COMMENTS ON THE DRAFT NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF AMPHETAMINE AND METHAMPHETAMINE

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1. SUMMARY

This document provides commentary on the NTP-CERHR amphetamine review, and adds substantial additional pharmacology and toxicology information for review by the expert panel.

Although data for amphetamine and methamphetamine are included together in the NTP-CERHR draft report, literature-derived information is provided here to demonstrate that these agents are very different pharmacologically and toxicologically. It is therefore advocated that the potential risks of reproductive and developmental toxicity of amphetamine and methamphetamine are considered separately. Differences in the basic pharmacology of the D- and L-enantiomers of these drugs should also be taken into account and may warrant the establishment of separate risk profiles for each moiety.

As acknowledged in the NTP-CERHR draft report, opposing pharmacological responses to amphetamine occur in ADHD sufferers compared to normal subjects and experimental animals, suggesting that a risk assessment based on studies in normal subjects may not extrapolate to an ADHD population. We have provided further literature support for this view, as well as summary data from a study demonstrating that D-amphetamine reduces hyperactivity in an animal model of ADHD (SHR rat).

Similarly, the risk assessments for therapeutic use of amphetamine in ADHD sufferers compared to use by drug abusers are completely different, and should take into account factors such as relative dose, product quality and data reliability.

Detailed summaries are provided here from additional regulatory developmental toxicology studies carried out to support clinical use of amphetamine in ADHD. These add to the safety database reviewed by the committee, and provide further reassurance for the continued safe therapeutic use of amphetamine containing products, such as Adderall XR™, in the treatment of ADHD. As with many medicines, caution with the use of amphetamines during pregnancy or nursing is clearly advised in the Adderall XR™ product label.

2. INTRODUCTION

The draft report by the NTP-CERHR expert panel provides an extensive review of the pharmacology, pharmacokinetics and toxicology of amphetamine and methamphetamine with particular emphasis on developmental and reproductive toxicology.

This document provides comments on the draft NTP-CERHR report. In particular, differences between amphetamine and methamphetamine are highlighted and non-clinical data from a pre- and post-natal study and a juvenile toxicity programme, previously unavailable to the CERHR, are summarised for consideration by the expert panel.

3. COMMENTS ON DRAFT REPORT

3.1 Differences between amphetamine and methamphetamine

Differences in pharmacology, behavioural effects and toxicity, and impurities and illicit drug use between amphetamine and methamphetamine are summarised in the following sections. More detailed information is presented in Appendix 1.

3.1.1 Pharmacology

The pharmacological profiles reported *in vitro* by Richelson & Pfenning (1984) and Rothman et al (2001) and *in vivo* by Kuczenski et al (1995) show that the d- and l-isomers of methamphetamine and amphetamine are all pharmacologically active. In addition, there are clear differences between the monoaminergic pharmacological profiles of methamphetamine and amphetamine, with even greater differences exhibited between the d- and l-isomers of the "amphetamines". Since each molecule is both structurally and pharmacologically distinct, the assumption that the reproductive and developmental toxicity risks associated with amphetamine can be predicted using clinical or human data obtained with methamphetamine and *vice versa* is invalid. Moreover, as both of the isomers of amphetamine and methamphetamine are pharmacologically active, some caveats will also need to be placed on predictions of the toxicity risk profile of a single isomer, eg d-methamphetamine, based on results generated with the corresponding racemate, ie methamphetamine. This caveat particularly applies to amphetamine where both the racemic form of the drug and the d-isomer are used clinically.

Rothman et al (2001) have reported *in vitro* data on monoamine release and reuptake inhibition for various other β-phenylethylamines related to the "amphetamines" eg MDMA, phentermine and fenfluramine, which reveal the marked differences in monoaminergic profile, which are evoked by small changes to the β-phenylethylamine chemical structure. The view of the Expert Panel not to consider evidence relating to phentermine in their evaluation is therefore strongly supported and it is recommended that this conclusion should apply equally to data generated with MDMA.

3.1.2 Behavioural effects and toxicity

Derlet et al, 1990a, 1990b reported that lethal doses of d-amphetamine or methamphetamine produced very different behavioural syndromes and incidence rates of seizures for comparable lethality rates. Characterisation using various monoamine and glutamate receptor antagonists revealed marked differences between the pharmacological mechanisms responsible for lethality evoked by these two "amphetamines" - lethality induced by d-amphetamine was dose-dependently attenuated by D_1 and D_2 dopamine receptor antagonists, the NMDA receptor antagonist, MK 801 and the β_1/β_2-adrenoceptor antagonist, propranolol; none of these antagonists attenuated methamphetamine-induced lethality. These results, therefore, add weight to the argument that amphetamine and methamphetamine are different both pharmacologically and toxicologically. Thus, reliable

predictions of the risks that they each pose for reproductive and developmental toxicity will be dependent on separate evaluations of these two “amphetamines”.

Scientific reports taken from a wide range of sources demonstrate that amphetamine, methamphetamine and various closely related β-phenylethylamine monoamine releasing agents, eg MDMA and the fenfluramines, each have distinctive neurotoxic finger-prints in the brain (Harvey & McMaster, 1975; Steranka & Sanders-Bush, 1979; Fuller & Hemrick-Luecke, 1980; Wagner et al 1980; Hotchkiss & Gibb, 1980; Ricaurte et al, 1980, 1982, 1988, 1991; Nwanze & Jonsson, 1981; Schuster et al, 1986; Battaglia et al, 1987; Finnegan et al, 1988; O’Hearn et al, 1988; Kleven et al, 1988; O’Callaghan & Miller, 1994). This finding is entirely consistent with the observation that small changes to the β-phenylethylamine chemical structure produce marked differences to their monoaminergic pharmacocological profiles (Richelson & Pfenning, 1984; Rothman et al, 2001; Kuczenski et al, 1995) and also their toxicological and behavioural profiles (Derlet et al, 1990a, 1990b). It has also been widely reported that methamphetamine, which is neurotoxic to both serotonergic and dopaminergic neurones in the brain (Wagner et al, 1980; Hotchkiss & Gibb, 1980; Ricaurte et al, 1980), has a wider spread of effect than amphetamine, which appears to be selectively neurotoxic to dopaminergic neurones when given at toxicological doses (Fuller & Hemrick-Luecke, 1980; Nwanze & Jonsson, 1981). Moreover, it has also been reported that whilst methamphetamine and amphetamine are both toxic to dopaminergic neurones, the former produces more profound and widespread damage to these neurones in the brain (Ellison & Switzer, 1993). Finally, these reports demonstrate that neurotoxicity only occurs at supra-pharmacological doses of the “amphetamines”. **Together, these data indicate that, in its deliberations of the potential reproductive and developmental risks of the “amphetamines”, the Expert Panel should evaluate methamphetamine separately from amphetamine and its isomers, and, in addition, should carefully consider those effects that are likely to derive from pharmacological/therapeutic doses as a different issue from those likely to derive from toxicological/abuse doses of these drugs.**

3.1.3 Impurities and illicit drug use

Major sources of potential error in using data from users of illicit drugs to assess the risks of the “amphetamines” to cause reproductive or developmental toxicity are two-fold. First, the reliability of the subjects as accurate witnesses of their history of drug abuse and second, the materials being abused being of sufficient chemical and enantiomeric purity to draw a valid conclusion on which compound is responsible for any observed adverse event or toxicity. The DEA has stated that the purity of seized methamphetamine varied between 71.9% in 1994 to as low as 30.7% in 1999; no statement was given in the NTP-CERHR draft report concerning the enantiomeric purity of the methamphetamine in question. In the current review, it has been demonstrated that both enantiomers of amphetamine or methamphetamine are pharmacologically active *in vitro* and *in vivo*, there are marked differences between their pharmacological and toxicological profiles, and several related β-phenylethylamines, eg phentermine, could be employed as substitutes for these “amphetamines” in illicit drug manufacture. **Thus, it is recommended to the Expert Panel that data from drug abusers can only provide a reliable prediction of the risks that either amphetamine or methamphetamine pose for reproductive and developmental toxicity if the drug history of the subjects can be accurately defined, either by drug source or chemical analysis of human samples.** For reasons given previously in this

review, we would contend that the evaluations of amphetamine or methamphetamine should be performed separately.

3.2 Previously available amphetamine reproductive data

Definitive reproductive studies with amphetamine, including a male and female fertility study and embryofetal development studies in rats and rabbits have been summarised in the draft NTP-CERHR report together with supporting toxicokinetic data based upon information in the FDA Adderall XR™ review (ref 36 in the NTP-CERHR draft report). In summary of these studies:

In the fertility study, the parental No Observed Adverse Effect Level (NOAEL) was judged to be 2mg free base/kg/day on the basis of clinical observations, body weight, and food consumption effects. However, the reproductive and developmental NOAELs were judged to be 20 mg free base/kg/day on the basis of no adverse effects on mating performance, fertility or early embryonic development.

Similarly, the maternal NOAEL in the rat embryofetal development study was less than 2mg free base/kg/day on the basis of mortality, clinical post-dose observations, body weight, and food consumption effects while the developmental NOAEL was considered to be 6mg free base/kg/day, the highest dose evaluated, on the basis of no significant adverse effects on embryo-fetal survival, growth or development. At this dose there was a possible increase in the incidence of incomplete ossification of cranial centers, a minor skeletal abnormality that would be expected to resolve spontaneously during postnatal development.

The maternal NOAEL in the rabbit embryofetal development study was considered to be 6mg free base/kg/day on the basis of mortality and clinical post-dose signs. The developmental NOAEL was considered to be 16mg free base/kg/day on the basis of no significant adverse effects on embryo-fetal survival, growth or development.

It can be concluded from these studies that administration of amphetamine is not associated with any significant effect upon fertility or embryofetal development.

3.3 Additional amphetamine developmental and reproductive data

Additional non-clinical studies that were not available to the CERHR were conducted with amphetamine as part of the non-clinical development programme for Adderall XR™. These comprised a pre- and post-natal study and a juvenile toxicity programme in rats; final reports from these studies were provided to FDA in June 2004. In order to provide the panel with additional information, these studies are summarised in the following sections and tabular summaries of the pre- and post-natal study and preliminary and main juvenile toxicity studies are presented in Appendix 2, Appendix 3 and Appendix 4 respectively.

3.3.1 Pre- and post-natal study

Administration of amphetamine (Adderall™ mixture) to pregnant rats at doses of 2, 6 or 10mg base/kg/day from Gestation Day (GD) 6 to Lactation Day (LD) 20 inclusive (Study No. R00093-SLI381-IIIC) was associated, at 6 and 10mg base/kg/day, with a variety of clinical signs including hyperactivity, excessive sniffing, head bobbing, cage licking and red nasal discharge or crust; self mutilation was also observed in a single animal in each of these two groups. One dam in each of the 2 and 10mg base/kg/day groups was killed in a moribund condition in the week prior to weaning following a period of severe weight loss; it is uncertain whether these isolated deaths were related to treatment. The death of a female at 6mg base/kg/day on LD 4 was unrelated to treatment with amphetamine.

Significant dose-related decreases in F0 dam body weight gain and food consumption were observed during gestation and lactation with bodyweight loss at 6 and 10mg base/kg/day between Days 6 and 8 of gestation.

Three total litter deaths occurred between LD 0 and LD 3 in each of the treated groups while offspring mortality was increased after LD 4 at 10mg base/kg/day only. Consequently offspring survival indices were significantly decreased in all groups. This effect upon offspring survival is likely to be a consequence of changes in maternal behaviour as amphetamines have previously been reported to adversely affect parturition, decrease offspring survival and increase the incidence of cannibalisation.

Treatment related clinical signs in F1 offspring included cold to touch, pale, evidence of cannibalisation, incomplete hair growth, thin, weak and no milk in stomach. Offspring bodyweight gain was reduced at 6 and 10mg base/kg/day and attainment of maturational landmarks was consequently delayed. Activity levels were increased at 10mg base/kg/day on Day 22 *post partum* but were subsequently unaffected at Week 5.

