: NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to describe more fully the methods of the assessment. NRC stated that they were "not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise statements of criteria used to exclude, include, and advance studies for derivation of [toxicity values]." Please comment on whether the new

Preamble provides a clear, concise, useful and objective description of the guidance and methods that EPA uses in developing IRIS assessments.

Comment: The SAB commended EPA for the progress made thus far in implementing the NRC's recommendations for the IRIS Program. The Panel observed that the Preamble is a "work in progress" that goes a long way to providing a clear, concise, useful, and objective summary of the complex set of guidance and methods that EPA uses in developing IRIS assessments. The SAB recommended that the Preamble should make clear that it does not establish new policy and that it is generic and some elements are not necessarily applicable to the ammonia assessment. Other specific recommendations for this assessment related to the Preamble included the following:

- Section 6 (Selection of studies for derivation of toxicity values) would benefit from elaboration and the addition of citations to relevant EPA guidance documents. EPA should clarify how the factors used to select the studies for the derivation of toxicity values are balanced against each other or against other factors not listed.
- EPA should confirm that all relevant guidance documents are included.
- EPA should describe the process for peer reviewing articles not previously peer reviewed.
- EPA should clarify which "ethical standards" are considered (Page xvi, lines 3-5).
- EPA should consider whether assessments should provide ranges for typical levels of exposure or intake for comparison to estimated doses or concentrations.
- The statement on page xx, line 26–30 needs to be revised such that the scientific quality of studies is foremost in assessing credibility.
- The role of NRC (2001, 2014) in the IRIS protocol development process should be mentioned.

Response: The IRIS program has substantially revised the Preamble based on: 1) experience with implementing the new document structure and systematic review procedures after the ammonia assessment was submitted for SAB review in 2013; 2) recommendations from SAB reports on the draft assessments for ammonia and trimethylbenzenes, and 3) comments from EPA's program and regional offices, other federal agencies and the Executive Office of the President, and the public.

The revised Preamble reflects recommendations for a shorter section, and some information previously in the Preamble is now discussed in the Toxicological Review (e.g., literature searching, screening, and study evaluation) or in the upcoming IRIS Handbook of Operating Procedures for Systematic Review of Environmental Health Hazards ("IRIS Handbook") being developed by the IRIS Program. The Preamble begins with a new statement that it summarizes general principles and systematic review procedures. Section 1 now states that "[t]his Preamble summarizes and does not change… EPA guidance," addressing the SAB recommendation that EPA make clear that the Preamble does not establish new policy. In place of summaries of specific citations to EPA guidance documents, the Preamble directs users to links to relevant guidance documents on the IRIS Program website. In response to the SAB recommendation that the Preamble clarify that it is generic and that some elements are not necessarily applicable to the

ammonia assessment, Section 9 of the Preamble states that "[t]he Preface also identifies assessment-specific approaches that may differ from the general approaches outline in this Preamble." New text in the Preface of the ammonia assessment describes features of the assessment that differ from those outlined in the Preamble. Finally, with a shorter, refocused Preamble, some of the text that was the subject of specific SAB recommendations no longer appears in the Preamble. Several of the specific SAB recommendations, including identification of relevant EPA guidance documents, reference to implementation of NRC recommendations, and extensive consideration of study quality as part of IRIS procedures for systematic review, are addressed in the upcoming IRIS Handbook.

Charge Question 2: NRC (2011) provided comments on ways to improve the presentation of steps used to generate IRIS assessments and indicated key outcomes at each step, including systematic review of evidence, hazard identification, and dose-response assessment. Please comment on the new IRIS document structure and whether it will increase the ability for the assessments to be more clear, concise, and easy to follow.

The SAB observed that the new format used for the ammonia assessment is a refreshing improvement over the old format, and evidence of EPA's commitment to a stepwise implementation of the NRC's recommendations for systematic review. The SAB further observed that the ammonia assessment has not fully implemented the systematic review envisioned by the NRC, but that the NRC/IOM approach is not a directive and is expected to need modification to address issues that EPA faces as implementation progresses. The SAB anticipated that refinements would be forthcoming in future assessments. Specific recommendations offered by the SAB related to the ammonia assessment are summarized below.

Comment: A clearer statement of how the main text reviews are intended to be different from the appendix summaries should be provided.

Response: A statement describing how the synthesis of health effects information in the main text relates to the study summaries provided in appendices in the Supplemental Information was added to the beginning of Section 1.2, Synthesis of Evidence.

Comment: The SAB observed that the bulk of study descriptions was presented in appendix summaries, and that it was cumbersome to refer back and forth between the main text and Supplemental Information when looking for specific details. The SAB suggested that hyperlinks between the main text and Supplemental Information be added to facilitate referring between the two documents.

Response: Ammonia health effect studies that appear in the Supplemental Information were developed as part of the draft assessment that used the "old" toxicological review format, i.e., the

format used by the IRIS Program before implementing the NRC recommendations for improving the structure of the toxicological review. Based on experience with the new document structure after the ammonia assessment was released for peer review, separate study summaries will not be included in the Supplemental Information in the future. For historical reasons, the EPA retained previously developed study summaries, without hyperlinks, for this assessment only.

Comment: A more detailed description and evaluation of the principal study, Holness et al. (1989), should be provided in the main assessment.

Response: The description of the Holness et al. (1989) study was expanded as described further in response to recommendations under Charge Question C1.

Comment: EPA should continue to work on efficiently summarizing and presenting data through tables and figures. It would be helpful to indicate study quality in the tables and figures, or present only studies that met clearly stated minimal criteria. By way of example, the SAB recommended that EPA tag the Anderson et al. (1964) study in Figure 1-1 as a weak study or omit the study from the figure.

Response: EPA is continuing to work on efficiently and transparently summarizing health effects evidence in tables and graphs; these changes will be reflected in future IRIS assessments. These changes include increased use of graphics to summarize health effect data and results of the systematic review of study evaluation for epidemiology studies. EPA is also exploring alternative approaches for documenting study quality, including the addition of study quality information to evidence tables. EPA notes that some methodologic features relevant to study quality (e.g., number of exposure groups, group sizes) are summarized in the current ammonia evidence tables.

