NATIONAL TOXICOLOGY PROGRAM
US Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of AMPHETAMINE and METHAMPHETAMINE

March 2005 NTP-CERHR- -

PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction and development caused by agents to which humans may be exposed.

Amphetamines were selected for expert panel evaluation because of widespread usage in children, availability of studies on developmental effects in children and experimental animals, and public concern about the effects of these stimulants on child development. Amphetamines evaluated were *d*- and *d,l*-amphetamine and methamphetamine. *d*- and *d,l*-Amphetamine are approved by the Food and Drug Administration for the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. *d*-Methamphetamine hydrochloride is used in pharmaceutical preparations in the United States and is approved for the treatment of ADHD and for short-term treatment of obesity. Methamphetamine is also manufactured and used as an illicit drug.

To obtain information about amphetamines for the CERHR evaluation, the PubMed (Medline) and Toxline databases were searched with CAS RNs for *d*- and *l*-amphetamine (51-64-9; 156-34-3) and *d*-methamphetamine (537-46-2) and its hydrochloride (51-57-0), and relevant keywords. The search was limited to studies indexed prior to December 31, 2004. References were also identified from databases such as REPROTOX®, HSDB, IRIS, and DART and from report bibliographies.

This evaluation resulted from the efforts of a thirteen-member panel of government and non-government scientists that culminated in a public expert panel meeting held January 10–12, 2005. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that amphetamines are reproductive or developmental toxicants based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk. This report has been reviewed by CERHR staff scientists, and by members of the Amphetamines and Methylphenidate Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies.

This Expert Panel Report will be a central part of the subsequent NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Amphetamines. This monograph will include the NTP-CERHR Brief, the Expert Panel Report, and all public comments on the Expert Panel Report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the web site (http://cerhr.niehs.nih.gov**) or from:**

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Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available on the CERHR web site (http://cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from those of the authors, and conversions or analyses of data conducted by the Panel.

Abbreviations

ACTH	adrenocorticotropic hormone
ADHD	attention/deficit-hyperactivity disorder
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AUC	area under the concentration versus time curve
BMD_{10}	benchmark dose, 10% effect level
BMDL	benchmark dose, 95th percentile lower confidence limit
BMI	body mass index
BUN	blood urea nitrogen
bw	body weight
CAS RN	Chemical Abstracts Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
CI	confidence interval
C_{max}	maximum concentration
CNS	central nervous system
CYP	cytochrome P450
DAPI	4',6-diamidino-2-phenylindole
DEA	Drug Enforcement Agency
DOPAC	3,4-dihydroxyphenylacetic acid
EEG	electroencephalogram
EKG	electrocardiograph
EPA	Environmental Protection Agency
Eq	equivalent
f	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
g	gram(s)
GABA	γ-amino-butyric acid
GC	gas chromatography
GD	gestation day(s)
GLP	Good Laboratory Practice
GSH	glutathione
h	hour(s)
HPLC	high performance liquid chromatography
HSDB	Hazardous Substances Data Bank
ip	intraperitoneal
iv	intravenous
kg	kilogram(s)
K_{ow}	octanol-water partition coefficient
L	liter(s)
LD_{50}	lethal dose, 50% mortality
LH	luteinizing hormone
LOAEL	low observed adverse effect level
m	male

M	molar
MAOI	monoamine oxidase inhibitor
max	maximum
mM	millimolar
mmol	millimole(s)
mol	mole(s)
mRNA	messenger ribonucleic acid
msec	millisecond
MTD	maximum tolerated dose
n or no	number
NCTR	National Center for Toxicological Research
ND	not determined
ng	nanogram(s)
NIA	National Institute on Aging
NIDA	National Institute on Drug Abuse
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NIOSH	National Institute of Occupational Safety and Health
nmol	nanomole(s)
NOAEL	no observed adverse effect level
NOEL	no observed effect level
ns	non-significant
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
PHS	Public Health Service
PND	postnatal day(s)
ppm	parts per million
PRL	Prolactin
RHA	Roman high-avoidance
RIA	radioimmunoassay
RLA	Roman low-avoidance
RR	relative risk
sc	subcutaneous
SCE	sister chromatid exchange
SD	standard deviation
SEM	standard error of the mean
$t_{1/2}$	half-life of elimination
T_{max}	maximum time
USP	United States Pharmacopoeia
v	volume
V_d	volume of distribution
VSD	ventricular septal defect
WISC	Wechsler Intelligence Scale for Children
wk	week(s)
µg	microgram(s)
µL	microliter(s)
µm	micrometer(s)
µM	micromolar
µmol	micromole(s)

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1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

This exposure section is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

1.1 Chemistry

1.1.1 Nomenclature

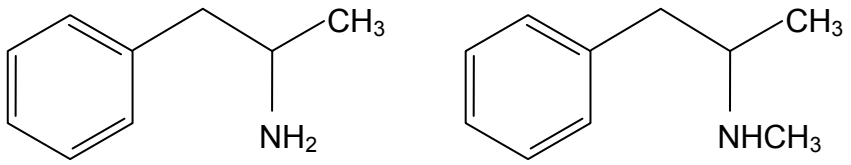
The term "amphetamines" is used to denote a class of chemicals with structural similarity to amphetamine. The amphetamines used in clinical practice include two distinct bases, amphetamine and methamphetamine, available in pharmaceutical preparations as various mixtures of enantiomers and as various salts. The compounds relevant to this report are identified in Table 1. Many of the trade names are no longer in use, although they remain in current lists of drug names *(1, 2*). The most commonly encountered proprietary amphetamine preparation is Adderall® (a mixture of amphetamine salts providing *d*-, and *l*-amphetamine in a 3:1 ratio). Generic equivalents of Adderall are also available. Generic *d*-amphetamine is often called dextroamphetamine and *l*-amphetamine is called levamfetamine **[spelling differences as per ChemID]**. In this report, the enantiomers will be designated by the *d*- or *l*- prefixes rather than by dextroamphetamine and levamfetamine. Methamphetamine in US pharmaceutical preparations is present as *d*-methamphetamine hydrochloride. *d*-Methamphetamine hydrochloride is also used recreationally and is the illicit stimulant most commonly meant by the term "speed." *l*-Methamphetamine can produce palpitations and gastrointestinal upset but does not produce the psychological effects desired by recreational users. There are no pharmaceuticals in the US that contain the *l*-enantiomer.

Many of the Hazardous Substance Data Bank (HSDB) proprietary names in Table 1 were not found on the Food and Drug Administration (FDA) web site (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/) and were presumed to be discontinued or foreign. Some proprietary names that were found on the FDA web site are for products listed as discontinued. The products that are currently marketed in the US are listed in Table 2.

1.1.2. Formula and molecular mass

The chemical formula for amphetamine is $C_9H_{13}N$ and the molecular mass is 135.20. The chemical formula for methamphetamine is $C_{10}H_{15}N$ and the molecular mass is 149.24. The structures are shown in Figure 1.

Figure 1. Amphetamine and Methamphetamine Structures.



Amphetamine Methamphetamine

Table 1. Amphetamine Nomenclature

Names and synonyms	CAS RN	Trade names			Street names
Amphetamine	300-62-9	Adderall[a]*	Isoamyne	Phenedrine	Speed[b]
(+-)-Benzedrine		Delcobese[a]*	Isomyn	Profamina	Bennies
(+-)-alpha-Methylbenzeneethanamine		Actedron	Mecodrin	Propisamine	
(+-)-alpha-Methylphenethylamine		Adipan	Mydrial	Protioamphetamine	
(+-)-alpha-Methylphenylethylamine		Allodene	Norephedrane	Psychedrine	
(Phenylisopropyl)amine		Anorexide	Novydrine	Raphetamine	
-Methyl-2-phenylethylamine		Anorexine	Obesin	Rhinalator	
1-Phenyl-2-aminopropane		Benzebar	Obesine	Simpatedrin	
1-Phenyl-2-aminopropane		Benzedrine*	Oktedrin	Simpatina	
1-Phenyl-2-propylamine		Benzolone	Ortedrine	Sympamine	
2-Amino-1-phenylpropane		Elastonon	Percomon	Sympatedrine	
3-Phenyl-2-propylamine		Fenopromin	Phenamine	Weckamine	
Amfetamine		Finam			
Desoxynorephedrine					
Amphetamine phosphate	139-10-6				
Amphetamine sulfate	60-13-9				
d-Amphetamine	51-64-9	Dexedrine*			Dexies
Dextroamphetamine		Amsustain			
Dexamphetamine		Dephadrin			
(+)-(S)-Amphetamine		Sympamin			
(+)-Amphetamine		Dextrostat*			
(+)-Phenaminum		Ferndex*			
(+)-alpha-Methylphenethylamine					
(+)-alpha-Methylphenylethylamine					
(2S)-(+)-Amphetamine					
(S)-(+)-Amphetamine					
(S)-(+)-beta-Phenylisopropylamine					
(S)-1-Phenyl-2-aminopropane					
(S)-1-Phenyl-2-propylamine					
(S)-Amphetamine					

Names and synonyms	CAS RN	Trade names			Street names
(S)-alpha-Methylphenethylamine					
(S)-alpha-Phenylethylamine					
D-(S)-Amphetamine					
D-1-Phenyl-2-aminopropane					
D-2-Amino-1-phenylpropane					
D-AM					
alpha-Methylphenethylamine, *d*-form					
d-(S)-Amphetamine					
d-1-Phenyl-2-aminopropane					
d-2-Amino-1-phenylpropane					
d-alpha-Methylphenethylamine					
d-Amphetamine sulfate	51-63-8	Acedron	DAS	Ephadren	Dexies
Dextroamphetamine sulfate	sulfate	Adjudets	Dadex	Eskatrol	Fastballs
Dextro-1-phenyl-2-amino-propane	D-	Adrizine	Dams	Evrodex	Oranges
sulfate	Betaphedrine	Afatin	Dasdel	Hetamine	
Dexamphetamine sulfate		Albemap	Dellipsoids	Lentanet	
Dextro-alpha-methylphenethylamine		Algo-dex	Dex ob	Lipsoids	
sulfate		Amdex	Dex-Sule	Lowedex	
Dextro-beta-phenylisopropylamine		Amphaetex	Dexaline	Maxiton sulfate	
sulfate		Ampherex	Dexalme	Medex	
		Amphetasul	Dexalone	Obesedrin	
		Amphex	Dexamed	Obesonil	
		Amptrerex	Dexamine	Pellcap	
		Amsustain	Dexamyl	Perke	
		Apetain	Dexten	Phetadex	
		Ardex	Dextenal	Pomadex	
		Betafedrine	Dextro-	Pro-Dexter	
		Betaphedrine	Profetamine	Psychodrine	
		Carrtime	Dextrosule	Recordati	
		Cradex	Diocurb	Revidex	
		Curban	Diphylets	Robese	
		D-Amfetasul	Ditab	Tempodex	
		D-Benzedrine	Domafate	Tuphetamine	

Names and synonyms	CAS RN	Trade names			Street names	
			Dura Dex Dynaphenyl	Tydex Zamine		
d-Amphetamine tartrate *d*-Amphetamine bitartrate	3994-11-4	Maxiton				
l-Amphetamine Levamphetamine (-)-Amphetamine (-)-Phenylisopropylamine (-)-alpha-Methylphenethylamine (R)-Amphetamine (R)-alpha-Methylbenzeneethanamine R)-alpha-Methylphenethylamine *l*-alpha-Methylphenethylamine	156-34-3					
l-Amphetamine succinate	5634-40-2	Cydril				
Methamphetamine (+)-N,alpha-Dimethyl-beta-phenylethylamine (+)-N,alpha-Dimethylphenethylamine 1-Phenyl-2-methylaminopropane Deoxyephedrine Desoxyephedrine Desyphed N-Methyl-beta-phenylisopropylamine	537-46-2		Norodin Stimulex			
d-Methamphetamine hydrochloride (+)-Methylamphetamine hydrochloride (+)-N,alpha-Dimethylphenethylamine hydrochloride Methedrine hydrochloride Methylisomin Semoxydrine hydrochloride	51-57-0	Adipex Chestox Deofed Desepin Desodex Desoxyn* Destim	Dexoval Dexstim Dosoxy Doxyfed Drinalfa Efroxine Eufodrinal	Isophen Norodin hydrochloride Obedrin-LA Pervitin Philopon Soxysympamine	Ice Crystal Glass Crank Meth Chalk Beenies	Wet White cross Wolminic Nasal spray Yellow bam Yellow powder Batu

Names and synonyms	CAS RN	Trade names			Street names	
N,alpha-dimethylbenzeneethanamine hydrochloride		Desyfed		Syndrox Tonedron	Blue mollies Crink Cris Croak Crossies Crypto Desocsins Desogtion Fire Go-fast Granulated orange Methlies quik Mexican crack Peanut butter Powder Quill Rose Speed	Cristy Hanyak Hiropon Hot ice Kaksonjae LA glass LA ice Quartz Super ice Lemon drop Soap dope Grimace Green monster Sketch Stove top Water Shabu Yaba
Methamphetamine hydrochloride (racemate)	300-42-5	Amdram	Amedrine	Normadrine		
(+-)-Methylamphetamine hydrochloride Deoxyephedrine Desoxyephedrine hydrochloride Methylpropamine N,alpha-Deimethylphenethylamine hydrochloride *d,l*-Desoxyephedrine hydrochloride		Amphedroxy Amphedroxyn Bombita Corvitin Daropervamin Depoxin Desamine Desfedran Desfedrin Desoxedrin Detrex Dexophrine Dopidrin Doxephrin	Fenyprin Kemodrin Lanazine Levetamin Madrine Mepho-D Methampex* Methamphin Methedrinal Methoxyn Methylbenzedrin Miller drine Neodrine Neopharmadrine	Norodrine Obesin Oxydess Oxydrene Oxydrin Oxyfed Phedoxe Phedrisox Premodrin Psichergina Psicopan Psychergine Psykoton Semoxydrine		

Names and synonyms	CAS RN	Trade names	Street names
		Estimulex Noradrin	
Amphetamine/dextroamphetamine resin complex	None	Biphetamine*	
Hydroxyamphetamine hydrobromide	306-21-8	Paredrine* Paremyd*	

Registration signs omitted from trade names.
*Identified in Drugs@FDA *(3)* as current US trade names (but not necessarily marketed).
[a]Adderall and Delcobese are 3:1 mixtures of *d*- and *l*-enantiomers containing a fixed ratio (1:1:1:1) of amphetamine aspartate, amphetamine sulfate, dextroamphetamine saccharate, and dextroamphetamine sulfate. Delcobese is no longer marketed.
[b]The term "speed" is used for any stimulant.
From references *(4, 5)*.

Table 2. Amphetamine Preparations Marketed in the US

Brand name (reference)	Manufacturer	Active ingredients	Inactive ingredients	Generic manufacturers
Adderall® *(6)*	Shire	Fixed weight ratio (1:1:1:1) of amphetamine aspartate, amphetamine sulfate, dextroamphetamine saccharate, and dextroamphetamine sulfate; total pill doses of 5, 7.5, 10, 12.5, 15, 20, or 30 mg (equivalent to 3.13 mg free based per 5 mg total pill dose).	Sucrose, lactose, corn starch, acacia, magnesium stearate, colors.	Abrika Pharmaceuticals, Barr, Corepharma, Eon, Mallinckrodt, Mutual Pharmaceutical, Watson Laboratories
Adderall XR® *(7)*	Shire	Extended release preparation of amphetamine aspartate, amphetamine sulfate, dextroamphetamine saccharate, and dextroamphetamine sulfate; total pill doses of 10, 20, or 30 mg (equivalent to 6.3 mg free based per 10 mg total pill dose).	Gelatin capsules, hydroxypropyl methylcellulose, methacrylic acid copolymer, Opadry beige, sugar spheres, talc, triethyl citrate, colors.	None
Desoxyn® *(8)*	Abbott	Methamphetamine hydrochloride 5 mg.	Corn starch, lactose, sodium paraaminobenzoate, stearic acid, talc.	Able
Dexedrine® *(9)*	GlaxoSmithKline	*d*-Amphetamine sulfate 5 mg tablets.	Calcium sulfate, gelatin,	Barr, Malinkrodt

			lactose, mineral oil, starch, stearic acid, sucrose, talc, colors.	
Dexedrine® Spansule® *(9)*	GlaxoSmithKline	*d*-Amphetamine sulfate sustained-release capsule 5, 10, or 15 mg.	Cetyl alcohol, dibutyl sebacate, ethylcellulose, gelatin, hydroxypropyl methylcellulose, propylene glycol, povidone, silicon dioxide, sodium lauryl sulfate, sugar spheres, colors.	Barr, Malinkrodt
DextroStat® *(10)*	Shire	*d*-Amphetamine sulfate 5 or 10 mg.	Acacia, corn starch, lactose monohydrate, magnesium stearate, sucrose, sodium starch glycolate (10 mg only).	Barr, Endo Pharmaceuticals, KV Pharmaceuticals, Malinkrodt
Paremyd® *(11)*	Akorn	Hydroxyamphetamine hydrobromide, USP 1.0%; Tropicamide, USP 0.25% ophthalmic solution.	Benzalkonium chloride, edetate disodium, sodium chloride, purified water. Hydrochloric acid and/or sodium hydroxide are added to adjust the pH.	None

1.1.3 Chemical and physical properties

Chemical and physical properties of amphetamine and methamphetamine are listed in Table 3.

Table 3. Chemical and Physical Properties of Amphetamine and Methamphetamine

Property	*d,l*-Amphetamine	*d*-Amphetamine	*d*-Methamphetamine
Form	Colorless liquid; the salts are white powders or crystals.		Colorless liquid; the hydrochloride is a white powder or clear crystal.
Boiling point	200–203ºC	203–204ºC	212ºC
Density	0.913	0.949	not located
pK_a	10.13	not located	9.9
log K_{ow}	1.76	1.76	2.07
Solubility	Slight in water; soluble in diethyl ether and ethanol. Amphetamine sulfate is insoluble in ether.		0.5 g/mL water, soluble in ethanol and diethyl ether; the hydrochloride is readily soluble in water.

Source: HSDB *(1)*.

1.1.4 Technical products and impurities

Table 2 summarizes active ingredient strength and lists the inactive ingredients in each marketed amphetamine and methamphetamine product. The ophthalmic solution (Paremyd®) is marketed as a mydriatic and will not be further considered in this report.

Illicit amphetamines, chiefly methamphetamine hydrochloride, can be synthesized by different methods (Section 1.2.1) with the potential for different contaminants. According to the US Drug Enforcement Administration (DEA) *(12)*, chemical supply houses internationally have restricted sales of the chemicals used to produce methamphetamine of high purity, resulting in substitution of other chemicals and a decrease in methamphetamine purity. In 1994, the average purity of methamphetamine seized by DEA was 71.9%, while in 1999, the average purity was 30.7%. Purity of seized methamphetamine increased thereafter to 35.3% in 2000 and 40.1% in 2001. The nature of the impurities was not discussed.

1.2 Use and human exposure

1.2.1 Production information

The methods of production used in the pharmaceutical manufacture of amphetamine and methamphetamine are not available; however, there are Internet sites that give a number of different methods for the synthesis of these compounds. Amphetamine can be synthesized by the sequential alkylation of methyl acetoacetate with dimethyl sulfate and benzyl chloride, followed by hydrolysis and deacetylation to give 2-phenylpropionic acid, which, through reaction with thionyl chloride and ammonia, forms 2-phenylpropionamide. Upon treatment with aqueous sodium hypochlorite, this amide undergoes Hofmann rearrangement to form racemic amphetamine (phenyl-2-aminopropane) *(13)*. Methamphetamine can be synthesized from ephedrine via reduction of chloroephedrine with hypophosphorous acid or by Birch reduction of pseudoephedrine. Pseudoephedrine is readily available in decongestant tablets and is the most common starting material for illicit methamphetamine *(12)*. The chemical synthesis information

on many web sites is interspersed with advice on avoiding explosion, arrest, and exploitation by professional criminals, suggesting that these sites are intended for illicit manufacturers.

Retail US distribution of amphetamines for calendar year 2002 is shown in Table 4. The United Nations reported that US manufacture of amphetamine **[assumed to be *d,l*-amphetamine]** was 18,586 kg in 2000, 9612 kg in 2001, and 7442 kg in 2002; US manufacture of *d*-amphetamine was 12,306 kg in 2000, 4919 kg in 2001, and 5962 kg in 2002; US manufacture of methamphetamine was 1306 kg in 2000, 1692 kg in 2001, and 1385 kg in 2002 *(14)*. Reported exports in 2002 were 9 kg amphetamine and 152 kg *d*-amphetamine; no value was given for methamphetamine. The DEA reported that 8000 clandestine methamphetamine laboratories were seized in 2001, and that 298 were so-called "super labs," capable of making >10 pounds (4.5 kg) of methamphetamine in a 24-hour period *(12)*. Most of these super labs were in northern Mexico and they were believed to be supplying the US In 2001, 1370 kg of methamphetamine was seized at the Mexico-US border.

Table 4. Retail US Distribution of Amphetamines in 2002

Drug (as base)	Amount (kg)
d,l-Amphetamine	2096
d-Amphetamine	3097
d-Methamphetamine	17

From DEA *(12)*.

1.2.2 Use

Amphetamine and methamphetamine are central nervous system (CNS) stimulants. The amphetamine preparations are indicated for the treatment of narcolepsy and attention/deficit-hyperactivity disorder (ADHD) *(6, 9)* and methamphetamine is indicated for the treatment of ADHD and the short-term treatment of obesity *(8)*. The Expert Panel is aware of off-label uses of amphetamines to treat depression, primarily as an adjunct to antidepressant medication, and to treat patients with post-stroke cognitive impairment (Scialli JV, Lusskin S, personal communication, September 22, 2004). While depression is common in men and women of reproductive age, strokes most often occur in older individuals. There is an increase in diagnosis and treatment of both ADHD and depression in adolescents and adults. Obesity is also common in individuals of reproductive age. More exposures in people of reproductive age can, therefore, be expected.

The DEA estimated that the number of amphetamine prescriptions written increased between 1992 and 2000 from fewer than 500,000 to nearly 8 million per year *(12)*. In calendar year 2001, more than 4000 kg of racemic and *d*-amphetamine were sold to pharmacies and 120 kg were sold to hospitals. By contrast, fewer than 17 kg of methamphetamine were sold to pharmacies and hospitals combined. **[The Expert Panel recognizes that therapeutic use of *d*-methamphetamine in the US is uncommon.]** The National Institute on Drug Abuse (NIDA) *(15)* states that addiction to stimulant medications does not occur when medicines are taken in the form and dosage prescribed. However, amphetamines and *d*-methamphetamine are used recreationally.

The DEA reported that during the year 2000, 4% of the US population reported trying methamphetamine at least once in their lives *(12)*. Illicit use is concentrated in the Midwest, Southwest, and Pacific coast regions of the country. The street price of methamphetamine ranges from $400 to $3000 per ounce (138 g). Methamphetamine hydrochloride is used recreationally as a powder by nasal inhalation or is mixed with water and injected intravenously (iv). The pure

crystalline form of methamphetamine, called ice, glass, or crystal, is typically smoked. A pill form called Yaba is made in Thailand and smuggled into the US; the methamphetamine content is 30–40 mg per pill *(12)*.

1.2.3 Human Exposure

The recommended starting doses of amphetamine for narcolepsy is 5 mg/day for children 6–12 years old and 10 mg for children older than 12 years and for adults. The maximum recommended dose is 60 mg/day. For ADHD, the starting dose of amphetamine is 2.5 mg/day for children 3–6 years old and 5 mg/day for children older than 6 years and for adults. The maximum dose recommended for ADHD is 40 mg/day. Amphetamine preparations are not recommended for children younger than 3 years. Amphetamines are taken every 4–6 hours or, for the sustained-release preparations, once/day *(6, 7, 9)*. The recommended starting dose for methamphetamine treatment of ADHD is 5 mg/day in individuals who are at least 6 years. The maximum recommended dose for ADHD is 25 mg/day. For obesity, the recommended methamphetamine dose is 5 mg before a meal. Methamphetamine is not recommended for the treatment of ADHD in children younger than 6 years old and is not recommended for obesity treatment in children younger than 12 years *(8)*.

Illicit methamphetamine use involves doses in the drug-naïve individual of about 30 mg; however, habitual use of methamphetamine to produce euphoria characteristically results in binges during which all available methamphetamine is used over a period of 3–15 days *(16)*. Cho et al. *(17)* reported a dose range of 20–250 mg or more per "hit" in methamphetamine abusers with total daily doses of up to several grams.

An abstract from the University of Utah reported that 0.2% of babies in a well-baby nursery and 1% of babies in a neonatal intensive care nursery have meconium samples positive for methamphetamine, presumably representing recent use during pregnancy by their mothers *(18)*. **[This information was presented to document prenatal exposures but will not be considered further since it is only an abstract.]** A report from the University of California Davis Medical Center indicated that at least 6% of pregnant women who were routinely screened for illicit drugs in urine (most of whom were presenting in labor) were positive for amphetamines *(19)*.

Numerous children in the US are exposed to methamphetamine or other toxic chemicals at clandestine laboratories. Toxic or hazardous chemicals used in methamphetamine production include solvents, caustics/irritants, and metals/salts *(20)*. Children at clandestine labs can be exposed to methamphetamine through inhalation of second hand smoke and can be exposed to both methamphetamine and other chemicals through vapors generated in the production process *(21)*. Additional exposures can occur through dermal contact with contaminated surfaces or clothing. Oral exposure to methamphetamine or other chemicals is possible through ingestion of contaminated foods or drinks that are often prepared with the same utensils and appliances used to manufacture methamphetamine *(22)*. In addition to toxicity risks, children at methamphetamine production sites face risks of fire or explosion and neglect or abuse associated with the methamphetamine lifestyle *(21)*. In 14,260 methamphetamine lab incidents in 2003, children were present at 1442 incidents, 3419 children were affected, 1291 children were exposed to toxic chemicals, 44 children were injured, and 3 children were killed *(23)*. Methamphetamine was detected in urine of 1/3 to 1/2 of tested children found at methamphetamine labs in Oregon *(20)*.

Clandestine laboratories have developed so-called designer amphetamines that contain modifications of the amphetamine or methamphetamine molecular structure in order to produce novel stimulant drugs. One of the most popular of these new chemicals is

methylenedioxymethamphetamine (MDMA; Ecstasy). These novel stimulants can be included in the class designation amphetamines, but are not used in therapeutics and will not be discussed in detail in this report.

1.3 Utility of Data

There are reliable data from the DEA on the amount of medicinal amphetamines available in the US. Estimates of the amount of amphetamines used for different indications by different groups of patients (children with ADHD and reproductive-age women with ADHD or depression) are not readily available. Information on illicit use of amphetamines, including populations using these drugs and the amount of drug used, appears to be approximate and of uncertain reliability.

1.4 Summary of Human Exposure Data

The term "amphetamines" denotes a class of compounds with structural similarity to amphetamine. The focus of this report is the amphetamines used in clinical practice, amphetamine and methamphetamine. Amphetamine is available as salts of the *d*- and *l*-enantiomers in a 3:1 ratio or as a salt of the *d*-enantiomer. Pharmaceutical methamphetamine is available as a salt of the *d*-enantiomer. The *d*- and *d,l*-amphetamine preparations are indicated for the treatment of ADHD and narcolepsy *(6, 9*), and methamphetamine is indicated for the treatment of ADHD and the short-term treatment of obesity *(8*). *d*-Methamphetamine hydrochloride is also used recreationally and is the illicit stimulant most commonly meant by the term "speed." It is believed that all human exposures occur through medication use and drug abuse. No information was identified on possible environmental or occupational exposure.

Recommended doses of amphetamine are 2.5–40 mg/day for treatment of ADHD in individuals 3 years of age and older and 5–60 mg/day for treatment of narcolepsy in individuals 6 years of age and older. Amphetamine preparations are not recommended for children younger than 3 years. Immediate-acting amphetamines are taken every 4–6 hours and sustained-release preparations once/day *(6, 7, 9*). Recommended methamphetamine doses are 5–25 mg/day for treatment of ADHD in individuals who are 6 years old or older, and 5 mg before meals for treatment of obesity in individuals 12 years old and older. Methamphetamine is not recommended for the treatment of ADHD in children younger than 6 years and is not recommended for obesity treatment in children younger than 12 years *(8*). Cho et al. *(17*) reported a dose range of 20–250 mg or more per "hit" in methamphetamine abusers with total daily doses of up to several grams.

DEA estimated that the number of amphetamine prescriptions written increased between 1992 and 2000 from fewer than 500,000 to nearly 8 million per year *(12*). In calendar year 2001, more than 4000 kg of racemic and *d*-amphetamine were sold to pharmacies and 120 kg were sold to hospitals. By contrast, fewer than 17 kg of methamphetamine were sold to pharmacies and hospitals combined. The DEA *(12*) reported retail US distribution of 2096 kg *d,l*-amphetamine, 3097 kg *d*-amphetamine, and 17 kg *d*-methamphetamine in 2002. **[The Expert Panel recognizes that therapeutic use of *d*-methamphetamine in the US is uncommon.]** The United Nations reported that 7442 kg amphetamine **[assumed to be *d,l*-amphetamine]**, 5962 kg *d*-amphetamine, and 1385 kg methamphetamine were manufactured in the US in 2002 *(14*). According to the DEA, 8000 clandestine methamphetamine laboratories, believed to be supplying the US, were seized in 2001 *(12*).

Amphetamines and *d*-methamphetamine are approved for the treatment of ADHD or narcolepsy and *d*-methamphetamine is approved for treatment of obesity. The Expert Panel is aware of the off-label use of amphetamines to treat depression primarily as an adjunct to antidepressant medication and to treat patients with post-stroke cognitive impairment. Inasmuch as depression, ADHD, and obesity are common in men and women of reproductive age, there is a potential for

amphetamine and *d*-methamphetamine exposure in that population. Diagnosis and treatment of ADHD in adults in increasing. There is no information on the numbers of pregnant or lactating women prescribed these drugs.

NIDA *(15)* states that addiction to stimulant medications does not occur when medicines are taken in the form and dosage prescribed. However, amphetamines and d-methamphetamine are used recreationally. The DEA reported that during the year 2000, 4% of the US population admitted trying methamphetamine at least once in their lives *(12)*. Illicit use is concentrated in the Midwest, Southwest, and Pacific coast regions of the country. Methamphetamine is used recreationally as a powder by nasal insufflation or is mixed with water and injected iv. The pure crystalline form of methamphetamine, called ice or crystal, is typically smoked. A pill form called Yaba is made in Thailand and smuggled into the US; the methamphetamine content is 30–40 mg per pill *(12)*.

2.0 GENERAL TOXICOLOGY AND BIOLOGIC EFFECTS

2.1 Pharmacodynamics and Pharmacokinetics

Consistent with Section 1, Section 2 will present information for the amphetamine class of drugs that is marketed as salts of *d,l*-amphetamine (Adderall), *d*-amphetamine (DextroStat or Dexedrine), and methamphetamine (Desoxyn).

Information in Section 2 is initially based upon reviews. Primary studies were reviewed if information in reviews was inadequate, if the information presented in the primary studies is highly relevant for the evaluation of developmental or reproductive effects, or if the studies were published subsequent to reviews. Some examples of highly relevant sources reviewed in detail are studies reporting amphetamine levels in breast milk or premature infants, studies examining species-related differences, or studies comparing dose- or route-related differences in pharmacokinetics.

2.1.1 Human

2.1.1.1 Pharmacodynamics

According to NIDA *(15*), amphetamine and methamphetamine are closely related, but methamphetamine has greater CNS effects.

Amphetamines cross the blood-brain barrier and the major site of pharmacological action is the brain (reviewed in *(24*)). Amphetamine and methamphetamine stimulate the CNS by acting as sympathomimetic drugs (reviewed in *(6, 8, 25*)). The therapeutic mode of action in treatment of ADHD with amphetamine or methamphetamine is not known *(6, 8*). Amphetamine and methamphetamine are substrates of the monoamine transporter found in cell and storage vesicle membranes of dopaminergic, noradrenergic, and adrenergic neurons (reviewed in *(25*)). Amphetamine is believed to block the reuptake of norepinephrine and dopamine by presynaptic neurons (reviewed in *(7*)). It is also thought that the transporter translocates amphetamines into dopaminergic neurons where the drug increases the release of dopamine *(26*) and norepinephrine (reviewed in *(27*)) into the extraneuronal space. Amphetamine may also inhibit monoamine oxidase (reviewed in *(27, 28*)). The actions of amphetamines increase the level of catecholamines in the synaptic space and overall catecholaminergic activity of the brain (reviewed in *(27*)). A review by Kraemer and Maurer *(25*) reported that amphetamines ". . . have no affinity to the adrenoceptors or dopamine receptors."

There is also evidence that amphetamines increase release and turnover of serotonin and it has been suggested that many of the behavioral effects of amphetamines are mediated through serotonin (reviewed in *(27*)).

Although stimulants decrease locomotor activity in children, an increase in activity is observed in experimental animal studies. A review by Solanto *(28*) discussed possible reasons for discordance between children and experimental animals. One theory is that reduced activity and increased attention in children compares to decreased activity as a secondary effect of stereotypy in animals given high doses. However, several studies examining divergent thinking and cognitive perseverance indicated no or inconsistent associations between therapeutic effects and cognitive constriction or stereotypic thinking (reviewed in *(28, 29*)). An alternate theory of mechanism of action in children is that stimulation of inhibitory pre-synaptic autoreceptors decreases dopamine activity, thus compensating for excessive dopamine activity in ADHD (reviewed in *(28*)).

Amphetamine *S*-(+) (*d*-) enantiomers have five times the stimulant activity of *R*-(–) (*l*-) enantiomers (reviewed in *(25)*).

Metabolism of amphetamine and methamphetamine is discussed in Section 2.1.1.4. Some metabolites of amphetamine and/or methamphetamine were reported to be active. 4-Hydroxyamphetamine, which has been used as an ophthalmologic drug, releases norepinephrine from postganglionic sympathetic nerves but has few if any CNS effects, possibly due to slowed passage through the blood-brain barrier as a result of its increased water solubility (reviewed in *(30)*). Additional metabolites, 4-hydroxynorephedrine, norephedrine (reviewed in *(31, 32)*), alpha-methyldopamine, and alpha-methylnorepinephrine (reviewed in *(30)*) were reported to act as false neurotransmitters. It has been postulated that false neurotransmitters are involved in habituation (reviewed in *(32)*). Alpha-methylnorepinephrine has CNS hypotensive activity (reviewed in *(30)*).

Anggard et al. *(33)* examined psychotic symptoms in stimulant abusers orally administered three 50-mg doses of *d,l*-amphetamine sulfate over 12 hours. In four subjects administered sodium bicarbonate to increase urinary pH, amphetamine excretion was reduced and as a result of prolonged exposure to amphetamine, higher levels of metabolites were excreted compared to three subjects administered ammonium chloride to acidify urine. There was no relationship between plasma amphetamine level and intensity of psychosis. However, there was a positive association between excretion of basic metabolites (4-hydroxyamphetamine, 4-hydroxynorephedrine, and norephedrine) and rating of psychosis ($r = 0.996$ in the 4 subjects with increased urinary pH). The authors interpreted the findings as suggesting that accumulation of metabolites and not amphetamine is responsible for psychotic symptoms. **[Methods for rating psychosis were not discussed, but a reference was provided. The Expert Panel notes that caution is required in the interpretation of this study due to the small group sizes. A more detailed explanation on the effects of urinary pH on amphetamine excretion and metabolism is included in Section 2.1.1.5.]**

2.1.1.2 Absorption

The Adderall brand of *d,l*-amphetamines is available as immediate-release tablets (Adderall IR) and sustained-release capsules (Adderall XR). Both products contain a 3:1 ratio of *d*- and *l*-amphetamine salts. The sustained-released capsules contain a mixture of immediate-acting pellets and enteric-coated delayed-released pellets. Plasma levels of *d*- and *l*-amphetamine peak at ~3 hours following oral intake of 10 mg Adderall IR by children (Table 5) or adults (Table 6) *(7, 34)*. The time to reach peak plasma concentrations of *d*- and *l*-amphetamines is ~5–7 hours following oral intake of 10–30 mg Adderall XR by adults (Table 6) or children **(**Table 5) *(7, 34)*. Plasma profiles of *d*- and *l*-amphetamine are similar following a single dose of 20 mg Adderall XR or two 10-mg doses of Adderall IR administered 4 hours apart *(7)*. Peak plasma *d*-amphetamine levels were reported at ~30, 50, and 75 ng/mL in children and ~15, 30, and 40 ng/mL in adults receiving *d,l*-amphetamine doses of 10, 20, and 30 mg *(34)*.

d-Amphetamine is also available in immediate-acting (DextroStat® and Dexedrine® tablets) and sustained-release (Dexedrine® Spansule capsules) formulations. The drug labels provide a limited amount of pharmacokinetics information obtained from studies in which healthy volunteers were administered two or three 5-mg doses of immediate-acting formulations or one 15-mg dose of a sustained-release formulation *(9, 10)*. The time to reach maximum blood levels was 2–3 hours for immediate-acting formulations and 8 hours for the sustained-release formulation. Maximum blood level was reported at 29.2 ng/mL in individuals administered 10 mg Dextrostat® in 2 divided doses. Maximum plasma levels were measured at 36.6 ng/mL in

volunteers given 15 mg Dexedrine® as 3 divided doses of immediate-acting formulation and 23.5 ng/mL in volunteers given 15 mg Dexedrine® as 1 dose of extended-release formulation.

Brown et al. *(35)* measured plasma amphetamine levels in 16 boys (60–144 months old) orally dosed with ~0.5 mg/kg bw *d*-amphetamine. Blood was collected at baseline and at 11 time points over a 30-hour time period for determination of plasma amphetamine levels by radioimmunoassay (RIA). Plasma amphetamine levels peaked between 3 and 4 hours with mean (±SEM) values 62.7 ± 3.8 ng/mL at 3 hours and 65.9 ± 3.6 ng/mL at 4 hours. The greatest inter-subject variations in plasma levels were observed during the absorption versus elimination phase. Apparent half-life of elimination was calculated at 6.8 ± 0.5 hours. The study was repeated in 6 of the boys who received a dose of ~0.5 mg/kg bw *d*-amphetamine 1 week following the first study. Analyses of interclass coefficients of variation by chi-square demonstrated no significant differences in apparent half-life of elimination or peak plasma amphetamine levels during the two different time periods.

In a second study, Brown et al. *(36)* reported plasma-amphetamine levels in 9 boys (60–144 months old) orally dosed with a sustained-released formula of *d*-amphetamine at ~0.5 mg/kg bw. Plasma levels of amphetamine were determined by RIA**. [Blood was collected over an unspecified time period and data were only shown for the first 6 hours following exposure.]** Plasma amphetamine levels peaked between 3 and 8 hours with mean ± SEM values in that time period ranging from 64.1 ± 9.5 to 70.2 ± 7.9 ng/mL.

Intake of Adderall XR with a high fat breakfast delayed T_{max} by 2–2.5 hours for both the *d*- and *l*-enantiomers, but did not affect extent of absorption (Table 6) *(7, 34)*. Rate and extent of *d*-amphetamine absorption were reported to be similar following administration of Dexedrine® sustained-release capsules to fasted or fed (58–75 g fat) volunteers *(9)*.

Table 5. Pharmacokinetics of *d*- and *l*-Amphetamine in Children

Study regimen and subjects	*d*-Amphetamine				*l*-Amphetamine			
	C_{max} (ng/mL)	T_{max} (hours)	$AUC_{0-\infty}$ (ng-h/mL)[a]	Half-life (hours)	C_{max} (ng/mL)	T_{max} (hours)	AUC[a] (ng-h/mL)	Half-life (hours)
10 mg Adderall IR (n = 9 and n = 12)[b]	28.4±6.50–33.8±11.1	2.5±1.2–3.3±1.3	384±109	7.47±0.97	9.64±2.35–10.6±3.5	2.5±1.2–3.2±1.5	146±51.6	8.55±1.57
10 mg Adderall XR (n = 8)	28.8±6.2	6.4±3.5	432±123 (AUC_{0-24})	NS	8.8±1.9	6.4±3.5	138±40 (AUC_{0-24})	NS
20 mg Adderall XR (n = 48)	48.8±13.5	6.8±3.2	704±190 (AUC_{0-24})	9.5±2.4	14.8±4.3	6.9±3.3	216±60 (AUC_{0-24})	10.9±3.1
3 x 10 mg Adderall XR (n = 20)	73.9±21.4	5.5±2.7	1255±231	8.0±1.7	22.7±6.3	5.6±2.7	425±95	9.0±1.6
30 mg Adderall XR (n = 20)	75.9±20.6	5.5±3.6	1338±281	8.6±1.9	22.7±5.9	5.6±3.5	454±123	10.2±2.8

From FDA *(34, 37)*.
[a]Values are for $AUC_{0-\infty}$ unless otherwise specified.
[b]Two separate studies were conducted, at least one of which was published *(38)*; a range of values is included when the endpoint was reported in both studies and a single value is included when reported in one study.
AUC area under the concentration versus time curve; C_{max} maximum concentration; NS not specified; T_{max} maximum time.
Errors (e.g., SEM, SD) were not specified.

Table 6. Pharmacokinetics of *d*- and *l*-Amphetamine in Adults

Study	*d*-Amphetamine				*l*-Amphetamine			
Regimen and subjects	C_{max} (ng/mL)	T_{max} (hours)	$AUC_{0-\infty}$ (ng-h/mL)[a]	Half-life (hours)	C_{max} (ng/mL)	T_{max} (hours)	$AUC_{0-\infty}$ (ng-h/mL)	Half-life (hours)
10 mg Adderall IR (n = 8)	14.5±4.1	3.3±0.9	300±116	11.9±3.9	4.7±1.4	3.5±1.2	124±71	15.2±7.8
10 mg Adderall XR (n = 8)	13.9±4.2	6.2±1.8	296±101	12.2±3.3	4.4±1.3	6.2±1.8	115±54	15.2±5.7
2 x 10 mg Adderall IR (n = 19)	28.3±7.1	6.9±1.3	530±114	10.9±2.0	9.3±2.4	7.1±1.4	203±49	13.2±2.7
20 mg Adderall XR (n = 19)	28.1±8.8	7.0±2.4	567±114	11.8±2.7	8.7±2.8	8.2±4.4	203±47	13.7±2.8
20 mg Adderall XR (n = 7) fasting	29.1±4.9	5.0±1.6	521±79	10.4±1.7	9.4±1.6	5.0±1.5	197±35	12.6±2.5
20 mg Adderall XR (n = 7) with high fat breakfast	28.0±4.4	7.1±2.1	557±97	10.8±2.3	8.7±1.4	7.4±2.2	205±39	13.4±2.7
30 mg Adderall XR (n = 19) fasting	44.3±11.1	5.2±2.0	851±214	10.4±2.3	13.3±3.7	5.6±2.1	289±79	12.7±3.3
30 mg Adderall XR (n = 19) high fat breakfast	39.7±8.8	7.7±2.3	823±200	10.3±2.0	12.0±2.9	8.3±2.9	274±69	12.5±2.6

From FDA *(34)*.
Errors (e.g., SEM, SD) were not specified.

2.1.1.3 Distribution

Following oral intake, amphetamines are rapidly distributed to major organ systems, including the brain *(24)*. Linear pharmacokinetics were demonstrated for Adderall XR at doses of 10–30 mg *(7)*. No unexpected accumulation occurred at steady state *(7)*.

The volume of distribution for methamphetamine was reported at 3.42 L/kg in humans (reviewed in *(17)*.

Villen et al. *(39)* measured blood, milk, and urine levels of amphetamine in a 36-year-old woman taking 20 mg/day racemic amphetamine in 4 divided doses between 10 AM and 4 PM. for treatment of narcolepsy. At 10 and 42 days after delivery, 2 samples of blood and milk were collected at 9:30 AM and 2 PM and a 24-hour urine sample was obtained for analysis by gas chromatography (GC). Amphetamine concentrations 42 days postpartum in plasma were 20–40 ng/mL, breast milk concentrations were 55–118 ng/mL, and milk:plasma ratios were 6.6–7.5. Maternal urinary amphetamine excretion was measured at 3.6 mg/24 hours 10 days postpartum and 8.9 mg/24 hours 42 days postpartum. Twelve-hour urine samples were collected from the infant and urinary amphetamine excretion was reported to be 1/1000 to 1/300 of maternal levels. No untoward effects on growth, neurological assessments, emotional development, or motor achievements were noted in the infant at up to 24 months of age.

Bost et al. *(40)* reported methamphetamine and amphetamine distribution in 2 premature twin boys born to a women who used methamphetamine by iv injection 5 hours prior to hospital admission; the infants died shortly after birth. Distribution of amphetamine and methamphetamine is reported in Table 7. Maternal blood levels were not reported.

Table 7. Methamphetamine and Amphetamine Distribution in Twin Boys Exposed to Methamphetamine In Utero Five Hours Before Birth).

Organ	Methamphetamine level	Amphetamine level
Kidney	6.34–7.38 mg/kg	0.95–0.97 mg/kg
Liver	9.20–11.0 mg/kg	0.18–1.43 mg/kg
Brain	4.53–5.71 mg/kg	0.61–0.76 mg/kg
Blood	6.3 mg/L **[6300 ng/mL]**	0.28 mg/L **[280 ng/mL]**
Placenta	8.66 mg/kg	1.18 mg/kg

Data from Bost et al. *(40)*.

Stewart and Meeker *(41)* reported postmortem methamphetamine and amphetamine blood concentrations from stillbirths or infant deaths when the mother was believed to have used methamphetamine (Table 8). Maternal blood concentrations were available in two cases. **[The ascertainment of maternal exposure was not discussed and the timing of maternal blood sampling was not given.]**

Table 8. Methamphetamine and Amphetamine Measured in Stillbirths and Infant Deaths

Gestational age	Blood concentration (mg/L)		Other measurements or comments
	Methamphetamine	Amphetamine	
Stillbirths			
6 months	0.34	0.05	Fetus had been stored frozen for 3–4 days.
20 weeks	0.20	0.08	
30–32 weeks	1.20	0.06	Maternal blood methamphetamine 0.18 mg/L, amphetamine 0.03 mg/L.
Not stated	0.36	0.01	Methamphetamine 0.54 µg/g in brain and 0.52 µg/g in liver; amphetamine 0.04 µg/g in brain and liver.
Infant deaths			
Full term	0.40	0.06	Maternal blood methamphetamine 0.21 mg/L, amphetamine undetectable.
32 weeks	0.57	0.06	
Full term	0.13	0.05	Infant died at 1 month of age.
Not stated	0.03	0	Infant died at 1 month of age.
28–32 weeks*	0.355	0.080	Infant died at 4 hours of age. Mother reported taking 30–45 mg/day methamphetamine in diet pills. Infant tissue levels (in µg/g):
			Methamphetamine / Amphetamine
			Bile 0.384 / 0.050
			Kidney 0.746 / 0.080
			Lung 0.857 / 0.120
			Brain 0.280 / <0.030
			Liver 0.246 / <0.020

From Stewart and Meeker *(41)*. *Added from Garriott and Sprull *(42)*.

2.1.1.4. Metabolism

Amphetamine is a metabolite of methamphetamine and the metabolic pathways for the two compounds are illustrated together in Figure 2.

The initial step of amphetamine metabolism is hydroxylation of the alpha, aromatic 4-, or beta carbon (reviewed in *(24, 31)*). As noted in Table 9, metabolism through each possible pathway varies according to species (reviewed in *(24)*). Oxidation of the alpha carbon leads to deamination and ultimately to the formation of benzoic acid, which can be conjugated with glycine to form hippuric acid (reviewed in *(24, 31)*). According to the NTP review, deamination appears to be the predominant pathway of amphetamine metabolism in humans, leading to urinary excretion of primarily benzoic acid and hippuric acid (Table 9). In contrast, the National Toxicology Program (NTP) *(24)* noted that the main metabolic pathway in rats is aromatic hydroxylation and the main urinary metabolite is *p*-hydroxyamphetamine (4-hydroxyamphetamine); both metabolites generated from the aromatic hydroxylation pathway are reported to have biological activity (reviewed in *(31)*). Hydroxylated metabolites can be excreted as sulfate conjugates (reviewed in *(31)*). Aliphatic beta carbon hydroxylation accounts for only a minor percentage of metabolism, but is considered important because the resulting metabolite, norephedrine, is reported to have biological activity (reviewed in *(31)*). Additional metabolic pathways such as nitrogen

hydroxylation, oxidation, and conjugation are mentioned in reviews *(24, 31*), but do not appear to be primary pathways based on metabolites detected in urine.

Table 9. Comparison of Amphetamine Urinary Metabolites in Various Species

Species (sex)	Dose (mg/kg bw)	Percent dose excreted in urine (48 hours for rats, 24 hours for other species)				Total percent of dose in urine
		Benzoic +hippuric acid	Phenylacetone	4-Hydroxy-amphetamine	Amphetamine	
Human (male)[a]	0.66[c]	45	2	9	37	66
Rhesus monkey (female)[b]	0.66[d]	31–38	0	0–11	3.8–31	42–73
Squirrel monkey (sex not given)[a]	2[c]	5	ND	1	23	34
Rat (female, Wistar)[a,b]	10[e]	3	0	60	13	85
Mouse (S.A.S./I.C.I, female)[b]	10[d]	31	0	14	33	78
Rabbit (female, New Zealand)[a,b,f]	10[e]	25	22	6	4	72
Dog (female greyhound)[a,b]	5[e]	28	1	6	30	75
Guinea pig (female)[b]	5[d]	62	0	0	22	83

ND = Not determined.
[a]From NTP *(24*).
[b]From Dring et al. *(43*).
[c]Enantiomers not specified.
[d]*d*-Amphetamine.
[e]*d,l*-Amphetamine.
[f]Rabbits also excreted 8% 1-phenylpropanol, a metabolite not seen in most other species.

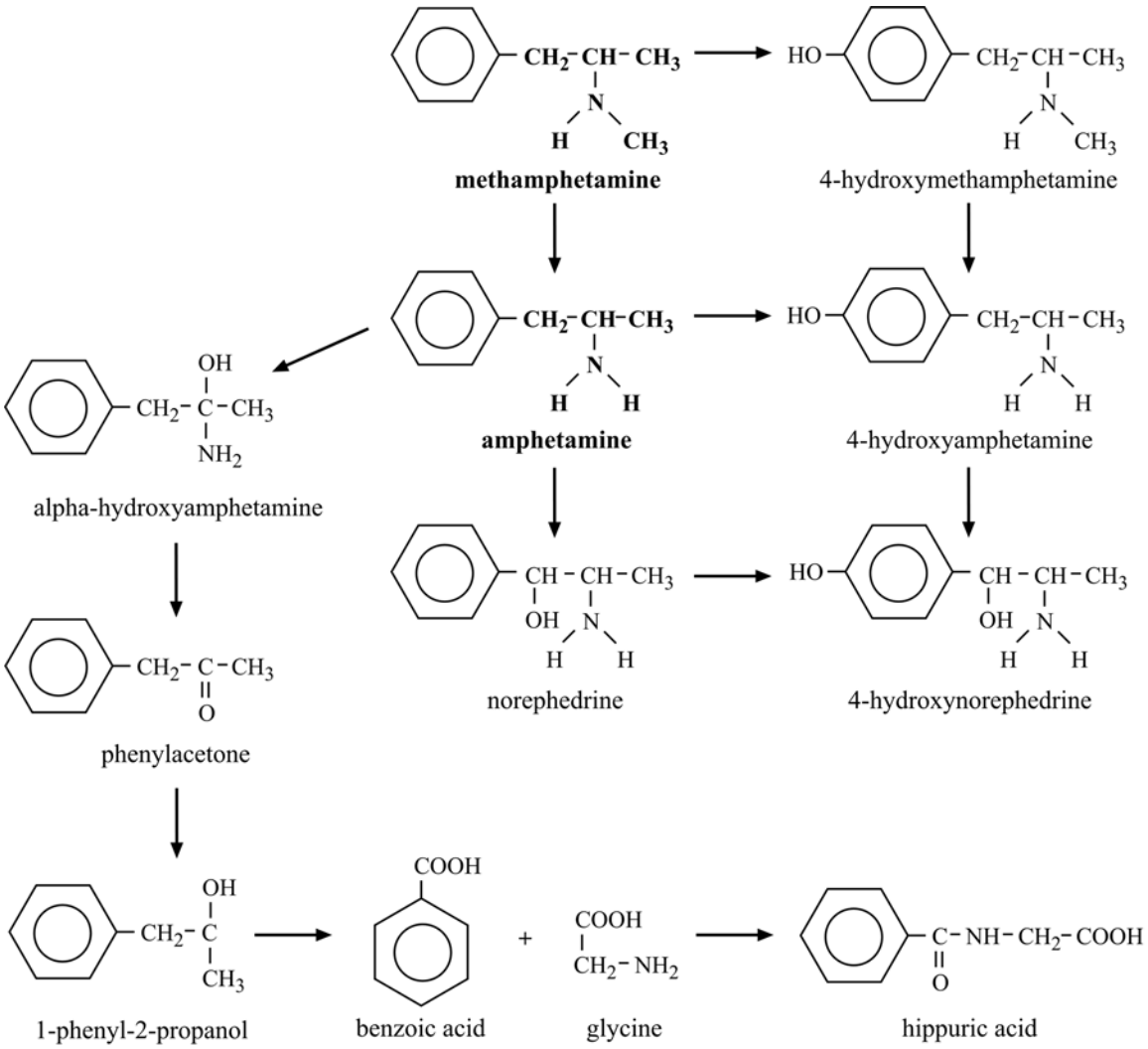


Figure 2. Metabolism of Amphetamine and Methamphetamine *(24, 31, 32)*.

Racemization does not occur during amphetamine metabolism (reviewed in *(25)*). Information on stereospecificity of amphetamine metabolism is conflicting. Dopamine beta-hydroxylase is the enzyme that catalyzes hydroxylation and is reported to react stereospecifically with the *d*-enantiomer in beta-carbon hydroxylation (reviewed in *(31)*). Due to the preferential beta-hydroxylation of *d*-amphetamine, less is excreted intact compared to the *l*-enantiomer. However, an FDA review *(34)* reported that metabolism and elimination do not appear to be stereoselective, since the ratio of systemic exposure to each enantiomer has been demonstrated to be equivalent to the composition of Adderall (3-*d*:1-*l*). **[It is possible that the preferential beta-hydroxylation of the *d*-enantiomer does not significantly affect systemic exposure because it is not expected to be a significant pathway with standard therapeutic dosing.]**

The two major pathways of methamphetamine metabolism are N-demethylation to form amphetamine, which can be metabolized through several pathways (see above), and aromatic hydroxylation to form 4-hydroxyamphetamine and then 4-hydroxynorephedrine *(25, 31, 32)*. Hydroxynorephedrine is described as a false neurotransmitter (reviewed in *(30)*) that may play a role in habituation *(32)*. Table 12 compares urinary methamphetamine metabolites and Table 13 compares the contribution of each metabolic pathway in humans, rats, and guinea pigs *(32)*. As noted in Table 12, humans excreted a larger percentage of unmetabolized methamphetamine than rats and guinea pigs, despite receiving lower doses. The two major pathways in humans are aromatic hydroxylation and demethylation, while deamination and beta-hydroxylation account for a smaller percentage of metabolism. In humans, aromatic 4-hydroxylation is much more extensive in the metabolism of methamphetamine compared to amphetamine (reviewed in *(30)*).

Nitrogen demethylation of racemic methamphetamine is reported to be stereospecific with more rapid biotransformation of the *d*-enantiomer (reviewed in *(31)*). During the first 16 hours following administration of racemic methamphetamine, both enantiomers were excreted at approximately equal levels, but excretion of *l*-methamphetamine was increased thereafter.

The role of cytochrome P450 (CYP) enzymes in amphetamine and methamphetamine metabolism in humans was discussed in a review by Kraemer and Mauer *(25)*. Studies using human liver microsomes, including those with poor CYP2D6 metabolic capability, demonstrated a major role of CYP2D6 in aromatic 4-hydroxylation of methamphetamine; results were replicated using recombinant CYP2D6 in yeast. A second investigator obtained the same results for amphetamine using microsomal preparations from cells expressing CYP2D6. Amphetamine and methamphetamine were reported to be substrates and competitive inhibitors of CYP2D6. In their review of human and animal studies (discussed in Section 2.1), Kraemer and Mauer *(25)* concluded ". . . there is convincing evidence about the role of CYP2D6 in the ring hydroxylation of amphetamine and methamphetamine."

A number of original studies were reviewed in detail.

Two studies conducted by Cook et al. *(44, 45)* provide information on pharmacokinetics in adults exposed to methamphetamine through different routes or following single versus repeat dosing. In 1 study, 8 healthy men received oral doses of 0.125 or 0.250 mg/kg bw *d*-methamphetamine HCl orally on study day 1, 10 mg/day of a slow-release, unlabeled *d*-methamphetamine medication on study days 2–14, and 0.125 or 0.250 mg/kg bw *d*-methamphetamine HCl on study day 15 *(44)*. Six volunteers were exposed to both doses and each value obtained was based on 3–6 volunteers. Pharmacokinetic parameters were examined on study days 1 and 15. In the second study, pharmacokinetic parameters were examined in 6 healthy men administered methamphetamine at 21.8±0.3 (SEM) mg **[0.26 mg/kg bw based on reported mean body weight of 83.8 kg]** through the inhalation route (smoking heated vapors) or 15.5 mg **[0.18 mg/kg bw]** through iv injection *(45)*. Mean free base doses were 17.5 mg for inhalation exposures and 12.42 mg for iv exposures. In both studies, collection of samples occurred

prior to dosing and at various time points after dosing for up to 48 hour for blood, 72 hours for urine, and 24 hours for saliva. Methamphetamine and amphetamine levels in samples were analyzed by GC.

Pharmacokinetic results for oral exposure are listed in Table 10 and for inhalation and iv exposure in Table 11. The following are observations or conclusions made by study authors based on results of these studies:

- No significant differences in pharmacokinetic parameters were observed when 0.125 mg/kg bw methamphetamine was administered orally, before versus after the 13-day oral exposure to the slow-release drug.
- Compared to study day 1, a slight but significant increase in maximum plasma methamphetamine level was observed when 0.250 mg/kg bw was administered on study day 15, following the 13-day subchronic exposure. **[The Expert Panel notes this finding is not likely to be of pharmacological relevance.]**
- Pharmacokinetic parameters were similar following inhalation and iv exposures.
- Bioavailability was higher following inhalation (90.3%) versus oral (67.2%) exposure.
- Percentage of dose excreted in urine was greater following oral exposure to 0.125 mg/kg bw compared to 0.250 mg/kg bw on study day 1 (statistically significant) and study day 15.
- AUC values for amphetamine (metabolite) were proportional between oral doses of 0.12 (AUC = 98.3 ng-h/mL) and 0.250 mg/kg bw (AUC = 224 ng-h/mL); the values were 30% of parent AUCs (330 ng-h/mL at low dose and 775 ng-h/mL at high dose).
- Renal excretion of parent drug following oral, iv, or inhalation exposure was dependent on urine flow and pH; dose was also a factor with oral exposure.
- Renal clearance rates following oral dosing (Table 10) exceeded the average renal filtration rate of ~125 mL/min, suggesting involvement of an active transport mechanisms in renal clearance.
- Dose-dependent differences in renal clearance between oral doses of 0.125 and 0.25 mg/kg bw suggest possible saturation of a renal active transport process.
- A comparison of oral, inhalation, and iv data suggested that renal elimination is decreased with increased bioavailability, also suggesting saturable excretion.
- Urinary amphetamine represented ~15% of the oral doses and ~7% of the inhalation or iv dose.
- Large variations in plasma to saliva ratios were observed with oral and inhalation dosing; saliva to plasma ratios of methamphetamine were similar with inhalation and iv dosing.
- Subjective (e.g., feeling "high") and cardiovascular effects following inhalation and iv exposure subsided before substantial decreases in plasma methamphetamine, suggesting development of acute tolerance.

Table 10. Pharmacokinetic Parameters in Men Orally Administered *d*-Methamphetamine HCl

Parameter	0.125 mg/kg bw		0.250 mg/kg bw	
	Study day 1	Study day 15	Study day 1	Study day 15
T_{max} (hour)	3.60±0.63	3.06±0.62	3.23±0.38	2.64±0.20
C_{max} (ng/mL)	19.8±2.7	20.3±3.0	37.2±1.3	41.8±1.7
Half-life (hours)	8.46±0.71	9.71±1.10	11.45±1.57	10.93±1.45
% dose in urine	54.1±5.8	50.0±12.4	34.6±4.3	30.0±3.1
Total clearance (mL/min)	446±66	404±60	381±66	357±57
Renal clearance (mL/min)	212±33	189±27	138±41	122±23
C_{max} (ng/mL) for amphetamine (metabolite)[a]	~1.6	~1.2	~3.9	~4.2

From *(44)*. All values are for methamphetamine, unless otherwise indicated; values presented as mean±SEM.

[a]Values estimated from a graph by CERHR.

Table 11. Pharmacokinetic Parameters in Men Administered *d*-Methamphetamine HCl through Inhalation or Intravenous Exposure

Parameter	Mean dose in mg (mg/kg bw)	
	21.8 (0.26) by Inhalation	15.5 (0.18) iv
Half-life (hour)	11.8±1.35	13.1±1.54
$AUC_{0-\infty}$ (ng-h/mL)	1013±141	787±29.7
Mean residence time (hour)	16.7±1.46	17.4±2.15
Total clearance in L/hour **[mL/min]**	15.9±0.73 **[265±12.2]**	NS
Renal clearance in L/hour **[mL/min]**	6.68±0.80 **[111±13.3]**	6.95±1.25 **[116±20.8]**
Volume of distribution (L/kg)	3.24±0.36	3.73±0.59
% Dose in urine	36.8±4.3	45.0±9.5
% Metabolic clearance	57.9±5.0	55.0±9.5
C_{max} (ng/mL) for amphetamine (metabolite)	4.2±0.56	4.0±0.63

From *(45)*. All values are for methamphetamine, unless otherwise indicated. Values presented as mean±SE. NS = Not specified.

Pharmacokinetic parameters in 8 male adult volunteers who smoked 40 mg methamphetamine or inhaled 50 mg methamphetamine in a mist (intranasal exposure) were examined in a study by Harris et al. *(46)*. Pharmacokinetic parameters were similar following smoking and intranasal exposure and were comparable to values reported by Cook et al. *(45)*. However, bioavailability with smoking exposure was reported at 67% by Harris, which was lower than the 90% value reported by Cook. Differences in pipe temperature and smoking techniques were discussed as possible reasons for the discrepancy between the two studies. Bioavailability was reported at 79% following intranasal exposure.

2.1.1.5 Excretion

The half-life for *d*-amphetamine was reported at ~7 hours in children taking 10 mg Adderall IR, ~8–9 hours in children taking 20–30 mg Adderall XR, and ~10–12 hours in adults receiving 10–20 mg Adderall IR or 10–30 mg Adderall XR. Dosing with the Adderall formulations described above resulted in *l*-amphetamine half-lives of ~9–11 hours in children (Table 5) and 13–15 hours in adults (Table 6) *(34)*.

Biological half-life for methamphetamine was reported at 4–5 hours *(8)*. Another review reported a half-life of 12 hours for methamphetamine in humans *(17)*.