Following maturation and pairing of the F1 generation animals, the number of animals mating and achieving pregnancy was unaffected by treatment. Although the number of implantations and consequent number of F2 offspring delivered was reduced compared to concurrent controls at 10mg base/kg/day, the numbers of implantations and offspring born in this group were greater than those of the F_0 Control group.

In conclusion, other than the transient increase in activity of offspring at 10mg base/kg/day, effects upon F1 offspring including decreased survival, reduced bodyweight gain and delayed development were likely to be indirect as a consequence of treatment related changes in maternal behaviour. The NOAEL for maternal toxicity was <2mg base/kg/day. In offspring, the NOAEL for survival was <2mg base/kg/day (as a consequence of changes in maternal behaviour), for development was 2mg base/kg/day and for locomotor activity was 6mg base/kg/day.

3.3.2 Juvenile toxicity programme

In a preliminary dose range finding study (Study No. R00091-SLI381-IIIA), groups of rats received amphetamine (Adderall™ mixture) by oral gavage once daily from Days 7 to 13 of age and twice daily for varying durations from Day 14 of age onwards. Dose levels (Days 7-

13/Day 14 onwards) were 6/12, 15/30, 20/40, 40/80 or 60/120mg base/kg/day. At 40/80 and 60/120mg base/kg/day clinical signs and marked reductions in bodyweight gain were apparent which precluded their use in the subsequent main study. Similar, but less severe, effects were apparent at 20/40mg base/kg/day and it was concluded that doses of 2/4, 6/12 and 20/40mg base/kg/day would be suitable for use in the subsequent main juvenile toxicity study.

Treatment of rats with amphetamine (Adderall™ mixture) in a main juvenile toxicity study (Study No. R00092-SLI381-IIIA) from Day 7 to 13 of age at dosages of 0, 2, 6, or 20mg/kg/day rising to dosages of 0, 4, 12 or 40mg/kg/day by twice daily dosing (approx 8 hours apart) from Day 14 of age to 8-9 weeks of age was well tolerated and there were no treatment related deaths. There was, however, a significant reduction in growth rate at 6/12 and 20/40mg/kg/day and a transitory impairment of bodyweight gain for females only at 2/4mg/kg/day during the early post-weaning period. Around the time of weaning a number of post-dosing observations consistent with the known effects of amphetamine became apparent. At 2/4mg/kg/day these were overactivity, repetitive movement of head and/or paws, licking of cage, piloerection, salivation and, in females only, increased vocalisation and irritable behaviour. These signs were also observed at higher dosages. At 6/12mg/kg/day the animals were also observed to be rubbing their chins on the bars of the cages and irritable behaviour and increased vocalisation were observed in males. Additional signs at 20/40mg/kg/day were partially closed eyelids and underactive behaviour (usually before dosing). The clinical sign of underactivity before dosing was confirmed by the automated motor activity data generated on Days 22 and 47 of age. Whilst this effect was questionable at Day 22 of age, by Day 47 of age the effect was well established and showed that although underactivity was only clinically appreciable at 20/40mg/kg/day it was present in all treated groups.

When animals were placed in automated motor activity meters during the treatment period before the first daily dose (approximately 14 hours after the previous dose), a reduction in activity was apparent for both sexes in all treated groups. Although data were variable at Day 22 of age (16 of treatment) by Day 47 of age this effect was well established and ambulatory and rearing activity was reduced in a dosage dependent manner in all treated groups. When these animals were tested during recovery this effect was much less marked although ambulatory and rearing activity for males and females at 20/40mg/kg/day and females at 6/12mg/kg/day still appeared lower than control.

When the animals were subjected to the Morris maze test of learning and memory before the first daily dose (approximately 14 hours after the previous dose) starting at Day 23/24 of age, a number of treatment-related findings were observed. One of the effects of treatment was that animals at 6/12 and 20/40mg/kg/day repeatedly jumped from the escape platform during the course of the test. The function of this test relies on each animal receiving visual cues from around the pool whilst standing on the platform in order to form a cognitive map and learn the relative position of the platform. It is therefore possible that the extent of this atypical behaviour at 20/40mg/kg/day contributed to the impaired performance in this group on this occasion where animals took longer to find the platform, failed more trials and entered more pool sectors than animals in the control group. Although performance at this dosage lagged behind controls, the rate of improvement in performance over the 4 days of the test was the same in treated and control groups. A further treatment-related finding was that on

the first day of the test, 1 male at 6/12mg/kg/day and 1 male and 1 female at 20/40mg/kg/day, all from the same litter, appeared to convulse after completing the test.

In order to investigate whether the refusal/inability to stay on the platform was due to a direct effect or rebound effect of treatment, naïve control and 20/40mg/kg/day animals were tested approximately 2 hours after dosing starting at Day 28 of age. The treated animals showed similar jumping behaviour to those tested before the first daily dose and demonstrated negligible improvement over the 4 days of the test while the control animals showed the expected pattern of improvement. The performance of these treated animals was considered to be compromised by acute pharmacological effects of amphetamine (in particular overactivity) and no further testing was performed after the first daily dose. Consequently further testing commencing on Day 48/49 was conducted before the first daily dose.

The animals that were previously tested at Days 23/24 of age were again exposed to the Morris maze test at Days 48/49 of age and Day 78 of age (Day 19 of recovery). Although no animals repeatedly jumped from the platform on Days 48/49, performance at 20/40mg/kg/day was still inferior to control over the 4 days of the test and there was a suggestion of impaired performance at 6/12mg/kg/day. Overall improvement in swimming times in all groups between Days 1 and 4 of the test was similar. On Day 19 of recovery all treated male groups showed significantly greater swimming times than the control group mirroring the increased times seen in these animals on the final day of testing during treatment. By the fourth day of this test males previously treated at 2/4 and 6/12 mg/kg/day showed similar performance to controls. Males previously treated at 20/40mg/kg/day still showed poorer performance than controls on the fourth day of the test and over the 4 days entered significantly more pool sectors than control. They did however show marked overall improvement over the 4 days and compared to test days during the treatment period. Females previously treated at 20/40 and 6/12mg/kg/day showed impaired performance on the first day of testing during the recovery period, again mirroring their performance on the last day of testing during the treatment period. By Day 4 of the test, group mean values for the females were similar in all groups. These deficits in Morris maze performance were not associated with any morphological changes in the CNS as histopathology did not detect any changes within the brain.

Time of sexual maturation (balano-preputial separation or vaginal opening) was significantly delayed at 20/40mg/kg/day. In the absence of organ weight or microscopic changes in the reproductive tract and as no functional effects on mating behaviour and fertility were apparent, this finding was considered to be of no long term biological significance and was likely to be an indirect effect of bodyweight reduction.

The smaller size of the animals at 6/12 or 20/40mg/kg/day also accounted for a number of apparent organ weight changes at necropsy. The only organ weight changes which could not easily be attributed to the bodyweight effect at 6/12 or 20/40mg/kg/day were increases in bodyweight relative adrenal and salivary gland weights of females at these dosages. The lack of histopathological findings in these tissues indicated that these changes were of no toxicological significance.

Reproductive assessments performed after the cessation of treatment indicated that treatment with amphetamine did not interfere with oestrous cycles, mating performance or fertility.

In conclusion, the NOAEL was considered to be 2/4mg/kg/day. Findings at this dosage were restricted to a transient reduction in bodyweight gain for females, post-dosing observations and reduced motor activity at Day 47 of age. After cessation of treatment there were no clinical signs and motor activity was similar to control.

3.4 Differences between experimental animals and humans

In the human pharmacodynamics section of the CERHR report (Section 2.1.1.1) the discordance between humans with ADHD and experimental animals is acknowledged; ie although stimulants decrease locomotor activity in children with ADHD, an increase in activity is observed in experimental animal studies.

The spontaneously hypertensive rat (SHR) is an animal model of ADHD, which shows hyperactivity, impulsivity and deficits in attention. In this animal model, amphetamine affects behaviour, reducing activity and impulsivity and increasing sustained attention, ie returning behaviour towards normal (unpublished data – see Appendix 5). **The relevance of findings in "normal" experimental animals and humans to the therapeutic use of amphetamines in the treatment of ADHD is therefore unknown.**

4. DISCUSSION

The draft report by the NTP-CERHR Expert Panel is an extensive review of the pharmacokinetics, general pharmacology (ie not related to the primary mechanism of therapeutic action) and toxicology of the "amphetamines" used in clinical practice, ie amphetamine and methamphetamine. The evaluation includes data obtained in animal and human studies with racemic methamphetamine, racemic amphetamine and their respective dextro-isomers; some consideration is also given to the l-isomer of amphetamine, but not to the l-isomer of methamphetamine. The focus of the draft report is an evaluation of the potential risks that d-methamphetamine and amphetamine and/or their isomers pose to human reproduction but it is unclear what will be the focus of the evaluation to be performed at the Expert Panel Meeting. On the basis of the data reviewed within the NTP-CERHR draft report, there are 2 alternative scenarios:

A) A final report that contains two separate evaluations. First, an evaluation of the potential reproductive and developmental toxicity risks associated with the therapeutic use of the "amphetamines". Second, the risks specifically associated with the misuse or abuse of the "amphetamines" by recreational drug users or drug-dependent subjects.

B) An amalgam of the above that assumes the risks of the "amphetamines" to cause reproductive and developmental toxicity are similar (or at least sit along a continuum), irrespective of whether these drugs are used appropriately in the clinical setting or are misused/abused.

If the approach given in Scenario B is adopted, it will equate the risks of therapeutic doses of the "amphetamines" with those associated with the supra-therapeutic quantities routinely taken by drug abusers often employing non-therapeutic routes of administration, ie intravenous injection or smoking. Since it is accepted that the side-effect/adverse event risks

associated with a drug when taken in overdose quantities are inevitably many times greater than those associated with its therapeutic use, it would seriously bias the outcome of the risk assessment if these two very different aspects of the potential toxicity of the “amphetamines” were considered as a single entity. Since, it is unclear what strategic approach will be taken at the Expert Panel Meeting, it is recommended that the clinical and animal data should be further analysed to define those risks for reproductive and developmental toxicity associated with therapeutic use of the “amphetamines” and to separate them from the more severe risks associated with misuse/abuse of these drugs.

Available data also suggest differences between the pharmacological and toxicological profiles of amphetamine and methamphetamine; it is therefore recommended that the evaluations of amphetamine or methamphetamine should be performed separately.

The non-clinical definitive reproductive toxicology studies summarised in the NTP-CERHR draft report together with the additional data from the pre- and post-natal study and juvenile toxicity studies described in this document provide reassurance for the absence of developmental and reproductive effects and therefore for the safe therapeutic use of amphetamine containing medications such as Adderall XR™. Reasons for this are as follows:

- Fertility, embryofetal development, pre- and post-natal development and juvenile toxicity studies have assessed the effects of administration of the amphetamine salts used in Adderall™ preparations.

- A twice-daily oral (gavage) dosing regimen was used to mimic clinical conditions and provide extended exposure.

- Studies were conducted to accepted designs and were in compliance with GLP regulations.

- There was no evidence of any teratogenic effect in the rat and rabbit embryofetal development studies and, despite maternal toxicity, only an equivocal effect upon fetal skeletal ossification was observed in the rat. In addition, in the rat pre- and post-natal study, no morphological changes were apparent in offspring.

- Mating performance and fertility of parental animals were unaffected by treatment with amphetamine in the definitive rat fertility study. Supplementary reproductive data generated in the pre- and post-natal and juvenile toxicity studies similarly did not reveal any adverse effects upon mating performance and fertility. Numbers of implantations and offspring were lower at the high dose level (10mg/kg/day) compared to concurrent controls in the pre- and post-natal study, but it is worthy of note that the values were greater than those seen in the F_0 Control animals. It is also worthy of note that no treatment-related microscopic changes in the CNS or reproductive organs were observed in the juvenile toxicity study.

Behavioural changes, as expected from the extensive, but not always consistent, published literature, were evident in rats in the definitive reproductive studies and included hyperactivity, and stereotypic behaviour in parental animals, transient hyperactivity in high dose group offspring in the pre-and post-natal study and pre-dose hypoactivity/post-dose hyperactivity and impaired memory/learning performance of animals in the juvenile toxicity

study. Following cessation of treatment there was evidence of recovery in these studies and no treatment-related microscopic changes were apparent. In contrast to these findings, in the SHR animal model of ADHD, behaviour is returned towards normal. This finding is analogous to the clinical situation where amphetamine products have proven efficacy in the treatment of ADHD.