The evaluation of animal toxicity studies of ammonia was revised to provide a more explicit framework by which individual studies were evaluated, including considerations related to test animals, experimental design, exposure characterization, endpoint evaluation, and results presentation (see Literature Search Strategy | Study Selection and Evaluation). Text documenting the outcome of this evaluation was added, including discussion of the limitations of the Anderson et al. (1964) study. The representation of this study in the evidence tables was revised to more accurately reflect the number of animals used. Anderson et al. (1964) was retained in the evidence tables and in the exposure-response array, but was given less weight in the synthesis of evidence, along with other studies with similar limitations.

Comment: Consideration should be given to moving appropriate kinetic or absorption/distribution/metabolism/elimination (ADME) information into the main text from the appendices if it is used in selection and weighing of studies, RfC/RfD derivation, or other key steps in the assessment.

Response: A new section (Section 1.1, Overview of Chemical Properties and Toxicokinetics) moves important information from the Supplemental Information document to the main document. In addition, an overview of key toxicokinetic information that provides useful context for evaluating the health effects of ammonia was provided in this new section. More detailed information on ammonia toxicokinetics was retained in the Supplemental Information.

Charge Question 3: NRC (2011) states that "all critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated" and that "strengthened, more integrative and more transparent discussions of weight of evidence are needed." NRC also indicated that the changes suggested would involve a multiyear process. Please comment on EPA's success thus far in implementing these recommendations.

Comment: The SAB observed that the ammonia assessment is "an excellent first step" in addressing NRC's recommendations, although there is "still terrain to cover." The NRC recommended that a standardized approach be adopted to provide more transparency and clarity for future assessments.

Response: The NRC anticipated that implementing their recommendations would be a multiyear process. EPA is continuing to make progress in fully implementing systematic review methods in new IRIS assessments that are in the problem formulation or early draft development steps. This includes the consistent application of study exclusion/inclusion criteria, methods to systematically evaluate study quality, and transparent integration of evidence. Assessments further along in the IRIS process, such as the ammonia assessment, incorporated elements of systematic review methods, as well as other improvements such as streamlining the document structure and increased incorporation of tables, figures, and exposure-response arrays. Future assessments will reflect greater implementation of systematic review.

Charge Question 4: EPA solicited public comments on the draft IRIS assessment of ammonia and has revised the assessment to respond to the scientific issues raised in the comments. A summary of the public comments and EPA's responses are provided in Appendix G of the Supplemental Information to the Toxicological Review of Ammonia. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed by EPA.

The SAB noted that, in general, EPA adequately and appropriately addressed the scientific issues raised by public commenters, and provided adequate scientific justification for the Agency's conclusions. Specific public comments that the SAB considered deserved further attention are summarized below.

Comment: EPA should attempt to obtain data from Dr. Holness in order to determine a

representative exposure concentration from the NOAEL study group, and then elaborate their response to this recommendation.

Response: EPA contacted the office of Dr. Linn Holness at St. Michael's Hospital in Toronto, Canada, in February 2015 and learned that no original data from the study were retained. In the absence of individual subject data, EPA re-analyzed the findings in the published paper to calculate a central estimate of the high-exposure group (see further discussion in response to recommendations related to the RfC under Charge Question E2).

Comment: EPA should consider expanding Appendix A to include other U.S. and international exposure guidelines (e.g., TLVs and AEGL-1 values), including their definition, purpose, and links to the assessments that explain the rationale for the guidelines and chemical-specific documentation that supports them.

Response: Table A-1 in Appendix A was expanded to include more information and links to toxicity values developed by other national and international health agencies.

Charge Question A1: Please comment on whether the conclusions have been clearly and sufficiently described for purposes of condensing the Toxicological Review information into a concise summary.

The SAB observed that the Executive Summary was too vague and unclear in some of the subsections. The SAB specifically recommended the following.

Comment: A section should be included at the beginning of the Executive Summary that provides information on the chemistry of ammonia, ammonium, and ammonium salts and the rationale for excluding or including ammonium salts.

Response: A brief summary of the chemical properties of ammonia was added to the Executive Summary. The scope of this assessment was revised to include the inhalation route of exposure only. A full evaluation of the complexities associated with ingestion of ammonium salts will be considered in a separate assessment (see Charge Question D1).

Comment: The discussion of noncancer effects from inhalation exposure should be placed before the discussion of oral exposures if an RfD is not derived, and the first sentence of the noncancer oral section should indicate that an oral RfD was not derived.

Response: As indicated above, an oral RfD will be considered in a separate assessment (see Charge Question D1).

Comment: A brief discussion of the weight of evidence of critical epidemiology studies should be provided by adding descriptors for the nature of effects measured (e.g., self-reported versus clinical examination) and a brief discussion of how each key epidemiology study used for RfC derivation controlled for potential confounding effects of co-exposures to other chemicals or particulate matter that might cause similar respiratory effects.

Response: The Executive Summary was revised by providing information on outcome measurement (e.g., self-report), magnitude of lung function changes, and potential co-exposures.

Comment: Description of the evidence that ammonia may act as a cancer promoter should be expanded.

Response: As indicated below under Charge Question C3, carcinogenicity will be addressed in a separate assessment on the oral route of exposure.

Comment: EPA should consider including parts of the discussion of the actual study data relevant for asthmatics as a susceptible population from Section 1.3.2.

Response: A brief summary of the nature and extent of the evidence for asthmatics as a susceptible population was added to the Executive Summary.

Comment: In the gray summary box of the Executive Summary, EPA should indicate that there is inadequate information to evaluate the carcinogenicity of ammonia or to derive an oral RfD for ammonia.

Response: Evaluation of the toxicity of ammonia via oral exposure, including carcinogenicity, will be addressed in a separate assessment (see Charge Questions C3 and D1).

Charge Question B1: The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection and Evaluation section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g., study quality, risk of bias) used for the selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of ammonia.

Comment: The SAB observed that, overall, the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment were fairly well described and supported,

and incorporated elements of systematic review; however, several areas needed further clarification and strengthening. The SAB encouraged EPA to incorporate and implement recommendations from both NRC reports as much as reasonably possible given time constraints. The SAB recognized that some of the weaknesses regarding the application of literature search and evaluation protocols identified by the panel may reflect EPA's progress in implementing past and more recent NRC recommendations, or insufficient clarity as to the extent and mechanisms for their application in the ammonia assessment. The SAB recommended that EPA accelerate the development of standardized, detailed literature search and evaluation protocols specific to IRIS objectives. Specific recommendations of the SAB related to literature search and study selection follow in the comments below.