Amphetamine, methamphetamine, and their metabolites are primarily excreted in the urine. Dosing with two 5-mg *d*-amphetamine tablets resulted in 45% urinary recovery in 48 hours *(10)*. In 2 male volunteers given 0.29 mg/kg bw radiolabeled methamphetamine, the percentages of radioactivity recovered in urine during the first 4 days following dosing were 55–69% at 24 hours, 78–90% at 48 hours, 86–94% at 72 hours, and 88–96% at 96 hours *(32)*. In 3–6 male volunteers given deuterated methamphetamine orally, urinary recoveries within 72 hours were ~50–55% at a dose of 0.125 mg/kg bw and ~30–35% at a dose of 0.25 mg/kg bw *(44)*.

On average, about 30–40% of an amphetamine or methamphetamine dose is eliminated unchanged and the remainder is eliminated as metabolites (Table 12) *(8, 31, 32)*. About 37–45% of a methamphetamine dose was eliminated unchanged and ~7% was eliminated as amphetamine in men given 22 mg through inhalation or 15.5 mg by iv exposure *(45)*. However the percentage of parent

compound excreted varies according to urinary pH (reviewed in *(31)*). Due to a pKa of ~9.9, amphetamines are primarily ionized under normal physiological pH and in acidic urine. Because the ionized form is not significantly reabsorbed by the kidney, large amounts are excreted unchanged. In alkaline urine, amphetamines exist primarily in the un-ionized form, and are readily reabsorbed by the kidney. Reabsorption results in prolonged half-life and increased biotransformation. Excretion of unmetabolized amphetamine and methamphetamine was reported to range from 1–2% in highly alkaline urine to 74–76% in strongly acidic urine. In a study by Anggard et al. *(33)*, the amount of amphetamine excreted unchanged in urine over 4 days was ~70% in 3 subjects with acidic urine (pH = 5.3–5.8) and ~20–56% in 4 subjects with neutral-to-basic urine (pH = 6.7–7.5); percentage of metabolites excreted increased at higher urinary pH. As noted in Section 2.2.1.3, numerous drugs can increase or decrease urinary pH.

Table 12. Comparison of Urinary Methamphetamine Metabolites in Humans, Rats, and Guinea Pigs

Compound	Percentage of methamphetamine dose in urine[a]			
	Humans (n = 2 males) given 0.29 mg/kg bw orally	Rats (n = 3) given 45 mg/kg bw orally	Guinea pigs given 10 mg/kg bw ip	Guinea pigs given 45 mg/kg bw ip
Methamphetamine	23	11	0.5	3
Amphetamine	3	3	4	13
Norephedrine	2	0	1	19
4-Hydroxymethamphetamine	15	31	0	0
2-Hydroxyamphetamine	1	6	0	0
4-Hydroxynorephedrine	2	16	0	0
Benzoic acid	5	4	63	31
Benzyl methyl ketone precursor	1	NS	11	2
Total	52	71	79.5	68

From *(32)*..
NS = not specified.
[a]Urine was collected for 1 day in humans and guinea pigs and 2 days in rats.

Table 13. Comparison of Methamphetamine Metabolic Pathways in Humans, Rats, and Guinea Pigs

Pathway	Percentage of methamphetamine dose metabolized through each pathway			
	Humans given 0.29 mg/kg bw orally (n = 2)	Rats given 45 mg/kg bw orally (n = 3)	Guinea pigs given 10 mg/kg bw ip (n = 3)	Guinea pigs given 45 mg/kg bw ip (n = 3)
Unmetabolized	23	11	0.5	3
Aromatic hydroxylation	18	53	0	0
Demethylation	14	28	79	64
Beta-hydroxylation	4	16	1	19
Deamination	6	4	74	33

From *(32)*.
[a]Urine was collected for 1 day in humans and guinea pigs and 2 days in rats.

2.1.2 Experimental Animal

2.1.2.1 Pharmacodynamics

A study using mice lacking the dopamine transporter examined the role of amphetamine in dopaminergic neurons *(47)*. The study suggested that amphetamine induced release of dopamine from neuronal vesicles to cytoplasm and then caused the reverse transport of dopamine from cytoplasm to extracellular space through the cytoplasmic dopamine transporter. Vesicle release was the rate limiting step, but both processes were necessary for amphetamine-induced extracellular dopamine release.

2.1.2.2 Pharmacokinetics

The volume of distribution for methamphetamine in rats was reported at 3.95 L/kg (reviewed in *(17)*). As in humans, amphetamine metabolic pathways in experimental animals can include hydroxylation of the alpha carbon, the aromatic 4-carbon, the beta carbon, or possibly the amine group *(24)*. As noted in Table 9, metabolism through each pathway varies according to species. Deamination can occur following alpha-carbon hydroxylation; according to Table 9, the pathway plays a minimal role in rats but is a major pathway in humans. Aromatic hydroxylation is the predominant pathway in rats and the primary urinary metabolite is 4-hydroxyamphetamine.

One review reported that 4-hydroxyamphetamine can be metabolized by a neuronal CYP to alpha-methyldopamine and then to alpha-methylnorepinephrine, a possible false neurotransmitter (reviewed in *(30)*). Studies in whole and striatal preparations of rat brain demonstrated *d*-amphetamine hydroxylation; following administration of *d*-amphetamine to experimental animals (mostly rats), 4-hydroxyamphetamine, 4-hydroxynorephedrine, alpha-methyldopamine, and alpha-methylnorepinephrine were found in rat brain. The half-life of hydroxyamphetamine was 1.5 days and the half-life of hydroxynorephedrine was 2.5 days in rat striatum.

Methamphetamine metabolism in rats exposed by gavage and guinea pigs exposed by intraperitoneal (ip) injection is qualitatively similar to that for humans, as described in Section 2.1.1.4. However, as noted in Table 12, the percentage of unchanged methamphetamine excreted in urine is lower in rats and guinea pigs compared to humans, despite the rats receiving higher doses than humans. Aromatic hydroxylation is the predominant metabolic pathway in rats, but substantial metabolism also occurs through demethylation and beta-hydroxylation. In guinea pigs, aromatic hydroxylation does not appear to occur and the main metabolic pathways are demethylation and deamination. Beta hydroxylation of amines was noted as a reaction of interest by Caldwell et al. *(32)*, since resulting metabolites, 4-hydroxynorephedrine and possibly norephedrine, can act as false neurotransmitters, postulated to be involved in habituation associated with chronic methamphetamine intake. Urinary excretion of norephedrine increased and benzoic acid decreased when the ip dose in guinea pigs was increased from 10 to 45 mg/kg bw. Caldwell et al. *(32)* suggested that deamination may be saturated at high doses in guinea pigs.

The role of CYP enzymes in amphetamine metabolism in experimental animals was discussed in a review by Kraemer and Mauer *(25)*. By administering quinidine (a specific CYP2D inhibitor) to rats dosed with amphetamine, it was demonstrated that CYP2D is involved in aromatic hydroxylation of amphetamine. CYP2D1/6 catalysis of methamphetamine 4-hydroxylation was demonstrated using liver microsomes from Sprague-Dawley and Dark Agouti rats (a poor CYP2D6 metabolizer phenotype); addition of anti-P450 BTL IgG, bufuralol (a CYP2D6 substrate), or quinine (a CYP2D6 inhibitor) blocked approximately 90% of the reaction in Sprague-Dawley rat microsomes. The reaction was mediated using reconstituted CYP2D isozymes purified from rat liver microsomes. A study measuring urinary elimination of 4-hydroxyamphetamine and amphetamine in Sprague-Dawley rats pre-dosed with cytochrome P450 inhibitors or inducers demonstrated significantly reduced elimination of 4-

hydroxyamphetamine in rats treated with inhibitors 1-aminobenzotriazole, carbon tetrachloride, quinidine, quinine, and primaquine. Based on debrisoquine metabolism, CYP2D1 was first thought to be the rat enzyme equivalent to CYP2D6 in humans, but it was later determined that the equivalent enzyme in rats is CYP2D2 (reviewed in *(48)*).

Kraemer and Mauer *(25)* reviewed a study demonstrating that quinidine, an inhibitor of CYP2D and CYP2C3, inhibited deamination by purified rabbit CYP2C3. It was concluded that CYP2C isozymes are greatly involved in amphetamine deamination.

A half-life of 87 minutes in plasma and 62 minutes in brain was reported following iv injection of rats with 0.5 mg/kg bw *d,l*-amphetamine (reviewed in *(24)*). Tissue half-life was reported at 5–9 hours following ip injection of rats with *d,l*-amphetamine sulfate (reviewed in *(24)*).

A half-life of 70 minutes was reported for an unspecified concentration of methamphetamine given to rats through an unspecified route (reviewed in *(17)*). Studies in rats given radiolabeled methamphetamine at 45 mg/kg bw orally or by ip injection demonstrated that ~75–85% of radioactivity was excreted in urine and ~1–3% in feces over 3 days *(32)*. Similar values were reported for guinea pigs dosed ip with 10 mg/kg bw methamphetamine, but at 45 mg/kg bw, urinary excretion of radioactivity was reported at 44–87% and fecal excretion at 5.5–29% over 4 days.

The FDA pharmacology review for Adderall *(34)* summarized fertility and reproductive studies that included pharmacokinetic data. Sprague-Dawley rats and New Zealand White rabbits were dosed by gavage with free amphetamine base **[the Expert Panel assumes a 3:1 mixture of *d*- and *l*-enantiomers, as in the marketed product]**. The results as given in the FDA summary are shown in Table 14 and Table 15. The developmental and reproductive endpoints are discussed in Sections 3.2 and 4.2.

Table 14. Pharmacokinetic Results in Rats Given Adderall

Sex	Gavage regimen	Dose (mg/kg bw/day)	$t_{1/2}$ (h)	C_{max} (ng/mL)		AUC (ng-h/mL)	
				1st dose	2nd dose	AUC_8	AUC_{24}
Male	Daily for 3 weeks	2	ND	39.1	46.5	100	216
		6	ND	233.9	203.4	648	1187
		20	1.9	880.4	976.1	2822	5689
Nonpregnant female	Daily for 1 week	2	2.7	81.6	81.3	236	555
		6	ND	212.2	236.0	888	1599
		20	ND	1080.7	1196.9	3727	8506
Pregnant female, GD 17	Divided doses GD 6–17	2	3.0	80.3	93.2	221	455
		6	2.3	248.6	233.8	700	1566

$t_{1/2}$ = serum elimination half-life, C_{max} = maximum serum concentration, AUC = area under the time-concentration curve (subscript denotes number of hours plotted after drug administration). ND = not determined. Data from FDA Pharmacology review *(34)*.

Table 15. Pharmacokinetic Results in Pregnant Rabbits Given Adderall

Dose (mg/kg bw/day)	C_{max} (ng/mL)		AUC (ng-h/mL)	
	1st dose	2nd dose	AUC_8	AUC_{24}
2	22.7	36.2	33.95	89.85
6	61.9	158.5	121.69	376.77
16	258.9	359.9	588.23	1464.42

Does were gavaged with 2 equal treatments separated by 8 hours on GD 6–19, and were sampled on GD 19. C_{max} = maximum serum concentration, AUC = area under the time-concentration curve (subscript denotes number of hours plotted after drug administration). Half-life was not reported. Data from FDA Pharmacology Review *(34)*.

[The Expert Panel noted a disproportionate increase in AUC_{24} in rats dosed with 2, 6, and 20 mg/kg bw/day Adderall and rabbits dosed with 2, 6, and 16 mg/kg bw/day Adderall, thus suggesting saturation or dose-dependent elimination and drug accumulation.]

A number of studies were reviewed in detail because they examined pharmacokinetic parameters in pregnant or immature animals and were thus highly relevant to a CERHR evaluation.

Acuff-Smith et al. *(49)* performed a developmental neurotoxicity study using pregnant Sprague-Dawley rats treated with *d*-methamphetamine (free base **[purity not given]**) twice daily by subcutaneous (sc) injection from gestation day (GD) 7 to 12 or from GD 13 to 18 (plug = GD 0). The developmental aspects of the study are discussed in Section 3.2. A satellite group of pregnant animals given 20 mg/kg bw/injection (40 mg/kg bw/day) was used for measurement of serum methamphetamine and amphetamine (4 dams at each of 5 time points over 8 hours after the 11th injection). Estimates of pharmacokinetic parameters for methamphetamine are presented in Table 16. Amphetamine serum concentrations rose over the first 2 hours after injection of methamphetamine, reaching a plateau of about 400 ng/mL that was maintained throughout the remainder of the 8-hour sampling period. Plasma and brain pharmacokinetic parameters for neonatal rats treated with sc methamphetamine were reported by Cappon and Vorhees *(50)* and are given in Table 17.

Table 16. Pharmacokinetic Parameters for Methamphetamine Administered to Pregnant Sprague-Dawley Rats.

Treatment period (GD)	t_{max} (hours)	C_{max} (ng/mL)	AUC (ng-h/mL)	$t_{1/2}$ (hours)
7–12	0.75	3600	16,200	6
13–18	0.75	3100	17,100	6

Estimated from figure in Acuff-Smith et al. *(49)*, presenting serum concentrations for 8 hours after the 11th twice/daily dose of methamphetamine 20 mg/kg bw/dose. AUC (8-hour) estimated using trapezoidal method.

Table 17. Pharmacokinetic Parameters for Methamphetamine Administered to Neonatal Sprague-Dawley Rats

		Plasma				Brain			
Dose regimen (sc)	PND	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_8 (ng-h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_8 (ng-h/mL)
15 mg/kg bw × 1 dose	1	5750	0.25	1.9	23,800	15.5	1.0	4.5	63
	11	5150	0.17	2.0	17,860	19.8	0.5	2.3	54
20 mg/kg bw × 2 (6 h apart)	1	7150	0.33	1.3	17,860	21.7	1.0	3.3	100
	11	6320	0.50	0.9	22,320	32.8	0.5	2.0	76
10 mg/kg bw × 4 (2 h apart)	1	7300	0.33	3.2	16,370	32.9	1.0	3.8	122
	11	6100	0.25	2.8	14,475	34.8	0.25	2.3	94

Data from *(50)*. AUC_8 data estimated from bar graphs in the original paper. Four to six pups were used for most data points, avoiding the use of littermates and including both males and females.

Won et al. *(51)* measured maternal and fetal brain levels of methamphetamine and amphetamine following sc injection of mouse C57B1/6 dams with 40 mg/kg bw *d*-methamphetamine hydrochloride on GD 14. In maternal striatum, methamphetamine levels peaked at ~510 ng/mg protein 1 hour following injection and rapidly declined over the remaining 3 hours of the experiment; amphetamine levels peaked at ~77 ng/mg protein at 2 hours and remained at that level for up to 4 hours. Methamphetamine levels in fetal brain peaked at ~22 ng/mg protein at 1 hour and declined over the next 3 hours. Amphetamine was only detected in fetal brain at 2 and 4 hours (~ 18 ng/mg protein) following injection of dams. A second experiment demonstrated that levels of methamphetamine were similar in maternal striatum (~335 ng/mg protein) and cortex (~294 ng/mg protein), but lower in brainstem (~236 ng/mg protein). Fetal brain levels of methamphetamine reported for 1 litter were ~99 ng/mg protein in striatum, ~102 ng/mg protein in brainstem, and ~57 ng/mg protein in cortex.

Burchfield et al. *(52)* studied the pharmacokinetics and pharmacodynamics of methamphetamine in pregnant sheep. On GD 125 (~85% of term), catheters were inserted in Grade Western sheep and the animals were given antibiotics during a 3-day recovery period. Following the recovery period, sheep received 1 or more iv treatments of methamphetamine that included 0.6 mg/kg bw over 12.5 minutes, 1.2 mg/kg bw over 12.5 minutes, or 1.2 mg/kg bw over 30 seconds. Blood was collected from 4 or 5 ewes/group and methamphetamine levels were measured in plasma by high performance liquid chromatography (HPLC) to determine pharmacokinetic parameters in ewes and fetuses. Results are listed in Table 18. Methamphetamine rapidly crossed the placenta. Though initial plasma levels were higher in ewes than fetuses, the longer elimination time in fetuses resulted in higher fetal AUC values. Fetal half-life was directly correlated with maternal half-life ($r = 0.78$) and inversely correlated with pretreatment fetal oxyhemoglobin saturation ($r = –0.79$). Methamphetamine levels were measured in 4 fetuses from ewes killed 2 hours following treatment. Methamphetamine levels were reported to be highest in lung > placenta > kidney > intestine > liver > brain >heart. Average fetal organ to plasma ratios for methamphetamine were ~19 in lung and ~6 in brain. Methamphetamine increased maternal and fetal blood pressure and reduced fetal oxyhemoglobin saturation and arterial pH (Section 3.2.1.1).

Table 18. Pharmacokinetic Parameters of Methamphetamine in the Pregnant Sheep and Fetus

Treatment of ewe	Measured in ewe or fetus	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng-min/mL)	Half-life (minutes)
0.6 mg/kg bw, iv	Ewe	300±72.5	8834±2585	22.3±3.4
infusion (n = 4)	Fetus	200±21.0	8888±2605	31.7±12.4
1.2 mg/kg bw, iv	Ewe	694±189	19,651±7023	29.7±8.0
infusion (n = 5)	Fetus	457±104	20,688±5870	33.8±9.2
1.2 mg/kg bw iv	Ewe	10,197±5552	30,590±10,573	38.8±9.4
bolus (n = 5)	Fetus	721±252	26,179±6728	39.5±11.1

Data presented as mean ± SD. From Burchfield et al. *(52)*.

Stek et al. *(53)* implanted catheters in 7 mixed-breed ewes and their fetuses at GD 107 (early third trimester). One mg/kg bw methamphetamine HCl **[purity not specified]** was injected into the maternal vena cava over 1.5 minutes and samples of maternal and fetal blood (n = 5 maternal-fetal pairs) were taken at timed intervals and amniotic fluid (n = 3 fetuses). Maternal serum methamphetamine peaked at 2 minutes (the first sampling time) at 2900 ± 120 ng/mL (mean ± SEM), with half-time disappearance from maternal serum of about 30 minutes **[estimated from a graph]**. Fetal serum methamphetamine peaked at 5 minutes at 1900 ng/mL, after which elimination from serum paralleled elimination in the dam at concentrations 200–300 ng/mL lower than maternal concentrations. Amniotic fluid methamphetamine peaked at 120 minutes at 1050 ± 100 ng/mL, after which concentrations were similar to those in maternal serum. Twenty-four hours after the methamphetamine dose, maternal serum methamphetamine was 150 ± 20 ng/mL, fetal serum methamphetamine was 210 ± 30 ng/mL, and amniotic fluid methamphetamine was 350 ± 140 ng/mL **[concentrations converted from µg/mL to ng/mL]**. Effects on the fetus are discussed in Section 3.2.1.1.

2.2 General Toxicity

2.2.1 Human

2.2.1.1 Side effects of medication therapy

Adverse events occurring in at least 1% of volunteers in a trial for sustained-release Adderall are listed in Table 19 *(7, 34)*. The events considered common and drug related by the FDA *(34)* included fever, loss of appetite, emotional lability, insomnia, and nervousness. As noted in Table 20, a dose-related pattern was noted for anorexia, weight loss, and insomnia.

Adverse effects listed in drug labels for *d,l*-amphetamine, *d*-amphetamine, and methamphetamine include heart palpitations, tachycardia, elevated blood pressure, over-stimulation, restlessness, dizziness, insomnia, euphoria, dyskinesia, dysphoria, tremor, exacerbation of motor and phonic tics and Tourette disorder, headache, dry mouth, unpleasant taste, diarrhea, other gastrointestinal disturbances, constipation, anorexia, weight loss, urticaria, impotence, and changes in libido *(6-10)*. The labels report that psychotic episodes are rare at recommended doses, but state that behavior disturbance and thought disorder may be exacerbated in psychotic patients. Isolated cases of cardiomyopathy following chronic amphetamine use were reported in labels for *d,l*- and *d*-amphetamines *(7, 9, 10)*. The American Academy of Pediatrics *(54)* also reported jitteriness and social withdrawal as common side effects associated with stimulant treatment.

Evidence of growth suppression and development of tics in amphetamine-treated children is discussed in detail in Section 3.1.

According to drug labels, use of *d,l*-amphetamine, *d*-amphetamine, and methamphetamine is contraindicated in individuals with advanced arteriosclerosis, symptomatic cardiovascular disease, moderate-to-severe hypertension, hyperthyroidism, glaucoma, hypersensitivity or idiosyncrasy to sympathomimetic amines, agitation, and history of drug abuse *(6, 10)*.

Table 19. Adverse Events in Volunteers Taking *d,l*-Amphetamine

		Percent with adverse events	
Body system	Effect	*d,l*-Amphetamine (n = 374)	Placebo (n = 210)
General	Abdominal pain (stomachache)	14	10
	Accidental injury	3	2
	Asthenia (fatigue)	2	0
	Fever	5	2
	Infection	4	2
	Viral infection	2	0
Digestive system	Loss of appetite	22	2
	Diarrhea	2	1
	Dyspepsia	2	1
	Nausea	5	3
	Vomiting	7	4
Nervous system	Dizziness	2	0
	Emotional lability	9	2
	Insomnia	17	2
	Nervousness	6	2
Metabolic/nutritional	Weight loss	4	0

Table 20. Dose-Response Relationship of Some Common *d,l*-Amphetamine Adverse Events

	Incidence (%)			
Adverse Event	Placebo	10 mg	20 mg	30 mg
Anorexia	11.4	16.3	23.1	26.6
Weight loss	0	1.6	2.5	8.9
Insomnia	1.9	11.6	19.0	19.4

2.2.1.2 Overdose symptoms

Amphetamine toxicity following overdose in humans is an extension of pharmacological activity that can affect the central nervous, cardiovascular, pulmonary, and gastrointestinal systems *(55)*. Gastrointestinal toxicity can be manifested by nausea, vomiting, and diarrhea.

Pulmonary symptoms of toxicity include dyspnea, hemoptysis, and pleuritic chest pain *(56)*. Pulmonary sequelae include noncardiogenic pulmonary edema and pulmonary hypertension caused by endothelial injury, direct spasm, and dysregulation of mediators of vascular tone.

CNS symptoms include agitation, hallucinations, psychosis, and seizures. Intracerebral hemorrhage and ischemic strokes have been reported with amphetamine compounds. Cerebral vasculitis as well as cerebral artery spasm and occlusion have been implicated as causes for both stroke and hemorrhage

(57-59). A case of transient cortical blindness in an infant exposed to methamphetamine has been reported *(60*).

Cardiovascular signs and symptoms of toxicity include chest pain, palpitations, tachycardia, hypertension, and ventricular dysrhythmias. Myocardial infarctions associated with amphetamine use are thought to be secondary to direct cardiac toxicity (myocarditis), vasospasm, and thrombus formation *(61, 62*). Profound hypotension, bradycardia, and metabolic acidosis have occurred with massive amphetamine overdoses. Both acute and chronic cardiomyopathy have been associated with amphetamine use *(63, 64*). Direct amphetamine toxicity and indirect hypertension have both been implicated as etiologies.

Severe systemic toxicity reported with overdoses of amphetamine compounds includes a pattern of fulminant hyperthermia, convulsions, disseminated intravascular coagulation, hepatocellular damage, rhabdomyolysis, acute renal failure, dysrhythmias, and refractory hypotension *(65, 66*).

2.2.1.3 Drug Interactions

Drug labels for *d,l*-amphetamine, *d*-amphetamine, and methamphetamine warn against taking monoamine oxygenase inhibitors (MAOI) within 14 days of amphetamine or methamphetamine use *(6-10*). Metabolism of amphetamines is slowed by MAOI antidepressants and a metabolite of furazolidone, thus potentiating the effect associated with monoamine release from adrenergic nerves. Potentiation can lead to potentially fatal hypertensive crisis, neurological toxicity, and malignant hyperpyrexia. Amphetamines and methamphetamine could enhance tricyclic or sympathomimetic drug activity resulting in marked and sustained increases in brain *d*-amphetamine levels and potentiation of cardiovascular effects.

The drug label for methamphetamine states that phenothiazines could inhibit stimulatory effects of amphetamines *(8*). In addition, the drug label warns that dietary regimen changes associated with methamphetamine use could alter insulin requirements in diabetics.

Additional drug interactions are discussed in labels for *d,l*- and *d*-amphetamine. Gastrointestinal acidifying agents (e.g., guanethidine, reserpine, glutamic acid HCl, ascorbic acid, fruit juices) inhibit absorption of amphetamines and urinary acidifying agents (e.g., ammonium chloride, sodium acid phosphatase, methenamine) increase ionization and therefore urinary excretion. Both groups of acidifying agents lower blood levels and efficacy of amphetamines. The opposite situation occurs with agents that alkalinize the gastrointestinal tract (e.g., sodium bicarbonate, antacids) or urine (e.g., acetazolamide, some thiazides). The effects of amphetamines may be potentiated by propoxyphene and inhibited by chlorpromazine, haloperidol, and lithium carbonate. Amphetamines could inhibit adrenergic blockers, sedative effects of antihistamines, and hypotensive effects of antihypertensive drugs. Amphetamines could potentiate analgesic effects of meperidine, the adrenergic effect of norepinephrine, and the anticonvulsant effects of phenobarbital and phenytoin. Intestinal absorption of ethosuximide could be delayed by amphetamines.

d-Amphetamine could interact with pimozide, which diminishes its stimulant effects, and with various antihypertensive agents *(67*). Effects of *d*-amphetamine can also be diminished by propranolol and metoprolol. Based on a case report, reactions with clonidine can result in syncope, hypotension, bradycardia, and sedation. *d*-Amphetamine can interact with guanethidine and bretylium **[drugs that are no longer clinically relevant]**.

2.2.1.4 Drug Abuse

Abuse of amphetamines and methamphetamines is associated with development of tolerance, psychological dependence, and social disability *(6, 8, 10*). There are reports of some patients greatly increasing dosages above recommended levels. Abrupt withdrawal following prolonged intake of high doses can result in extreme fatigue, mental depression, and sleep electroencephalogram (EEG) changes. Toxicity associated with chronic intoxication can include dermatoses, insomnia, irritability, hyperactivity, and personality changes. Though rare with oral intake, psychosis that is indistinguishable from schizophrenia has been reported. Repetitive and stereotypic behavior that can advance to self-injurious behavior has been observed with high or repeated dosing with amphetamines (reviewed in *(27*)). Animal models used to investigate self-injurious behavior in humans are discussed below in Section 2.2.2.

Studies in humans who abused methamphetamine and possibly other substances revealed persistent reductions in striatal dopamine transporter as observed by imaging techniques (reviewed in *(27, 68*)) and deficits in striatal dopamine, tyrosine hydroxylase activity, and dopamine transporters during autopsy (reviewed in *(27*)). The effects in autopsy subjects were described as likely markers of damage to the striatal dopaminergic system, but it was noted that there were no obvious behavioral symptoms. Dopaminergic effects in humans receiving therapeutic doses of amphetamines are not known.

2.2.2 Experimental Animal

Symptoms of acute amphetamine toxicity in rats and mice include hyperactivity, piloerection, salivation, and hyperpnea (reviewed in *(24*)); dilated pupils and convulsions have also been reported *(69*). Symptoms of acute methamphetamine toxicity in rodents were reported as excitement, convulsions, and changes in seizure threshold *(70*). The oral LD_{50} for *d*-amphetamine in rats is reported at 96.8 mg/kg bw. LD_{50} values for *d,l*-amphetamine and methamphetamine in various species are summarized in Table 21 and Table 22. **[The Expert Panel questions the accuracy of mouse LD_{50} values reported for *d,l*-amphetamine (Table 21). It does not make sense that the oral LD_{50} would be less than the iv LD_{50}, nor that the sc LD_{50} would be lowest of all. The discrepancies may be due to the use of animals of different ages. In addition, while the LD_{50} for oral administration of *d,l*-amphetamine in mice is listed as 24 mg/kg in Table 21, the NTP carcinogenicity studies *(24, 71*) described in Section 2.4.2 used an estimated 19 (female) and 30 (male) mg/kg bw/day dose for 103 weeks and had no differences in survival of treated groups.]**

Table 21. LD_{50} Values for *d,l*-Amphetamine

Species	Exposure route	LD_{50} (mg/kg bw)
Mouse	oral	24[a]
	ip	13[a]
	iv	31.8[a]
	sc	7[a]
Rat	oral	55
	ip	125
	sc	160
Dog	oral	23
	iv	6
Guinea pig	ip	50
	sc	105
Rabbit	iv	22

Data from NIOSH *(69*) for all routes and from NTP *(24*) for oral route.
[a]**[The Expert Panel questions the accuracy of the mouse LD_{50} data, as explained in text above.]**

Table 22. LD_{50} Values for Methamphetamine

Species	Exposure route	LD_{50} (mg/kg bw)
Mouse	ip	15
	iv	6.3
	sc	7.56
Rat	sc	10.93
Guinea pig	oral	90

Data from Registry of Toxic Effects of Chemical Substances (RTECS) database *(70)*.

Continuous iv infusion of rats with amphetamines 1 mg/kg bw/hour for 12 days resulted in reduced brain norepinephrine and cardiac catecholamine levels and increased motor activity and stereotypic behaviors, manifested as grooming, scratching, rearing, limb flicks, and biting (reviewed in *(24)*). Behavioral effects were reversed upon cessation of dosing. Amphetamine effects following repeated oral dosing are reported below in the description of an NTP study. Repeated parenteral dosing of rodents with methamphetamine has caused excitement, weight loss or decreased weight gain, alteration in classical conditioning, changes in pancreas, and hemorrhage *(70)*. Higher doses **[not specified]** of amphetamines were reported to induce brain lesions such as hyperemia, hemorrhage, and glial proliferation in monkeys and enlarged and chromatolytic medulla oblongata neurons in cats (reviewed by *(71)*).

A review by Kita et al. *(27)* reported that parenteral dosing with methamphetamine or amphetamine concentrations exceeding therapeutic levels has resulted in persistent regional (mainly striatal) depletion of brain dopamine levels in rats, mice, cats, guinea pigs, and monkeys, and decreased tyrosine hydroxylase activity in rats and cats. Decreased numbers of dopamine transport pumps were reported in mice and rats. Catecholamine turnover was found to be increased in rats. Reductions in brain norepinephrine levels were observed in monkeys and cats. Decreases in serotonin concentration and tryptophan hydroxylase activity were reported in rats and cats and reduced numbers of serotonin transporter pumps were reported in one rat study. Some recovery of neurotransmitter levels was observed at lower doses in monkeys (~2.0 mg/kg bw) and rats (≤ 12.5 mg/kg bw), but at higher doses, the effects persisted for months. Studies examining effects of amphetamines on neurochemical indices in developing animals are discussed in detail in Section 3.2.3.

Neuroanatomical evidence of amphetamine-induced toxicity was also reported. Swollen nerve fibers and terminals were observed in rats and mice dosed with amphetamine or methamphetamine (reviewed in *(27)*). Methamphetamine treatment has resulted in reductions of brain dopaminergic axons and axon terminals in rats and monkeys and serotonergic axons and axon terminals in rats (reviewed in *(72)*). Development of neurotoxicity depends on dose, number of exposures, intervals between dosing, and duration of neuron exposure (reviewed in *(72)*). It has been reported that administration of reuptake blockers with amphetamines prevents neuronal damage (reviewed in *(27, 72)*). Apoptosis was reported as a possible mechanism of neurotoxicity in mice and rats (reviewed in *(27)*).

A review by Kita et al. *(27)* discusses the animal literature and modeling that supports self-injurious behaviors in humans. Kita et al. state "The acute appearance of self-injurious behavior associated with administration of amphetamines to rodents may accurately predict the later neurotoxicity and at the same time serve as a model for the study of this disabling symptom in humans." Animal literature does not provide a neurochemical basis for self-injurious behavior although it points to involvement of central dopamine neurons. Reversal of some self-injurious behaviors by dopamine antagonists provides evidence of dopaminergic involvement.

NTP toxicity studies were reviewed in detail because reproductive organs were examined in a subchronic and a carcinogenicity study and growth was measured in the subchronic study. The carcinogenicity study is described in Section 2.4.2.

The NTP *(24)* conducted 14-day and 13-week studies to determine the toxicity of *d,l*-amphetamine sulfate in F344/N rats and B6C3F$_1$ mice. Rats and mice were randomly assigned to groups and administered USP grade *d,l*-amphetamine sulfate (99% purity) through feed. Drug stability and concentration in feed were verified. Survival data were analyzed using Kaplan Meier, Cox, or Tarone life table test methods. Continuous data were analyzed using Dunn, Shirley, or Jonckheere tests.

In the 14-day study, 7-week-old rats (5/sex/group) were fed diets containing 0, 47, 94, 188, 375, or 750 ppm *d,l*-amphetamine sulfate. Nine-week-old mice (5/sex/group) were fed diets with 0, 125, 250, 500, 1000, or 2000 ppm *d,l*-amphetamine sulfate. Animals were observed daily and weighed before, during, and after the study. Following kill, animals were necropsied and organ weights were measured. Major organs were fixed in 10% neutral buffered formalin for a histological analysis. Included among organs analyzed were mammary gland, prostate, testis, seminal vesicle, ovary, and uterus. Doses at which histological evaluation was conducted in each species are discussed below.

In the 14-day study, all rats survived. Final mean body weights in the 375 and 750 ppm groups were 7–9% lower for males and 5–16% lower for females compared to controls. Reduction in final body weight was statistically significant for females at 750 ppm. Feed intake was reduced in groups exposed to ≥94 ppm during the first week of the study and was marginally reduced in males from the 750 ppm group during the second week of the study. Hyperactivity **[determined by general observations, not standard measures]** was observed in rats exposed to ≥375 ppm. According to the results section, absolute heart weight was reduced in females exposed to ≥375 ppm. **[The only organ weight effect data shown in Table J1 of the NTP study is for liver. According to that table, relative (to body weight) liver weights were increased in males exposed to 750 ppm and females exposed to ≥188 ppm.]** Histopathologic evaluation was conducted on animals from the control and 750 ppm groups; no lesions were observed **[data not shown]**.

Four males in the 1000 ppm group and 1 male in the 2000 ppm died in the 14-day mouse study. According to study authors, the deaths were not clearly related to treatment. Weight loss occurred in males exposed to ≥500 ppm. Mean final body weights of females exposed to ≥250 ppm were significantly lower than controls by 12–13%. Feed intake was similar in all groups, although it was noted that the 2000 ppm males scattered their feed. Mice in the 1000 ppm group were either hyperactive or lethargic and hyporesponsive. According to the results section, relative liver weights were increased in males exposed to ≥ 250 ppm and females from the 2000 ppm group. Other significant organ weight changes listed in Table J3 of the NTP report included decreased absolute brain and thymus weight and increased relative kidney weights in males of the 2000 ppm group; absolute heart weight was increased in females exposed to ≥125 ppm. Histopathologic evaluation was conducted in controls, the 2000 ppm group, and males in the 1000 ppm group. No lesions were observed **[data not shown].**

The 13-week studies were conducted according to FDA Good Laboratory Practice (GLP) procedures. At 7–8 weeks of age, 10 rats/sex/dose were fed diets containing 0, 47, 94, 188, 375, or 750 ppm *d,l*-amphetamine sulfate. At 8–9 weeks of age, 10 mice/sex/group were fed diets containing 0, 125, 250, 500, 1000, or 2000 ppm *d,l*-amphetamine sulfate. Doses were based upon results observed in the 14-day studies. Animals were observed daily and weighed before, during, and after the study. Following kill, animals were necropsied and organs were weighed. Major organs were fixed in 10% neutral buffered

formalin for histological analysis. Included among organs analyzed were mammary gland, prostate, testis, ovary, and uterus. Doses at which histologic evaluation was conducted in each species are discussed below. Thymus and spleen were also examined in some rats from lower dose groups.

All rats survived in the 13-week study. Final body weights were significantly reduced in both sexes of all treated groups. Final mean body weights in the 188, 375, and 750 ppm groups were 11, 18, and 38% lower in males and 15, 26, and 32% lower in females. Feed intake by the 750 ppm males was 20% lower than controls. Hyperactivity was observed in all dose groups and severity increased according to dose. According to study authors, organ weight changes were due to body weight reductions. **[The study authors' statement is confirmed by dose-related reductions in absolute weight and increases in relative organ weights observed in many organs. The exceptions that appeared to be dose-related were an increase in absolute brain weight (≥94 ppm females) and decreases in relative (to body weight) liver (750 ppm males) and thymus (≥94 ppm males, ≥375 ppm females) weights.]** A histopathological examination was conducted in controls and the 750 ppm group. No treatment-related lesions were observed **[data not shown]**.

In the 13-week mouse study, 8 males of the 500 ppm group died, 3 males of the 1000 ppm group died, and 7 females and 6 males from the 2000 ppm group died. Mean final body weights of all treated groups were lower than controls. At doses ≥250 ppm, body weights were 18–30% lower than controls in males and 13–19% lower than controls in females. All treated mice were hyperactive and severity increased according to dose. Fighting was observed in males exposed to ≥250 ppm. According to study authors, increases in relative organ weights were due to reduced body weights. **[Decreases in absolute organs weights were also consistent with reduced body weights.]** A histological evaluation was conducted in the control and 2000 ppm groups, males in the 1000 ppm group, and all mice that died during the study. No treatment-related lesions were observed **[data not shown]**.

2.3 Genetic Toxicity

Details of study protocols and results for in vitro genetic toxicity testing of *d,l*-amphetamine sulfate are listed in Table 23. Mutagenicity testing in *S. typhimurium* produced negative results except in strain TA98 in the presence of hamster but not rat S9 activation *(24)*. The response was classified as equivocal by the NTP. Negative results were obtained for sister chromatid exchange and chromosomal aberration in Chinese hamster ovary cells. Although chromosomal aberrations were increased in the first trial with metabolic activation, the result was not repeated in two additional trials and the NTP classified the results as negative. An unpublished study reported in the FDA Pharmacology Review of Adderall *(34)* reported that Adderall was negative for mutagenicity in an *E. coli* reversion test at concentrations up to 5000 µg base/plate, with and without S9 activation.

Details of study protocols and results for in vivo genetic toxicity assays are listed in Table 24. The FDA Pharmacology Review *(34)* indicated that Adderall was negative in the mouse micronucleus test at doses up to 10 mg/kg bw with a mean ± SD amphetamine plasma concentration up to 433.4 ± 72.4 ng/mL. However, the FDA review also cited a published study demonstrating an increase in micronucleated polychromatic erythrocytes in mice given 2 oral doses of ≥12.5 mg/kg bw *d,l*-amphetamine *(73)*.

Table 23. Results of In Vitro Genetic Toxicity Testing of *d,l*-Amphetamine

Reference	Concentration	Testing with metabolic activation	Species or cell type/strain	Endpoint	Results
NTP *(24)*	100–10,000 µg/plate	Yes	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537	Mutagenicity at the histidine operon	↑ mutagenicity in TA98, with rat but not hamster S9 metabolic activation; ↔ in all other strains; results judged equivocal by NTP
FDA *(34)*	≤ 5000 µg/plate	Yes	*Escherichia coli*	Mutagenicity	↔
NTP *(24)*	50–1600 µg/L	Yes	Chinese hamster ovary cells	Sister chromatid exchange	↔
NTP *(24)*	300–1000 µg/L	Yes	Chinese hamster ovary cells	Chromosomal aberration	↔

↔ = no change, ↑ = statistically significant increase.

Table 24. Results of In Vivo Genetic Toxicity Testing of *d,l*-Amphetamine

Reference	Species	Dose (route)	Cell type	Endpoint	Results
FDA *(34)*	Mouse	2.5–10 mg/kg bw (oral)	bone marrow erythrocytes	micronucleus	↔
Tariq et al. *(73)*	Mouse	6.25–25.0 mg/kg bw (2 oral doses)	bone marrow erythrocyte	micronucleus	↑ at ≥12.5 mg/kg bw

↔ = no change, ↑ = statistically significant increase.

2.4 Carcinogenicity

2.4.1 Human
No data were identified.

2.4.2 Experimental animal
The NTP *(24, 71)* examined the carcinogenicity of *d,l*-amphetamine sulfate in F344/N rats and $B6C3F_1$ mice in a study conducted according to FDA GLP procedures. Rats and mice were randomly assigned to groups and administered USP grade *d,l*-amphetamine sulfate (99% purity) through diet. Fifty rats (7–8 weeks old) and mice (8–9 weeks old)/sex/group were given diets containing 0, 20, or 100 ppm *d,l*-amphetamine sulfate for 103 weeks. Study authors estimated doses of ~0, 1, and 5 mg/kg bw/day in rats. Doses were estimated at 0, 4, or 30 mg/kg bw/day in male mice and 0, 3, or 19 mg/kg bw/day in female mice. However, the dose estimate in high-dose male mice may have been affected by feed scatter. Dose selection was based upon results of the 13-week studies described in Section 2.2.2. Drug stability and concentrations in feed were verified. Animals were observed daily and weighed before,

during, and after the study. Following sacrifice, all animals were necropsied and major organs from animals in all dose groups were fixed in 10% neutral buffered formalin for a histological analysis. Included among organs analyzed were mammary gland, prostate, testes, seminal vesicles, ovaries, and uterus. Survival data were analyzed using Kaplan Meier, Cox, or Tarone life table test methods. Continuous data were analyzed using the Dunn, Shirley, or Jonckheere test. Tumor incidence data were assessed using logistic regression analysis, Cox life table test, Fisher Exact test, or Cochran-Armitage trend test.

No difference in survival was observed in the rat carcinogenicity study. Body weights were 10–34% lower in female rats exposed to 20 or 100 ppm and male rats exposed to 100 ppm. **[Compared to control animals, CERHR calculated body weight reductions of 11% in 20 ppm males, 10% in 20 ppm females, and 20% in 100 ppm females, based on body weights presented in Table F1 and F2 of the NTP report. The Panel notes that body weight reductions >10% indicate that maximum tolerated dose was exceeded.]** Feed intake of the 100 ppm females was 84% of control levels. An increase in testicular interstitial cell adenomas in male rats was not considered to be treatment-related by study authors due to the common occurrence of that tumor. **[The results section listed the interstitial cell adenoma incidence as 34/50 in controls, 43/50 in the 20 ppm group, and 48/50 in the 100 ppm group. However, Table A3 of the NTP reported listed the incidence as 43/50 in controls, 43/50 in the 20 ppm group, and 48/50 in the 100 ppm group.]** Thyroid follicular cell adenomas were not dose-related and hyperplasia was not observed prior to development of adenoma. An increase in cataracts and retinal atrophy in females was believed to be due to excessive light exposure resulting from cage placement. Because amphetamines can cause hyperthermia, pupil dilation, and increased activity, it was also postulated by study authors that the drug could have indirectly contributed to the formation of eye lesions. An increase in bone myelofibrosis was thought to be due to loss of adipose tissue secondary to weight loss. The study authors concluded that none of these lesions were directly caused by drug exposure. Dose-related reductions were observed for neoplasms in adrenal gland, pituitary gland, mammary gland, and uterus.

In the mouse study, there were no differences in survival of treated groups. Mean body weights of males were reduced by 10–36% in the 100 ppm group and 10–19% in the 20 ppm group. Mean female body weights were reduced by 10–34% in the 100 ppm group and 10–19% in the 20 ppm group. Final body weights in the 100 ppm groups were 60–70% of control values. **[Based on information presented in NTP Tables F3 and F4, CERHR estimated that compared to controls, body weights of animals in the 100 ppm group were reduced by ~25%; this suggests that maximum tolerated dose was exceeded.]** Average feed consumption was not reduced in treatment groups, but the authors stated that the values were not corrected for scatter, which may have led to overestimation in males. Seventeen females in the 100 ppm group were mistakenly bypassed during sacrifice at the end of the study. They received water but no feed for 5 days, at which time they were killed, necropsied, and apparently included in histological analyses. A slight increase in thyroid follicular cell neoplasms in male mice was not considered treatment-related by study authors due to lack of precursor follicular cell hyperplasia, a lack of increase in follicular cell adenomas in female mice, and a reduced incidence of follicular cell hyperplasia in female mice. Treated mice displayed dose-related reductions in neoplasms of the liver, pituitary, lung, and harderian gland. Ovarian atrophy was increased in females of the 100 ppm group, but it was not stated if the effect was statistically significant. The incidences of ovarian atrophy were 14/49 in the control group, 12/48 in the 20 ppm group, and 25/46 in the 100 ppm group **[*P* = 0.0054 chi-square performed by CERHR]**. Study authors postulated that ovarian atrophy may have been due to reduced body weight rather than a direct drug effect.

The study authors concluded that there was no evidence of carcinogenic activity of *d,l*-amphetamine sulfate in male or female F344/N rats or $B6C3F_1$ mice.

In a review of the NTP studies, the FDA noted that the high dose in rats (5 mg/kg bw = 30 mg/m^2) is equivalent to the maximum advised dose for children, "30 mg/25 kg × 25 = 30 mg/m^2," and that the high doses in male (30 mg/kg bw = 90 mg/m^2) and female (19 mg/kg bw = 57 mg/m^2) mice are only 3 and 2 times the maximum advised children 's dose *(34)*.

2.5. Potentially susceptible populations

2.5.1 Pharmacogenetics

CYP2D6 is involved in the aromatic hydroxylation of amphetamines in humans and rats *(25)*. The gene for CYP2D6 is located on the long arm of human chromosome 22. Polymorphisms for CYP2D6 are associated with at least 12 variants that alter enzyme activity (reviewed in *(74, 75)*). People with the usual CYP2D6 activity are called extensive metabolizers and people with lower levels of activity are called poor metabolizers. Poor metabolizer phenotypes occur in 5–8% of whites and 2–10% of blacks and Asians. There is considerable variation within racial groups; for example, there is a higher incidence in African Americans (8.5%) than in Zimbabweans (1.8%) of 1 of the inactive CYP2D6 alleles and up to 29% of Ethiopians carry duplicated or multiduplicated CYP2D6 alleles.

CYP2C3 is involved in amphetamine deamination reactions in rabbits *(25)*. **[The enzyme is not a member of the of the CYP2C human gene family *(76)*, but the Expert Panel finds it reasonable to assume that one or more members of the CYP2C family (CYP2C8, CYP2C9, CYP2C18, or CYP2C19) may be involved in deamination reactions in humans.]** The gene family is highly polymorphic with functional variants identified at CYP2C9 and CYP2C19 loci. Two of three known defective alleles of CYP2C9 result in decreased activity; CYP2C19 polymorphisms resulting in slow metabolizer phenotypes are present in 3% of Caucasians and nearly 20% of Asians *(77)*.

Vorhees et al. *(78)* performed a study, sponsored by an NIH grant, to explore the effects of methamphetamine metabolism on developmental neurotoxicity, summarized in Table 39 in Section 3.2.3. Two strains of rat were used: ACI Black (Dark) Agouti, the females of which are poor metabolizers of debrisoquine, and Sprague-Dawley, which are extensive metabolizers of debrisoquine. In rats, debrisoquine oxidation differences are due to polymorphisms of CYP2D1, believed also to metabolize methamphetamine. The authors tested the hypothesis that there would be differences between these strains in behavioral testing of adult females after administration of methamphetamine 30 mg/kg bw sc twice daily on postnatal day (PND) 11–20. The test battery consisted of an acoustic startle test, straight-channel swimming, and a Morris maze, which is a test of spatial memory. The behavioral test results did not differ by strain, arguing against the hypothesis. There was, however, a large difference in mortality during the treatment period: 59.4% of ACI Black Agouti and 18.8% of Sprague-Dawley female offspring died ($P < 0.01$, Fisher test). The authors postulated that there may have been an increased sensitivity to methamphetamine toxicity in the ACI Black Agouti strain, but that a difference in sensitivity would have been obscured by testing only the survivors of the treatment period (i.e., that the cohort of survivors were not representative of overall strain susceptibility). The authors questioned whether metabolizer status could explain the mortality differences between the strains inasmuch as the inter-strain difference in mortality was also seen among treated males, which do not manifest the poor-metabolizer phenotype.

A second study suggested a possible increased susceptibility in rats with poor or intermediate methamphetamine metabolizer phenotypes *(48)*. Dark Agouti female rats (poor metabolizers) that were sc injected with 15 mg/kg bw methamphetamine on PND 11–20 performed less effectively on the Morris Maze test of spatial learning and memory and had greater acoustic startle amplitudes compared to female Sprague-Dawley rats receiving the same treatments. Treated male Dark Agouti rats

(intermediate metabolizers) also displayed increased startle amplitudes compared to treated male Sprague-Dawley rats. Details of this study are included in Table 39.

Inhibition of methamphetamine 4-hydroxylation in rats was reported to enhance stereotyped behavior in rats, but reviewers noted that metabolic differences in humans compared to rodents complicate the application of the study findings to humans with inborn CYP2D6 deficiencies (reviewed in *(30)*).

2.5.2 Sex-related differences

Amphetamine pharmacokinetic parameters in men and women were compared in an FDA review *(34)*. Higher systemic exposure of women was attributed to weight differences. According to the FDA, the difference was reduced when the doses were normalized for body weight. Table 25 outlines the dose-normalized values for *d*-amphetamine in men and women administered *d,l*-amphetamine.

Table 25. Dose-Normalized Comparison of *d*-Amphetamine Pharmacokinetic Parameters in Men and Women Given *d,l*-Amphetamine

Dosing regimen	Sex	Dose (mg/kg bw)	C_{max}/dose (ng/mL)/(mg/kg bw)	$AUC_{0-\infty}$/dose (ng-hr/mL)/(mg/kg bw)
Single dose of 30 mg	M	0.354	106.6	2237
	F	0.486	104.8	1811
30 mg/day for 7 days	M	0.398	145.0	2109
	F	0.493	154.2	2063

Data from 10 subjects/sex; from FDA review *(34)*.

Some studies suggest that male mice are more susceptible than female mice to methamphetamine-induced depletion of striatal dopamine, and some but not all studies suggest that estrogen may act as a neuroprotectant (*(79)* and reviewed in *(27, 80)*). Slight effects were replicated in rats (reviewed in *(27)*, but one study demonstrated that methamphetamine-induced reductions in striatal dopamine and serotonin were similar in male and female rats when hyperthermia was maintained at a consistent level in both sexes *(80)*.

2.5.3 Age-Related Differences

Ontogeny of hepatic CYP2D6 in humans was reviewed by Hines and McCarver *(76)*. One study found no evidence of hepatic CY2D6 expression in 11–13-week-old fetuses. Another study reported that CYP2D6 protein and mRNA were detected in 30% of liver samples from fetuses <30 weeks old at 5% of adult levels. In fetuses more than 30 weeks old, CYP2D6 protein and mRNA were detected in 50% of liver samples and activity was 15% of adult levels. CYP2D6 protein was reported to increase at birth, regardless of gestational age (i.e., time period of gestation completed at time of delivery), and reach 50–75% of adult levels during the neonatal period.

Hines and McCarver *(76)* also reviewed the ontogeny of hepatic CYP2C enzymes. CYP2C8, CYP2C9, and CYP2C18 transcripts were detected in human fetal livers at 10% of adult levels, but there was no evidence of CYP2C activity in 16–40-week-old human fetuses. CYP2C expression appears to be activated at birth and is independent of gestational age. Activity in human neonates is ~30% of adult levels and remains constant up to 1 year of age.

Table 5 and Table 6 in Section 2.1.1.2 illustrate some differences in *d,l*-pharmacokinetic parameters in adults and children. At equivalent doses, the half-life is shorter, but systemic bioavailability is greater in children compared to adults. The FDA attributed lower body weight to the increased bioavailability in children *(34)*. When normalized to body weight, children had lower C_{max} and AUC values than adults. Table 26 outlines the dose-normalized values for *d*-amphetamine in children and adults administered *d,l*-amphetamine.

Table 26. Dose-Normalized Comparison of *d*-Amphetamine Pharmacokinetic Parameters in Children and Adults Given *d,l*-Amphetamine Repeated Dosing at 30 mg/day

Population	Dose (mg/kg bw)	C_{max}/dose (ng/mL)/(mg/kg bw)	$AUC_{0-\infty}$/dose (ng-hr/mL)/(mg/kg bw)
Adults (n = 19)	0.45	148.7	2064
Children (n = 20)	0.88	101.1	1550

From FDA review *(34)*.

Rapoport et al. *(81)* performed a study at NIH that evaluated differences between boys and men in response to a single dose of *d*-amphetamine. The study included 14 normal boys and 15 hyperactive boys (some of whom had been on medication) with a mean age of about 10 years in both groups (SD = 2.1). The boys were given ~16 mg *d*-amphetamine. The adult group consisted of 15 men given a mean of 34 mg *d*-amphetamine and 16 men given a mean of 17 mg *d*-amphetamine (SD = 1–2 mg to adjust for weight differences; the high dose was about the same as the boys' dose on a mg/kg bw basis). The mean age ± SD of the men was 22 ± 3 years. Baseline measures were performed on a Monday. On Wednesday, subjects were randomized to receive either amphetamine or placebo followed by a battery of tests. On Friday, each subject received the opposite treatment (amphetamine or placebo) followed by the same tests. Motor activity, evaluated using a sensor attached to the trunk and expressed by comparing the *d*-amphetamine to the placebo sessions, was decreased 44% in hyperactive boys, 24% in normal boys, and 9% in men given the low dose of *d*-amphetamine. There was no effect of the high dose of *d*-amphetamine on motor activity in men. Adults reported feeling euphoria and feeling less tired on *d*-amphetamine while boys reported feeling cranky and more tired. Interviewer behavior evaluations (blind to treatment condition) of hyperactive boys rated them less hyperactive, less fidgety, and less silly on *d*-amphetamine, whereas men on the high dose of *d*-amphetamine were rated as more fidgety. Other tests of vigilance, learning, and speech communication showed similar effects in boys and men, although the hyperactive boys had more exaggerated responses to *d*-amphetamine than normal boys or men in many domains.

Alhava and Mattila *(82)* examined heart and brain distribution of 50 mg/kg bw *d,l*-amphetamine administered by ip injection to adult male or developing (either sex) NMRI mice at the ages of 3–5, 13–15, or 32–35 days. In adults and developing mice ≥13 days of age, amphetamine peaked within 30 minutes in both brain and heart, and levels were higher in brain than in heart. In infant mice (3–5 days old), amphetamine levels peaked in brain within 2 hours, were initially lower than heart levels, which peaked within 30 minutes, and were much lower than adult brain levels. When doses were adjusted according to surface area (i.e., twice the dose given to infant versus adult mice), peak brain levels were similar in adults and infants; the time to reach peak ranged from 30 minutes to 2 hours. Heart levels of amphetamines were higher in infants than in adults when doses were adjusted to surface area. In vivo results were not replicated in in vitro experiments that measured distribution in heart and brain slices. The study authors suggested that the increased time to reach peak in infant brains may have been a result of circulatory factors and brain immaturity.

A number of studies compared neurological endpoints in adult versus developing experimental animals exposed to amphetamine or methamphetamine.

Laviola et al. *(83)* ip injected male and female adult and periadolescent (30–45-day old) mice (8 or 9/sex/group; strain not specified) with saline or 2 mg/kg bw amphetamine **[purity and enantiomer not specified]**, returned them to their cages for 100 minutes, subjected them to mild stress by removal of

sawdust from the cage, and then killed them 20 minutes later for measurement of plasma corticosterone levels. Amphetamine treatment significantly increased plasma corticosterone levels in male and female periadolescents but had no effect on adult mice.

Lanier and Isaacson *(84*) treated Long-Evans hooded rats with *d,l*-amphetamine 0, 2, 5, or 10 mg/kg bw ip during 3 different juvenile age periods (PND 18–22, PND 34–38, PND 45–49) and during adulthood. An increase in locomotor activity in open field testing occurred at all ages except PND 34–38. Lesioning the hippocampus on PND 21 or 26 resulted in greater sensitivity to the lower doses of amphetamine. The authors postulated that the hippocampus becomes functionally capable of suppressing locomotor activity during the PND 34–39 interval, accounting for the lack of inhibition when amphetamine was given during the PND 18–22 period. By PND 45, they argued, the catecholamine system had matured sufficiently so that stimulatory effects of amphetamine overrode hippocampal suppression.

Gazzara et al. *(85*) evaluated a possible dopamine-mediated age-related difference in behavioral response to amphetamine treatment in rats. They noted that adult rats demonstrate increased locomotor activity when treated with low doses (0.5–1.5 mg/kg bw amphetamine) and stereotyped behavior (e.g., sniffing, licking) when given high doses (10 mg/kg bw amphetamine), but that prior to 35 days of age, rats exhibit only increased locomotion, regardless of dose. The increased locomotor behavior is attributed to dopamine release from neurons in the nucleus accumbens, while the stereotyped behavior is due to dopamine release in the caudate-putamen. These authors used electrochemical recordings from electrodes stereotactically placed in the caudate-putamen of PND 21–22, PND 35–36, and adult Sprague-Dawley rats. After baseline recordings were made, animals were treated with 0.1 or 1.0 mg/kg bw *d*-amphetamine sulfate (HPLC or reagent grade) sc and voltammetric recordings were made every 10 minutes. Adult and 35–36-day-old rats showed an increase in caudate-putamen dopamine release after treatment with 1.0 mg/kg bw amphetamine, whereas 21–22-day-old animals showed a decrease in dopamine release after treatment with either dose (except for a transient elevation after the high dose). The authors proposed that amphetamine may decrease the firing rate of nigrostriatal neurons in young rats due to local release of dendrite dopamine from neurons in the substantia nigra with inhibition of neuronal firing through autoreceptors in these neurons. This mechanism may be more sensitive in immature animals.

Trent et al. *(86*) examined age-related differences in nigral dopaminergic neuronal firing following treatment of Sprague-Dawley rats with *d*-amphetamine sulfate **[purity not specified]**. In the first set of experiments, 5 mg/kg bw *d*-amphetamine was given by ip injection to 6 adult male rats or immature rats on PND 1–6, 7–15, or 16–28 (n = 11–20 rats/age group). A paradoxical response was noted in 1–6-day-old rats with increased firing in 45%, reduced firing in 25%, and no effect in 30% of dopaminergic neurons examined. Either inhibition or no effect on neuronal firing was observed in the older groups of rats following *d*-amphetamine treatment. Percent dopaminergic neurons with inhibited firing in response to amphetamine treatment was 50% at 7–15 days of age, 82% at 16–28 days of age, and 100% in adulthood. **[Although the authors present values as percentage of neurons examined, it actually appears that the data are presented as percentage of animals examined. Considering that extracellular recordings were taken, the Panel noted that firing would represent a sum of all surrounding neurons and data therefore reflect information from each animal.]** In order to determine route of exposure effects, *d*-amphetamine was given by iv injection to 12 pups and 4 adult males at doses of 0.25, 0.5, 1.0, and 2.0 mg/kg bw administered sequentially at 1-minute intervals. Consistent with ip data, iv administration of *d*-amphetamine resulted in atypical responses that differed significantly from adult responses during the first 2 weeks of life. **[Results were only shown for an 8-day-old rat and an 18-day-old rat.]** Injection ip or iv with apomorphine significantly inhibited dopaminergic neuron activity in all age groups, thus demonstrating that dopamine autoreceptors are

functional in rats at birth. Haloperidol, a D_1/D_2 antagonist, consistently reversed apomorphine-induced inhibition at all ages.

Other authors evaluated the age-related differences in amphetamine and methamphetamine sensitization *(87, 88*), noting that adult rats pretreated with amphetamines display an augmentation of locomotor response when subsequently rechallenged with an amphetamine dose. This sensitization response does not occur until 3–4 weeks of age. The authors suggested that the appearance of mature presynaptic dopamine autoreceptors may be necessary for sensitization *(88*) or that maturation of dopamine reuptake sites is the limiting factor in the development of sensitization *(87*). **[Since the publication of these studies, amphetamine sensitization in preweanling rats has been demonstrated (reviewed in Table 39); however, the interval between sensitizing treatments and subsequent challenge dose must be much shorter than in mature animals; therefore, this model continues to be an example of age-related differences in response to amphetamines.]**

Tsuchida et al. *(89, 90*) examined the ontogeny of amphetamine-induced effects on dopamine and its metabolites in the striatum of Sprague-Dawley rats. On PND 7 (earlier study only), 14, 21, 28, and 56, striatal perfusate levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid were measured using an in vivo microdialysis technique and HPLC for up to 180 minutes in 4–6 male rats/group treated with 4 mg/kg bw methamphetamine HCl **[purity not specified]**. Data were analyzed by Kruskall-Wallis test, Mann-Whitney *U*-test, ANOVA, and Fisher protected least significant difference test. Baseline levels of dopamine did not differ by age but DOPAC and homovanillic acid levels increased with age. Following amphetamine treatment, dopamine levels were increased in all age groups. At 20 minutes, the dopamine increase in PND 14 rats was smaller compared to the other age groups, while at 40–60 minutes, dopamine increases in the PND 56 group were larger compared to the other groups. DOPAC levels were reduced at all ages, but the reduction in PND 14 rats was significantly smaller than in the other groups at 40–100 minutes following treatment. Homovanillic acid levels were increased in the PND 14 group but reduced in all other age groups.

Ehrlich et al. *(91*) used Western blot techniques to examine ΔFosB and dopamine-related protein levels in response to ip injection of saline, 5 mg/kg bw/day amphetamine, or 20 mg/kg bw/day cocaine for 7 days in adult (60-day-old), periadolescent (33-day-old), or postweanling (24-day-old) male CD-1 mouse brain. Each dose group contained 10–12 animals. Amphetamine significantly increased ΔFosB protein expression in nucleus accumbens **[~225% of control levels]** and caudate putamen **[~175% of control levels]** of periadolescent, but not adult or preweanling mice. Amphetamine had no effect on dopamine transporter or dopamine and cAMP regulated phosphoprotein (DARPP-32) expression. Findings in cocaine-treated groups were similar except that cocaine treatment significantly increased ΔFosB levels in caudate putamen of all groups. Age-related effects of ΔFosB and dopamine transporter expression were also examined. Expression of ΔFosB was similar in preweanling and periadolescent mice but was significantly higher in caudate putamen **[~3 times higher]** and nucleus accumbens **[~2 times higher]** of adult mice. Dopamine transporter expression did not vary by age. The study authors concluded that changes in ΔFosB induction in adolescent mice may be related to increased tendency for addiction in adolescents versus adults.

Pu and Vorhees *(92*) examined the effects of methamphetamine exposure on brains of developing and adult Sprague-Dawley rats. Male rats were administered methamphetamine HCl at 0 (saline control), 10, or 20 mg/kg bw/dose (expressed as free base) by ip injection, 4 times daily at 20, 40, 60, and 80 days of age. Each age group contained 10 rats, 2 of which were treated with saline and 4/group of which were treated with the low and high methamphetamine dose. Three days following the last treatment, the animals were killed and brains were sectioned and stained with antibodies to glial fibrillary acid protein and tyrosine hydroxylase. Rats treated with both 10 and 20 mg/kg bw/dose methamphetamine displayed agitation, fine body tremors, and piloerection. One adult in the 20 mg/kg

bw/dose group died. In all 60- and 80-day-old rats treated with either dose of methamphetamine, tyrosine hydroxylase-positive cells were severely depleted and glial cell hypertrophy and proliferation were dramatically increased in the caudate-putamen. Glial cell proliferation was increased in the ventral lateral region of the striatum in 40-day-old rats treated with either methamphetamine dose, but tyrosine hydroxylase-positive terminals were unaffected. Neither endpoint was affected by methamphetamine treatment in 20-day old rats.