Taken collectively these studies provide reassurance for the continued safe therapeutic use of amphetamines at recommended clinical doses.

Although there are considered to be no significant adverse treatment-related effects upon reproduction and embryofetal development in the studies presented above, the prescribing information for Adderall XR™, in addition to summarising the above data, also reflects published literature and advises caution with the use of amphetamines during pregnancy or nursing as follows:

"Pregnancy: ……A number of studies in rodents indicate that prenatal or early postnatal exposure to amphetamine (d- or d, l-) at doses similar to those used clinically can result in long-term neurochemical and behavioural alterations. Reported behavioural effects include learning and memory deficits, altered locomotor activity and changes in sexual function. There are no adequate and well-controlled studies in pregnant women. ……Amphetamines should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Usage in Nursing Mothers: Amphetamines are excreted in human milk. Mothers taking amphetamines should be advised to refrain from nursing."

5. CONCLUSION

This document provides commentary on the NTP-CERHR amphetamine review, and adds substantial additional pharmacology and toxicology information for review by the expert panel.

Although data for amphetamine and methamphetamine are included together in the NTP-CERHR draft report, literature-derived information is provided here to demonstrate that these agents are very different pharmacologically and toxicologically. It is therefore advocated that the potential risks of reproductive and developmental toxicity of amphetamine and methamphetamine are considered separately. Differences in the basic pharmacology of the D- and L-enantiomers of these drugs should also be taken into account and may warrant the establishment of separate risk profiles for each moiety.

As acknowledged in the NTP-CERHR draft report, opposing pharmacological responses to amphetamine occur in ADHD sufferers compared to normal subjects and experimental animals, suggesting that a risk assessment based on studies in normal subjects may not extrapolate to an ADHD population. We have provided further literature support for this view, as well as summary data from a study demonstrating that D-amphetamine reduces hyperactivity in an animal model of ADHD (SHR rat).

Similarly, the risk assessments for therapeutic use of amphetamine in ADHD sufferers compared to use by drug abusers are completely different, and should take into account factors such as relative dose, product quality and data reliability.

Detailed summaries are provided here from additional regulatory developmental toxicology studies carried out to support clinical use of amphetamine in ADHD. These add to the safety database reviewed by the committee, and provide further reassurance for the continued safe therapeutic use of amphetamine containing products, such as Adderall XR™, in the treatment of ADHD. As with many medicines, caution with the use of amphetamines during pregnancy or nursing is clearly advised in the Adderall XR™ product label.

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APPENDIX 1 DIFFERENCES BETWEEN AMPHETAMINE AND METHAMPHETAMINE

BACKGROUND

The draft report by the NTP-CERHR Expert Panel is an extensive review of the pharmacokinetics, general pharmacology (ie not related to the primary mechanism of therapeutic action) and toxicology of racemic methamphetamine, racemic amphetamine and their respective dextro-isomers; some consideration is also given to the l-isomer of amphetamine, but not to the l-isomer of methamphetamine. The focus of this review is an evaluation of the potential risks that d-methamphetamine and amphetamine and/or its isomers pose to human reproduction, ie genotoxicity and prenatal and developmental toxicity, with particular attention placed on neurotoxicity. As stated by the Expert Panel, the scientific sources employed were review articles and the primary scientific sources if they were considered to be relevant to the NTP-CERHR evaluation of developmental and reproductive toxicity. The Expert Panel has made its evaluation as methodical and consistent as is possible, bearing in mind the very disparate sources and quality of scientific information that were available to them, by summarising individual studies or closely related groups of studies using a *"Strengths/Weaknesses"* analysis. In this analysis, they have considered factors such as the predictive validity of the methodologies employed, robustness of the experimental findings, appropriateness of the statistical evaluation, relevance of the route of drug administration to the clinical situation and the interpretation of the results by the authors. The Expert Panel has also attempted to provide an additional degree of rigour to its evaluation by restricting it to what the participants have termed are the *"amphetamines"* described as *"a class of chemicals with structural similarity to amphetamine"* (Section 1.1.1, *Nomenclature:* page 1, para 1). It is further stated that *"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."* (Section 1.1.1, *Nomenclature:* Page 1, para 1).

Commentary
The Expert Panel has restricted its evaluation to *"The amphetamines used in clinical practice ….. amphetamine and methamphetamine …"* (Section 1.1.1, *Nomenclature:* page 1, para 1), but significantly it also cites the extensive misuse/abuse especially of methamphetamine, eg *"d-Methamphetamine hydrochloride is also used recreationally and is the illicit stimulant most commonly meant by the term 'speed' "* (Section 1.1.1, *Nomenclature:* page 1, para 1). This illicit aspect of methamphetamine use is clearly very different from the legitimate medical use of the "amphetamines" in the treatment of ADHD or narcolepsy because misuse/abuse comes with its own specific portfolio of risks and adverse events, eg drug overdose. It is unclear from the introduction to the NTP-CERHR draft report, what will be the focus of the evaluation to be performed at the Expert Panel Meeting. On the basis of the data reviewed within the NTP-CERHR draft report, there are 2 alternative scenarios:

A) A NTP-CERHR Final Report that contains two separate evaluations. First, an evaluation of the potential reproductive and developmental toxicity risks associated with the therapeutic use of the "amphetamines" when they are used under the supervision and guidance of a qualified practitioner, in appropriate patient populations, at clinically relevant doses and in strict accordance with the approved Regulatory labelling for the products. Second, the risks specifically associated with the misuse or abuse of the "amphetamines" by recreational drug users or drug-dependent subjects.

B) An amalgam of the above that assumes the risks of the "amphetamines" to cause reproductive and developmental toxicity are similar (or at least sit along a continuum), irrespective of whether these drugs are used appropriately in the clinical setting or are misused/abused recreationally or to alleviate the severe symptoms of psychological dependence associated with these drugs.

If the approach given in Scenario 2 is adopted, it will equate the risks of therapeutic doses of the "amphetamines", eg, *"…the maximum recommended dose of methamphetamine for ADHD is 25 mg/day… and for obesity is 5 mg before each meal."* (Section 1.2.3, *Human Exposure*: page 10, para 1) with those associated with taking amounts of the "amphetamines" routinely consumed by drug abusers, eg *"…the doses of methamphetamine to induce euphoria in a drug-naïve individual are ~30 mg, however, habitual use to produce euphoria requires the drug to be taken in binges over 3-15 days in the dose range of 20-250 mg or more per "hit" with total daily doses of up to several grams."* (Section 1.2.3, *Human Exposure*: page 10, para 2). It is obvious that in the latter situation, where drug overdose is a frequent adverse event, the misuse/abuse of the "amphetamines" has much closer similarity to "OVERDOSE" than to "INDICATIONS AND USAGE" as defined in the FDA approved Drug labels for the "amphetamines". There is another confound because abusers with severe dependency problems often resort to maximising the "high" produced by the "amphetamines" by employing non-therapeutic routes of administration, ie intravenous injection and smoking (methamphetamine as "ice" or "glass"; Section 1.2.2 *Use*: page 10, para 2). Since it is accepted that the side-effect/adverse event risks associated with a drug when taken in overdose quantities are inevitably many times greater than those associated with its therapeutic use, it would seriously bias the outcome of the risk assessment if these two very different aspects of the potential toxicity of the "amphetamines" were considered as a single entity. Since, it is unclear what strategic approach will be taken at the Expert Panel Meeting, we would strongly recommend that the clinical and animal data should be further analysed to define those risks for reproductive and developmental toxicity associated with

therapeutic use of the "amphetamines" and to separate them from those rather more severe risks associated with misuse/abuse of these drugs.

Clinical use of amphetamine, methamphetamine and related β-phenylethylamine drugs
The common factor linking all of the compounds under discussion, ie amphetamine, methamphetamine, phentermine, fenfluramine (and its more serotonin selective dextro-isomer) and MDMA, is they all have been used clinically to treat obesity; in addition, all but MDMA, have been approved for this therapeutic indication by the Food and Drugs Agency (FDA).

A concise overview on the FDA's perspective on the history of anti-obesity drugs and its current position on their utility was recently provided by Dr Coleman from the FDA Division of Metabolic and Endocrine Drugs (Coleman, 2004). In 1973, the FDA restricted the treatment of obesity with amphetamine, methamphetamine and amphetamine-like drugs to "a few weeks". The FDA approved Product Labels for amphetamine, methamphetamine and phentermine, each contain statements mandating short-term use of the drugs in obesity; hence, their use throughout pregnancy is clearly "off-label". Moreover, with several of these compounds, eg Benzedrine™ (dl-amphetamine), Desoxyn® (d-methamphetamine) and Adipex-P® (phentermine), there are clear statements in the labeling informing patients to seek medical advice before using these drugs during pregnancy (Benzedrine™ Product Label, 1978; Desoxyn® Product Label, 1995; Adipex-P® Product Label, 2000). These drugs are also generally contra-indicated whilst breast feeding (Desoxyn® Product Label, 1995; Adipex-P® Product Label, 2000). Thus, careful consideration needs to be given to data from human studies where the "amphetamines" have been taken to treat obesity during pregnancy without adequate medical supervision, or for prolonged periods, as this does not constitute appropriate USE as defined by the approved drug labelling.

Extensive clinical experience with the anti-obesity drugs, fenfluramine (or its dextro-isomer) and phentermine, highlight how differences in cardiotoxicity and serious adverse events occur with two structurally similar β-phenylethylamine, monoamine releasing agents. Thus, the use of fenfluramine or d-fenfluramine has been clearly linked to an increased risk of primary pulmonary hypertension (PPH; Brenot et al, 1993; Abenhaim et al, 1996), whereas no clear association has been established for the use of phentermine (*"rare cases of PPH in patients who reportedly have taken phentermine alone"*; Adipex-P® Product Label, 2000). Similarly, a 20-30% incidence of cardiac valvulopathy occurred in patients taking the fenfluramines either alone or in combination with phentermine (often called "fen/phen") (Connolly et al, 1997; Gardin et al, 2000). This observation led to the voluntary global withdrawal of the fenfluramines, but no association with cardiac valvulopathy has been established for phentermine (*"there have been rare cases of valvular heart disease in patients who reportedly have taken phentermine alone"*; Adipex-P® Product Label, 2000) and it is still widely prescribed in the USA.

The other major use for the "amphetamines" and other psychostimulants, eg methylphenidate, is in the treatment of attention deficit hyperactivity disorder (ADHD). The use of psychostimulants to treat ADHD is much less controversial than their use as anti-obesity agents, because long-term maintenance of efficacy in ADHD is not disputed. In the case of amphetamine, despite the fact that the d- and l-isomers have quite different monoaminergic profiles (Table 1), both isomers have been shown to be equally effective in

alleviating the symptoms of ADHD when given alone (Arnold et al, 1972, 1973); albeit d-amphetamine has a more rapid onset of maximum efficacy (Arnold et al, 1972, 1973).

Chemistry and Pharmacology of the "Amphetamines"
The descriptor of "amphetamines" as applied by the Expert Panel to amphetamine and methamphetamine (and their isomers) is a common term used to describe various members of the β-phenylethylamine chemical class of compounds. The β-phenylethylamine substructure is shown in Figure 1.

Amphetamine and methamphetamine are two of the simplest of the β-phenylethylamine class of compounds. Amphetamine is substituted with a methyl group on the α-carbon of the side-chain of the substructure, whilst methamphetamine has the additional substitution of a methyl group on the terminal nitrogen atom. The replacement of one of the two hydrogen atoms on the α-carbon atom by a methyl group in amphetamine and methamphetamine creates an asymmetric carbon residue (Figure 1). This asymmetry results in amphetamine and methamphetamine existing as 2 isomers, ie d-amphetamine (or s(+)-amphetamine), l-amphetamine (or r(-)-amphetamine), d-methamphetamine (or s(+)-methamphetamine) and l-methamphetamine (or r(-)-methamphetamine). Since almost all physiological processes, eg receptors, ion channels, transporters, enzymes etc, exhibit stereoselectivity to a greater or lesser extent, it can be predicted that the d- and l-isomers of amphetamine and methamphetamine will have different pharmacological and toxicological profiles.