Response: As already noted, future assessments will reflect greater implementation of systematic review. IRIS assessments that are currently in the problem formulation or early draft development steps will include the development and application of protocols for literature searching, literature screening, and evaluating studies, and transparent documentation of the results of the literature search, literature screening, and study evaluation. The literature search strategy section of the ammonia assessment was revised to more transparently present the approach for study identification and screening. (See responses to specific recommendations below for more information on enhancements to documentation of the literature search strategy for ammonia).

Comment: The list of databases included in the literature search should be expanded. The SAB agreed with EPA's objective of using the literature supporting ATSDR's *Toxicological Profile* to reduce unnecessary duplication of effort across agencies, but stated that it was unclear if and to what extent ATSDR's literature search strategy incorporated principles of systematic review. As such, ATSDR's literature search should not be deemed directly transferrable to the EPA's assessment without further clarification.

Response: The reference list in ATSDR's Toxicological Profile (ATSDR, 2004) was examined to ensure that the search using on-line databases did not miss any health effect studies. In addition, ATSDR's *Toxicological Profile for Ammonia* was used, in particular, to identify toxicokinetic studies. The literature on ammonia toxicokinetics is extensive because of ammonia's importance in nitrogen homeostasis and acid-base balance. ATSDR's Toxicological Profile was used to facilitate the identification of key toxicokinetic literature. Use of ATSDR's Toxicological Profile in the literature search section was clarified.

The initial literature search had included other databases and resources (e.g., EPA's Office of Pesticide Program chemical search, Organization of Economic Co-operation and Development's High Production Volume (HPV) chemical database, EPA's High Production Volume Information System (HPVIS), and the NIOSH Registry of Toxic Effects of Chemical Substances database) to augment the search of the core computerized databases (PubMed, Toxline, TSCATS, HERO, WOS, and Toxcenter), but failed to include documentation of these databases and resources.

Documentation of these searches was added to the Supplemental Information (see Appendix B, Table B-2), and reference to the search of these databases/resources was added to the Literature Search Strategy | Study Selection and Evaluation section.

Comment: The SAB recommended that inclusion/exclusion criteria be made more transparent, to provide insight as to why some apparently relevant publications were not included or cited (e.g., Mirabelli et al. (2007)). In addition, the SAB encouraged EPA to consider publications beyond March 2013 (e.g., Hovland et al. (2014)).

Response: The literature search section was rewritten to more clearly describe the approach used to identify and screen the ammonia literature. Figure LS-1 (literature search and screening flow diagram) was revised, adding a table (Table LS-1) of inclusion and exclusion criteria used to screen studies. Also included was a modified set of criteria for the post-SAB literature search update. Discussion of the focused search of literature on cleaning and hospital workers was moved from the Supplemental Information to the main document, and documentation of this search was added to the Supplemental Information. Also included in this table is a disposition of each study based on inclusion/exclusion criteria. As noted in this table, the study by Mirabelli et al. (2007) was previously identified, but excluded because of a lack of ammonia-specific data. An updated literature search was conducted in September 2015. Eight new epidemiology studies were retrieved in the literature search update and screen; five of the eight were excluded from further consideration because they were reviews that did not contain primary data or were determined to be uninformative. The three studies studies added to the assessment did not substantively changed conclusions about ammonia hazard. No new animal toxicity studies were identified in the literature search update. Documentation of the post-SAB updated literature search, including the disposition of epidemiological studies identified in this updated search, was added to the Supplemental Information (Table B-5).

Comment: The exclusion of ammonium salts should be supported by a thorough and systematic review of the relevant literature. If a systematic search was done, EPA should indicate this clearly in the description of search criteria and in Appendix C of the Supplemental Information. The SAB suggested that the rationale for excluding ammonium salts could be buttressed by adding data on LC_{50} and LD_{50} values for various ammonium salts to show the variability in response.

Response: The scope of the current assessment was revised to include inhalation only. A systematic review of the ammonium salts literature will be conducted as part of a separate oral assessment (see also Charge Question D1).

Comment: The description of studies in Appendix C (previously Appendix E in the revised external review draft) should be made uniform across all types of studies. The SAB also noted that it would be useful to provide hyperlinks between citations and Appendix C (previously Appendix E in the

revised external review draft) summaries in electronic versions of the assessment and supporting information.

The outline for describing key study characteristics according to the criteria and major limitations (as listed in Tables D-2 to D-4) that was applied to studies of health care/cleaning and livestock farming settings (pp. xlii-xliii) should also be applied to the industrial studies. The SAB also recommended that the outline for the narrative be made uniform, with attention paid to describing the range of different co-exposures present in the various types of study settings.

Response: The formats of the summary tables of human evidence in the Supplemental Information (Tables C-7, C-8, and C-9 in Appendix C; previously Appendix E in the revised external review draft) were revised to be consistent. Based on experience gained with the new structure for IRIS toxicological reviews, such detailed study summaries will not be provided in future IRIS assessments, and the EPA retained previously developed study summaries, without hyperlinks, in this assessment (see also response under Charge Question 2).

The evaluations of studies of industrial settings, health care/cleaning settings, and agricultural settings in the Literature Search Strategy | Study Selection and Evaluation section were based on the same key study characteristics. To make it clearer that these same characteristics were evaluated across all studies, subheadings corresponding to each evaluation aspect (e.g., participant selection, exposure parameters, outcome measurements, confounding) were added to the evaluation of studies in cleaning and agricultural settings consistent with the evaluation of industrial studies. Information on potential co-exposures is summarized in the evaluation of individual epidemiology studies in Tables B-6 to B-8 in Appendix B and is discussed in the study evaluation section and in the synthesis of effects of ammonia on the respiratory system (Section 1.2.1).

Comment: The potential contribution to ammonia exposure from cigarette smoke and the varying levels of ammonia in tobacco and cigarette smoke should be described. The panel specifically cited Seeman and Carchman (2008).

Response: A brief description of the potential contribution of ammonia exposure from tobacco smoke and the varying levels of ammonia in tobacco and cigarette smoke was added to the discussion of confounding in the Literature Search Strategy | Study Selection and Evaluation section. As discussed in this section, potential confounding by smoking of ammonia-containing tobacco or by inhaling tobacco smoke was not considered to be a major limitation of the occupational epidemiology studies because smoking as a potential confounder was adequately addressed in the studies that examined effects on the respiratory system.

Comment: The criteria by which EPA determines the acceptability of studies and the significance of specific study limitations should be clarified. The SAB recommended including a summary of the consistency of exposures, confounders, and outcomes across categories of studies, including

relevant findings from the epidemiology studies.