Pu et al. *(93*) examined the effects of methamphetamine exposure on glutamate-positive neurons in the somatosensory cortex of adult and developing Sprague-Dawley rats. Male 20-day-old, 40-day-old, and adult rats were given 4 ip injections of saline or 10 mg/kg bw/dose *d*-methamphetamine HCl every two hours. Eight adult and five immature animals were used in each dose group. Three days following the last treatment, the animals were killed and brains were sectioned and stained with antibody to glutamate-glutaraldehyde and glial fibrillary acid protein. Methamphetamine treatment of adult rats resulted in marked-to-moderate depletion of glutamate-positive neurons in the somatosensory cortex in five of eight animals. Astrogliosis was observed in three methamphetamine-treated animals. The somatosensory cortex of 20- and 40-day old animals was unaffected by methamphetamine treatment. The study authors postulated that increased resistance of immature animals may be related to dopaminergic and glutamatergic system ontogeny. Evidence that these systems are still developing in the 20- and 40-day old rats was suggested by cited studies demonstrating that N-methyl-D-aspartate channels in the frontal cortex reach adult levels after 45 days of age, in vitro glutamate-stimulated striatal dopamine release remains below adult levels at 45 days of age, and striatal dopamine release in response to electrical stimulation of dopaminergic tracts of the medial forebrain bundle increases 3-fold between 33 and 114 days of age.

The ontogeny of methamphetamine-induced neurotoxicity in the rat was reviewed by Vorhees and Pu *(94*). They observed that adults lack behavioral manifestations of methamphetamine toxicity, in spite of decreases in striatal dopamine and forebrain serotonin and in spite of gliosis of the striatum, whereas developmental exposure produces smaller neurochemical changes and an absence of anatomic disruption while at the same time being associated with clear behavioral alterations when these rats are evaluated as adults. The authors believed the age-related differences in neurotoxicity might be related to a shift in sensitivity of tyrosine hydroxylase between PND 40 and 60 and a shift in glial fibrillary acid protein responsiveness between PND 20 and 40. Since that review was written, additional work has suggested that alterations in dorsal striatal protein kinase A activity may underlie the sensitivity of preweanling rats to amphetamine-induced behavioral alterations (discussed in Section 3.2.3).

Fukui et al. *(95*) conducted an in vitro study to investigate methamphetamine effects on dopamine signaling in neostriatal slices from young (14–15- or 21-22-day-old) or adult (6–8-week-old) male C57Bl/6 mice. The slices were incubated in 100 μM methamphetamine **[14.9 μg/mL assuming value is for the free base and not the salt]** for up to 5 minutes and an immunoblotting technique was used to measure dopamine and cAMP-regulated phosphoprotein M_r 32 kDA (DARPP-32) phosphorylation at the Thr34 and Thr75 sites. In all three age groups, methamphetamine increased Thr34-DARPP-32 phosphorylation but reduced Thr75-DARPP-32 phosphorylation. Similar effects were noted by incubating slices in SKF81297, a dopamine D1 receptor agonist, but methylphenidate and cocaine only increased Thr34-DAPP-32 phosphorylation in adult animals.

2.6 Summary of General Toxicology and Biological Effects

2.6.1 Pharmacodynamics and pharmacokinetics

2.6.1.1 Pharmacodynamics

Amphetamine and methamphetamine stimulate the CNS by acting as sympathomimetic drugs (reviewed in *(6, 25)*). The therapeutic mode of action in treatment of ADHD with amphetamine or methamphetamine is not known. It is believed that amphetamines increase levels of catecholamine in the synaptic space by blocking reuptake of norepinephrine and dopamine by presynaptic neurons (reviewed in *(7)*), by releasing dopamine *(26)* and norepinephrine (reviewed in *(27)*) from dopaminergic neurons, and possibly by inhibiting monoamine oxidase (reviewed in *(27, 28)*). Dopamine receptors and adrenoceptors reportedly have no affinity for amphetamines (reviewed in *(25)*). There is also evidence that amphetamines increase release and turnover of serotonin and it has been suggested that many of the behavioral effects of amphetamines are mediated through serotonin (reviewed in *(27)*). Amphetamine *d*-enantiomers have five times the stimulant activity of *l*-enantiomers (reviewed in *(25)*).

Although stimulants decrease locomotor activity in children, an increase in activity is observed in experimental animal studies. A review by Solanto *(28)* discussed possible theories for discordance between children and experimental animals.

2.6.1.2 Pharmacokinetics

d,l-Amphetamine and *d*-amphetamine are available as immediate- and sustained-release formulations. Pharmacokinetic parameters reported to FDA are summarized in Table 5 and Table 6. In studies by Brown et al. *(35, 36)*, administration of 0.5 mg/kg bw *d*-amphetamine to boys resulted in peak blood levels of ~63–70 ng/mL within 3–8 hours. Bioavailability for methamphetamine has been reported at 67.2% following oral dosing of adults with 0.125–0.250 mg/kg bw *d*-methamphetamine *(44)* and 67–90.3% following inhalation of 15.5–40 mg vapors *(45, 46)*.

Absorption of amphetamine and methamphetamine was also demonstrated in animal studies. In gavage studies in rats and rabbits, maximum blood levels of amphetamines were dependent on dose and ranged from ~39 to 1080 ng/mL in rats dosed with 2–20 mg/kg bw and 23 to 259 ng/mL in rabbits dosed with 2–16 mg/kg bw *d,l*-amphetamine *(34)*. Similar blood levels were observed following dosing of pregnant and non-pregnant rats. Methamphetamine dosing of rats through the sc route resulted in maximum blood levels of 3100–3600 ng/mL in pregnant rats given 20 mg/kg bw *(49)* and 5150–7300 ng/mL in neonates given 14–40 mg/kg bw *(50)*.

Following oral intake by humans, amphetamines are rapidly distributed to major organ systems, including brain *(24)*. Linear pharmacokinetics were demonstrated for extended-release *d,l*-amphetamine at doses of 10–30 mg *(7)*. No unexpected accumulation occurred at steady state *(7)*. The volume of distribution for methamphetamine was reported at 3.42 L/kg in humans (reviewed in *(17)*).

In a lactating 36-year-old woman taking 20 mg/day racemic amphetamine in 4 divided doses, amphetamine concentrations at 42 days postpartum were 20–40 ng/mL in plasma and 55–118 ng/mL in breast milk; milk:plasma ratios were 6.6–7.5. Maternal urinary amphetamine excretion was measured at 3.6 mg/24 hours 10 days postpartum and 8.9 mg/24 hours 42 days postpartum. Urinary amphetamine excretion in the infant was reported to be 1/1000 to 1/300 of maternal levels.

Three studies reported methamphetamine and amphetamine levels in blood and or tissues of infants who had been exposed to methamphetamine in utero and were stillborn or died after birth *(40-42)*.

Methamphetamine and amphetamine were distributed to infant blood, brain, liver, kidney, lung, and bile. Respective blood levels of methamphetamine and amphetamine were measured at 0.2–1.2 and 0.01–.08 mg/L in 4 stillborn infants, 0.355–6.3 and 0.08–0.28 mg/L in 3 infants dying shortly after birth, and 0.03–0.13 and 0–0.05 mg/L in 2 infants dying at 1 month of age. Methamphetamine brain levels were reported at 0.28–5.71 μg/g and amphetamine brain levels at <0.03–0.76 μg/g in 3 infants dying shortly after birth. A sc exposure study in mice demonstrated transfer of methamphetamine from dam to fetus on GD 14, with fetal brain levels of methamphetamine that were ~2–5 times lower than concentrations in dam brain *(51)*; amphetamine was also detected in brains of dams and fetuses at a concentration at least 4 times higher in dams. Studies in pregnant sheep also demonstrated transfer of methamphetamine from the ewe to the fetus following dosing of the ewe; fetal elimination paralleled maternal elimination *(52, 53)*. One study reported a methamphetamine plasma:brain ratio of 6 in the sheep fetus *(52)*.

The initial step of amphetamine metabolism in humans is hydroxylation of the alpha, aromatic 4-, or beta carbon (reviewed in *(24, 31)*). Oxidation of the alpha carbon leads to deamination and ultimately to the formation of benzoic acid, which can be conjugated with glycine to form hippuric acid (reviewed in *(24, 31)*). According to the NTP review, deamination appears to be the predominant pathway of amphetamine metabolism in humans. Smaller amounts of amphetamine are metabolized through aromatic hydroxylation. Aliphatic beta carbon hydroxylation accounts for only a minor percentage of metabolism in humans, but can be important due to the generation of norephedrine, a metabolite with possible biological activity (reviewed in *(31)*). An FDA review *(34)* reported that metabolism and elimination of amphetamine do not appear to be stereoselective since the ratio of systemic exposure to each enantiomer has been demonstrated to be equivalent to racemic amphetamine formulations (3-*d*:1-*l*).

As in humans, amphetamine metabolic pathways in animals can include hydroxylation of the alpha carbon, the aromatic 4-carbon, the beta carbon, or possibly the amine group *(24)*. Deamination can occur following alpha-carbon hydroxylation. While deamination is a major metabolic pathway in humans, it plays a minimal role in amphetamine metabolism in rats (Table 9). Aromatic hydroxylation is the predominant pathway in rats and the primary urinary metabolite is 4-hydroxyamphetamine *(24)*. One review reported that 4-hydroxyamphetamine can be metabolized by a neuronal CYP to alpha-methyldopamine and then to alpha-methylnorepinephrine, a possible false neurotransmitter (reviewed in *(30)*). Studies in rats administered *d*-amphetamine demonstrated the presence of 4-hydroxyamphetamine, 4-hydroxynorephedrine, alpha-methyldopamine, and alpha-methylnorepinephrine in rat brain.

The two major pathways of methamphetamine metabolism in humans are N-demethylation to form amphetamine, which can be metabolized through several pathways (see above), and aromatic hydroxylation to form 4-hydroxyamphetamine and then 4-hydroxynorephedrine *(25, 31, 32)*. Humans excreted a larger percentage of unmetabolized methamphetamine than rats or guinea pigs, even at lower doses. The two major pathways in humans are aromatic hydroxylation and demethylation, while deamination and beta-hydroxylation account for a smaller percentage of metabolism. In humans, aromatic 4-hydroxylation is much more extensive in the metabolism of methamphetamine compared to amphetamine (reviewed in *(30, 44)*).

Half-lives for *d*-amphetamine ranged from ~7 to 9 hours in children and from 10 to 12 hours in adults taking 10–30 mg extended- or sustained-release *d,l*-amphetamine formulations; half-lives for the *l*-enantiomer were slightly longer: ~9–11 hours in children and 13–15 hours in adults (Table 5 and Table 6) *(34)*. Biological half-life for methamphetamine was reported at 4–5 hours *(8)*. Another review reported a half-life of 12 hours for methamphetamine in humans *(17)*. In humans, amphetamine, methamphetamine, and their metabolites are primarily excreted in urine.

Gavage studies in rats dosed with up to 20 mg/kg bw *d,l*-amphetamine reported half-lives of ~2–3 hours *(34)*. Half-lives of 87 minutes in plasma and 62 minutes in brain were reported following iv injection of rats with 0.5 mg/kg bw *d,l*-amphetamine (reviewed in *(24)*). Studies in rats given radiolabeled methamphetamine at 45 mg/kg bw orally or by ip injection demonstrated that ~75–85% of radioactivity was excreted in urine and ~1–3% in feces over 3 days *(32)*.

2.6.2 General Toxicity

2.6.2.1 Humans

Side effects that the FDA considers common and related to treatment with *d,l*-amphetamine include fever, loss of appetite, emotional lability, insomnia, and nervousness *(34)*. Dose-related patterns were noted for anorexia, weight loss, and insomnia. Additional side effects reported in drug labels for or reviews of *d,l*- and *d*-amphetamine and methamphetamine are heart palpitations, tachycardia, elevated blood pressure, over-stimulation, restlessness, dizziness, insomnia, euphoria, dyskinesia, dysphoria, tremor, exacerbation of motor and phonic tics and Tourette disorder, headache, dry mouth, unpleasant taste, diarrhea, other gastrointestinal disturbances, constipation, anorexia, weight loss, urticaria, impotence, changes in libido, jitteriness, and social withdrawal. Psychotic episodes and cardiomyopathy were reported as rare and isolated events. Drug labels warn that behavior disturbance and thought disorders may be exacerbated in psychotic patients.

Amphetamine toxicity following overdose in humans is thought to be an extension of pharmacological effects on the central nervous, cardiovascular, and gastrointestinal systems *(24)*. As noted in Section 2.2.1.2, overdose symptoms include restlessness, irritability, tension, weakness, insomnia, chills, fever, angina, dry mouth, gastrointestinal cramps and diarrhea, stimulation followed by fatigue and depression, tremor, hyperreflexia, rapid respiration, confusion, hallucinations, panic, rhabdomyolysis, arrhythmias, hypertension or hypotension, circulatory collapse, nausea, and vomiting. Overdose can lead to convulsions, coma, and death. There is great variation in toxic response to amphetamine *(6)*. Idiosyncratic toxicity has been reported at doses as low as 2 mg but is rare at doses below 15 mg. Severe reactions have been noted at 30 mg but doses of 400–500 mg do not necessarily result in fatality.

Amphetamine and methamphetamine abuse is associated with development of tolerance, psychological dependence, and social disability *(6, 8, 10)*. Abrupt withdrawal following prolonged intake of high doses can result in extreme fatigue, mental depression, and sleep EEG changes. Toxicity associated with chronic intoxication can include dermatoses, insomnia, irritability, hyperactivity, and personality changes. Though rare with oral intake, psychosis that is indistinguishable from schizophrenia has been reported. Repetitive and stereotypic behavior that can advance to self-injurious behavior has been observed with high or repeated dosing with amphetamines (reviewed in *(27)*.

2.6.2.2 Experimental Animals

As noted in Section 2.2.2, symptoms of acute amphetamine toxicity in rats and mice include hyperactivity, piloerection, salivation, hyperpnea, dilated pupils, and convulsions. Symptoms of acute methamphetamine toxicity in rodents were reported as excitement, convulsions, and changes in seizure threshold. The oral LD_{50} for *d*-amphetamine in rats is reported at 96.8 mg/kg bw. LD_{50} values for *d,l*-amphetamine and methamphetamine in various species are summarized in Table 21 and Table 22.

Neuroanatomical evidence of toxicity has also been reported following amphetamine or methamphetamine exposure. Findings have included swollen nerve fibers and terminals in rats and mice (reviewed in *(27)*), reduction of brain dopaminergic axons and axon terminals in rats and

monkeys, and reduction of serotonergic axons and axon terminals in rats (reviewed in *(72)*). Continuous iv infusion of rats with amphetamines at 1 mg/kg bw/hour for 12 days resulted in reduced brain norepinephrine and cardiac catecholamine levels and increased motor activity and stereotypic behaviors (reviewed in *(24)*). Repeated parenteral dosing of rodents with methamphetamine has caused excitement, weight loss or decreased weight gain, alteration in classical conditioning, changes in pancreas, and hemorrhage *(70)*.

Effects of repeated dietary exposure of rats and mice to *d,l*-amphetamine were reported in an NTP study *(24)*. Hyperactivity, reduced feed intake, and decreased body weight were commonly observed in rats and mice. Following 13 weeks of exposure, final body weights were more than 10% lower compared to controls at dietary amphetamine concentrations of ≥188 ppm in rats and at 125 (females) and 250 (males) ppm in mice.

2.6.3 Genetic toxicity

With the exception of an equivocal mutagenicity response in *S. typhimurium* strain TA98, *d,l*-amphetamine produced negative results in mutagenicity testing of additional *S. typhimurium* strains and *E. coli* and in sister chromatid exchange and chromosomal aberration assays in Chinese hamster ovary cells *(24, 34)*. In in vivo micronucleus assays in mice, micronucleus frequency was not increased in 1 study with dosing up to 10 mg/kg bw *(34)*, but was increased in a second study at doses ≥12.5 mg/kg bw *(73)*.

2.6.4 Carcinogenicity

In a 2-year GLP dietary carcinogenicity study, there was no evidence of neoplasia at *d,l*-amphetamine doses up to 100 ppm (~5 mg/kg bw/day in rats and 19–30 mg/kg bw/day in mice) *(24, 71)*. An increase in lesions such as cataracts, retinal atrophy, and bone myelofibrosis in rats and ovarian atrophy in mice did not appear to have been directly caused by drug exposure. The study authors concluded that there was no evidence of carcinogenic activity of *d,l*-amphetamine sulfate in male or female F344/N rats or $B6C3F_1$ mice.

2.6.5 Potentially susceptible populations

Polymorphisms for CYP2D6, the enzyme involved in the aromatic hydroxylation of amphetamines in humans and rats, are associated with at least 12 variants that alter enzyme activity (reviewed by *(74, 75)*. Poor metabolizer phenotypes occur in 5–8% of whites and 2–10% of blacks and Asians. CYP2C3 is involved in amphetamine deamination reactions in rabbits *(25)*. The enzyme is not a member of the CYP2C human gene family *(76)*, but the Expert Panel finds it reasonable to assume that one or more members of the CYP2C family may be involved in deamination reactions in humans. The gene family is highly polymorphic with functional variants identified at CYP2C9 and CYP2C19 loci. Two of three known defective alleles of CYP2C9 result in decreased activity. CYP2C19 polymorphisms resulting in slow metabolizer phenotypes are present in 3% of Caucasians and nearly 20% of Asians *(77)*.

Two studies examined the effects of methamphetamine on Dark Agouti rats, which have a CYP2D1 polymorphism that results in poor methamphetamine metabolism in females and intermediate metabolism in males *(48, 78)*. Administration of methamphetamine 30 mg/kg bw sc twice daily on PND 11–20 did not affect behavioral testing results in offspring of Dark Agouti versus Sprague-Dawley rats, but mortality was higher in male and female Dark Agouti rat offspring compared to Sprague-Dawley rats. In a second study, sc injection with 15 mg/kg bw methamphetamine on PND 11–20 resulted in less effective performance on the Morris Maze test of spatial learning and memory in female Dark Agouti rats and greater acoustic startle amplitudes in male and female Dark Agouti rats compared to Sprague-Dawley rats. Inhibition of methamphetamine 4-hydroxylation in rats was reported to enhance stereotyped behavior in rats, but reviewers noted that metabolic differences in humans versus

animals complicate the application of the study findings to humans with inborn CYP2D6 deficiencies (reviewed in *(30)*).

Comparison of *d,l*-amphetamine pharmacokinetics parameters in adults versus children indicated that half-life is shorter in children and bioavailability is lower in children when adjusted for weight differences *(34)*. A comparison of *d*-amphetamine responses in hyperactive boys and men demonstrated that activity was decreased to a greater extent in hyperactive boys, and that while hyperactive boys reported feeling cranky and more tired, the men reported feeling euphoric and less tired *(81)*.

Eleven studies compared neurological effects following amphetamine or methamphetamine exposure in developing versus adult animals in an attempt identify mechanisms responsible for discordant responses in adults versus children. Summaries of those studies and their references are included in Section 2.5.3. Amphetamine studies in rats suggested age-related alterations in dopamine release, firing of dopaminergic neurons, and sensitization following challenge dosing, while mouse studies suggested alterations in corticosterone release and ΔFosB expression. Methamphetamine studies in rats demonstrated age-related alterations in dopamine release or turnover, tyrosine hydroxylase activity, susceptibility to glial cell proliferation, toxicity to glutamate positive neurons, or alterations in dorsal protein kinase A activity.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

3.1.1 Exposure During Pregnancy

3.1.1.1 Case Reports and Case Series

Case reports and series are presented in this section in the order of publication.

Briggs et al. *(96)* described a normal child born to a woman who used 100–180 mg/day *d*-amphetamine for narcolepsy. The child was followed to the age of 18 months.

Strengths/Weaknesses: A strength is the report of a high-dose exposure in a woman who appeared to be at low risk for other drug exposures. No information was provided, however, on length of use of the medication or on specific developmental and neurologic assessments.

Utility (Adequacy) for CERHR Evaluation Process: This single case report is of limited utility.

A group from **the Department of Pediatrics at the Karolinska Institute (St. Göran's Hospital)** in Stockholm followed a cohort of amphetamine-addicted pregnant women and their newborn children for the first year of the children's life. Several publications described aspects of this project; a summary description was published by Larsson *(97)*.

The first paper in the series was a retrospective review of the records of 23 infants whose mothers were identified as having been addicted to amphetamine *(98)*. Six of the mothers discontinued amphetamine use by the beginning of the second trimester and 17 women continued to use amphetamine throughout pregnancy. The authors noted that the mothers made fewer prenatal visits than is typical of pregnant women in Sweden. There were 3 pregnancies complicated by preeclampsia and 6 preterm births (< 37 weeks gestation). Three children were ≥2 standard deviations below the mean birth weight for gestational age. Two children were drowsy and required tube feeding, suggesting amphetamine withdrawal. These complicated pregnancies were among women who continued to use amphetamine.

Prospective evaluation and follow-up of pregnancies in amphetamine-addicted women began in 1976 and included deliveries in the Stockholm metropolitan area between June 1, 1976 and December 31, 1977. There were 71 children born to 69 women; 1 woman delivered twice during the study period and 1 woman delivered twins. Psychosocial and medical characteristics of the mothers *(99, 100)* and outcomes of the newborns *(100)* were described. Of the 69 women, 17 stopped using amphetamine upon learning of their pregnancy during the first trimester, and 52 continued to use amphetamine throughout pregnancy. It was noted that many of the mothers themselves came from "multiproblem families," and many of them had experienced foster care as children. Women who stopped amphetamine use early in pregnancy came for prenatal care earlier and more reliably than women who continued to use amphetamine and 14 of the 17 women who gave up amphetamine use identified the pregnancy as a life-changing experience that contributed to their decision to discontinue illicit drug use. Three (18%) of the 17 women who stopped using amphetamine continued to use alcohol. Of the 53 women who continued to use amphetamine during pregnancy, 17 (32%) also used alcohol.

One child with malformations was born to a woman who had stopped using amphetamine in the first trimester. This child died at 3 weeks of age. Two infants were stillborn; both mothers had continued to use amphetamine. One quarter of the amphetamine-using women delivered preterm and two of the preterm infants died in the first week of life. Among the infants born to women on amphetamine, there

were nine who were transferred to a ward for observation due to maternal drug abuse; five of the nine developed symptoms that were interpreted as amphetamine withdrawal (e.g., somnolence).

Billing et al. *(101)*, supported by the Swedish Medical Research Council and the Första Majblomman Foundation, published a 1-year follow-up of children born to Swedish women who used amphetamine during pregnancy. Of the 69 infants born alive, 66 were alive at the time of follow-up. Sixteen children were born to women who stopped their drug use in the first trimester (two of whom restarted drug use after pregnancy). The remaining 50 children were born to women who continued to use amphetamine throughout pregnancy; 37 of these children were discharged with their mothers after birth and 13 were discharged to foster care. This report was based on interviews with the mothers or foster parents, reviews of medical and social welfare records, and evaluations by a psychologist who was unaware of the children's background.

The health and psychological status of the children is summarized in Table 27. The authors noted that postnatal maternal amphetamine abuse appeared to be an important predictor of abnormal "emotional" development (autism, delayed speech, or lack of wariness of strangers). There were two children with abnormal emotional development in the group exposed to amphetamine only during the first trimester, and in both cases, the mothers had resumed amphetamine abuse postpartum. The authors believed that prompt fostering of children born to women who continued to use amphetamine was important in childhood development. They questioned whether health and psychological problems in children exposed prenatally to amphetamine were due to effects of the drug or due to the poor environment provided by the drug-abusing mother.

Table 27. Twelve-Month Health and Psychological Status of Children Born to Amphetamine-Using Women

	Amphetamine exposure/initial caregiver		
	1st trimester	Entire pregnancy	
Number of children (%)	Mother	Mother	Fostered
Total sample	16	37	13
Neonatal hospital admissions	2 (12.5)	12 (32.4)	10 (76.9)
Children admitted in first year	3 (18.8)	15 (40.5)	3 (23.1)
Abnormal motor development[a]	2 (12.5)	6 (16.7)[b]	1 (7.7)
Abnormal emotional development	2 (12.5)	10 (27.8)[b]	1 (7.7)
In foster care at the end of year 1	1 (6.2)	12 (33.3)[b]	13 (100)

[a]Delay of ≥2 months; [b]36 children evaluated. From *(101)*.

A 4-year follow-up on 65 of these children born to 63 of the women *(102, 103)* included evaluation by a single psychologist using the Terman-Merrill Test, an IQ test, and observation of 2 hours of free play. The psychologist reviewed a parental questionnaire on psychomotor and emotional adjustment. Based on the psychologist's observations and his review of the questionnaires, he rated adjustment in each child as very poor, poor, fair, good, or very good. Sixty percent of the children were judged fair, good, or very good. A number of potential predictors of adjustment were examined one at a time; adjustment was significantly correlated with length of maternal ethanol abuse during pregnancy, while correlations with length of drug abuse before or after delivery of the child were lower and did not achieve statistical significance **[multiple regression results were not presented]**. Physical characteristics of the children at 4 and 8 years of age were published separately *(104, 105)* and as a part of the 10-year follow-up report, discussed below.

A 10-year follow-up of the 69 children born to 71 women who had abused amphetamines during pregnancy tested and examined the children at ages 1, 4–5, and 8–9 *(106)*. The tests used to examine the children are summarized in Table 28.

Table 28. Evaluations of the Offspring of Amphetamine-Abusing Women over 10 Years of Life

Age (years)	Evaluations
1	Maternal interview, Observation, Gesell, Growth, Health
4	Maternal interview, Observation, Terman Merril, Goodenough, Bender, Koh Block Design, Growth, Health
8	Maternal interview, Observation, Terman Merril, Goodenough, Bender, Koh Block Design, School Achievement, Growth, Health
10	School achievement, Growth, Health

From Eriksson and Zetterström *(106)*. Details of assessment methods were not provided.

Data obtained in these subjects were compared to Swedish general population statistics because the authors stated, "From the start we were aware of the fact that a control group could not be found and followed in Sweden." In the amphetamine-exposed group, perinatal mortality was 5.6%, infant mortality was 5.8%, malformations were diagnosed in 3% of children, 18% of children had a birth weight lower than 2500 g, 20% were born at a gestational age less than 37 weeks, and 38% had to be transported to a neonatal unit. There were no significant differences in weight and length at birth or at 1, 4, 8, or 10 years of age in boys born to mothers who used amphetamine during pregnancy compared to general population figures for Swedish boys. Girls born to mothers who had abused amphetamine were found to have lower weights and lengths at birth and at ages 1 and 10 years and increased weights at age 4 years. **[The Expert Panel notes the apparent sexually dimorphic response, which is not reported elsewhere.]**

The authors stated that all of the children were found to have a normal intellectual capacity, except for 1 child (IQ of 60–80 at 4 years of age) who they classified as mentally retarded. Mean IQ of the amphetamine-exposed children was 103 at 4 years of age, which was said to be lower than the Swedish mean of 110. At 10 years of age, 8 children (12%) attended a class a year below the norm for their age level. A negative correlation at 8 years of age was reported between an index **[not otherwise explained]** of psychometric test results (Bender, Koh Block Design, and Goodenough) and increased exposure to amphetamine during fetal life **[data not presented]**.

Behavioral problems at 4 and 8 years of age were found to be frequent in the study group **[compared to a control group of Swedish children evaluated 25 years earlier]**. Among amphetamine-exposed children, 35% demonstrated aggressive behavior and 59% had peer-related problems at age 4. At age 8, 23% of the children showed aggressive behavior and 13% show peer-related problems. There was a significant correlation between aggressive behavior at ages 4 and 8 and amphetamine exposure during fetal life **[methods not explained, data not presented]**. A psychologist found that 40% of the children at ages 4 and 8 were poorly adjusted. The authors noted that by 10 years of age, only 30% of the amphetamine-exposed children were with their biological mothers, the rest having been placed in foster homes or adopted.

Subsequent reports presented 14-year follow-up on 65 children from the original cohort *(107, 108)*. By age 14, 80% of the children had been placed in foster homes or adopted. By age 14–15, 10 (15%) of the children were a class below their age level. There was a significant correlation between IQ level at age 8 and grades at age 14 **[data not presented]**. Compared to 494 Stockholm children born in 1976, amphetamine-exposed boys were an average of 5 cm taller and girls an average of 2 cm shorter. Boys were also an average of about 10 kg heavier. The authors speculated that alterations in timing of

puberty might be an explanation for height and weight differences, but did not have data on puberty events. The authors concluded that amphetamine exposure during the fetal life affects the development of children up to age 14. **[The Expert Panel notes the apparent sexually dimorphic response, which is not reported elsewhere.]**

Strengths/Weaknesses: Although these reports are presented together because they deal with the same cohort of children, the quality of the papers is highly variable. The strengths and weaknesses of the individual papers are summarized in Table 29.

Table 29. Strengths and Weaknesses of the Papers on the Karolinska Institute Cohort of Amphetamine-Exposed Children

Study	Strengths	Weaknesses
Eriksson et al., 1978 *(98*)	Trimester data presented (negative consequences appeared only when amphetamine use was throughout pregnancy).	Risk factors other than amphetamine addiction were not mentioned; differences between women who stopped and women who continued amphetamines during pregnancy were not indicated; there was no reference to general population rates of the negative outcomes seen in the sample.
Larsson et al, 1979 *(99*)	Had access to social data; recognized that addicted pregnant women come from "problem families;" differences recognized between women who did and did not stop amphetamine use during pregnancy.	Role (or absence) of father not considered; ethanol use identified as differentiating women who did and did not stop amphetamines, but patterns of drinking not described.
Eriksson et al., 1981 *(100*)	Lists outcomes found in neonates of amphetamine-using women; mentions possible effects of maternal-child separation.	No detail on other risk factors, making a causal link with amphetamines problematic; inadequate attention to tobacco use and clinic attendance.
Billing et al, 1980 *(101*)	Considered pre- and postnatal amphetamine use; blinded evaluations; contrasting of fostered and non-fostered children; recognized the effects of multiple separations; included data on parent/partner and other people in the home.	Lassitude may have been due to problems with foster homes or problems encountered prior to fostering, even during the first 2 months of life; the hospitalization rate in fostered children may depend on unreported factors such as how many other children are in the home and who provides transportation for the child; the determinants used to diagnose "emotional" problems are an odd mixture, raising questions about their reliability.
Billing et al., 1985 and 1988 *(102, 103*)	Blinded evaluations; data from 4-year-olds; prenatal ethanol identified as an important contributor to outcomes; identification of father criminality as important is in agreement with other studies.	Not clear how fostered children differed from non-fostered children; determination of alcohol as a contributing factor was not clear; pattern of alcohol use (e.g., binging, chronic) not explored; collection of prenatal data may have been retrospective and unreliable; the general assessment of the children's emotional well-being appears subjective and

		unverifiable.
Eriksson et al., 1985, 1989, 1994 *(104-106)*	Presentation of outcome by child's sex; useful outcome measures (growth, cognitive, behavioral).	Comparison of exposed children with 25-year-old general population statistics; pre- and postnatal risk factors other than amphetamines were not mentioned; it is not clear how fostered children (13/69 at birth and 70% by age 10) were treated analytically; the timing of being placed in foster care was likely to be a surrogate for continued amphetamine use and therefore important; an index describing the mothers' emotional and abuse problems was not explained; the implication that longer amphetamine exposures were causally associated with aggression was not appropriate because there are too many intervening psychosocial variables by age 8.
Eriksson et al., 1994, 2000; Cernerud, 1996 *(107-109)*	Comparison of amphetamine cohort to a similar-age population; evaluation by child's sex; introduces the concept of exposure possibly leading to vulnerability to psychosocial problems.	Although the authors reported that 80% of the cohort was in foster care by age 14, there was no apparent consideration of fostering in the analysis or interpretation of the data. It must be assumed that 52 of the 65 children were removed from their homes because of continuing maternal drug addiction, which would have an important impact on the outcomes considered (performance in school, growth); school performance was evaluated using grades from different teachers; no investigation was reported of the children's possible substance abuse.

Utility (Adequacy) In the CERHR Evaluation Process: This set of reports is generally useful in the evaluation process, although individual reports taken by themselves may be of limited or no utility. The most useful of these reports is the study of Billing et al. *(101)*. The utility of the studies in the evaluation process extends only to an evaluation of the amphetamine-using lifestyle. Because of the psychosocial factors and the exposure to ethanol that accompanied prenatal amphetamine exposure in this cohort, conclusions relevant to the amphetamine exposures per se are not possible.

Dominguez et al. *(110)*, support not indicated, presented 10 cases of brain and ocular abnormalities in children born to women who had been exposed during pregnancy to amphetamine, cocaine, phenylpropanolamine, or heroin. The authors considered these cases together due to the vasoconstricting properties of the drugs of exposure and due to similarities among the outcomes. There was a single child exposed to amphetamine. This infant, whose mother received no prenatal care, was born at 27 week gestation, with a birth weight of 1070 g. The child was said to have had a long nursery stay due to prematurity. Examination at birth showed motor delay with truncal hypotonia, and neuroimaging at an unspecified gestational age showed dysgenesis of the corpus callosum with enlarged occipital horns.

Strengths/Weaknesses: The listing of brain and ocular abnormalities may help other health care workers to identify similar outcomes in their patients; however, polydrug users are not useful in evaluating possible effects of a single agent. This report represents a single retrospectively ascertained case report of amphetamine use during pregnancy and adverse perinatal outcome. The maternal interviews were characterized as extensive but were not necessarily structured. Because the neonates were identified based on urine drug screening, the mothers may have been interviewed while under the influence of the abused drugs. There was no evidence of screening for tobacco, ethanol, or other drugs of abuse.

Utility (Adequacy) for CERHR Evaluation Process: This report is not useful for the evaluation process.

Bays *(111)* published a letter-to-the-editor describing seven children with limb reduction defects, two of whom were born to women who admitted illicit methamphetamine use. One of these two mothers plus an additional mother in the series used cocaine. The author believed the vasoconstrictive effects of methamphetamine and cocaine may have been responsible for the limb defects in these cases.

Strengths/Weaknesses: This letter could alert clinicians to look for other instances of limb defects in exposed populations; however, this case series involves only two methamphetamine-exposed pregnancies, one of which was also exposed to cocaine. There is no indication of evaluation for other drugs and ethanol.

Utility (Adequacy) In the CERHR Evaluation Process: This report is not useful in the evaluation process.

Joffe and Kasnic *(112)* presented a case report of a pregnant methamphetamine addict who was prescribed *d*-amphetamine beginning at 28 weeks gestation as part of an addiction treatment program. The initial dose of *d*-amphetamine was 100 mg once or twice/day; this dose was decreased and by 32 weeks gestation, the woman was not taking any prescribed or illicit amphetamines. At 40 weeks gestation, she delivered a healthy infant who did not have withdrawal symptoms or evidence of intracranial bleeding.

Strengths/Weaknesses: The strength of this report is the novel clinical exposure. A weakness is the lack of information on assessment beyond signs of withdrawal and the presence of intracranial bleeding.

Utility (Adequacy) In the CERHR Evaluation Process: This report is of minimal utility in the evaluation process.

Finnegan and Ehrlich *(113)* described neonatal abstinence symptoms and treatment among the offspring of 300 drug-abusing women. The drugs of abuse were opioids and non-opioids, including methylphenidate and methamphetamine. The statement was made in this paper that fewer infants required treatment for abstinence among those exposed to non-opioids **[with no data presented on infant condition by individual drug exposure]**.

Strengths/Weaknesses: The strength of this report is the large number of women; however, the weaknesses include the evaluation of polydrug using women and the lumping of diverse substances as non-opioids.

Utility (Adequacy) In the CERHR Evaluation Process: This report is not useful in the evaluation process.

Furara et al. *(114)* reported pregnancy outcome of 28 of 33 women who said they used amphetamines when they booked for prenatal care over a 23-month period at a UK hospital. Fifteen of these women admitted to use of multiple illicit drugs. Amphetamine and other illicit drug use was ascertained purely by maternal report; testing of biologic samples was not employed. Fourteen women said they stopped amphetamine use during pregnancy. Five women had previous obstetric complications. Mean gestational age was 37.8 weeks (range 23–42 weeks), with 28.6% of the pregnancies ending before 37 completed weeks of gestation. The mean birth weight was 2920 g (range 1680–3997 g), with 25% of the babies weighing less than 2500 g. There was one stillbirth and one neonatal death, both in women who continued amphetamine use during pregnancy. Seven children were admitted to the neonatal intensive care unit **[it is not stated whether illicit drug exposure was a criterion for admission]**. The authors did not identify an effect of self-reported amphetamine cessation during pregnancy on birth weight or the incidence of preterm labor.

Strengths/Weaknesses: The authors' impression that stopping amphetamines during pregnancy was associated with a decrease in perinatal death was not statistically evaluated. Weaknesses include use of polydrug abusing women, lack of objective determination of abstinence, lack of consideration of ethanol, lack of determination of the timing of amphetamine cessation, and lack of consideration of non-amphetamine risk factors for adverse perinatal outcome.

Utility (Adequacy) In the CERHR Evaluation Process: This report is not useful in the evaluation process.

Sherman and Wheeler-Sherman *(115)* published an abstract describing the offspring of 202 women who admitted to the illicit use of amphetamine, methamphetamine, or similar drugs during pregnancy. There were 33 infants (16.3%) with major congenital anomalies among which were 13 cardiac defects (chiefly septal), 12 gastrointestinal defects (especially gastroschisis and imperforate anus), 11 genitourinary malformations (most commonly hydronephrosis), and 5 CNS malformations. There were no details on the ascertainment of exposure or outcome information.

Strengths/Weaknesses: Strengths include the exclusion of tobacco use. Weaknesses include the possible influence of ethanol use on the results, the lack of detail on methods, and the lack of explanation for a rate of anomalies that is much higher than reported elsewhere in the literature.

Utility (Adequacy) In the CERHR Evaluation Process: This abstract is not useful in the evaluation process.

van Tonningen-van Driel et al. *(116)* and McElhatton et al. *(117)* represent two reports from teratology information services providing follow-up information after recreational pregnancy exposure to methylenedioxymethamphetamine (MDMA; Ecstasy). The first is a paper from Holland *(116)* **[published in Dutch; reviewed in abstract]** that identified 49 pregnancies about which a physician or midwife had called with concerns about a patient's exposure to MDMA **[the authors note that these women often have additional drug exposures as well]**. Follow-up was available by questionnaire for 43 pregnancies. There were three elective terminations of pregnancy and two spontaneous abortions. There was one set of triplets. Of the 40 live-born babies, 1 had a congenital cardiac malformation. The second report, from the UK National Teratology Information Service *(117)*, was a follow-up on inquiries about 302 pregnancies exposed to MDMA, of which 31 were ongoing and 135 (45%) were lost to follow-up. Information on the pregnancies with follow-up was obtained by questionnaire from health care providers. There were 11 spontaneous abortions and 48 elective abortions (1 elective abortion for fetal anomalies). The spontaneous abortion rate (8%) was within the expected range, but the elective termination rate (35%) was higher than the UK average. Delivery prior to 37 weeks

gestation occurred in 7 pregnancies, 1 of which was a twin pregnancy. There was one neonatal death of a child who appeared to be morphologically normal (although no autopsy was reported). The mother had taken MDMA, heroin, and methadone. Birth weight in the sample was described as normal with only 3 term infants weighing less than 2500 g. Of the 78 live born infants, 12 had congenital malformations, for an incidence of 15.4% (95% CI 8.2–25.4%), compared to an expected rate of 2–3%. The authors noted an incidence of talipes equinovarus (clubfoot) that was greater than expected based on national statistics. There were 3 infants with this malformation for a rate of 38/1000 births (95% CI 8–109) compared to an expected rate of 1 per thousand births. In addition, all three affected infants were girls whereas idiopathic talipes equinovarus has a 3:1 male predominance in the UK. The authors also noted that 2 infants had ventricular septal defects (one of whom may also have had an atrial septal defect) for a rate of 26/1000 births (95% CI 3–90/1000). The expected incidence of congenital heart disease was 5–10/1000 births, with 1/4 to 1/3 of these cases including ventricular septal defect. Due to the small size of the case series, the authors declined to draw conclusions regarding a possible causal connection between MDMA exposure in pregnancy and congenital anomalies.

Strengths/Weaknesses: Both reports are limited by being derived from a self-selected population of individuals who inquired about MDMA exposures. In the van Tonningen-van Driel et al. study *(116*), no indication was given on frequency and timing of exposure and it is not clear what assessments were performed. The McElhatton et al. study *(117*) gave a useful comparison to general population statistics, including sex-related rates, and the authors were appropriately reserved about the conclusion that MDMA exposure was causally involved in the adverse outcomes.

Utility (Adequacy) In the CERHR Evaluation Process: The McElhatton et al. study *(117*) is useful in the evaluation process to the extent that data on MDMA can be applied to amphetamine and methamphetamine; the report by van Tonningen-van Driel et al. study *(116*) is less useful based on lack of detail.

McElhatton et al. *(118*) presented an abstract from the UK National Teratology Information Service in which they reported follow-up information on 281 pregnancies for which inquiries had been made about pregnancy exposures to amphetamine-like drugs. Of the 281 women, 41 abused amphetamines alone, 18 abused amphetamines and other drugs (including MDMA), and 136 abused MDMA with or without other drugs (except other amphetamines). **[The Expert Panel assumes that that these women were also reported in *(117*).]** There were 86 women with exposure to anorectic drugs, 77 of whom used phentermine. There were 21 children with congenital anomalies born to the 195 women who abused amphetamines with or without other drugs. There were 64 elective abortions, 14 spontaneous abortions, and 2 stillbirths/neonatal deaths in this group. The anomaly rate was 10.8% of live-born children, which the authors described as elevated compared to an expected rate of 2–3%. Musculoskeletal defects (7.5% of live births) and congenital heart disease (2.5% of live births) were also said to be elevated compared to an expected rate of 0.5–1%. **[The methods of exposure and outcome ascertainment were not described.]**

Strengths/Weaknesses: The breakdown of usage of amphetamine, MDMA, and other drugs is a strength, although it is not clear what the other drugs were. The comparison of outcomes to general population statistics is also a strength, but it is not clear what outcome assessments were performed, other than anomaly determination, or how elective and spontaneous abortions were distributed among drug-using subgroups. Among women exposed to licit drugs, most used phentermine, which is not likely to produce relevant information on amphetamine exposure.

Utility (Adequacy) In the CERHR Evaluation Process: Abstracts are not used directly to develop conclusions in the evaluation process, but this abstract is useful as supplemental information.

3.1.1.2 Controlled studies

Nora et al. *(119)*, supported by March of Dimes and NIH, reported in a letter-to-the-editor a comparison of 184 children with congenital heart disease and 108 children without congenital heart disease **[children seen in the well-baby clinic of the same hospital]**. Exposure to *d*-amphetamine was determined by asking the mother within 1 year of birth whether she had been exposed. Of the 184 children with congenital heart disease, 33 (18%) had maternal exposure to *d*-amphetamine, compared to 9 (9%) of the children without congenital heart disease ($P < 0.05$ **[OR 2.40, 95% CI 1.06–5.95, calculated by CERHR using chi-square feature of CDC's SABER program]**). When exposure to *d*-amphetamine was restricted to the "vulnerable period" (not otherwise specified) 20 (11%) of the children with congenital heart disease and 3 (3%) of children without congenital heart disease were identified ($P = 0.025$ **[OR 4.27, 95% CI 1.22–22.89]**). A family history of congenital heart disease was found in 49 (27%) of the children with congenital heart disease and 6 (6%) of the control children ($P < 0.001$ **[OR 6.17, 95% CI 2.49–18.23]**). Maternal exposure during the vulnerable period plus family history of congenital heart disease was identified in 9 (5%) of the children with congenital heart disease and 1 (1%) of the children without congenital heart disease (*P* value not calculated by authors **[CERHR calculation: $P = 0.07$; OR 5.50, 95% CI 0.74–243]**). The authors indicated that they wished to modify the conclusion expressed in a previous letter *(120)*, which stated that the probability of an association between *d*-amphetamine exposure during pregnancy and congenital heart disease appeared to be low. **[This communication is described in this section because its design appears is that of a case-control study, although the letter format lacks the level of detail typically included in a study report.]**

Strengths/Weaknesses: This letter has the strengths of considering family history as a risk factor for cardiovascular malformation and of including a large group of affected children. Weaknesses include failure to indicate the reasons for maternal use of *d*-amphetamine, lack of information on other exposures such as ethanol, and lack of detail on study design or on the study population. It does not appear that estimates were adjusted for potential confounders.

Utility (Adequacy) In the CERHR Evaluation Process: This letter is not useful in the evaluation process.

Nelson and Forfar *(121)*, supported by Ciba Ltd. and the Distillers Company Ltd., interviewed 458 mothers who gave birth to children with congenital malformations and mothers in each of 2 control groups. One control group consisted of 500 women who gave birth to normal babies immediately after a case child and a second group consisted of an additional 411 women who gave birth to normal children and who were matched to case mothers on age, parity, and infant sex. Women were interviewed before discharge from the maternity unit and were asked about medication use. Reported medication exposure was confirmed with the general practitioner, hospital records, or prescription records. A discrepancy in records occurred for 11.3% of the 4731 reported prescriptions **[the authors state that these prescriptions were "rejected" but do not indicate if the mothers were considered unexposed or were removed from consideration]**. Pregnancy dating relied on last menstrual period and associations were tested by specific exposure periods. These exposure periods were described as the prefertilization period (first 14 days), the period of "maximum organogenesis" (first 56 days), and the period of "total organogenesis" (first trimester). A category called "whole of pregnancy" was designated for exposure at any time of pregnancy, including during one of the more specific periods. The associations between prescriptions for *d*-amphetamine products and congenital anomalies are shown in Table 30. A significant association with *d*-amphetamine was shown when all children with malformations were compared to children without malformations, regardless of the time period studied. For children with minor malformations, a significant association was shown only for exposures during the first 15 days (prior to fertilization) and the first 56 days. Details were given for the 10 children with

malformations after maternal exposure in the first 56 days. Four children had urogenital anomalies; other anomalies **[assumed to be one per child]** included congenital heart disease, cleft lip, limb deformity, ear abnormality, dislocated hip, and pilonidal sinus.

Table 30. Number of Exposures to *d*-Amphetamine among Women Delivering Malformed and Normal Babies

	Malformations			Control	ORs, 95% CI for
Exposure period	All (n = 458)	Major (n = 175)	Minor (n = 283)	(n = 911)	Total Malformation
Whole of pregnancy	13*	5	8	10	**[2.63, 1.07–6.52]**
First trimester	11*	4	7	8	**[2.78, 1.03–2.61]**
First 56 days	10*	3	7*	5	**[4.04, 1.27–13.65]**
First 14 days	8*	2	6*	2	**[8.08, 1.59–55.25]**.

Data from Nelson and Forfar *(121)*.
*$P < 0.05$ according to the authors, statistical method not indicated.

Strengths/Weaknesses: Strengths include the attempt to match mothers for age and parity, efforts to confirm maternal reports using a second source such as pharmacy records, analysis by different periods of exposure, and division of malformations into major and minor, inasmuch as the latter may be more prone to under-ascertainment. Weaknesses include the lack of consideration of confounds such as maternal socioeconomic status, nutritional status, and tobacco and ethanol use. No information is provided on why women were taking the medication. If these women were using the medication for weight control, it is possible that excess maternal weight was an independent risk factor for malformations. It is also a weakness that the information on amphetamines is based on only 13 cases. The apparent inattention to a multiple comparison problem is also a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This report is of limited utility in the evaluation process.

Levin *(122)* presented 11 cases of biliary atresia presenting in infants between January 1, 1969 and June 1, 1970. Prenatal drug use histories were solicited from the infants' mothers and compared to histories obtained from the mothers of 50 control infants of the same age **[details of the methods not given]**. Four mothers of affected infants had used amphetamines during the second and third month of pregnancy, and a fifth mother used amphetamines during the last trimester. Other medications were also used by some of these women. Three of the 50 control mothers had used amphetamines. The authors reported that the difference between exposure rates in affected and unaffected pregnancies was significant at $P < 0.05$ **[OR 8.95, 95% CI 1.20–70.92 calculated by CERHR using CDC SABER program].**

Strengths/Weaknesses: The focus on a specific defect and the inclusion of information on timing of exposure are strengths; however, weaknesses include the retrospective nature of the report, the lack of consideration of covariates, and the lack of detail on aspects of the methods, including the selection of controls.

Utility (Adequacy) for CERHR Evaluation Process: This report is not useful in the evaluation process.

The National Collaborative Perinatal Project *(123)* reported on 50,282 mother-child pairs in which pregnancy had lasted at least 5 lunar months. Information on medication exposure during pregnancy was collected at the time of the first prenatal visit and recorded prospectively thereafter. Outcome

information was based on physical examination of the child up to the age of 1 year in 91% of the sample and for up to 4 years of age in an unspecified proportion of the sample. There were 29 malformed children from 367 exposures to *d*-amphetamine during the first 4 lunar months, 17 malformed children from 215 exposures to "amphetamines" **[not otherwise specified]**, and 5 malformed children from 89 exposures to methamphetamine. Relative risks were calculated using the entire sample as a reference group (3248 malformed children from 50,282 pregnancies, less the specific exposed individuals). The crude relative risk **[95% CI, calculated by CERHR using the CDC SABER program]** was 1.23 **[0.82–1.82]** for *d*-amphetamine, 1.23 **[0.72–2.05]** for amphetamines, and 0.87 **[0.31–2.22]** for methamphetamine. Standard relative risks were calculated using a multiple-logistic-risk function analysis procedure (with confounders tailored to each exposure group). Evaluation of 12 different malformation categories for all sympathomimetic drug exposures together showed no significant difference of the standardized relative risk from unity for any category. For the 367 *d*-amphetamine exposures during the first 4 lunar months, the standardized relative risk for any malformation was 1.08 (95% CI 0.65–1.68), for major malformations 1.29 (95% CI 0.73–2.10), and for minor malformations 1.46 (95% CI 0.59–2.98). For exposures anytime during pregnancy and any malformation, standardized relative risks were statistically not different from unity for *d*-amphetamine (1069 exposures), amphetamines (509 exposures), and methamphetamine (320 exposures).

Strengths/Weaknesses: This study included a large multi-site sample with prenatal recruitment and the report specified the background characteristics of the women who were recruited, not recruited, and who refused participation. There was also a well-defined methodology for identification of the substances used and at least two medical record sources were used to determine malformations. The authors thoughtfully addressed the issue of subjective differences in the analysis of certain malformations and analyzed these malformations separately from those that are more "uniform." Another strength is sensitivity to potential ethnic differences in malformations of interest. There was a well-specified multivariate model and a tight targeting of the period of possible exposure to the first trimester of pregnancy. Major weaknesses of this study include failure in the Collaborative Perinatal Project to identify prenatal alcohol exposure and a lack of information about dose of exposure to amphetamines or indications for use. There were multiple comparisons and lack of adjustment for covariates. A family history of birth defects was not considered. A final weakness is conflation of the drugs of interest in the current review (methamphetamine and *d*-amphetamine) with a range of exposures to related but not identical substances commonly found in patent cold medicines that contribute the largest number of observations to the analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process with a recognition of the limitations in interpretation associated with the weaknesses of the study.

Naeye *(124)*, supported by the US Public Health Service (PHS), presented an analysis of birth weight, length, and head circumference stratified by maternal prepregnancy weight and pregnancy weight gain from the National Collaborative Perinatal Project. Only term infants without congenital anomalies were considered. Infants whose mothers took *d*-amphetamine for weight control (n = 237) were compared to infants whose mothers did not take *d*-amphetamine (n ≈41,774 **[an unspecified number of women who took *d*-amphetamine for psychotropic purposes was excluded]**). Drug-using women were matched to all non-users who had the same race, parity, and smoking habits during pregnancy **[the number of these matched controls was not indicated]**. There was no difference in birth weight among infants born to women who discontinued *d*-amphetamine by 28 weeks of pregnancy compared to control women. Among infants born to women who continued *d*-amphetamine use into the third trimester, birth weights were decreased compared to controls if the mother gained >12 kg during pregnancy and had a pre-gravid weight < 45 kg. Among infants born to women with a pre-pregnancy weight ≥45 kg, birth weight was lower than in controls if the woman gained at least 8 kg during pregnancy. Birth length, head circumference, perinatal mortality, and incidence of maternal diastolic

blood pressure >85 mm Hg did not show a significant association with *d*-amphetamine exposure. The magnitude of the birth weight decrement **[estimated from a graph]** was 100–400 g, depending on the pre-pregnancy weight and pregnancy weight gain category. The author indicated that a decrease in birth weight without a change in birth length or head circumference was consistent with a drug-associated reduction in placental blood flow due to vasoconstriction.

Strengths/Weaknesses: Strengths include the large sample of women who took amphetamines for the same reason (weight control); the clear exclusion criteria; the matching for race, parity, and smoking; the attempt to analyze results by gestational age of exposure (leading to the biologically plausible weight effects with third trimester exposure); and the inclusion of information about birth weight and head circumference. Weaknesses include the lack of information on medication dose, and the lack of inclusion of covariates such as maternal age in the analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process although limited by the identified weaknesses.

Milkovich and van den Berg *(125)*, supported by the National Institute of Child Health and Human Development, evaluated pregnancy outcome of nearly all the white women insured by Kaiser Health Plan who delivered infants in the San Francisco East Bay Area between 1959 and 1966. The authors evaluated associations between anorectic drugs used by the mother during pregnancy (from treatment records) and congenital anomalies in the offspring. Congenital anomalies were assessed based on diagnosis at birth or at a Kaiser clinic through 61 months of age. A diagnosis of "severe congenital anomaly" was made in 3.4% of children of 8989 women who did not use anorectic drugs and 3.4% of 1694 women who reportedly used amphetamines during pregnancy **[crude RR 1.01, 95% CI 0.76–1.32]**. The authors noted an apparent increase in oral clefts, reporting 3 affected children of 175 women who were believed to have used amphetamines during the first 56 days of pregnancy and 5 of the 1694 women reported to have used amphetamines anytime during pregnancy. The unexposed group gave birth to 21 affected children of 10,213 total children. **[The authors do not present a statistical analysis. The Expert Panel calculated for 5 affected children of 1694 compared to 21 affected children of 10,213, the point estimate is 1.43 (95% CI 0.54–3.8), *P*=0.41.]** There was no increase in congenital heart disease incidence in children born to exposed women (14 affected children/1694 exposed) compared to unexposed women (rate given only as 0.9%).

Strengths/Weaknesses: Strengths include the use of a Kaiser population, which indicates good access to health care, good ascertainment of exposure, and a broad socioeconomic base. The sample size was large and 90% of the exposures were for appetite suppression. It is a strength that specific defects were evaluated and that the mother's weight gain during pregnancy was considered. Weaknesses include the lack of detail identified in the summary above, the lack of information about family history and about syndrome diagnosis, and failure to control for tobacco use, which may be a risk factor for oral clefts. The identified increase in cleft palate is a weak finding based on few cases.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process, but its utility is decreased by the lack of details and the small number of children with oral clefts.

Oro and Dixon *(126)*, supported by NIH, presented 104 infants enrolled over an 18-month period due to maternal or infant urine toxicology screening positive for cocaine, methamphetamine, or opioids. Medical records were reviewed and comparisons made between a pooled group of 46 infants exposed to cocaine or methamphetamine (28 of whom were exposed to methamphetamine and 5 of whom were exposed to both drugs), a group exposed to opioids (n = 49), and a comparison group of drug-free mother-infant pairs (n = 45). Gestational age, birth weight, length, and head circumference were lower in the groups exposed to cocaine or methamphetamine. Mean gestational age in the

cocaine/methamphetamine group was 37.9 ± 3.0 weeks compared to 39.4 ± 1.4 week in the drug-free control group ($P < 0.05$). Mean birth weight was 2901 ± 711 g in the cocaine/methamphetamine group and 3246 ± 552 g in the drug-free control group ($P < 0.05$). Mean length was 48.0 ± 5.1 cm in the cocaine/methamphetamine group and 50.7 ± 2.8 in the drug-free control group ($P < 0.05$). Mean head circumference was 33.2 ± 2.7 cm in the cocaine/methamphetamine group and 34.4 ± 1.5 cm in the control group ($P < 0.05$) **[the errors are assumed to be SD]**.

There were more perinatal complications in the cocaine/methamphetamine group than in the drug-free control group (28% compared to 9%). Complications in the cocaine/methamphetamine group consisted of prematurity, decreased head circumference, smallness for gestation age, fetal bradycardia, seizure, premature rupture of membranes, hyperbilirubinemia, placental hemorrhage, and anemia. Of these complications, only the last two were identified as occurring in a statistically larger proportion of drug-exposed than control infants.

Neurologic and behavioral abnormalities were reported to occur in a majority of cocaine/methamphetamine-exposed infants and consisted of abnormal sleep patterns (81%), tremors (71%), poor feeding (58%), hypertonia (52%), vomiting (51%), sneezing (45%), high-pitched cry (42%), frantic fist sucking (42%), tachypnea (19%), loose stools (16%), fever (19%), yawning (12.9%), hyperreflexia (15%), and excoriation (6%).

Based on comparisons with the group of infants exposed to opioids, the authors concluded that adverse effects on neonatal condition were due to cocaine or methamphetamine rather than maternal socioeconomic characteristics or poor attendance at prenatal visits.

Strengths/Weaknesses: It is a strength that illicit drug use was documented by testing maternal and infant urine. This study includes important endpoints such as gestational age, birth weight, head circumference, fetal growth, and behavior. Weaknesses include: poor explanation for the selection of controls; mixing of amphetamine and cocaine exposure; lack of consideration of ethanol exposure, malnutrition, or living situation; lack of consideration of maternal disease; and poor description of the statistical analysis with failure to show the results of the regression analysis. Dose information was considered unreliable.

Utility (Adequacy) In the CERHR Evaluation Process: This study is not useful in the evaluation process.

Dixon and Bejar *(127)*, supported by March of Dimes, collected anthropomorphic and cranial ultrasound data on three groups of newborns at the University of California, San Diego. After excluding preterm infants and infants with other possible causes of neurologic abnormalities, a stimulant-exposed group of 81 infants was identified based on infant urine toxicology screening. This group included 27 infants who had been exposed to methamphetamine. A neurologically-at-risk comparison group consisted of 87 drug-free infants suspected on clinical grounds of having hypoxic-ischemic encephalopathy. An additional comparison group consisted of 19 normal, drug-free infants. The cranial ultrasound findings were made with knowledge of group membership.

Stimulant-exposed infants were smaller than the infants in the other groups. Mean birth weight was 2904 ± 475 g in the stimulant-exposed group, 3346 ± 687 g in the neurologically-at-risk group, and 3351 ± 420 g in the normal control group. Mean birth length was 48.9 ± 3.1 cm in the stimulant-exposed group, 50.5 ± 4.8 cm in the neurologically-at-risk group, and 51.8 ± 2.5 in the normal control group. Mean head circumference was 33.4 ±1.5 cm in the stimulant-exposed group, 34.4 ± 1.8 in the neurologically-at-risk group, and 34.3 ± 1.1 in the normal control group (all differences between stimulant-exposed and other groups significant at $P < 0.05$ or greater, ANOVA followed by corrected *t*-

test). **[The errors are assumed by the Expert Panel to be SD. The stimulant-exposed group included cocaine-exposed babies and anthropomorphic data were not separated by individual stimulant exposures. Some of the infants in this study were previously presented by Oro and Dixon *(126)*; the reported measurements are similar although not identical in the two studies.]**

Mean gestational ages were 4–5 days lower than in the comparison groups (38.9 ± 1.3 weeks in children exposed to stimulants, 39.6 ± 1.3 weeks in the neurologically-at-risk children, and 39.4 ± 0.7 weeks in control children); the effect in stimulant-exposed children was statistically significant compared to both groups. The authors stated that the gestational age difference did not explain the anthropomorphic differences. Stimulant-exposed infants were more likely than infants in the other groups to be Black and growth-restricted (19.8% intrauterine growth restriction among stimulant-exposed babies compared to 8.0% of neurologically-at-risk babies and 5.3% of normal controls, $P < 0.01$ for comparison of stimulant group to either of the other groups).

Abnormal cranial ultrasound findings were identified in 26 (35.1%) of the stimulant-exposed infants, 24 (27.6%) of the neurologically-at-risk infants, and 1 (5.3%) of the normal control infants. The abnormality in the normal control infant was subependymal hemorrhage. Nine (37.5%) of the 24 infants exposed to methamphetamine had abnormal results consisting of 1 (2.4%) with white matter cavities, 3 (12.5%) with white matter densities, 4 (16.7%) with intraventricular hemorrhage, 4 (16.7%) with subarachnoid hemorrhage, 3 (12.5%) with subependymal hemorrhage, and 2 (8.3%) with ventricular enlargement. Of stimulant-exposed infants, 22% had multiple findings (not further broken down by specific stimulant). There was no association between the cranial ultrasound findings and the birth weight or length by **[unspecified]** univariate analysis.

The authors found the ultrasound findings consistent with a presumed vasoconstriction mechanism of stimulant toxicity. They believed maternal lifestyle factors to be less likely explanations because a cocaine + opioids subgroup of the stimulant-exposed infants had fewer lesions than a cocaine-only subgroup, but would have been expected to have less favorable maternal social conditions.

Strengths/Weaknesses: This study has strengths and weaknesses similar to that of Oro and Dixon *(126)*, with the added strength that a single radiologist evaluated all ultrasound examinations and the additional weaknesses of failure to match for gestational age (which was shorter in the exposed group) and failure to consider ethanol, trauma history, and medical conditions.

Utility (Adequacy) In the CERHR Evaluation Process: This study is not useful in the evaluation process.

Little et al. *(128)*, supported by NIH, over a 10-month period identified 52 pregnant women who reported that they had abused iv methamphetamine. Pregnancy outcome in these women was compared using medical record review with that of a control group of 52 women not known to have abused drugs, selected by their deliveries having occurred next after those of the methamphetamine-abusers. Thirty-eight of the 52 methamphetamine abusers admitted to other recreational drug use during pregnancy. The most common other drugs used by methamphetamine-exposed women during pregnancy were tobacco (46%), marijuana (39%), and cocaine (27%). Among the control women, tobacco use was identified in 11% and marijuana use in 2%.

Comparisons were made between the groups using unspecified nonparametric tests and ANOVA. There was no difference between the groups in pregnancy complications. In the methamphetamine group, there were six reports of pregnancy complications consisting of pregnancy-induced hypertension, peripartum hemorrhage, and syphilis. In the control group there were 14 reports of pregnancy complications including pregnancy-induced hypertension, peripartum hemorrhage, syphilis,

chorioamnionitis, and hepatitis. No differences were found in neonatal complications. Eleven methamphetamine-exposed neonates had records of complications consisting of meconium-stained fluid, fetal heart rate decelerations, tachypnea, fetal tachycardia, and withdrawal symptoms. Among control infants, there were nine complications consisting of meconium-stained fluid, tachypnea, and congenital syphilis. There was no difference between the two groups in the proportions with congenital abnormalities detected at birth. The methamphetamine group had six reports of infant abnormalities consisting of cleft lip/palate, low body fat, Mongolian spots, systolic murmur, undescended testes, and ventricular septal defect. There were seven infants with abnormalities in the control group including one case each of undescended testes, hairy nevus, hip/knee click, natal teeth, supernumerary nipple, umbilical hernia, and vaginal tag. The authors indicated that their sample size had a 39% power to detect a 4-fold increase in malformations.

Infants in the methamphetamine group had a lower mean (± SD) birth weight (2957.0 ± 574.0 g) compared to control infants (3295.8 ± 433.3 g, $P < 0.001$). Mean birth length (± SD) was lower in the methamphetamine-exposed infants (48.1 ± 2.0 cm) compared to the control group (49.8 ± 2.3 cm, $P < 0.001$). Mean head circumference (± SD) was also reduced in methamphetamine-exposed infants (33.2 ± 1.0 cm) compared to control infants (33.9 ± 1.2 cm, $P < 0.001$). There was no difference between the groups in estimated gestational age (39.1 ± 1.5 weeks in the exposed group and 39.3 ± 2.0 in the control group).