This assertion is fully supported by the data provided in Table 1 reporting the potencies of the individual isomers of amphetamine and methamphetamine as inhibitors of the uptake of [^{3}H]monoamines, ie dopamine, noradrenaline (or norepinephrine) or 5-hydroxytryptamine (5-HT) or serotonin. Thus, Table 1 shows that d-amphetamine has approximately equal potency as an inhibitor of dopamine and noradrenaline uptake with weak effects on 5-HT. On the other hand, l-amphetamine is 4-fold more potent as a reuptake inhibitor of noradrenaline than of dopamine; it has little or no efficacy against 5-HT. d-Methamphetamine is 3-fold more potent as a reuptake inhibitor of noradrenaline than of dopamine, with potency approximately equivalent to the d-isomer of amphetamine as a 5-HT reuptake inhibitor. Although not considered in the review by the Expert Panel, l-methamphetamine is pharmacologically active and is likely to contribute to the toxicological effects of racemic methamphetamine, especially when the racemate is given at high dose to animals or when methamphetamine of unknown enantiomeric purity is taken by drug abusers. In respect of the latter point, the NTP-CERHR draft report notes that *"In 1994, the average purity of methamphetamine seized by the 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."* (Section 1.1.4 *Technical products and impurities*: page 8, para 4). l-Methamphetamine is a noradrenaline reuptake inhibitor with weak or non-existent actions as a reuptake inhibitor of dopamine or 5-HT (this lack of dopaminergic activity is almost certainly the reason why l-methamphetamine is neither used as a prescription pharmaceutical, nor abused as a psychostimulant).

Table 1 Profiles of the isomers of amphetamine and methamphetamine as inhibitors of monoamine reuptake

Drug	Reference	Inhibition of [^{3}H]monoamine uptake into synaptosomes (Ki = nM)		
		Dopamine	Noradrenaline	5-HT
d-Amphetamine	1	82	50	1,840
	2	34	39	3,830
l-Amphetamine	1	380	90	10,000
d-Methamphetamine	2	114	48	2,137
l-Methamphetamine	2	4,840	234	14,000

1 = Richelson & Pfenning (1984); 2 = Rothman et al (2001)

As stated in the NTP-CERHR draft report (Section 2.1.1.1, *Pharmacodynamics*: page 13, para 2), it is widely accepted that the "amphetamines" produce their pharmacological effects by increasing the synaptic release of monoamines, inhibiting their reuptake, and by preventing their metabolism via inhibition of monoamine oxidase (MAO). The data reported in Table 1 refer only to one of these mechanisms, ie reuptake inhibition, and in addition, the results have been obtained in vitro. Consequently, it could be argued that such differences are not relevant to the in vivo situation. Intracerebral microdialysis in freely-moving animals can be used to determine the extraneuronal concentration of monoamine neurotransmitters. A given drug's effect on the extraneuronal concentration of monoamine is due to the sum of its pharmacological actions, ie on monoamine release, reuptake and metabolism. Therefore, extraneuronal monoamine concentrations are a well accepted, dynamic, surrogate marker of the synaptic concentration of these neurotransmitters and also of the level of monoaminergic activation. Kuczenski et al (1995) have used intracerebral microdialysis to profile the effects of the individual isomers of amphetamine and methamphetamine on dopamine and 5-HT efflux in the striatum and noradrenaline efflux in the hippocampus of the rat brain. These four compounds were all tested at pharmacologically equivalent doses based on behavioural activation. The results confirm and extend the findings provided in Table 1. The d- and

l-isomers of amphetamine and methamphetamine are all pharmacologically active (Kuczenski et al, 1995). Moreover, each isomer had its own distinct “finger-print” of effects on the central monoamines, ie dopamine: d-amphetamine = d-methamphetamine > l-amphetamine > l-methamphetamine; noradrenaline: l-methamphetamine > l-amphetamine = d-amphetamine > d-methamphetamine; 5-HT: d-methamphetamine = l-methamphetamine > d-amphetamine = l-amphetamine (Kuczenski et al, 1995). The situation is further complicated when more than one brain region is examined. Thus, Shoblock et al (2003) used intracerebral microdialysis to compare the effects of d-amphetamine and d-methamphetamine on dopamine efflux in the nucleus accumbens and prefrontal cortex of the rat brain. Although both amphetamines were equally effective in elevating dopamine efflux in the nucleus accumbens, d-amphetamine evoked much greater increases in the prefrontal cortex. Concomitantly, d-amphetamine increased glutamate efflux in the nucleus accumbens, but was without effect in the prefrontal cortex, whilst d-methamphetamine produced exactly the opposite effect.

Overall, the pharmacological profiles provided in Table 1 show that the d- and l-isomers of methamphetamine and amphetamine are all pharmacologically active. In addition, there are clear differences between the monoaminergic pharmacological profiles of methamphetamine and amphetamine, with even greater differences exhibited between the d- and l-isomers of the “amphetamines”. Since each molecule is both structurally and pharmacologically distinct, I would argue the assumption that the reproductive and developmental toxicity risks associated with amphetamine can be predicted using clinical or human data obtained with methamphetamine and *vice versa* is invalid. Moreover, as both of the isomers of amphetamine and methamphetamine are pharmacologically active, some caveats will also need to be placed on predictions of the toxicity risk profile of a single isomer, eg d-methamphetamine, based on results generated with the corresponding racemate, ie methamphetamine. This caveat particularly applies to amphetamine where both the racemic form of the drug and the d-isomer are used clinically.

Structure-activity relationships of the monoaminergic profiles of various β-phenylethylamine drugs

When considering the potential risks of compounds for causing reproductive and developmental toxicity, it is obvious that the pharmacological actions of each specific compound have a significant role to play in the outcome. In the previous section, biologically-relevant differences between the chemical and pharmacological profiles of the isomers of amphetamine and methamphetamine were illustrated. In this section, this argument is extended by an examination of the chemical structures and monoaminergic pharmacological profiles of various β-phenylethylamines related to amphetamine and methamphetamine that have been considered in the NTP-CERHR draft report, including MDMA (eg Section 3.1.1.1, *Case Reports and Case Series:* pages 57-59) and phentermine (eg Section 3.1.1.1, *Case Reports and Case Series:* pages 58-59).

Because the catecholamines, dopamine and noradrenaline, are both simple β-phenylethylamines, many variants to this substructure, apart from amphetamine and methamphetamine, have been made. Some of the closest variants are shown in Figure 2.

Phentermine is the closest analogue to amphetamine with the substitution of the hydrogen on the α-carbon by a methyl group, yielding a non-chiral β-phenylethylamine. Substituted secondary amines, cf methamphetamine, include fenfluramine and its dextro-isomer,

d-fenfluramine, where the phenyl ring is substituted with a trifluromethyl group and there is an ethyl residue on the amine nitrogen. MDMA has the phenyl ring substituted with a methylenedioxy group; like methamphetamine, the secondary amine contains a methyl group. Like amphetamine and methamphetamine, MDMA is a chiral molecule existing as d- and l-isomers.

As reported in Table 2, phentermine is 5 to 6-fold more potent as a reuptake inhibitor and releaser of noradrenaline than of dopamine, with much weaker effect on 5-HT. In contrast, dl-fenfluramine is approximately 5-fold more potent as an inhibitor of 5-HT than of noradrenaline, but equipotent as a releaser of both of these monoamines; dl-fenfluramine has no pharmacologically relevant actions as a dopamine reuptake inhibitor or releaser. On the other hand, MDMA has a spread of reuptake inhibitory and releasing actions across all of the monoamines with its most potent effects exerted on 5-HT.

Table 2 Monoamine reuptake inhibition and release profiles of various β-phenylethylamine drugs

	Inhibition of [^{3}H]monoamine uptake in synaptosomes (Ki = nM)			Release of [^{3}H]monoamines from synaptosomes (IC_{50} = nM)		
Drug	Dopamine	Noradrenaline	5-HT	Dopamine	Noradrenaline	5-HT
Phentermine	1580	234	14,000	262	39	2511
dl-Fenfluramine	23,700	1987	269	>10,000	77	79
dl-MDMA	1572	462	238	376	302	57

Data taken form Rothman et al (2001)

In the NTP-CERHR draft report, the Expert Panel has for example concluded "*The McElhatton et al study (119) is useful in the evaluation process to the extent that data on MDMA can be applied to amphetamine and methamphetamine;"* (Section 3.1.1.1, *Case Reports and Case Series:* page 58, para 3). In contrast, the Expert Panel in reviewing the study of McElhatton et al (120) have stated that *"Among women exposed to licit drugs, most used phentermine, which is not likely to produce relevant information on amphetamine exposure."* (Section 3.1.1.1, *Case Reports and Case Series:* page 58, para 5).

In view of the data presented in the previous sections highlighting the marked differences in pharmacological action that are evoked by small changes to the β-phenylethylamine chemical structure, we would, therefore, strongly support the view of the Expert Panel not to consider data relating to phentermine in their evaluation, and would add that this conclusion

would apply equally to data generated with MDMA. Moreover, we would contend based on pharmacologically significant differences in their monoaminergic profiles that the reproductive and developmental toxicity risks of amphetamine cannot be determined with any degree of confidence from data obtained in animals or humans using methamphetamine (either as the racemate or its more dopaminergic dextro-isomer). This rationale would also dictate that findings obtained using the racemate of a drug with two pharmacologically active isomers, eg amphetamine and methamphetamine, will have some degree of predictive validity for each isomer of the parent compound, with the caveat that therapeutic benefit may derive from both isomers, but toxicity may be specific to one of them.

DIFFERENCES BETWEEN THE PHARMACOLOGICAL MECHANISMS RESPONSIBLE FOR AMPHETAMINE- AND METHAMPHETAMINE-INDUCED LETHALITY

In earlier sections of this document, evidence has been presented to demonstrate that there are significant differences between the monoaminergic pharmacological profiles of amphetamine, methamphetamine and their respective isomers. Derlet and co-workers have provided experimental data to support the hypothesis that such pharmacological differences are biologically important (Derlet et al, 1990a, 1990b). Lethal doses of d-amphetamine or methamphetamine were administered to rats by an intraperitoneal injection. As noted by the authors, these two amphetamines produced very different behavioural syndromes in the animals "*d-Amphetamine treated vehicle control animals became hyperactive and tachypneic with intermittent tonic-clonic convulsions lasting 30-60 seconds. The behaviour of animals receiving d-amphetamine contrasted sharply to those receiving methamphetamine. Methamphetamine treated vehicle control animals demonstrated within 5 minutes, arching of backs, marked piloerection, and fine body tremor, with very brief tonic-clonic convulsions that lasted 5-15 seconds"* (Derlet et al, 1990a). The incidence of seizures was also very different with these two "amphetamines", being 95% for d-amphetamine, but only 40% for methamphetamine (Derlet et al, 1990a). Lethality rates were, however, similar at 95% and 90% for d-amphetamine and methamphetamine, respectively (Derlet et al, 1990a). Characterisation using various receptor antagonists revealed marked differences between the pharmacological mechanisms responsible for lethality evoked by d-amphetamine and methamphetamine. Lethality induced by d-amphetamine was dose-dependently attenuated by various dopamine receptor antagonists, ie SCH 23390 (D_1; Derlet et al, 1990a) and haloperidol (D_2; Derlet et al, 1990b), the NMDA receptor antagonist, MK 801 (Derlet et al, 1990b), and the β_1/β_2-adrenoceptor antagonist, propranolol (Derlet et al, 1990b); none of these antagonists attenuated methamphetamine-induced lethality in this side-by-side experiment (Derlet et al, 1990a, 1990b).

These results, therefore, add weight to the argument that amphetamine and methamphetamine are different both pharmacologically and toxicologically. Thus, reliable predictions of the risks that they each pose for reproductive and developmental toxicity will be dependent on separate evaluations of these two "amphetamines".

