CENTER FOR DRUG EVALUATION AND RESEARCH

ADVISORY COMMITTEE: ENDOCRINOLOGIC AND METABOLIC DRUGS ADVISORY COMMITTEE

DATE OF MEETING: 11/16/95

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SLIDES (BRIEFING PACKAGE)

CONFIDENTIAL

Background Package

Endocrinologic and Metabolic Drugs Advisory Committee Meeting

November 16, 1995

for
NDA #20-344
Dexfenfluramine HCl 15 mg Capsules

Interneuron Pharmaceuticals, Inc.
99 Hayden Avenue, Suite 340
Lexington, MA 02173
(617) 861-8444

October 26, 1995

EXECUTIVE SUMMARY

At the previous Endocrinologic and Metabolic Drugs Advisory Committee Meeting held on September 28, 1995, there appeared to be four important issues that the committee needed to consider: (1) the efficacy of the drug, (2) the possible association with the extremely rare but serious disease, primary pulmonary hypertension, (3) the relevance of long-term brain serotonin reduction in animals treated with large doses of dexfenfluramine or fenfluramine, and (4) the overall risk/benefit profile.

The committee appeared to be satisfied with the efficacy of the drug and comfortable that the benefits of the drug in treating obesity outweigh the very small possible risk of pulmonary hypertension. Therefore, we will only briefly revisit these issues in this document. This background package will instead concentrate on the issue of serotonin reduction in animal brains, the area which seemed to be unresolved, and will briefly discuss the overall risk/benefit.

Please refer to the background document (green binder) dated September 8, 1995 from Interneuron for details regarding dexfenfluramine's mechanism of action as a serotoninergic agent and its effects on satiety; this same background package also summarized the efficacy data from 19 double-blind, placebo-controlled studies of 3 to 12 months duration which showed dexfenfluramine to be consistently superior to placebo in producing greater weight losses. The efficacy data showed that dexfenfluramine plus diet therapy produce clinically significant weight loss, compared to diet therapy alone. Significantly larger proportions of patients lose clinically significant amounts (5, 10 or 15%) of weight on drug when compared to those on placebo.

The September 8, 1995 background package also described the evidence for the need to treat obesity and the risk reduction seen with weight loss.

Most NDAs have safety assessments in a few thousand patients. In the case of this NDA, we *also* have one of the larger post-marketing experience databases from which to assess safety. Approximately 10 million patients have been exposed to dexfenfluramine in the 10 years that it has been marketed outside the U.S. It is currently approved and marketed in 65 countries. In addition, over 30 million patients have been exposed to fenfluramine in the 25 years it has been on the market including millions of Americans, since the drug was approved in the U.S in 1973. It is important to realize that fenfluramine contains equal parts of dexfenfluramine and levofenfluramine. The usual daily dose of fenfluramine is

60 to 120 mg/day, so patients who take fenfluramine are receiving at least 30 mg/day of dexfenfluramine, our recommended daily dose.

At the September 28, 1995 meeting Dr. Lutwak (FDA Medical Reviewer) showed a slide of serious and non-serious events reported from spontaneous post-marketing surveillance since 1984 with dexfenfluramine. The nature of such surveys often leads to reports of serious adverse events that may or may not be associated with the use of a drug. Such surveys can however, serve as early sentinels of potential rare toxicities. Given that approximately 900 serious and non-serious CNS adverse reactions have been reported in 10 million patient exposures, with a drug whose mechanism of action is CNS-based, the risk of some heretofore unrecognized toxicity seems remote. Indeed, other approved serotoninergic agents produce similar spontaneous reporting incidences of CNS adverse events.

Brain serotonin levels are reduced following administration of large doses of dexfenfluramine in animals. Whether or not this reduction of serotonin in animals is described as neurotoxicity may not be the relevant question or issue. What is important is the *clinical relevance* of these high-dose animal toxicity studies. There are several ways to understand these observations. Consider giving 30 times the recommended dose of any drug that you commonly prescribe. Although you may not have personal experience with doses this high, you do know the probable consequences of such an experiment.

Another way to put into perspective these very large doses used in the animal studies and compare them to humans is to calculate a "margin of safety or exposure". This is a technique commonly used by toxicologists, and especially by the FDA, to understand human relevance. Dr. Contrera, in his report of September 1, 1995, states that there is at least a 15 fold "margin of safety" observed for the changes seen with serotonin reduction in animals. (This could be as high as 30-fold if you take into consideration the variation observed in the MRS validation experiments in the monkey.) His assessment implies a level of comfort with this margin. An explicit statement to that effect was made by Drs. Lutwak and Troendle in the FDA's background package dated September 28, 1995.

Many of the slides shown by Drs. Molliver and Seiden on September 28, 1995 were from MDMA experiments, <u>not</u> experiments with fenfluramine or dexfenfluramine . It is unfair and invalid to extrapolate adverse findings from this drug of abuse on the basis of perceived similarity in chemical structure (i.e. MDMA) to dexfenfluramine or fenfluramine. As you will see later in this dossier,

chemically related compounds such as MDMA produce a variety of specific changes that are indicative of neurotoxicity that are not seen with dexfenfluramine. Using the "chemical structure" line of reasoning might lead one to suspect that all phenethylamines such as ephedrine, epinephrine, or norepinephrine are potential neurotoxins. As many of the committee members know, the toxicities observed with phenformin kept metformin, a chemically-related compound, off the market in the U.S. for many years, even though the toxicities were different. Chemically-related compounds do not necessarily produce similar toxicities.