Response: A discussion of what constitutes major and minor limitations was added to the Literature Search Strategy | Study Selection and Evaluation section in the Considerations for Evaluation of Epidemiology Studies subsection.

EPA considered the consistency of findings across three categories of studies (industrial, cleaner, and agricultural settings) that differed in population characteristics, level and pattern of exposure, and potential confounders as adding strength to the evidence for an association between respiratory effects and ammonia exposure. Rather than add this observation to the Literature Search Strategy | Study Selection and Evaluation section, discussion of the consistency in respiratory findings across different categories of studies was added to the synthesis of evidence of ammonia as a respiratory hazard (Section 1.2.1) and to the Executive Summary.

As discussed in response to recommendations under Charge Question 2, the critical evaluation of animal toxicity studies of ammonia was revised by providing a more explicit framework by which individual studies were evaluated (e.g., considerations related to test animals, experimental design, exposure characterization, endpoint evaluation, and results presentation). EPA is developing approaches to systematic evaluation and documentation of study quality, and these will be reflected in future assessments.

Comment: EPA should clarify why requests for additional data from the public were not extended beyond 2009.

Response: Federal Register notices specifically soliciting public input on ammonia were published in 2007 and in 2009. In addition, EPA encourages the public to submit information throughout the assessment development process for all IRIS assessments. For example, the announcement of the 2012 IRIS agenda (77 FR 26751, May 7, 2012) reiterated that the public may submit information on any chemical substance at any time. The text in the literature search section was revised to indicate that the request for data from the public was broader than the two Federal Register notices published in 2007 and 2009.

Charge Question C1: A synthesis of the evidence for ammonia toxicity is provided in Chapter 1, Hazard Identification. Please comment on whether the available data have been clearly and appropriately synthesized for each toxicological effect. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically supported.

The SAB stated that the scientific evidence for respiratory effects is sufficiently robust to support the conclusion that ammonia induces respiratory effects in humans and animals. Recommendations related to improving the synthesis of evidence were as follows.

Comment: The SAB recommended more precise documentation of how evaluation criteria were applied to individual studies and ultimately integrated into the weight of evidence analysis, and suggested that these revisions be included in tabular summaries. Additionally, the SAB recommended including a more detailed description of Holness et al. (1989) in support of the RfC, and a brief summary of acute and short-term studies that identify ammonia as an irritant and toxicant to the upper respiratory tract (and the eye).

Response: In the peer review draft of the assessment, specific methodological features of individual epidemiology studies were systematically evaluated (including selection of study participants, outcome measurement, exposure parameters, confounding, and statistical analysis) (see Literature Search Strategy | Study Selection and Evaluation section). Documentation of the evaluation of animal toxicity studies was expanded in the Study Selection and Evaluation section by adding Table LS-3, which provides the framework used to evaluate individual animal studies, and text that reflects the application of this framework (including considerations related to test animals, experimental design, exposure, endpoint evaluation, and results presentation) to the ammonia literature.

Section 2.1.1 was revised to provide more detailed descriptions of the Holness et al. (1989) study and the three other cross-sectional studies that provided information useful for evaluating the relationship between chronic ammonia exposure and respiratory effects, as well as further discussion of key strengths and limitations in the individual studies considered for quantitative analysis for the RfC. The evidence pertaining to ammonia as a respiratory tract irritant following acute exposure is discussed in Section 1.2.1 under "Respiratory Symptoms."

Comment: The SAB recommended that the biological bases for tolerance/adaptation be considered as part of the evaluation, and discussed in the context of exposure to ambient ammonia (NH_3) gas. The integration of tolerance into the evaluation should be differentiated from "healthy worker" issues or independent host factors also known to influence the response and sensitivity to inhalable irritants. Three papers on ammonia tolerance were identified for consideration: Von Essen and Romberger (2003), Lavinka et al. (2009), and Petrova et al. (2008).

Response: Section 2.1.4, Uncertainties in the Derivation of the Reference Concentration, was revised to include a discussion of the potential for underestimation of response to ammonia in the general population based on findings in worker-exposed populations as a result of development of tolerance and "healthy worker" bias. The discussion of potential for developing tolerance following repeated exposure to ammonia relied on studies by Ihrig et al. (2006) and Ferguson et al. (1977), two papers that specifically addressed habituation to ammonia. The contribution of Von Essen and Romberger (2003), Lavinka et al. (2009), and Petrova et al. (2008) in examining tolerance to ammonia was limited compared to Ihrig et al. (2006) and Ferguson et al. (1977). As a result, these papers were not included for the following reasons: (1) Von Essen and Romberger (2003) focused on adaptation of workers to repeated exposure to endotoxin in swine confinement barns, and did

not specifically address effects of ammonia; (2) Lavinka et al. (2009) examined the natural lack of neuropeptides in naked mole-rats as a mechanism for adaptation to a subterranean environment with high levels of ammonia; this paper was considered less relevant than the available human studies; and (3) Petrova et al. (2008) evaluated the irritation potential of ammonia in asthmatics and healthy volunteers, but did not examine habituation to ammonia in either population.

Comment: The SAB recommended that gastrointestinal effects of ammonia be re-examined as part of a more integrated evaluation of the *in vivo* biological properties of ammonia (e.g., Bodega et al. (1993)).

Response: The evidence for an association between ammonia exposure and gastrointestinal effects will be re-examined as part of a separate health assessment of ingested ammonia (see Charge Question D1).

Charge Question C2: Does EPA's hazard assessment of noncancer human health effects of ammonia clearly integrate the available scientific evidence (i.e., human, experimental animal, and mechanistic evidence) to support the conclusion that ammonia poses a potential hazard to the respiratory system?

Comment: The SAB observed that the scientific evidence supporting the conclusion that ammonia poses a potential hazard to the respiratory system was well-integrated. However, the SAB recommended expanding the evaluation of the chemical reactions and ammonia generation that may impact gastrointestinal endpoints and their impact on the decision not to derive an RfD.

Response: As noted above, EPA agrees with expanding the evaluation of ammonia's oral toxicity to include a systematic review of the ammonium salts literature. This will be conducted as a separate assessment (see Charge Question D1).

Charge Question C3: Does EPA's hazard assessment of the carcinogenicity of ammonia clearly integrate the available scientific evidence to support the conclusion that under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is "inadequate information to assess the carcinogenic potential" of ammonia?