DIFFERENCES IN THE NEUROTOXOCITY PROFILES OF AMPHETAMINE, METHAMPHETAMINE AND RELATED B-PHENYLETHYLAMINE RELEASING AGENTS

The data described in the following section demonstrate that the neurotoxicity profiles of the substituted amphetamines are very different. Also, they highlight the findings that small changes to the β-phenylethylamine chemical structure substantially alter the neurotoxic profile.

In humans, who abuse methamphetamine, dopaminergic deficits revealed by positron emission tomography (PET) imaging, eg Volkow et al (2001a; 2001b); Sekine et al (2003), have been found, together with impairments in motor function and memory. When given to animals at toxicologically-equivalent doses, amphetamine selectively damages dopaminergic neurones, eg Fuller & Hemrick-Luecke (1980); Nwanze & Jonsson (1981), whilst MDMA, eg Battaglia et al (1987); Finnegan et al (1988); O'Hearn et al (1988) and fenfluramine, eg Harvey & McMaster (1975); Steranka & Sanders-Bush (1979); Schuster et al (1986); Kleven et al (1988); Ricaurte et al (1991), selectively damage serotonergic neurones. On the other hand, methamphetamine is reported to be toxic to both dopaminergic and serotonergic neurones, eg Wagner et al (1980); Hotchkiss & Gibb (1980); Ricaurte et al (1980). Thus, methamphetamine has a much broader spectrum of neurotoxic actions than amphetamine. The neurotoxic effects of the "amphetamines" in rodents appear to be confined to the axon terminals and regenerative sprouting can occur, eg Ricaurte et al (1982); O'Hearn et al (1988); Molliver et al (1990); DeSouza et al (1990). However in primates, nerve cell bodies are affected and the toxic effects of the "amphetamines" are longer lasting and possibly permanent, eg Ricaurte et al (1988).

Even for dopaminergic neurones, where amphetamine and methamphetamine both cause damage, the latter has been reported to be considerably more toxic. d-Amphetamine (5.45mg/day), given continuously to rats over 5 days via a subcutaneous pellet, produced substantial dopaminergic neurodegeneration in the striatum. On the other hand, even when administered only acutely, methamphetamine (6mg/kg x 4 given at 2 hourly intervals) caused pronounced degeneration not only in the striatum, but also in many other brain regions, eg cerebellum and corpus callosum (Ellison & Switzer, 1993).

A very recent study by Armstrong & Noguchi (2004) compared the neurotoxic effects of dl-methamphetamine and dl-MDMA in rat brain. Rats were given continuous infusions of equimolar doses of dl-methamphetamine (32mg/kg/day) and dl-MDMA (40mg/kg/day) for 5 days via subcutaneuous osmotic minipumps. Neurodegeneration in brain sections was assessed using autoradiography with the selective serotonin reuptake inhibitor [^{3}H]paroxetine (to measure SERT sites), the dopamine uptake inhibitor [^{3}H]mazindol (to measure DAT sites), the combined D_2 and 5-HT_2 antagonist and [^{3}H]methylspiperone (a measure of postsynaptic 5-HT and dopamine receptor binding). dl-Methamphetamine and dl-MDMA both induced significant reductions in the Bmax values of [^{3}H]paroxetine, [^{3}H]mazindol and [^{3}H]methylspiperone binding demonstrating changes in both pre- and postsynaptic neurotransmitter function. However, the results demonstrated that dl-methamphetamine-treated animals had greater deficits in [^{3}H]paroxetine, [^{3}H]mazindol and [^{3}H]methylspiperone binding in all brain areas compared with the dl-MDMA-treated group. These observations indicated that dl-methamphetamine was more toxic than dl-MDMA to 5-HT terminals in forebrain regions, including the anterior cingulate nucleus, caudate nucleus, nucleus accumbens and septum. Moreover, dl-methamphetamine was more toxic than dl-MDMA to dopaminergic terminals in the habenula and posterior retrosplenial cortex. Hence, this study clearly demonstrated the differential neurotoxicity of dl-methamphetamine and dl-MDMA in specific brain regions at both pre- and post-synaptic sites.

O'Callaghan & Miller (1994) compared the neurotoxicity profiles of the substituted amphetamines, d-methamphetamine (10mg/kg), d-MDMA (20mg/kg) and d-fenfluramine (25mg/kg) after dosing subcutaneously 4 times to mice. d-Methamphetamine and d-MDMA treatment increased striatal and cortical GFAP (ie gliosis, indicative of neural damage).

d-Methamphetamine and d-MDMA also produced large (50-75%) decreases in dopamine and tyrosine hydroxylase that did not resolve within a 3 week period. Neurotoxicity to striatal fibres and nerve terminals degeneration was confirmed by silver staining. The effects of d-methamphetamine and d-MDMA were blocked by pretreatment of the animals with the NMDA receptor antagonist, MK-801. d-Fenfuramine did not increase glial fibrillary acidic protein (GFAP) or neuronal degeneration, but did produce a prolonged decrease of cortical 5-HT. These data suggest that d-methamphetamine and d-MDMA, but not d-fenfluramine, produce cell damage in mouse striatum and cortex.

Scientific reports taken from a wide range of sources demonstrate that amphetamine, methamphetamine and various closely related β-phenylethylamine monoamine releasing agents, eg MDMA and the fenfluramines, each have distinctive neurotoxic finger-prints in the brain (Harvey & McMaster, 1975; Steranka & Sanders-Bush, 1979; Fuller & Hemrick-Luecke, 1980; Wagner et al 1980; Hotchkiss & Gibb, 1980; Ricaurte et al, 1980, 1982, 1988, 1991; Nwanze & Jonsson, 1981; Schuster et al, 1986; Battaglia et al, 1987; Finnegan et al, 1988; O'Hearn et al, 1988; Kleven et al, 1988; O'Callaghan & Miller, 1994). This finding is entirely consistent with the observation that small changes to the β-phenylethylamine chemical structure produce marked differences to their monoaminergic pharmacocological profiles (Richelson & Pfenning, 1984; Rothman et al, 2001; Kuczenski et al, 1995) and also their toxicological and behavioural profiles (Derlet et al, 1990a, 1990b). It has also been widely reported that methamphetamine, which is neurotoxic both to serotonergic and dopaminergic neurones in the brain (Wagner et al, 1980; Hotchkiss & Gibb, 1980; Ricaurte et al, 1980), has a wider spread of effect than amphetamine, which appears to be selectively neurotoxic at dopaminergic neurones when given at toxicological doses (Fuller & Hemrick-Luecke, 1980; Nwanze & Jonsson, 1981). Moreover, it has also been reported that whilst methamphetamine and amphetamine are both neurotoxic to dopaminergic neurones, the former produces more profound and widespread damage to these neurones in the brain (Ellison & Switzer, 1993). Finally, these reports demonstrate that neurotoxicity only occurs at supra-pharmacological doses of the "amphetamines". Together, these data indicate that, in its deliberations of the potential reproductive and developmental risks of the risks of the "amphetamines", the Expert Panel should evaluate methamphetamine separately from amphetamine and its isomers, and in addition, should carefully consider those effects that are likely to derive from pharmacological/therapeutic doses as a different issue from those likely to derive from toxicological/abuse doses of these drugs.

CONTRADICTION BETWEEN THE BEHAVIOURAL PHARMACOLOGY OF THE "AMPHETAMINES" AND OTHER PSYCHOSTIMULANTS IN ADHD PATIENTS VERSUS NORMAL SUBJECTS AND ANIMALS

Attention deficit hyperactivity disorder (ADHD) as the name implies is a behavioural and cognitive disorder that is characterised by developmentally inappropriate hyperactivity, impulsivity and inattention (American Psychiatric Association, 1994). This disorder has been successfully treated using psychostimulant drugs, including racemic amphetamine, d-amphetamine, l-amphetamine, d-methamphetamine or methylphenidate, for over 60 years (see reviews by Elia, 1993; Spencer et al, 1996; Elia et al, 1999). Thus, in this predominantly juvenile disorder, the psychostimulants evoke not an increase in behavioural activity, but a paradoxical decrease in hyperactivity (see reviews by Elia, 1993; Spencer et al, 1996; Elia et al, 1999). On the other hand, in normal animals, amphetamine, methamphetamine or the individual isomers thereof induce locomotor activation at low to moderate doses and at higher doses behavioural stereotypy, including sniffing, grooming, chewing, licking and biting,

eg Scraggs & Ridley (1978); Derlet et al (1990a); Kuczenski et al (1995); Halford et al (1998). Similarly, in man as the doses of amphetamine or methamphetamine are increased, so the degree of behavioural activation increases also, eg Angrist et al (1971). Since increased locomotor activity and/or stereotypy has a major effect to decrease food intake (Halford et al, 1998) and to elevate energy expenditure through increased muscular work, these pharmacological and physiological consequences are likely to have a negative influence on reproduction and development.

In summary, the "amphetamines" induce hyperactivity in normal subjects and animals, but paradoxically reduce hyperactivity in ADHD sufferers. Since ADHD patients account for the majority of the amphetamines produced for legitimate medical use there are serious questions concerning the predictive validity of conclusions on the reproductive and developmental toxicity risks for ADHD patients modelled on studies performed in animals or human subjects, who have been made hyperactive by exposure to the "amphetamines".

PREDICTIVE VALIDITY OF THE DATA OBTAINED IN HUMAN SUBJECTS MISUSING/ABUSING "AMPHETAMINES" FROM NON-LEGITIMATE SOURCES

Two major sources of potential error in using data from users of illicit drugs to assess the risks of the "amphetamines" to cause reproductive or developmental toxicity are, first, the reliability of the subjects as accurate witnesses of their history of drug abuse, eg the dependability of the testimony that he/she is a user exclusively of methamphetamine and/or amphetamine, and second, assuming the witness statement of his/her drug history is accurate, the risk assessment is dependent on the material being abused being of sufficient chemical and enantiomeric purity to support a conclusion that one of the compounds being evaluated in the NTP-CERHR draft report is responsible for any adverse event(s) or toxicological outcome that has been reported. It was noted in the NTP-CERHR draft report that *"In 1994, the average purity of methamphetamine seized by the 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."* (Section 1.1.4 *Technical products and impurities*: page 8, para 4). Whilst impurities were not discussed in the DEA report, it has to be accepted based on their figures that ~30% of the material being taken as methamphetamine was not in fact this "amphetamine", and this figure rose to ~60% in 2001 after the DEA had acted to prevent access to the starting materials for the illegal manufacture of this drug. Based on the statements in the NTP-CERHR draft report, the enantiomeric purity of the samples does not appear to have been determined by the DEA when these methamphetamine seizures were analysed. It is also well accepted that drug dealers will prepare cocktails of psychoactive drugs, and/or substitute active ingredients whilst still passing the material off to their "clients" as being pure. In view of these caveats, the data obtained from drug abusing populations are of dubious value, unless the subject has been part of a long-term rehabilitation programme and has been receiving supplies of pharmaceutical grade amphetamine or methamphetamine of known enantiomeric quality. An alternative approach to ensure that the drug history of the subject could have be realistically linked to the adverse event or toxicity outcome is to have performed analyses on hair and/or urine samples from individuals as part of the data reported from the investigation.

In summary, it has previously been demonstrated that while both enantiomers of amphetamine and methamphetamine are pharmacologically active in vitro and in vivo, there are marked differences in their pharmacological and toxicological profiles, and several

related β-phenylethylamines, eg phentermine, could be employed as substitutes for these "amphetamines" in illicit drug manufacture. Thus, we would recommend that data from drug abusers can only provide a reliable prediction of the risks that either amphetamine or methamphetamine pose for reproductive and developmental toxicity if the drug history of the subjects can be accurately defined, either by drug source or chemical analysis of human samples. For reasons given previously in this review, we would contend that the evaluations of amphetamine or methamphetamine should be performed separately.