The authors indicated that they found no separate effect of cigarette smoking on anthropomorphic parameters **[analysis not shown]**. There was said to be no difference in the proportion of infants in each group with Apgar scores ≤ 6 **[data not shown]**.

The authors identified as limitations in their study the reliance on self-reported illicit drug use and the difficulty of separating effects of methamphetamine from lifestyle factors such as nutritional status. They concluded, however, that the restricted fetal growth was associated with methamphetamine abuse during pregnancy.

Strengths/Weaknesses: A strength is the ability to identify 52 iv methamphetamine users; however, a weakness is that this number is too small to perform multivariate analysis to control for pregnancy exposure to other substances. The selection of controls to represent the population served by the hospital is a strength. Some of the stated abnormalities (e.g., Mongolian spots) are normal ethnic variations and should not have been counted as abnormalities. Other weaknesses were identified by the authors of the article: the reliance on self-reported illicit drug use and the difficulty of separating effects of methamphetamine from lifestyle factors such as nutritional status.

Utility (Adequacy) In the CERHR Evaluation Process: This study is not useful in the evaluation process.

Gillogley et al. ***(19)***, support not indicated, evaluated pregnancy outcome among women with positive urine drug screens delivering at the University of California, Davis Medical Center, compared to a group of drug screen-negative women matched for race and nearest discharge date. Women were excluded from the control group if they gave a history of illicit drug use or if screening of their babies identified illicit drug exposure. Screening was conducted for opioids, cocaine, and amphetamines. Information on exposure and pregnancy outcome was obtained by medical record review. Most of the urine toxicology screens were performed on women presenting in labor as part of the hospital's routine. Infant screening was performed only if the mother had a positive screen or if the neonate was clinically suspected to have been exposed to illicit drugs. Among 1643 women with known toxicology screen results, 379 (23%) were positive for 1 or more of the tested substances and 106 (6%) were positive only for amphetamines. An additional 35 women had positive screens for multiple substances (not

specified), and 39 were positive for something (details not given). Prenatal care had not been received by 40% of women who were positive for amphetamines compared to 11% of the 293 control women. A history of smoking was present in 80% of amphetamine-positive women and 32% of control women, and a history of alcohol use was present in 22% of amphetamine-positive women and 11% of control women (both comparisons $P < 0.05$ by Fisher Exact or chi-square test). Pregnancy complications and mode of delivery were not different between amphetamine-positive and control women. There were no differences between the amphetamine and control groups in gestational age, proportion born prematurely (< 37 weeks), and proportion with low birth weight (< 2500 g), but mean birth weight and head circumference were reduced in the amphetamine group **[the data table indicates the mean birth weight in the amphetamine group was 294.7 g, which is assumed to be a misprint]**. Mean head circumference was 32.8 cm in the amphetamine group and 33.5 cm in the control **[variances not given]**. There was only one child in the amphetamine-positive group with a congenital anomaly (hypospadias) and the congenital anomaly rate was not different from the control sample. Regression analysis on the entire sample of illicit drug-positive women (exposed to amphetamines, opioids, cocaine, or multiple chemicals) showed illicit drug use to make an independent contribution to birth weight and head circumference when smoking and gestational age were controlled **[there was no regression on the individual drugs of abuse]**. The authors drew conclusions about illicit drug use in general, but did not offer conclusions specifically on amphetamine use.

Strengths/Weaknesses: The identification of women by substance of abuse and separate analysis of the women positive only for amphetamines is a strength; there was, however, an insufficient number of amphetamine-only women for use in the regression analysis. The use of record review and computerized discharge information for exposure and outcome determination is an important weakness, in part because all information on cigarette smoking and alcohol consumption would have come from these sources. Medical records are known to be inconsistent in reliably providing this kind of information. The paper noted that information on quantity or frequency of alcohol consumption was not collected, and it is not clear whether information was collected on the amount of cigarette smoking as opposed to a dichotomous determination of use (smoker/nonsmoker). Information on past drug use was also problematic; of women with a positive drug screen, 38% denied illicit drug use at any time. In addition, drug screening was performed at only one point in time. No information on nutrition during pregnancy was collected. Another weakness is the screening of newborns based on a criterion (suspicion for illicit drug exposure) that may not have been uniformly applied. The typographical error in the table is a problem because the magnitude of the deficit in birth weight can only be guessed. The statistical analysis was inadequate in that the regression analysis on birth weight, head circumference, and length did not adjust for any sociodemographic factors and the multivariate approach was used for one set of analyses that combined all drug exposure groups. As a result, conclusions about amphetamine exposure were based on data without consideration of the possible role of other exposures (e.g., smoking or alcohol) or demographic factors.

Utility (Adequacy) In the CERHR Evaluation Process: This paper is of minimal utility in the evaluation process in adding evidence for an effect of prenatal amphetamine abuse on fetal growth. Independence from effects of tobacco, ethanol, or other lifestyle factors cannot be assumed.

Smith et al. ***(129)***, funded by NIH, evaluated 12 children with a history of intrauterine methamphetamine exposure and 14 control children without a history of illicit drug exposure. Six of the methamphetamine-exposed children had also been exposed to cigarette smoking **[presumably in utero]**. The children were 6–9 years old at the time of the study. The exposed children were identified through a state-funded drug-treatment program in which their mothers had been enrolled. Control children came from the same population of lower- and middle-income urban residents **[recruitment methods not indicated]**. Parents completed the Child Behavior Checklist and children underwent magnetic resonance imaging studies of the brain and localized ^{1}H-magnetic resonance spectroscopy of

the right frontal white matter and basal ganglia. The Child Behavior Checklist did not show any problems in the control group. The methamphetamine-exposed group included two children with social problems and delinquent behavior and one child with thought problems, aggressive behavior, and anxiety. There were no anatomic abnormalities in the brains of any of the children by magnetic resonance imaging. Magnetic resonance spectroscopy showed increased concentrations of creatine and glutamate/glutamine in the basal ganglia of methamphetamine-exposed children without a significant difference in *N*-acetyl containing compounds, which are markers of neuronal loss. The authors believed the increased creatine concentration could represent gliosis, although there was no increase in the concentration of myoinositol, which would be expected with gliosis. Alternatively, the increased creatine concentration could have represented an alteration in energy metabolism. The authors noted that previous studies had shown similar increases in creatine associated with past cocaine use in adults and with prenatal cocaine exposure in children. They suggested that multiple episodes of decreased placental blood flow associated with the vasoconstrictive effects of methamphetamine could have resulted in long-lasting alterations in energy metabolism in the brain.

Strengths/Weaknesses: The use of DSM-IV criteria for methamphetamine dependence is a strength, as is the exclusion of women with other illicit drug dependence and women medicated for chronic illness. The sample size is too small to control for other risk factors and there was no prenatal ethanol exposure in the controls. As the authors point out, the magnetic resonance findings cannot be considered specific to methamphetamine exposure because other potential exposures, such as lead, were not evaluated.

Utility (Adequacy) In the CERHR Evaluation Process: This study is correctly described by its authors as preliminary and provides only supplemental information.

Smith et al. ***(130)***, sponsored by NIH, retrospectively evaluated neonatal outcome in pregnancies identified as methamphetamine-exposed by urine toxicology screen of the mother or a "history of drug use" in the mother. Toxicology screens were triggered by a record of fewer than 4 prenatal visits, first prenatal visit after 30 weeks gestation, maternal history of drug abuse, symptoms or behaviors suspicious for drug abuse, placental abruption, and out-of-hospital birth. A comparison group of unexposed infants was identified using newborn logbooks and matching for maternal parity and month of birth. Unexposed status was based on negative urine toxicology screen or negative maternal history. Comparisons were made between infants using *t*-tests and Fisher exact test; however, because more mothers in the exposed group smoked cigarettes, comparisons were repeated using two-way ANOVA with smoking as the second factor. Results were presented for 135 methamphetamine-exposed infants and 160 controls **[the discrepancy in numbers was not explained]**. Methamphetamine-exposed mothers were older, more likely to smoke cigarettes, and more likely to be white. Ethanol was used by 44% and marijuana by 59% of methamphetamine-exposed mothers, compared to none of the control mothers. Mean gestational age was lower in methamphetamine-exposed pregnancies than controls (mean ± SD 37.5 ± 1.0 compared to 39.7 ± 1.5 weeks, $P < 0.001$). **[The Expert Panel notes that by design, neither group included babies born prior to 37 weeks gestation.]** There were no differences between groups in mean birth weight, length, head circumference, or incidence of obstetrical complications. There were 13 methamphetamine-exposed infants identified as small for gestational age compared to 2 control infants ($P < 0.001$ **[statistical test and control for smoking not indicated; crude RR 7.70, 95% CI 2.08–46.20 calculated by CERHR using CDC SABER program]**. Mean birth weight and head circumference were significantly lower in infants exposed to methamphetamine in all trimesters compared to those exposed for only the first or second trimesters. Methamphetamine exposure and smoking were associated with deficits in birth weight and head circumference compared to methamphetamine exposure alone **[although it is not clear whether the effect of smoking explains the decrease in birth weight and head circumference in infants exposed to methamphetamine for three trimesters of pregnancy compared to control]**. Withdrawal symptoms were recorded in 49% of methamphetamine-exposed infants, but only 4% required pharmacologic treatment for withdrawal. The

authors concluded that methamphetamine exposure during pregnancy is associated with intrauterine growth restriction in infants born at term, but that other factors such as alcohol, tobacco, and marijuana "likely contributed" to the findings.

Strengths/Weaknesses: The identification of exposure by trimester and the inclusion of smoking status in the comparisons are strengths; however, it is a weakness that exposure to substances (including tobacco) were not controlled in a dose-related manner. Differences in race/ethnicity between groups and failure to interpret smallness for gestational age in a race-normed manner are also weaknesses. The restriction to children at term has the advantage of providing a group for which gestational age effects on weight should have been reduced; however, even within term infants, weight should have been adjusted for gestational age. The finding that 44% of methamphetamine-using women drank alcohol and 84% smoked make the weight findings difficult to interpret, although impairments in growth with amphetamines are biologically plausible. The behavioral findings are uninterpretable because there is no indication that the raters were masked to exposure history.

Utility (Adequacy) for CERHR Evaluation Process: This report is not useful in the evaluation process with the limitations noted.

Hansen et al. ***(131)***, supported by the University of California at Davis, performed visual-evoked potential measurements on eight infants with a history of prenatal stimulant exposure and eight control infants. Stimulant-exposed infants were recruited from a special clinic for prenatally drug-exposed infants and controls were selected from a well-baby clinic and matched for ethnicity. All children were born at 37–41 weeks gestation. Maternal drug history was ascertained by medical record review, interview, or urine toxicology screen at the time of delivery. Of the eight babies with prenatal stimulant exposure, four were exposed to cocaine, two were exposed to cocaine and amphetamines, and two were exposed only to amphetamines. Three mothers in the drug-exposed group and three mothers in the control group had used ethanol during pregnancy. Six drug-exposed and two control mothers smoked cigarettes. **[Results were presented as a group for stimulant-exposed infants and for controls without regard to which stimulant was used and without regard to cigarettes or ethanol use.]** The mothers using stimulants were significantly older (25.5 ± 5.1 years, mean ± SD) than control mothers (19.3 ± 3.3 years), and birth weight was significantly lower among drug-exposed infants than controls (3100 ± 454.9 g compared to 3600 ± 331.3 g, mean ± SD). Visual-evoked potentials to an alternating checkerboard pattern were recorded between 4 and 5 months of age. There was no difference between drug-exposed and control infants in the mean latency, amplitude, or duration of evoked waves. Visual recognition memory was tested between 6 and 12 months of age using the Fagan Test of Infant Intelligence. In this test, a picture was shown to the infant for a period of time to permit familiarization. The familiar picture was then paired on a screen with a novel picture. The proportion of time the infant spent looking at the novel picture was recorded. The full test used the mean of 10 such pairings to derive a novelty preference score. Children with low scores **[cut-off not given]** were considered to be "at risk." The mean ± SD Fagan score in the drug-exposed group (59.15 ± 6.2) was lower than the score in the control group (64.64 ± 3.6). Four children in the exposed group were considered at risk, compared to none in the control group ($P < 0.05$). Because the assessment of visual-evoked potentials showed no drug-associated deficits, the authors concluded that the impaired performance on the Fagan test may indicate cognitive impairment in drug-exposed children and could not be attributed to delayed maturation of visual pathways. The authors could not exclude altered visual processing or attention as explanations for the poorer performance on the Fagan test.

Strengths/Weaknesses: The strength of this study is the masking of the neurologist to exposure status. Weaknesses include the small size of the convenience sample, failure to match for gestational age, use of a one-tailed *P* value, and conflation of cocaine and amphetamines.

Utility (Adequacy) for CERHR Evaluation Process: This report is not useful for the evaluation process.

Boe et al. ***(132)*** presented an abstract from the University of California, Davis in which 774 pregnancies were identified as methamphetamine-exposed and 562,799 pregnancies were identified as unexposed. Pregnancy outcome information was linked to exposure information and relative risks were calculated. **[Exposure and outcome ascertainment were based on hospital discharge data and birth certificates; no other detail was provided.]** Statistically significant increases in relative risk were shown for associations between methamphetamine exposure and placental abruption, chorioamnionitis, premature rupture of the membranes, delivery before 36 weeks, transient tachypnea of the term newborn, respiratory distress syndrome at term, meconium aspiration, intraventricular hemorrhage, and neonatal death.

Strengths/Weaknesses: The strength of this report is the sample size. Weaknesses include the reliance on birth certificates for history of other exposures and failure to control for maternal demographic factors and amount of prenatal care. The most important weakness is absence of a full report **[confirmed with authors (Gilber WM, personal communication, October 26, 2004)]**.

Utility (Adequacy) for CERHR Evaluation Process: Abstracts are not carried forward into the evaluation process.

Felix et al. ***(133)*** presented an abstract from the California Teratogen Information Service in which follow-up was reported on 228 pregnant women who had abused amphetamines between the years 1979 and 1999. These women were compared to a group of 337 women who had only been exposed to low grade fever or other exposures not considered to be teratogenic. **[The abstract does not identify the source of these women; however, the Expert Panel assumes that these women telephoned the information service seeking advice about their exposures. No information was given on whether any of the amphetamine-exposed women used prescribed as opposed to illicit amphetamines.]** The authors reported a high rate of tobacco, alcohol, and other illicit drug use in the amphetamine groups as well as a 24% loss to follow-up in this group compared to 3% loss to follow-up in the control group.

There were 3 stillbirths (1.3%) in the exposed group and none in the control group. There was no difference in the incidence of major structural defects between the groups (amphetamine-exposed 5%, control group 3.4%). The defects in the amphetamine-exposed group included tracheal-esophageal fistula, pyloric stenosis, transposition of the great vessels, cystic adenomatoid malformation, vascular ring constricting the trachea, unilateral inguinal hernia, and multiple malformations associated with a chromosomal anomaly. Among the amphetamine-exposed children, there was a 21% incidence of minor anomalies compared with 11% in the control group. Of the 80 amphetamine-exposed infants examined blindly after 1 week of life, 5 (6.2%) had neurologic signs including abnormal tone and irritability. None of the control infants demonstrated neurologic abnormalities **[denominator not given for control group]**.

Strengths/Weaknesses: Strengths include use of a masked neurologic examiner and consideration of tobacco use. Weaknesses include failure to specify maternal ethnicity and lack of definition of minor malformations. The most important weakness is the absence of a full report **[confirmed with authors (Chambers C, personal communication June 24, 2004)]**.

Utility (Adequacy) In the CERHR Evaluation Process: Abstracts are not carried forward into the evaluation process.

3.1.2 Adverse Effects in Children

3.1.2.1 General Side Effects
Adverse effects of amphetamine preparations listed in the product labels are given in Section 2.2.1.1.

Greenberg et al. *(134)* supported by NIH, PHS, and Pfizer, randomized 61 of an initial group of 76 hyperactive African American children to an 8-week trial of placebo (n = 10), chlorpromazine, (n = 17), hydroxyzine (n = 17), or *d*-amphetamine (n = 17). The mean age of the children was 8.7 years (range 6.5–11 years), and the mean Wechsler Intelligence Scale for Children (WISC) full-scale IQ was 85. Side effects were assessed **[when and how assessed is not indicated]** and efficacy evaluations were undertaken. Side effects that were increased by *d*-amphetamine compared to placebo (at $P < 0.1$) included decreased appetite (76% compared to 20%), insomnia (53% compared to 10%), increased depression (49% compared to 10%), irritability (29% compared to 0), headache (41% compared to 10%), and stomachache (41% compared to 0). **[Fisher exact test performed by CERHR shows a significant difference at P ≤ 0.05 between *d*-amphetamine and control proportions for decreased appetite and insomnia.]** Palpitations and elevated blood pressure did not occur more often with *d*-amphetamine than placebo. Mean WISC IQ increased with *d*-amphetamine therapy to 96.2, while there was no change in the placebo group. The individual dose of *d*-amphetamine was adjusted based on each child's response; the mean daily dose was 25 mg. The authors expressed surprise that depression worsened in more children in the *d*-amphetamine group than the control group (*d*-amphetamine 8/17, control 1/10 **[*P* = 0.09, Fisher Exact test by CERHR]**). **[The Expert Panel notes that side effects appeared dose-dependent.]**

Strengths/Weaknesses: Strengths of this study include double-blinding, random assignment, use of multiple raters, and the 8-week time period. Issues of dose were considered, but the sample is too small for ready interpretation. The sample was limited to a single ethnicity, African American, which is underrepresented in other samples. Results were valid and consistent with those of other studies. Use of a control psychoactive substance (chlorpromazine) that increases appetite and weight gain, made the *d*-amphetamine effect more dramatic. A weakness is the low pretreatment IQ of subjects. In addition, the study was conducted in 1972 and thus used what are now considered antiquated forms of psychiatric diagnoses (e.g., "neurosis"). It is therefore also unclear if any of the children in the "hyperactive" group, including the one who became psychotic, was in fact suffering from juvenile bipolar disorder, a diagnosis that did not exist at that period.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited-to-moderate adequacy for the evaluation process.

Efron et al. *(135)*, supported by a hospital research fund, randomized 114 boys and 11 girls with ADHD to a 2-week trial of *d*-amphetamine or methylphenidate, followed by a 24-hour wash-out period, followed by a 2-week trial of the other stimulant. The mean age of the subjects was 104.8 months (range 60–179 months). The *d*-amphetamine dose was 0.15 mg/kg bw/dose and the methylphenidate dose was 0.30 mg/kg bw/dose, both given twice/day, rounded to the nearest tablet size. Investigators, subjects, teachers, and family members were blinded to the identity of the medication. Evaluations included effectiveness endpoints **[not discussed here]** and side effects, derived from a behavior questionnaire completed by parents at the end of each 2-week treatment period. A list of 17 common side effects was presented on the questionnaire with a ranking scale for the evaluation of severity ranging from 0 (not present) to 9 (severe). Side effects were evaluated with regard to whether they were present or absent at baseline and on treatment and with regard to mean of the severity ranks. Trouble sleeping and poor appetite were more commonly present after 2 weeks of *d*-amphetamine than at baseline (poor sleeping: 54% at baseline and 70% on *d*-amphetamine; poor appetite: 34% at baseline, 69% on *d*-amphetamine). Fingernail biting and unusual happiness were reported to be more common at

baseline than on *d*-amphetamine therapy (fingernail biting: 50% at baseline, 40% on *d*-amphetamine; unusual happiness 42% at baseline, 26% on *d*-amphetamine). The remainder of the symptoms were identified as present in similar proportions of children at baseline and on *d*-amphetamine. The authors concluded that many side effects identified on stimulant medication may be side effects associated with the underlying disorder rather than due to the medication therapy.

Strengths/Weaknesses: A strength of this study is the double-blind crossover design, with children used as their own controls. The pre-medication period served as the non-stimulant comparison and *d*-amphetamine and methylphenidate were compared to each other. Other strengths include use of DSM-IV criteria to diagnose ADHD, as well as a standardized child behavioral measure. Side effects were rated not only in a yes/no manner but also by level of severity. Statistical methods were clearly specified. A weakness, as noted by study authors, is potential bias introduced by parental reporting. For example, many of the potential "side effects" (behaviors on the Side Effects Rating Scale) decreased significantly while on one or both stimulants, possibly indicating that parents could have been "motivated" to have their child get better on the trial. Also noted by study authors is that the moderate doses administered twice daily in this study cannot be generalized to regimens of different dose levels and frequencies. Another weakness is that each phase of drug exposure was 2 weeks followed by "wash-out" and then exposure to the next drug for 2 weeks. It would have been better to test one drug for a longer period of time. In addition, children were of a wide age range, spanning from preschool to post-pubertal (5–15 years). Because the study was done in Australia, the children may have been facing somewhat different environmental challenges than those in the United States.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for use in the evaluation process.

Hemmer et al. *(136)*, supported by the Crown Family, performed EEGs on 179 males (3–20 years old) and 55 females (3–19 years old) with ADHD. The mean age of the subjects was 9–10 years. Epileptiform EEGs were obtained in 36 subjects prior to stimulant therapy or up to 8 weeks after the initiation of therapy. The decision to accept or decline stimulant therapy was made by parents and did not appear to be influenced by the EEG results. There were 175 subjects treated with stimulants of whom 30 (17%) had had an epileptiform EEG. Of the 29 subjects who declined stimulant therapy, 6 (21%) had had an epileptiform EEG. Seizures occurred in four subjects **[follow-up period not specified]**. All of the subjects with seizures were in the stimulant group, although one child had a seizure after being off stimulant medication for 2 months. Three of the four children with seizures had prior epileptiform EEGs. The authors concluded that a normal EEG prior to stimulant therapy was reassuring that seizures would not occur on therapy. They were not convinced that the stimulant therapy was the cause of the seizures that occurred based on the timing of the seizures with respect to the start of stimulant therapy, and based on the low overall incidence of seizures (2%) in the stimulant-treated population. **[The specific stimulants were not named except in the four cases of seizure. The stimulants used in these cases included methylphenidate and *d*-amphetamine.]**

Strengths/Weaknesses: Strengths include use of DSM-III and IV diagnoses as well as parent and teacher reports. However, several weaknesses preclude this study from determining if stimulants are associated with seizures in children with epileptiform EEGs. Authors state the obvious—if the EEG is normal, the child is at low risk for a seizure; if the EEG is abnormal, the child with ADHD is at considerable risk for the eventual occurrence of a seizure—without attributing the effect to medication. The EEGs were performed anywhere from before to 8 weeks after initiation of stimulant therapy, thus making it difficult to ascertain whether the EEG findings in fact reflected neurological issues preceding the treatment. Major weaknesses also include lack of information regarding masking of EEG readers, current treatment of child, and family history of epilepsy. The incidence of epileptiform EEGs in this

study (15.4%) is much higher than the estimated incidence of EEG abnormalities in an unselected population of children (2%).

Utility (Adequacy) for CERHR Evaluation Process: This study is of marginal adequacy for the evaluation process.

3.1.2.2 Onset or Worsening of Tics

Tourette disorder is a chronic neurologic disorder characterized by repeated and involuntary body movements (tics) and uncontrollable vocal sounds. Tics can include eye blinking, repeated throat clearing or sniffing, arm thrusting, kicking movements, shoulder shrugging, or jumping. A large proportion of children with Tourette disorder have comorbid ADHD (reviewed by Leckman *(137)*). In 1974, a case report was published describing a 9-year-old boy treated with methylphenidate for hyperactivity who developed Tourette disorder on therapy *(138)* and since that report, additional papers have described tics or Tourette disorder in association with stimulant therapy (Table 31). There is only one controlled study of amphetamine exposure and development of tics *(135)*.

Table 31. Reports of Tics in Children Treated with Stimulant Medication

Medication	Stimulant dose (mg/day except where indicated)	Characteristics of the children	Outcome	Comments[a]	Reference
Methylphenidate, *d*-amphetamine, methamphetamine	Not reported	32 Tourette disorder patients who had been exposed to stimulants (unspecified ages)	17/32 experienced worsened symptoms when on stimulants	This report is descriptive, with no doses or ages given. Other medications in the mix were not considered. Omitted from consideration were 39 of 45 subjects with pre-existing Tourette disorder that did not worsen.	*(139)*
d-Amphetamine, methylphenidate *d*-amphetamine/ pemoline methylphenidate/ pemoline	Not reported	4 boys, 8–11 years old	Tourette disorder developed and continued after medication	This paper indicates the independence of Tourette disorder from medication, but includes only 4 children who used 3 medications alone and in combination with no indication of dose.	*(140)*
Methylphenidate, *d*-amphetamine, pemoline	Not reported	200 children, 48 with Tourette disorder	8/48 tics worsened	Strengths: Inclusion of a fairly homogeneous population of 48 children with pre-existing Tourette disorder. Weaknesses: Retrospective review, dose range not given; there was no independent diagnosis; follow-up duration was variable.	*(141)*
Methylphenidate, pemoline, *d*-amphetamine	Not reported	170 twins and individuals	50% with worsening of tics, some developed tics	Strength: Twin study (In 6 monozygotic twins, discordant for medications; other twin developed Tourette disorder suggesting genetic basis). Weaknesses: Doses were not given; Tourette disorder was pre-existing; the relationship of tic worsening to therapy was vague.	*(142)*
Methylphenidate, *d*-amphetamine	Up to 90 mg/day methylphenidate; up to 45 mg/day *d*-amphetamine	45 hyperactive boys age 6–12 years	10/45 had increase in tics or development of tics only on methylphenidate; 6/45 only on *d*-amphetamine, and 11 on both	Strengths: Compared methylphenidate and *d*-amphetamine; high doses used. Weakness: Tics were not clearly distinguished from other behavioral changes and disorders (e.g., obsessive-compulsive disorder) that developed over the course of the study.	*(143)*
Methylphenidate,	Methylphenidate	122 children	Tic/dyskinesias occurred in	Strengths: Large study with standarized	*(144)*

Medication	Stimulant dose (mg/day except where indicated)	Characteristics of the children	Outcome	Comments[a]	Reference
d-amphetamine, pemoline	dose (mean ± SD): 21.1 ± 11.7 with tics, 24.4 ± 17.2 without tics; *d*-amphetamine dose (mean ± SD): 14.2 ± 5.2 with tics, 15.8 ± 6.8 without tics	with ADHD age 3.6–15.8 years	8.2% of children treated with medication	diagnosis of ADHD (DSM-IIIR). Weaknesses include retrospective design, unknown weight-adjusted doses, and the reliance on chart review for parental assessments of unusual tic-like movements such as "eye bugging." Prior medication history not detailed.	
Methylphenidate, *d*-amphetamine	Methylphenidate 35–90; *d*-amphetamine 10–45	20 children, mean age ± SD 9.4 ± 2.0 years	Reversible increase in tics, particularly with *d*-amphetamine. Methylphenidate-associated tics returned to placebo baseline with continued methylphenidate treatment.	Strengths: Two drugs were compared; wide range of doses; double blind. Weakness: Small sample size	*(145)*
Methylphenidate, *d*-amphetamine	Methylphenidate 0.6 mg/kg bw/day, *d*-amphetamine 0.3 mg/kg bw/day	125 children with ADHD, mean age ± SD 104.8 ± 27.6 months; range 60–179 months (5–15 years)	Tics or "nervous movements" present in 35% of subjects at baseline and 26–28% of subjects after 2 weeks on either drug	Strengths: Moderately high doses of two drugs were evaluated. Used Barkley scale as in *(146)* Weaknesses: Lack of placebo control; the item scored was "tics or nervous movements," (in this case the rating scale may overestimate prevalence of tics at baseline, confounding analysis); the duration of the study was only 2 weeks on each drug; no notation of whether subjects were "drug naïve."	*(135)*
Methylphenidate, *d*-amphetamine, pemoline	Not reported	374 children on methylphenidate, 126 children on *d*-amphetamine, 13 on pemoline	Tics present in 7.8% of children; not more frequent in any medication category	Strength: Comparison of 3 medications. Weakness: Retrospective chart review.	*(147)*

[a]None of the studies screened for substance abuse.

Strengths/Weaknesses: The overall data set is weak. The one controlled study *(135*) has serious weaknesses (see Table 31).

Utility (Adequacy) for CERHR Evaluation Process: The data set is not useful in the evaluation process.

3.1.2.3 Substance Abuse Disorder

Biederman et al. *(148*), in a study supported by the NIMH and NIDA, evaluated the risk of substance abuse disorders associated with psychotropic medication for treatment of ADHD. Data were obtained and reanalyzed from an ADHD longitudinal genetics study conducted in 260 families. Females were not evaluated because most medicated subjects were male and subjects younger than 15 were excluded due to significantly younger age of the medicated versus unmedicated subjects. Subject groups consisted of Caucasion males who were ≥15 old years and had previously received medication for ADHD (n = 56), had ADHD but were not medicated (n = 19), or did not have ADHD (n = 137). The average duration of treatment was 4.4 years. **[The types of medications used were not specified.]** Multiple logistic regression was used to correct confounding by age, socioeconomic status, lifetime risk of conduct disorder, and substance use disorders in parents. Substance use disorders were examined for alcohol, marijuana, hallucinogens, cocaine/stimulants, and tobacco. ADHD subjects who had been medicated had a significantly reduced risk of any substance use disorder compared to unmedicated subjects with ADHD (OR 0.15, 95% CI 0.04–0.6). Unmedicated subjects with ADHD had a significantly increased risk of any substance abuse disorder compared to controls without ADHD (OR 6.3, 95% CI 1.8–21.4). With the exception of tobacco use, the medicated group had reduced risk of all other individual substance use disorders compared to the unmedicated ADHD group, but the sample size was too small to evaluate statistical significance for individual substances. The study authors concluded that pharmacotherapy is associated with an 85% reduction in risk for substance abuse disorders in youths with ADHD.

Strengths/Weaknesses: Strengths of this study include well articulated competing hypotheses and longitudinal design, as well as masked assessment and careful definition of the sample restricted to white males older than 15 years. Authors pay considerable attention to quality control and structured DSM-IIIR interviews were used to establish the diagnosis of substance use disorders (because of small numbers, abuse and dependence were analyzed as a single category as substance abuse disorder). Important variables including comparisons of treated and untreated ADHD children were considered in analyses. The study addresses, implicitly, the issue of self-medication by determining that treated groups had a diminished odds ratio of substance abuse. Other strengths include control of parental substance use disorder and presentation of outcomes as an aggregate of any substance use disorder and disaggregated by substance. Limitations include use of an exclusively tertiary-referred white sample, so findings may not apply to less privileged or lower income risk groups. Other weaknesses include lack of specification of the drugs that the treated members of the cohort received. The authors correctly identified lack of power as diminishing confidence in null findings. Other weaknesses are that the age ranges of subjects and the time period they had been off medication were not specified.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation process.

Barkley et al. *(149*) (also reported in **Fischer and Barkley *(150*)**), in a study supported by the NIMH, examined possible associations between stimulant medication therapy during childhood or adolescence and substance use during adolescence and young adulthood. During each evaluation period, subjects or their parents were asked about stimulant therapy, behavior, mental

health, illicit drug use, and education history; psychological tests were conducted and subjects were rated according to scales. Groups consisted of 91% males and 9% females. Racial distribution was 94% white, 5% black, and 1% Hispanic. Subject ages were 12–20 years at adolescent evaluation and 19–25 years at adult evaluation. During the adolescent evaluation, 119 hyperactive subjects were available for interview and parents were questioned about stimulant therapy during childhood. Ninety-eight were treated with stimulants during childhood, 21 were not. Percentages treated with each type of stimulant during childhood were 80% methylphenidate, 3% *d*-amphetamine, and 20% pemoline. Some subjects received more than 1 type of stimulant; *d*-amphetamine was given to 2% and pemoline to 22% of the children in the methylphenidate group. All children in the pemoline group had also received *d*-amphetamine. Mean durations of treatment during childhood were 44.8 months for methylphenidate, 32.8 months for amphetamine, 13.3 months for pemoline, and 40.2 months for stimulants in general. During the adult evaluation, 147 hyperactive subjects were questioned about stimulant treatment during high school, but were not asked to identify the specific stimulant medication taken. Thirty-two subjects were treated with stimulants and 115 were not treated with stimulants during high school. Mean duration of stimulant treatment during high school was 26.6 months. Seven of the subjects were receiving stimulant treatment at the time of the interview. **[Severity of ADHD symptoms and conduct disorders were the only potentially confounding factors considered.]**

At the adolescent evaluation, subjects were asked if they had ever tried cigarettes, alcohol, marijuana, hashish, cocaine, heroin, hallucinogens, unprescribed stimulants, sedatives, or tranquilizers. **[Information about frequency of use was not obtained and substance abuse/dependency was not considered.]** The proportions of hyperactive subjects who had ever tried any of the substances were similar in the stimulant-treated and untreated groups by chi-square analysis. No significant differences were found when all stimulants (cocaine, amphetamines) were combined or when duration of stimulant therapy was considered.

Subjects were questioned in adulthood about their use of alcohol, marijuana, cocaine, amphetamines/speed, any stimulant, hallucinogens, narcotics, sedatives, or other drugs. Frequencies of substance use were log transformed, due to high standard deviations, and compared by ANOVA. Stimulant treatment in childhood did not significantly increase the frequency of any type of substance use in early adulthood. The frequency of cocaine use was significantly higher ($P = 0.043$) in subjects who were treated with stimulant medications in high school, but the results were no longer significant when corrected for severity of ADHD and conduct disorder. **[Table 3 of the study, which presents effects of high school stimulant treatment, lists group numbers for childhood treatment (n = 21 untreated, 98 treated) instead of high school treatment (n = 115 treated, 32 untreated).]** The proportion of subjects who ever used each of the substances was analyzed by chi-square. If statistically significant findings were observed, a binary logistic analysis was conducted to adjust for severity of ADHD symptoms and conduct disorders. A greater percentage of adults who were treated with stimulants in childhood and in high school used cocaine at least 1 time (5% untreated compared to 26% treated in childhood, $P = 0.037$ and 20% untreated compared to 40% treated in high school, $P = 0.016$). Due to increased cocaine use, the use of any stimulant was also increased in adults treated during high school (25% in untreated compared to 47% in treated, $P = 0.018$). Additional analyses indicated that risk of cocaine use was primarily mediated by severity of conduct disorder and not by use of stimulant medication. Increased duration of stimulant treatment was not found to adversely affect risk of substance use. No significant differences in adult substance abuse/dependence rates (diagnosed by DSM-III-R criteria) were noted in hyperactive subjects who were or were not treated with stimulants in childhood or during high school. **[There was no statistical analysis for abuse/dependency in adults.]** The study authors concluded that there was no compelling evidence that stimulant treatment of children or adolescents with ADHD leads to increased risk of substance experimentation, use, dependence, or abuse by adulthood.

Strengths/Weaknesses: A strength of this study is that substance abuse was defined by DSM-IIIR criteria. This study considered not only substance use but also frequency/quantity and distinguished experimentation from problem use. Initiation and experimentation did not differ by stimulant medication exposure status. Another strength is consideration of duration of treatment, with considerable detail provided on length of time subjects received different medications. Two time frames of stimulant medication use and drug use were examined; uniquely, drug use was examined while a few subjects were still receiving medication. Importantly, the study showed that cocaine use was related to adolescent treatment, but that this relationship was lost when severity of ADHD was statistically controlled; this finding emphasizes the need for such control in other studies. In addition to the paucity of control variables (including family history), a major weakness noted by authors on page 100 of the *Pediatrics* article is that the assessor was not masked to stimulant exposure history. It is both a strength and a weakness that the authors specify the medications to which the children were exposed, but because of small cell sizes and a predominance of methylphenidate, stimulants were only evaluated as a single generic exposure. However, the authors did use standard instruments. Weaknesses include the fact that tobacco use was not adequately evaluated. The authors were correct in noting that it is difficult to ascertain whether the weak association between high school stimulant treatment and cocaine use was an artifact of multiple comparisons. However, another conceptual weakness they did not consider is that perhaps children who are more deviant and therefore, with or without treatment, more prone to substance use disorders are more likely to continue to be treated into high school. Though important, it was not stated whether subjects treated in high school received stimulants at both ages, especially as findings were mediated by severity of ADHD. The authors themselves point out that their study design did not permit them to identify the temporal sequences of conduct disorder and substance use disorder, leading to difficulties in interpretation particularly of this worrisome finding of possible connection of stimulant treatment to cocaine use.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the evaluation process.

Lambert and Hartsough *(151)* and Lambert *(152)*, supported by the Tobacco-Related Disease Research Program, examined the effects of ADHD and stimulant treatment on tobacco use and substance dependency in a longitudinal sample of 492 adults. According to information provided in the Lambert study *(152)*, subjects were born in the San Francisco area between 1962 and 1968. About 22% of the subjects were female and 23% represented ethnic minority groups. The authors reported that among subjects using stimulant medications, 69% used only methylphenidate, 16% used combinations of methylphenidate and other stimulants, and 15% used other CNS stimulants (amphetamines, pemoline). At various stages throughout their lives, the subjects were questioned about their use of tobacco, alcohol, marijuana, stimulants, and cocaine. A total of 399 subjects were said to be available for interview. **[These studies appear to have numerous discrepancies or mathematical errors in text compared to tables or in different parts of tables. In adding numbers presented in some study tables, it appears that either mathematical errors were made or more than 399 subjects were evaluated for some endpoints (i.e., Table 3 in Lambert and Hartsough *(151)*). In other cases, fewer than 399 subjects were included in analyses and it is not clear if or why some subjects were excluded (Table 5 in Lambert and Hartsough *(151)*).]**

In the Lambert and Hartsough study *(151)* subjects were placed into hyperactive or control groups. According to information presented in Lambert *(152)*, there were 217 hyperactive subjects (136 with primary hyperactivity with no causal explanation, 31 with secondary hyperactivity possibly due to organic factors, and 50 with untreated hyperactivity). There were 182 controls (141 age controls and 41 with non-ADHD behavioral problems). Information in

Lambert *(152)* indicates that only 80% of the primary hyperactive group and 66% of the secondary hyperactive group received stimulant treatment. **[It is not clear why untreated subjects in the primary and secondary hyperactive group were not put into the untreated hyperactive control group.]** It appears that about 3% of controls received stimulant treatment. Subgroups of individuals were grouped together based on similarity of health, familial, educational, and social background factors. **[There was no discussion of adjustment for additional confounding factors such as severity of ADHD.]** The rate of smoking in adults who had ADHD as children and who never used stimulant medication (n = 47) was 37.0%; for adults who had used stimulant medication for up to 1 year (n = 28), the rate of smoking was 22.0%; and for adults who had used stimulant medication for ≥1 year (n = 52), the rate of smoking was 40.9% ($P < 0.03$ for never-used compared to use ≥ 1 year, by chi-square). The Mantel-Haenszel test for linear trend was also significant for duration of stimulant use ($P < 0.01$). Significant linear trends ($P < 0.03$) were noted for rates of tobacco dependency in adults who had ADHD as children and who never used stimulant medication (n = 81; 32.1% rate) or had used stimulant medication for up to 1 year (n = 9; 38.5% rate) or ≥1 year (n = 84; 48.8% rate). Significant linear trends ($P < 0.05$) were also noted for cocaine dependency in adults who had ADHD as children and who never used stimulant medication (15.0%) or had used stimulant medication for up to 1 year (17.9%) or ≥1 year (27.4%). **[The text states that statistical significance by chi-square was obtained for cocaine dependency, but the legend of Table 7 in the study indicates that results of chi-square analyses were not significant for either tobacco or cocaine dependency.]** A comparison of subjects who had ADHD as children with subjects who did not have ADHD as children showed that subjects with ADHD began smoking regularly at a younger age, had a higher rate of smoking as adults, and had higher cocaine dependency rates. The study authors concluded that there is a possible link between stimulant medication and rates of smoking and tobacco and cocaine dependency in adulthood.

In the Lambert study *(152)*, subjects were divided into groups of 268 who received no CNS stimulant treatment and a group of 131 who received stimulant treatment. **[According to Table 18.2 in the paper, the group with no stimulant treatment was comprised of 162 subjects without ADHD and 106 with ADHD (41 severe, 25 moderate, and 40 mild). The stimulant treatment group was comprised of 10 subjects without ADHD and 121 subjects with ADHD (62 severe, 48 moderate, and 11 mild.]** The percentage of subjects who had not yet become regular smokers was significantly higher ($P \leq 0.05$ by Lee Desu statistic) in the untreated group (~60%) compared to the treated group (45%). The same subjects were evaluated according to the age when stimulant treatment was ended: age 10, age 11–13, or after age 14. Stimulant treatment appeared to protect against smoking during childhood. However in adulthood, smoking rates were significantly higher ($P < 0.001$ by chi-square) in treated groups (41%) compared to the untreated group (19%). Adjusted ORs were calculated. **[The confounding factors considered in the analyses are not clearly identified, but it appears that childhood conduct disorders were considered in addition to socioeconomic status, cognitive ability, and ethnicity. It is not clear how many subjects were included and how the subjects were classified in calculating the ORs. It is assumed that, as in previous analyses, subjects with and without ADHD were collapsed into the same groups based on stimulant exposure.]** In the group treated with stimulants for more than 1 year, ORs were described as significant for daily smoking (2.817) and cocaine dependency (2.251) in adulthood. In subjects exposed for less than 1 year, a significant OR (3.951) was obtained for daily smoking in adulthood **[95% CIs were not given]**. ADHD severity was found to be significantly related to tobacco, cocaine, and stimulant dependency in adulthood.

Strengths/Weaknesses: A strength of these studies is the emphasis on cigarette consumption, which possibly indicated self-medication, as higher rates of smoking were found in untreated ADHD subjects. However, the inconsistencies in sample sizes and inaccuracies in study tables

make conclusions tenuous. Other weaknesses include the very inadequate description of sample in terms of ethnicity, social class, parental substance use, severity of ADHD, and many other potential confounds. In addition, the authors tended to make sweeping conclusions on the basis of univariate analyses. All of these weaknesses make interpretation of reported findings problematic.

Utility (Adequacy) for CERHR Evaluation Process: These studies are not useful for the evaluation process.

Paternite et al. *(153)* and Loney et al. *(154)*, from the same group, examined the effects of stimulant medication in childhood on substance use in adulthood. One of the reports *(153)*, partially supported by the NIMH, indicates that the medicated subjects were treated with methylphenidate, but the other report *(154)* refers generically to "CNS stimulants such as [methylphenidate]." **[The Expert Panel recognizes in reviewing these reports that few, if any, of the subjects may have been treated with amphetamines.]** Subjects were selected from 219 **[listed as 285 in 1 study, but this figure appears to be an error]** boys (98% white) who were referred to the University of Iowa child psychiatric clinic at 4–12 years of age. Boys were diagnosed as having hyperkinetic reaction (70%) or minimal brain dysfunction (30%). By more current standards, ~70% of the boys would have been diagnosed with ADHD and the term ADHD is used in the later paper for convenience. Aggressiveness was noted in 7% of the boys who would have likely received a diagnosis of oppositional defiant disorder according to more recent terminology. Based on treatment preferences of 3 different physicians, 182 of the boys received stimulant medication and 37 were not given medication. At follow-up during adulthood (21–23 years old), 97 of 121 subjects medicated with methylphenidate in childhood were available for evaluation. **[It appears that the 121 medicated subjects were selected from the group of 182 medicated subjects. The number of untreated subjects available for evaluation in adulthood was not specified.]** The medicated subjects were treated between 1967 and 1972 at a mean age of 8.8 years. Mean methylphenidate dose was 32 mg/day (range 8–80 mg/day) and mean duration of treatment was ~30 months **[reported as 30.4 and 36 months in the 2 papers]** with a range of 1–76 months. **[It was not stated how many untreated subjects were included in analyses.]**

In the Paternite et al. study *(153)*, regression analyses were conducted to determine associations between methylphenidate dose, response, or treatment duration and alcoholism, drug abuse disorder, psychiatric conditions, and measurements of social function and IQ. Child age, symptom dimensions, and the two other medication variables were held constant in each analysis. Neither alcoholism nor drug abuse disorders were significantly associated with methylphenidate treatment, although the authors concluded that there was a trend between increased dose and fewer diagnoses of alcoholism ($r = –0.2$, $P < 0.10$). **[Most data were not shown; only values approaching or reaching statistical significance were listed in tables.]** The only negative finding related to methylphenidate treatment was an association between better response to treatment and reduced likelihood of high school graduation ($r = –0.34$, $P < 0.01$). Additional findings included associations between increased dosage and reduced suicide attempts; better medication response with improved psychiatric outcomes and social functioning; and longer treatment duration with improved psychiatric outcomes, higher IQ, and better reading scores. Significant associations or trends were noted between inattention-overactivity and unemployment and adverse outcomes on some psychiatric or behavioral measures. Associations or trends noted for aggression were drug abuse disorder, antisocial personality disorder, and adverse outcomes on some psychiatric or behavioral measurements. **[The Expert Panel notes that a number of unique positive associations with medication were noted (e.g., reduced suicide attempts). Only one adverse significant association with medication was reported and it is surprising: "better response to treatment and reduced likelihood of high school graduation."]**

In the study by Loney et al. *(154*), rates of involvement (experimentation, continuation, or escalation of use) with alcohol, tobacco, barbiturates, tranquilizers, stimulants, marijuana, glue, cocaine, LSD, and opioids were compared between ADHD subjects who either were or were not treated with methylphenidate. The analyses controlled for year of birth and inattention, overactivity, or aggressive defiance symptoms. In unmedicated compared to medicated subjects, adult involvement was significantly increased ($P < 0.05$) for tobacco, stimulants, glue, and opioids. **[The unit on the Y axis of involvement graphs (Figures 17.1 and 17.2 of the study) is not specified and it is not clear what kind of analysis was conducted.]** According to the study authors' interpretation of the data, medicated subjects progressed less far along the path from experimentation to continued use. Significantly fewer ($P < 0.05$) medicated versus unmedicated subjects (respective percentages) had experimented with glue (~22 vs. 38%), stimulants (38 vs. 58%), LSD (~30 vs. 49%), and opioids (~23 vs. 42%). Medicated versus unmedicated subjects (respective percentages) had significantly lower rates of alcoholism (27 vs. 56%, $P = 0.002$) and antisocial personality disorder (24 vs. 44%, $P = 0.004$). Drug abuse rates were similar between the two groups (17 vs. 19%). Loney et al. *(154*) concluded that their studies did not indicate a negative effect of childhood methylphenidate treatment on future drug use, but suggested that further research is needed. **[The Expert Panel notes evidence of self-medication, as non-treated subjects were more likely to be 'involved' with tobacco and stimulants.]**

Strengths/Weaknesses: Strengths include a relatively lucid exposition of the technical problems in this field and an ethnically homogenous sample that consisted of all preadolescent subjects at the time of intake. Other strengths were that both treated and untreated subjects had ADHD and that inattention/hyperactivity and aggression were explored separately. In the Paternite et al. study, *(153*), regression analyses were applied to consider many putative associations, including some that were unique (e.g., social function). Weaknesses include the need to reclassify now outdated clinical measures to fit modern criteria and the use of other outdated measures for outcomes, as well as lack of consideration of family risk factors, both genetic and environmental. The small size of the unmedicated subgroup (n=37) would tend to bias the evaluation against finding a negative effect in the unmedicated group. It is not clear how the follow-up medicated subjects were selected or how many untreated subjects were followed to adulthood. For example, in these two articles that are considered together, authors fail to describe clearly how an initial sample of 182 treated subjects became 121 and then 97.

Some weaknesses in the interpretation of the Loney et al. *(154*) study were noted. The main finding was that medicated subjects were less likely to go from 'experimentation to continued use' (terms not defined). Drug abuse (not defined) was reported to be similar among treated and non-treated groups, but medicated subjects were less likely to 'experiment' with most drugs. Therefore, the conclusion that drug abuse rates are not impacted by medication is problematic. As fewer medicated subjects experimented, it appears that the proportion of medication subjects who experimented and went on to continuous drug use was higher than the proportion of non-medicated subjects. A statistical control is needed for this finding.

Utility (Adequacy) for CERHR Evaluation Process: The Paternite et al. *(153*) study is of limited utility; the Loney et al. *(154*) study is not useful for the evaluation process.

Wilens et al. *(155*) conducted a meta-analysis of studies examining possible associations between long-term medication for treatment of ADHD and substance use disorders. The studies reviewed in the analysis are listed in Table 32 and included published reports identified in a PubMed search, data presented at scientific meetings, and unpublished findings. **[Published studies are reviewed in detail above.]** Included in the analysis were prospective studies examining subjects during adolescence (*(148*); Molina and Pelham 1999 abstract cited in *(155*)) and young adulthood *(151, 153, 154*). One retrospective study examined subjects during adulthood (Huss 1999 abstract

cited in *(155)*). A total of 674 medicated and 360 unmedicated subjects with ADHD were included in the meta-analysis. The analysis did not examine nicotine use. ORs for drug and alcohol substance abuse disorders are listed in Table 32. An OR > 1 indicates a protective effect of medication, while an OR < 1 indicates an adverse effect of medication. **[According to the ORs and CIs listed in Table 32, none of the studies demonstrated a significant adverse effect of medication.]** The pooled OR of 1.9 (95% CI 1.1–3.6) was consistent with a nearly 2-fold reduction in risk of substance abuse disorders in youths medicated versus unmedicated for treatment of ADHD. Additional analyses indicated that no one study heavily influenced outcome. Studies that controlled for baseline severity of ADHD were found to have larger ORs **[statement not consistently supported by drug data in Table 32]**. A greater protective effect of medication was found in studies examining adolescent (OR 5.8) verus adult subjects (OR 1.4) **[95% CIs were not presented]**. The study authors concluded that results suggested an association between stimulant treatment in childhood and reduced risk of subsequent drug and alcohol disorders.

Strengths/Weaknesses: This paper reviewed numerous studies, some of which were not published. Strengths of this study include statistical analyses (albeit of data of heterogeneous quality and composition) and care in checking that no one study heavily influenced the combined estimates, as well as attention to publication bias. Other strengths were largely conceptual. The authors raised an important issue about baseline severity of ADHD in moderating impact of stimulant treatment; unfortunately, part of this analysis was based on unpublished observations (Barkley et al.). Another interesting point is that children from families with a history of substance may be more resistant to stimulant treatment on the one hand. On the other hand children with more severe oppositional and aggressive disorders (and thus at greater risk of later substance use disorder whether treated or untreated) are more likely to receive stimulant treatment than children at lower baseline risk. It can either be regarded as a strength or a weakness that samples were heterogeneous in the age of follow-up with two examining adolescents who were presumably quite early in the substance use disorder trajectory and the remainder examining adults. Another weakness is that the studies reviewed used differing measures of varying validity to document substance use disorder. Problems also include conflation of prospective and retrospective studies and exclusion of cigarette/tobacco use as an outcome when it was a primary outcome of a limited study that found an adverse effect of childhood stimulant treatment *(151)*.

Utility (Adequacy) for CERHR Evaluation Process: This study is of marginal utility for the evaluation process.

Table 32. Meta-Analyses for Studies Examining Substance Abuse in Subjects Who Were or Were Not Medicated for ADHD

Reference	Similar baseline severity?	Number of ADHD subjects		ORs (95% CI)	
		Medicated	Unmedicated	Drugs	Alcohol
Lambert and Hartsough, 1998 *(151)*	No	93	81	0.47 (0.22–1.0)	0.6 (0.32–1.1)
Biederman et al., 1999 *(148)*	Yes	145[b]	45[b]	3.9 (1.8–8.1)	8.1 (3.9–17.2)
Huss, 1999 abstract cited in *(155)*	No	98	21	2.2 (0.99–5.1)	No data
Loney et al., 2002 *(154)*	Yes	182	37	1.1 (0.46–2.8)	3.6 (1.7–7.4)
Molina and Pelham, 1999 abstract cited in *(155)*	Yes	53	73	4.6 (1.5–14.5)	6.6 (1.4–30.2)
Barkley unpublished data cited in *(155)*[a]	Yes	NS	NS	0.83 (0.29–2.3)	0.98 (0.36–2.7)

[a]This study may have been published later as *(149)*.
[b]According to CERHR review of this study, there were 56 medicated and 19 unmedicated subjects with ADHD.
From *(155)*.
NS=not specified

As noted above, some studies examining the effects of ADHD medications also found associations between ADHD and/or conduct disorders and substance use *(151, 153, 156)*. Numerous studies examined possible associations between ADHD, independent of treatment, and substance abuse **[not considered here]**. A review by Wilens *(157)* concluded, "There is a robust literature supporting a relationship between ADHD and SUD [substance use disorders]. Noncomorbid ADHD appears to confer an intermediate risk factor for SUD, although conduct and bipolar disorder appear to heighten the risk of early onset of SUD. Both family-genetic and self medication influences appear to be operational in the development and continuation of SUD in ADHD subjects."

[The Expert Panel noted that in general the studies examining substance use disorders are complicated by the well known association in pedigrees of substance use disorders, ADHD, and other psychiatric disorders and by the studies' varying sophistication in measuring true substance use disorder compared to simple experimentation or initiation. A weakness of all the studies is the use of self-report only to measure substance use without any confirmation by biologic markers such as urine or hair, which might enhance accurate identification of users.]

3.1.2.4 Effects on Height and Weight

Aarskog et al. *(158)* examined the acute effects of *d*-amphetamine treatment on growth hormone levels in children before and after 6–8 months of treatment with 5–35 mg/day methylphenidate. Mean ±SEM serum growth hormone levels in 7 children given 15 mg *d*-amphetamine were

3.1±0.9 ng/mL at baseline and 14.1±5.0 ng/mL at peak prior to methylphenidate treatment and 8.6±1.5 and 10.0±3.6 ng/mL following methylphenidate treatment. The mean time for achieving maximum growth hormone levels following *d*-amphetamine administration was 60 minutes before methylphenidate treatment and 90 minutes after treatment.

Studies on growth in children treated with amphetamines are summarized in Table 33. The reviewed studies investigated amphetamine alone, compared amphetamine to a control group, compared amphetamine and methylphenidate, or compared amphetamine to another medication. Some of these studies lacked information on the magnitude and timing of effects, thereby contributing to inconsistent results. There are several methodologic problems and limitations in all of the studies making it difficult to compare results among studies and determine definitive conclusions. Some of the limitations and problems include:

- Imprecise height measurements with measurement errors comparable in magnitude to the effect being studied.
- Use of cross-sectional rather than longitudinal analysis of growth data.
- Height deficits in ADHD children could be related to genetic influences, i.e., parental stature (no studies take into account the contribution of parental height).
- Comorbid conditions that may influence growth.
- Use of old reference standards inappropriate to age contemporaneous standards.
- Imprecise or absent description of inclusion criteria of children both on and off medication.
- Inclusion of children from different socioeconomic groups.
- Inability to document compliance with medication.
- Use of different age groups within the same study including pubertal ages, which could significantly influence both height and weight.
- Absence of control groups of unmedicated children.
- Different durations of medication treatment.
- Stimulant exposure prior to study intervention and measurements.

It is also important to note that statistically significant differences in height and weight parameters are not necessarily clinically significant differences.

Table 33. Studies on Growth in Children Treated with Amphetamines

Parameters[a]	Results and author conclusions	Comments	Reference
n = 29 (sex unspecified) Control: Unmedicated ADHD controls (n = 7); Anthropometric scale (Historical control) Age (years): Elementary school-aged Duration: Group 1 ≥ 9 months; Group 2 ≥ 2 years Dose: 10 or 15 mg/day	Methylphenidate and *d*-amphetamine combined results -The group that discontinued medication after 9 months (n = 13) gained twice as much weight in the summer as the group staying on medication (n = 7). However, this weight rebound was not sufficient to compensate for the initial weight suppression. -Over 2 years, medicated group (n = 9) had a percentile weight change of –20.38, compared to +6.79 in the control group (n = 7). For height, the medicated group percentile change was –13.45 compared to +1.29 in controls. -Tolerance did not develop for weight-gain suppression in the 2-year group; these children had a mean weight gain of 1.8 kg, compared to the expected 3.1 kg. -Percentile height decrease correlated with percentile weight decrease, but was not significant compared to baseline. *d*-Amphetamine results: -Significant difference in weight gain between group discontinuing medication in the summer (0.47 kg/mo) and group continuing medication (0.14 kg/mo). -Suppressive effect on weight gain was significant and not dose dependent.	Strength: Control group Weaknesses: First data set: Most were in special classes for learning and behavior problems; measurements only 3 times in 9 months; 13/20 taken off medication during summer, making exposures non-uniform. Second data set: Data obtained retrospectively from school nurse records; medication information obtained from parent or nurse reports; different medication doses for children; small number for subjects (9) and controls (7); use of percentiles. It is difficult to draw conclusions from these data.	*(159)*
n = 29 (sex unspecified for d-amphetamine group, however 44 of 49 in study were male) Control: 14 non-medicated ADHD males Age (years): 6.7 Duration ≥ 2 years (mean = 2.9) Dose: unspecified	<table><tr><th></th><th>N</th><th colspan="2">Percentile change in growth</th></tr><tr><th></th><th></th><th>Weight</th><th>Height</th></tr><tr><td>*d*-Amphetamine</td><td>29</td><td>–20.38</td><td>–13.45</td></tr><tr><td>Control</td><td>14</td><td>+6.79</td><td>+1.29</td></tr></table> -Summer continuance not significant for weight; marginally significant for height (t = 1.20, n = 28, $P < 0.2$). -Significant decrease in average percentile weight loss each year indicates development of tolerance for weight suppression. -Most important factors were duration of treatment and frequency of medication.	Strength: Use of a control group Weakness: Evaluation performed once/year; growth was evaluated by total change in the normative percentiles for weight and height.	*(160)*
n = 34 (sex unspecified; 4:1 male:female ratio for study as a whole) Control: Anthropometric scale	<table><tr><th rowspan="3">Medication status during the summer (n)</th><th colspan="4">Monthly growth</th></tr><tr><th colspan="2">Weight (kg)</th><th colspan="2">Height (cm)</th></tr><tr><th>School year</th><th>Summer</th><th>School year</th><th>Summer</th></tr></table>	Strength: 3-year study period Weaknesses: Measurements twice/year; measurement with a	*(161)*

Parameters[a]	Results and author conclusions					Comments	Reference
(historical control) Age (years): 10.3 (8–13) Duration ≥ 3 years Dose: 12 (5–20) mg/day	On medication (8)	0.19±0.06	0.11±0.13	0.40±0.11	0.42±0.15	yardstick is unlikely to be sensitive to 0.01 cm, as the data are expressed; potential inclusion of puberty in age group; use of historical control. General comment: Results suggest growth rebound.	
	Off medication (26)	0.15±0.15	0.42±0.27	0.35±0.17	0.64±0.39		
	Expected	0.28		0.52			
	-Growth rate differences are statistically significant regardless of dose, which indicates rebound for both height and weight.						
n = 24 (17 male) Control: Pre-treatment percentiles Age (years): 9.0 (6–12.4) Duration: 5.5 (2–9) years Dose: 16.5 (5–32.5) mg/day		Mean change in percentile[a]				Strength: Longer treatment period (at least 2 years). Weaknesses: Data partly retrospective; measurements converted to percentiles for comparison. Comments: The utility of this study is in showing that height and weight deficits are compensated in long-term treatment.	*(162)*
	Time after onset	Weight	Height	Dose, mg/day[b]			
	1 year All 24 on medication	–5.9 ($P < .05$)	–1.8 (P = NS)	12.2			
	Final follow-up 12 on medication, 12 off	+16.0 ($P < .02$)	+10.9 ($P < .01$)	19.6			
	[a] For all patients [b] Mean dose for patients still on medication						
	-Data shows some weight loss in the first 3 years compared to expected norms (statistically significant only in the first year), which recovered in later years. Both height and weight exhibited a statistically significant percentile increase at final follow-up. -Patients who had discontinued medication at final follow-up showed a "larger" height (not statistically significant) and weight ($P < .06$) rebound than those still on medication.						