Comment: The SAB stated that the scientific evidence supported the conclusion that there is inadequate information to assess the carcinogenic potential of ammonia, and agreed that the evidence presented by Tsujii et al. (1993) suggesting ammonia exhibits tumor-promoting properties is limited. The SAB recommended that the EPA expand on the strengths and weaknesses of the following two relevant lines of evidence: (1) an epidemiologic study regarding promoter influences Fang et al. (2011); and (2) an animal study reporting increased numbers of

adenocarcinomas following exposure to ammonium acetate via intra-rectal infusions (Clinton et al., 1988).

Response: Information on the carcinogenic potential of ammonia comes from oral exposure studies. Therefore, the assessment of ammonia carcinogenicity, including the two studies identified for consideration by the SAB, will be addressed in a separate assessment of the health effects of ingested ammonia (see Charge Question D1).

Charge Question D1: Please comment on whether the rationale for not deriving an RfD is scientifically supported and clearly described (see Section 2.1). Please comment on whether data are available to support the derivation of an RfD for ammonia. If so, please identify these data.

Comment: The SAB offered the following recommendations related to EPA's decision not to derive an RfD:

- EPA should thoroughly re-evaluate the publications to determine if they should continue to exclude ammonium salts from the IRIS assessment, or explicitly expand the scope of the assessment to include the ammonium ion with ammonia. The rationale and presentation of data to support their conclusions need to be strengthened.
- EPA should evaluate the relevant toxicity studies of ammonium salts as studies that could additionally inform consideration of gastrointestinal effects and to determine if they offer valuable information for the derivation of an RfD. In particular, the panel pointed to the study by Lina and Kuijpers (2004), which included a Cl^- control.
- A decision to address ammonium salts would require further evaluation of the RfC and the impact of the inhalation of ammonium-containing airborne particulate matter.

Response: EPA agrees with expanding the evaluation of ammonia's oral toxicity to include a systematic review of the ammonium salts literature. This will be conducted as a separate assessment. Specific SAB recommendations related to the evaluation of the health effects of ingested ammonia will be addressed in this separate assessment.

Response to the SAB recommendation to evaluate the impact of inhaling ammonium-containing airborne particulate matter on the RfC is addressed in response to comments on the RfC (see response to recommendations under Charge Question E1).

Charge Question D2: As described in the Preface, data on ammonia salts were not considered in the identification of effects of the derivation of an RfD for ammonia and ammonium hydroxide because of concerns about the potential impact of the counter ion on toxicity outcomes. Please comment on whether the rationale for this decision is scientifically supported and clearly described.

Comment: The SAB recommended that the rationale for the decision not to derive an RfD be better supported and more clearly detailed, especially given lack of clarity about the chemistry of ammonia/ammonium, and given the existence of at least one study of ammonium that appears to have adequately controlled for the possible toxicity of the counter ion (Lina and Kuijpers, 2004).

Response: As discussed in response to recommendations provided under Charge Question D1, this will be addressed in a separate assessment.

Charge Question E1: Please comment on whether the evaluation and selection of studies and effects for the derivation of the RfC is scientifically supported and clearly described (see Section 2.2.1). Please identify and provide the rationale for any other studies or effects that should be considered.

The SAB observed that the evaluation of studies was clearly described in the supplementary materials and concisely summarized in the main assessment. Specific comments and recommendations related to study selection and evaluation for deriving the RfC were the following.

Comment: A better description of the controlled human studies should be provided and the rationale for their exclusion strengthened.

Response: Section 2.1.1 was expanded to include the rationale for not using controlled human exposure studies for dose-response analysis, i.e., that the short exposure durations used in these studies (15 seconds to 6 hours) make them inappropriate for evaluating the effects of chronic exposure to ammonia.

Comment: Further discussion of the potential implications of reversibility and long-term attenuation of effects through acclimatization and/or the healthy worker effect (e.g., self-selected attrition due to respiratory symptoms) should be added.

Response: As noted under Charge Question C1, Section 2.1.4, Uncertainties in the Derivation of the Reference Concentration, was revised to include a discussion of the potential for underestimation of response to ammonia in the general population as a result of development of tolerance and "healthy worker" bias in worker-exposed populations.

Comment: The EPA should elaborate on its rationale for the selection of self-reported respiratory symptoms and small subclinical changes in lung function measures as "adverse" health outcomes.

Response: Discussion of the use of self-reported respiratory symptoms and small subclinical changes in lung function measures as adverse health outcomes was expanded in Section 2.1.1 by referring to what the American Thoracic Society considers as adverse respiratory health effects in

the context of air pollution. These considerations distinguish between lung function changes that may be clinically significant at the individual level and those that may be significant at the population level. Small changes in the distribution of pulmonary function can result in a proportion of the exposed population shifted down into the lower "tail" of the pulmonary function distribution.

Comment: It was unclear if the quality of the 1989 Holness study overrode other factors listed in the Preamble for selection of a key study, especially considering that the Ballal et al. (1998) and Rahman et al. (2007) studies could be used to derive BMDs, which the Preamble indicates is preferred over the NOAEL/LOAEL approach. The role in study selection of any differences in outcome measures and of confounding controls among these studies was also unclear.

Response: Documentation of the factors considered in evaluating the quality of individual epidemiologic studies is provided in Appendix B, Tables B-6 to B-8, and discussed in the study evaluation section. For dose-response analysis, EPA determined that the overall coherence in the set of industrial studies of ammonia supported derivation of an RfC. Factors considered in selecting the NOAEL from Holness et al. (1989) as the basis for the RfC included exposure characterization, outcome measures, and potential for confounding. Specifically, the Holness et al. (1989) study was selected over the studies by Ballal et al. (1998) and Rahman et al. (2007) because of higher confidence in measurement of ammonia exposure, evaluation of both respiratory symptoms and lung function parameters, smaller potential for co-exposures to other workplace chemicals, and the fact that the estimated NOAEL for respiratory effects was the highest of the NOAELs estimated from the candidate principal studies. Section 2.1.1 was revised to provide a more transparent discussion of considerations weighed in selecting studies for dose-response analysis (in particular differences in outcome measures and control for confounding).

Comment: A brief discussion of the possible deleterious effects of airborne particulate ammonia should be added to the assessment based on a recent study (Paulot and Jacob, 2014) that found that ammonia gas emanating from farming practices can form aerosols that adversely affect human health.