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Figure 1 The β-phenylethylamine substructure

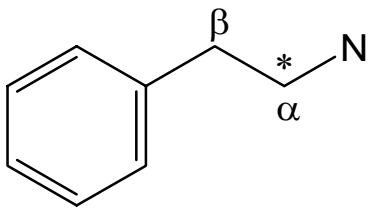


Figure 2 The 3-dimensional structures of the d- and l-isomers of amphetamine and methylamphetamine

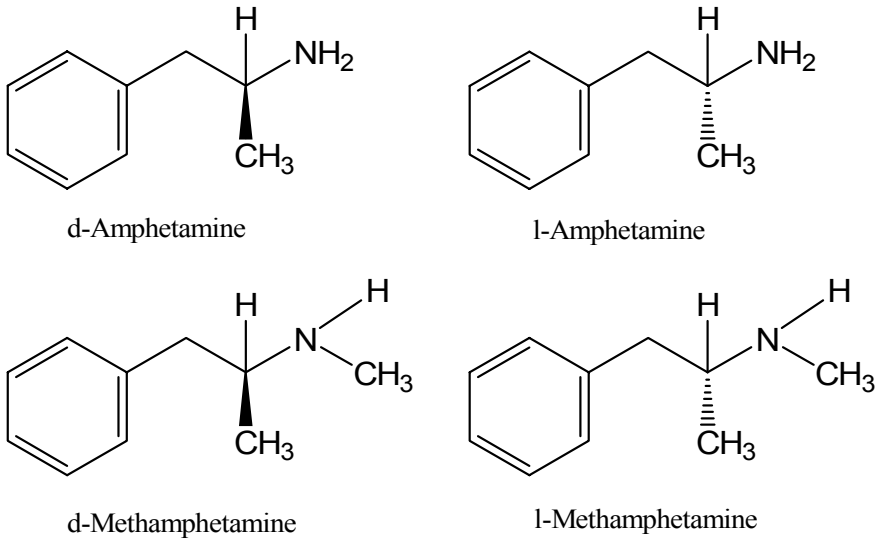
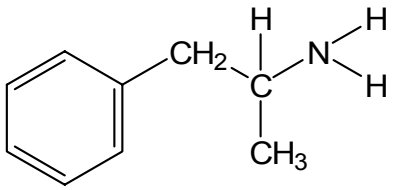
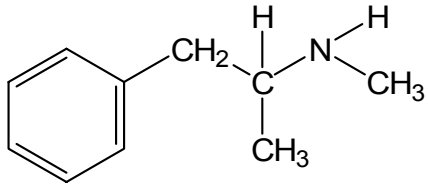


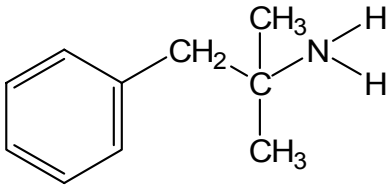
Figure 3 The "amphetamines" and related β-phenylethylamine monoamine releasing agents



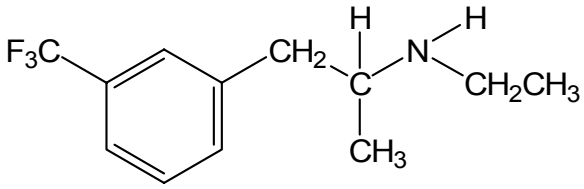
Amphetamine



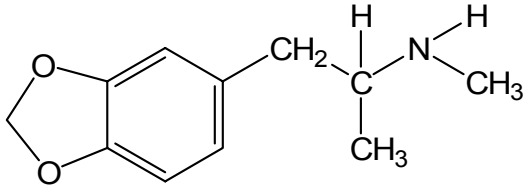
Methamphetamine



Phentermine



Fenfluramine



Methylenedioxymethylamphetamine (MDMA)

APPENDIX 2 AMPHETAMINE (ADDERALL™ MIXTURE): ORAL (GAVAGE) PRE-AND POST-NATAL STUDY IN THE RAT.

Objective
The objective of this study was to assess the effects of daily oral (gavage) administration of the amphetamine constituents of Adderall™ on pregnant rats treated from the time of implantation to weaning. The study assessed effects on offspring survival, growth and development and included neurobehavioural examinations and assessment of mating performance and fertility.

The study was conducted in accordance with the ICH guideline on Detection of Toxicity to Reproduction for Medicinal Products.

Justification for the treatment regimen
The oral (gavage) route of administration was selected to simulate the conditions of clinical administration.

Dose level selection was based upon a previous embryo-fetal development study in which pregnant rats dosed at 20mg/kg/day exhibited self-mutilation and had to be terminated after 3 days of dosing (Gestation Day 9). One female at 6mg/kg/day also exhibited self-mutilation although this finding was unique at this dose level. Bodyweight losses of 4 and 11g were observed for dams at 2 and 6mg/kg/day between Gestation Days 6 and 7. Mean bodyweight gain for the 6mg/kg/day dose group for Gestation Days 6-20 was 7% less than controls, while weight gain at 2mg/kg/day was comparable to controls. A high dose of 10mg/kg/day was therefore selected for this pre- and post-natal study with mid and low doses of 6 and 2mg/kg/day respectively.

Results
A tabular summary of the results is presented in the attached table (see Table 1).

Table 1: Amphetamine (Adderall™ mixture): oral (gavage) pre-and post-natal study in the rat

Reproductive and Developmental Toxicity: Effects on Rat Pre- and Post-natal Development: Amphetamine (Adderall™ mixture)				
Design similar to ICH 4.1.2?: Yes	**Initial Age**: 10-12 weeks		**Study No**.: R00093-SLI381-IIIC	
Species/Strain: Rat/ Crl:CD®(SD)IGS BR	**Day of Mating**: Day 0 of gestation		**Location in CTD**:	
Date of First Dose: 15 July 2002	**Method of Administration**: Oral (Gavage)		**GLP Compliance**: Yes	
Vehicle/Formulation: Reverse osmosis water; 0.2, 0.6, 1.0mg/mL			**Special Features**: Dosed bid – one half of the total daily dose given twice daily at least 8 hours apart. Litters culled to 4/sex/litter	
Duration of Dosing: Gestation Day (GD) 6 to Lactation Day (LD) 20 inclusive				
No Observed Adverse Effect Level: F_0 females: < 2mg/kg; F_1 pup viability: < 2mg/kg; F_1 pup development: 2mg/kg; F_1 males and females - reproductive parameters, learning and memory: 10 mg/kg, - locomotor activity: 6mg/kg				
Daily Dose (mg base/kg/day)	0 (Control)	2	6	10
F_0 females				
Toxicokinetics: AUC_{0-24} (ng.h/mL) GD 6/LD 20	-/-	724/623	2199/2147	4208/4285
Number of Animals	25	25	25	25
No. Pregnant	25	25	25	25
No. Died or Sacrificed Moribund	0	1	1	1
No with Total Litter Resorption	0	0	0	0
Clinical Observations	-	++	+++	+++
Necropsy Observations	-	-	-	-
Gestation Body Weight gain (%†) Days 6-8	9.3	-95%**	-182%**	-206%**
Gestation Body Weight (%†) Day 20	365.2	-4%*	-9%*	-9%*

(continued)

Reproductive and Developmental Toxicity: Effects on Rat Pre- and Post-natal Development: Amphetamine (Adderall™ mixture)				
Daily Dose (mg base/kg/day)	0 (Control)	2	6	10
Lactation Body Weight (%[†]) Day 21	275.5	-3%	-5%	-1%
Gestation Food Consumption (%[†]) Days 6-8	24.3	-26%**	-40%**	-43%**
Gestation Food Consumption (%[†]) Days 6-20	27.5	-8%**	-13%**	-12%**
Mean Duration of Gestation (days)	21.6	21.9	21.8	21.7
Abnormal Parturition	-	-	-	-
F_1 Litters (Pre-Weaning):				
No. Litters Evaluated	25	25	25	25
Mean No. of Implantations	13.64	12.96	12.72	13.52
Mean No. Pups/Litter	12.76	12.04	11.12	11.44
Mean No. Liveborn Pups/Litter	12.76	12.08	10.96	11.38
No. of Litters with Stillborn Pups	0	2	1	1
Post-natal Survival to Day 4	12.56	12.27	10.95*	10.14**
Post-natal Survival to Weaning	8.00	8.00	7.71	7.29**
No. of Total Litter Losses	0	4	4	4
Change in Pup Body Weights (Birth to Weaning) (g)	45.56	43.46	37.22	31.01
Pup Sex Ratios (% males)	51	48	47	49
Pup Clinical Signs	-	-	-	+
Pup Necropsy Observations	-	-	-	-

(continued)

Reproductive and Developmental Toxicity: Effects on Rat Pre- and Post-natal Development: Amphetamine (Adderall™ mixture)				
Daily Dose (mg base/kg/day)	0 (Control)	2	6	10
F_1 Males (Post-Weaning):				
No. Evaluated Post-Weaning	25	25	25	25
No. Died or Sacrificed Moribund	0	0	1	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight Change (g) - Weaning to Mating	371.7	369.8	353.2	334.1**
Mean Age of Preputial separation (days)	40.80	41.51	43.70**	45.88**
Motor Activity – Total Day 22 post partum	403	506	571	781**
Learning and Memory	-	-	-	-
Mean No. Days Prior to Mating	3.54	4.04	3.96	2.61
No. of Males that Mated	24	25	25	23
F_1 Females (Post-Weaning):				
No. Evaluated Post-Weaning	25	25	25	25
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Pre-Mating Body Weight Change (g) Weeks 0 to 7	180.2	193.9	174.1	176.6
Gestation Body Weight Change (g)	148.5	151.4	149.4	135.2*
Mean Age of Vaginal Patency (days)	31.96	31.86	34.94**	36.60**

(continued)

Reproductive and Developmental Toxicity: Effects on Rat Pre- and Post-natal Development: Amphetamine (Adderall™ mixture)				
Daily Dose (mg base/kg/day)	0 (Control)	2	6	10
Motor Activity – Total Day 22 post partum	409	332	519	679
Learning and Memory	-	-	-	-
Mean No. Days Prior to Mating	3.54	4.04	3.96	2.61
No. of Females Sperm +ve	16	16	16	13
No. of Pregnant Females	25	24	22	24
Mean Duration of Gestation (days)	21.9	21.7	21.7	21.6
Abnormal parturition	-	-	-	-
F_2 Litters:				
No. Litters Evaluated	25	24	22	24
Mean No. Implantations	15.84	16.67	15.00	13.92**
Mean No. Pups/Litter	15.32	16.33	14.36	13.42**
Mean No. Liveborn Pups/Litter	15.24	16.25	14.36	13.42**
Pup Sex Ratios (% males)	55	50	48	52
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	-

- No noteworthy findings. + Mild ++ Moderate +++ Marked

* $p<0.05$ ** - $p<0.01$

† For controls, group means (g or g/rat/day) are shown. For treated groups, percent of control is shown. Statistical significance is based on actual data.

APPENDIX 3 AMPHETAMINE (ADDERALL™ MIXTURE): ORAL (GAVAGE) PRELIMINARY TOXICITY STUDY IN THE JUVENILE RAT.

Objective

The objective of this preliminary study was to assess the feasibility of repeated daily oral administration of Amphetamine (Adderall™ mixture) in young rats from Day 7 of age, and to determine a high dose level for use in a subsequent main juvenile toxicity study. Initially, it had been intended to treat juvenile rats for up to 15 consecutive days but the study was modified and extended in response to ongoing findings. In order to refine the choice of dose levels appropriate to the dosing regimen for use in a subsequent Main juvenile toxicity study, some animals were treated up to Day 40 of age and then retained untreated until Day 47 of age in order to investigate effects following the cessation of treatment. The study was designed to assess general effects such as survival, clinical condition and growth rate. Toxicokinetic assessment on Day 21 of age was also performed as part of the study.

Justification for the test system

The rat was chosen because it is a species of choice for neonatal toxicity studies and has been used extensively for general toxicity and reproduction studies. The IGS:CD strain was used because of the historical control data available.

Justification for the treatment regimen

The oral (gavage) route of administration was selected to simulate the conditions of clinical administration.

Juvenile rats were dosed once daily on Days 7-13 of age in order to minimise disturbance of feeding behaviour and growth. From Day 14 of age, the potential for disturbance of feeding behaviour is reduced and offspring were dosed twice daily, for consistency with other toxicity studies performed with this compound, (except when toxicokinetic blood sampling was performed after the first dose on Day 21 of age).