Other government agencies have also wrestled with the clinical relevance of this issue. The UK Medicines Control Agency recently reviewed this issue and concluded: "We have now completed our assessment of the report prepared by Professor C.K. Atterwill, and have reviewed the spontaneous reports of neurological adverse drug reactions associated with dexfenfluramine and fenfluramine, received to date. We conclude that no action is required in relation to this aspect of the drugs' safety profile at present." [see page 5]

It is our primary intent to review for you in this supplemental background package the available animal behavioral and human data addressing whether there is any evidence that the observations in animals have *any relevance* to humans.

As you will read, the neurological and neuropsychological assessments that have been collected have found no evidence of "neurotoxicity," subtle or otherwise, during treatment periods ranging from 3 to 12 months or post-treatment follow-up periods of 1 to 12 months.

Despite the lack of any indication of serious neurological adverse effects, we have included a section which expresses our intent to continue to examine this issue in post-marketing studies. We have committed to these studies and will continue to work closely with Dr. Sobel and his staff for agreement of acceptable designs, duration, size and measurements for these studies, including studying special obese populations (i.e. obese diabetic and obese hypertensive patients) and to expand our assessment of the clinical relevance of the neurochemical changes observed in animals by conducting long-term studies and examining a variety of additional neuropsychological parameters.

In summary:

1) An analysis of neurologic, psychometric, behavioral and cognitive parameters in 17 placebo-controlled trials in our NDA database failed to reveal any clinically significant differences between dexfenfluramine-treated patients and placebo-treated patients.

2) Whether or not one chooses to accept long-term serotonin depletion as a marker for neurotoxicity, the doses of dexfenfluramine required to produce a theoretical long-term serotonin depletion in man would be about 900 mg/day vs. the proposed dose of 30 mg/day.

3) Many neuroscientists do not believe that dexfenfluramine has any neurotoxic potential, including leading neurotoxicologists from the Environmental Protection Agency (EPA).

4) In 10 years of dexfenfluramine use outside of the U.S. with an estimated 10 million patients treated, there has been no epidemiologic signal indicating neurological or behavioral problems in actual clinical use. The same is true for fenfluramine, which has been used by an estimated 30 million people around the world, including several million in the U.S. European regulatory authorities, including the Medicines Control Agency in the UK, have examined the question and have concluded that no regulatory action is warranted.

5) We and our marketing partner, Wyeth-Ayerst, have committed to a Phase IV program

Finally, as everyone is aware, Interneuron and the Committee Members were caught unaware by the enormous emphasis on neurotoxicity claims during the FDA and outside experts' presentations. As an insight into the procedural irregularities during the meeting we found the enclosed BioCentury article of interest and we have enclosed it for your review. [see pages 6-8]

Department of Health
MEDICINES CONTROL AGENCY
Market Towers 1 Nine Elms Lane London SW8 5NQ
Telephone 0171-273 0274
Facsimile 0171-273 0675

Mr C. Vix,
Les Laboratories Servier,
6, Place des Pléiades,
92415 Courbevoie Cedex,
France.

5 September, 1995

Dear Mr Vix,

Re: Assessment update of the neurotoxic potential of dexfenfluramine

We have now completed our assessment of the report prepared by Professor C. K. Atterwill, and have reviewed the spontaneous reports of neurological adverse drug reactions associated with dexfenfluramine and fenfluramine, received to date. We conclude that no action is required in relation to this aspect of the drugs' safety profile at present.

Yours sincerely,

APPEARS THIS WAY
ON ORIGINAL

Dr E M Cockburn PhD
Senior Scientific Officer
Pharmacovigilance Assessment Group
Post Licensing Division
MCALETHD.DOC

October 9, 1995

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BioCentury this week

BioCentury has been faxed in two separate parts this week. Pages A1-A5 comprise Part I. Pages B1-B13 comprise BioCentury Part II.

Cover Story

Interneuron's FDA advisory committee meeting fell apart in the face of agenda surprises, a long-standing scientific dispute, and conflicting agency decisions.

Commentary

Emerging Company Profile

Noteworthy

Departments

Interneuron's FDA meeting

Behind the mess

By Steve Usdin
Contributing Editor

WASHINGTON — Interneuron Pharmaceuticals Inc.'s FDA advisory committee meeting for Redux dexfenfluramine combined elements of surprise, a long-running scientific dispute and potential conflict with previous FDA decisions to result in the bizarre outcome of the meeting, at which committee members voted both to recommend and not to recommend approval of the drug to treat obesity.

The chaos at the deliberations of the Endocrinologic and Metabolic Drugs Advisory Committee on Sept. 28, (see *BioCentury Extra* Sept. 28 *and BioCentury* Oct. 2) also can be attributed to the fact that the committee members were confronted by scientific data outside their area of expertise, for which the agency apparently did little to prepare them.