Response: Mention of Paulot and Jacob (2014) was added to the Preface in the context of ammonia from agricultural sources as a contributor to fine inorganic particular matter ($PM_{2.5}$). Paulot and Jacob (2014) used a chemical transport model to estimate the impact of U.S. agricultural sources of ammonia (NH_3) on the concentration of fine inorganic particulate matter ($PM_{2.5}$) present in the atmosphere as ammonium–sulfate–nitrate salts. These authors examined the health benefits that could be achieved by reducing NH_3, SO_2, and NO_X emissions and thereby reducing $PM_{2.5}$ mass, but did not investigate the health effects of airborne particulate ammonia itself.

A growing body of literature has attempted to identify whether individual components of PM are more strongly associated with morbidity or mortality compared to PM mass alone. This literature was evaluated in EPA's Integrated Science Assessment for Particulate Matter (PM ISA)

(U.S. EPA, 2009a), which reviews the extensive literature on sources of PM and components that react to produce PM, atmospheric chemistry and transport models, exposure, and health effects. Based on an evaluation of studies of various components and sources of PM, including NH_4^+, the 2009 PM ISA concluded that "many constituents of PM can be linked with differing health effects and the evidence is not yet sufficient to allow differentiation of those constituents or sources that are more closely related to specific health outcomes" (U.S. EPA, 2009a). Thus, the PM literature does not support analysis of NH_4^+ as a component of PM and health outcomes. Further, literature on particulate ammonia other than as a contributor to PM was not identified.

Given the fact that the literature on airborne particulate ammonium is limited to ammonia as a source of $PM_{2.5}$, a topic covered in the scientific review that supports the PM National Ambient Air Quality Standard (NAAQS), and the lack of evidence to support associations between specific constituents of PM (including NH_4^+) and health outcomes as per U.S. EPA (2009a), consideration of the health effects of airborne particulate ammonia was not added to the IRIS assessment of ammonia. An updated ISA for PM is under development and the study by Paulot and Jacob (2014) will be considered in the context of that review.

Charge Question E2: The NOAEL/LOAEL approach was used to identify the point of departure (POD) for derivation of the RfC (see Section 2.2.2). Please comment on whether this approach is scientifically supported and clearly described.

The SAB observed that the approach for RfC derivation was reasonable and clearly described, but offered the following recommendations.

Comment: EPA should attempt to obtain individual-level data and/or the mean/median exposure concentrations for the high-exposure group from Dr. Holness in order to identify a better supported point of departure. The SAB suggested that EPA consider using a central estimate (i.e., mean or median) of the high-exposure group ammonia concentration rather than the minimum. If individual data are unavailable, EPA should consider whether there is sufficient information available in the Holness et al. (1989) study to estimate the mean concentration for the high-exposure group, e.g., assuming a lognormal or other skewed distribution for the measured concentrations. The SAB noted that the Holness et al. (1989) study should be used whether the individual data are obtained or not.

Response: EPA was informed by the office of Dr. Linn Holness (call from Susan Rieth, U.S. EPA, to Charmaine Clayton, administrative assistant to Dr. Holness, St. Michael's Hospital, Center for Research Expertise in Occupational Health, Toronto, Canada, February 11, 2015) that no original data from the study were retained. In the absence of individual subject data, the frequency distribution information provided in Holness et al. (1989) was used to estimate the parameters of the lognormal distribution that best fit the data. This frequency distribution is provided in Table C-12 in Appendix C. Assuming a lognormal distribution for the measured concentrations, EPA

estimated the mean concentration for the high-exposure group (17.9 mg/m^3). The 95% lower confidence bound on the mean exposure concentration, or 13.6 mg/m^3, was used as the POD for deriving the RfC to reflect the statistical uncertainty around the estimate of the mean.

Comment: The SAB recommended clarifying and strengthening the evidence that supports the idea that the reported respiratory and lung function effects of ammonia result from cumulative exposure rather than acute exposure. The SAB observed that some support is provided in Table 3 of the Ballal et al. (1998) study.

Response: As discussed in response to recommendations provided in Appendix B of the SAB report (Comments on the Supplemental Information), the study by Rahman et al. (2007) provides evidence of contributions from both immediate (acute) exposure and length of exposure (cumulative exposure) to ammonia's respiratory effects. In addition, Ballal et al. (1998) found a significant correlation between respiratory symptoms (cough, phlegm, and wheezing) and duration of service (a proxy for exposure duration). Section 2.1.2 was revised to acknowledge the potential contribution of both immediate (acute) exposure and length of exposure to ammonia's respiratory effects. In the absence of clear evidence that respiratory effects in occupationally-exposed populations are an acute response, and given evidence for the contribution of exposure duration (cumulative exposure) to the respiratory effects of ammonia, the standard adjustment to continuous exposure was applied.

Comment: The SAB recommended that the source of exposure values and the rationale for their use be clarified. Specifically, the SAB suggested that EPA clarify the assumed inhalation rates of 10 m^3/8-hour workday and 20 m^3/24-hour day, noting that inhalation rates provided in the assessment differ from those referenced in the 2011 Exposure Factors Handbook (U.S. EPA, 2011). If a breathing rate of 20 m^3/day is meant to be an upper bound, EPA should cite its data source and discuss whether incorporation of this aspect of inter-individual pharmacokinetic variability at the NOAEL determination stage has implications for later selection of an uncertainty factor.

Response: The ratio of the workday to daily average inhalation rate of 10 m^3/20 m^3 (or 0.5) was retained, but the reference to support the value was corrected to U.S. EPA (1994), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*. The inhalation rate values are consistent with inhalation rates from a 2009 study conducted by U.S. EPA (2009b) and as cited in the 2011 *Exposure Factors Handbook* (U.S. EPA, 2011). Section 2.1.2 was revised to include a discussion of the consistency in inhalation rates between U.S. EPA (1994) and U.S. EPA (2009b). By using average values for both occupational and daily inhalation rates, there should be no significant implications for interindividual uncertainty or for the selection of the intraspecies uncertainty factor of 10.

Charge Question E3: Please comment on the rationale for the selection of the uncertainty

factors (UFs) applied to the POD for the derivation of the RfC (see Section 2.2.3). Are the UFs appropriate based on the recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

Comment: The SAB observed that the selection of uncertainty factors was appropriate, clearly described, and consistent with the 2002 EPA recommendations.

Response: No response needed.

Charge Question F1: Quantitative cancer estimates were not derived for ammonia because of inadequate information. Please comment on whether the rationale for not deriving quantitative cancer estimates for ammonia is scientifically supported and clearly described (see Section 2.3). Please comment on whether data are available to support a quantitative cancer assessment. If so, please identify these data.