Parameters[a]	Results and author conclusions					Comments	Reference
n = 7 (all male) on *d*-amphetamine Control: 8 boys on phenothiazines Age (years): 6–9.5 Duration: 1 year Mean dose: 0.88 mg/kg bw/day		Weight		Height			Strengths: Prospective study; no prior stimulant treatment; compliance was monitored by prescription renewals; height velocity was evaluated; measurements every 3 months. Weaknesses: Small numbers of subjects; control group exposure to phenothiazines; study period of only 1 year.	*(163, 164)*
		At start	Change	At start	Change	Velocity		
		mean percentiles [± SD assumed]				(cm/year)		
	Amphetamine (n = 7)	31.86 ± 34.22	–15.8 ± 15.0	36.43 ± 29.32	–11 ± 12.9	3.186 ± 1.499		
	Control[a] (n = 8)	23.38 ± 32.46	3.0 ± 9.55	28.75 ± 27.81	4 ± 9.32	5.525 ± 2.393		
	[a] Controls were on phenothiazines							
	-At 1 year, significant decline in weight percentage ($P < 0.02$). Also a significant decline in height percentage ($P < 0.02$) and drop in growth velocity ($P < 0.05$). - Noted that the control, phenothiazine, may promote growth in children, which could have increased the apparent growth deficit for the *d*-amphetamine group.							
n = 13 (all male) Control: Pre-treatment percentiles Age (years): 6–9.5 Duration: 12–21 months Dose: 21 (10–30) mg/day	-After 1 year, height and weight both decreased significantly. Weight decreased by 16 percentile points and height decreased by 10 percentile points. Height and weight velocities both decreased significantly. The trend also occurred in patients on medication for 21 months, with weight decreasing by 12.1 percentile points and height decreasing by 14.8. -Dose negatively correlated with height and weight velocity. -Weight deficits were greater than height deficits. -Results did not suggest rebound for height or weight. -Noted that growth deficits were not severe, while behavioral benefits were substantial.						Strengths: Prospective design; findings consistent with previous studies; rigorous growth-measurement techniques with measurements performed every 3 months (agreement to within 1 mm for height and 0.1 kg for weight); used weight adjusted for height, height and weight velocities, and comparisons with expected velocities. Weaknesses: Small number of subjects; control exposure to phenothiazines.	*(165)*
n = 28 (25 male) Age (years): 9.3 (4.0–15.3) Duration ≥ 5 months; mean duration of follow-up = 10.8 months Mean dose: 14.9 mg/day (weight-adjusted dose = 0.5 mg/kg bw/day)	Height and weight measurements represented as *z*-scores: Initial weight 0.5 Initial height 0.1 Weight change at follow-up –0.6 Height change at follow-up –0.4 -Children were divided into 2 groups to analyze effects of pre-treatment weight; 84% of the heavy group (n = 19) experienced decreased BMI from expected compared to 56% of the thinner group (n = 9). -BMI slope analysis showed significant difference in growth deficit between heavy and thin groups.						Strengths: No prior drug treatment; used weight-for-height curves (BMI). Weaknesses: Retrospective study; data on weekend treatment only partially available; BMI curves were from a Caucasian sample and not generalizable to non-white children.	*(166)*

Parameters[a]	Results and author conclusions	Comments	Reference
	-Major predictor of decreased BMI was pre-treatment weight. No significant effect of dose, duration of follow-up, or age on degree of weight loss. -Retrospective study; did not account for drug holidays.		
n = 124 (all male) Control: 109 normal male controls Age (years): 14.5 (617) Dose: methylphenidate equivalent dose (twice the *d*-amphetamine and half the pemoline dose) = 38 (5–120) mg/day	-Of the 124 ADHD children, 110 were medicated at some time in their lives with either methylphenidate, *d*-amphetamine, or pemoline. At the time of the study, 53 had been treated in the preceding 2 years with a mean methylphenidate equivalent dose of 38 mg/day -ADHD children were 4 kg lighter and 3 cm shorter than controls, neither of which was statistically significant. However, height deficit was statistically significant ($P = 0.03$) when height was converted to a *z* score. Significantly more ADHD children were at least 2 SDs shorter than the mean ($P = 0.02$) when corrected for age and parental heights. -No significant difference was found for height or weight between unmedicated and stimulant-treated children with ADHD. -Modest height deficits were unrelated to weight deficits or stimulant treatment, and only evident in ADHD children in early adolescence. This result indicates that ADHD children may experience delayed growth, rather than permanent stunting of growth. -No evidence of weight suppression or delayed pubertal development. -No association between height and drug treatment, drug class, duration of treatment, or dose regimen was identified.	Strengths: Normal controls; used *z* scores; corrected for parental heights; performed pubertal assessments. Weaknesses: Measurements only at 4-year follow-up; missing data.	*(167)*
n = 68 (all male) Control: Norwegian population sample Age (years): 3–13 Duration: 1–5 years Dose: Range 2.532.5 mg/day; mean dose (mg/day): Year N Dose 0–1 68 11.9 1–2 53 13.6 2–3 44 14.7 3–4 33 14.5 4–5 24 15.4	-Compared 23 boys on methylphenidate to 68 boys on a racemic mixture of *l*- and *d*-amphetamine -No statistically significant difference between methylphenidate- and amphetamine-treated children in height or weight except for a lower weight gain in amphetamine-treated children during the first year ($P < 0.05$). -21 boys (31%) on amphetamine and 4 on methylphenidate (17%) either lost or did not gain weight during the first year, with losses ranging from 0 to 9.5 kg. There was no significant dose difference between those who lost weight in the first year and those who did not. All boys who had lost weight in the first year experienced subsequent sufficient weight gains. -Children above the 50th percentile in weight prior to treatment had significantly increased weight loss compared to those below the 50th percentile ($P < 0.05$), suggesting a slimming effect.	Strengths: Large number of subjects followed for an extended time period; results and conclusions are consistent with previous studies. Weaknesses: Large age range of subjects; inclusion of pubertal subjects; once-yearly height and weight data, collected retrospectively; included developmentally-delayed children without reference to whether they had a growth-retarding syndrome. General comments: Standard	*(168)*

Parameters[a]	Results and author conclusions	Comments	Reference
	-No effect on height was observed. -No effect of cumulative dose or age. -Concluded that methylphenidate and amphetamine do not have adverse growth effects for most children. -Retrospective study, some missing data, broad age range, measurement reliability uncertain.	Norwegian population sample comparison. Multiple regression showed that neither cumulative dose nor age had a significant effect on growth when initial weight and height were controlled.	
n = 51 (44 male) Control: National Centre for Health Statistics Age (years): 7.2 (3.1–11.4) Duration: 6–42 months Dose: 0.5 ± 0.13 (mean ± SD) mg/kg bw/day; 12.5 (7.5–25) mg/day	Data reported as mean ± SD of Standard Deviation Score (a *z* score). -Initial height = 0.24 ± 1.05; initial weight = 0.61 ± 1.13. -After 6 months, weight was 1.7 kg less than expected; 76% lost weight. After 30 months there was a 3.0-kg deficit. -During the first 2 years, the height deficit was approximately 1 cm/year; the average height deficit after 42 months was 2.4 cm. -In the first 6 months, 86% had a decreased height velocity. After 30 months, the deficit was attenuated and most children had normal height velocity. -31% experienced weight deficit even without reported appetite suppression. -Average weight deficit was 2.4 times the height deficit after 30 months. -Significant decrease in height and weight after 6 and 18 months ($P < 0.001$) and after 30 months ($P < 0.01$). -Retrospective study; results do not separate methylphenidate (n = 19) from *d*-amphetamine (n = 32) treatment; 10 patients were also on clonidine; did not account for drug holidays.	Strengths: Rigorous measurements, obtained every 6 months; height, weight, and height velocity corrected for age and sex using SD scores. Weaknesses: Retrospective study; no control group; no long-term follow-up. General comments: Height velocity was lowest during the first 6 months, but in most cases normalized after 3 years; results consistent with other findings.	*(169)*

[a]Data presented as mean (range) unless otherwise specified.

Overall Assessment of Height and Weight Data: While the observations in this area are consistent with most clinical experience, the quality of data in the older papers is suboptimal. These articles have variable but generally marginal-to-moderate utility, with incomplete documentation of compliance or actual dosing regimens and with failure to consider (in most cases) basic factors that are usually assessed in growth studies, such as mid-parent height and parent BMI, family history of timing of puberty onset, the child's actual physical or endocrinologic level of puberty at start of treatment (some of the youngsters in were as old as 15 when the studies were conducted), and measurement of skeletal maturity (bone age), which particularly in school-aged children is considered a useful indication of expected growth potential. The seasonal differences in expected growth (in the northern hemisphere, children grow faster in summer) are not accounted for by designs that compare children whose families chose to leave them on stimulants through the summer and children whose families did not leave them on medication during the summer. Thus, it cannot be ruled out that those who remained on the medicines also had other conditions or behavioral patterns (like fetal alcohol effects) that motivated their parents to continue the medication and might also decrease growth. It is not known whether final adult height and weight are affected by current treatment regimens, which frequently include continuous use and use beyond childhood.

In addition, assessments of growth do not appear to be masked to stimulant exposure history. For example, in the reports of Safer et al. *(159-161*), the nurse who obtained the measurements was not masked to the children's drug histories, and in many cases actually administered the drugs herself. The studies did not control for potential confounders such as intrauterine exposure to tobacco, ethanol, and illicit drugs, or parental mental health.

Findings overall seem to suggest that appetite and growth suppression are less with methylphenidate than with amphetamines, but these findings are not conclusive. There are interesting and clinically relevant issues of mechanism that have not been fully elucidated. It is unclear whether the growth alterations that are noted are primarily related to appetite suppression (as might be expected given the widespread use of amphetamines by dieters) or by endocrine alterations as well. If the issue is only appetite suppression, it is possible to test a number of useful clinical interventions, such as feeding the child a high-calorie supplement before the first daily dose and monitoring whether this intervention alters the patterns of growth. The possible role of stimulant-associated endocrine changes cannot be addressed with the current data set because the endocrinologic data are outdated and use comparison drugs that increase the release of prolactin, creating a possible artifact of lower hormone levels with stimulants.

3.2 Experimental Animal Data

3.2.1 Prenatal toxicity endpoints

Studies examining endpoints in prenatally-exposed animals or models of prenatal development are discussed in this section. The section is divided into studies conducted in mammalian species in vivo, mammalian species in vitro, and in chickens. Amphetamine studies are presented before methamphetamine studies. Oral exposure studies are presented prior to parenteral exposure studies.

3.2.1.1 In Vivo Mammalian studies

The FDA Pharmacology Review for Adderall *(34*) summarized a developmental toxicology study in Sprague-Dawley rats, submitted to the agency as part of the approval process. Pregnant rats (22/dose group) were gavaged with amphetamine free base in water at total daily dose levels of 0, 2, 6, or 20 mg/kg bw/day in 2 equal divided doses 8 hours apart **[the Panel assumes that**

the test article was *d*- and *l*-amphetamine in a 3:1 ratio, as in the marketed product]. Treatments were given on GD 6–17 **[blood for pharmacokinetic evaluation was taken at intervals for 24 hours after a treatment on GD 17; the Panel assumes that only a single treatment was given on this day]**. Dams were killed on GD 20 and uterine contents evaluated. Fetuses were given external examinations. Half the fetuses were sectioned for evaluation of visceral abnormalities and the other half were processed for skeletal evaluation.

Dose-related clinical signs occurred in the middle- and high-dose groups, with a low incidence of clinical signs in the low-dose group. The severity of clinical signs in the high-dose group led to termination of this group on GD 9. Dam body weight gain was decreased by 5 and 13% in the low- and middle-dose groups over the dosing period. There were no treatment-related effects on litter parameters (implantations, resorptions, live young, pre- or post-implantation loss, or sex ratio) or on placental, fetus, or litter weights. There was no increase in malformations or in variations except for a possible increase in delayed cranial ossification sites. When analyzed by number of litters containing at least 1 fetus with cranial ossification delay, there were no differences between groups. There was an increase in the number of litters in which there were 3 or more fetuses with delayed cranial ossification, 1, 4, and 6 of 22 litters in the control, low-dose, and mid-dose groups, respectively. **[Benchmark dose[1] calculations performed by CERHR for this endpoint gave a BMD_{10} of 3 mg/kg bw/day and a BMDL of 2 mg/kg bw/day.]**

The same review *(34)* included a summary of a developmental toxicity study performed using pregnant New Zealand White rabbits (22/dose group) given divided amphetamine gavage doses totaling 0, 2, 6, or 16 mg/kg bw/day on GD 6–19. Periodic blood samples were taken on GD 19 for pharmacokinetic studies. **[As for the rat study, the Expert Panel assumes a 3:1 ratio of *d*-,*l*-amphetamine and a single dose of the test article on GD 19.]** Does were killed on GD 29 and uterine contents examined. Fetuses were evaluated for external, visceral, and skeletal abnormalities.

Clinical signs were noted in the high-dose group. There were no treatment-related effects on weight gain or feed consumption. There were no effects on litter parameters including implantations, resorptions, live young, sex ratio, and pre-implantation loss. Post-implantation loss was described as slightly increased in the high-dose group; statistical evaluation was not provided. The post-implantation loss rate was 17.2% in the high-dose group compared to 12.0% in the control group **[SD or SEM not given]**. There were no alterations in placental, fetal, or litter weights. There was no increase in malformations in any dose group. There was reported to be an increase in fetuses with 13 ribs (a variation in this species), which appeared only on a per fetus evaluation (at least one fetus was affected in nearly all litters in all dose groups). Litter incidence of 13^{th} ribs was high in all dose groups with 100% incidence in control litters. **[Statistical analysis was not provided. Evaluation by CERHR does not show a significant difference between groups in fetuses with 13 ribs by chi-squared testing; group percentages of fetuses with 13 ribs ranged from 57 to 69%. The Expert Panel notes that litter analysis is preferred; however, data given in the FDA review do not permit such an analysis.]**

The FDA reviewer concluded that there was no clear evidence of developmental toxicity but noted that based on pharmacokinetic data (presented in Section 2), exposures in these

[1] The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% confidence interval around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

experimental animal studies were lower than exposures in children (based on AUC) or only 2–3 times higher (based on C_{max}). The FDA reviewer also noted that although congenital malformations were not increased in these studies, other studies in the literature identified neurobehavioral abnormalities associated with developmental exposures of rodents to amphetamines. **[The FDA reviewer specifically cited three references *(49, 92, 170)*; the Expert Panel reviewed these and other studies, discussed in Section 3.2.3.]**

Strengths/Weaknesses: Several strengths were noted, including the standard design under GLP using multiple doses administered orally. The dosing paradigm (2 equal divided doses 8 hours apart) was designed to better mimic clinical exposure patterns. Blood samples were collected for toxicokinetic evaluations. Group sizes were adequate, appropriate controls were used, and the study included external, visceral, and skeletal examinations of term fetuses. A weakness is that dosing was not continued into the latter part of pregnancy, which is a more recently introduced regulatory design. Statistical analyses were not described nor were statistical results presented. It is not clear that the Staples technique for evaluating fetal morphology would have detected cardiovascular problems. In rats, the high dose exceeded the maximum tolerated dose (MTD), so animals in this group had to be removed from the study on GD 9. Thus, only two dose groups were available for evaluation. In rabbits, the significance of increased fetal incidence of 13^{th} ribs is difficult to determine given the high litter incidence (100%) of 13^{th} ribs in control animals. As stated in the report summary, the safety margins provided by this study were minimal-to-nonexistent with mean total exposures lower than those seen clinically, and peak levels approximately 2-fold higher in rats and 3-fold higher in rabbits than levels measured clinically.

Utility (Adequacy) for CERHR Evaluation Process: This study is potentially useful for the evaluation process. However, the panel was not able to use the study because a complete report was not available.

Summaries of unpublished studies on Adderall were provided to the Expert Panel by Shire Pharmaceuticals Group *(171)*. A pre- and postnatal GLP study, performed in accordance with ICH guidelines (similar to ICH 4.1.2), used gavage administration to Sprague-Dawley rats from GD 6–lactation day 20 (mating = GD 0). Dose levels of amphetamine base equivalents were 0, 2, 6, and 10 mg/kg bw/day, n = 25/group. Clinical signs and a significant decrease in gestational feed consumption and body weight gain during specific periods were seen in all amphetamine-treated groups. Postnatal survival to PND 4 was reduced at 6 and 10 mg/kg bw/day and postnatal survival to weaning was decreased at 10 mg/kg bw/day. Offspring preputial separation and vaginal opening were delayed in the 6 and 10 mg/kg bw/day groups. There were no apparent alterations in F_1 mating success or pregnancy length. Dams from the high dose group had a decrease in implantations, pups/litter, and live pups/litter. **[These summaries were noted but were not used by the Expert Panel in the Evaluation Process]**.

Yasuda et al. *(172)*, in a study funded by NIH, examined maternal and fetal toxicity in ICR-JCL mice treated with amphetamine sulfate **[enantiomers and purity not specified]**. Beginning on GD 0 (day plug detected) and continuing through pregnancy, dams were gavaged with 50 mg/kg bw/day amphetamine sulfate or with distilled water. **[It is not clear if control dams were treated and examined concurrently with the dosed animals.]** The dams were killed on GD 18.5 for examination of fetotoxicity. **[Methods of statistical evaluation were not discussed and it does not appear that the litter was considered in those evaluations.]**

Amphetamine-treated dams showed excitement for 6–7 hours following dosing. Four of 69 treated dams died during the study. Maternal body weight of treated dams was significantly lower than controls after GD 16. Feed and water intake were reduced only during the first 2 days of dosing. There were no differences in implantation rates between amphetamine-treated and control

animals. Litters from 24 treated and 10 control dams were available for an examination of total implantations, resorptions, fetal mortality, weight, and external malformations. The only statistically significant effect was an increase in dead fetuses (17.5% in treated versus 9.5% in control). There were no significant effects on fetal body weight or gross malformations.

Strengths/Weaknesses: The use of a high oral dose with adequate group sizes is a strength. Initiation of dosing the day after mating demonstrated that 50 mg/kg bw/day amphetamine did not affect implantation. Several weaknesses were noted, such as the assessment only of gross malformations. In addition, this study used only a single dose level, so dose-response comparisons were not possible. Pregnant mice were given a single daily gavage dose of amphetamine sulfate, not 2 doses a day as is more relevant to clinical use patterns. Toxicokinetics were not determined. Apparently, the control mice were not gavaged. Statistical methods were not identified. There were 20 litters in the control group and 47 litters in the amphetamine-treated group, yet only half of these litters (10 and 24) were evaluated for total implants, live/dead fetuses, fetal body weights, and malformations.

Utility (Adequacy) for CERHR Evaluation Process: This study is not very useful for the evaluation process.

Ramirez et al. ***(173)*** examined the effects of prenatal amphetamine exposure on estrous cyclicity, sexual behavior, and hypothalamic monoamine levels in rats **[strain and number treated not specified]**. Animals were sc injected with saline or 0.5 mg/kg bw/day *d*,*l*-amphetamine **[purity not specified]** during the entire gestation period. Litters were culled to four females and four males at birth. At 3 months of age, estrous cycles were monitored by taking vaginal smears on 5 days/week for at least 4 cycles in 30 control and 20 treated offspring. **[The number of litters from which the offspring were obtained was not specified.]** On the last day of estrus, the offspring were ovariectomized and ova in tubes were counted. Three weeks later, sexual behavior in response to a male rat was monitored following priming with various doses of estradiol (25–200 μg/kg) or estradiol (10 μg/kg) in combination with progesterone (2 mg/kg bw) (n = 10–24/group). Four weeks following the sexual behavior studies, rats were primed with estradiol (100 μg) or estradiol (10 μg) in combination with progesterone (2 mg) and killed for measurement of hypothalamic dopamine, noradrenalin, serotonin, and 5-hydroxyindolacetic acid levels (n = 6–9/group). Ovulation and cyclicity data were analyzed by Fisher exact probability test, and catecholamine and behavioral data were evaluated by Student *t*-test.

Prenatal *d*,*l*-amphetamine treatment had no effect on cyclicity or ovulation. Sexual receptivity, as measured by lordosis response, was significantly higher in the amphetamine group primed with estradiol and progesterone. In the estradiol dose-response experiment, sexual receptivity in the amphetamine group was significantly higher than controls at estradiol doses of 100 and 200 μg/kg. The only significant effect on monoamines was a reduction in medial hypothalamic serotonin levels in the amphetamine group **[67% of control levels]** primed with estradiol and progesterone, but not estradiol alone. The study authors concluded that prenatal exposure to amphetamines has long-lasting effects on sexual behavior in female rats.

Strengths/Weaknesses: The evaluation of unique and important endpoints is a strength. Pregnant rats were dosed with amphetamine from the beginning of gestation until term and aspects of reproduction were examined in female offspring, including ovulatory function and sexual receptivity. The authors tried to correlate behavioral changes with hypothalamic monoamine levels. A weakness is the use of a single dose level given by sc injection, while humans typically are exposed by oral or iv routes. With only one dose level, dose-response relationships cannot be evaluated. Parental animals were selected based on their performance in an active avoidance test, and the potential influence of such selection on study outcome is unclear. It is also a weakness

that housing of adult females singly or as a group is not specified. There is no information on the number of exposed dams and the number of litters from which the offspring originated; consequently, there is no indication as to whether the authors controlled for litter effects. There is insufficient experimental detail to track animals throughout this study (e.g., Table 1 of the study references 30 controls and 20 in utero amphetamine-treated animals, which were subsequently ovariectomized for the sexual receptivity experiments. However, Figure 1 of the study [sexual receptivity with estradiol and progesterone] references 18 controls and 24 in utero amphetamine-treated animals). Furthermore, the authors' statistical methods were inadequate in some cases (i.e., using a Student *t*-test to compare sexual receptivity across different doses of estradiol as shown in Figure 2).

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited usefulness for the evaluation process.

Adams et al. *(174)* at the National Center for Toxicological Research (NCTR) gave *d*-amphetamine sulfate 0 or 2.0 mg/kg bw/day sc to Sprague-Dawley rats on GD 12–15 (n = 9/dose group; plug = GD 0). Dosing solution concentrations were verified by GC. Dams were killed on GD 20 and uterine contents evaluated. Live fetuses were decapitated and heads fixed in Bouin solution for free-hand razor-blade sectioning. Visceral evaluation was performed by dissection under a stereomicroscope. Carcasses were cleared and stained for skeletal examination with Alizarin Red S. A companion behavior study (summarized in Table 39 in Section 3.2.3) contributed culled pups on PND 1 for teratologic evaluation by the same methods. There was a 32% decrease in body weight gain during the dosing period, but the difference was not statistically significant; differences between the amphetamine and control groups in dam weight gain, implantation sites/litter, live fetuses/litter, resorptions, sex ratio, and fetal weight were also not statistically significant. There were no malformations in any of the amphetamine-exposed fetuses or pups. The authors recognized that a larger number of litters may have revealed a low malformation rate, but they concluded that the 2.0 mg/kg bw/day amphetamine dose at mid-gestation had a low likelihood of producing this kind of developmental toxicity.

Strengths/Weaknesses: Strengths include the relatively low dose and the good postnatal data sets. Concentration verification was conducted on dose solutions using GC and the groups were counterbalanced during dosing. The high-dose group (2.0 mg/kg bw/day on GD 12–15) was evaluated for effects on uterine weight, live/dead fetuses, resorption sites, and fetal structures (external, visceral, and skeletal examinations). The major weakness is the small sample size (n=9/group) leading to reduced sensitivity. In addition, only one dose group was available, so that dose-response assessments could not be made. Malformations were examined in the culled pups from litters used in the postnatal study (examined on the day of birth), reducing the likelihood that a low incidence ,malformations was missed. For neurobehavioral assessments, nested litter analyses show more sophisticated statistical analyses. The weakness of this study is the summarizing of the teratology data rather than more detailed reporting. In addition, rats were dosed with amphetamine by sc injection, while humans typically are exposed by oral or iv routes. The objective of this study was to examine primarily the neurobehavioral effects of prenatal amphetamine exposure (not developmental toxicity); thus, not all intermediate dose levels were included for fetal analyses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

Nora et al. *(175, 176)*, in two studies funded by NIH, the National Research Council of Canada, and/or the March of Dimes, examined teratogenicity in A/Jax mice and C57Bl/6J mice dosed with *d*-amphetamine sulfate **[purity not specified]**. On GD 8 **[plug day not specified]** the mice

received a single ip dose of 50 mg/kg bw *d*-amphetamine sulfate in water, a dose that was stated to be 200 times higher than the usual human dose. Controls were ip injected with physiological saline. Fetuses were removed 1–2 days before term, fixed in Bouin solution, and the thorax was sectioned and examined. **[There was no mention of methods used for statistical analyses and the litter was not considered in statistical evaluations.]**

In the first study *(175*) conducted in A/Jax mice, there were 21 treated and 10 control dams. There were 130 living fetuses in the treated group and 70 in the control group. The findings reported to affect more treated compared to control fetuses were (percent incidence in treated compared to controls) resorptions (29 vs. 8%), malformations (38 vs. 7%), cardiac malformations (12 vs. 0%), cleft lip (18 vs. 4%), and eye abnormalities (8 vs. 3%). Cardiac malformations included defects of ventricular and atrial septum, defects of the right aortic arch, and coarctation of the aorta and right aortic arch. **[Fisher exact test performed by CERHR on the fetal data shows statistical significance for total malformations (*P* < 0.001), cardiac malformations (*P* = 0.0015), and cleft lip (*P* = 0.0074). The Expert Panel notes that litter analysis is preferred; however, data given in the paper do not permit such an analysis.]**

In the second study, there were 34 treated and 32 control A/Jax dams and 26 treated and 27 control C57Bl/6J dams *(176*). For the A/Jax mice, there were 283 living treated fetuses and 233 living control fetuses. There were 95 resorbed embryos in the treated group and 26 resorbed embryos in the control group. Cleft palate was the most frequently occurring malformation in the A/Jax strain **[incidence not stated]**. Findings that were significantly increased in fetuses from the treated versus control group were (percent incidence in treated compared to controls) total malformations **[22 compared to 8.6%]** and cardiac anomalies (13% compared to 1.3%. **[The percentage should be 8% if the authors' figures are correctly printed (24/283); however, it is possible that the denominator should have been printed 183, which would give 13%]**). The most common cardiac anomalies in treated and control A/Jax fetuses were atrial septal defects. For the C57Bl/6 mice, there were 161 living treated and 202 living control fetuses. The number of resorbed fetuses was 63 in the treated group and 19 in the control group. Microphthalmia was the most frequently occurring malformation in C57Bl/6J fetus **[incidence not stated]**. Findings that were significantly increased in C57Bl/6J fetuses for the treated compared to the control group were (percent incidence in treated compared to controls) total malformations **[32 compared to 7.9%]** and cardiac anomalies (11 compared to 1%). The most common cardiac anomalies in treated and control C57Bl/6J fetuses were ventricular septal defects. The study authors concluded that amphetamine treatment increased the frequency of spontaneously occurring cardiac malformations in each strain of mouse.

Strengths/Weaknesses: The use of a high dose is a strength in increasing sensitivity, but the use of multiple doses would have been superior. The use of two strains is a strength. In addition, fetal examiners were blind with respect to treatment group in the original study *(175*). These studies evaluate a reasonable margin of safety at 50 mg/kg bw *d*-amphetamine, which is approximately 200 times the usual clinical dose. Several weaknesses were noted. Pregnant mice were dosed with *d*-amphetamine at a single dose level by ip injection, which is not considered a relevant route of exposure due to a) possible direct adverse effects of the drug and/or solvent on the uterus and secondarily on embryo-fetal development and b) the possibility that the drug might be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism and distribution. With only one dose level, dose-response relationships cannot be evaluated. The dosing period was unusually long (10 AM to 4 PM to inject 21 mice), which could result in confounding due to diurnal variations. Furthermore, there is insufficient experimental detail to interpret these studies. For example, there was no information given on randomization method, maternal and fetal body weight during the study, or litter data (number of litters/group, average litter size, litter weights, etc.). Although there were only 2 additional dams in the *d*-amphetamine-

treated versus control A/J mice (34 treated females vs. 32 controls) in the 1968 study *(176*), there were 50 more live fetuses and 69 more resorbed fetuses than were seen in the control group, which may be further evidence that the total number of fetuses should have been 183 rather than 283, as printed. There was no mention of the statistical methods employed and litter-based analyses were not conducted.

Utility (Adequacy) for CERHR Evaluation Process: These two reports are of limited usefulness for the evaluation process.

Fein et al. *(177*), in a study conducted at an Israeli Medical Center, examined teratogenicity and cardiac effects of *d*-amphetamine in ICR mice. During GD 9–11 (plug day = GD 0), mice were ip injected with 50 or 100 mg/kg bw/day *d*-amphetamine sulfate **[purity not specified]**. Controls were ip injected with saline. **[It is clear that 1 group of mice was injected with amphetamine on GD 9, 10, and 11. Other groups were injected on 1 or 2 days between GD 9 and 11. The saline controls were injected on GD 9 or 10 or 11.]** Three to 15 dams/group treated during GD 9–11 were killed on GD 15 and electrocardiograms (EKG) were recorded in 17–39 fetuses/group. Other surviving dams (n = 5–19/group) were killed on GD 19. Implantation and resorption sites and fetal survival and external malformations were examined. EKG recordings were obtained from fetuses without malformations from the GD 9–11 group (n = 5–15 dams/group and 40–71 fetuses/group). Following the EKG recordings on GD 19, fetuses were weighed, killed, and examined for internal malformations **[method not specified]**. Hearts were fixed in 4% formaldehyde, sectioned, and stained with hematoxylin and eosin. Data were evaluated by Student *t*-test.

The discussion of results is limited to mice treated on GD 9, 10, and 11. As noted in Table 34, amphetamine treatment reduced fetal weight and increased maternal and embryonic mortality and fetal malformations. The types of malformations observed were microphthalmia, amelia, exencephaly, and cleft lip. There were no internal malformations observed. No differences in EKG patterns were observed on GD 15. On GD 19, mean Q-T intervals in the 50 mg/kg bw group (445 msec) and 100 mg/kg bw group (467 msec) were significantly longer than the mean Q-T interval in the control group (215 msec). EKG patterns in GD 19 treated embryos were similar to those observed in control embryos around GD 14–16. Histodifferentiation of myocardial tissue was delayed in GD 19 embryos with prolonged Q-T intervals. The study authors concluded that high doses of *d*-amphetamine retard general embryonic development and histodifferentiation of cardiac tissue.

Table 34. Mortality and Malformations in Offspring of Mice Treated with *d*-Amphetamine Sulfate on GD 9–11

Endpoint	Dose (mg/kg bw/day)		
	0 (GD 9, 10, or 11)	50 (GD 9–11)	100 (GD 9–11)
No. of surviving dams	10 (100%)	5* (62%)	15* (60%)
No. of live fetuses	96 (95%)	44 (88%)	60* (42%)
No. resorption sites	6 (5%)	6 (12%)	84* (58%)
No. malformed embryos	0	4* (9%)	9* (15%)
Fetal weight, g (mean±SD)	1.3256±0.084 (n = 102)	Not specified	1.1529±0.104* (n = 51)

*$P < 0.01$ compared to control **[Original states *t*-test, but the Expert Panel assumes that a test of proportions was used for comparisons other than fetal weight].** From Fein et al. *(177*).

Strengths/Weaknesses: The unique focus on cardiovascular effects and the evaluation of sensitive periods are strengths of this report. EKGs and histology were used to demonstrate a delay in development of the heart on GD 19 in mouse fetuses exposed to 50 or 100 mg/kg bw/day *d*-amphetamine on GD 9, 10 and/or 11. Prolonged Q-T intervals and undifferentiated cardiac

myocytes supported the conclusion of incomplete maturation of cardiac muscles at term. Although not specifically stated in the text, the tables list n values as the "number of dams/litters evaluated," suggesting that there was some control for litter effect. Adequate sample sizes were used during most segments of this study. Several weaknesses were noted. Pregnant mice were dosed by ip injection, which is not considered a relevant route of exposure due to a) possible direct adverse effects of the drug and/or solvent on the uterus and secondarily on embryo-fetal development and b) the possibility that the drug might be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism, and distribution. This study lacks the proper controls for some dose groups as controls were dosed on GD 9, 10, or 11 and some *d*-amphetamine-treated animals were treated on GD 9 and 10, or GD 9–11. The MTD was exceeded as evidenced by maternal mortality (37–42%) in all dose groups and the high resorption rates (20–58%) in the high-dose mice. Data on maternal body weights before, during, and after dosing were not presented. Statistical methods were unclear and possibly inappropriate.. It is not clear why the n values varied so much in the experiments measuring cardiac Q-T interval (n = 3–15) and whether this impacted the outcome of this measurement.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited usefulness for the evaluation process.

Kasirsky and Tansy *(178)*), in a study supported by NIH, evaluated the teratogenicity of methamphetamine in mice and rabbits. Fifty CF1 mice/group were iv dosed with 5 or 10 mg/kg bw/day methamphetamine HCl **[purity not specified]** on GD 9–11, 9–12, 12–15, or 9–15 (plug day = GD 1). One control group of 50 mice was not treated and a second group of controls was given saline on GD 9–15. Mice were killed on GD 19 and implantation sites were examined. Fetuses were weighed and examined by Wilson procedure. Every third fetus was cleared for skeletal examination. Twelve New Zealand White rabbits/group were iv dosed with 1.5 mg/kg bw/day methamphetamine HCl on GD 12–15, 15–20, or 12–30. One control group of rabbits (n = 12) was not treated and a second control group was treated with saline **[day of treatment not specified]**. Rabbits were killed on GD 30. Fetuses were examined for gross abnormalities, weighed, and sexed. **[The method for examination of visceral and skeletal malformations was not discussed.]** Data for weight and malformation rate were analyzed by chi-square. **[The litter was not considered in the statistical evaluations. Chi-square tests are not appropriate for analysis of weight]**

Mice and rabbits showed signs of excitement from 5 minutes to 6–7 hours following methamphetamine administration. Maternal weights were significantly reduced in every treatment group in both species. Feed and water intake were reduced on the first 2–3 days of exposure **[data not shown]**. Fetal weights were significantly decreased in all treatment groups in mice and rabbits. There were no significant effects on resorptions in either species. **[However, the Expert Panel noted that the resorption rate in mouse controls was ~4% and in treated groups, the resorption rate was ~ 15–22%.]**

In mice, the only significant increase in malformations occurred in fetuses from the group treated with 10 mg/kg bw/day methamphetamine on GD 9–15; the malformation rate in that group was 13.6% compared to 1% in either control group. Malformations (numbers of fetuses affected) included exencephaly (25), cleft palate (10), microphthalmia (8), and anophthalmia (6).

The malformation rate in both groups of control rabbits was 3%. A significant increase in malformation rate occurred in fetuses of rabbits dosed with 1.5 mg/kg bw/day methamphetamine on GD 12–15 (12% compared to 3% in controls) and the most common malformations (number of fetuses affected) were cyclopia (5) and exencephaly (2). A significant increase in malformation rate also occurred in fetuses from rabbits treated with 1.5 mg/kg bw/day methamphetamine on

GD 12–30 (15.5% compared to 3% in controls). The most common malformation was exencephaly, which affected four fetuses. Heart malformations were not observed in either species. The study authors concluded that high iv doses of methamphetamine can induce congenital anomalies in mice and rabbits. Benchmark doses[2] (Table 35) and dose-response curves (Figure 3) for the mouse study were derived using EPA Benchmark Dose Software. **[Calculation of the benchmark dose and estimation of the dose-response curves is particularly limited given the use of only 3 dose levels (0, 5, and 10 mg/kg bw/day) in this study.]**

Table 35. Benchmark Doses Calculated from the Mouse Study of Kasirsky and Tansy *(178)*

	Benchmark dose (mg/kg bw/day, GD 9–15)	
Endpoint	BMD_{10}	BMDL
Maternal weight	7.8	7.2
Fetal weight	2.1	1.5
Resorptions (per implant)	0.6	not meaningful
Malformations (per live implant)	9.2	8.4

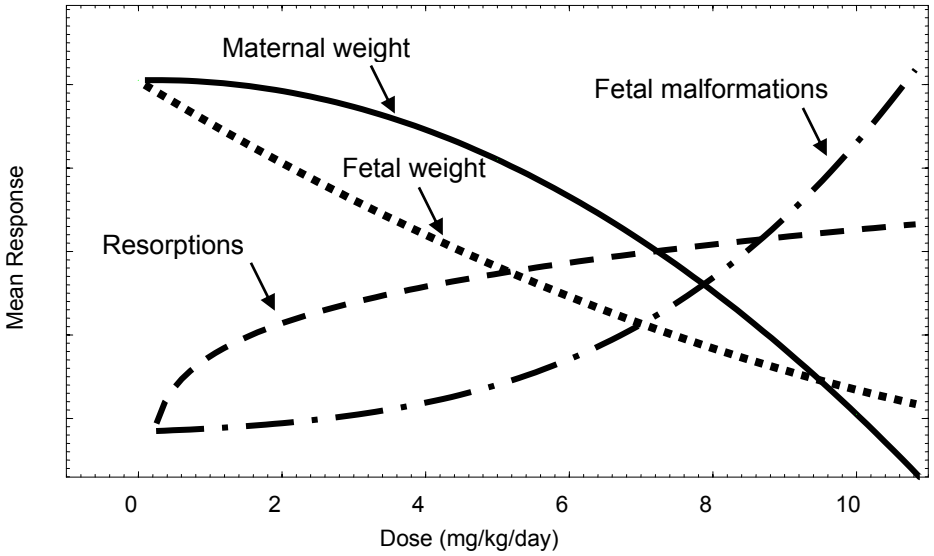


Figure 3. Dose-Response Curves for the Mouse Study Reported by Kasirsky and Tansy *(178)*.

Data points and variances removed. Y-axis is an arbitrary scale with non-zero origin. Tested doses were 0, 5, and 10 mg/kg bw/day on GD 9–15. Both doses of methamphetamine were effect levels by pairwise comparison except for malformations, for which 5 mg/kg bw/day was a NOAEL. Graphs were drawn using the EPA Benchmark Dose Software. Maternal and fetal weight were plotted as distributions (mean ± SD). Resorptions and fetal malformations were plotted as proportions of total and live implants, respectively.

[2] Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% confidence interval around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

Strengths/Weaknesses: The high-dose iv administration protocol provides unique information that is realistic for drugs of abuse. The use of multiple doses and a sensitive period approach are strengths. This study identified the most critical periods for methamphetamine-induced teratogenesis in the mouse (GD 9–15) and the rabbit (GD 12–15/12–30). Although there were only three dose levels, mouse data were presented in sufficient detail to permit benchmark dose calculations. The incomplete reporting in this older study is a weakness. In addition, there was no indication that the statistical analyses were litter-based. Although methamphetamine-treated mice and rabbits had marked effects on maternal and fetal body weights, which may have contributed to developmental toxicity, there were no pair-fed controls.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

Yamamoto et al. ***(179)***, support not indicated, treated Jcl:ICR mice with a single dose of ip methamphetamine HCl **[purity not indicated]** in saline on GD 8 (plug = GD 0). Methamphetamine doses and number of dams used were 0 (n = 13), 11 (n = 10), 13 (n = 11), 14 (n = 10), 15 (n = 16), 17 (n = 19), 19 (n = 17), or 21 (n = 26) mg/kg bw. More animals were used for the higher dose groups in anticipation of treatment-induced maternal death. Maternal death occurred in 3 of 16 dams at 15 mg/kg bw, 5 of 14 dams at 17 mg/kg bw, 6 of 17 dams at 19 mg/kg bw, and 13 of 26 dams at 21 mg/kg bw. Restlessness and agitation were described after dosing **[presumably in all dose groups]**. Maternal feed consumption and weight gain were not reported. Fetal body weights at hysterotomy on GD 18 were not affected by treatment. Fetuses were examined for external malformations and about one-third of randomly selected fetuses were cleared, stained with Alizarin Red S, and evaluated for skeletal abnormalities. **[Visceral abnormalities were not mentioned.]** Maternal mortality data and litter data for fetal death and external and skeletal malformations are shown in Figure 4. The litter percent affected at 21 mg/kg bw methamphetamine was lower than at 19 mg/kg bw, and these high-dose data were ignored in graphing the dose-response data in Figure 4. **[The authors proposed that the 50% maternal mortality at the 21 mg/kg bw dose would have led to a decrease in crowding of the dams, which were initially housed 3 to a cage during pregnancy. This decrease in crowding was believed to have improved pregnancy outcome in the high-dose group. The Expert Panel notes that group housing of pregnant animals is not conventional and finds it difficult to evaluate these data given the likelihood that there were different numbers of dams per cage in all the dose groups (none of the group sizes are evenly divisible by 3). NOAELs, LOAELs, and benchmark doses are provided in Figure 4 to facilitate comparison of the data produced in this study. The Expert Panel does not mean to imply that this study should be used as the basis for regulation.]**

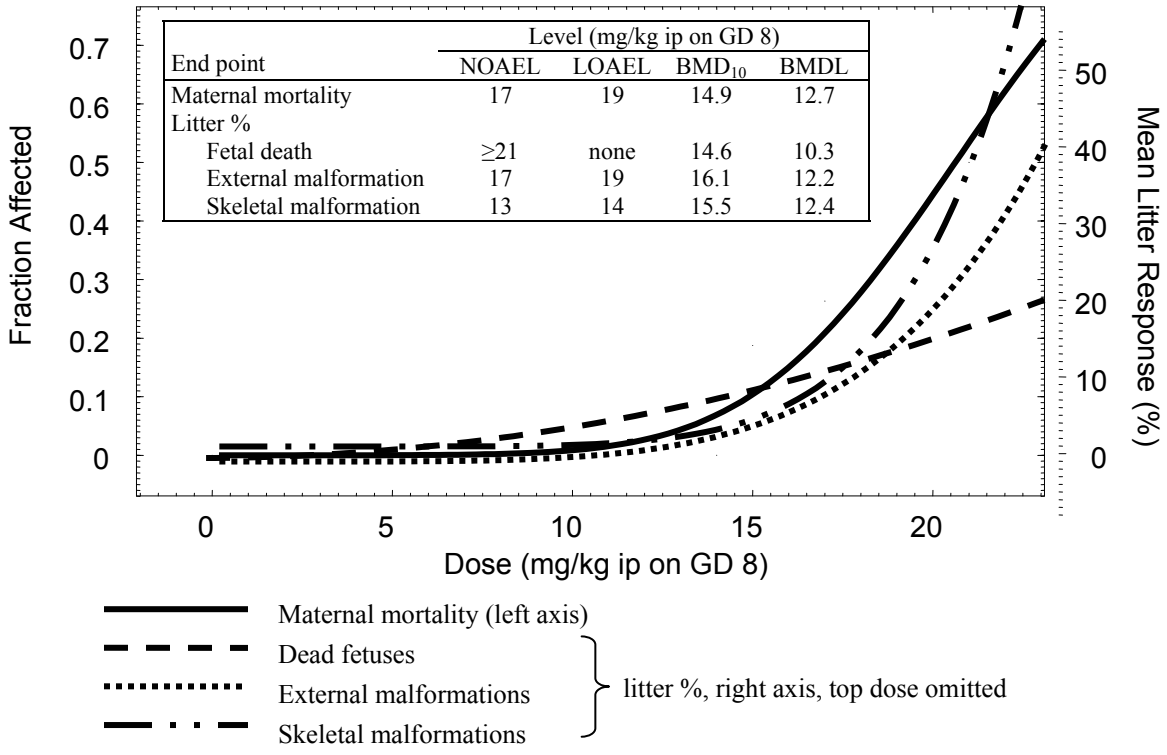


Figure 4. Dose-Response Curves from the Data of Yamamoto et al. *(179)*.

The curves in Figure 4 were drawn using EPA Benchmark Dose software. Data points and variances have been omitted for clarity. The NOAELs and LOAELs were based on author assessment of significance on pair-wise comparison with control, except for maternal mortality, which is based on pair-wise comparison by CERHR. The benchmark dose corresponding to a 10% effect level (BMD_{10}) and the benchmark dose corresponding to the lower bound of the 95% confidence interval at this effect level (BMDL) were calculated by CERHR using EPA Benchmark Dose software version 1.3.2.

Strengths/Weaknesses: The use of multiple doses and standard methodology is a strength. Litter-based statistics were used to evaluate fetal mortality and malformation rates, whereas fetal-based statistics were used for the percentage of malformed fetuses. Study data were given in sufficient detail to calculate benchmark doses. The authors concluded that methamphetamine has a steep dose-response for teratogenesis and that it does not appear to be a selective fetal toxicant, because the difference between the single teratogenic dose (19 mg/kg bw) and the maternal LD_{50} dose (21 mg/kg bw) was very small. Several weaknesses were noted. Pregnant mice were dosed with methamphetamine by ip injection, which is not considered a relevant route of exposure due to a) possible direct adverse effects of the drug and/or solvent on the uterus and secondarily on embryo-fetal development and b) the possibility that the drug might be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism and distribution. The MTD was exceeded as evidenced by maternal mortality (19–50%) at doses $\geq$15 mg/kg. A pair-fed control group was not included. Maternal body weights and body weight gains were not reported. Fetuses were not evaluated for visceral alterations. Animals were group housed during pregnancy, which may confound pregnancy outcomes, particularly given unequal numbers of animals co-housed in the different treatment groups.

Utility (Adequacy) for CERHR Evaluation Process: The utility of the study is decreased by the potentially important effects of housing. The lack of effect consistency at the top dose makes the

results of this study difficult to interpret.. This study is of limited utility for dose-response modeling.

Burchfield et al. *(52)* studied the pharmacokinetics and pharmacodynamics of methamphetamine in pregnant sheep. On GD 125 (~85% of term), catheters were inserted in Grade Western sheep and the animals were given antibiotics during a 3-day recovery period. Following the recovery period, sheep received 1 or more iv treatments of methamphetamine that included 0.6 mg/kg bw over 12.5 minutes, 1.2 mg/kg bw over 12.5 minutes, or 1.2 mg over 30 seconds. Blood was collected from 4–5 ewes/group and methamphetamine levels were measured in plasma by HPLC to determine pharmacokinetics in ewes and fetuses (discussed in Section 2.1.2.2). Arterial blood gas samples and maternal and fetal blood pressure and heart rate were evaluated periodically. Methamphetamine rapidly crosses the placenta in sheep, and fetuses ultimately achieve higher methamphetamine concentrations than adults due to a longer elimination half-life. Maternal and fetal blood pressure was increased by maternal methamphetamine exposure and the magnitude of the increase was independent of dose regimen. Fetal heart rate was decreased in response to the 1.2 mg/kg bw maternal bolus dose of methamphetamine. Fetal oxyhemoglobin saturation was decreased by all maternal treatments and fetal arterial pH was decreased by maternal methamphetamine at 1.2 mg/kg bw. There was a greater decrease in fetal arterial pH after bolus than slow administration of methamphetamine to the mother. The authors believed their results to be consistent with a decrease in placental perfusion after maternal methamphetamine administration. **[The Expert Panel noted that the most interesting elements of this study were the correlation between fetal methamphetamine disposition and fetal oxyhemoglobin levels, as well as the maternal/fetal physiological changes (blood pressure, fetal oxyhemoglobin saturation, fetal pH, etc.) that accompany methamphetamine exposure. The authors hypothesized that decreased maternal vasodilation may account for fetal hypoxia and maternal and/or fetal hypertension could lead to poor fetal outcome.]**

Strengths/Weaknesses: Strengths of this study include the evaluation of possible mechanisms of toxicity, the real-time evaluation of the fetal sheep, and documentation of some important pharmacokinetic principles for methamphetamine administered iv to pregnant sheep. Doses used in this study were reportedly at or below those used recreationally (60–100 mg; Hall et al. *(180)*), yet maternal ovine blood concentrations were reported to be approximately equivalent to those achieved in humans 1 hour after receiving 160–200 mg amphetamine iv *(181)*. A weakness is the possibility that instrumentation could modify the treatment effects. In addition, sheep were used for multiple samples, meaning that data across samples are not independent trials. Aside from maternal methamphetamine elimination half-life and fetal pre-exposure oxyhemoglobin saturation levels, other factors affecting fetal elimination half-life are unknown. Limited data were available with which to compare levels observed in maternal and fetal sheep with humans. There are a number of factors that can affect cross-species extrapolation of these data (differences in placental structure, cross-species differences in plasma protein binding or fetal metabolic capacity, etc.). There are no corresponding developmental toxicity studies in sheep to demonstrate that these fetal changes result in adverse developmental outcome.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

Dickinson et al. *(182)*, support not indicated, evaluated the effects of methamphetamine **[purity not specified]** on pregnant sheep **[breed unspecified]** and their fetuses. Catheters were surgically placed in maternal and fetal blood vessels on GD 125–128 (third trimester). After a 5–7-day recovery period, methamphetamine was given iv (maternal femoral vein) over 2–3 minutes at 1.25 mg/kg bw. Fetal blood was sampled 15, 30, 60, 90, 120, and 180 minutes after methamphetamine administration. Mean arterial blood pressure and heart rate increased in mother and fetus after methamphetamine. There were increases in maternal plasma glucose, insulin,

epinephrine, and norepinephrine after treatment. Maternal blood gases were not altered. Fetal plasma glucose, insulin, lactate, epinephrine, and norepinephrine increased after maternal methamphetamine administration. Fetal arterial pO_2 decreased from a mean ± SEM of 21.4 ± 1.9 mm Hg to 15.3 ± 1.3 mm Hg by 60 minutes after maternal methamphetamine. There were no significant changes in fetal arterial pH or pCO_2 by pair-wise comparison with the baseline values **[trend testing by CERHR showed a significant linear trend (*P* = 0.016) for pH, which declined from a baseline mean ± SEM of 7.35 ± 0.01 to a 180-minute value of 7.29 ± 0.02]**. The authors concluded that changes in release of catecholamines after methamphetamine treatment may have caused glucose changes and alterations in placenta perfusion leading to fetal hypoxemia.

Strengths/Weaknesses: Strengths include the collection of data that bear on mechanism, the use of real-time cardiovascular data, the use of a standard experimental model, and the collection of third trimester information. The authors present useful gestational pharmacokinetic data and confirmed many of the observations reported by Burchfield et al. *(52)* (i.e., increased blood pressure, heart rate, decreased fetal pO_2, etc.). Maternal and fetal catecholamines (epinephrine and norepinephrine) were also measured, along with increased fetal glucose, insulin, and lactic acid levels. The resulting profile was consistent with alterations in fetal sympathoadrenal activity and fetal hypoxemia. There is concern that instrumentation may have altered the response to treatment. A weakness of the study is that multiple samples were collected from each sheep, increasing the likelihood of confounding results (i.e., data across samples are not independent trials). Limited data were available with which to compare levels observed in maternal and fetal sheep with humans. There are a number of factors that can affect cross-species extrapolation of these data (differences in placental structure, cross-species differences in plasma protein binding, or fetal metabolic capacity, etc.). There are no corresponding developmental toxicity studies in sheep to demonstrate that these fetal changes result in adverse developmental outcome.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

Stek et al. *(53)* implanted catheters in the maternal and fetal vessels of 7 mixed-breed sheep at 107 days gestation (early third trimester). After a 1-week recovery period, methamphetamine HCl **[purity not specified]** 1 mg/kg bw was administered into the maternal vena cava over 1.5 minutes. This dose was characterized as similar to a recreational dose of methamphetamine in humans. Maternal and fetal serum and amniotic fluid samples were drawn periodically for measurement of methamphetamine concentration (discussed in Section 2), and measurements were made of cardiovascular parameters periodically over 3 hours. Results are shown in Table 36. The authors concluded that methamphetamine crosses the ovine placenta and produces significant and prolonged maternal and fetal cardiovascular changes that they presumed would be potentially detrimental to maternal and fetal health.

Strengths/Weaknesses: Strengths include the collection of data that bear on mechanism, the use of real-time cardiovascular data, the distinction between direct and indirect effects, the use of a standard experimental model, and the collection of third trimester information. Time course data for maternal and fetal methamphetamine are useful. To ensure the integrity of placental transfer prior to methamphetamine dosing, fetuses were required to meet minimum standard values for pO_2 and pH. The dose level used (1 mg/kg) approximates typical human doses of methamphetamine users. The authors used appropriate statistical models (repeated-measures analysis of variance) where possible. When not possible due to limited degrees of freedom, they decreased the *P*-value for paired *t*-tests to reduce the possibility of type I error. Results from this study are consistent with those reported by Burchfield et al. *(52)*. There is concern that instrumentation may have altered the response to treatment. A weakness is that sheep were used

for multiple samples with at least 2 days of recovery between experiments; thus, data across samples are not independent trials. Amniotic fluid samples for the determination of methamphetamine levels were not available from all fetuses at each time point. Limited data were available with which to compare methamphetamine concentrations and kinetics in humans to those observed in maternal and fetal sheep. There are a number of factors that can affect cross-species extrapolation of these data (differences in uteroplacental structure, cross-species differences in plasma protein binding or fetal metabolic capacity, etc.). There are no corresponding developmental toxicity studies in sheep to demonstrate that these fetal changes result in adverse developmental outcome.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

Table 36. Cardiovascular Parameters (Mean ± SEM) in Early Third-Trimester Sheep after Administration of 1 mg/kg bw over 1.5 Minutes to the Vena Cava of the Ewe

Parameter	Baseline	Maximum response	Minutes to maximum response/resolution[a]	*P* value[b]
Maternal				
Mean arterial blood pressure, mm Hg	69 ± 4	148 ± 9	2/> 180	0.03
Heart rate, beats/minute	102 ± 5	161 ± 19	5/> 180	0.012
Cardiac output, L/minutes	5.9 ± 1.1	8.0 ± 1.0	5/> 180	0.09
Systemic vascular resistance (calculated), mm Hg/L/minute	13.6 ± 3.6	18.9 ± 2.6	2/60	NS
Uterine blood flow, mL/minute	672 ± 48	983 ± 65	2/5	0.009
Uterine vascular resistance (calculated), mm Hg/L/minute	0.10 ± 0.01	0.39 ± 0.17	5/120	0.05
Fetal				
Blood pressure, mm Hg	49 ± 3	64 ± 3	5/180	0.04
Heart rate, beats/minute	157 ± 5	213 ± 11	120/> 180	0.03
Umbilical blood flow, mL/minute	503 ± 40	412 ± 44	5/10, with overshoot thereafter to ~550 mL/minute	not given **[0.15 by CERHR]**
Umbilical vascular resistance (calculated), mm Hg/mL/minute	0.10 ± 0.01	0.17 ± 0.02	5/60	0.02
Arterial pH	7.334±0.017	7.286±0.016	10/not stated	NS
Arterial pO_2, mm Hg	22.4 ± 0.6	17.4 ± 1.5	5/not stated	0.06

n = 7 ewes/fetuses except for maternal cardiac output and systemic vascular resistance where n = 4.
[a]Resolution estimated from graph and not based on statistical testing.
[b]Repeated measures ANOVA for change over time, as per authors.

Stek et al. ***(183)*****,** supported by NIH, in a follow-up to their previous study *(53)*, evaluated whether a methamphetamine-associated decrease in fetal arterial pO_2 could be attributed to a reduction in uterine blood flow. Nine mixed-breed ewes underwent surgical placement of maternal and fetal catheters and uterine artery flow probes on GD 110–115. Animals were permitted to recover for at least 5 days before experimentation. Methamphetamine HCl **[purity not specified]** was given in increasing doses of 0.03, 0.1, 0.3, and 1.0 mg/kg bw. Each dose was given by iv infusion **[possibly through a vena cava catheter]** over 60–90 seconds. Infusions were separated by 30–35 minutes and recordings were made 5 minutes after each infusion. On a different day, fetal infusions were given using methamphetamine doses of 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg estimated bw at 30–35 minute intervals **[the fetal vessel used for the infusion was not specified; both the aorta and the vena cava were cannulated]**. Maternal mean arterial blood pressure increased in a dose-dependent manner, peaking at a mean of 76% over baseline after the 1.0 mg/kg bw dose. Total uterine blood flow decreased in 4 animals by a mean of 33% at the 1.0 mg/kg bw dose and was not statistically changed in 2 animals, with the seventh animal excluded due to probe malfunction. The net effect in the 6 animals was not significantly different from baseline. Uterine vascular resistance increased in a dose-dependent manner in the 6 evaluable animals to a maximum of 140% of control after methamphetamine 1.0 mg/kg bw. Fetal mean arterial pressure increased in a dose-dependent manner after maternal methamphetamine administration, with a mean 28% increase after the 1.0 mg/kg bw dose. Umbilical blood flow increased by a mean of 15% from baseline after the 0.3 mg/kg bw dose to the mother, without additional increase at 1.0 mg/kg bw. Umbilical vascular resistance was described by the authors as having increased after the 1.0 mg/kg bw maternal dose, but statistical testing showed no significant change from baseline. Mean fetal arterial pO_2 decreased in a dose-dependent manner from a mean ± SEM of 21.2 ± 2 mm Hg at baseline to 16.3 ± 2.2 mm Hg after the maternal 1.0 mg/kg bw dose of methamphetamine. There were no statistically significant changes in maternal or fetal pH **[presumably arterial]**, or in maternal or fetal arterial pCO_2. After fetal administration of methamphetamine, there was no significant alteration in fetal arterial pO_2, although there was a significant increase in fetal mean arterial blood pressure and a decrease in fetal pH. The authors concluded that the alteration in fetal arterial pO_2 after maternal methamphetamine exposure was likely due to effects of the drug on placental or uterine vasculature because no alteration occurred in fetal arterial pO_2 after direct administration to the fetus. They also concluded that alterations in fetal blood pressure and pH after maternal administration of methamphetamine were likely due to direct effects on the fetus after placental transfer. **[The Expert Panel notes, however, that there were no significant effects on fetal pH after maternal administration of methamphetamine in this experiment; the authors concluded a decrease in fetal pH despite of the lack of statistical significance. The Expert Panel also notes that significant decreases in ovine fetal arterial pH were noted in another study *(52)*.]**

Strengths/Weaknesses: Strengths include detailed examination of the pH issue, the use of a standard model, the addressing of maternal-fetal influences, and the use of multiple low doses. Another strength is that integrity of placental transfer prior to methamphetamine dosing was ensured by requiring fetuses to meet designated pO2 and pH values. A weakness is that sheep were used for multiple samples with doses administered at 30- to 35-minute intervals, meaning that data across samples are not independent trials. Limited data were available with which to compare levels observed in maternal and fetal sheep with humans. There are a number of factors that can affect cross-species extrapolation of these data (differences in uteroplacental structure, cross-species differences in plasma protein binding or fetal metabolic capacity, etc.). There are no

corresponding developmental toxicity studies in sheep to demonstrate that these fetal changes result in adverse developmental outcome.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process.

3.2.1.2 In Vitro Studies

Yamamoto et al. ***(184)*** examined developmental effects in Wistar rat embryos exposed to *d*-methamphetamine HCl in vitro. GD 10.5 embryos (plug day = GD 0; n = 8–26/group) were incubated for 24 hours in media containing 0, 0.1, 0.2, 0.4, 0.6, or 0.8 mM methamphetamine (0, 15, 30, 60, 90, or 120 µg/mL, according to author calculations) and examined for mortality, growth, and malformations. Exposure to methamphetamine did not affect embryo viability. Methamphetamine treatment (concentration of effect) significantly reduced crown-rump length, number of somites, and protein content (≥0.4 mM), developmental score (≥0.6 mM), and yolk sac diameter (0.8 mM). The number of malformed embryos was significantly increased at ≥0.6 mM. The most common malformations included microcephaly, neural tube closure defects, incomplete rotation of body axis, and tortuous spinal cord. Derangement and necrosis in neuroepithelial tissues were observed at 0.8 mM. Based on the observation that a 70 mg/kg bw ip dose results in a peak blood level of <50 µg/mL in rats, the study authors suggested that the methamphetamine concentrations inducing malformations in this in vitro study are likely to be much higher than the plasma level obtained with a single teratogenic dose of 19 mg/kg bw in a mouse study. **[It was not explained how values were extrapolated from rats to mice. According to a study by Acuff-Smith et al.** ***(49)*****, methamphetamine serum levels peaked at 3.1–3.6 µg/mL in rats sc exposed to 40 mg/kg bw/day methamphetamine (Section 2.1.2.2), a dose that caused increased fetal mortality but no increase in external malformations. Methamphetamine blood levels were reported at 0.03–6.3 µg/mL in infants exposed to methamphetamine in utero (see Section 2.1.1.3).]**

Yamamoto et al. ***(185)***, supported by the Japanese government, cultured 9–10 somite Wistar rat embryos (about GD 10) with *d*-amphetamine **[purity not specified]** at concentrations of 0, 0.1, 0.4, 0.8, 1.2, and 1.6 mM **[14, 54, 108, 162, and 216 µg/mL]**. There were 20 control embryos and 10–13 embryos in each of the amphetamine-exposed groups. Embryos were evaluated after 24 hours in culture. There were no apparent adverse effects on embryo development at amphetamine concentrations of 0.8 mM or lower. At 1.2 mM and higher, there was a significant decrease in yolk-sac diameter, crown-rump length, somite number, and protein content, and an increase in the proportion of abnormal embryos. One embryo each in the 1.2- and 1.6-mM groups died during the incubation period. The authors concluded that amphetamine was less potent in producing abnormal embryo development than methamphetamine based on a comparison of the lowest effective concentration (1.2 mM) in this study and the lowest effective methamphetamine concentration (0.6 mM) in their prior study *(184)*, and based on histologic evidence of necrosis in the neuroepithelium at 1.2 mM amphetamine in this study that was comparable in effect to 0.8 mM methamphetamine in the previous study.

Strengths/Weaknesses: Strengths of the in vitro studies by Yamamoto et al. are that they examine the direct effects of amphetamine and methamphetamine on embryonic development during a limited developmental stage and allow comparisons between in vitro and in vivo dosing. The whole embryo culture techniques appeared to be sound and the investigators controlled for initial developmental stage by limiting the study to embryos with 9–10 pairs of somites. A

weakness is that a limited period of development can be studied with embryo culture. There is no indication that the authors controlled for litter effect by dividing embryos from each litter across the various treatment groups. It is unclear why the authors used twice as many embryos in the control group as in the treatment groups (20–26 control embryos vs. 8–14 embryos in the treated groups). Embryos were not scored blind with respect to treatment group. This whole embryo culture system circumvents maternal absorption, distribution, and metabolism, therefore potentially exposing embryos for a longer period of time. Furthermore, the concentrations used in these studies were excessive. The authors estimate that the *in vitro* teratogenic concentration of methamphetamine was much higher than the plasma concentrations that could be achieved with a single teratogenic dose given to ICR mice in a previous study. Furthermore, the *in vitro* teratogenic concentration was greater than the methamphetamine plasma concentration seen in fatal cases of human overdose.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility. However, i*n vitro* studies can be used as supplemental information in the evaluation process.

Won et al. *(186)*, in a study funded by several government and industry grants, examined the effects of methamphetamine in a three-dimensional, rotation-mediated reaggregate tissue culture system. In the assay, single cells from specific fetal brain regions are incubated in a rotation culture system where they could develop and interact with each other. For this study, cells were obtained from the rostral mesencephalic tegmentum and corpus striatum of embryonic mice and cultured in monoamine-free media for 15 days. Methamphetamine was added to the culture at concentrations of 0 or 10^{-7}–10^{-4} M **[14.9–14,900 ng/mL assuming the values given are for methamphetamine free base and not the salt]** for 7 days (culture days 15–22) and then levels of endogenous dopamine and serotonin were measured. Methamphetamine treatment resulted in significant dose-related reductions of dopamine at $\geq 10^{-6}$ M and serotonin at $\geq 10^{-5}$ M. A time-course experiment demonstrated that 10^{-4} M methamphetamine reduced dopamine and serotonin levels following 3 days of exposure, but that no further reduction followed at 5 and 7 days of exposure. A recovery experiment using 15-day-old aggregates exposed to 0 or 10^{-4} M methamphetamine for 7 days demonstrated that dopamine levels rose from 28.8 to 73.7% of control levels and serotonin levels rose from 15.1 to 42.5% of control levels during the first 9 days of recovery. During the next 11 days of recovery, dopamine levels remained at 75% of control values and serotonin levels at ~50% of control values, indicating that complete recovery did not occur. **[Thus, longer term effects on axonal growth and/or cell maturation during the recovery experiments are possible.]**

An earlier study from the same group *(187)* used a tyrosine hydroxylase immunochemistry method to demonstrate that methamphetamine-induced (10^{-4} M) reduction of serotonin in a reaggregate tissue culture was not due to loss of dopamine-containing cell bodies.

Strengths/Weaknesses: A strength of this study is its applicability to mechanism considerations. Other strengths are that prior to culturing neurons, the authors dialyzed the culture sera to remove exogenous serotonin and that experiments were adequately replicated. A weakness is that the embryonic age of the donor animals is not specified. The *in vitro* cell culture system circumvents maternal and fetal absorption, distribution, metabolism, and excretion. The authors mentioned that the concentrations used in this study were similar to peak brain concentrations (1.65×10^{-4} M) achieved during systemic administration of a methamphetamine congener; however, there is

no indication that this value is representative of embryonic brain concentrations, the system examined in these experiments.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility. However, i*n vitro* studies can be used as supplemental information in the evaluation process.

3.2.1.3 Chicken studies

Cameron et al. *(188)*, in a study funded in part by the American Heart Association, examined mechanisms of *d*-amphetamine-induced malformations in White Leghorn chicken embryos. The first stage of the study involved dose range-finding studies for *d*-amphetamine sulfate **[purity not specified]**, alpha-methyl-*p*-tyrosine (a catecholamine synthesis blocker), metoprolol (a beta-adrenergic blocker), and phentolamine (an alpha-adrenergic blocker). **[The rational for selecting doses for further study was not clear.]** In the second stage of the study, embryos were exposed to *d*-amphetamine in combination with one of the other drugs. Normal embryos were exposed to the drugs at 96 hours, incubated for 15 days, and then autopsied under a dissecting microscope. Embryos were exposed to alpha-methyl-*p*-tyrosine 6 hours before *d*-amphetamine treatment, and were exposed to the other 2 drugs 1 hour following *d*-amphetamine treatment. Controls consisted of eggs that were opened but untreated or embryos treated with saline or a hyperosmotic solution to match the osmolarity of *d*-amphetamine. In the mechanistic studies, controls treated with only *d*-amphetamine or *d*-amphetamine and saline were included. Eighteen to 96 embryos were used per group. Data were evaluated by chi-square.

Malformations were increased at *d*-amphetamine doses of ≥0.25 mg/embryo and survival was decreased at *d*-amphetamine doses of ≥0.50 mg/embryo. All malformations involved the heart and great vessels and included abnormal persistence of the left fourth aortic arch and ventricular septal defects. **[The Expert Panel notes that cardiac malformations were consistent with those in the study by Nora *et al. (176)*.]** The 0.25 mg dose was selected for the mechanistic studies; that dose resulted in a malformation rate of ~30%, a level significantly greater than the rate observed in controls (< 10%). The incidence of *d*-amphetamine-induced malformation was significantly reduced to rates of ≤ 10% by co-treatment with alpha-methyl-*p*-tyrosine (0.50 mg, the high dose), metoprolol (0.1 mg, the mid dose), and phentolamine (0.005 mg, the low dose). None of the drugs improved survival following *d*-amphetamine treatment. The study authors postulated that chicken embryos may be able to respond to the alpha- and beta-adrenergic properties of *d*-amphetamine through release of endogenous catecholamines and that the process may have a causal relationship to malformations.