Try again Nov. 16

At the meeting, the panel voted 5-3 against recommending approval of Redux based on doubts about the safety of dexfenfluramine (DF) as a long-term treatment for obesity raised by expert witnesses citing their research in animals. However, after three members of the panel had departed the meeting, a rump group voted 3-2 in favor of DF, after FDA officials asked them to consider whether the drug was approvable on a risk-benefit basis.

Late last week, the FDA decided to give IPIC the opportunity to present more safety data at a Nov. 16 committee meeting, reversing its initial decision to send transcripts of the earlier meeting to the three absent committee members and to take their votes over the telephone.

The September meeting was thrown into disarray when a debate broke out between the sponsor and outside experts invited by the FDA. Mark Molliver, neuroscience and neurology professor at Johns Hopkins School of Medicine, and Lewis Seiden, pharmacology professor at the University of Chicago, made lengthy presentations of evidence they said demonstrated the neurotoxicity of DF.

Joseph Contrera, assistant director of the office of research resources at FDA, also presented data on the drug's neurotoxic effects.

The presentations took the committee and the company by surprise. IPIC strongly rebutted the presentations and attacked the underlying scientific rationale.

Unexpected

According to Bobby Sandage, IPIC's senior vice president for R&D, the company initially was told only to expect testimony from Contrera, who was acting as an in-house consultant on the DF NDA. A week later, which was three weeks prior to the meeting, the agency notified IPIC that Molliver and Seiden would be testifying.

The agenda "was not balanced," said Sandage. "We got 90 minutes to present on all issues and (Molliver and Seiden) got an hour between the two of them to present on one issue."

Sandage said the company had been led to believe that neurotoxicity would not be a major issue at the meeting.

In fact, two committee members contacted last week by BioCentury confirmed that no evidence of potential neurotoxicity was included in the briefing documents they received from FDA prior to the meeting.

It is customary for the FDA to provide committee members with data supporting arguments it intends to make and conclusions it will draw at the meetings.

Advance provision of data to the panel was especially important in the case of DF. The committee is composed of experts in endocrinology and metabolic disorders, not psychopharmacology, who were unlikely to know the history of a 20-year contro-

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The tangled web at FDA meeting

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versy about the potential neurotoxicity of the compounds. The committee members interviewed by BioCentury last week were not aware of the long-running dispute.

The neurotoxicity issue was debated in the mid-1970s, when FDA was considering approval of fenfluramine, the racemic form of DF.

The agency approved fenfluramine in 1973, rejecting arguments from researchers who said their experiments pointed to neurotoxicity. The agency and other scientists said the evidence might have been an artifact of the experiments, and there was no functional evidence of neurotoxicity.

Dexfenfluramine is the active portion of fenfluramine. DF's profile of adverse effects is smaller than fenfluramine's, but any neurotoxic effects of the two compounds would be identical, according to IPIC and papers published by its critics.

An Oct. 12, 1994, article in *The Journal of the American Medical Association* quotes Alex Jordan, supervisory pharmacologist in the FDA's metabolic and endocrine drug products division, as saying: "There's no reason not to believe that if there is toxicity associated with dexfenfluramine, then fenfluramine also has toxicity."

Hard to undo

Although rejecting DF based on neurotoxicity would suggest that fenfluramine should be removed from the market, Jordan said that it is "easier to keep one (drug) off the market than it is to withdraw one that is already approved. First, we have to find out whether dexfenfluramine is toxic."

According to IPIC, more than 10 million patients have received DF, which is approved in 65 countries, since 1985. An additional 40 million have taken fenfluramine. "We have extensive post-market surveillance and there's nothing in the database to suggest a (neurotoxicity) problem," Sandage said.

The issue was revived in the early 1980s when researchers published papers on the neurotoxicity of amphetamine analogs using new methodologies. Other researchers picked up on the thread and investigation of neurotoxic effects resumed, including that of fenfluramine and dexfenfluramine.

Molliver and Seiden have been among the researchers keeping the issue alive.

Prior to joining FDA, Contrera worked at the National Institute of Drug Abuse laboratory in Baltimore, where he was credited as a co-author of at least 15 papers about the neurotoxicity of fenfluramine and dexfenfluramine from 1980 to 1991.

At the advisory committee meeting, Contrera outlined the preclinical evidence for neurotoxicity of DF in terms similar to those used by Molliver and Seiden. Committee members told BioCentury they initially thought the two independent scientists were FDA employees, and were left with the impression that the agency endorsed their conclusions.

BioCentury was unable to reach Contrera either directly or through FDA press channels.

Molliver, a highly respected scientist, has conducted research on fluoxetine, the serotonin reuptake inhibitor marketed as Prozac by Eli Lilly and Co. for depression.

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BioCentury Company Index
October 9, 1995

Commentary

Dysfunctional teamwork

The highly publicized resignation last week of Apple Computer Inc.'s chief financial officer, Joseph Graziano, reminded us of conversations we've had over the past few years about the frustrations of CFOs and the representation of shareholder interests at biotech companies.

Graziano's resignation was widely ascribed to his assessment, apparently rejected by Apple's chief executive and board, that the struggling personal computer maker be put up as an acquisition candidate. One does not have to think more than a nanosecond to conclude that such an issue is not foreign to the biotech sector, where advocates of consolidation most often blame the egos of CEOs, followed by the relative passivity of boards of directors, for the relative snail's pace of mergers and acquisitions.