Comment: The SAB agreed with EPA's conclusion that the existing data are inadequate to reach a conclusion on the carcinogenicity of ammonia, and thus it would not be scientifically justified to develop quantitative cancer risk estimates for this chemical. The SAB further observed that the rationale for not deriving quantitative cancer estimates was described clearly and supported scientifically.

Response: As noted in response to recommendations under Charge Question C3, the assessment of ammonia carcinogenicity will be addressed in a separate assessment of the health effects of ingested ammonia.

Charge Question G1: Ammonia is produced endogenously and has been detected in the expired air of healthy volunteers. Please comment on whether the discussion of endogenous ammonia in Section 2.2.4 (currently 2.1.4) of the Toxicological Review is scientifically supported and clearly described.

Comment: The SAB considered the description of endogenous ammonia production to be generally appropriate. The panel recommended providing a clearer understanding of the pathways for ammonia generation and the health effects associated with increased ammonia levels, and expanding the section to include all sources of endogenous ammonia.

The SAB provided information on the relationship (or lack of relationship) between endogenous ammonia, concentrations of ammonia in inhaled, expired, and alveolar air, the lung metabolic pool of ammonia, and ammonia in the oral cavity. The panel observed that the concentration of ammonia in the mouth is not a major contributor to either the systemic or inhaled

concentration of ammonia. The panel also observed that exhaled ammonia concentrations are likely higher than inhaled concentrations even for mouth breathers, much as exhaled CO_2 is higher than inhaled CO_2. The panel recommended that the assessment clearly state that exhalation of air and ammonia is a clearance mechanism of an otherwise toxic contaminant.

Response: A summary of the pathways for the production of endogenous ammonia in Appendix C, Section C.1.3 (Metabolism/Endogenous Production of Ammonia) was expanded. The discussion of disease states that can lead to hyperammonemia was expanded in Section 1.3.2 (Susceptible Populations and Lifestages).

The endogenous ammonia discussion in Section 2.1.4, Uncertainties in the Derivation of the Reference Concentration, provides a comparison of the range of ammonia concentrations in exhaled breath with the RfC, noting that ammonia in exhaled breath has, under certain conditions, been measured at concentrations that exceed the RfC. The intent of this uncertainty section was to provide context for this comparison, and not to be a broad review of endogenous ammonia and its sources. The section was rewritten to clarify the objective of the section, focusing on the following points:

- Ammonia is produced endogenously; one route of elimination is exhalation.
- Ammonia concentrations exhaled through the mouth are higher than concentrations exhaled through the nose. Concentrations in the nose generally do not exceed the RfC, better represent levels at the alveolar interface of the lung, and are thought to be more relevant to understanding systemic levels of ammonia.
- Concentrations in breath cannot be correlated with blood ammonia concentrations or with previous exposure to environmental (ambient) concentrations of ammonia.
- The exhalation of ammonia is a clearance mechanism for a product of metabolism that is otherwise toxic in the body at sufficiently high concentrations. Exhaled concentrations may be higher than inhaled concentrations, particularly when compared to exhaled air from the mouth or oral cavity, although ammonia concentrations in exhaled air from the respiratory tract are generally lower than the RfC.

In addition, the title of the section was changed to "Comparison of Exhaled Ammonia to the RfC" to more transparently identify the uncertainty addressed in this section. Section C.1.3 in Appendix C was revised to include an expanded discussion of sources of endogenous ammonia and the relationship between ammonia concentrations in different internal compartments. A reference to Appendix C for further information on endogenous ammonia was included in the uncertainties discussion.

Comment: EPA should consider including (in Section 2.1.4 and the Executive Summary) ammonia concentration ranges for typical indoor and ambient air to provide context for the potential contributions of endogenously generated ammonia to NH_3 inhalation doses, and for placing the RfC in the context of expected concentrations in non-industrial, residential, and office indoor environments, and in outdoor air.

Response: Concentration ranges for ammonia in indoor and ambient air were added to the Preface and the Executive Summary. EPA considers these sections to be more appropriate locations for background information on ammonia, including typical air concentrations, than Section 2.1.4.

Appendix B of the SAB Report

In Appendix B of their report, the SAB provided a number of specific recommendations for changes to improve the clarity or accuracy of the Toxicological Review; these changes were adopted by EPA in revising the Toxicological Review. Summaries of these specific recommendations and responses to these recommendations are not addressed further in this appendix. Specific recommendations pertaining to the evaluation of oral health effects data, derivation of the oral RfD, and evaluation of cancer data will be considered in a separate assessment of the health effects of ammonia following oral exposure and are not further addressed in this appendix. Other changes made in response to SAB comments that were not already addressed as recommendations under charge questions to the SAB are summarized below.

Comments on the Executive Summary and Toxicological Review of Ammonia

- The section of the Preface that described major uses of ammonia was expanded to include a summary of the major sources of ammonia exposure. Information on ammonia concentrations in ambient air based on measurements from the National Atmospheric Deposition Program's Ammonia Monitoring Network was added to the Preface and Executive Summary.
    - Evidence for effects on the adrenal gland and kidney, based largely on inhalation studies, was reconsidered. EPA concluded that the evidence of possible effects of ammonia on the adrenal gland (i.e., one guinea pig study (Weatherby, 1952) that was limited in design and reporting) was insufficient to evaluate hazard. Findings of effects on the kidney come from three inhalation studies in multiple animal species; these studies, all from the toxicological literature published between 1952 and 1970, were also limited in design and reporting. For example, none of the three studies provided incidence of histopathologic lesions, and characterization of lesions in the Weatherby (1952) study (e.g., "congestion of the kidneys) was non-specific. The summary of evidence for an association between inhaled ammonia and effects on the kidney was revised to more clearly describe these limitations in the evidence.

Comments on the Supplemental Information

- Appendix C, Section C.1.1, Absorption, was revised to more accurately describe absorption of ammonia from the intestines. More current references were added to address the SAB comment that the better quality data suggest/support that the small intestine also contributes to intestinal ammoniagenesis via the use of amino acids as an energy source.
- Normal blood ammonia levels from more recent sources were added to the text in Appendix

C, Section C.1.2, Distribution; the statement pertaining to blood ammonia levels based on papers from the older ammonia literature (i.e., Conn (1972), Brown et al. (1957)) was deleted in light of the lower reliability of assays used at that time.