Because the response of juvenile rats treated with Amphetamine (Adderall™ mixture) was unknown, a cautious approach was adopted with the study performed in phases: initially, 12 male and 12 female juvenile rats (3 litters) were dosed once daily at 6 mg free base/kg/day (Group 1). This dosage was chosen based on the results of a fertility and early embryonic development study in the CD rat (Shire Study No. R00036-SLI381-IIIB) in which treatment of adult male and female rats at 20 and 6mg free base/kg/day induced some general adverse toxicological effects, notably overactivity, retarded bodyweight gain and decreased food consumption. Based on the results after 7 days of treatment, once daily dosing of juvenile rats in Group 2 commenced at 20mg free base/kg/day. Based on the results from Groups 1 and 2, once daily dosing of juvenile rats in Group 3 commenced at 40mg free base/kg/day and once daily dosing of animals in Group 4 commenced at 15mg free base/kg/day. The rationale for these latter two doses was as follows: three female pups (one from each of 3 different litters) had been found dead following the start of once daily dosing at 20mg free base/kg/day. Necropsy did not reveal any evidence for mal-dosing and it was suspected that these deaths might have been related to treatment; if further deaths had occurred among animals treated at 40mg free base/kg/day, then this would have been taken as confirmation

that the deaths were related to treatment. If the deaths at 20/40mg free base/kg/day were treatment related, then the use of 15/30mg free base/kg/day allowed assessment of effects at a slightly lower dosage.

In the final phase of the study, juvenile rats in Group 5 commenced treatment at 60mg free base/kg/day based on initial results at 40mg free base/kg/day.

The original intention of the study was that juvenile rats would be dosed once daily on Days 7-13 of age then twice daily on Days 14-20 of age. On Day 21 of age, it was intended that the animals would be dosed once and then bled for toxicokinetic assessment and killed. However, based on the initial results it was clear that juvenile rats dosed once per day did not show the same extent of bodyweight loss and post dosing clinical signs observed in adult animals and hence some juvenile rats in the 20/40, 40/80 and 60/120mg free base/kg/day groups were dosed once daily on Days 7-13 of age and then twice daily on Days 14-40 of age, to investigate effects following removal of the influence of the parent female on Day 21 of age and of a longer treatment period. The animals were then retained untreated until Day 47 of age in order to investigate effects following the cessation of treatment.

Results
A tabular summary of the results is presented in the attached table (see Table 2).

Discussion
Treatment of male and female juvenile rats at 60/120 or 40/80mg free base/kg/day was associated with marked reductions in bodyweight gain following once and twice daily dosing prior to weaning. In addition, clinical signs were observed at both dosages during the period of twice daily dosing and included overactivity, underactivity, piloerection and repetitive movement of the head/forelimbs. The latter sign was not apparent until after Day 21 of age. The marked reduction in bodyweight gain prior to weaning contraindicates the use of 60/120 or 40/80mg free base/kg/day on a main juvenile toxicity study as it could prejudice assessment of later development.

At 20/40mg free base/kg/day, there were slight (ca 20%) initial reductions in bodyweight gain of males and females in comparison with the group receiving 6/12 mg free base/kg/day during the once daily dosing phase with signs of overactivity, underactivity, piloerection and repetitive movement of head/forelimbs observed during the period of twice daily dosing. All of the signs except for overactivity were not apparent until after Day 21.

At 15/30mg free base/kg/day, there was a slight initial reduction in bodyweight gain in comparison with animals receiving 6/12mg free base/kg/day.

At 6/12mg free base/kg/day, there were no overt effects of treatment.

The clinical signs observed at dosages of 20/40mg free base/kg/day and above, showed good consistency both within and between litters and along with the bodyweight effects provide a good basis for the choice of dosages for the main juvenile toxicity study.

Conclusion

It is concluded that 20/40mg free base/kg/day would be a suitable high dosage for a main juvenile toxicity study and is likely to be associated with a reduction in bodyweight gain, and overactivity, piloerection and repetitive movement of head/forelimbs.

Table 2 Amphetamine (Adderall™ mixture): preliminary juvenile toxicity study

	Study Number: R00091-SLI381-IIIA **Report Date :** 25 March 2003					**Duration of Treatment:** Days 7 to 21 or 40 of age. Necropsy on Day 21 or Day 47 of age.				
Species/Strain: Rat/Crl:CD® (SD) IGS BR	**Route:** Oral (gavage)					**Dosing Frequency:** For animals killed on Day 21 of age: once daily on Days 7-13 and 21 of age and twice daily on Days14-20. For animals killed on Day 47 of age: once daily on Days 7-13 of age and twice daily on Days14-40.				
Weight Range on Day 7 : Males 11.6 – 18.4 g Females 10.7 – 18.5 g	**Test Material** : Amphetamine (Adderall™ mixture) **Batch No :** 013198; 013425; 013322; 013251									
Age on Day 1 : 7 days	**Vehicle :** Water For Formulation					**Necropsy Dates** : 24 July - 4 September 2002				
Dose Volume : 5 ml/kg/occasion (10 ml/kg/occasion at 60/120 mg/kg/day)	**Treatment of controls :** Vehicle at 5 ml/kg/occasion									
No Observable Adverse Effect Level (NOAEL) : 6/12 mg free base/kg/day – males and females	**Study in Compliance with GLP :** No									
Study Design:	Male					Female				
Dose (mg free base/kg/day)[a]	6/ 12	15/ 30	20/ 40	40/ 80	60/ 120	6/ 12	15/ 30	20/ 40	40/ 80	60/ 120
No. animals	12A	12D	20B	17C	8E	12A	12D	20B	17C	8E
Pharmacokinetic parameters (Day 21 of age (Day 15 of treatment)**:**										
C_{max} (ng/ml)	350	1833	1942	3020	-	390	928	1535	4263	-
AUC_8 (ng.h/ml)	1095	6834	7382	16489	-	1069	3895	6382	17780	-
Important Findings : Treatment related findings are shown in bold										
No. unscheduled deaths	0	0	0	2	1	1	1	3	0	1
Clinical observations	**Overactive, underactive, piloerection and repetitive movement of head/forelimbs at 20/40 mg free base/kg/day and above. Chin rubbing on cage and eyelids partially closed - 60/120 mg free base/kg/day only.**									
Body weight gain, mean (g):										
Days 7-10	6.9	**6.0**	**5.7**	**3.6**	**2.7**	6.9	**5.3**	**5.4**	**4.0**	**2.9**
Days 10-14	10.4	9.4	10.2	**9.0**	**7.8**	9.5	9.3	9.8	**8.5**	**8.7**
Days 14-21	17.1	**13.0**	**14.8**	**10.6**	**8.7**	15.6	12.3	14.9	**11.2**	**9.5**
Days 22-40	-	-	107.8	**96.1**	**87.7**	-	-	81.2	**73.6**	**69.1**
Days 40-47	-	-	76.4	**66.0**	**60.2**	-	-	40.7	46.0	42.5
Days 7-21	34.4	**28.3**	**30.8**	**23.2**	**19.3**	32.0	**27.0**	**30.1**	**23.7**	**21.2**
Days 7-40	-	-	138.0	**120.7**	**109.2**	-	-	109.4	**98.2**	**91.8**
Macroscopic pathology - no adverse findings										

a - lower dosage administered once daily, higher dosage administered as two equal sub- doses given twice daily 8 hours apart.

A - juvenile rats in litters 3, 4 and 5 were killed on Day 21 of age

B - juvenile rats in litters 10, 11 and 19 were killed on Day 21 of age; juvenile rats in litters 12 and 20 were killed on Day 47 of age

C - juvenile rats in litters 23 and 24 were killed on Day 21 of age; juvenile rats in litters 17 and 25 were killed on Day 47 of age

D - juvenile rats in litters 15, 16 and 18 were killed on Day 21 of age

E - juvenile rats in litters 21 and 22 were killed on Day 47 of age

APPENDIX 4 AMPHETAMINE (ADDERALL™ MIXTURE): ORAL (GAVAGE) TOXICITY STUDY IN THE JUVENILE RAT.

Objective
The objective of this study was to assess the effects of daily oral (gavage) administration of the amphetamine constituents of Adderall™ on neonatal/juvenile rats treated from Day 7 of age to at least Day 59 of age. The study assessed effects on general condition, development and maturation up to attainment of puberty and included routine toxicological investigations as well as neurobehavioural examinations. Some animals underwent a recovery period after Day 59 of age, during which mating performance and fertility were assessed and further toxicological and neurobehavioural investigations performed. Toxicokinetic assessment was also performed as part of the study in order to investigate systemic exposure after 8 and 47 days of administration.

Justification for the test system
The rat was chosen because it is a species of choice for neonatal/juvenile toxicity studies and has been used extensively for general toxicity and reproduction studies. The IGS:CD strain was used because of the historical control data available.

Justification for the treatment regimen
The oral (gavage) route of administration was selected to simulate the conditions of clinical administration.

The high dosage level was based on the findings of a preliminary study (Study No. R00091-SLI381-IIIA) in which the recommended maximum repeatable daily oral dosage was 20 mg free base/kg/day administered on Days 7-13 of age increasing to 40 mg free base/kg/day by twice daily administration from Day 14. Higher dosages induced marked reduction in bodyweight gain and marked clinical signs including overactivity, underactivity, piloerection and repetitive movement of head/forelimbs.

The low dosage was expected to be a small multiple of the expected human dose and the intermediate dose is the approximate geometric mean of the low and high dosages.

Offspring were dosed once daily on Days 7-13 of age in order to minimise disturbance of feeding behaviour and growth. From Day 14 of age, the potential for disturbance of feeding behaviour is reduced and offspring were dosed twice daily for consistency with other toxicology studies performed with this compound.

Results
A tabular summary of the results is presented in the attached table (see Table 3).

Table 3: Amphetamine (Adderall™ mixture): Oral (gavage) toxicity study in the juvenile rat

Studies in Juvenile Animals: Oral (Gavage) Toxicity Study in the Juvenile Rat: Amphetamine (Adderall™ mixture)

Species/Strain: Rat; Crl: CD® (SD) IGS BR
Initial Age: 7 days
Date of First Dose: 26 November 2002
Vehicle/Formulation: Water for formulation; 0.4, 1.2, 4.0mg/mL
No Observed Adverse Effect Level: 2/4mg/kg/day – males and females
Duration of Dosing: Days 7 to 59/61-65[a]
Duration of Post-dose: ~ 7 weeks[a]
Method of Administration: Oral (gavage)
Study No.: R00092-SLI381-IIIA
GLP Compliance: Yes
Special Features: Dosed once daily from Days 7-13 of age; twice daily approx. 8 h apart from Day 14. Day of Caesarian Section = Gestation Day 14

	Male				Female			
Daily Dose (mg base/kg/day)	0 (Control)	2/4	6/12	20/40	0 (Control)	2/4	6/12	20/40
Number of Animals Toxicity Phase Reproductive Phase Satellites for Day 14 Toxicokinetics	10 10 0	10 10 20	10 10 20	10 10 20	10 10 0	10 10 20	10 10 20	10 10 20
Toxicokinetics: C_{max} (ng/mL) Day 14	-	146.2	378.0	2430.9	-	143.7	534.6	2126.6
AUC_{0-24} (ng.h/mL) Day 14	-	1651	4352	26319	-	1832	4616	30471
Toxicokinetics: C_{max} (ng/mL) Day 53	-	91.4	472.7	2006.8	-	159.3	519.0	3616.3
AUC_{0-8} (ng.h/mL) Day 53	-	264	1228	6534	-	540	1808	14570
Noteworthy Findings								
No. Died or Sacrificed Moribund	1	0	0	0	1	0	0	0
Body Weight Gain (g) Days 7-24	54.8	52.2	**45.9****	**37.9****	52.6	**47.9****	**42.9****	**35.0****
Body Weight Gain (g) Days 7-59	338	332	**298****	**254****	213	211	**197***	**173****

(continued)