On a more fundamental level, biotech is quite like the other post-industrial age sectors where the primacy of technology, entrepreneurship, dependence on uniquely skilled human capital and the broad spectrum of perceived risk between management and investors can conspire to push shareholder interests into the background.

This perhaps is exaggerated in biotech, where time to market and capital consumption requirements force management to concoct what amount to long-term survival strategies, and stories to go along with them, in the hope of persuading investors to fund very abstract concepts for a very long time. The situation is such that it has become rational for biotech management to gobble up capital at every opportunity. This creates a situation where the next dollar to come in can become more important than protecting the value of the investment capital that preceded it.

The question is when does shareholder interest become so subordinate that the partnership of the board of directors, the CEO and the CFO becomes unraveled.

Donald Hawthorne of Biocircuits Corp. is one of the thoughtful biotech CFOs who thinks about these issues a lot. In Hawthorne's view, "the CEO is expected to have a vision for the company. That vision can be driven both by technology and market issues as well as by a series of personal beliefs."

By comparison, he says, "the CFO should have a vision for the business. As long as there is some general compatibility between the two visions, then you have philosophical compatibility between the two players as they think about how to run the business."

Managing the dichotomy

In this dichotomy, one important role of the CEO is to market the company vision to others, among them the board members. On the other hand, the CFO has to help both the CEO and the board think through the economics and the business structure of the vision — as Hawthorne points out, to address the reality of the situation.

To accomplish this, the CFO acts on two dimensions. The first is modeling the business going forward, or "what it's going to take to get there." The second is monitoring actual progress versus the global vision.

The potential for conflict, of course, comes when the CFO sees a disconnect between the model of the vision and the reality of the company's situation. At this point, the important question is whether the CFO can carry out an open and frank discussion with the CEO and the board. "If the board is asking for these kinds of discussions," says Hawthorne, "then you can

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In 1994, Lilly withdrew its NDA for use of a high-dose of fluoxetine, which it calls Lovan, for treating chronic obesity, citing new FDA requirements for long-term trials of anti-obesity agents. However, the high dose formulation is approved in the U.S. for treatment of bulimia, and in other countries for obesity.

Medical literature on treatments for obesity mention off-label use of fluoxetine. For example, an article in the March 1993 *Archives of Family Medicine*, "A Practical Approach to Treatment of the Obese Patient," states: "While pharmacotherapy with serotoninergic agents such as fenfluramine hydrochloride or fluoxetine (weight loss is an unlabeled use for fluoxetine) may be effective for some obese patients, rapid regaining of lost weight after discontinuation makes its short-term use a problem."

A July 1994 article in the *Journal of Family Practice*, stated that "In this study, fluoxetine was prescribed more often to obese patients. This prescribing pattern may indicate that primary care physicians perceive overweight patients as good candidates for fluoxetine regardless of inconclusive evidence about the effectiveness of this drug for weight loss."

It also found a greater number of depressed obese women were given fluoxetine than other antidepressants, suggesting "that primary care physicians view overweight female patients as good candidates for fluoxetine therapy, even though the use of this medication to achieve weight loss" has not been documented.

Those familiar with the neurotoxicity debate generally seem aware of an association between Molliver and Lilly researchers, which was documented in a photograph in Lilly's 1987 annual report.

The caption reads: "Laboratory studies by Ray W. Fuller, Ph.D. (right), provided crucial data supporting the therapeutic potential of Prozac. Kenneth W. Perry was Dr. Fuller's associate in the laboratory studies. Dr. Fuller and his Lilly colleagues later worked with scientists at The Johns Hopkins University School of Medicine, including Mark E. Molliver, M.D. (left), professor of neuroscience, and J. Raymond DePaulo, Jr., M.D. (center), director of the Affective Disorders Clinic."

However, Molliver told the advisory committee, and BioCentury, that he has never had a financial interest in Prozac, nor has he been employed by Lilly to conduct research on it.

"Doctors Molliver and Seiden are good and respected scientists, however, they do seem to have an obsession with this," said IPIC's Sandage. "Maybe it would have been better if the FDA had found someone in a neutral corner instead of pitting them against us."

OVERVIEW: NEUROCHEMICAL CHANGES IN ANIMALS

The reduction in brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content following the administration of high doses of fenfluramine or dexfenfluramine to animals has been known for over 20 years. These findings vary markedly across species and by route and regimen of dosing. Some investigators (e.g., Drs. Molliver, Seiden and Contrera (FDA)) have interpreted the reduced brain indole levels and the correlate observation of diminished visualization of neurons by 5-HT immunohistochemistry, as surrogate indicators of neurotoxicity. It is this interpretation that has been the subject of years of contentious debate. We disagree with this point of view; we believe that the reductions in brain indole levels following the administration of high doses of fenfluramine or dexfenfluramine to animals are no more than an extension of the pharmacological effects of this class of serotonergic drugs. Regardless of which interpretation you choose to accept, significant evidence will be presented to demonstrate that a large margin of exposure exists between brain levels of dexfenfluramine and its active metabolite d-norfenfluramine that produce changes in specific markers of neurotoxicity in animals and those achieved in obese patients receiving therapeutic doses of dexfenfluramine. In addition, significant evidence will be presented that demonstrate a lack of significant behavioral/functional changes in animals, and more importantly, a lack of significant clinical neuropsychological findings with dexfenfluramine in controlled trials and in extensive post-marketing experience over the last 10 years in an estimated 10 million patients (40 million patients when fenfluramine is considered). The data summarized in this document demonstrate that the neurochemical changes produced by high dose dexfenfluramine administration in animals does not represent a significant health risk to humans.