- Appendix C, Section C.1.2, Distribution, was updated to include the relative amounts of NH_4^+ and NH_3 at physiological pH as reported by Weiner and Verlander (2013) (see footnote 3).
- The text in Appendix C, Section C.1.3, Metabolism/Endogenous Production of Ammonia, was revised to clarify that intestinal ammonia production can exceed hepatic metabolism capacity, leading to increased blood ammonia levels, under conditions of abnormal liver function.
- Appendix C, Sections C.1.3, Metabolism/Endogenous Production of Ammonia, and C.1.4, Distribution, were revised to more accurately describe the kidney's role in the production and elimination of ammonia, noting that the kidneys actually add ammonia to the body, as renal vein ammonia content exceeds renal artery ammonia content.
- Appendix C, Section C.1.4, Distribution, was revised to more accurately characterize the mechanisms of ammonia elimination. As noted by the SAB, characterization of renal ammonia transport is highly complex, involving proteins such as the ammonia transporter proteins Rhbg and Rhcg, and is beyond the scope of this assessment. Citations for recent review papers were added to provide readers with a source of more detailed information.

Selection of the RfC

- Findings related to hemoptysis in Ballal et al. (1998) were added to the summary in Appendix C, Section C.2.1.
- The findings in Table 3 of Ali et al. (2001) were further evaluated in response to SAB comments on the value of FEV_1%. FVC% predicted was statistically significantly higher in the exposed workers than in the control group; FEV_1% predicted was approximately 1.5% higher in the exposed workers than the control, but the difference was not statistically significant and was not considered consistent with a beneficial effect of exposure. Comparison of the values for FVC% predicted in Tables 3, 4, and 5 of the paper suggests that the value for FVC% predicted of 105.65 in Table 3 may be incorrect. The basis for this determination was added to the summary of the Ali et al. (2001) study in the Supplemental Information. Given the concerns regarding the FVC% predicted value in Table 3, only study results from Tables 4 and 5 of the Ali et al. (2001) study were presented in the Toxicological Review.
- Results from the Rahman et al. (2007) study were re-evaluated in response to SAB observations comparing pre-shift values between the ammonia and urea plants in this study. EPA agreed with the SAB that results in Table 5 of Rahman et al. (2007) provide evidence of an immediate effect of ammonia exposure on lung function. Specifically, mean preshift FVC and FEV_1 values in ammonia and urea plant workers were similar (suggesting similar lung function in low- and high-exposure workers upon arrival at work), and cross-shift changes in FVC and FEV_1 in the urea plant workers (i.e., the more highly-exposed

workers) were statistically significantly decreased. However, other findings from the Rahman et al. (2007) study suggest contributors to lung function changes other than daily (immediate) exposure. The study authors applied a multiple regression model to data from 23 workers (from both the ammonia and urea plants) with concurrent measurements of ammonia exposure and lung function; both the concentration of ammonia and duration of exposure (using years of employment as a proxy for duration) contributed to percentage cross-shift decrease in FEV_1% (ΔFEV_1%) (Table 6). Rahman et al. (2007) reported that each year of work in a production section was associated with a decrease in ΔFEV1% of 0.6%. These findings were added to Table 1-2. It should be noted that a limitation of the multiple regression analysis was the failure to explore the age parameter, since there was a high correlation between age and years of work (Pearson correlation coefficient 0.97). The evidence from Rahman et al. (2007) for contributions of both immediate exposure and length of exposure to ammonia's respiratory effects was discussed in Section 2.1.2 in the context of adjustment of noncontinuous (occupational) exposure to continuous (general population) exposure in deriving the RfC.

- Chapter 2 was revised to include bulleted summaries of the studies considered for dose-response analysis, with a focus on the contribution of each to the understanding of the dose-response relationship between ammonia exposure and respiratory effects.
- In response to other SAB comments on Chapter 2.2.1, the summary of outcomes in Rahman et al. (2007) was expanded to include the magnitude of cross-shift decline in FEV_1 and FVC in the high-exposure group; support for self-reported respiratory symptoms as well accepted outcomes for evaluating respiratory health was provided by reference to the American Thoracic Society guidelines and EPA's Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry; and consideration of potential co-exposures as they relate to selection of studies for dose-response analysis was added.

Other Comments

- Discussion of a focused literature search of studies of cleaning and hospital workers, undertaken to address a new area of research identified during the 2013 literature search update, was added to the Literature Search Strategy | Study Selection and Evaluation section. More detailed documentation of the focused search was included in Appendix B of the Supplemental Information, Table B-3.

Appendix C of the SAB Report

In Appendix C of their report, the SAB provided suggestions for additional studies relevant to selection of the RfD, neurotoxic effects from exposure to ammonia, and endogenous production of ammonia. Specific recommendations pertaining to the oral RfD will be considered in a separate assessment of the health effects of ammonia following oral exposure and are not further addressed in this appendix. Observations pertinent to inhaled ammonia were considered in revising this

assessment. Specific SAB recommendations were addressed as follows:

Comment: The SAB recommended further development of the discussion regarding measurement of ammonia in exhaled air and how it may impact the RfC, and the relevance to hyperammonemia, ingested ammonia, or long-term exposure to gaseous ammonia.

The SAB recommended that, in addition to the cited references that evaluated the relationship between ammonia concentration in exhaled breath and systemic ammonia levels (i.e., (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977)), the recent paper by Solga et al. (2013) should also be cited; these authors found that the amount of ammonia in expired air was influenced by temperature of the breath sample and breath analyzer, the pH of a mouth rinse, and open versus closed mouth breathing.

Response: Discussion of ammonia levels in exhaled breath and the relationship of exhaled ammonia to the RfC in Section 2.1.4 was revised as discussed in response to Charge Question G1.

Hyperammonemia has not been associated with exposure to ammonia at environmental concentrations. The discussion of ammonia in exhaled breath in cases of disease states resulting in hyperammonemia is included in Appendix C, Section C.1.4, Ammonia Elimination. As the SAB observed in addressing Charge Question G1, correlating prior chronic exposure with alveolar concentrations is challenging. This point was added to the Toxicological Review in Section 2.1.4, Comparison of Exhaled Ammonia to the RfC.

A summary of the paper by Solga et al. (2013) was added to Table C-1; discussion of the findings from this study were added to Appendix C, Section C.1.4. Because this study involved a single volunteer and did not report ambient ammonia concentrations, this study did not contribute substantially to the existing discussion of ammonia in expired air.

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