Strengths/Weaknesses: A strength is the evaluation of mechanisms of cardiovascular effects. All embryos were examined to verify that they were at normal stage 24 prior to use in these experiments, and adequate controls were included (i.e., in single-treated experiments: opened-untreated controls, saline-treated controls, and controls treated with Na_2SO_4 to control for the hyperosmotic environment created by 0.25 mg *d*-amphetamine; in double-treatment experiments: untreated, saline + saline, *d*-amphetamine alone, and *d*-amphetamine + saline). Treatment periods for alpha-methyl-p-tyrosine, phentolamine and metoprolol were designed so that the maximum effectiveness of these treatments coincided with *d*-amphetamine treatment. Concentrations used in this study (0.1–1.00 mg = $2.7 \times 10^{-7} – 2.7 \times 10^{-6}$ moles) were reported to be within the concentration range seen in humans in other studies. A weakness is the lack of a maternal system inherent in this experimental model. There is no indication that fetal examiners were blind with respect to treatment group. The hyperosmotic control was designed to mimic the 0.25 mg *d*-

amphetamine group and may have been inadequate to control for the higher *d*-amphetamine exposures (0.5, 0.75, 1.0 mg). Saline-treated controls for single experiments had only a 72% survival rate and those used in double experiments (saline-saline) had only a 64–67% survival rate. Aside from alpha-methyl-p-tyrosine (the catecholamine synthesis inhibitor), both the alpha and beta blockers (phentolamine and metoprolol, respectively) were effective at decreasing *d*-amphetamine-induced malformations. This lack of specificity is unusual. The authors suggest that adrenergic receptors may not be as specific in 4-day-old chick embryos, but there are no data to support this claim. It is not always clear why the selected doses were designated as effective (e.g., the lowest dose of phentolamine was deemed effective at decreasing mortality and malformations; however, the next dose level (0.010 mg), did not affect survival (67 vs. 68% in controls) or malformation rates (10%, a non-significant change). These inconsistencies make the selection of effective doses seem somewhat arbitrary. None of these agents improved embryonic survival.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility but can add supplemental information in the evaluation process.

Cameron et al. ***(189)***, in a study supported in part by the American Heart Association, measured heart rate and mean ventricular blood pressure in 4-day-old White Leghorn chicken embryos exposed to a teratogenic dose (0.25 mg) of *d*-amphetamine sulfate **[purity not specified]**. Eggs containing control embryos were either unopened or injected with saline. A total of 16–34 embryos/group were examined hourly at 1–7 hours following exposure. Heart rate was significantly lower in the amphetamine compared to the control groups at 2, 3, and 5 hours following exposure. Mean ventricular blood pressure was significantly increased by 30% in the amphetamine group compared to the control groups at 4 hours following exposure. The study authors concluded that increased embryonic blood pressure during a critical period of aortic arch and ventricular septum development may be a causal factor for the malformations observed in these structures following amphetamine exposure. **[The Panel notes that increases in mean ventricular blood pressure recorded in chick embryos are consistent with the findings of other investigators, who have shown increases in blood pressure in *d*-amphetamine-exposed fetuses (e.g., sheep studies discussed previously in Section 3.2.1.1).]**

Strengths/Weaknesses: A strength of this study is the addition of a functional dimension to the study of amphetamine effects on the embryonic chicken heart. All embryos were examined to verify that they were at normal stage 24 prior to use in these experiments and adequate controls were used in the experiments (i.e., opened-untreated controls, saline-treated controls, and controls treated with Na_2SO_4 to control for the hyperosmotic environment created by 0.25 mg *d*-amphetamine). The *d*-amphetamine concentrations used in this study (0.25 mg = 6.8×10^{-7} moles) were reported to be similar to human concentrations seen in other studies. A weakness is the lack of a maternal system inherent in this experimental model. Blood pressure analyses were conducted using a one-way ANOVA (not two-way ANOVA). Graph lines in Figure 2 of the study appear to be mislabeled. Furthermore, the authors state that "a significant increase in blood pressure was seen 4 hours after *d*-amphetamine treatment, despite a lower heart rate;" in fact, there was no difference in heart rate in *d*-amphetamine-treated chicks at 4 hours. While the investigators show that blood pressure increased after *d*-amphetamine treatment, they do not establish that this 1-hour increase in ventricular blood pressure is sufficient in magnitude or duration to cause persistent left fourth aortic arch and ventricular septal defects.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility but can add supplemental information in the evaluation process.

Kolesari and Kaplan *(190)* examined amphetamine and methamphetamine effects on development of White Leghorn chicken embryos. Eggs containing embryos at 45–49 hours of development (Hamilton-Hamburger stage 12) were injected subgerminally with 0.5 mg *d*-amphetamine sulfate (n = 17–21/final stage) or 1.0 mg methamphetamine HCl (n = 19/final stage). Control eggs were either left unopened (n = 20–21/final stage) or injected with saline (n = 7–12/final stage). At 24 hours following injection (stage 17 or 18), embryos were examined for hematomas and growth. Statistical significance of effects was determined by Student *t*-test. Compared to either control group, *d*-amphetamine and methamphetamine treatment significantly reduced crown–rump length and cross sectional area of aorta, notochord, neural tube, and whole body. Fewer structures were affected at stage 18 versus stage 17. Embryos treated with *d*-amphetamine and methamphetamine had increased numbers of caudal hematomas.

Strengths/Weaknesses: A strength of this study is the provision of detailed data on growth beyond overall weight. Eggs were selected at stage 12, the maximum time of susceptibility to hematoma formation. Data were presented for both untreated (unwindowed) and saline-treated controls. Treatment with amphetamine and methamphetamine resulted in reductions in embryonic size, a finding reported in other studies as well. A weakness is the lack of a maternal system inherent in this experimental model. This study used only a single dose level, so dose-response comparisons were not possible. Embryos were not scored blind with respect to treatment group. There were no controls for osmotic changes that were shown to occur in chick embryos in the study by Cameron et al. *(188)* (10 µL volume in isotonic saline; 494 mOsm/L for a 0.25 mg dose (6.8×10^{-7} moles) vs. 20 µL volume of sterile saline with 0.5 mg *d*-amphetamine or 1.0 mg methamphetamine in the present study). For crown-rump length, windowing the eggs had a greater impact on size than amphetamine treatment. The authors suggest that proximity of the caudal dorsal aorta to the underlying yolk containing *d*-amphetamine/methamphetamine may contribute to hematoma formation. There are interspecies differences in the proximity of aorta and yolk sac. Caudal hematomas in chick embryos are a precursor to rumplessness in chick embryos and are considered a model of human caudal dysplasia syndrome; however, this syndrome has not been associated with human prenatal amphetamine exposure.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility but can add supplemental information in the evaluation process.

3.2.2 Postnatal development endpoints (non-neurological)

This section presents non-neurological endpoints in experimental animals exposed during prenatal or postnatal development. Testing was conducted to examine neurological endpoints in many of the studies; the neurological findings are discussed in Section 3.2.3. With the exception of one oral exposure study conducted in monkeys, the studies summarized in this section examined rats exposed parenterally. The order of presentation is amphetamine studies followed by methamphetamine studies.

Summaries of unpublished studies on Adderall were provided to the Expert Panel by Shire Pharmaceuticals Group *(171)*. A GLP-compliant juvenile toxicity study in Sprague Dawley rats was performed using amphetamine base-equivalent doses of 0, 2, 6, or 20 mg/kg bw/dose. Animals were given a dose by gavage once/day from PND 7 to 13 and twice/day from PND 14 to

59 (20 animals/group; 10 animals/group were continued for up to an additional 6 days). Body weight gain was decreased in the highest two dose groups and, in females, in the low dose group from PND 7 to 24. Clinical signs were present in all amphetamine-treated groups. Preputial separation and vaginal opening were delayed in the highest dose group. Motor activity was decreased at all amphetamine doses with testing on PND 22 and 47. Mating and fertility were normal. There were no effects of treatment on the proportion of females with regular estrous cycles or on numbers of corpora lutea, implantations, or conceptuses, or on post-implantation loss. Behavioral effects were not described. **[These summaries are noted but were not used by the Expert Panel in the Evaluation Process.]**

Ching and Tang *(191)*, supported by PHS and the Human Growth Foundation, treated pregnant Sprague-Dawley rats by gavage with *d*-amphetamine sulfate **[purity not specified]** in water at 0 (n = 6), 1 (n = 7), 2 (n = 3), or 5 (n = 7) mg/kg bw/day from 3 days after a sperm-positive smear until pups were weaned on PND 25. **[The Expert Panel assumes that pups were reared by their own dams and that culling was not employed.]** Pup weight and length were measured on the day of birth. Sexual development was assessed in female offspring by recording the day of vaginal opening. Offspring were decapitated on PND 25 and hypothalami dissected and homogenized. A methanol extract of two hypothalami per sample was evaporated and stored under refrigeration (6ºC) until tested for growth hormone releasing-activity. **[The authors do not indicate whether the two hypothalami that were combined were from littermates or from animals of the same sex.]** Growth hormone-releasing activity was evaluated by incubation of rat anterior pituitary cell cultures with reconstituted hypothalamus extract and subsequent radioimmunoassay for rat growth hormone.

The 5 mg/kg bw dose of amphetamine caused a reduction in feed and water consumption over an unspecified 2 days **[not specified whether during pregnancy or lactation; no data were provided for the 2 mg/kg bw dose group]**. There was no treatment effect on litter size or pup mortality at birth. Newborn body weight was increased compared to control in offspring in the 1 and 2 mg/kg bw groups, and decreased in the 5 mg/kg bw group. Body weights in the lower 2 amphetamine groups were 106–116% of control, and body weights in the high dose group were 92–97% of control **[estimated from a graph; the graph shows males to weigh less than females in all groups, including the control group; the Expert Panel assumes the graph was mislabeled]**. At 1 week of age, pup mortality was increased in the 5 mg/kg bw group (27.1% of pups in 5 of 7 litters compared to the control rate of 1.5% of pups in 1 of 5 litters). **[Benchmark dose[3] was calculated based on per-offspring analysis: BMD_{10} 3.2 mg/kg bw/day, BMDL 2.2 mg/kg bw/day.]** Vaginal opening was reported to have been delayed in 15% of offspring whose dams were treated with 2 mg/kg bw/day amphetamine **[no data shown for other groups]**. Growth hormone release by cultured anterior pituitary cells was decreased by hypothalamic extracts from the 1 and 5 mg/kg bw amphetamine groups **[data were not presented for the 2 mg/kg bw group]**. The control value (µg growth hormone/mL/2.5 hours; mean ± SEM) was 0.59

[3]Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% confidence interval around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

± 0.06. The value associated with the low-dose amphetamine treatment was 0.32 ± 0.07 and the value associated with the high-dose amphetamine treatment was 0.25 ± 0.03 (n = 8 cultures per treatment group, $P < 0.01$, Duncan test). The authors concluded that chronic administration of "relatively high doses" of amphetamine during pregnancy and lactation can alter the survival, growth, and development, and the hypothalamic growth hormone releasing activity of offspring.

Strengths/Weaknesses: A strength of this study is that rats were exposed orally by gavage (a relevant route) to multiple doses (0, 1, 2, or 5 mg/kg bw/day *d*-amphetamine) from GD 3 to PND 25. Unfortunately, the shortcomings of the study design and data analyses make this study uninterpretable. There are no maternal data on body weight, body weight gains, gestational clinical observations with which to assess maternal toxicity. There is a reference to altered maternal behavior in the discussion that suggests that decreased postnatal survival in the high-dose *d*-amphetamine group was, at least partially, related to altered maternal caregiving. There is no explanation given for the small number of litters (3) in the 2 mg/kg bw *d*-amphetamine group. There is no indication that litter-based statistical analyses were conducted. This is critical as pups from the same litter are not independent samples. The authors mention that data were analyzed using a Student *t*-test or modified *t*-test, which is inappropriate when there are four dose groups. Figure 1 of the study shows the body weights and lengths of newborn offspring ranging in age from 0 to 1 day. It is unclear why body weights were mixed across these 2 days when pups gain weight quickly postnatally. Also, it is not clear why there is a relatively large deviation between male and female body weights and lengths in the water control group, whereas these values are similar in the saline control group. The body weight and length values for the water controls should have been discussed. For vaginal opening, the authors state that 15% of female offspring in the 2 mg/kg bw *d*-amphetamine group showed delayed vaginal opening, but the authors do not define "delayed." Also, females within the same litter are not independent samples. Their ages at vaginal opening should be averaged to yield a litter mean for statistical analyses. With only 3 litters in the 2 mg/kg bw *d*-amphetamine group, it is not clear how the authors determined that 15% had delayed vaginal opening. The age of vaginal opening was not presented for the water controls. The body weights in the water controls vs. the 2 mg/kg bw *d*-amphetamine females at the time of vaginal opening were not reported. Additionally, there are no data for the 1 and 5 mg/kg bw *d*-amphetamine doses, thus there was no opportunity to look for dose-related trends in age at vaginal opening. For experiments examining growth hormone-releasing activity, there is insufficient experimental detail to determine how pups were sampled. The fact that there are 8 animals per group suggests that there was no control for litter effect, as the largest number of litters in the *d*-amphetamine groups was 7. There is no indication as to whether hypothalamic samples were from males or females. Growth hormone-release data were similar for controls in both the presence and the absence of hypothalamic extract. The authors speculate that there is a growth hormone inhibitory activity in the hypothalamic extracts, but offer no supporting evidence. The authors should have provided some context for these control values (e.g., previous growth hormone release values). The fact that growth hormone-releasing activity was decreased in both the 1 mg/kg bw *d*-amphetamine group and 5 mg/kg bw *d*-amphetamine group to nearly the same degree when there were marked differences in pup body weights/sizes on PND 1 merits careful consideration. There were no data for the 2 mg/kg bw *d*-amphetamine group. Body weight data from these groups on PND 25 would have been useful to assist in the interpretation of these data (i.e., Would body weights support decreased growth hormone-releasing activity?). The authors also show that food and water consumption were decreased in 5 mg/kg bw *d*-amphetamine dams over a 2-day period; however, they do not specify which 2 days (gestation?

lactation?). The lack of experimental detail make the feed and water consumption data uninterpretable. Again, 2 mg/kg bw *d*-amphetamine group data are missing.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

Hitzemann et al. *(192)*, supported by US PHS, treated pregnant Sprague-Dawley rats with *d*-amphetamine sulfate **[purity not specified]** 0, 1, or 3 mg/kg bw sc twice daily from GD 5 through parturition on GD 19–22. Litters were culled randomly to eight at birth. Culled pups were "examined for gross pathological abnormalities" **[method of examination not otherwise specified]**. The number of treated dams was not given. **[There were 80 control, 88 1-mg/kg bw, and 56 3-mg/kg bw pups followed from PND 4, implying that 10, 11, and 7 dams, respectively, contributed litters to the experiment.]** Litter size and weight were said to be similar among groups and there were no gross malformations **[data not shown]**. The authors noted 25% pup mortality in the 3-mg/kg bw amphetamine group, compared to 15% in the 1-mg/kg bw group and 6% in the control group **[percent mortality estimated from a figure]**. None of the dead pups was abnormal on necropsy. To evaluate the possibility of lactation abnormality, 24 pups with antenatal exposure to amphetamine **[dose not specified]** and 24 control pups were cross-fostered to dams of the opposite treatment group **[number of dams and number of pups/litter not specified]**. Mortality was 25% among pups with antenatal amphetamine exposure and 8.3% among pups without antenatal amphetamine exposure. **[The data suggest that altered maternal caregiving was not the primary factor in decreased neonatal survival.]** The authors concluded that because neonatal mortality is not noted among humans with antenatal exposure to amphetamine, the effect may be peculiar to rats.

Strengths/Weaknesses: Strengths include use of relatively low doses (1 and 3 mg/kg given twice daily) targeted to be pharmacologically relevant and relatively non-toxic and the cross-fostering design to determine whether altered maternal caregiving contributed to neonatal mortality. A weakness is that rats were dosed with *d*-amphetamine by sc injection, while humans typically are exposed by oral or iv routes. It is unclear whether dams were dosed from GD 5 to GD 19–22 (as stated in the text) or from GD 5 to GD 19–20 (as stated in the legend for Figure 1). There were no data on gestation length or whether altered gestation length contributed to increased neonatal mortality. Although some summary statements were made, data on maternal body weights, body weight gains, clinical signs, litter sizes, and litter weights were not presented. Litters were randomly adjusted to eight pups, but there is no indication that there were attempts to balance gender within the litters (e.g., four males and four females). Presenting pup body weights as "percent change in pup weight from control" is unusual and difficult to interpret, particularly given that the amphetamine-exposed pup body weights fluctuate above and below the control mean and there is no information on variance. For biogenic amines and motor activity, there is no information about sample selection (i.e., one male and one female/litter/dose group at the appropriate ages?). There is no indication that the authors controlled for either litter or gender bias. Biogenic amines and motor activity were only examined in animals from the 0 and 3 mg/kg bw/day dose levels, so dose-response relationships could not be evaluated. Statistical analyses were not well defined. Student *t*-test is mentioned, but this would not be appropriate for variables collected at all three dose levels. There is no indication that analyses were litter based.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited adequacy for use in the evaluation process.

Vorhees et al. *(193)*, of NCTR and the Cincinnati Children's Hospital Research Foundation, administered *d*-amphetamine sulfate **[purity not specified]** to pregnant Sprague-Dawley rats at 0 (n = 12), 0.5 (n = 11), or 2.0 (n = 10) mg/kg bw/day sc on GD 12–15 (plug = GD 0). There was also an uninjected control group (n = 13). Litters were culled to 8 (4/sex) on PND 1 and weaned on PND 21. The primary purpose of the study was to evaluate offspring for neurodevelopmental endpoints, reported in Section 3.2.3 (Table 39). Litter parameters were analyzed by ANOVA, except for frequency data, which were analyzed by Fisher test.

There were no significant effects of treatment on gestation length, dam body weight during pregnancy or lactation, or offspring weight pre- or post-weaning. The number of pups born per litter was significantly reduced in the amphetamine groups at $P = 0.06$ (0 mg/kg bw: 13.8 ± 0.4; 0.5 mg/kg bw: 12.4 ± 0.6; 2.0 mg/kg bw: 12.2 ± 0.5; mean ± SEM **[trend test by CERHR gave $P = 0.03$; BMD_{10} = 2.0 mg/kg bw/day, BMDL 1.1 mg/kg bw/day[4]]**). Post-weaning pup mortality was increased in the 0 mg/kg bw group (5.2%) and the 2.0 mg/kg bw group (3.4%); this finding was not considered drug related. Sex ratio was decreased to 0.52 in the low-dose amphetamine group as a result of fewer males. There were no significant drug effects on incisor eruption or vaginal patency. Eye opening was delayed a mean of 0.5 days in the low-dose amphetamine group. The 2.0 mg/kg bw dose had no effect on eye opening.

Strengths/Weaknesses: This study is well conducted with a strong design and litter-based analysis. Other strengths are the low doses and the provision of some physical maturation data. Sample sizes were adequate and proper statistical analyses were employed. A weakness is that rats were dosed with *d*-amphetamine by sc injection, while humans typically are exposed by oral or iv routes.

Utility (Adequacy) for CERHR Evaluation Process: This study is very useful in the evaluation process.

Martin *(194)*, supported by NIH, treated pregnant Sprague-Dawley rats with methamphetamine HCl **[purity not specified]** 0, 1, 3, or 5 mg/kg bw sc twice/day from GD 1 (the morning after insemination) to GD 21. **[Total daily doses were 2, 6, or 10 mg/kg bw.]** There were six dams/group except in the high-dose group, which included seven dams. Litters were culled to 8 **[sex not specified]** on PND 7. Behavioral effects, which were the focus of the paper, are summarized in Section 3.2.3 in Table 39. Four of 7 dams in the 5 mg/kg bw group failed to deliver and 1 dam in this dose group delivered only 1 pup. One 3 mg/kg bw dam cannibalized her litter. Maternal weight gain and litter size were decreased by treatment. Gestation length was decreased by a mean 0.5 days by all doses of methamphetamine. The percentage of pups with eyes open on PND 14 was decreased by methamphetamine treatment **[analyzed on a per fetus basis]**. The percentages with eyes open were 71% in the control group, 38.8% in the 1 mg/kg bw group, 17.5% in the 3 mg/kg bw group, and 25% in the 5 mg/kg bw group (which contained only

[4] The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% confidence interval around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

2 litters). **[Many of the effects observed in this study have been reported above for methamphetamine (e.g., decreased maternal weight gain, decreased litter size, delayed eye opening and decreased number of litters at high doses). There were no gross malformations in surviving offspring in this study. Other findings (e.g., decreased gestation length) were novel. Pair-wise comparisons with the control group were not reported for fetal effects.]**

Strengths/Weaknesses: A strength is the provision of physical maturation data. Another strength is that the dosing interval (twice daily) was selected based on the half-life of amphetamines in humans, which is 14 hours. A weakness is the small group size (n=6–7 dams/dose level) and limited statistical analysis. Statistics were used to identify differences between saline-treated control animals and methamphetamine-treated animals; however, post hoc comparisons were not conducted to determine which dose levels of methamphetamine were significantly different from the controls. It is not clear whether litter-based analyses were used when needed (although some variables such as birth weights were analyzed by litter). Rats were dosed by sc injection, while humans typically are exposed by oral or iv routes. The doses of methamphetamine were based on GD 1 body weights and were not adjusted for increasing body weights during pregnancy.

Utility (Adequacy) for CERHR Evaluation Process: This study is somewhat adequate for use in the evaluation process.

Martin at al. *(195)*, supported by NIH and March of Dimes, treated pregnant Sprague-Dawley rats with methamphetamine HCl **[purity not specified]** 0 or 5 mg/kg bw given sc twice/day from GD 1 (the morning after insemination) to GD 21 and PND 2–21. **[CERHR assumes that the total daily dose was 10 mg/kg bw.]** There were 25 dams treated with methamphetamine, 13 dams injected with saline, and 13 uninjected controls. Litters were culled to 8 (males preferred) on PND 7, and weaned on PND 21. Behavioral tests were performed using 1 male/litter (summarized in Table 39 in Section 3.2.3). Methamphetamine-exposed dams gained less weight during pregnancy than either control, and neonates were lighter on PND 7, 14, 21, and 28, and through the end of the experiment at 16 months of age. Mean birth weight was numerically lower **[statistical significance could not be evaluated from the information given]**. Gestation length was decreased. The percentage of pups with ear opening on PND 4 and incisor eruption on PND 7 was not affected by treatment. There was a delay in eye opening assessed on PND 14.

Strengths/Weaknesses: A strength is the extended treatment and evaluation period, with dosing interval (twice daily) selected as a realistic exposure scenario. The litter was the unit for statistical analyses and adequate sample sizes were used. This study monitored rats into adulthood. A weakness is the use of a high single dose administered through sc injection, while humans typically are exposed by oral or iv routes. With only one dose level, dose-response relationships cannot be evaluated. The doses of methamphetamine were based on GD 1 body weights and were not adjusted for increasing body weights during pregnancy.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited adequacy for use in the evaluation process.

Martin et al. *(196)*, in a study supported by NIA/NIH, examined lifespan and pathology of rats exposed to either methamphetamine or nicotine in utero. Twenty-five Sprague-Dawley rats were sc injected twice daily with 5.0 mg/kg bw methamphetamine HCl **[purity not specified]** during 21 days of gestation and the last 19 days of lactation. **[CERHR assumes that the total daily**

dose was 10 mg/kg bw.] Thirteen control dams were injected with the saline vehicle and another group of 13 dams was not injected. Rats receiving 3 mg/kg bw nicotine were also examined. The dams were allowed to deliver their litters. On PND 4, litters were culled to 8 pups, maintaining as many males as possible. Statistical analyses included Kruskal-Wallis rank test for lifespan and Kendall Tau Correlations and ANOVA for organ histopathology. Control and treated offspring were placed into subgroups for an examination of lifespan and pathology endpoints *(196)*. Mortality was unaffected by methamphetamine treatment in one set of offspring (n = 12/treatment group), the body weights of which were reduced to 80% of their normal weight over a 2-week period. No treatment-related differences in lifespan were observed in a group (n = 15) that was fed ad libitum, but methamphetamine treatment resulted in the steepest death curve. Methamphetamine-treated rats remained lighter throughout most of the adult period until 16 months of age. In an "autopsy" study, four rats/group were randomly selected for necropsy with histological evaluation of brain, heart, lung, liver, spleen, kidney, and adrenals when an animal from the same group died. Sixteen animals were necropsied in each group. Incidence and severity of pathology and lifespan were similar in methamphetamine and control groups. **[The study authors made some observations about tumors in the autopsy studies. However, CERHR notes that the study was not designed to examine tumorgenicity and the observations were complicated by the sacrifice of animals at different time periods, as also noted by study authors.]**

Strengths/Weaknesses: Strengths include the extended evaluation period and the use of standard experimental methods. This is the only study to examine the long-term effects of in utero and neonatal methamphetamine exposures. In many cases (necropsy, pathology reports, etc.), animals were coded so that evaluations were conducted blind to treatment group. A weakness is the limited evaluations (e.g., mortality and some organ weights). Rats were dosed with methamphetamine at a single dose level (5.0 mg/kg bw/day) by sc injection, while humans typically are exposed by oral or iv routes. With only one dose level, dose-response relationships cannot be evaluated. The doses of methamphetamine were based on GD 1 body weights and were not adjusted for increasing body weights during pregnancy. This study was somewhat difficult to interpret because of the variety of ages at which the animals died or were necropsied. Despite this, no alterations in lifespan, organ pathology, or tumor incidence were definitively attributed to methamphetamine treatment.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited adequacy for use in the evaluation process.

Cho et al. *(197)*, of the Korean National Institute of Safety Research, treated pregnant Wistar rats with methamphetamine HCl **[purity unspecified]** at 0, 1, 2, 3, or 4.5 mg/kg bw/day sc on GD 7–20 (n = 13 or 14/dose group). Caffeine 90 mg/kg bw/day was administered as a positive control. At birth, pups were examined for external malformations **[not further discussed and results not given]**. On PND 4, four pups/sex/litter were selected for evaluation of the acquisition of physical developmental landmarks and for behavioral testing (summarized in Table 39 in Section 3.2.3). **[It is not stated whether the unselected pups were retained in the litter or culled; the Expert Panel assumes they were culled.]** The male and female offspring were mated after the 14th week postpartum and evaluated for reproductive performance **[evaluation methods unstated]**.

Maternal body weight gain during gestation was suppressed at ≥2 mg/kg bw methamphetamine. Only one high-dose dam delivered live pups. Male offspring body weight gain during lactation

and post-weaning was decreased in the 3 and 4.5 mg/kg bw groups. There was no methamphetamine treatment effect on age at pinna detachment or abdominal hair appearance. Incisor eruption and eye opening were delayed in the 3 mg/kg bw group; the magnitude of the delay was a mean of 0.1–0.2 days. Testicular descent was delayed a mean of 1.2 days in this group. Day of vaginal opening was not affected. Results are summarized in Table 37. **[The Expert Panel notes that only eyelid opening was affected in the 4.5 mg/kg bw group; however, because only 1 litter was produced in this dose group, the findings in this group are not useful.]** Mating performance of male and female offspring was not affected by treatment **[data not shown]**. The authors concluded that prenatal exposure to methamphetamine was associated with growth impairment and developmental delay. **[Delays in eye opening, negative geotaxis reflex, mid-air righting reflex, incisor eruption, and testis descent are consistent with the decreased body weight gain in methamphetamine-treated dams and subsequent decreases in F_1 male body weights at these dose levels. Many of the effects observed in this study have been reported previously by Martin et al. *(194, 195*). Transient, but significant, decreases in motor activity and latency time for development of avoidance responses were seen at 2.0 and 4.5 mg/kg bw/day methamphetamine; however, dose-response relationships for these parameters were not maintained at 3.0 mg/kg bw/day and effects were not significant at this dose level. Total distances traveled were decreased at 2.0, 3.0, and 4.5 mg/kg bw/day.]**

Table 37. Dose Levels Affecting Physical Development in Rats Exposed Prenatally to Methamphetamine

Response description	Incisor eruption	Eye opening	Testicular descent
	dam dose (mg/kg bw/day sc) on GD 7–20		
NOAEL	2	2	2
LOAEL	3	3	3
BMD_{10}	5.1	15.7	3.8
BMDL	3.1	3.2	3.3

NOAEL: No observed adverse effect level. LOAEL: Lowest observed adverse effect level. BMD_{10}: Dose corresponding to a 10% increase in response over control, calculated from fitted dose response curve (power model). BMDL: Dose corresponding to lower bound of the 95% CI around the 10% response. BMD calculations by CERHR using EPA Benchmark Dose software version 1.3.2. **[Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.]**
Data from Cho et al. *(197*).

Strengths/Weaknesses: Strengths include good physical maturation data, including data on puberty. Sample sizes were adequate (n=13 or 14 dams/dose level). A weakness of this study is the lack of detail on mating evaluation. In contrast to other studies, rats were dosed once per day with methamphetamine. Rats were dosed by sc injection, while humans typically are exposed by oral or iv routes. Breeding was somewhat confounded by pairing one male with two study females. The authors do not provide any information on the types of statistics used to analyze their data, nor do they describe any methods to control for litter effects. The 4.5 mg/kg bw/day methamphetamine dose level exceeded the MTD as there was only 1 live litter from the 13 dams in this group. The limited group size (n=1 litter) at the highest dose likely accounts for the lack of

dose-response with some of the endpoints (e.g., maternal body weights, age at incisor eruption, age at testis descent, and the acquisition of avoidance response).

Utility (Adequacy) for CERHR Evaluation Process: This paper is of limited adequacy for use in the evaluation process.

Vorhees and Acuff-Smith *(198)* reported in a Letter to the Editor that preliminary experiments identified anophthalmia associated with prenatal methamphetamine exposure on GD 7–12 at a maternal dose of 50 mg/kg bw sc twice/day. Among the 3 litters treated on these days, 45.5–88.9% of pups had eye abnormalities (almost all anophthalmia). The authors stated that there were no affected litters among the 4 dams treated with this dose regimen on GD 13–18. A subsequent, more complete report of this observation was published as **Acuff-Smith et al. *(199)***, supported by PHS. According to the full report, pregnant Sprague-Dawley rats were treated with *d*,*l*-methamphetamine hydrochloride **[purity not given]** 0 (n = 6) or 50 (n = 15) mg/kg bw twice daily by sc injection from GD 7 to GD 12 or from GD 13 to GD 18 (plug = GD 0). The 50-mg dose was expressed as the free-base equivalent. The control animals were pair-fed to the methamphetamine animals. With methamphetamine treatment, maternal weight gain was reduced, 2 of 15 dams died during dosing, 2 litters were completely resorbed, 1 litter died on the day of delivery, and 8.6% of live-born pups died during the lactation period. There were no control dam or offspring deaths. There were no alterations in gestation length, litter size, or sex ratio. Live-born pups were raised by their dams and weaned on PND 28. Two males and two females from each litter were killed on PND 28 and their brains removed. Striatum and hippocampus were dissected and frozen for later determination of monoamines as summarized in Section 3.2.3, Table 40. The remainder of the pups were used for behavioral testing, as summarized in Table 39. Offspring were evaluated for eye abnormalities at the time of unscheduled death, at sacrifice on PND 28, or at the end of the experiment (PND 98). Eye defects were reported in 19/114 offspring (16.7%) with prenatal methamphetamine exposure and in 0/85 control offspring. Eight of the defects were anophthalmia or microphthalmia and 11 of the defects were folded retinas in eyes not affected by anophthalmia or microphthalmia. **[Possible litter effects were not addressed, nor were eye defects analyzed with regard to the degree of dam toxicity. This study is presented here because it appears preliminary to the study that immediately follows. Anophthalmia seen in offspring of dams exposed on GD 7–12 but not of dams exposed on GD 13–18 is consistent with the critical period for eye development *(200, 201)*.]**

Strengths/Weaknesses: A pair-fed control was included in the full study report *(199)* and proper statistical analyses were used. Blood samples were collected from half the dams per group on GD 12 to assess methamphetamine and amphetamine levels. A weakness is that rats were dosed with *d,l*-methamphetamine at a single dose level (50 mg/kg bw/day) by sc injection, while humans typically are exposed by oral or iv routes. With only one dose level, dose-response relationships cannot be evaluated. The 50 mg/kg dose exceeded the MTD as evidenced by the loss of 5 of 15 litters (2 maternal deaths, 2 totally resorbed litters, and 1 total litter loss that occurred postnatally). The methamphetamine blood levels were bimodally distributed and are discussed in the context of litter outcome (i.e., footnotes in Table 2 of the study). While the levels on GD 12 may be relevant to litter resorptions, this may not be the most critical gestation stage for methamphetamine-induced fetal loss. Furthermore, there is no indication that methamphetamine levels on GD 12 are representative for other stages of gestation. Inclusion of ad libitum controls would have been useful, particularly for neurobehavioral assessments.

Utility (Adequacy) for CERHR Evaluation Process: The full study *(199*) is of limited usefulness in the evaluation process.

Acuff-Smith et al. *(49*), supported by NIH, treated pregnant Sprague-Dawley rats with *d*-methamphetamine (free base **[purity not given]**) 0, 5, 10, 15, or 20 mg/kg bw twice daily by sc injection from GD 7 to GD 12 or from GD 13 to GD 18 (plug = GD 0). **[Following the authors' practice, the treatment groups are identified here by the amount of methamphetamine in each sc dose rather than by the daily dose, which was twice the individual sc dose.]** Rats in the 15 and 20 mg/kg bw groups and one 0 mg/kg bw group were fed and watered ad libitum. Another 0 mg/kg bw group and the 5 and 10 mg/kg bw groups were pair-fed and watered using the rats in the 15 mg/kg bw group as a reference. There were 14–16 litters/group, except for 12 litters in the ad libitum control group. Litters were standardized on PND 3 to 8 pups, balanced for sex. Litters were weighed weekly and weaned on PND 28. One male and female from 8 litters each in the 20 mg/kg bw group and both control groups were decapitated on PND 70. Serial sections 2 mm apart were taken from the brain and specific brain regions dissected (medial frontal cortex, caudate-putamen, nucleus accumbens, hippocampus). Sections were frozen until assayed by HPLC for dopamine, serotonin, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindole-3-acetic acid, and homovanillic acid (See Section 3.2.3, Table 40). Behavioral testing was performed using the remaining offspring (discussed in Section 3.2.3, Table 39). The adult offspring and culled pups from the 20 mg/kg bw and both control groups were evaluated for eye defects. Data were evaluated using fixed-effect factorial analysis of variance with post hoc Duncan multiple range testing. A satellite group of pregnant animals at the high dose was evaluated for serum methamphetamine and amphetamine concentrations (discussed in Section 2.1.2.2).

All dams had a decrease in body weight compared to ad libitum-fed and watered control animals during the treatment period. Maternal mortality was increased and gestation length increased in the 15 and 20 mg/kg bw groups treated on GD 13–18. Gestation length was also increased in the 10-mg/kg bw group treated on GD 13–18. Post-weaning offspring body weights showed no consistent difference by group after GD 7–12 exposure, but showed continued decreases in the two high-dose groups after GD 13–18 exposure. Litter size was decreased at all dose levels of methamphetamine given on GD 13–18 in comparison with both control groups. On a per fetus basis, stillbirth and postnatal mortality (PND 1–3) were increased in 20 mg/kg bw group after exposure of GD 7–12 and were increased in the 10, 15, and 20 mg/kg bw groups after treatment on GD 13–18. The proportion of offspring with anophthalmia or microphthalmia was numerically higher on a per fetus basis after GD 7–12 exposure in the 15 and 20 mg/kg bw groups, but there were no statistically significant differences. The frequency of folded retina was increased in the 20 mg/kg bw groups after exposure on GD 7–12 and 13–18, but the frequency in the earlier exposure period was similar to that in the pair-fed and watered control. Neurochemical data showed no significant differences in the 20 mg/kg bw group animals compared to the pair-fed and watered controls, although there were several significant differences compared to the ad libitum control group. **[The Expert Panel notes that the authors conclude that dopamine was reduced in the caudate nucleus in female offspring exposed on GD 13–18, but that the data table does not support this conclusion.]**

The developmental endpoints for which data were suitable for benchmark dose[5] analysis are shown in Table 38 **[benchmark dose calculations performed by CERHR using US EPA benchmark dose software]**. The authors noted that the 15 and 20 mg/kg bw groups experienced significant maternal toxicity and associated effects on offspring viability. Their principle conclusions were directed to the behavioral test results, summarized in Table 39.

Strengths/Weaknesses: This is a well conducted and comprehensive study. Strengths of this study include use of multiple doses within the range of realistic human exposures, a design that evaluated sensitive windows for specific effects (e.g., eye development and dopamine and serotonin neurotransmitters in the brain), use of pair-fed and watered controls, and the uniqueness of the folded-retina data. Sample sizes (12 in *ad-libitum* controls, 14–16 dams in other groups) were adequate. Pups from each litter were assigned to the various test methods and proper statistical analyses were employed. Pharmacokinetics (time course at 0.5, 0.75, 1, 2, and 8 hours post-treatment and peak concentrations) of *d*-methamphetamine and amphetamine were determined. To facilitate data interpretation, the 5 and 10 mg/kg bw methamphetamine groups were matched to the pair-fed controls. Aside from some developmental parameters, a comprehensive assessment of neurobehavioral endpoints and a pharmacological challenge with methamphetamine or fluoxetine were conducted. The data support the authors' conclusions. Weaknesses are that rats were dosed by sc injection, and only the control and high-dose methamphetamine groups were tested on the water maze.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for use in the evaluation process.

[5] Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% confidence interval around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

Table 38. Benchmark Dose Estimates from Acuff-Smith et al. *(49)*

Treatment period (GD)	Endpoint	Benchmark dose (mg/kg bw/day)	
		BMD_{10}	BMDL
7–12	Stillborn per number pups born	91	58
	Postnatal mortality per live-born pup (PND 1–3)	48	40
	Anophthalmia/microphthalmia per live pup	48	42
13–18	Dam mortality	27	20
	Gestation length	112	75
	Litter size	38	20
	Stillborn per number pups born	36	31
	Postnatal mortality per live-born pup (PND 1–3)	53	40
	Maternal body weight on GD 19 (estimated from graph)	91	56

Pair-fed and watered group was used to represent 0 mg/kg bw/day exposure. Data are expressed as total daily dose, which represents twice the amount of methamphetamine in each of the twice-daily sc injections. BMD_{10}: Exposure level associated with a 10% response, estimated from a mathematical dose–response model. BMDL: Exposure level associated with the lower bound of the 95% CI around the BMD_{10}. EPA software version 1.3.2.

Williams et al. *(202)*, supported by NIH, investigated a possible role for the hypothalamic-pituitary-adrenal axis in mediating the developmental neurotoxicity of methamphetamine in neonatal Sprague-Dawley rats. Animals were derived from litters standardized on PND 1 to 10 pups, with 4–6 pups of each sex. One or two pups/litter were fostered if necessary to give at least four pups/sex/litter. Treatments consisted of 4 daily sc injections of 0 or 15 mg/kg bw methamphetamine (free base **[purity not specified]**) on PND 11, 11–15, or 11–20, ages during and after the stress hyporesponsive period, which lasts from PND 0–14. A handled but uninjected control was also used. On the last day of each treatment period, 1 animal/sex/litter was decapitated 15 minutes before or 15, 30, or 60 minutes after the fourth injection of the day, and trunk blood was collected for measurement of corticosterone and adrenocorticotropic hormone (ACTH). Brains were dissected and bilateral hippocampus weights were recorded. Ten litters were used for each of the 9 conditions (methamphetamine, saline-control, and uninjected control for each of the 3 treatment periods: PND 11, PND 11–15, or PND 11–20). Methamphetamine-treated pups were lighter than either control beginning on PND 13. Relative but not absolute hippocampus weight was increased by methamphetamine treatment **[statistical analysis by CERHR showed a difference in relative hippocampus weight only on PND 20]**. Plasma corticosterone was increased by methamphetamine treatment at all ages, although the PND 15 and 20 pups did not show this increase until 30 minutes after the injection. ACTH was also increased by methamphetamine treatment at all ages; however, there were several time points on PND 15 and 20 when ACTH and corticosterone results were discordant, particularly among females. The authors postulated that the adrenal gland may have been sensitive to small changes in ACTH (resulting in large changes in corticosterone in the absence of statistically detectable changes in ACTH) or the corticosterone levels may have suppressed the ACTH plasma levels. The authors concluded that changes in the hypothalamic-pituitary-adrenal axis may represent a mechanism for the long-term changes in spatial learning and memory associated with neonatal methamphetamine exposure.

Strengths/Weaknesses: A strength is that the study included two control groups: a non-injected handheld group and a saline-injected control. The litter was the unit of analyses for statistical purposes. A weakness of this study is the small number of endpoints evaluated. Rats were dosed with *d*-methamphetamine at a single dose level by sc injection, while humans typically are exposed by oral or iv routes. With only one dose level, dose-response relationships cannot be evaluated. Some cross-fostering of pups was performed, making it difficult to control for litter effects. Elevations in ACTH do not always mirror increases in corticosterone (e.g., P20 at 60 min). Authors targeted postnatal hippocampal development in rats as a period comparable to hippocampal development during the third trimester in humans; however, there is no evidence that the pharmacokinetics of third trimester maternal/fetal exposures are the same as sc exposures in rat pups at 2-hour intervals. Furthermore, the authors adopted the procedure of dosing rats 4x/day instead of 2x/day to induce greater neurotoxicity for the same overall dose; however, the critical element is the exposure scenario which most mimics the human situation.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited adequacy for use in the evaluation process.

Courtney and Valerio *(203)* examined developmental toxicity of methamphetamine HCl in *Macaca mulatta* monkeys in a study supported by the A. H. Robins Co. Additional drugs were examined and sponsored by other companies. Monkeys were mated during the 10^{th}–14^{th} day of the menstrual cycle, as determined by daily vaginal swabs. When pregnancy was obtained, the 12^{th} day of the menstrual cycle was considered GD 1. Five monkeys were given 0.5 mg/kg bw/day methamphetamine HCl **[purity not specified]** from implantation to term (from about GD 11 to GD 167). **[Medications were administered in honey, a sugar cube, or by gavage, but the particular method used for methamphetamine was not specified. There was no mention of control colony treatment.]** The monkeys were allowed to give birth, although some required cesarean section **[not specified by treatment]**. Upon birth, infants were examined for general state of health, growth, and development.

With the exception of 1 monkey that lost 0.4 kg, all pregnant monkeys gained weight during the study. Periodic hematologic evaluations during pregnancy revealed all values to be within normal ranges. Four normal births and one autolyzed stillbirth occurred. Noting a 12% rate of abortion and stillbirth in their control colony, the authors concluded that the stillbirth may not have been related to methamphetamine treatment. Gestation lengths and birth weights were within normal ranges. Growth and development was normal in the four surviving infants. At 2–6 months of age, infants were killed, and macroscopic and microscopic examinations revealed normal tissues and organs. **[Methods were not specified. With the exception of birth weight, no data were presented. There was no evidence of teratogenicity in the surviving offspring.]**

Strengths/Weaknesses: Weaknesses include small sample sizes and no mention of a concurrent control group. Monkeys were given a single dose level of methamphetamine, so dose-response relationships cannot be evaluated. One monkey's offspring was stillborn, but it cannot be determine whether this is related to methamphetamine treatment or part of the spontaneous background of abortions and stillbirths in this laboratory (~12%). The dose level used in this study, 0.5 mg/kg bw/day, is low compared to estimated human exposures.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited usefulness for the CERHR process.

3.2.3 Developmental Neurotoxicity

Amphetamine developmental rat studies with neurobehavioral outcomes are summarized in Table 39. Studies in which treatments were given to the dam during gestation involved sc administration of amphetamine or methamphetamine on GD 12–15 *(174, 193, 204-206*), GD 7–12 *(49, 199*), GD 13–18 *(49*), or during all or most of the gestation period *(192, 194, 197, 207-211*). In one study *(212*), the treatment period during gestation was not specified, and the study is included only for completeness. The treatments in many of the studies resulted in significant decreases in maternal weight gain, associated with a decrease in maternal feed intake. One study reported loss of maternal weight during the treatment period among methamphetamine-treated animals and pair-fed controls *(49*). Some studies reported adverse effects of treatment on pup birth weight *(213*) or viability *(49, 192, 199*). One study was reviewed but not included in the table due to very low delivery rates among the animals, including the controls *(214*).

Results of behavioral studies after prenatal exposures generally suggest developmental delay *(193, 194, 197, 199*) and increased motor activity *(49, 174, 192, 199, 208*). One study *(49*) found prenatal exposure to methamphetamine to be associated with impairment of learning and memory in adult offspring. The lowest effect level reported with gestational treatment of the dam was 0.5 mg/kg bw/day for both *d*-amphetamine *(174*) and for *d*,*l*-amphetamine *(207, 208*).

Studies involving direct treatment of rat neonates have used methamphetamine administered sc on PND 1–10 *(170, 215, 216*) or PND 11–20 *(48, 78, 170, 215, 217-221*). Both administration periods have been associated with a decrease in pup weight compared to vehicle-treated controls. The decrement in pup weight persisted until at least PND 42 and in some cases until PND 70. Pup mortality during the treatment period was also increased by neonatal methamphetamine treatment in many of the studies *(48, 78, 170, 215-217*). Treatment of neonates was associated with increased reactivity on acoustic startle testing and with deficits in associative processes and memory when animals were tested as adults. The lowest effective dose reported for neonatal treatment with *d*-methamphetamine was 30 mg/kg bw/day *(48*). **[The Expert Panel notes that studies involving direct treatment of neonates typically used a single methamphetamine dose level and a vehicle-treated control. No attempt was made to ascertain a NOAEL in these studies.]** The memory deficits in adult rats after treatment of PND 11–20 pups involve spatial tasks such as locating a submerged escape platform in a swimming tank *(48, 49, 78, 215, 217, 221*). The deficits have been shown to be due to impairments in reference (long-term) memory and not to general motor or cognitive impairment or to deficits in working (short-term) memory, and have been attributed to alterations in hippocampus development that would be expected to correspond to developmental events in the third trimester of human pregnancy. Treatment of young rats with amphetamine or methamphetamine is also associated with behavioral sensitization to subsequent challenge with amphetamine or methamphetamine. This sensitization can persist for months after the last treatment (reviewed in Section 2.5.3).

There were three reports in which methamphetamine *(195, 222*) or *d*-amphetamine *(213*) were given during gestation and continued through the lactation period. The two methamphetamine reports, which present different endpoints from the same experiment, involved sc treatment of dams. The amphetamine study used administration in drinking water **[probably resulting in direct treatment of pups during the last week of the lactation period]**. Adverse effects on dam and pup body weight were noted in these studies, and both studies showed developmental delay

and increased motor activity in offspring, consistent with the studies that used only gestational exposures. Effective doses were 2 mg/kg bw/day *d*-amphetamine **[the lower of 2 dose levels used]** and 10 mg/kg bw/day methamphetamine **[the only dose level used]**.

Several studies evaluated persistent anatomical or biochemical changes in the brains of rodents after prenatal or juvenile exposure to amphetamine or methamphetamine and those studies are summarized in Table 40. With the exception of a drinking water exposure study *(223*), exposures occurred through parenteral routes, mainly sc. Chemical purity was not reported in any of the studies.

As discussed in Section 2.5.3, treatment of young (preweanling) animals is associated with persistent changes in behavior, with little if any anatomic alteration in the brain. However, other studies summarized in Table 40 reported a transient increase in prefrontal cortex neuronal density in 14-day-old rats exposed to 10 mg/kg bw/day amphetamine on GD 8–22 *(224*) and reduced hippocampal volume formation in 30-day-old male rats exposed to 25 mg/kg bw/day amphetamine on PND 1–30 *(225*). Increases in dendritic length and branching and spine density of pyramidal cells of the prefrontal cortex were reported in 90-day-old male gerbils that received 50 mg/kg bw methamphetamine on PND 14 *(226*). Behavioral effects were not reported in these studies.

Most of the studies examining biochemical effects focused on measurement of monoamine levels in the brain. Three of the studies were conducted in mice *(227-229*) and the rest in rats *(199, 223, 224, 230-234*). Studies of both amphetamine and methamphetamine in rats and mice demonstrated variable effects on brain levels of dopamine, norepinephrine, and serotonin. One study demonstrated a reduction in serotonin-mediated renin secretion following prenatal methamphetamine exposure *(235*) and other studies demonstrated inconsistent changes in dopamine receptor density following postnatal exposure to amphetamine *(231*) or methamphetamine *(232*). Direct comparison of studies examining monoaminergic endpoints is precluded by differences in species, dose level and duration, developmental period of exposure, age of evaluation, and brain region examined. No obvious patterns were noted.

A series of studies from Crawford and colleagues examined effects on protein kinase A, a critical enzyme in signal transduction cascades of several different receptors, including dopamine receptors *(231, 232, 236*). Prenatal exposure to amphetamine was shown to reduce protein kinase A activity in immature and adult rats, and prenatal methamphetamine exposure was shown to reduce protein kinase A activity in adults. Other studies demonstrated changes in brain GABA levels *(230*), reduced density of brain alpha adrenergic receptors *(237*), and changes in nerve conduction *(238*) following prenatal amphetamine exposure. Changes in brain tyrosine hydroxylase mRNA expression following prenatal methamphetamine exposure were also reported *(239*). These findings are not known to have been replicated by other investigators.
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Table 39. Developmental Neurotoxicity Testing of Amphetamines in Rats

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
		Gestational Exposure of Dams		
Long-Evans hooded. Pregnant animals (n = 20) treated once ip with epinephrine, 3.0 mg/kg bw *d*-amphetamine sulfate **[purity not given],** or saline on either one of GD 6–9 or 12–15 **[number of animals in each treatment and time group not given]**. Dams and offspring left undisturbed until PND 45.	Body weight was said not to vary by treatment **[possibly referring to offspring body weight on PND 45]**. *Open-field activity (PND 45)*: Amphetamine-exposed animals were said to enter fewer squares when tested before and after a 48-hour immobilization stress. **[Date presented as mean number of squares without indication of variance or n and without statistical analysis.]**	"The results indicate modified emotionality as a function of the prenatal treatments…"	Utility limited by lack of experimental detail, use of obsolete design, and suboptimal exposure route.	*(240)*
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not specified]** 0 or 1 mg/kg bw/day sc on GD 12–15; n = 4/dose group. Litters culled at birth to 3 of each sex, weaned on PND 21.	Dam and litter parameters not given. Offspring body weight not affected by treatment at any age. *Locomotor activity (PND 13, 15, 18, 21, 46, 60):* Amphetamine associated with decreased activity on PND 21. *Maze learning (PND 15+):* Amphetamine-exposed offspring reached goal more quickly than controls. *Operant conditioning (PND 37+):* No effect of treatment. **[No control for multiple comparison/repeated measures.]**	"Amphetamines may have a different influence on behavior in developing organisms than in adults."	Utility limited by lack of experimental detail, use of obsolete design, inadequate sample size, and suboptimal exposure route.	*(204)*
Strain not given. *d,l*-Amphetamine given sc once/daily at 0 or 0.5 mg/kg bw/day from the day of sperm positivity	No information on maternal or pup weights. No alteration in day of eye opening or vaginal opening. *Conditioned avoidance (PND 45 and 90)*: Amphetamine-exposed animals had better	Adrenergic system might be affected by amphetamine; full development of this system not affected by amphetamine until PND 90.	Utility limited by lack of experimental detail, use of obsolete design, and suboptimal exposure route.	*(207)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
until delivery. Pups redistributed to produce litters of 8, equal sex when possible. Offspring weaned at "one month."	acquisition and retention than controls at PND 90. No treatment effect at PND 45. *Seizure activity to direct hippocampal application of potassium (PND 90+)*: Amphetamine-treated animals had seizure activity after a shorter duration of exposure to potassium.			
Sprague-Dawley. Methamphetamine HCl **[purity not specified]** 0, 1, 3, or 5 mg/kg bw was given sc twice/day from GD 1 (the morning after insemination) to GD 21. There were 6 dams/group except 7 rats in the high-dose group. Litters culled to 8 **[sex not specified]** on PND 7, weaned on PND 21.	4 of 7 dams in 5 mg/kg bw group failed to deliver and 1 dam delivered only 1 pup. One 3-mg/kg bw dam cannibalized her litter. Maternal weight gain and litter size were decreased by treatment. Gestation length was decreased by a mean 0.5 days by all doses of methamphetamine. *Eye opening (PND 14)*: Delayed in methamphetamine-exposed groups. *Conditional avoidance response (PND 100–120)*: No statistically significant treatment effect; mean avoidance response number was increased in the 5-mg/kg bw group at $P = 0.09$.	"The [5 mg/kg bw] dose level was somewhat effective in increasing shuttle-box performance. These results are only tentative in that the numbers were small and the study not replicated."	Utility limited by lack of experimental detail, use of obsolete design, and suboptimal exposure route.	*(194)*
Osborne-Mendell. *d*-Amphetamine **[purity not specified]** sc at 0, 5, or 10 mg/kg bw/day on GD 5–9 or 12–16 (GD 0 = a 24 hour cohabitation period). Neonates weaned on PND 28 **[culling not mentioned]**. Testing was performed on 1 pup/litter/replicate **[the number of dams or pups is not indicated, except to say that 165 dams were initially mated]**.	No information given on clinical signs, feed consumption, weight gain, litter size, viability, or other possible indicators of general toxicity. *Water wading (PND 74):* There was no treatment effect on the number of fecal boli dropped when animals were placed in 3 cm deep water. *Audiogenic seizure (PND 84)*: There was no treatment effect on the number of defecations in the seizure box. "Wild running" and seizures in response to bell ringing were increased among animals whose mothers were given placebo during early pregnancy, compared to the other groups.	Intrauterine exposure to amphetamine was probably stressful to the offspring, and this early exposure to stress resulted in habituation, making these animals less emotional in later life.	Utility limited by lack of experimental detail, use of obsolete design, and suboptimal exposure route.	*(241)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not specified]** 0, 1, or 3 mg/kg bw sc twice daily from GD 5 through parturition on GD 19–22. Litters culled randomly to 8 at birth. Weaning not specified. **[Number of dams not given.]**	Increased mortality among amphetamine-exposed pups. *Activity (PND 36 and 84)*: Increased by 3 mg/kg bw amphetamine exposure during intervals in the firs 30 minutes in the activity cage. No overall alteration over 2-hour period. *Brain amines (PND 84)*: Norepinephrine was decreased in the diencephalon and brainstem and dopamine was decreased in the brainstem **[The paper appears to use per fetus analysis; methods were not detailed.]**	There was a time-dependent increase in motor activity in amphetamine-exposed animals unfamiliar with the testing cage. The significance of this effect is not clear.	Utility limited by lack of experimental detail, use of obsolete design, and suboptimal exposure route.	*(192)*
Wistar. *d,l*-Amphetamine **[purity not specified]** 0 or 0.5 mg/kg bw/day sc from first day of pregnancy until parturition. Litters culled to 8 (equal sexes when possible) on day of delivery and weaned at 1 month of age. Number of dams not given. Eleven offspring of each sex tested per treatment except 10 males in the amphetamine group.	No assessments of general toxicity were given. *Open field testing (PND 65–85)*: Motor activity of males was increased by amphetamine treatment. *Lashley III maze (PND 90–100)*: Amphetamine-treated animals made more errors than controls.	Prenatal amphetamine treatment increased the excitability of adult offspring. This increased excitability may be due to an increased release of potassium by stimuli.	The experimental conditions were better controlled than in previous studies but the single sc dose is a weakness and the hypothesis is speculative.	*(208)*
Sprague-Dawley. *d*-Amphetamine sulfate 0, 0.5, 1.0, or 2.0 mg/kg bw/day (concentrations verified by GC) sc on GD 12–15 (n = 9/dose group). Litters culled to 8 (3–5/sex) on PND 1, weaned	Control and high-dose group did not differ in dam weight gain, implantation sites/litter, live fetuses/litter, resorptions, sex ratio, or fetal weight. There were no differences across all dose groups in postnatal pup body weights. *Y-water maze (PND 38-41)*: A smaller proportion of amphetamine-exposed than control animals failed to learn to escape; there was no difference	The animals failing to learn to escape demonstrated prolonged floating, suggesting an immobilization response. The mechanism of prenatal amphetamine increasing responsiveness to postnatal amphetamine is not known.	The use of multiple doses is a strength, as is the controlled exposure and the control for toxicity.	*(174)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
on PND 21.	by amphetamine dose. There were no treatment-related differences in retention or reversal. *Spontaneous and amphetamine-induced activity (PND 45–50):* No statistical difference in baseline activity. Amphetamine-stimulated activity was accentuated in rats previously exposed to amphetamine in utero.			
Albino, strain not specified. Amphetamine **[purity, salt, enantiomer not specified]** 0 or 2 mg/kg bw/day sc from GD 7 until delivery, n = 5/dose group. Litters redistributed to equalize number of offspring **[day of redistribution, final litter size, and sex ratio not given]**.	Amphetamine-exposed offspring were heavier than controls on PND 15 and 21. There was no influence of treatment on litter size. *Open field activity (PND 8, 15, 21)*: Amphetamine exposure was associated with increased locomotor activity on PND 15 and decreased locomotor activity on PND 21.	The changes in locomotor activity may reflect altered activity of the GABA system.	This report is limited by inadequate experimental detail and by use of a single sc dose level.	*(230)*
Sprague-Dawley. Methamphetamine HCl **[purity not specified]** 0 or 5 mg/kg bw sc twice/day at unspecified time of gestation. Culling and weaning of litters not mentioned. Animals maintained at 80% of free-feeding weight from PND 72.	Preference for saccharin-containing solution over tap water was lost at an earlier age when tested from 6 to 36 months of age; however, differences from placebo group were only apparent during some of the trials.	Treatment with methamphetamine during pregnancy alters patterns of taste preference, but "effects are neither simple nor readily predictable."	There is inadequate detail in this report. The saccharin preference test is not indicative of developmental neurotoxicity.	*(212)*
Roman high- (RHA) and low-avoidance (RLA) rats treated with *d*-amphetamine **[purity not**	Amphetamine-treated dams of both genetic lines weaned 6 of 8 litters born. The RHA saline group weaned 4 of 7 litters, and the RLA saline group weaned 4 of 4 litters. Litter size at birth and	These results are consistent with amphetamine augmentation of basal arousal level in RHA rats and decreased basal arousal level in RLA rats.	The use of genetically selected rats is not helpful. There is inadequate detail in the	*(209)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
specified] 0 or 3 mg/kg bw/day sc (1 mg/kg bw in the morning and 2 mg/kg bw in the afternoon), GD 7–20. Culling not mentioned. Weaned on PND 28. RHA amphetamine n = 8 litters, saline n = 7 litters; RLA amphetamine n = 8 litters, saline = 4 litters.	weaning appeared reduced in the RHA saline group, but variances were not provided. Pup birth and weaning weight also may have been reduced in this group, but again, variances were not provided. The authors stated that there were no statistical differences "in any aspect of the data." *Avoidance training (PND 70+)*: RHA animals showed more improvement over time if exposed prenatally to amphetamine; RLA animals showed better performance over time if not exposed to amphetamine.		report.	
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not specified]** 0 (n = 12), 0.5 (n = 11), or 2.0 (n = 10) mg/kg bw/day sc on GD 12–15. There was also an uninjected control (n = 13). Litters culled to 8 (4/sex) on PND 1, weaned on PND 21.	Pup mortality slightly increased in vehicle control and 2.0 mg/kg bw groups, not considered drug-related. Decrease in litter size in amphetamine groups was significant at $P < 0.1$. *Surface-righting (PND 3–12)*: No treatment effects. *Negative geotaxis (PND 6, 8, 10, 12)*: No treatment effects. *Pivoting locomotion (PND 7, 9, 11)*: No treatment effects. *Auditory startle onset*: No treatment effects. *Olfactory orientation (PND 9, 11, 13)*: No treatment effects. *Swimming ontogeny (PND 6–24)*: Amphetamine groups had impaired performance on PND 6. On PND 10, the 2.0 mg/kg bw group had impaired performance. *Figure-8 activity (PND 15–17; 38–43)*: no treatment effects. *Straight channel swimming and Biel water maze (PND 48–53)*: No treatment effects.	Little evidence of amphetamine-induced behavioral teratogenesis was found. The few decrements in swimming performance probably represent transient delays in development.	The use of multiple doses and control for general toxicity are strengths of this study.	*(193*)
Sprague-Dawley. *d*-Amphetamine sulfate	Maternal weight gain was decreased during the dosing period. Offspring body weight affected at	The purpose of the Collaborative Behavioral Teratology Study was not to	The overview nature of the overview reports	*(205, 242-246*)