Several important points can be made regarding the neurochemical changes produced by high doses of dexfenfluramine (or fenfluramine) in animals. Each point outlined below is discussed in detail in the accompanying pages. These points in support of the safety of dexfenfluramine are: 1) reductions in brain serotonin produced by high doses of dexfenfluramine in animals are pharmacologic in nature and common to other serotonin reuptake inhibitors (e.g., fluoxetine); 2) differences between species in the reduction in 5-HT content are pharmacokinetic in origin and directly related to the brain concentrations of dexfenfluramine and d-norfenfluramine achieved. Following therapeutic doses (15 mg bid), human brain concentrations of dexfenfluramine and d-norfenfluramine rapidly reach steady-state, are low and do not accumulate; 3) acute reductions in brain serotonin content are reversible; 4) long-term administration of high doses of dexfenfluramine produces no long-term reductions in brain serotonin content or paroxetine binding, suggesting that there is something unique to the acute, high dose pulsed dose regimen; 5) techniques that use a measure of 5-HT as their basis (e.g., 5-HT neurochemical assays of tissue homogenates or immunohistochemical techniques that depend upon 5-HT content) are not suitable indices of neurotoxicity of a serotonergic

drug; 6) acute reductions in brain serotonin produced by high doses of dexfenfluramine in animals are not accompanied by specific markers of neurotoxicity (i.e., altered silver staining, gliosis, impaired retrograde axonal transport in 5-HT neurons), as is observed with known neurotoxins (e.g., 5,7-dihydroxytryptamine); and finally, 7) comparisons of brain concentrations of dexfenfluramine plus d-norfenfluramine in animals receiving doses that have no effect on specific markers of neurotoxicity with those in obese patients receiving therapeutic doses (15 mg bid) reveal that the **margin of exposure is at least 16- to 25-fold**. This margin of exposure is similar to the clinical margin of safety of 15 estimated by Dr. Contrera in his report of September 1, 1995, to the Committee.

Based on a review of the effects of dexfenfluramine and fenfluramine on animal behavior (locomotor activity, learning and memory, social behavior/aggression), endocrine and immune function, it can be concluded that these drugs do not produce any significant or persistent adverse functional or behavioral effects. As an expected consequence of their acute pharmacology, high doses of dexfenfluramine/fenfluramine produce sedative effects in animals that have been reported to reduce locomotor activity, social behavior and acquisition of learning paradigms. These effects are not observed once high dose drug treatment is discontinued, and are thus not consistent with any interpretation of neurotoxicity. Similarly, as an expected consequence of their acute pharmacology, both direct and indirect serotonin agonists, including fenfluramine/dexfenfluramine, cause the release of several releasing factors and hormones. The repeated daily administration of dexfenfluramine leads to the rapid development of tolerance to these endocrinological effects and is certainly not an indicator of neurotoxicity.

Critical to the issue of neurotoxicity is whether or not behavioral or functional deficits are observed with prolonged treatment. In this regard, the subchronic and chronic (>6 months) administration of dexfenfluramine/fenfluramine fail to produce any significant or persistent adverse functional or behavioral effects. Sensitive memory processes in aged rats, for example, are unaffected by subchronic and chronic dexfenfluramine administration. Furthermore, the subchronic and chronic administration of dexfenfluramine has been found to actually restore the normal hormonal response to stress and to maintain the NK and T cell arms of the immune system of aged rats at youthful levels.

Small, extracellular calcifications were observed in several organs, including the brain, of rats and mice receiving dexfenfluramine in feed for at least two years. Dr. Contrera has suggested that brain calcification may be a manifestation of neurotoxicity. A number of observations make these concerns unwarranted. These observations include: 1) small, extracellular calcifications were observed in brain, as well as other organs (e.g., cornea , prostate), of control and dexfenfluramine-treated animals; 2) the apparent increase in the number of calcifications in dexfenfluramine-treated animals was not dose-dependent; 3) the spontaneous incidence of these extracellular

calcifications varies widely; spontaneous calcifications in $B_6C_3F_1$ mice have been reported to range from the observed calcification was not of neural origin and did not appear to adversely affect adjacent neurons (i.e., the neighboring neuronal parenchyma was normal, without cell necrosis, gliosis or inflammatory infiltrate); 5) neurochemical indices of serotonergic function directly related to the pharmacologic actions of dexfenfluramine were unaffected by this long-term, high-dose drug treatment in mice (at brain levels at least 12-times the human therapeutic levels); 6) mineralization similar to that observed in rodents is common and well-documented in monkeys and humans (without functional consequences); and, 7) neither disturbed calcium homeostasis (e.g., tetany) nor secondary hyperparathyroidism has been reported in man following the administration of dexfenfluramine. In summary, the observed increase in brain calcium deposits in rodents appears to have no effects on neuronal structure or function, certainly none that can be extrapolated to humans. Furthermore, attempts to relate the present findings to human brain function or pathology are unwarranted and scientifically invalid.