Studies in Juvenile Animals: Oral (Gavage) Toxicity Study in the Juvenile Rat: Amphetamine (Adderall™ mixture)								
	Male				Female			
Daily Dose (mg base/kg/day)	0 (Control)	2/4	6/12	20/40	0 (Control)	2/4	6/12	20/40
Clinical Observations	**2/4mg/kg/day: overactivity, repetitive movement of head and/or paws, licking of cage, piloerection, salivation and, in females only, increased vocalisation and irritable behaviour** **6/12mg/kg/day: as above – all observations for both sexes + rubbing of chin on bars of cage** **20/40mg/kg/day: as above + partially closed eyelids, underactive behaviour (usually before dosing)**							
Mean Age of Preputial separation (days)	44.6	43.5	44.0	**46.4***	-	-	-	-
Mean Age of Vaginal Patency (days)	-	-	-	-	35.0	35.7	35.8	**38.8****
Motor Activity (days of age):								
Day 22 – total low beam breaks	282.4	**137.0****	**105.6****	**157.6****	246	**152.3**	**121.4**	**188.6**
Day 22 – total high beam breaks	41.6	**25.7**	**15.9***	**20.9***	46.4	**14.9***	**16.4***	**34.8****
Day 47 – total low beam breaks	899.1	**671.9****	**438.7****	**230.1****	1060.8	**762.8****	**432.2****	**222.3****
Day 47 – total high beam breaks	137.4	**105.3***	**59.2****	**25.8****	188.3	**105.0****	**48.5****	**28.8****
Day 77 (18R) – total low beam breaks	920.4	905.4	903.7	**802.8**	1013.7	937.6	**825.1**	**608.3****
Day 77 (18R) – total high beam breaks	167.0	171.4	153.2	**116.0**	154.7	124.4	**114.3***	**77.2****
Learning and Memory (Morris Maze/ days of age):								
Day 23/24 – Mean swimming time Day 1	62.5	66.7	68.9	**76.7**	71.1	70.8	71.6	**88.6****
Day 23/24 – Mean swimming time Day 4	18.9	23.4	16.3	**35.0**	29.4	17.0	28.3	**46.4**
Day 48/49 – Mean swimming time Day 1	29.7	38.3	**53.5***	**63.2****	31.6	34.1	**42.9**	**60.8**
Day 48/49 – Mean swimming time Day 4	10.4	16.5**	**15.0****	**26.4****	9.4	13.5	**18.2**	**24.5***

(continued)

Studies in Juvenile Animals: Oral (Gavage) Toxicity Study in the Juvenile Rat: Amphetamine (Adderall™ mixture)								
	Male				Female			
Daily Dose (mg base/kg/day)	0 (Control)	2/4	6/12	20/40	0 (Control)	2/4	6/12	20/40
Day 78 (19R) – Mean swim. time Day 1	8.4	20.9*	**21.3***	**34.1****	12.2	14.1	**33.7****	**40.4****
Day 78 (19R) – Mean swim. time Day 4	5.7	7.6	7.1	**12.0***	7.8	6.3	9.9	10.8
Blood Chem. after 8-9 wks treatment								
Alkaline phosphatase (u/L)	605	659	668	**816****	414	456	461	**512***
Aspartate aminotransferase (u/L)	94	100	**114***	**118****	87	88	99	96
Creatinine (μmol/L)	43	**40***	**39****	**39****	45	45	**42***	**42***
Triglycerides (mmol/L)	0.80	0.70	0.61	**0.48***	0.70	**0.35****	**0.37****	**0.39****
Potassium (mmol/L)	4.1	4.1	4.2	4.3	3.7	3.9	**4.0****	**4.0****
Phosphorus (mmol/L)	2.49	2.38	2.43	2.50	2.17	2.21	**2.43***	**2.47****
Calcium (mmol/L)	2.70	2.68	2.65	2.68	2.78	**2.68***	**2.73***	**2.72***
Total protein (g/L)	62	61	**59****	**59****	64	**60****	**61****	**60****
Blood Chem. - 41-51 days recovery								
Phosphorus (mmol/L)	1.93	1.88	1.91	1.98	1.75	1.78	1.85	**1.96***
Organ Weight after 8-9 wks treatment								
Bwt relative (%) adrenal glands	0.0179	0.0180	0.0190	0.0190	0.0298	0.0315	**0.0349***	**0.0360****
Bwt relative (%) salivary glands	0.1815	0.1875	0.1946	0.2032	0.1743	0.1848	**0.2135****	**0.2102****
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

(continued)

Studies in Juvenile Animals: Oral (Gavage) Toxicity Study in the Juvenile Rat: Amphetamine (Adderall™ mixture)

	Male				Female			
Daily Dose (mg base/kg/day)	0 (Control)	2/4	6/12	20/40	0 (Control)	2/4	6/12	20/40
Reproductive phase								
Oestrous cycles – Regular 4/5 days (%)	-	-	-	-	100	100	100	90
% Mating	100	100	100	100	100	100	100	100
% Fertile Males/Pregnant Females	89	100	90	90	89	100	90	90
Mean No. Corpora Lutea	-	-	-	-	15.5	16.6	15.1	15.7
Mean No. Implantations	-	-	-	-	16.0	16.3	15.4	15.0
Mean % Pre-implantation Loss	-	-	-	-	0.7	4.1	1.3	4.0
Mean No. Live Conceptuses	-	-	-	-	15.4	14.8	15.0	14.1
Mean No. Resorptions	-	-	-	-	0.6	1.5*	0.4	0.9
Mean % Post- implantation loss	-	-	-	-	3.9	9.3	3.0	5.7

\- No noteworthy findings. + Mild ++ Moderate +++ Marked

* $p<0.05$ ** - $p<0.01$

a Reproductive phase animals treated Days 7 to 59 of age followed by a recovery period before mating and necropsy; Toxicity phase animals treated from Days 7 to 61-65 of age with necropsy on Days 62-66 of age

APPENDIX 5 EFFECTS OF D-AMPHETAMINE IN A RAT MODEL OF ATTENTION DEFICIT HYPERACTIVITY DISORDER (UNPUBLISHED DATA)

Introduction

The spontaneously hypertensive rat (SHR) provides an animal model of ADHD. These rats show hyperactivity, impulsivity and deficits in attention (Sagvolden et al 2004). The control strain is usually the Wistar Kyoto Rat (WKY) as this rat is the progenitor strain and it's behaviour is similar to that of other strains when tested in operant tasks. Drugs used in ADHD (amphetamines and methylphenidate) have been shown to have desirable effects in this model that mimic those exhibited by ADHD patients. Data from studies with d-amphetamine are presented below.

***Overactivity**:* The pronounced SHR overactivity was reduced by amphetamine (Figure 1). Following the highest dose, the general activity level of the SHR approached that for the WKY control. For d-amphetamine, the ANOVA showed a main effect of group, $F(1, 29) = 145.8$, $p<0.001$ and a Group x Dose interaction effect, $F(3.2, 92.4) = 19.2$, $p<0.001$).

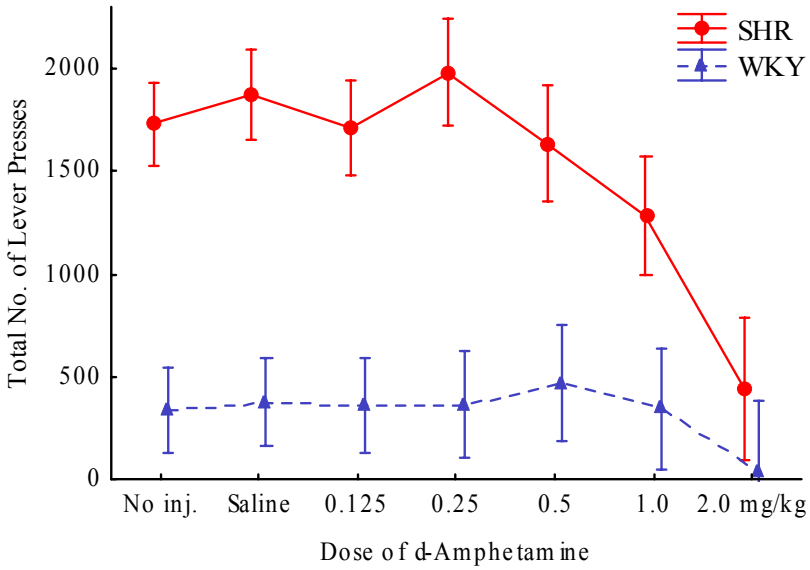


Figure 5. Effect of d-amphetamine on general activity level (± SEM with 95% confidence intervals.)

Impulsiveness: The SHR showed a pronounced impulsiveness that was reduced by d-amphetamine. Following the highest dose, the impulsiveness of the SHR approached that for the WKY control (Figure 2). For d-amphetamine, the ANOVA showed a main effect of group, $F(1, 29) = 19.5$, $p<0.001$ and a Group x Dose interaction effect, $F(1.9, 55.5) = 9.2$, $p<0.001$).

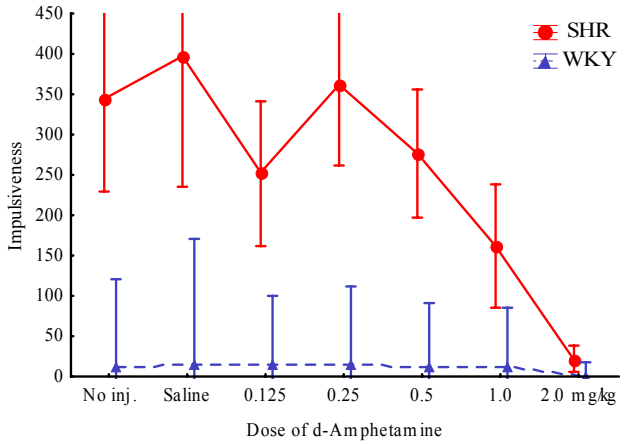


Figure 2. Effect of d-amphetamine on impulsiveness (± SEM with 95% confidence intervals.)

Sustained attention: Without treatment, the SHR showed poorer sustained attention than WKY controls. In contrast to the effects on activity and impulsiveness where the normalisation was seen following the highest doses used, sustained attention appeared to improve in the SHR following low doses of d-amphetamine but was impaired at higher doses (Figure 3). The ANOVA of effects of d-amphetamine with saline as baseline showed a significant improvement in the SHR group. For d-amphetamine there was a group x dose interaction: (F(3,84)=4,36717, p<0,007; p<0,02 following Greenhouse-Geisser corrections).

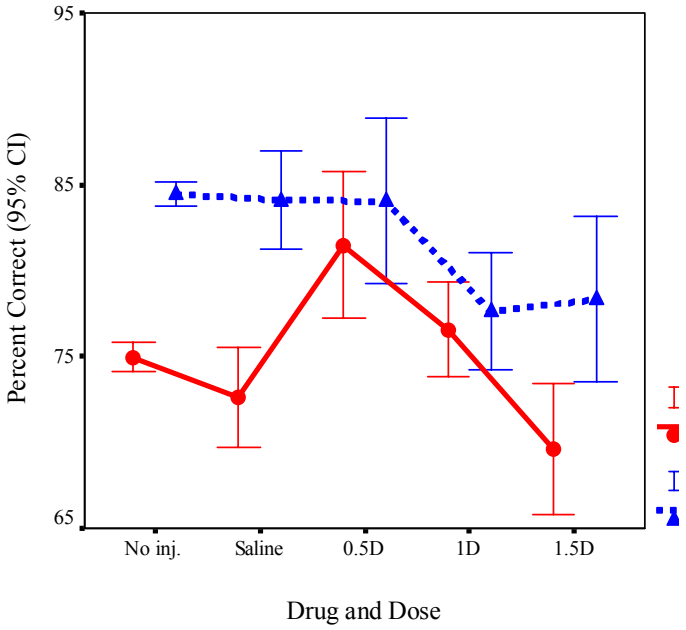


Figure 3. Effects of d-amphetamine on sustained attention. ± SEM.

Circle continuous line- SHR

Triangle dotted line - WKY

Reference

Sagvolden,T, Aase,H, Johansen,EB, Farshbaf,M (2004): Animal Models of Attention-Deficit/Hyperactivity Disorder. In Barch, D, editor. *Cognitive and Affective Neuroscience of Psychopathology*. Oxford: Oxford University Press.