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
[purity not specified] 0, 0.5, or 2.0 mg/kg bw/day sc GD 12–15, plus an untreated control. There were 7 dams/dose group/replicate in order to generate at least 4 litters/dose group/replicate. There were 4 replicates per lab and 5 labs, so at least 80 litters per dose group would have been generated. Culled on PND 1 to 8/litter (3–5/sex). Weaned on PND 21.	two labs on PND 1. *Eye opening (PND 12–16)*: No treatment effect. *Incisor eruption (PND 7–13)*: No treatment effect. *Testes descent (PND 21–26)*: No treatment effect. Vaginal opening (PND 30–40)*:* No treatment effect. *Negative geotaxis (PND 7–10)*: No treatment effect. *Olfactory discrimination (PND 9–11)*: No treatment effect except in one lab. *Auditory startle habituation (PND 1–19, 57–58)*: No treatment effect except in males in one lab. *1-Hour Activity (figure-8 maze; PND 21, 60)*: No treatment effect. *23-Hour activity (figure-8 maze; PND100–108)*: No treatment effect. *Activity after amphetamine challenge (PND 120–131)*: No treatment effect. *Operant visual discrimination task (PND 75–89)*: No treatment effect.	generate more toxicologic data on *d*-amphetamine, but to evaluate the use of a standardized, partly automated test scheme across different laboratories. There were few adverse behavioral effects at the low amphetamine dose levels used in these studies.	(without detailed experimental data) reduces their utility. The report that includes the results of the study is useful. The study was very well-controlled with multiple behavioral endpoints. It is a weakness that only two dose levels of amphetamine were used.	
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not specified]**. Experiment 1: 0, 0.5, 2.0, or 3.0 mg/kg bw/day sc GD 12–15 (n = 12 dams per dose group). Experiment 2: 0 (n = 9) or 3.0 (n = 12) mg/kg bw/day. Experiment 3: 0 (n = 21) or 3.0 (n = 23) mg/kg bw/day. Litters culled to 8 (3–5/sex) on PND 1; weaned on PND 21. Two-day response	Transient decrease in maternal weight gain during dosing in 2.0 and 3.0 mg/kg bw/day groups. No difference in pregnancy weight gain or offspring weights. *Auditory startle habituation (Experiment 1: PND 47–48, 57–58, 120–121; Experiments 2 and 3: PND 19)*: Increased startle in females of the 3.0 mg/kg bw/day group on PND 47–48 and 120–121. Response in males suppressed by 2.0 but not 3.0 mg/kg bw/day treatment when tested on PND 57–58. *Figure 8 maze activity (Experiment 1: PND 47–48, 120–121, 135–137; Experiment 2: PND 47–48)*: No treatment effects except possible inconsistent effects with short-term exposure to	There was no evidence of an effect of prenatal amphetamine exposure on long-term activity measures, but there was "a suggestion of an effect at higher doses on short-term reactivity to novel fields." Behavioral effects of treatment occur at 3 mg/kg bw/day, but are sex-specific and without a discernible pattern. The inconsistency in results among studies may not be accidental: the behavioral test results may depend on the psychological state of the dam during dosing and the offspring at testing.	The use of multiple doses with several endpoints and male/female data are strengths.	*(206)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
testing included administration of methamphetamine challenge on the second day.	the maze (novel stimulus effects). *Activity in single photo-cell chamber (Experiment 1: PND 47–48, 120–121, 133)*: No treatment effects. *Emergence (Experiment 1: PND 128)*: Amphetamine-exposed females in top two dose groups were more likely to emerge. Male effects were said to follow similar pattern although not statistically significant. *Open-field activity (Experiment 2: PND 70–72)*: No treatment effects. *Intake of sweet solution (Experiment 2: PND 85–105, 215–217)*: Preference for sweet solution was increased in 3.0 mg/kg bw/day group.			
Sprague-Dawley. Methamphetamine **[purity unspecified]** 0 or 2 mg/kg bw "for 3 weeks during pregnancy." **[interval not stated; presumed daily]**. Control dams reared 8 methamphetamine-exposed and 8 vehicle-exposed pups **[number of litters not specified].** Weaned PND 21.	General toxicity information not provided. *Total and vertical activity (PND 5–34)*: total motor activity decreased and vertical activity increased by prenatal methamphetamine treatment. *Activity in response to methamphetamine challenge (5th week of life)*: No effect of treatment. *Activity in response to sound (3–4 weeks after birth)*: Activity in controls was suppressed after noise. Vertical activity in prenatally methamphetamine-exposed offspring was stimulated by noise during the dark period. *Brain catecholamines*: frontal cortex dopamine decreased in methamphetamine-exposed pups on PND 35 but not PND 21. Decreased spiperone binding in frontal cortex (reflecting decreased serotonin receptors) on PND 35 in methamphetamine group.	Prenatal methamphetamine exposure impairs reactivity to environmental stimuli but does not sensitize the offspring to postnatal methamphetamine exposure.	The attempt to correlate behavior and neurochemistry are strengths. The lack of experimental detail and the use of a single sc dose level are weaknesses.	*(210)*
Wistar. Methamphetamine HCl **[purity unspecified]**	Maternal body weight gain during gestation was suppressed at 2 mg/kg bw methamphetamine and	"[M]ethamphetamine might induce some behavioral teratogenicity in rats." There	The use of multiple dose levels and several	*(197)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
0, 1, 2, 3, or 4.5 mg/kg bw/day sc on GD 7–20 (n = 13 or 14/dose group). On PND 4, 4 pups/sex/litter were selected for behavioral testing. **[Culling and time of weaning not specified.]**	higher. Only 1 high-dose dam delivered live pups. Male offspring body weight gain during lactation and post-weaning was decreased in the 3 and 4.5 mg/kg bw groups. *Surface righting (from PND 4)*: No treatment effect. *Cliff avoidance (from PND 4)*: No treatment effect. *Negative geotaxis (PND 6–12)*: Delayed in the 3 and 4.5 mg/kg bw groups *Mid-air righting (from PND 12)*: Delayed in the 3 and 4.5 mg/kg bw groups. *Spontaneous motor activity (from 3 weeks of age)*: 3–5-week-old methamphetamine-exposed pups (≥ 2 mg/kg bw/day) had a decrease in distance traveled. *Conditioned avoidance (from 7 weeks of age)*: No consistent effects of treatment, although authors state that all methamphetamine-exposed offspring "tended to have higher avoidance response rates." Conditional avoidance response was increased on day 2 in the 2 and 3 mg/kg bw groups but not on days 3–5.	was an increase in conditioned avoidance response, especially at the 3 mg/kg bw dose.	behavioral endpoints are strengths. The small number of animals is a weakness.	
Sprague-Dawley. *d,l*-Methamphetamine HCl **[purity not given]** 0 (n = 6) or 50 (n = 15) mg/kg bw (free base equivalent) twice daily by sc injection on GD 7–12. Controls were pair-fed. Litters raised by dam, not culled, weaned on PND 28.	With methamphetamine treatment, maternal weight gain was reduced, 2 of 15 dams died during dosing, 2 litters were completely resorbed, 1 litter died on the day of delivery, and 8.6% of live-born pups died during the lactation period. There were no control dam or offspring deaths. *Olfactory orientation (PND 9, 11, 13):* Impaired by methamphetamine. *Early locomotion (PND 10, 12, 14):* Impaired by methamphetamine at $P < 0.1$. *Acoustic startle (PND 27):* Increased by methamphetamine.	Offspring exhibit long-term consequences of prenatal exposure to high doses of methamphetamine, including a decrease in early locomotion and olfactory orientation and an increase in adult locomotion and acoustic startle.	This study was well controlled and included multiple endpoints and male/female analysis. The single sc dose level and the lack of information on compound purity are weaknesses.	*(199)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
	Adult locomotor activity (PND 63–64, 70–71): Increased by methamphetamine. *Straight channel swimming (PND 84):* Impaired by methamphetamine. *Cincinnati maze (PND 85+)*: No effect.			
Sprague-Dawley. *d,l*-Methamphetamine HCl [**purity not given]** 0, 2, or 10 mg/kg bw sc twice/day. Treatment started 1–3 weeks before mating, and mating occurred only after feed intake returned to control levels. Treatment was continued throughout pregnancy. There were 2 low-dose, 2 high-dose, and 3 control litters. All pups were fostered to different saline-treated dams. Weaned on PND 26. The pup was taken as the statistical unit (29 control pups, 17 low-dose pups, 9 high-dose pups).	No difference by treatment in number of days to righting reflex or eye opening. No other information on general toxicity. *Open field (PND 30)*: Fewer squares crossed by high-dose methamphetamine group. Less rearing by both methamphetamine-exposed groups. *Morris water maze (PND 44)*: No treatment difference in latency to find platform. *Brain monoamine uptake:* Variable by brain region and methamphetamine dose.	Results may indicate a depressed response to novelty or reduced locomotion.	The attempt to correlate behavioral and biochemical change is a strength; however, the use of only two dose levels and the limited behavioral testing are weaknesses.	*(247)*
Sprague-Dawley. *d*-Methamphetamine (free base **[purity not given]**) 0, 5, 10, 15, or 20 mg/kg bw twice daily by sc injection from GD 7–12 or from GD 13–18 (plug = GD 0). Rats in the 15 and 20 mg/kg bw groups and one 0 mg/kg	All dams had a decrease in body weight compared to ad libitum fed and watered controls during the treatment period. Maternal mortality was increased and gestation length increased in the 15 and 20 mg/kg bw groups treated on GD 13–18. Gestation length was also increased in the 10-mg/kg bw group treated on GD 13–18. Post-weaning offspring body weights showed no consistent difference by group after GD 7–12	Prenatal exposure to methamphetamine early in gestation resulted in delayed development of early locomotion and impaired performance on tests of learning and memory. The Morris water maze results suggested impairment of spatial memory and perseveration (inability to extinguish an old response and begin a new search).	The use of multiple dose levels and endpoints are strengths of this well-controlled study.	*(49)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
bw group were fed and watered ad libitum. Another 0 mg/kg bw group and the 5 and 10 mg/kg bw groups were pair-fed and watered using the rats in the 15 mg/kg bw group as a reference. Litters standardized on PND 3 to 8, balanced for sex. There were 14–16 litters/group (except for 12 litters in the ad libitum control group).	exposure, but showed continued decreases in the 2 highest dose groups after GD 13–18 exposure. *Olfactory orientation (PND 9, 11, 13)*: No treatment effects. *Early locomotion (PND 10, 12, 14)*: Decreased in 15 and 20 mg/kg bw groups after exposure on GD 7–12, but not GD 13–18. *Locomotion (PND 15, 30, 45, 60):* No pattern of effects. *Spontaneous alternation (T-maze; PND 16, 31, 46, 61)*: Female 20 mg/kg bw offspring exposed on GD 7–12 alternated less often than pair-fed/watered group when tested on PND 31. More 15 mg/kg bw female offspring than either control failed to perform the task in the allotted time when tested on PND 31 and 46. *Passive avoidance (PND 17, 20, 32, 35, 47. 50, 62, 65)*: Training latency not affected by treatment. Retention was decreased in 20 mg/kg bw male offspring. *Straight channel swimming trials (within a week following PND 50)*: No treatment effect. *Cincinnati water maze (within a week following PND 50)*: 15 and 20 mg/kg bw groups took longer to solve maze than ad libitum controls (but not compared to pair-fed/watered controls). *Morris maze (within 1 week following PND 57)*: Some effects were found in the 15 and 20 mg/kg bw groups, especially with treatment on GD 7–12.			
Wistar. *d*-Amphetamine sulfate **[purity not specified]** given sc at 0, 5, or 10 mg/kg bw/day from GD 8–22 (plug = GD 1), n = 10 dams/dose group.	Birth weight lower in amphetamine 10 mg/kg bw/day group than other groups. Litter size not affected by treatment. *Stereotyped behavior after amphetamine challenge (PND 23, 60)*: Prenatal amphetamine at either dose increased stereotyped behavior on	Prenatal amphetamine exposure causes behavioral sensitization in rats, with different profiles of behavioral reactivity depending on maternal exposure level, amphetamine challenge dose, and age at testing.	This study addressed behavioral sensitization, but its utility is reduced by lack of specification of compound purity.	*(211)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
Dams in 0 and 5 mg/kg bw/day groups were pair-fed and watered to the 10 mg/kg bw/day rats. On the day of birth, 4 males and females per litter were retained and fostered to surrogate mothers. Pups were weaned on PND 22.	PND 23 after challenge dose of 2.5, 5.0 or 7.5 mg/kg bw amphetamine. Prenatal amphetamine increased stereotyped behavior on PND 60 only after a challenge dose of 60 mg/kg bw. *Acoustic startle (PND 90)*: Prenatal exposure at maternal dose of 10 mg/kg bw/day resulted in greater startle amplitude after saline challenge and after amphetamine 1 mg/kg bw challenge. Prepulse inhibition was impaired by both prenatal amphetamine doses.			
	Postnatal Exposure of Pups			
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not specified]** 10 mg/kg bw ip on one of PND 1, 2, 4, 7, 10, 12, 14, 16, 18, 20–30, 35, or in adulthood (7–9 weeks of age). Pups were kept with their dams **[weaning and culling not addressed]**.	Body weight and feed consumption not evaluated. Animals were observed for forward and reverse locomotion and stereotyped behavior, such as licking, tongue protrusion, and sniffing. Bursts of locomotion were noted on PND 1. Stereotyped behavior began on PND 2 and was similar to that in adults by PND 18.	As early as 2 days of age, there is functional integrity of the neurologic system subserving stereotyped behavior.	The use of a single dose level, the absence of experimental detail, and the lack of controlled testing limit the utility of this paper.	*(248)*
Sprague-Dawley. *d*-Methamphetamine **[purity not given]** 30 mg/kg bw sc twice daily on PND1–10 (early) or PND 11–20 (late). Animals were given sc water on the days during PND 1–20 when they did not receive methamphetamine and controls received sc water twice daily during PND 1–	Pup body weight was decreased during treatment period and after weaning. Animals given methamphetamine on PND 1–10 remained lighter than controls through the end of the experiment on PND 70; animals given methamphetamine on PND 11–20 were similar in weight to controls by PND 49. Mortality was increased in the group given methamphetamine on PND 1–10: 20% of the pups died compared to 1.7% of the control pups and 0.9% of pups given methamphetamine on PND 11–20. *Spontaneous alternation (PND 31, 46, 61)*: More	Methamphetamine exposure is associated with increased reactivity, deficits in associative or memory processes. Findings in late-exposed animals suggest multiple forebrain effects on cognitive function. Early-exposed animals demonstrated predominantly effects on startle. The decrease in activity on PND 30 associated with neonatal methamphetamine treatment is in contrast to the increase in activity associated with prenatal methamphetamine exposure in	This well-controlled study used adequate numbers of animals and multiple endpoints. The single dose level is a weakness.	*(170, 215)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
2; n = 15 litters/dose group. Litters culled to 4/sex on PND 1; weaned on PND 28. One pup/sex/litter was used for each behavioral testing session.	pups failed to make a choice after late treatment when tested on PND 31 only. *Passive avoidance (PND 32, 47, 62)*: No treatment effect on learning or retention. *Acoustic startle (PND 36, 51, 66)*: Methamphetamine treatment increased reactivity in females averaged across ages and decreased prepulse inhibition in both sexes (only with early treatment in males). *Straight channel swimming (age not given)*: No treatment effect. *Cincinnati water maze (age not given)*: Increased errors in late-treated rats was significant at $P < 0.1$. There were more trial failures among early-treated males. *Morris water maze (age not given)*: Increased latency to find platform in late-treated animals. *Locomotor activity (PND 30, 45, 60)*: Activity was decreased with methamphetamine treatment, particularly on PND 30 and particularly after PND 11–20 treatments. Fluoxetine challenge resulted in a decrease in activity that was accentuated in males by PND 1–10 neonatal methamphetamine exposure. Methamphetamine challenge increased activity in all groups, which was accentuated (according to the authors visual inspection of the data) by neonatal methamphetamine.	other studies.		
Sprague-Dawley. *d*-Methamphetamine **[purity not given]** 0 or 20 mg/kg bw twice daily by sc injection to female rat neonates on PND 1–10. On PND 1, litters were	62 of 119 (52%) offspring in methamphetamine group died, compared to 2 of 45 control offspring. Offspring body weight was decreased in the methamphetamine group during treatment and thereafter (to PND 49). *Acoustic startle (PND 50)*: Methamphetamine-treated animals showed a decrease in startle	Neonatal methamphetamine facilitates startle probably by augmentation of the basic reflex rather than an effect on other processes such as habituation or prepulse inhibition.	The single dose level with very high mortality limits the utility of this report.	*(216)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
culled to 8, preferentially retaining females. Weaned on PND 28.	habituation at $P < 0.1$ and a decrease in startle inhibition by a prepulse stimulus ($P < 0.05$ for average response across all prepulse intensities).			
Sprague-Dawley. *d*-Amphetamine sulfate **[purity not given]** 0 (saline), 1.0, 2.5, or 5 mg/kg bw/day ip × 4 days or *R*-propylnorapomorphine (a dopamine agonist) 1 mg/kg bw/day ip × 4 days. Treatments began on PND 11 or PND 17. Animals were challenged 2 or 8 days later with the same treatment or, in the saline treated animals, with saline or 1 of the amphetamine doses (the 8-day challenge experiments used only 5 mg/kg bw for 11-day-olds and 2.5 mg/kg bw for 17-day-olds); n = 8 rats/treatment group.	No general toxicity information given. Challenge after 2 days: *Line crosses in open-field (5 minutes after the challenge dose of saline, amphetamine, or* R-*propylnorapomorphine)*: Increased by amphetamine, but sensitization shown only for 1 mg/kg bw dose. *Stereotyped sniffing (same timing)*: Increased at highest amphetamine dose; sensitization occurred only in the younger animals. *Vertical activity (same timing)*: Increased by amphetamine, with sensitization in both age groups. Challenge after 8 days: *Line crosses in open-field (5 minutes after the challenge dose of saline, amphetamine, or* R-*propylnorapomorphine)*: Rats given amphetamine were more active than rats given only saline. There was no difference between saline and amphetamine pretreatment groups at either age. *Stereotyped sniffing (same timing)*: Not increased by amphetamine, although increased by *R*-propylnorapomorphine. *Vertical activity (same timing)*: Increased by amphetamine, but not more so in animals pretreated with amphetamine.	Sensitization occurs in pre-weanling rats, but only short-term, suggesting a difference in mechanism between short- and long-term behavioral sensitization.	This study addresses sensitization. The multiple dose levels and endpoints are a strength. The lack of information on compound purity is a weakness.	*(249)*
Sprague-Dawley. Experiment 1: On PND 17–20, pups received daily ip injections of saline or 0.3 mg/kg bw dizolcipine	No general toxicity results were given. Experiment 1: *Open field testing (PND 17–20)*: Rats pretreated with dizolcipine followed by amphetamine had an increase in line-crosses compared to pretreatment	The *N*-methyl-*d*-aspartate receptor modulates sensitization of the locomotor response to amphetamine in preweanling rats.	The complex experimental design detracts from the ability to generalize the findings of this study.	*(250)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
(a non-competitor antagonist at the *N*-methyl-*d*-aspartate receptor), followed 30 minutes later by ip saline, 2.5 mg/kg bw *d*-amphetamine sulfate **[purity not given]** or 1 mg/kg bw *R*-propylnorapomorphine (a dopamine agonist). On PND 22, rats that had been pretreated with only saline received a challenge of one of the other treatments ip at the same dose. Rats that had been given a non-saline treatment on PND 17–20 received a challenge of the same treatment on PND 22. Experiment 2: PND 17–20 treatments were saline, 0.3 mg/kg bw dizolcipine, 2.5 mg/kg bw *d*-amphetamine sulfate, or 1 mg/kg bw *R*-propylnorapomorphine. PND 22 challenge was with amphetamine or *R*-propylnorapomorphine.	with saline or amphetamine alone. Amphetamine alone increased stereotyped sniffing. *Open field testing (PND 22)*: Pre-exposure to amphetamine on PND 17–20 was associated with increased line-crosses, sniffing, and rearing compared to pretreatment with saline or dizolcipine. Experiment 2: *Open field testing (PND 17–20)*: Amphetamine increased sniffing and rearing but not line crosses compared to saline. *Open field testing (PND 22)*: Pre-treatment with amphetamine caused an increase in line crosses and rearing but not sniffing in animals challenged with amphetamine. Pretreatment with amphetamine increased line crosses, sniffing, and rearing in response to challenge with *R*-propylnor-apomorphine.			
ACI Black Agouti (presumed to be a poor metabolizer of methamphetamine) and Sprague-Dawley	59.4% of ACI Black Agouti and 18.8% of Sprague-Dawley offspring died during the treatment period. Body weight was suppressed during the dosing period with persistent body weight deficits through PND 49 (ACI Black	The lack of effect of treatment on acoustic startle was not consistent with earlier experiments and was unexplained. The effect of methamphetamine on spatial memory (tested by the Morris maze) was	None.	*(78)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
(presumed to be an extensive metabolizer of methamphetamine). On PND 1, litters were culled to 8, preferentially retaining females. *d*-Methamphetamine (free base **[purity not given]**) 0 or 30 mg/kg bw was given sc twice/day on PND 11–20. Litters were weaned on PND 28. Only females were used for behavioral tests because only females manifest the poor metabolizer phenotype. ACI Black Agouti: 25–26 females from 25–26 litters in each group; Sprague-Dawley: 65–69 females from 10–12 litters in each group.	Agouti) and PND 42 (Sprague-Dawley). *Acoustic startle(PND 50)*: No treatment effect. *Straight-channel swimming (PND 54)*: No treatment effect. *Morris maze (unstated age)*: Poorer performance in methamphetamine groups of both strains; no strain-dependent difference in performance.	consistent with previous results. The lack of influence of strain may mean that metabolizer status is not relevant; however, the high mortality rate among ACI Black Agouti may have led to a survivor population that did not represent the behavioral susceptibility of this strain to methamphetamine.		
Dark Agouti (a poor metabolizer of methamphetamine) and Sprague-Dawley (presumed to be an extensive metabolizer of methamphetamine). On PND 1, litters were culled to 8 (balanced for sex). *d*-Methamphetamine (free base **[purity not given]**) 0 or 15 mg/kg bw was given sc twice/day on PND 11–	One-third of methamphetamine-treated Dark Agouti and 13.3% of methamphetamine-treated Sprague Dawley offspring died. Methamphetamine-treated offspring weighed less than controls and the weight difference persisted after the treatment period. *Acoustic startle (PND 50)*: Methamphetamine increased startle amplitude in Dark Agouti but not Sprague-Dawley rats. *Straight channel swimming (PND 54):* Methamphetamine-treated Sprague-Dawley males took longer than controls to swim in 2 of 4 trials. There were no treatment-related differences	The increased susceptibility of Dark Agouti females to methamphetamine toxicity in the Morris maze is consistent with the unmetabolized drug being the active toxicant. The acoustic startle results were unexpected because males were also affected; male Dark Agouti rats have an intermediate metabolizer status. Different brain regions are responsible for spatial learning (hippocampus) and acoustic startle (brainstem) and these different regions may have different sensitivity to methamphetamine.	This study was well planned with multiple endpoints and addresses the issue of possible genetic susceptibility. A weakness is the use of a single dose level.	*(48)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
20. Litters were weaned on PND 28. Dark Agouti: 20 litters given methamphetamine, 15 litters given vehicle; Sprague-Dawley: 15 litters given methamphetamine, 13 litters given vehicle. The additional Dark Agouti litters had been added to the planned n = 15 due to mortality.	among females or in either sex in the Dark Agouti strain. *Morris maze (PND 57)*: Dark Agouti males and females and Sprague-Dawley males had an increased latency to finding the hidden platform after methamphetamine treatment. In memory testing, methamphetamine-treated females of both strains spent less time in target quadrant than controls; the Dark Agouti females were more affected than the Sprague-Dawley females. Reacquisition followed the same sex and strain pattern as memory. Dark Agouti females treated with methamphetamine showed less competence in searching for a new platform location in reversal trials.			
Sprague-Dawley. On PND 1, litters were culled to 8, balanced for sex. *d*-Methamphetamine (free base **[purity not given]**) 0 or 40 mg/kg bw/day was given sc on PND 11–20. Animals received 4 daily sc injections at 2-hour intervals: Controls received 4 saline injections, 1 methamphetamine group received 4 injections of 10 mg/kg bw each, and a second methamphetamine group received methamphetamine 20 mg/kg bw followed by 2 saline injections followed	Mortality was increased by methamphetamine administration, with 15 and 21% of pups dying during the treatment period in the 2 methamphetamine groups. Body weight was decreased in the methamphetamine groups from PND 15 to 42. *Straight-channel swimming (PND 50–56)*: No treatment-related effects. *Morris maze (age not specified)*: Methamphetamine treatment was associated with poorer performance. The results of cued learning tests (in which a visible marker was mounted above the underwater platform of the Morris maze) did not explain the poorer performance on the uncued Morris maze when cued learning was used as a covariate in an ANCOVA.	Developmental exposure to methamphetamine produces spatial learning and memory defects.	This well-controlled study focused on cognitive effects. The single dose level and high mortality are weaknesses.	*(217)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
by a second methamphetamine injection of 20 mg/kg bw. There were 15–18 litters per dose group.				
Sprague-Dawley. Experiment 1: On PND 11–15, 32 male and 32 female rats were given *d*-amphetamine **[purity not given]** 0 or 2.5 mg/kg bw/day ip. On PND 23 or PND 90, rats were given a challenge of amphetamine 0 or 2.5 mg/kg bw ip. Experiment 2: On PND 11–15, 24 male and 24 female rats were given *d*-amphetamine **[purity not given]** 0, 2.5, or 5.0 mg/kg bw/day ip. On PND 23 or PND 90, rats were given a challenge of amphetamine 2.5 mg/kg bw ip.	No information given on general toxicity. Experiment 1 and 2: *Open field testing(PND 23 and 90)*: Amphetamine challenge produced an increase in line crossing without regard to treatment regimen on PND 11–15. *Protein kinase A activity*: There were changes in dorsal striatal and accumbal protein kinase A activity (described in the text).	The identified changes in protein kinase A activity may not be relevant to locomotor behavior. Whether these changes impact learning, addiction, or memory is not known.	The use of only two dose levels and the limited behavioral testing decrease the utility of this report.	*(236)*
Sprague-Dawley. On PND 1, litters were culled to 8, with at least 6 males/litter. *d*-Methamphetamine (free base **[purity not given]**) 0 or 40 mg/kg bw/day was given sc on PND 11–20 to male offspring. Animals received 4 daily sc 10 mg/kg bw injections at 2-	Methamphetamine treatment was associated with a decrease in body weight gain from PND 12 to 35. By PND 42, there was no significant body weight difference between the treatment groups. *Nonspatial Morris water maze pretraining (PND 50+)*: There was no effect of treatment on learning to swim and to find a submerged escape platform. *Spatial learning (PND 50+)*: Methamphetamine-treated animals that were naïve to the test had	Spatial learning is impaired by neonatal methamphetamine exposure. No such impairment occurs in animals with test experience. These results are consistent with hippocampal response to stressors as a mediator of impaired learning and memory.	The single dose level and limited behavioral testing detract from the utility of this report.	*(202)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
hour intervals. There were 12 litters in each treatment arm, each of which contributed 2 males for testing.	impaired spatial learning. Pretrained animals did not differ by methamphetamine exposure.			
Sprague-Dawley. On the day after delivery, litters culled to 4/sex. *d*-Methamphetamine HCl **purity not specified]** given sc at 0 or 10 mg/kg bw every 2 hours for a total of 4 doses/day on PND 11–15 or on PND 16–20 (with saline vehicle given on the other days in the PND 11–20 treatment period). Litters (n = 16) were split so that 1 male and 1 female per litter was treated with methamphetamine for each time period and 2 animals/sex/litter received only saline. Day of weaning not specified.	Pup weight gain was decreased during methamphetamine treatment. In the group treated late, body weight reached control levels by PND 42. In the group treated early, body weight reached control levels by PND 63. *Zero maze (~PND 50)*: No significant effect of treatment. *Straight-channel swimming (1 day after zero maze)*: No treatment effect. *Morris water maze (~PND54)*: Learning impaired with methamphetamine exposure on PND 11–15 but not PND 16–20.	The sensitivity of this early time period may be due to treatment during the stress hyporesponsive period. Methamphetamine-associated corticosterone release during this period may injure neurons in the hippocampus. This explanation is not entirely satisfactory because PND 1–10 treatment is also during the stress hyporesponsive period but has not been shown to produce the same learning deficits.	The use of a single dose level and the pup weight effects limit the utility of this report.	*(220)*
Sprague-Dawley. On the day of delivery, litters culled to 5/sex. *d*-Methamphetamine HCl **[purity not specified]** given sc at 0, 5, 10, or 15 mg/kg bw every 2 hours for a total of 4 doses/day	Two litters discontinued due to mortality in 3 of 4 high-dose pups. Pup body weight was decreased by all doses of amphetamine on PND 16 and 21, and was at control levels on PND 49. *Straight channel swimming (PND 50):* No effects of treatment. *Cincinnati water maze (PND 53–57)*: No effects of treatment.	Methamphetamine treatment on PND 11–20 results in adult spatial learning and memory deficits despite prior swimming training and maze experience. Reference memory was specifically impaired while working memory was unaffected. The effects are selective and not due to generalized cognitive or motor	This study used multiple dose levels and focused on cognitive effects. The high mortality is a weakness.	*(221)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
on PND 11–20. Litters (n = 19) were split so that 1 male and 1 female per litter received each dose, except 2 animals/sex/litter received the high dose. Day of weaning not specified.	*Morris water maze, cued (platform visible above water; PND 60–65*): No effects of treatment. *Morris water maze, hidden platform (PND 67–145)*: Acquisition impaired in a dose-dependent manner in all amphetamine-exposed groups. When platform position was shifted, performance was impaired in high-dose group. Working memory was not adversely affected by treatment.	impairment.		
Sprague-Dawley. On the day after delivery, litters culled to 4/sex. *d*-Methamphetamine HCl **[purity not specified]** given sc at 0 or 5 mg/kg bw every 2 hours for a total of 4 doses/day on PND 11–20. Litters (n = 16) were split so that 2 males and 2 females per litter were treated with methamphetamine or vehicle. Day of weaning not specified.	Preweaning but not post-weaning body weight gain was reduced by methamphetamine treatment. *Barnes maze (PND 60–65)*: Methamphetamine impaired performance when aversion to light was the motivator but not when a food reward was the motivator. *Forced swim (PND 90–97)*: Methamphetamine associated with less elevation of plasma corticosterone after forced swim. *Cliff-avoidance(~PND 90)*: After 20 mg/kg bw dose of methamphetamine, neonatally amphetamine-treated rats avoided cliff more than controls.	The Barnes maze results with the aversive stimulus support the deficit in spatial learning seen with the Morris maze. The lack of methamphetamine effect on Barnes maze performance with a food reward may reflect the relatively low dose of methamphetamine used for the neonatal treatments.	This study used a good experimental design to focus on spatial learning. The single dose level is a weakness.	*(219)*
	Exposure During Gestation and Lactation Periods			
Sprague-Dawley. Methamphetamine HCl **[purity not specified]** 0 or 5 mg/kg bw was given sc twice/day from GD 1 (the morning after insemination) to GD 21 and PND 2–21. There were 25 dams treated with	Methamphetamine-exposed dams gained less weight during pregnancy than either control and neonates were lighter on PND 7, 14, 21, and 28, and through 16 months of age. Mean birth weight was numerically lower, but statistical significance cannot be evaluated from the information given. Gestation length was decreased by methamphetamine. *Ear opening (PND 4), Incisor eruption (PND 7),*	Methamphetamine resulted in decreased gestation length and decreased maternal weight. Methamphetamine reduced offspring weight and the reductions persisted through 16 months of life. Methamphetamine treatment was associated with developmental delay.	The lack of experimental detail and the use of a single dose level detract from the utility of this paper.	*(195)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
methamphetamine, 13 dams injected with saline, and 13 uninjected controls. Litters culled to 8 males preferred) on PND 7, weaned on PND 21. Behavioral tests performed using 1 male/litter.	*distance traveled quadripedally (PND 7, 11)*: No significant effect of treatment. *Eye opening (PND 14, 18)*: Delayed by methamphetamine treatment. *Wheel-running activity (PND 90+)*: Increased in methamphetamine group compared to injected control but no difference from uninjected control.			
Sprague-Dawley. Methamphetamine HCl **[purity not specified]** 0 (n = 13) or 5 (n = 25) mg/kg bw sc twice/day GD 1–21 and lactation day 3–21 (weaning). Culling not mentioned. One male/litter used for testing, n = 12/dose group. Rats were feed-restricted from PND 72 to 80% of free-feeding weight.	Offspring of methamphetamine group were lighter than control (both groups were feed-restricted, but free-feeding littermates were said also to be lighter in the methamphetamine group, with citation to *(195)*. *Wheel-running activity (PND 90 until death)*: Methamphetamine exposure was associated with increased activity. Correction of activity for body weight was said to attenuate differences, but the degree and significance of the attenuation was not discussed.	Maternal methamphetamine treatment is responsible for increased locomotor activity from adulthood through death.	This paper is somewhat useful in addressing long-term effects of developmental exposures.	*(222)*
Sprague Dawley. *d*-Amphetamine sulfate **[purity not specified]** 0, 2, or 5 mg/kg bw/day was administered in drinking water. Water restriction was used to ensure consumption of entire dose. There were 16 dams per treatment group. Mating occurred after 30 days of treatment, permitting dam body	Maternal body weight gain was decreased by both doses of amphetamine. Litters were produced by only 6 control dams, 13 low-dose dams, and 8 high-dose dams. Litter size was not affected by treatment, but pup body weight was decreased by both amphetamine doses on PND 1. *Righting reflex (PND 1, 3, 6)*: Significant delay in turning over in both amphetamine groups on PND 3. *Eye opening (PND 14)*: Delayed by both doses of amphetamine. *Vaginal opening (PND 37)*: Delayed by both doses of amphetamine.	Prenatal amphetamine treatment is associated with a delay in maturation and altered preweaning behavior. Water restriction may have had effects on these parameters, and there may have been an amphetamine-water restriction interaction.	None.	*(213)*

Strain and dose regimen	General toxicity and neurobehavioral findings	Author conclusions	Comments	Reference
weights to return to normal after an initial decrease. Pups were culled to a maximum of 8 at birth, weaned on PND 22. Untreated ad libitum water supply was initiated at weaning of pups.	*Open field (PND 7, 14, 21)*: Amphetamine-exposed animals were more active than controls on PND 7. *Bridge crossing (PND 7, 14, 21)*: Both amphetamine-exposed groups showed impaired ability on PND 14; only high-dose animals were impaired on PND 21.			

Barnes maze: A circular surface with regular holes every 12°. One of the holes leads to the goal box.
Biel maze: A network of pathways, one of which leads to the goal and the others of which are blind alleys. The original Biel maze was a water maze, but dry versions have been described.
Cincinnati maze: A series of nine interlocking T-mazes, can be run with water or dry.
Lashley III maze: A box divided into four alleys with doors permitting entry into one alley from another. The subject must exit the start box, run down alley one to the door, turn right into alley two, left into alley three, right into alley four, and left into a goal box.
Morris maze: A circular pool of water with a submerged platform that permits escape. The subject must find the platform and remember its location.
Zero maze: An elevated ring with alternating quadrants of high dark walls and low transparent walls (the open area). Avoidance of the open areas represents fearfulness or anxiety.

Table 40. Results of Non-Behavioral Developmental Neurotoxicty Testing

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
	Prenatal Exposure			
Albino rat **[strain not specified]**. Animals sc injected with 0 or 2.0 mg/kg bw/day amphetamine **[salt and enantiomer not specified]** from GD 7 until delivery. Offspring killed on PND 1, 8, 15, and 21 for measurement of brain monoamine and metabolite levels **[litter representation not discussed].** Results of locomotor testing are presented in Table 39.	*Serotonin and 5-HIAA:* Increased on PND 1 with amphetamine treatment. *GABA:* Amphetamine treatment resulted in small but significant decrease on PND 15 and increase on PND 21. Levels inversely related to the activity of glutamate decarboxylase **[methods of measurement not detailed]**. *Norepinephrine:* No change. *Dopamine:* No change.	Development of different transmitter systems appears to be affected by indirect drug effects.	This paper lacks experimental detail and is not useful.	*(230*)

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
	Locomotive activity results summarized in Table 39.			
Wistar rats. Animals were sc injected with 10 mg/kg bw/day *d*-amphetamine sulfate on GD 8–22. Saline, pair-fed, and unhandled controls were included. **[Numbers of dams treated were listed as 6 but it was later stated that there were 12 litters/group.]** Litters were culled to 4 male and 4 female pups. Effects on prefrontal cortex were evaluated on PND 14, 30, and/or 90. **[It appears that only male offspring were examined, and that 2–6 offspring/group were evaluated, although it is not clearly stated for each endpoint.]**	Maternal weight gain in amphetamine-treated dams was lower than saline group. No effect on gestation length, litter size, male:female ratio, mean birth weight of males, or postnatal growth of males. *Forebrain:body weight relationship to PND 90:* No effect. *Prefrontal cortex volume:* No effect on PND 14 or 30. *Morphometric analyses of prefrontal cortex:* Amphetamine increased neuronal density on PND 14 but not PND 30. *Levels of dopamine, serotonin, and their metabolites in prefrontal cortex:* Amphetamine decreased serotonin level and increased serotonin:5-HIAA ratio on PND 30, but not PND 14. Dopamine and its metabolites were unaffected.	"These changes, whether permanent or transitory, raise the possibility that some of the effects of prenatal exposure to amphetamine may be due to modifications in the neurotransmitter levels of serotonin."	The experimental design is confusing. The maternal toxicity is a weakness.	*(224)*
Wistar rats. Animals sc injected with saline or 0.5 mg/kg bw/day *d,l*-amphetamine sulfate throughout entire pregnancy **[number of animals treated not specified]**. Litters were culled to 8 pups at birth. Adult male offspring **[7 months old]** were evaluated. **[Numbers of treated litters**	In utero amphetamine treatment resulted in reduced density of brain alpha adrenergic receptors (22% less than controls) but not beta receptors. There was no effect on alpha or beta receptor affinity.	"This change may be a consequence of long lasting alterations in the metabolism of brain catecholamines produced by amphetamine administration at fetal age, and may account for the behavioral alterations described in these animals." **[See *(207)* and *(208)* in Table 39**	The receptor measurements were acceptable for the time but are not current. The use of a single dose level is a weakness.	*(237)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
represented not specified.]		**for description of behavior effects].**		
Albino rats **[strain not specified]**. Animals sc injected with saline or 0.5 mg/kg bw/day *d,l*- amphetamine sulfate throughout entire pregnancy **[number of animals treated not specified]**. Adult female offspring (8–9/group) were evaluated. **[Numbers of treated litters represented not specified.]**	Following application of conditioning trains of pulses to the locus coeruleus, potentiation of population spike amplitude in gyrus dentate was 2–3 times greater in rats exposed to amphetamine vs. saline in utero.	"This effect may be due to the changes in catecholamine metabolism and (or) catecholamine receptors observed in these animals and could help explain the behavioral alterations caused by amphetamine exposure in utero." **[See *(207)* and *(208)* in Table 39 for description of behavior effects.]**	None.	*(238)*
C57Bl/6 mice. Animals were unhandled or ip injected with *d*-amphetamine sulfate 0 or 5 mg/kg bw/day for the last 6 or 7 days of pregnancy. **[Number treated not specified.]** Pups were killed at PND 0, 3, 7, 14, 21, and 30 for evaluation of dopamine and norepinephrine in brain. **[Number of pups and distribution by sex and litter not stated except that a minimum of three litters was represented for each mean. It is not clear whether the n values given in the data table represent individual brains or pooled brain samples. Different numbers of brains were pooled at different ages and a different "n" is used for different treatment groups on each day of evaluation.]**	*Birth*: Amphetamine-exposed pups had lower brain norepinephrine than either control. *PND 21*: Amphetamine-exposed pups had lower brain dopamine levels than the vehicle-treated controls. *PND 30*: Amphetamine-exposed pups had higher brain norepinephrine levels than either control. There were no other significant differences between vehicle- and amphetamine-treated groups. A second experiment with same treatment paradigm, but evaluation on PND 75 found no differences in brain catecholamines **[data not shown]**. Results of behavioral testing are listed in Table 39.	Prenatal exposure to amphetamine could alter brain catecholamines in young animals and produce long-lasting effects on behavior.	There is inadequate detail in this report and the experimental design is confusing.	*(227)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
Wistar rats. Animals given drinking water with ascorbic acid (control), 80 mg/L methamphetamine HCl **[chirality not specified],** or 80 mg/L methamphetamine HCL plus 200 mg/L chlorpromazine HCl for 4 weeks prior to mating and throughout gestation and lactation **[number of dams treated not specified]**. Male offspring killed at 3, 6, and 9 months after weaning for measurement of neurotransmitters and their metabolite levels in brain **[results only reported for 9 months, although authors stated there were no age-related effects; it is not clear how many litters were represented].**	There were no behavioral disturbances but treated rats were more difficult to handle. *Norepinephrine:* Methamphetamine increased levels in hippocampus, hypothalamus, and corpora quadrigemina and decreased level in thalamus. *Normetanephrine:* Methamphetamine increased levels in cortex and hippocampus and decreased level in pons medulla. *Dopamine:* Amphetamine increased level in cortex. Methamphetamine plus chlorpromazine decreased norepinephrine in hypothalamus and increased norepinephrine in pons medulla.	Methamphetamine changes catecholamines, primarily norepinephrine, concentrations in brain. ". . . the fact that normetanephrine concentrations are also altered suggests that the whole metabolism of [norepinephrine] may be disturbed."	It is a strength that methamphetamine was given orally, but the exposure through drinking water resulted in imprecise exposure information.	*(223)*
Sprague-Dawley rats. Animals sc injected with *d,l*-methamphetamine HCl at 0 (pair-fed, n = 6), or 50 (expressed as free base, n = 15) mg/kg bw twice daily from GD 7–12 or GD 13–18 (plug = GD 0). Two pups/sex/dose killed on PND 28 and striatum and hippocampus removed for monoamine measurement. Remainder of pups used for behavioral testing as summarized in Table 39. Non-neurological developmental effects	Methamphetamine treatment did not affect striatum levels of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxytryptamine (serotonin), or 5-HIAA or hippocampus levels of norepinephrine, 3-methoxy-4-hydroxyphenyl glycol, 5-hydroxytryptamine, or 5-HIAA.	"Monoamine metabolism in the hippocampus and neostriatum was not affected following in utero exposure to [*d,l*-methamphetamine] when measured on [PND 28]."	This well-controlled study used multiple endpoints, male-female analysis, and evaluation of neurotransmitter metabolites. The single sc dose level and lack of information on compound purity are weaknesses.	*(199)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
discussed in Section 3.2.2.				
Sprague-Dawley rats. Animals (n = 9) sc injected with 5 mg/kg bw/day *d,l*-methamphetamine HCl in 2 divided doses on GD 13–20. Two control groups were saline injected: 1 fed ad libitum (n = 5) and 1 pair-fed (n = 8). At birth, litters culled to 5 males and 4 females, then fostered to untreated dams. On PND 30 (female offspring) and PND 70 (male offspring), 1–2 pups/litter/group ip injected with saline or 8 mg/kg bw *p*-chloroamphetamine (a serotonin releaser).	Methamphetamine-treated and pair-fed dams gained less weight during treatment, but there was no effect on maternal cortical serotonin uptake site density. Methamphetamine treatment had no effect on litter size, male/female ratio, pup length, anogenital distance, or birth weight. p-*Chloroamphetamine response in PND 30 females:* Methamphetamine attenuated *p*-chloroamphetamine-induced increases in plasma renin concentration, but had no effect on ACTH or corticosterone. p-*Chloroamphetamine response in PND 70 males:* Methamphetamine attenuated *p*-chloroamphetamine-induced increases in plasma renin activity (also observed in pair-fed group) and plasma renin concentration, but had no effect on ACTH, corticosterone, or prolactin. *Serotonin uptake sites in cortex and hypothalamus and 5-HT_1 and 5-HT_2 receptors in cortex in PND 70 males:* No effect on density.	"These data, which demonstrate long-term postnatal deficits in [serotonin] mediated renin secretion, suggest selective functional alterations of brain [serotonin] systems in male and female progeny exposed in utero to methamphetamine."	None.	*(235)*
Wistar rat. Animals sc injected with 5 mg/kg bw/day *d,l*-methamphetamine HCl in 2 divided doses on GD 8–22. Controls were pair-fed and saline injected. **[Numbers treated not specified.]**	*Substantia nigra:* Non-significant trend for decreased tyrosine hydroxylase mRNA expression in female offspring on PND 14. *Ventral tegmental area:* Decreased tyrosine hydroxylase mRNA	"Collectively, the present results indicated that gestational [methamphetamine] exposure affects [tyrosine hydroxylase] gene expression in the postnatal life, a phenomenon that appears to be	This report demonstrates the transient nature of treatment effects; however, utility is limited by the lack of	*(239)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
Litters culled to 4 male and 4 female pups at birth. Offspring evaluated on PND 7, 14, and 30. **[Two to eight determinations/group but the number of pups examined and litters represented not specified.]**	expression in female offspring on PND 7 and 14. No effects in male offspring or in female offspring at the other time points.	transient, since it is no longer evident by the end of the first month of life in the rat."	experimental detail and the single sc dose level.	
C57B1/6 mouse. Animals (6–7/ group) sc injected twice daily with saline or 40 mg/kg bw *d*-methamphetamine HCl on GD 7–13 or 7–15. Dams killed on GD 16 for examination of dopamine levels in fetal brain.	Methamphetamine treatment during both gestational periods increased dopamine levels in fetal corpus striatum (60% for GD 7–13 and 32% for GD 7–15 compared to controls) and rostral mesencephalic tegmentum (48% for GD 7–13 and 25% for GD 7–15 compared to controls).	"This increase in fetal dopamine is consistent with our findings that exposure to methamphetamine in utero results in adult dopaminergic neurons which are more responsive in terms of methamphetamine induced release of the neurotransmitter and more sensitive to the neurotoxic effects of the drug." **[Based on previous study.]**	This study is useful in demonstrating prenatal effects. The addition of postnatal follow-up would have been helpful.	*(228)*
C57B1/6 mouse. Animals (8/group) sc injected twice daily with saline or 40 mg/kg bw *d*-methamphetamine HCl from GD 7–18 (plug = GD 1). Adult offspring (4/sex from different litters) received 2 sc injections separated by 2 hours of methamphetamine HCl 0, 5, 10, 15, or 20 mg/kg bw. Seven days later monoamines and metabolites were measured in brain.	Compared to mice exposed to saline in utero and challenged with methamphetamine in adulthood, in utero methamphetamine exposure and adult methamphetamine challenge resulted in decreased striatal levels of dopamine, DOPAC, homovanillic acid, and ventral brainstem dopamine level in males, and reduction of striatal 3-methoxytyramine in both sexes. Temperature response (increase correlated with decreased striatal dopamine) following methamphetamine challenge of adults did not vary with prenatal	Prenatal exposure to methamphetamine produces region- and gender-specific alterations in the adult brain that may signal increased susceptibility to neurotoxicity of male methamphetamine abusers who were also exposed prenatally to this drug.	This study is useful in investigating the effects of adult methamphetamine challenge following prenatal exposure. The single sc dose level is a weakness.	*(229)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
	treatment.			
		Postnatal Exposure		
Wistar rat. Males were sc injected with saline or 25 mg/kg bw/day *d*-amphetamine sulfate administered in 2 divided doses on PND 1 (day after birth) to PND 30. Growth was evaluated from PND 6 to 30 and brain weight on PND 30 (in males selected from 9 different litters containing 4 males/litter). Hippocampal volumetric parameters (n = 6/group) were measured on PND 30.	Amphetamine treatment inhibited body weight gain, especially on PND 6–12 and 24–30, and resulted in lower brain weight. Amphetamine treatment reduced mean volume of hippocampal formation with significant difference at the level of the dentate gyrus.	"The functional repercussions of the body weight gain allied to the decreased volume of the dentate gyrus molecular layer after early psychostimulant exposure are likely to be noteworthy since, whether permanent or simply representing a developmental delay, they will induce a reduction in the input information towards the hippocampus. . ."	This paper is not useful based on the grossness of the brain measurements. The single sc dose level is a weakness. The conclusions of the authors are speculative.	*(225)*
Sprague-Dawley rats. *Experiment 1:* Males and females (16/sex/group) ip injected with saline or 2.5 mg/kg bw/day *d*-amphetamine sulfate on PND 11–15 (pretreatment period), then received challenge with either saline or *d*-amphetamine on PND 23 or 90 (n = 4/sex/group). Following challenge, locomotor activity was tested (see Table 39) and	Compared to rats pretreated with saline, rats pretreated with *d*-amphetamine on PND 11–15 had lower dorsal striatal protein kinase A activity following challenge with saline (80.3% of saline pretreatment group) or *d*-amphetamine (66.7% of saline pretreatment group) on PND 23 and lower dorsal striatal protein kinase A activity following challenge with saline (67.3% of saline pretreatment) or *d*-amphetamine (80.5% of saline pretreatment group) on PND 90.	Behavioral relevance of reduced phosphokinase A activity is uncertain since locomotor sensitization did not occur following amphetamine challenge (see Table 39).	The single ip dose level is a weakness and the relevance of the findings is unclear.	*(236)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
dorsal striatum (caudate-putamen) removed for measurement of striatal protein kinase A activity.				
Experiment 2: Animals (male and female, 8/sex/group) ip injected with saline or 2.5 or 5.0 mg/kg bw/day *d*-amphetamine sulfate on PND 11–15, then challenged with 2.5 mg/kg bw *d*-amphetamine on PND 23 or 90. Rats were tested for motor activity (Table 39) and killed to measure protein kinase A activity in dorsal striatum and nucleus accumbens.	*PND 23:* Protein kinase A activity was lower in the dorsal striatum of the 2.5 mg/kg bw/day *d*-amphetamine pretreatment group (59.3% of saline pretreatment group). *PND 90:* In both the 2.5 and 5.0 mg/kg bw/day amphetamine pretreatment groups, protein kinase A activity was lower in dorsal striatum (76.2 % of saline control levels at high dose and 80.6% of saline control levels at the low dose) and nucleus accumbens (77.2 and 70.3% of saline control levels at the high and low dose).			
Sprague-Dawley rats. Males and females ip injected with saline or 2.5 mg/kg bw/day *d*-amphetamine sulfate on PND 11–17. Rats killed on PND 90 for evaluation of protein kinase A activity and dopaminergic endpoints in dorsal striata (5–6/group) **[not clear if n was per sex or total]**.	*Protein kinase A:* Activity in amphetamine group was decreased to 77% of control values in males and 88% of control values in females. *Dopamine:* Content in amphetamine group was decreased to 43% of control values in males and 75% of control values in females. *D_2-like receptors:* Density significantly increased with amphetamine treatment (176% of control values in males and 129% of control values in females), but there was no effect on affinity. *D_1-like receptors:* Density and affinity not significantly affected.	It was speculated that reduced dopamine levels may have caused the up-regulation of striatal D_2-receptors that may then have inhibited protein kinase A activity through the cAMP signal transduction pathway. **[Statement later retracted. See *(232)* below.]**	The evaluation of dopamine receptor changes is a strength. The single ip dose level is a weakness.	*(231)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
Sprague-Dawley rats. Males and females sc injected with saline or 10 mg/kg bw methamphetamine HCl (as free base) **[chirality not specified]** 4 times daily, every 2 hours, on PND 11–20 (day of birth = PND 0). Rats killed on PND 90 for evaluation of protein kinase A activity and dopaminergic endpoints in dorsal striatum (6/group, although not clear if the number was per sex or total).	Methamphetamine significantly reduced protein kinase A activity in males **[73.4% of control value]** and in males and females combined **[82.7% of control value]**; decreased density of D_2-like receptors in males and females combined (87.7% of control value) with no effect on receptor affinity; and reduced dopamine (86.8% of control value) and DOPAC (82.8% of control values) in both sexes. Males were more sensitive to effects on protein kinase A activity and DOPAC content.	Based on the decrease in D_2-like receptor binding sites, the authors retracted their previous suggestion that up-regulation of striatal D_2 receptors may be responsible for the inhibition of protein kinase A activity (see *(231)*, above). As an alternative, they suggested that desensitization of D_1-like receptors may be responsible for the decline in protein kinase A activity. They also noted the comparability of the response in this study to methamphetamine 40 mg/kg bw/day and the response in their prior studies to amphetamine 2.5 or 5 mg/kg bw/day. Given the greater neurotoxicity potency of methamphetamine, the authors concluded that the decrease in protein kinase A activity was not due to a neurotoxicity mechanism.	This study was better designed than previous studies, and evaluated long-term effects.	*(232)*
Sprague-Dawley rat. Animals sc injected twice daily on PND 10–40 with total doses of 0, 12.5, 25, or 50 mg/kg bw/day methamphetamine HCl **[chirality not specified]** or *d*-amphetamine sulfate. Each drug administered to at least 2 litters of 10 that had been constructed from pooled and redistributed	Caudate dopamine was reduced by *d*-amphetamine 50 mg/kg bw/day and by methamphetamine 25 and 50 mg/kg bw/day. There were no alterations in norepinephrine levels in caudate, midbrain, hypothalamus, pons-medulla, or telencephalon.	Findings were consistent with effects of the amphetamines on adult rat brains.	The multiple dose levels permit determination of a NOEL.	*(233)*

Strain and dose regimen	Amphetamine or methamphetamine findings	Author conclusions	Comments	Reference
PND 3 pups (without regard to sex). Two weeks after treatment, neurotransmitter levels were measured in brain.				
Sprague-Dawley rats. Animals sc injected with methamphetamine HCl **[chirality not specified]** from PND 7 to PND 10 (0, 50, or 100 mg/kg bw/day) or PND 17 to PND 20 (0 or 100 mg/kg bw/day). Treatments given in 2 equal doses per day separated by 12 hours. Pups **[sex not specified]** killed 2 weeks after the last dose and neurotransmitter levels in brain measured.	Methamphetamine-treated animals demonstrated significant reductions in caudate dopamine concentrations, ranging from 67–75% of control values. There were no alterations in norepinephrine or serotonin levels in caudate, pons-medulla, or telencephalon.	Caudate dopamine was reduced after neonatal methamphetamine to a lesser extent than in adults **[based on comparisons with previously published experiments; no adult data were presented in this paper].**	The use of only two dose levels is a weakness.	*(234)*
Male gerbil. Animals received a single ip injection of saline or 50 mg/kg bw methamphetamine on PND 14. Brains from 7–8 gerbils/group were examined on PND 90.	Methamphetamine treatment increased total dendritic length, slightly increased dendritic branching, and increased spine density in pyramidal cells of the prefrontal cortex.	"The present results show that early postnatal treatment with methamphetamine significantly interferes with morphogenesis of prefrontal pyramidal cells."	This paper used a novel species without a clear rationale. The use of a single ip injection is a weakness. The use of more detailed morphologic evaluation is a strength.	*(226)*

Strengths/Weaknesses: Strengths of the data set taken as a whole include the large number of studies conducted over more than a 40-year time span, the good experimental design and excellent statistics in some of the studies (particularly important is controlling the number of litters used), and the similarities noted in a large number of these studies. Summarizing across studies suggests that developmental exposure will produce developmental delays, alter motor function (activity), and yield deficits in cognitive functions. Biochemical studies, while not entirely confirmatory of one another, do support the hypothesis that amphetamine and methamphetamine alter catecholamines. Weaknesses include the inability to tell from many of the reports whether body weight and/or lethality confounded interpretation of behavioral changes and lack of information on how the high doses used in early postnatal exposures relate to early human exposures. Many studies, especially early reports, did not contain adequate statistical information concerning the number of litters and number of animals tested from each litter. Many studies used statistical procedures (e.g., *t*-tests) that can bias towards false positives.

Utility (Adequacy) for CERHR Evaluation Process: Overall, the data provide useful information for the evaluation process. Exposures are prenatal and postnatal. Confidence is moderate due to unresolved questions about the relationship between maternal and/or pup toxicity and the behavioral effects. This level of confidence could be increased with more detailed analyses of the data.

3.3 Utility of Developmental Toxicity Data

There are data from humans and experimental animals to evaluate the potential developmental toxicity of amphetamine. The experimental animal data set can be evaluated for methamphetamine. Most of the publications on human developmental toxicity involve illicit use for which the identity and purity of the drug being used is uncertain, making this literature of no utility for the evaluation of methamphetamine developmental toxicity in humans.

There are several case reports describing a variety of adverse outcomes among infants exposed antenatally to various amphetamines. These case reports are not useful in assessing the potential developmental effects of the amphetamines. There is a case series from Sweden involving 14 years of follow-up of children born to amphetamine-abusing women; the utility of this study is limited by the presence of other prenatal and postnatal liabilities for abnormal development. Three case-control and eight cohort studies of varying utility on amphetamine exposure during human pregnancy are available. Some of the exposures in these studies were to pharmaceutical preparations for weight loss and some were recreational exposures to methamphetamine. None of these studies involved amphetamines prescribed for ADHD.

There are five experimental animal developmental studies from which assessment of dose-response is possible, though limitations were noted in each of the studies. Two of these studies (one rat, one rabbit) were available only as FDA summaries *(34)*, precluding detailed review of the data by the Expert Panel. Three of the multidose-level studies were conducted in mice *(177-179)*. Additional developmental neurotoxicity studies featured treatment of pregnant or neonatal rodents. Of the 37 studies with behavioral endpoints (Table 39), 23 included a single dose level plus a control, 7 included 2 dose levels plus a control, and 7 included 3 or more dose levels plus a control. Quantitative evaluation of the behavioral endpoints was possible using this data set, but limited by the presence in many instances of high administered doses with significant uncontrolled effects on body weight. There were 19 papers with non-behavioral neurodevelopmental endpoints (Table 40), some of which also had behavioral endpoints. Of these 19 studies, 15 included a single dose level plus the control, and 2 each contained 2 dose levels

and 3 or more dose levels plus a control. Quantitative evaluation of neurodevelopmental endpoints was possible using this data set, but limited by the presence in many instances of high administered doses with significant uncontrolled effects on body weight.

3.4 Summary of Developmental Toxicity Data

3.4.1 Human Data

An uncontrolled case series from Sweden involved 71 children born to amphetamine addicts, 65 of whom were followed to age 14 *(107-109*). Abnormalities of growth and school performance were noted in these children; however, prenatal exposure to ethanol, a high rate of foster care, and other socioeconomic factors were not excluded as causes or contributors to the outcomes in these children. Another case series *(114*) reported that 28.6% of pregnancies in women with self-reported amphetamine use ended prior to 37 weeks gestation and 25% had newborns weighing less than 2500 g.

Controlled studies on pregnancy outcome in women exposed to amphetamines, or exposed to stimulants including amphetamines, are summarized in Table 41 and Table 42. Of the case-control studies in Table 41, one report *(121*) was evaluated by the Expert Panel as being of limited utility and two reports *(119, 122*) were evaluated as not being useful based on methodologic problems or lack of detail. Of the cohort studies reported in Table 42, those concerning illicit drug abuse were not considered useful in the evaluation process due to uncertainty about the constituents of the drug being used and for some studies, other methodological problems.

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Table 41. Case-Control Studies on Human Pregnancy Outcome after Maternal Exposure to Amphetamines

Cases and controls	Exposure assessment	Risk estimate	Reference
Cases = 184 children with congenital heart disease. Controls = 108 children without congenital heart disease.	Mothers asked within a year of the birth about *d*-amphetamine use.	18% of case mothers and 9% of control mothers were exposed ($P < 0.05$) **[OR 2.40, 95% CI 1.06–5.95].**	*(119)**
Cases = 458 mothers of children with congenital malformations. Control 1 = 500 mothers of normal children born immediately after each case. Control 2 = mothers of 411 mothers of normal children matched to cases on maternal age and parity and infant sex.	Mothers were asked about *d*-amphetamine use, with confirmation of use by general practitioner, hospital records, or prescription records.	For total malformations, exposure during all of pregnancy occurred in 13/458 cases and 10/911 controls **[OR 2.63, 95% CI 1.07–6.52]**, exposure in the first trimester occurred in 11/458 pregnancies and 8/911 controls **[OR 2.78, 95% CI 1.03–2.61]**, exposure during the first 56 days occurred in 10/458 pregnancies and 5/911 controls **[OR 4.04, 95% CI 1.27–13.65]**, and exposure during the first 14 days occurred in 8/458 pregnancies and 2/911 controls **[OR 8.08, 95% CI 1.59–55.25]**.	*(121)*
Cases: 11 infants with biliary atresia. Controls: 50 normal infants of the same age.	Drug use histories were solicited from mothers for amphetamines.	4/11 case mothers used amphetamines during the first trimester and 3/50 control mothers used amphetamines in pregnancy **[OR 8.95, 95% CI 1.20–70.92].**	*(122)**

ORs and 95% CIs were calculated by CERHR using the CDC SABER program. *This report was judged not to be useful in the evaluation process due to methodologic problems or lack of detail, and is presented here for completeness only.

Table 42. Cohort Studies on Human Pregnancy Outcome after Maternal Exposure to Amphetamines

Exposed and unexposed	Outcome assessment	Risk estimate/comparisons	Reference
Therapeutic use			
50,282 mother-child pairs, retrospective and prospective record of pregnancy exposures; exposures in first 4 lunar months: *d*-Amphetamine n = 367; "Amphetamines" n = 215 Methamphetamine n = 89.	Physical examination of children up to 1 year of age in 91% of the sample.	Comparison of children with specific exposure of interest to all children without exposure of interest during first 4 lunar months: *d*-Amphetamine crude RR 1.23 **[95% CI 0.82–1.82]**; standardized RR for any malformation 1.08 (95% CI 0.65–1.68), for major malformation 1.29 (95% CI 0.73–2.10), for minor malformation 1.46 (95% CI 0.59–2.98). Amphetamines crude RR 1.23 **[95% CI 0.72–2.05]**. Methamphetamine crude RR 0.87 **[95% CI 0.21–2.22]**.	*(123)*
		In a separate analysis, birth weight was 100–400 g lower among *d*-amphetamine-exposed non-malformed babies if the mother took the medication after 28 weeks of gestation and gained >12 kg during pregnancy or had a prepregnancy weight ≥45 kg.	*(124)*
White women insured by Kaiser Health Plan who delivered in the San Francisco East Bay area; 1694 used amphetamines for weight loss, 10,213 did not use anorectant drugs.	Diagnosis of a congenital malformation at birth or at a Kaiser clinic through 61 months of age.	For exposures during pregnancy, "severe congenital anomaly" in 3.4% of each exposure group **[crude RR 1.01 95% CI 0.76–1.32]**. Also, cleft palate in 5/1694 exposed, 21/10,213 unexposed **[crude RR 1.43, 95% CI 0.54–3.8]**. For exposures during the first 56 days of pregnancy: Cleft palate 3/175 exposed.	*(125)*
Illicit Use (Evaluated by the Expert Panel as not useful in the evaluation process due to uncertainty about the constituents of the drug being used, and, for some studies, other methodologic problems.)			
46 infants exposed antenatally to cocaine or methamphetamine identified by maternal or infant toxicology screens compared to 45 infants not exposed to tested illicit drugs.	Gestational age, birth weight, length, head circumference, perinatal complications. **[SD assumed in interpreting errors.]** Cocaine and amphetamine exposure not separated.	Exposed* / Unexposed Gestational age (weeks) 37.9 ± 3.0 / 39.4 ± 1.4 Birth weight (g) 2901 ± 711 / 3246 ± 552 Length (cm) 48.0 ± 5.1 / 50.7 ± 2.8 Head circumference (cm) 33.2 ± 2.7 / 34.4 ± 1.5 Perinatal complications (%) 28 / 9	*(126)*

Exposed and unexposed	Outcome assessment	Risk estimate/comparisons	Reference
		*Differences all statistically significant	
81 stimulant-exposed infants (27 infants exposed to methamphetamine) based on infant urine toxicology screening, 87 drug-free infants suspected of having hypoxic-ischemic encephalopathy (HIE), 19 normal drug-free infants. All born at term.	Presumably record review. Cocaine and amphetamine exposure not separated. **[SD assumed in interpreting errors]**.	Exposed / HIE / Control Birth weight (g) 2904 ± 475[a] / 3346 ± 687[b] / 3351 ± 420[b] Length (cm) 48.9 ± 3.1[a] / 50.5 ± 4.8[b] / 51.8 ± 2.5[ab] Head circumference (cm) 33.4 ± 1.5[a] / 34.4 ± 1.8[b] / 34.3 ± 1.1[b] Abnormal cranial ultrasound (%) 37.5[a] / 27.6[a] / 5.3[b] Gestational age (weeks) 38.9 ± 1.3[a] / 39.6 ± 1.3[b] / 39.4 ± 0.7[b] Intrauterine growth restriction (%) 19.8[a] / 8.0[b] / 5.3[b] [ab]Within row, different superscripts are significantly different $P < 0.05$.	*(127)*
52 methamphetamine-abusing pregnant women compared using record review with 52 women not known to have abused drugs whose babies were born next in the delivery unit.	Record review.	No difference between groups in rate of pregnancy complications or congenital anomalies. Exposed / Unexposed Gestational age (weeks) 39.1 ± 1.5 / 39.3 ± 2.0 Birth weight (g) 2957.0±574.0 / 3295.8±433.3 Length (cm) 48.1 ± 2.0 / 49.8 ± 2.3 Head circumference (cm) 33.2 ± 1.0 / 33.9 ± 1.2 All comparisons except gestational age were significant at $P < 0.001$.	*(128)*
135 methamphetamine-exposed infants identified by maternal urine toxicology or history, 160 matched controls, all ≥ 37 weeks gestation.	Record review.	Gestational age reduced a mean 2.2 weeks ($P < 0.001$) in methamphetamine group. Small for gestational age increased in methamphetamine group to 13/135 compared to 2/160 ($P < 0.001$) **[crude RR 7.70, 95% CI 2.08–46.20]**.	*(130)*
8 infants with history of maternal methamphetamine use during pregnancy, 8 unexposed infants matched for ethnicity.	Fagan Test of Infant Intelligence, visual-evoked potentials.	Birth weight 500 g lower in exposed group. Visual-evoked potentials not affected by exposure status. Fagan performance poorer in exposed infants with 4/8 "at risk" based on their scores, compared to 0/8 unexposed infants.	*(131)*

General side effects in children treated with *d*-amphetamine were discussed in two controlled studies *(134, 135*). Both studies reported reduced appetite and insomnia as the most common side effects. Headache, stomachache, and irritability were also reported in one study *(134*). While one study reported that an increase in depression was a surprising finding *(134*), the other study reported that "unusual happiness" was reduced by *d*-amphetamine therapy *(135*). One author noted that some side effects reported on stimulant therapy may actually be related to the underlying disorder *(135*).