Based on the large margin of exposure that exists between the brain levels of dexfenfluramine and its active metabolite d-norfenfluramine that produce changes in specific markers of neurotoxicity in animals and those achieved in obese patients receiving therapeutic doses of dexfenfluramine, the lack of any significant or persistent behavioral/functional changes in animals, and more importantly, the lack of significant clinical neuropsychological findings with dexfenfluramine in controlled trials and in extensive post-marketing surveillance over the last 10 years in an estimated 10 million patients (40 million patients when fenfluramine is considered), it can be concluded that there is no risk of neurotoxicity when dexfenfluramine is used for the treatment of obesity.

MECHANISM OF ACTION

The actions of dexfenfluramine are mediated through changes in serotonergic neurotransmission; in contrast, d-amphetamine-like stimulants act through changes in catecholaminergic (norepinephrine, dopamine) neurotransmission. The effects of dexfenfluramine and its active metabolite d-norfenfluramine on monoamine mechanisms *in vitro* are summarized in Table 1.

Table 1. Effects of fenfluramine isomers and metabolites on monoamine mechanisms *in vitro

Mechanism	dexfenfluramine	d-norfenfluramine
Uptake (IC_{50} in μM)		
5-HT	0.5	1.4
NE	10	1.8
DA	20	17
Release (SC_{25} in μM)[a]		
5-HT	5	1
5-HT (reserpine)[b]	>100	0.2
NE	>10	-
DA	10	-
Binding (IC_{50} in μM)		
5-HT_1	7	4.2
5-HT_2	>30	2.2
α_1	>30	>30
α_2	25	17
β	>30	10
D_2	>30	>30

* Adapted from Garattini et al., Clin Neuropharmacol 11 (Suppl 1): S8-S32, 1988
[a] SC_{25} is the drug concentration stimulating [^{3}H]-5-HT release by 25% of basal outflow
[b] Animals received reserpine (10 mg/kg, ip) 18 hr before synaptosome preparation

Dexfenfluramine inhibits the reuptake of serotonin (5-HT) by axon terminals. In this regard, dexfenfluramine is pharmacologically similar to other selective serotonin reuptake inhibitors (e.g., fluoxetine, sertraline, paroxetine). Its sole active metabolite d-norfenfluramine not only inhibits serotonin reuptake, but is also a potent releaser of serotonin. Release of serotonin by d-norfenfluramine appears to be from a reserpine-insensitive, extravesicular pool. d-Norfenfluramine also binds directly to serotonin receptors, particularly those of the 5-HT_2 type. Thus, dexfenfluramine and its active metabolite appear to act relatively selectively through serotonergic mechanisms. This is in sharp contrast to amphetamine-like stimulants that release and inhibit the reuptake of catecholamines, particularly dopamine. Furthermore, dexfenfluramine does not have stimulant effects in humans; on the contrary, this drug produces mild CNS depressant effects, such as somnolence.

Substantial evidence indicates that serotonin is involved in the regulation of food intake (see Garattini et al., Clin Neuropharmacol 11 (Suppl 1): S8-S32, 1988 for a review). Agents that enhance serotonergic neurotransmission, like dexfenfluramine, reduce food intake. Agents that block serotonin receptors or reduce serotonin availability at presynaptic terminals inhibit this effect.

Dexfenfluramine is effective in reducing food intake in a number of species across a broad range of feeding paradigms. Paradigms that involve overfeeding rather than deprivation show a greater effect of the drug (see Rowland and Carlton, Clin Neuropharmacol 11 (Suppl 1): S33-S50, 1988 for a review). Furthermore, both stress-induced eating and food-motivated response (running) are particularly sensitive to inhibition by dexfenfluramine (ibid). Importantly, dexfenfluramine is particularly effective in reducing weight in rats once obesity had been established (as opposed to weight loss during the growth phase) (Blundell and Hill, In: *Metabolic complications of human obesities*, Vague J et al. (Eds), pp 199-206, 1986). In general, the dose of dexfenfluramine that reduces food intake by 50% (ED_{50}) across a broad range of feeding paradigms in rats was approximately 1.0 mg/kg (Rowland and Carlton, Clin Neuropharmacol 11 (Suppl 1): S33-S50, 1988). These anorectic doses of dexfenfluramine are <u>not</u> associated with altered motor function or alertness and are <u>not</u> associated with depletion of brain serotonin (Garattini, In: *Disorders of eating behavior: A psychoneuroendocrine approach*, Ferrari E (Ed), pp 327-341, 1986; Rowland and Carlton, ibid). In fact, Contrera and colleagues (Zaczek et al., J Pharmacol Exp Ther 253: 104-112, 1990) have demonstrated that equivalent doses of fenfluramine (1.0 and 2.0 mg/kg/day, bid, sc x 4 days) actually increase brain serotonin content in rats. Therefore, the high doses of dexfenfluramine (e.g., 10 mg/kg/day, sc x 4 days) used to affect brain serotonin content are well above the effective anorectic dose in most animal feeding models.

Dexfenfluramine acts on various components of eating behavior. It modifies the 24 hr

eating pattern by slowing eating during the meal, reducing meal size and increasing the duration of between-meal intervals (Blundell, Appetite 7: 39-56, 1986). Dexfenfluramine also decreases the motivation to eat (Kirkham and Blundell, Pharmacol Biochem Behav 25: 123-128, 1986) and has a specific inhibitory effect on supplementary food intake due to non-energetic needs such as stress-induced food intake (Garattini, In: *Disorders of Eating Behavior: A Psychoneuroendocrine Approach*, Ferrari E and Brambilla F (Eds), pp 327-341, 1986) or dessert intake (Rowland and Carlton, ibid, pp 367-374). Moreover, dexfenfluramine appears to have a selective effect on macronutrient choice, sparing protein intake while decreasing high carbohydrate intake and associated fat (Leibowitz et al., Clin Neuropharmacol 11 (Suppl 1): S51-S71, 1988; Wurtman and Wurtman, In: *Anorectic Agents: Mechanisms of Action and Tolerance*, Garattini S and Samanin R (Eds), pp 169-182, 1981).

NEUROCHEMICAL EFFECTS OF HIGH DOSES OF DEXFENFLURAMINE

Introduction

The reduction in brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content following the administration of high doses of fenfluramine or dexfenfluramine to animals has been known for over 20 years. These findings vary markedly across species and by route and regimen of dosing. Some investigators have interpreted the reduced brain indole levels, and the correlate observation of diminished visualization of neurons by 5-HT immunohistochemistry, as surrogate indicators of neurotoxicity. It is this interpretation that has been the subject of years of contentious debate. The Advisory Committee has received information from Drs. Molliver, Seiden and Contrera (FDA) expressing their point of view. Presented below are some important considerations that are critical to your evaluation and interpretation of the animal findings. In addition, data are summarized which directly address the Committee's concerns.

The critical issue is whether or not the neurochemical changes produced by high dose dexfenfluramine administration in animals represent a significant health risk to humans. The data summarized in this and the following sections addressing this issue demonstrate that concerns about clinical neurotoxicity are unwarranted.

Reductions in brain serotonin are pharmacologic and common to all serotonin reuptake inhibitors

Dexfenfluramine is believed to produce its anorectic effects through interactions with the serotonergic neurotransmitter system (see previous section). Many widely available antidepressant agents (e.g., fluoxetine (Prozac), sertraline (Zoloft) and paroxetine (Paxil)) share with dexfenfluramine the ability to inhibit the reuptake of serotonin. These agents also share with dexfenfluramine the ability to release serotonin directly from presynaptic nerve terminals through their active metabolites (Caccia et al., Br J Pharmacol 110: 355-359, 1993). The repeated administration of anorectic (ED_{50}) doses of fluoxetine, sertraline and paroxetine in rats produces acute reductions in cortical 5-HT (Table 2). Furthermore, when measured one week after discontinuation of treatment with the anorectic dose of fluoxetine, significant reductions in brain 5-HT content were observed (ibid). Differences between these serotonergic agents in the duration of their effects on brain serotonin content is related, at least in part, to the pharmacokinetics of the parent drug and active metabolite in brain. As noted previously, anorectic doses of dexfenfluramine in rats under similar conditions (ED_{50} = 1.29 mg/kg, ip) are not associated with significant reductions in cortical 5-HT levels (Garattini, In: *Disorders of eating behavior: A psychoneuroendocrine approach*, Ferrari E (Ed), pp 327-341, 1986). In general, acute reductions in cortical 5-HT levels (4 hrs after the last dose) in rats following the repeated administration of dexfenfluramine are seen at doses of (po) or greater. Therefore, the high doses of dexfenfluramine (e.g., 10 mg/kg/day, sc x 4 days) typically used to affect brain serotonin

content are well above the effective anorectic dose in most animal feeding models.

Table 2. Cortical 5-HT content after repeated anorectic doses of fluoxetine, sertraline and paroxetine in rats

Drug	Dose (mg/kg, bid, ip x 14 days)	5-HT Content (% pair-fed control)[a]
fluoxetine	12.0	47 ± 13*
sertraline	16.7	80 ± 6*
paroxetine	7.9	55 ± 7*

*$p<0.01$; [a]determined 1.0 hr after the last dose

The reduction in serotonin content produced by dexfenfluramine and other serotonin reuptake inhibitors is dose- and time-dependent; higher doses produce greater and longer lasting reductions in brain indole content. It is important to note that fenfluramine/dexfenfluramine have been particularly useful research tools in this regard because of their low toxicity. Extremely high doses of dexfenfluramine can be administered for long periods of time (e.g., no effect level in rats - po x 78 weeks; no effect level in dogs ≈ 10 mg/kg/day x 26 weeks (NDA 20-344, vols 17 & 24)). In contrast, attempts to reduce brain 5-HT content with fluoxetine by greater than 50% in rats is lethal at doses greater than 50 mg/kg (po), only a few multiples greater than its oral anorectic dose in rats (Garattini, personal communication).