There are no useful data with which to evaluate the risk of tics or movement disorders in association with amphetamine or *d*-methamphetamine treatment. There is only one controlled study *(135*), which has weaknesses that preclude its use in the evaluation process.

Concerns have been raised that stimulant treatment in childhood can increase the risk for developing substance abuse disorders later in life. Numerous studies examining possible associations between ADHD, independent of treatment, and substance abuse were not considered by the Expert Panel. The Panel notes a review by Wilens *(157*) that concluded, "There is a robust literature supporting a relationship between ADHD and SUD **[substance use disorders]**. Noncomorbid ADHD appears to confer an intermediate risk factor for SUD, although conduct and bipolar disorder appear to heighten the risk of early onset of SUD. . ." In studies found to be useful or to have limited usefulness for evaluating risks of substance abuse, the type of stimulant treatment was not specified in 1 study *(148*); *d*-amphetamine was given to 3% of subjects and to 2% of subjects who also received treatment with methylphenidate in the second study *(149*); and methylphenidate was probably used in 100% of subjects in the third study *(153*). None of the studies found evidence that prolonged treatment of ADHD with stimulants in childhood increased the risk of tobacco or cigarette use in adolescence *(148, 149*) or alcohol or substance abuse in adolescence *(148, 149*) or adulthood *(149, 153*). One of the studies found associations between ADHD and/or conduct disorders and substance use *(153*). Two studies reported associations between stimulant treatment (15% of subjects treated with amphetamines or pemoline) and tobacco or cocaine dependency in adulthood *(151, 152*). However, the Expert Panel found the studies to be unreliable for drawing conclusions due to limitations such as inadequate consideration of severity of ADHD, apparent errors in the reporting of data, and questionable statistical procedures.

The effects of *d*- or *d,l*-amphetamine on growth of children were evaluated in ten studies summarized in Table 33. Studies have reported variable findings; however, the weight of evidence suggests that amphetamine treatment is associated with an initial decrease in height and weight gain in children. It is not known whether final adult height and weight are affected by current treatment regimens, which frequently include continuous use and use beyond childhood. The quality of data in the older papers is suboptimal. These articles have variable but generally marginal-to-moderate utility with lack of masked assessments; incomplete documentation of compliance or actual dosing regimens; and failure to consider (in most cases) basic factors that are usually assessed in growth studies, such as mid-parent height and parent BMI; family history of timing of puberty onset; the child's actual physical or endocrinologic level of puberty at start of treatment (some of the youngsters were as old as 15 when the studies were conducted); and measurement of skeletal maturity (bone age), which particularly in school-aged children is considered a useful indication of expected growth potential. The seasonal differences in expected growth (in the northern hemisphere, children grow faster in summer) are not accounted for by designs that compare children whose families chose to leave them on stimulants through the summer and children whose families did not leave them on medication during the summer. Thus, it cannot be ruled out that those who remained on the medicines also had other conditions or

behavioral patterns (like fetal alcohol effects) that motivated their parents to continue the medication and might also decrease growth. The studies did not control for potential confounders such as intrauterine exposure to tobacco, ethanol, and illicit drugs or parental mental health.

3.4.2 Experimental Animal Data

Developmental toxicity studies providing dose-response information are summarized in Table 43.

A GLP rat and rabbit developmental toxicity study reviewed in the FDA Pharmacology Report *(34*) was not available to the Expert Panel, but is summarized here because it is the only study conducted with oral exposure at multiple dose levels. Pregnant rats (22/dose group) were gavaged on GD 6–17 with free amphetamine base in water at total daily dose levels of 0, 2, 6, or 20 mg/kg bw in 2 equal divided doses 8 hours apart. **[The Panel assumes that the test article was *d*-,*l*-amphetamine in a 3:1 ratio, as in the marketed product.]** The high-dose animals were killed on GD 9 due to excessive toxicity. There were clinical observations and a decrease in dam body weight gain in the low and middle dose. There were no treatment-related effects on implantations, resorptions, live young, pre- or post-implantation loss, sex ratio, or placental, fetus, or litter weights. There was an increase in the number of litters with 3 or more fetuses exhibiting delayed cranial ossification (1, 4, and 6 of 22 litters in the control, low-dose, and mid-dose groups, respectively **[Benchmark dose calculations performed by CERHR for this endpoint gave a BMD_{10} of 3 mg/kg bw/day and a BMDL of 2 mg/kg bw/day]**).

In the same FDA review *(34*), New Zealand White rabbits (22/dose group) were given divided amphetamine **[assumed 3:1 ratio of *d*- and *l*-enantiomers]** gavage doses totaling 0, 2, 6, or 16 mg/kg bw/day on GD 6–19. Clinical signs were noted in the high-dose dams. There were no treatment-related effects on weight gain or feed consumption. There were no effects on implantations, resorptions, live young, sex ratio, pre-implantation loss, or alterations in placental, fetal, or litter weights. A slight increase in post-implantation loss in the high-dose group (17.2 vs. 12% in controls) was not statistically analyzed and could not be done so by CERHR due to lack of SD or SEM. A reported increase in fetuses with 13 ribs was statistically analyzed by CERHR and found not to be significant. The FDA reviewer concluded that there was no clear evidence of developmental toxicity but noted that pharmacokinetic data (presented in Section 2) indicated that exposures in these experimental animal studies were lower than exposures in children (based on AUC) or only 2–3 times higher (based on C_{max}).

Fein et al. *(177*) ip injected ICR mice with 50 or 100 mg/kg bw/day *d*-amphetamine on GD 9 and GD 11 and control mice were injected with saline on 1 or more days between GD 9–11. EKGs were recorded in fetuses on GD 15 and 19, while other litters were evaluated for implantation and resorption sites, fetal survival, and external and internal malformations on GD 19. The number of surviving dams was reduced in both dose groups to 58–63% versus 100% in controls. Malformations were increased in fetuses from both dose groups and included microphthalmia, amelia, exencephaly, and cleft lip. EKG patterns in both dose groups suggested delayed histodifferentiation of cardiac tissue. At the high dose, resorptions were increased and fetal body weights (only reported for high dose) were reduced. **[The Expert Panel noted that this study is limited by the exceeding of MTD in dams and improper statistical procedures.]**

Kasirsky and Tansy *(178*) iv dosed 50 mice/group with 5 or 10 mg/kg bw/day methamphetamine HCl **[purity not specified]** on GD 9–11, 9–12, 12–15, or 9–15 (plug day = GD 1). One control group of 50 mice was not treated and a second group of controls was given saline on GD 9–15. Maternal weights were significantly reduced in every treatment group and feed and water intake were decreased on the first 2–3 days of exposure **[data not shown]**. Fetal weights were

significantly decreased in all treatment groups, but there were no significant effects on resorptions. **[The Expert Panel calculated a BMD_{10} of 2.1 mg/kg bw/day and BMDL of 1.5 mg/kg bw/day for fetal body weights on GD 9–15.]** The only significant increases in malformations (exencephaly, cleft palate, microphthalmia, and anophthalmia) occurred in fetuses from the group treated with 10.0 mg/kg bw/day methamphetamine on GD 9–15 (rate was 13.6 vs. 1% in either control group). **[The Expert Panel estimated a BMD_{10} of 9.2 mg/kg bw/day and BMDL of 8.4 mg/kg bw/day for malformations/live implant.]** The Expert Panel noted that this study is relevant for evaluation of abuse scenarios but is limited by incomplete reporting and lack of litter-based analyses.

Yamamoto et al. *(179*), administered mice (n=10–26/group) a single ip dose of methamphetamine HCl **[purity not indicated]** in saline on GD 8 (plug day = GD 0) at dose levels of 0, 11, 13, 14, 15, 17, 19, or 21 mg/kg bw. Larger numbers of animals were used for the higher dose groups in anticipation of treatment-induced maternal death, which occurred in 3 of 16 dams at 15 mg/kg bw, 5 of 14 dams at 17 mg/kg bw, 6 of 17 dams at 19 mg/kg bw, and 13 of 26 dams at 21 mg/kg bw. Maternal feed consumption and weight gain were not reported. Fetal body weights and mortality were not affected by treatment. External and skeletal malformations (visceral malformations not examined) occurred at doses ≥14 mg/kg bw. **[The Expert Panel calculated BMD_{10}s of 14.6 for fetal death, 16.1 for external malformations, and 15.5 for skeletal malformations; BMDLs were estimated at 10.3 for fetal death, 12.2 for external malformations, and 12.4 for skeletal malformations.]** The Expert Panel noted that the ip dose during pregnancy is not relevant for human therapeutic exposures due to a) possible direct adverse effects of the drug and/or solvent on the uterus and secondarily on embryo-fetal development and b) the possibility that the drug might be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism and distribution. The result interpretation is very limited by unconventional group housing of dams.

Other developmental toxicity studies included only a single dose level. Many of the studies dosed the animals by sc or ip injection, while humans typically are exposed by oral or iv routes,, and most studies had significant methodological limitations, such as inadequate statistical analysis. However, the studies are briefly described here since they provided qualitative information. No increase in malformations, prenatal mortality, or adverse effects on body weight gain were observed in offspring of rats sc dosed with 2 mg/kg bw/day *d*-amphetamine on GD 12–15 *(174*). An increase in dead fetuses was observed in mice gavage dosed with 50 mg/kg bw/day amphetamine throughout pregnancy, but there was no increase in malformations *(172*). Mortality and decreased body weight were observed in amphetamine-treated dams in this study. An increase in malformations (cardiac, cleft lip, eye) was observed in mice ip dosed with 50 mg/kg bw *d*-amphetamine on GD 8 *(175, 176*). There were no effects on estrous cyclicity or ovulation, but there was increased sexual receptivity in offspring of rats sc injected with 0.5 mg/kg bw/day *d*,*l*-amphetamine during the entire gestation period *(173*). Malformations (cyclopia and exencephaly) were increased in rabbits iv dosed with 1.5 mg/kg bw/day methamphetamine on GD 12–15 *(178*).

Sheep in the third trimester of pregnancy have been given iv doses of methamphetamine to evaluate effects on maternal and fetal hemodynamics, oxygenation, and acid-base balance. Treatment with 1.0–1.25 mg/kg bw in these studies has been associated with elevations of maternal and fetal blood pressure and decrements in fetal oxygenation consistent with impaired uteroplacental perfusion *(52, 53, 182, 183*). The Expert Panel determined that the studies in sheep were well conducted, but their application for evaluation of human toxicity is uncertain.

A number of studies reported non-neurological postnatal effects following gestational exposure to *d*-amphetamine or methamphetamine in rats. The only study with oral exposure was judged to be uninterpretable by the Expert Panel due to numerous design and analyses limitations *(191)*. The remaining studies examined effects in offspring of rats dosed sc during gestation with multiple dose levels. Though the route differs from human exposures that typically occur orally or iv and limitations were noted in most studies (e.g., analysis on a per fetus versus per litter basis), the studies do provide qualitative information and allow a dose-response assessment. Multiple-dose studies examining postnatal development in rats are summarized in Table 44. Studies with *d*-amphetamine provided evidence of decreased litter size at a dose ≥0.5 mg/kg bw/day and increased pup mortality at doses ≥2 mg/kg bw/day. Methamphetamine studies suggested that types and magnitude of effects can vary according to period of gestational exposure, so a direct comparison of studies is difficult. However, the studies suggest that prenatal methamphetamine exposure can result in decreased litter size (≥10 mg/kg bw/day), delayed eye opening (≥3 mg/kg bw/day), reduced postnatal body weight gain (≥3 mg/kg bw/day), and increased stillbirth or postnatal mortality (≥20 mg/kg bw/day). One study demonstrated that increases in stillbirths and postnatal mortality are greater with late- (GD 13–18) versus mid- (GD 7–12) gestational exposures *(49)*. Reductions in the number of dams delivering litters was observed at ≥4.5 mg/kg bw/day, and increases in eye defects were noted at ≥30 mg/kg bw/day. A delay in testicular descent was noted in one study where pups had other evidence of development delays *(197)*, but none of the reliable studies reported delays in vaginal opening. A more detailed explanation of study details and results, LOAELs, benchmark dose values, and complete references are included in Table 44.

The Expert Panel reviewed 37 rat studies with developmental neurotoxicity endpoints, which are summarized in Table 39. Prenatal studies involved sc administration of amphetamine or methamphetamine to dams during various time periods in gestation. The treatments in these studies resulted in significant decreases in maternal weight gain associated with a decrease in maternal feed intake. The use of pair-fed control animals was unusual *(49)*. Some studies reported adverse effects of treatment on pup birth weight *(213)* or viability *(49, 192, 199)*. Results of behavioral studies after prenatal exposures have not been consistent among studies, but generally suggest developmental delay *(193, 194, 197, 199)* and increased motor activity *(49, 174, 192, 199, 208)*. One study *(49)* found prenatal exposure to methamphetamine to be associated with impairment of learning and memory in adult offspring. These studies did not use ranges of doses and were not suitable for dose-response modeling. The lowest effect level reported with gestational treatment of the dam was 0.5 mg/kg bw/day for both *d*-amphetamine *(174)* and *d*,*l*-amphetamine *(207, 208)*.

Studies involving direct treatment of rat neonates used methamphetamine administered sc on PND 1–10 or PND 11–20. Both administration periods have been associated with a decrease in pup weight compared to vehicle treated controls. The decrement in pup weight persisted until at least PND 42 and in some cases until PND 70. Pup mortality during the treatment period was also increased by neonatal methamphetamine treatment in many of the studies. Treatment of neonates was associated with increased reactivity on acoustic startle testing and with deficits in associative processes and memory when animals were tested as adults. Single-dose studies were the rule and dose-response modeling was not possible. The lowest effective dose reported for behavioral alterations after neonatal treatment with *d*-methamphetamine was 30 mg/kg bw/day *(48)*. Behavioral deficits in adults after neonatal treatment are due to impairments in reference (long-term) memory and not to general motor or cognitive impairment or to deficits in working (short-term) memory, and have been attributed to alterations in hippocampus development that would be expected to correspond to developmental events in the third trimester of human pregnancy. Treatment of young rats with amphetamine or methamphetamine is also associated with

behavioral sensitization to subsequent challenge with amphetamine or methamphetamine. This sensitization can persist for months after the last treatment (reviewed in Section 2.5.3).

There were three reports in which methamphetamine *(195, 222*) or *d*-amphetamine *(213*) were given during gestation and continued through the lactation period. The two methamphetamine reports, which present different endpoints from the same experiment, involved sc treatment of dams. The amphetamine study used administration in drinking water **[probably resulting in direct treatment of pups during the last week of the lactation period]**. Adverse effects on dam and pup body weight were noted in these studies, and both studies showed developmental delay and increased motor activity in offspring, consistent with the studies that used only gestational exposures. Effective doses were 2 mg/kg bw/day *d*-amphetamine **[the lower of 2 dose levels used]** and 10 mg/kg bw/day methamphetamine **[the only dose level used]**.

The Expert Panel reviewed 17 experimental animal studies evaluating persistent anatomical or biochemical changes in the brains of rodents after prenatal or juvenile exposure to amphetamine or methamphetamine. The studies are summarized in Table 40. With the exception of a drinking water exposure study *(223*), exposures occurred through parenteral routes, mainly sc. Treatment of preweanling animals is associated with persistent changes in behavior, with little anatomic alteration in the brain. Some studies reported a transient increase in prefrontal cortex neuronal density in 14-day-old rats exposed to 10 mg/kg bw/day amphetamine on GD 8–22 *(224*) and reduced hippocampal volume formation in 30-day-old male rats exposed to 25 mg/kg bw/day amphetamine on PND 1–30 *(225*). Increases in dendritic length and branching and spine density of pyramidal cells of the prefrontal cortex were reported in 90-day-old male gerbils that received 50 mg/kg bw methamphetamine on PND 14 *(226*). Behavioral effects were not reported in these studies.

Most of the studies examining biochemical effects focused on measurement of monoamine levels in the brain. Studies of both amphetamine and methamphetamine in rats and mice demonstrated variable effects on brain levels of dopamine, norepinephrine, and serotonin. Direct comparison of studies examining monoaminergic endpoints is precluded by differences in species, dose level and duration, developmental period of exposure, age of evaluation, and brain region examined. No obvious patterns were noted. A series of studies from Crawford and colleagues examined amphetamine and methamphetamine effects on protein kinase A, a critical enzyme in signal transduction cascades of several different receptors, including dopamine receptors *(231, 232, 236*).

Cardiovascular defects, particularly ventricular septal defects (VSD), have been reported in a variety of study types reviewed by the Expert Panel. In these studies, amphetamine was administered during organogenesis. Early single-dose studies *(175, 176*) administering *d*-amphetamine ip to mice on GD 8 found a statistically significant increase in VSDs in fetuses. This effect was demonstrated in two strains of mice. Another single-dose study *(177*) administered 50 or 100 mg/kg ip on GD 9–11 to mice. This study identified amphetamine effects on fetal EKG (longer Q-T intervals) on GD 19 and delayed development of cardiomyocytes as determined histologically on GD 19. Studies in chick embryos *(188*) also demonstrated structural (including VSD) and functional (blood pressure) effects of amphetamine exposure during organogenesis. FDA summaries *(34*) of data from developmental toxicity studies using gavage administration of 2 and 6 mg/kg amphetamine (3:1 *d*- to *l*- amphetamine ratio) in rats and rabbits reported several individual fetuses with VSD defects, 1 rat in the 6 mg/kg dose group, 3 rabbits in the 2 mg/kg dose group, and 3 rabbits in the 6 mg/kg dose group. Although all of these individual studies have limitations, together they suggest that the heart is a target organ of concern for embryonic exposure to amphetamine. Data presented in a Letter to the Editor addressed

cardiovascular effects of prenatal amphetamine exposure in humans *(119*) and reported increased risk for congenital heart disease. Limitations of this report included limited detail on study design and population, lack of information on other exposures, such as ethanol, and lack of information on the reason for the amphetamine exposure.

Expert Panel Conclusions – Amphetamine

Human data are insufficient for an evaluation of the developmental toxicity of amphetamine following prenatal exposure. There are two studies that indicated no statistically significant increase in the overall rate of major or minor congenital malformations, although the data do not allow an evaluation of the risk of individual specific malformations. A study on birth weight reported an effect of amphetamine therapy but did not control for alcohol use and other confounds.

Data are insufficient for an evaluation of amphetamine effects on growth in children and adolescents. Growth studies in these children demonstrate an association of reduced growth and amphetamine treatment; however, a causal association with the medication is not possible due to a lack of control of potential confounding factors. These potential confounders could be causing the observed growth effects.

Data are insufficient to evaluate whether amphetamine therapy alters the risk of tobacco use, problematic alcohol consumption, and illicit substance abuse in adolescents and adults.

Data are insufficient to conclude whether amphetamine treatment of children at standard therapeutic doses increases the risk of tics or movement disorders.

Data are sufficient to demonstrate developmental toxicity of *d*- and *d,l*-amphetamine manifested as abnormal neurobehavioral testing in rats exposed during gestation to maternal doses of 0.5 mg/kg bw/day throughout pregnancy or 2 mg/kg bw/day on GD 12–15. There is insufficient evidence to conclude that there are morphological effects of amphetamine treatment on the brains of these animals. The experimental animal data are assumed relevant to humans.

There are sufficient data to conclude that ip injection of amphetamine in pregnant mice at doses of 50 mg/kg bw/day GD 9-11 increases the incidence of malformations in the offspring. Malformation data on other species or other routes were not available for the Expert Panel to review. The mouse data were not considered relevant to humans because of the use of ip dosing during pregnancy. Drugs administered ip in pregnancy can a) have a possible direct adverse effect on the uterus and secondarily on embryo-fetal development and b) possibly be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism and distribution.

Several rat and mouse studies reported effects on fetal or neonatal viability, but the evidence is not sufficient to permit conclusions due to limitations of the studies.

The experimental animal data are assumed relevant to humans.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/news/guidelines.html.

Expert Panel Conclusions – Methamphetamine

There are no interpretable human data on methamphetamine developmental toxicity.

Data are sufficient to demonstrate developmental toxicity of *d,l*-methamphetamine manifested as abnormal neurobehavioral testing in rats exposed during gestation to maternal doses of 2 mg/kg bw/day throughout pregnancy or GD 7–20 for methamphetamine (unspecified enantiomer) and 5 mg/kg bw/day for *d*-methamphetamine. There is insufficient evidence to conclude that there are morphological effects of methamphetamine treatment on the brains of these animals.

There is sufficient evidence that methamphetamine produces developmental toxicity in rats manifested as behavioral alterations after treatment of neonates with 30 mg/kg bw/day.

There is sufficient evidence that methamphetamine produces developmental toxicity in rats manifest as decreased pup weight at maternal doses of 3 mg/kg bw/day sc on GD 7–20 and decreased litter size at maternal doses of 10 mg/kg bw/day sc on GD 13–18. A number of studies examining fetal effects of gestational methamphetamine administration by the sc route in rats reported effects on litter size and postnatal pup weight.

The experimental animal data are assumed relevant to humans.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/news/guidelines.html.

Table 43. Summary of Multiple-Dose Experimental Animal Prenatal Developmental Toxicity Studies

Species/strain	Exposure	Maternal effect level, mg/kg bw or mg/kg bw/day	Critical developmental effects	Developmental effect level, mg/kg bw or mg/kg bw/day	Reference
Sprague-Dawley rat	*d,l*-Amphetamine. Gavage 0, 2, 6, or 20 mg/kg bw/day[a] on GD 6–17.	LOAEL = 2 mg/kg bw/day (decreased body weight gain)	Litters containing fetuses with delayed cranial ossification.	**[BMD_{10}[b] = 3; BMDL = 2]**	*(34)*
New Zealand White rabbit	*d,l*-Amphetamine. Gavage 0, 2, 6, or 16 mg/kg bw/day[a] on GD 6–19.	LOAEL = 16 (clinical signs)	Increased post-implantation loss.	LOAEL = 16	*(34)*
ICR mouse	*d*-Amphetamine. ip 50 or 100 mg/kg bw/day GD 9–11.	LOAEL = 50 (decreased survival)	Increased malformations and EKG patterns suggesting delayed myocardial differentiation.	LOAEL = 50	*(177)*
			Increased resorptions and decreased fetal weights.	LOAEL = 100	
Jcl:ICR mouse	Methamphetamine. ip 11, 13, 14, 15, 17, 19, or 21 mg/kg bw on GD 8.	LOAEL 19 (mortality) [**BMD_{10}[b] = 14.9, BMDL = 12.7**]	Fetal death	NOAEL 21 **[BMD_{10}[b] = 14.6; BMDL = 10.3]**	*(179)*
			External malformations	LOAEL 19 **[BMD_{10}[b] = 16.1; BMDL = 12.2]**	
			Skeletal malformations	LOAEL 14 **[BMD_{10}[b] = 15.5; BMDL = 12.4]**	
CF1 mouse	Methamphetamine iv 5.0 or 10.0 mg/kg bw/day on GD 9–11, 9–12, 12–15, or 9–15.	LOAEL = 5.0 (decreased body weight for all treatment days)	Resorptions	LOAEL = 5 **[BMD_{10}[b] = 0.6**	*(178)*
			Decreased fetal weight (all treatment days)	LOAEL = 5 **[BMD_{10}[b] = 2.1; BMDL = 1.5]**	
			Increased malformations (GD 9–15 treatment only)	LOAEL = 10 **[BMD_{10}[b] = 9.2; BMDL = 8.4]**	

[a]Doses were given in two divided doses and the values are presented as total daily dose.
[b]Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% CI around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making

Table 44. Summary of Multiple-Dose Experimental Animal Pre- and Postnatal Developmental Toxicity Studies

Species / strain	Exposure	Maternal effect level, mg/kg bw or mg/kg bw/day	Critical develop-mental effects	Developmental effect level, mg/kg bw/day	Reference
Sprague-Dawley rat	*d*-Amphetamine. sc 0, 2, or 6 mg/kg bw/day[a] on GD 5 through parturition	Not reported	Increased pup mortality	LOAEL = 2	*(192)*
Sprague-Dawley rat	*d*-Amphetamine. sc 0, 0.5, or 2.0 mg/kg bw/day on GD 12–15	NOAEL = 2	Decreased pups/litter at birth	LOAEL = 0.5 [**BMD_{10} = 2.0, BMDL = 1.1]**	*(193)*
Sprague-Dawley rat	Methamphetamine sc 0, 2, 6, or 10 mg/kg bw/day[a] GD 1–21	LOAEL = 2 (decreased gestation length and body weight gain). No litters delivered in 4 of 7 dams at high dose.	Decreased litter size and delayed eye opening	Pair-wise comparison with the control group was not reported.	*(194)*
Wistar rat	Methamphetamine. sc 0, 1, 2, 3, or 4.5 mg/kg bw/day on GD 7–20	LOAEL = 2 (decreased body weight gain). Only 1 litter delivered at 4.5 mg/kg bw/day.	Decreased male pup body weight gain during lactation and post-weaning; delayed testicular descent, incisor eruption, and eye opening	LOAEL = 3 **[Testicular descent: BMD_{10} = 3.8 ; BMDL = 3.3; incisor eruption: BMD_{10} = 5.1; BMDL = 3.1; eye opening: BMD_{10} = 15.7; BMDL = 3.2]**[a]	*(197)*
Sprague-Dawley rat	Methamphetamine. sc 0, 10, 20, 30, or 40 mg/kg bw/day[a] on GD 7–12 or GD 13–18.	LOAEL = 10 (decrease in body weight) **[BMD_{10} = 91 and BMDL = 56 for GD 13–18 exposure]**; dam mortality increased at 30 **[BMD_{10} = 27 and BMDL = 20 for GD 8–13 exposure]**.	Decreased litter size (GD 13–18 exposure)	LOAEL = 10 **[BMD_{10} = 38 and BMDL = 20**]	*(49)*
			Increased stillbirth and postnatal mortality on PND 1–3 (GD 13–18 exposure)	LOAEL = 20 **[Stillborn/pup: BMD_{10} = 36 and BMDL = 31; postnatal mortality/live-born pup: BMD_{10} = 53 and BMDL = 40]**	
			Increased stillbirth and postnatal mortality on PND 1–3 (GD 7–12 exposure)	LOAEL = 40 **[Stillborn/pup: BMD_{10} = 91 and BMDL = 58; postnatal mortality/live born pup: BMD_{10} = 48 and BMDL = 40]**	
			Decreased post-weaning offspring body weights (GD 13–18 exposure)	LOAEL = 30	
			Increased pups with anophthalmia or microphthalmia (GD 7–12 exposure).	LOAEL = 30 (not statistically significant); **[BMD_{10} = 48 and BMDL = 42**]	

[a]Doses were given in two divided doses and the values are presented as total daily dose.
b Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% CI around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human data were located on reproductive effects of amphetamines. The Expert Panel is aware of reports of sexual dysfunction in amphetamine addicts *(251, 252*). These reports are largely of an anecdotal nature and not suitable for an evaluation of amphetamine-related reproductive effects.

4.2 Experimental Animal Data

4.2.1 Female reproduction

Ramirez et al. *(173*), funding not indicated, examined the effects of prenatal amphetamine exposure on estrous cyclicity, sexual behavior, and hypothalamic monoamine levels in rats. Because the exposure was prenatal, this study assesses developmental toxicity and is discussed in Section 3.2.1.1.

4.2.2 Male reproduction

Cates and Jozeforicz *(253*), funding not indicated, reported that 200 µg/mL of *d*-amphetamine had no effect on *in vitro* motility of frog (*Rana pipiens*) sperm. **[Data were not shown.]**

Strengths/Weaknesses: Weaknesses of this study include the use of a single dose, a non-mammalian species, and an *in vitro* design. No rational was given for selection of test concentration.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Schneiden *(254*), funding not indicated, evaluated the *in vitro* effect of *d*-amphetamine exposure on rabbit sperm motility as compared to a glycine acetate control. Ten control samples and five or six samples per *d*-amphetamine concentration were evaluated. Sperm motility was inhibited at a *d*-amphetamine concentration of 500 µg/mL, but there was no effect at 25 µg/mL. **[Although it was stated that sperm motility was evaluated according to the method of Emmens, the results presentation was somewhat unclear, making it difficult to verify the author's conclusion.]**

Strengths/Weaknesses: Strengths of this study include the identification of a dose-response relationship and the use of standard protocols. Weaknesses include no rationale given for selection of test concentrations, small sample size, and insufficient experimental detail (e.g., age and body weights, order of sample collection versus treatment and analysis, maintenance of samples at a constant temperature, etc.).

Utility (Adequacy) for CERHR Evaluation Process: This *in vitro* study can be used as supplemental information in the evaluation process.

Larez et al. *(255*) examined dominant lethality in rats treated with *d*-amphetamine. Male Sprague-Dawley rats were gavage dosed with saline (n = 10) or 2 mg/kg bw/day *d*-amphetamine sulfate (n = 8) **[purity not specified]** for 5 days. Males were mated to 3 virgin rats per week over a period of 5 weeks. On GD 14, the females were killed for an examination of corpora lutea, resorptions, and implants. Mutagenic index was calculated by determining the percentage of early fetal deaths relative to total implants. Statistical significance was determined using the method of binomial distribution of proportions and percentages. Mutagenic index was significantly increased at week 3 ($P < 0.05$), and borderline significance ($p \approx 0.05$) was obtained for increases occurring during weeks 2 and 4. The study authors concluded that *d*-amphetamine was mutagenic under the conditions of this study.

Strengths/Weaknesses: Although typically performed as a mutagenesis assay, this study provides information on male reproductive toxicity by looking at pregnancy outcome when males are treated prior to mating. Strengths of the study include an oral route of administration with vehicle control. A weakness is use of a mating period shorter than the sperm cycle, limited pregnancy outcome endpoints (only resorptions),limited detail on methods and results, and lack of litter-based statistics.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility for the evaluation process.

Kasirsky and Tansy *(178)*, supported by NIH, evaluated the offspring of 6 male rabbits iv treated with methamphetamine HCl in saline at doses of 0, 1.5, 3.0, or 5.0 mg/kg bw/day for 3 months prior to mating. **[Very limited protocol details were provided, but it is assumed that procedures were similar to those conducted in female rabbits discussed in Section 3.2.1.1.]** After mating with treated males, untreated female rabbits were killed on GD 30 for examination of fetuses. There were no significant effects on whole litter resorptions, offspring survival, malformations, or fetal weight.

Strengths/Weaknesses: Strengths of this study are the route and duration of exposure, which are realistic for drugs of abuse. A weakness is the lack of clear information on sample size. Sample size is necessary to determine the power of the study to detect an adverse effect of treatment. In addition, insufficient experimental detail is given to adequately assess outcomes (e.g., no data on clinical signs, body weight, etc.).

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited.

Yamamoto et al. *(256)*, funding not indicated, examined the effects of methamphetamine exposure on reproductive toxicity in male mice. Eight-week-old male ICR mice were given a single ip injection of *d*-methamphetamine HCl **[purity not specified]** in saline at 0 (n = 30), 3.75 (n = 20), 7.5 (n = 20), or 15 (n = 60) mg/kg bw. Authors mention that 15 mg/kg bw is about 10 times the maximal dose in Japanese abusers of methamphetamine. Twenty-four hours after injection, mice were paired 1:1 with untreated female mice until a plug was detected or for 14 days. Dams were allowed to litter and at birth, litter size was noted, and pups were weighed and examined for external malformations. The same mating procedure was conducted in half the mice from the 15 mg/kg bw/day group 48 hours following injection. Additional mice (5–7/group) treated with saline or 15 mg/kg bw *d*-methamphetamine were evaluated for testicular and epididymal weight, serum testosterone level, sperm motility, sperm morphology, testicular and epididymal histology, and serum methamphetamine and amphetamine levels. The number of matings resulting in vaginal plugs was evaluated by chi-square test. Data on litter-based mortality was evaluated by Wilcoxon-Mann-Whitney test. Other data were evaluated by Student *t*-test.

Methamphetamine serum levels peaked at 7.5 µg/mL **[7500 ng/mL]** at 5 minutes and amphetamine levels peaked at 0.5 µg/mL **[500 ng/mL]** at 15 minutes. Serum levels of both compounds were below the detection limit 24 hours later, the time period between treatment and test initiation. Clinical signs in the 15 mg/kg bw/day group included hyperlocomotion and salivation, which peaked 15 minutes following injection. About 37% of the animals in the 15 mg/kg bw/day group died within 20 hours of treatment. The number of vaginal plugs and births were significantly reduced in the 15 mg/kg bw/day group mated 24 hours after treatment, but the effects were not observed 48 hours after treatment. **[In Table 1 of the study, it is not clear if the numbers of plugs and births are expressed in terms of the number of animals mated and evaluated in each group. The number of animals listed in Table 1 is the same as the number of animals that were said to be treated in the methods section. The death of 37% of males in the 15 mg/kg bw/day group suggests that the numbers mated would**

be well below the numbers treated, unless the numbers given in the methods section were for total evaluated instead of treated.] Weights of testes and epididymides were described as "slightly lower" in the 15 mg/kg bw/day group 24 hours after treatment, but it is not clear that statistical testing was performed **[*t*-test by CERHR showed no significant difference in weights of the caudae epididymides or left testes. There was a significant 7% decrease in right testis weight in the 24-hour 15 mg/kg bw group. Organ weights were not measured at lower methamphetamine doses. Variances were not specified, but were assumed to be SEM.]** Sperm motility was lower in males treated with 15 mg/kg bw/day and examined 24 and 48 hours later. The text identifies sperm motility as 57 and 62% of control values at 24 and 48 hours post-treatment with 15 mg/kg methamphetamine. Serum testosterone level was higher in the 15 mg/kg bw/day males examined at 24 hours, but lower in males examined at 48 hours. There were no significant effects on litter size, pup body weight, sex ratio, postnatal mortality, sperm morphology, or testicular or caudal epididymal histology at any dose level.

Strengths/Weaknesses: This study provides a good spectrum of information on male reproductive effects. The authors rightly concluded that there was very little difference between the dose that affected reproductive behavior and the dose that was toxic to the animal. Weaknesses are the limitations in endpoint information and analysis, use of the ip route, exceedance of MTD at the high concentration, no data on male body weights, use of inexperienced breeders, no indication that female mice were examined to verify normal estrous cyclicity, no verification of female fertility, no confirmation of mating by vaginal lavage samples, no measurement of sperm motility in animals mated 48 hours after treatment, and no definition of sperm motility parameters (e.g., number evaluated, number of microscopic fields). Weaknesses associated with testosterone analyses are no indication that diurnal variations were considered, insufficient sample size (n=5) considering variations in this measurement, and analysis only in high-dose animals.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited adequacy for use in the evaluation process.

Saito et al. ***(257)***, funding not indicated, examined copulatory behavior in 10-week-old male Wistar-Imamichi rats exposed to methamphetamine HCl **[chirality and purity not indicated]**. In the first of 2 experiments, male rats were given a single ip dose of methamphetamine in distilled water at 0, 1, 2, or 4 mg/kg bw and immediately tested for either copulatory behavior with a sexually receptive female (n = 6/group) or spontaneous motor activity (n = 7–10/group). Copulatory behavior data were analyzed by Fisher exact probability test and the Mann Whitney U test, while motor activity data were analyzed by the Duncan multiple *t*-test. In the high-dose group (4 mg/kg bw), the number of mounts, intromissions, and ejaculations over a 90-minute period was significantly reduced compared to the control group. Compared to a 100% rate in controls, 3 males in the 4 mg/kg bw group did not ejaculate and 2 of those males had no intromissions. Frequency of spontaneous motion was significantly increased at 2 and 4 mg/kg bw, and stereotypic behaviors (e.g., compulsive gnawing and sniffing) were observed in the 4 mg/kg bw group. In the second experiment, rats were ip administered 0 (n = 7) or 1 (n = 5) mg/kg bw methamphetamine HCl once/week for 8 weeks. Copulatory behavior during a 90-minute period was observed 5 times at 2-week intervals. A significant reduction in percentage of rats ejaculating over a 90-minute period reached statistical significance during the 4th and 5th testing. None of the treated rats ejaculated during the 90-minute period of the 5th testing, while all of the control rats ejaculated. Rats were housed overnight and a vaginal plug was found for all females the next morning, thus indicating delayed ejaculation. Percentage of treated rats intromitting was also significantly reduced during the 5th test. In treated rats, number of mounts was increased only during the 3rd testing and mount latency was increased only during the 4th testing. Body weight gain was not affected. Based on these findings, the study authors concluded that methamphetamine inhibits intromission and ejaculation in rats. **[Based on proportion of males ejaculating, the NOAEL is 2 mg/kg bw and the LOAEL is 4 mg/kg bw,**

according to the authors' results. Benchmark dose[6] calculations by CERHR using the EPA Benchmark Dose Software give a BMD_{10} of 2.0 mg/kg bw, and a BMDL of 1.1 mg/kg bw.]

Strengths/Weaknesses: A strength is that authors used estradiol- and progesterone-treated females to avoid differences in estrous cyclicity and/or receptivity. Sample sizes were sufficient in experiment 1, but small for behavioral assessments in experiment 2. A weakness is that rats were dosed with amphetamine by ip injection, while humans typically are exposed by oral or iv routes. The study would have been stronger if observational data were collected without knowledge of the treatment groups. In experiment 2, it is not clear when copulatory behavior was tested in relation to the weekly doses of methamphetamine. Motor activity was not examined in the rats dosed for 8 weeks (1x/week) with methamphetamine. This seems like an omission given that stereotypic behavior was observed at the 5th testing.

Adequacy/Utility for CERHR Evaluation Process: This study is of limited adequacy for the evaluation process.

4.2.3 Mating studies in concurrently treated males and females

The FDA Pharmacology Review for Adderall *(34)* summarized an oral fertility and early embryo development study in Sprague-Dawley rats, submitted to the agency as part of the approval process. Male and female rats (22/sex/dose group) were gavaged with free amphetamine base in water at total daily dose levels of 0, 2, 6, or 20 mg/kg bw in 2 equal divided doses 8 hours apart **[the Panel assumes that the test article was *d*-,*l*-amphetamine in a 3:1 ratio, as in the marketed product]**. Males were treated for 29 days prior to mating and females were treated for 15 days prior to mating. Males and females were paired for up to 3 weeks with continuation of treatment throughout the mating period and until GD 7 in females. Males were treated for a total of 8 weeks after which they were killed and reproductive organs weighed and examined macroscopically. Females were killed on GD 14 and uterine contents evaluated.

Dose-related clinical signs occurred in the middle- and high-dose groups, with few and transient clinical signs in the low-dose group. Body weight was dose-dependently decreased in the middle-and high-dose group as well. No effects of treatment were seen on estrous cyclicity, mating, fertility, live young, resorptions, or pre- or post-implantation loss. Absolute but not relative male reproductive organ weight changes occurred in high-dose males. **[Absolute epididymal weight, which is typically conserved in the presence of moderate body weight decrements *(258)*, was decreased by 6% in the high-dose males; it is not known whether this value was statistically identified.]** In an accompanying pharmacokinetic study after ~21 days of dosing (males) or ~7 days of dosing (females) with 20 mg/kg bw/day in a twice daily gavage regimen, blood samples were collected after the first daily dosing. The C_{max} in males was 880–976 ng/mL and C_{max} in females was 1081–1197 ng/mL. The mean AUC_{24} value in males was 2139 ng-h/mL and in females was 4909 ng-h/mL. At the 20-mg/kg bw/day dose, the mean AUC_{24} in males was 5689 ng-h/mL and in females was 8506 ng-h/mL. The FDA reviewer noted for comparison purposes that children receiving 30 mg/day amphetamine therapy have a mean AUC_{24} of 1800 ng-h/mL. The total exposure at the high dose in the fertility study was only 3 times that of children on therapy for male rats and only 5 times that of children on therapy for female rats. The FDA reviewer called attention to the use of a different dosing regimen in the range-

[6]Benchmark dose estimates performed using EPA software version 1.3.2. The BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL represents the dose associated with the lower 95% CI around this estimate. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

finding study (once daily dosing) versus the definitive study (two divided doses/day), and the use of nonpregnant animals for the range-finding and the pharmacokinetic studies.

Strengths/Weaknesses: This is a full fertility study using standard methods. Strengths are sufficient group sizes, appropriate controls, and sufficient duration of dosing prior to breeding. The dosing paradigm (oral administration in 2 equal divided doses 8 hours apart) was designed to better mimic clinical exposure patterns. Blood samples were collected prior to mating for toxicokinetic evaluations. The lack of detailed data is a weakness (i.e., no data or mean values for estrous cycles, time to mating, organ weights, etc.). The high dose exceeded the MTD as determined by body weights and body weight gains. Pair-fed controls were not included. Statistical analyses were not described nor were statistical results presented. Male reproductive organs were weighed and examined macroscopically rather than by histopathological examination. As stated in the report summary, the safety margins provided by this study were minimal, with mean total exposures in rats equal to 3–5 times the levels measured in children given 30 mg, the maximum clinical dose.

Utility (Adequacy) for CERHR Evaluation Process: This study could not be used in the evaluation process because a full report of the study was not available for review.

4.3 Utility of data

There are no data from controlled human studies with which to evaluate possible reproductive toxicity of amphetamine or methamphetamine. The animal data are insufficient for an evaluation of possible reproductive toxicity following exposure to amphetamine or methamphetamine. A one-generation fertility study of *d,l*-amphetamine in rats was reviewed by the FDA, but the original study was not available to the Expert Panel. There are no methamphetamine studies that examine fertility in female rats. Limited studies in male rats exposed to methamphetamine examine sexual function and fertility parameters.

4.4 Summary of Reproductive Toxicity Data

4.4.1 Human data

No human data from controlled studies were located.

4.4.2 Experimental Animal data

Repeat dosing studies are summarized in Table 45.

In a developmental toxicity study that is described in detail in Section 3, rats were sc injected with 0 or 0.5 mg/kg bw/day *d,l*-amphetamine over the entire gestation period *(173*). Female offspring exposed to amphetamine in utero were more sexually receptive, as determined by lordosis response, but exhibited no effects on estrous cyclicity or ovulation.

In a rat dominant lethality test, percent early fetal deaths relative to total implants was significantly increased in fetuses sired by males treated with 2 mg/kg bw/day *d*-amphetamine sulfate and mated 3 weeks after dosing; borderline significance was obtained at 2 and 4 weeks after dosing *(255*).

The FDA *(34*) reviewed a fertility study conducted with *d,l*-amphetamine in rats. The study was not available to the Expert Panel, but is described in this section, due to the paucity of amphetamine reproductive toxicity data. In that study, male and female rats (22/sex/dose group) were gavaged with free amphetamine base in water at total daily dose levels of 0, 2, 6, or 20 mg/kg bw administered in 2 equal divided doses 8 hours apart. Males were treated for 29 days prior to mating and throughout a 3-week mating period. Following treatment, males were killed for weighing and macroscopic evaluation

of reproductive organs. Females were treated for 15 days prior to mating, during the 3-week mating period, and through GD 7. Females were killed on GD 14 for evaluation of uterine contents. Reduction in body weight and clinical signs occurred in the middle- and high-dose groups. Amphetamine treatment had no effect on estrous cyclicity, mating, fertility, live young, resorptions, or pre- or post-implantation loss. Absolute but not relative male reproductive organ weight changes occurred in the high-dose males. Based on preliminary pharmacokinetic studies in rats, the FDA estimated that respective total exposures in high-dose male and female rats were 3 and 5 times that of children on therapy.

Saito et al. *(257*), dosed male Wistar-Imamichi rats with a single ip dose of methamphetamine at 0, 1, 2, or 4 mg/kg bw and immediately tested copulatory behavior with a sexually receptive female. At 4 mg/kg bw, the number of mounts, intromissions, and ejaculations over a 90-minute period was significantly reduced compared to the control group. Frequency of spontaneous motion increased at 2 and 4 mg/kg bw, and stereotypic behavior increased at 4 mg/kg bw group. In a second experiment, rats were ip administered 0 (n = 7) or 1 (n = 5) mg/kg bw methamphetamine HCl once/week for 8 weeks. Copulatory behavior was observed 5 times at 2-week intervals. In the 1 mg/kg bw group, a decrease in percentage of rats ejaculating reached statistical significance during the 4th and 5th testing and percentage of treated rats intromitting was reduced during the 5th test. **[Based on proportion of males ejaculating, the NOAEL is 2 mg/kg bw and the LOAEL is 4 mg/kg bw, according to the authors results. Benchmark dose calculations by CERHR using the EPA Benchmark Dose Software version 1.3.2 gave a BMD_{10} of 2.0 mg/kg bw and a BMDL of 1.1 mg/kg bw.]**

Yamamoto et al. *(256*), treated mice with a single ip injection of *d*-methamphetamine HCl in saline at 0 (n = 30), 3.75 (n = 20), 7.5 (n = 20), or 15 (n = 60) mg/kg bw. **[It is not clear if numbers are for total numbers treated or evaluated.]** Twenty-four hours after injection, mice were paired 1:1 with untreated female mice until a plug was detected or for 14 days. The same mating procedure was conducted in half the mice from the 15 mg/kg bw/day group 48 hours following injection. Dams were allowed to litter and at birth, litter size was noted, and pups were weighed and examined for external malformations. Clinical signs were observed in the 15 mg/kg bw/day group and about 37% of the animals died within 20 hours of treatment. The number of vaginal plugs and births were significantly reduced in the 15 mg/kg bw/day group mated 24 hours after treatment, but the effects were not observed 48 hours after treatment. There were no significant effects on litter size, pup body weight, sex ratio, or postnatal mortality at any dose level. Additional mice (5–7/group) were treated with 0 or 15 mg/kg bw *d*-methamphetamine and evaluated for testicular and epididymal weight, serum testosterone level, sperm motility, and serum methamphetamine and amphetamine levels. Weights of testes and epididymides were described as "slightly lower" in the 15 mg/kg bw/day group 24 hours after treatment. **[*t*-Test by CERHR showed no significant difference in weights of the caudae epididymides or left testis. There was a significant 7% decrease in right testis weight in the 24-hour 15-mg/kg bw group. Variances were not specified but were assumed to be SEM.]**
Sperm motility was lower in males treated with 15 mg/kg bw/day and examined 24 and 48 hours later. Serum testosterone level was higher in the 15 mg/kg bw/day males examined at 24 hours, but lower in males examined at 48 hours. The Expert Panel noted that reproductive competency of untreated female mice was not verified, and measurements of testosterone levels were inadequate since factors such as diurnal variations were not considered.

In a study that provided limited protocol details, 6 male rabbits were iv treated with methamphetamine HCl in saline at doses of 0, 1.5, 3.0, or 5.0 mg/kg bw/day for 3 months prior to mating *(178*). The rabbits were mated with untreated females that were killed on GD 30 for examination of fetuses. There were no significant effects on whole litter resorptions, offspring survival, malformations, or fetal weight.

Expert Panel Conclusions - Amphetamine

There are no human reproductive data.

Experimental animal data are insufficient to evaluate male and female reproductive toxicity of amphetamine. Only one study was found in the open literature on male reproduction and one study on the effects of prenatal exposure on the female reproductive system. Reproductive toxicity studies submitted to FDA were not available in sufficient detail for review.

Expert Panel Conclusions- Methamphetamine

There are no human reproductive data.

There are no experimental animal female reproductive toxicity studies or male or female fertility studies.

Data are insufficient to conclude that methamphetamine affects male mating behavior. In one study rats injected with 1 mg/kg methamphetamine for a 5-week period failed to ejaculate during a 90-minute test

Table 45. Summary of Multiple-Dose Amphetamine and Methamphetamine Reproductive Toxicity Studies

Sex/Species/Strain	Drug/Exposure	Critical effect	Effect level	Reference
Male and female Sprague-Dawley rat	*d,l*-Amphetamine. Gavage (free base) 0, 2, 6, or 20 mg/kg bw in 2 equal divided doses 8 hours apart for 29 days prior to mating through a 3-week mating period in males and 15 days prior to mating through GD 7 in females.	No effects on estrous cyclicity, mating, fertility, live young, resorptions, or pre- or post-implantation loss.	NOAEL = 20 mg/kg bw/day	FDA *(34)*
Male Wistar-Imamichi rat	Methamphetamine HCl. Ip 0, 1, 2, or 4 mg/kg bw, single dose.[a]	Decreased number of mounts, intromissions, and ejaculations over a 90-minute period.	LOAEL = 4 mg/kg bw NOAEL = 2 mg/kg bw/day **[BMD_{10} of 2.0 mg/kg bw, and a BMDL of 1.1 mg/kg bw]**	Saito et al. *(257)*
Male ICR mouse	*d*-Methamphetamine HCl. Ip 0, 3.75, 7.5, or 15 mg/kg bw, single injection.	Decreased vaginal plugs and births in mice mated 24 hours after treatment, but not 48 hours after treatment. Decreased sperm motility at 24 and 48 hours after treatment; increased serum testosterone level at 24 hours, but lower at 48 hours after treatment **[Sperm and testosterone were only examined in animals treated with 15 mg/kg bw/day].**	LOAEL = 15 mg/kg bw/day NOAEL (for vaginal plugs and births) = 7.5 mg/kg bw/day	Yamamoto et al. *(256)*
		No significant effects on litter size, pup body weight, sex ratio, or postnatal mortality.	NOAEL = 15 mg/kg bw/day	
Male rabbit	Methamphetamine HCl. Iv 0, 1.5, 3.0, or 5.0 mg/kg bw/day for 3 months prior to mating.	No significant effects on whole litter resorptions, offspring survival, malformations, or fetal weight.	NOAEL = 5 mg/kg bw/day	Kasirsky and Tansy *(178)*

[a]Results of a single-dose study with multiple exposures over a period of weeks is discussed in the summary text.

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Developmental Toxicity

5.1.1 Human Data

The Expert Panel concluded that there was insufficient evidence to evaluate the developmental toxicity of therapeutic use of amphetamines in humans. There were two cohort studies in which pregnant women were prescribed amphetamines to limit weight gain during gestation that did not show statistically significant increases in overall malformations, but their power for studying malformations was low. These studies are not sufficient to draw conclusions about risk of specific malformations. There is insufficient evidence for a conclusion on the effect of gestational exposure to amphetamine on birth weight.

There are insufficient data to evaluate whether amphetamine treatment in children is associated with depressed growth, increased tics, or altered risk of tobacco use, problematic alcohol use, or illicit substance use in childhood, adolescence, and adulthood.

The Expert Panel concludes that the data regarding illicit methamphetamine are insufficient to draw conclusions concerning developmental toxicity in humans.

5.1.2. Experimental Animal Data

5.1.2.1 Amphetamine

There is insufficient information to evaluate the effects of oral amphetamine on developmental toxicity in animals. Although there are limitations in most available studies, a weight of evidence approach suggests patterns of developmental effects following in utero amphetamine exposure. Increased malformations, which included cardiac malformations, cleft lip, eye abnormalities and exencephaly, were observed in 3 mouse studies involving ip administration of $\geq$50 mg/kg bw/day amphetamine on GD 8-11. However, these data were not considered relevant to humans due to use of ip dosing during pregnancy. Drugs administered ip in pregnancy can a) possibly have a direct adverse effect on the uterus and secondarily on embryo-fetal development and b) possibly be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism and distribution The majority of other developmental toxicity studies used sc exposures to examine the developmental effects of amphetamine. Data from several studies in mice and rats suggest that decreased fetal and/or neonatal viability may occur with prenatal amphetamine exposure; however, this finding was inconclusive based on limitations in the animal studies and/or excessive maternal toxicity.

Although limited by parenteral administration of single dose levels in most studies, rat data are sufficient to demonstrate developmental toxicity of *d*- and *d,l*-amphetamine. Toxicity is manifested as abnormal neurobehavioral function in rats exposed during gestation to maternal doses of 0.5 mg/kg bw/day throughout pregnancy or 2 mg/kg bw/day on GD 12–15. The data are insufficient for making conclusions about effects on monoamine levels and morphological effects of amphetamine treatment on the brains of these animals.

5.1.2.2 Methamphetamine

There were no methamphetamine developmental toxicity studies in experimental animals that used the oral route of exposure. Available studies employed different routes of exposure, but sc injection was most common. A qualitative evaluation of these studies indicates that decreased litter size and postnatal body weight gains were observed in pregnant rats exposed to methamphetamine by sc injection. Four rat studies supported the conclusion of altered litter size and/or decreased postnatal survival with methamphetamine treatment. These effects were seen at similar doses to those altering maternal body weights, although decreased offspring survival was also seen in studies that included a pair-fed control. A similar number of studies support alterations in pup body weights. In some cases, these body weight effects persisted beyond cessation of treatment. Additional comparisons of methamphetamine studies were complicated by exposures during varying periods of gestation.

Although limited by parenteral administration of single-dose levels in most studies, rat data are sufficient to demonstrate developmental neurotoxicity of *d,l*-methamphetamine. Toxicity is manifested as abnormal neurobehavioral testing in rats exposed during gestation and/or lactation to maternal doses of 2 mg/kg bw/day throughout pregnancy or GD 7–20 for methamphetamine (enantiomer unspecified) and 5 mg/kg bw/day for *d*-methamphetamine. There is insufficient evidence to conclude that there are effects on monoamine levels or morphological effects of methamphetamine treatment on the brains of these animals.

There is sufficient evidence that methamphetamine produces developmental toxicity in rats manifested as behavioral alterations after treatment of neonates with 30 mg/kg bw/day.

5.2 Reproductive Toxicity

There are no human reproductive toxicity data for amphetamine or methamphetamine.

The Expert Panel concluded that there are insufficient experimental animal amphetamine data to evaluate reproductive toxicity. Only two relevant studies were available in the open literature, both of which were very limited in scope. Guideline reproductive toxicity studies conducted for submission to FDA as preclinical toxicity data were not available in sufficient detail for the Expert Panel to review.

The Expert Panel concluded that data are not sufficient to evaluate reproductive toxicity of methamphetamine in animals. There are no female reproductive toxicity studies or male or female fertility studies. Delayed ejaculations occurred in rats injected with methamphetamine for a 5-week period, but the data were judged to be insufficient for evaluating male mating behavior.

5.3 Summary of Human Exposures

The focus of this report is the amphetamines approved for clinical practice: *d*-, and *d,l*-amphetamine and *d*-methamphetamine. The number of amphetamine prescriptions written increased between 1992 and 2000 from fewer than 500,000 to nearly 8 million per year *(12)*. Treatment of ADHD in teenagers and adults is increasing. More people of reproductive age may be taking amphetamine, but there is no information on the numbers of pregnant or lactating women who are taking the drug(s).

The *d* and *d*,*l*-amphetamine preparations are indicated for the treatment of ADHD and narcolepsy *(6, 9)*, and methamphetamine is indicated for the treatment of ADHD and short-term treatment of obesity *(8)*. The Expert Panel recognizes that therapeutic use of *d*-methamphetamine in the US is

uncommon. Recommended doses of amphetamine are 2.5–40 mg/day for treatment of ADHD in individuals 3 years of age and older and 5–60 mg/day for treatment of narcolepsy in individuals 6 years of age and older.

The Expert Panel is aware of off-label uses of amphetamines to treat depression, primarily as an adjunct to antidepressant medication, and to treat patients with post-stroke cognitive impairment.

d-Methamphetamine hydrochloride is used as a recreational drug. Routes of administration include inhalation, nasal, iv, and oral. There is also potential for abuse of *d*- and *d,l*-amphetamine.

5.4 Overall Conclusions

The Expert Panel was impressed with the paucity of interpretable toxicity data relevant to human therapeutic use. The decision to use the medication must be made by the responsible health care provider, the patient, and the family if the patient is a minor.

5.4.1 Amphetamine

There is a substantial published database of studies designed to investigate the potential adverse reproductive and developmental effects of amphetamine exposure in both humans and laboratory animals. However, thorough review of these studies led the Expert Panel to judge that within all of the human domains and some of the animal domains, the data were generally insufficient to reach valid scientific conclusions.

Specifically, the Expert Panel found data were available but insufficient to evaluate:

- developmental toxicity in children following intrauterine exposure;
- growth in children and adolescents receiving medication;
- altered risks of tobacco use, problematic alcohol consumption, or illicit substance abuse in adolescents or adults;
- altered risks of tics or movement disorders in treated children;
- fetal and/or neonatal viability in laboratory animals exposed prenatally;
- morphological effects in brains of laboratory animals; and
- male and female reproductive toxicity in experimental animals.

There were no human reproductive data available.

The Expert Panel judged the data sufficient to conclude that there was evidence for the following:

- neurobehavioral alterations following prenatal exposure in rats to maternal sc doses of 0.5 mg/kg bw/day throughout pregnancy or 2 mg/kg bw/day on GD 12–15; and
- increased incidence of congenital malformations in mice following ip injections of 50 mg/kg bw/day. The data were not considered relevant to humans because of the route of administration during pregnancy and the corresponding lack of information on pharmacokinetics. Drugs administered ip in pregnancy can a) possibly have a direct adverse effect on the uterus and secondarily on embryo-fetal development and b) possibly be physically transported directly to the embryo/fetus, bypassing maternal absorption, metabolism, and distribution.

Thus, the Expert Panel concluded that, based on the experimental animal findings, there was some concern with regard to potential neurobehavioral alterations due to prenatal amphetamine exposure in humans both in therapeutic and non-therapeutic settings.

5.4.2 Methamphetamine

There is a substantial published database of studies designed to investigate the potential adverse reproductive and developmental effects of methamphetamine exposure in both humans and laboratory animals. However, the Expert Panel felt that the studies that focused upon humans were uninterpretable due to such factors as a lack of control of potential confounding factors and the issue of the purity and contaminants of the methamphetamine used by the drug abusers.

The Expert Panel found data were available but insufficient to evaluate the following:

- morphological effects in brains of laboratory animals; and
- male mating behavior in laboratory animals. There were no experimental animal female reproductive toxicity or male or female fertility studies.

The Expert Panel judged the data sufficient to conclude that there was evidence for the following:

- neurobehavioral alterations following pre- and/or postnatal exposure in laboratory animals; and
- effects on litter size and postnatal pup weight.

Thus, the Expert Panel concluded that, based on the experimental animal findings, there was concern with regard to potential adverse perinatal outcomes and neurobehavioral alterations due to prenatal methamphetamine exposure in humans both in therapeutic and non-therapeutic settings.

5.5 Critical Data Needs

Critical data needs are defined as research or studies that would provide information to substantially reduce uncertainty and increase confidence in assessing human reproductive and developmental risks. Although studies documenting effects of childhood exposures were available, the Expert Panel found the studies were generally limited due to inadequate design, use of outdated methods or standards, and insufficient numbers of subjects treated only with amphetamines. Therefore, the Expert Panel concluded that better quality studies are required to effectively evaluate toxicity concerns associated with amphetamines. There are a number of considerations that should be applied in the design of quality human studies for amphetamines. The studies need to use current techniques and age-standardized norms. Confounding factors such as prenatal and/or postnatal exposure to tobacco, alcohol, and illicit drugs, parental psychiatric disorders, and care-giving environment need to be noted and adequately controlled. Subpopulations that are susceptible to development of ADHD (e.g., children born prematurely) need to be considered in study design and interpretation. The studies need to consider currently under-represented populations such as children born prematurely, non-White individuals, and females. Current trends in treatment such as infrequent use of drug holidays and durations of exposure that often extend through adolescence and into adulthood need to be considered. The studies should compare endpoints in individuals within the same developmental stage (e.g., childhood versus adolescence) and use appropriate controls.

5.5.1 Amphetamines

5.5.1.1 Human Studies

- Pharmacokinetic data related to therapeutic use during pregnancy and lactation are needed.
- Data on possible reproductive effects of amphetamines in humans are needed
- Growth data (height and weight) in both prepubertal and pubertal children and adolescents are needed.
- Rates of progression through pubertal stages in boys and girls with ADHD treated with amphetamines are needed.
- Studies are needed to characterize the effects of amphetamines on tic and other movement disorders.
- Because of the wide age spectrum in which the drug is used, studies are needed to identify possible developmental variations that can affect toxicity.
- Toxicity data are needed for under-represented populations of children and adolescents including girls, non-Caucasians, children with dysmorphic and genetic syndromes and global mental retardation, and children born prematurely.
- Data on neurobehavioral outcomes from infancy to adulthood following prenatal exposure in humans are needed.
- Data on long-term neurobehavioral outcomes across the life cycle following therapeutic postnatal exposure in humans are needed.
- Studies are needed to evaluate possible effects of amphetamine treatment on tobacco use, problematic use of alcohol, and illicit substance use in children, adolescents, and adults.
- Epidemiological data are needed regarding the extent of therapeutic and off-label exposure during periconceptual period, pregnancy, and lactation among women with ADHD, narcolepsy, and/or depression.
- Data are needed to determine the numbers of amphetamine prescriptions for teenagers, adults, and children < 3 years old.
- Studies are needed to characterize the long-term effects of amphetamine treatment on heart rate and blood pressure. The studies need to consider all populations, including the under-represented populations denoted above.

5.1.1.2 Experimental Animal Studies

- Data are needed on developmental and reproductive toxicity of amphetamines in experimental animals. The studies should include relevant routes of exposure, multiple dose levels and pharmacokinetic endpoints.
- Data are needed on the effects of amphetamines during pregnancy with detailed pharmacokinetic data on transfer of the drugs and their metabolites to the fetus.
- Experimental animal data from studies submitted to regulatory agencies should be made available to the public.
- Studies are needed to evaluate possible effects of amphetamine on pubertal timing and quality.
- Studies are needed to model data obtained by non-oral routes of administration so these data can be more useful in evaluating human oral exposures.
- Valid animal models of ADHD need to be used in studies of amphetamine toxicity in order to evaluate toxicity in a system that more closely approximates the human patient population.
- Nonhuman primate or guinea pig studies would be useful to evaluate effects of gestational amphetamine use in the second and third trimester.

5.5.2 Methamphetamine

The Expert Panel recognizes that pure toxicology studies in humans are not possible in evaluating the developmental and reproductive impact of illicitly synthesized methamphetamine. However, the Panel recognizes an urgent scientific and public health need for epidemiologic studies to evaluate, with covariate control, the potential short- and long-term effects of in utero exposure and passive postnatal exposure to illicit methamphetamine and its contaminants. In addition, evaluation of reproductive toxicity of exposure to illicit methamphetamine and its contaminants would be of interest.

The following data needs were identified for experimental animal studies:

- Data are needed on developmental and reproductive toxicity of methamphetamine in experimental animals. The studies should include relevant routes of exposure, multiple dose levels, and pharmacokinetic endpoints.
- Data are needed on the effects of methamphetamine during pregnancy, with detailed pharmacokinetic data on transfer of the drugs and their metabolites to the fetus.
- Studies are needed to evaluate possible effects of methamphetamine on pubertal timing and quality.
- Nonhuman primate or guinea pig studies would be useful to evaluate effects of gestational methamphetamine use in the second and third trimester.

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