Reduction in food intake alone alters brain serotonergic neurochemistry
Recently, reduction in food intake alone has been found to produce changes in the serotonergic system. For example, two weeks of food restriction in young rats causes a 32% reduction in paroxetine binding (a measure of the serotonin transporter) (Huether et al., Biol Psych, submitted, 1995). In humans, dieting has been reported to lower the plasma tryptophan levels, thereby reducing brain serotonin synthesis and upregulating the responsiveness of $5\text{-}HT_{2C}$ receptors (Cowen et al., Nature 376: 557, 1995). Thus, dieting alone can influence serotonergic neurotransmission. Moreover, these recent observations underscore the importance of including "pair-fed"control groups in animal experiments which measure neurochemical changes in the serotonergic neurotransmitter system.

Species differences are pharmacokinetic
As noted above, wide interspecies variations in the effects of dexfenfluramine administration on brain indole concentrations have been observed. At similar doses, the reduction of indoles (5-HT & 5-HIAA) in mice is rather weak compared to rats, while in non-human primates the reduction appears to be greater. On this basis, several

PHASE IV COMMITMENT

Wyeth-Ayerst and Interneuron have discussed with FDA their commitment to a Phase IV Clinical Program.

STUDY 1: Glycemic Control

This is a randomized, double-blind, placebo-controlled study of diet plus dexfenfluramine for weight control and glycemic control in patients with obesity and non-insulin dependent diabetes (NIDDM). There will be three phases to the study. Phase I will consist of a single-blind placebo and diet run-in for 2 weeks. Patients will then be randomized to dexfenfluramine, 15 mg bid, or placebo and followed for 1 year (Phase II). Patients will be evaluated at 1, 3, 6, 9, and 12 months following randomization. Phase III will consist of a 6 month post-therapy follow-up with evaluations at 3 and 6 months after termination of treatment.

The patient population will consist of approximately 200 patients with NIDDM and obesity (BMI between 27 and 40 kg/m^2) who are suboptimally controlled on diet alone or sulfonylurea therapy. Patients will have a hemoglobin (Hbg_{A1C}) of >6.5% and ≤10% and fasting plasma glucose of ≤260 mg/dl to qualify for the study. Randomization will be stratified by diet or sulfonylurea to assure equivalent patient assignment to dexfenfluramine and placebo treatment.

Primary endpoints will be the change in Hgb_{A1C} and weight from baseline to 1 year between treatment groups. Among the secondary endpoints to be assessed will be the effect at 6 months post-therapy on measures of neuropsychological function such as: *Global Measure*: Mini-Mental State Exam (MMSE): standard test of mental status; *Memory measure*: Alzheimer's Disease Assessment Scale (ADAS): cognitive subscale; *Functional Measure*: Blessed Dementia Rating Scale (BDRS): activities of daily living; *Mood Measure*: Hamilton Depression Rating Scale (HAM-D).

STUDY 2: Blood Pressure Control

This is a randomized, double-blind, placebo-controlled study of diet plus dexfenfluramine for weight control and blood pressure control in patients with mild hypertension and obesity. There will be 3 phases to the study. Phase I will consist of a single-blind placebo and diet run-in for 2 weeks. Patients will then be randomized to dexfenfluramine, 15 mg bid, or placebo and followed for 1 year (Phase II). Patients will be evaluated at 1, 3, 6, 9 and 12 months following randomization. Phase III will consist of a 6 month post-therapy follow-up with evaluations at 3 and 6 months after termination of treatment.

The patient population will consist of approximately 200 patients with obesity (BMI between 27 and 40 kg/m^2) and mild hypertension who are currently receiving no anti-hypertension medication. Patients will have a diastolic blood pressure (DBP) between 90 mmHg and 104 mmHG to qualify for the study. At randomization DBP will be <100 mmHg and systolic blood pressure (SBP) <160 mmHg.

Primary endpoints will be the change in mean DBP and weight from baseline to 1 year between treatment groups. Among the secondary endpoint(s) to be assessed will be the effect at 6 months post-therapy on measures of neuropsychological function such as:
Global Measure: Mini-Mental State Exam (MMSE): standard test of mental status;
Memory measure: Alzheimer's Disease Assessment Scale (ADAS): cognitive subscale;
Functional Measure: Blessed Dementia Rating Scale (BDRS): activities of daily living;
Mood Measure: Hamilton Depression Rating Scale (HAM-D).

Interneuron Pharmaceuticals Incorporated

One Ledgemont Center
99 Hayden Avenue, Suite 340
Lexington, MA 02173
Tel: (617) 861-8444
Fax: (617) 862-3859

Glenn L. Cooper, M.D.
President and
Chief Executive Officer

September 29, 1995

Solomon Sobel, M.D.
Division Director
U.S. Food and Drug Administration
Metabolism and Endocrine Drug Products Division

Dear Dr. Sobel:

Reference is made to the September 28th meeting of the Endocrinologic and Metabolic Drugs Advisory Committee which considered whether to recommend for approval the New Drug Application, No. 20-344, submitted by Interneuron Pharmaceuticals, Inc.

We are pleased that the Committee definitively resolved questions related to the clinical importance of the weight reduction demonstrated by dexfenfluramine and the impact of the risk of primary pulmonary hypertension on the overall analysis of safety and efficacy.
raised at the C

We are ready to meet with you expeditiously to design a mutually agreeable Phase IV trial to address the issues raised at the September 28th Advisory Committee meeting. Our intention would be to aim toward receipt of an approvable letter from the Agency as soon as possible.

Sincerely,

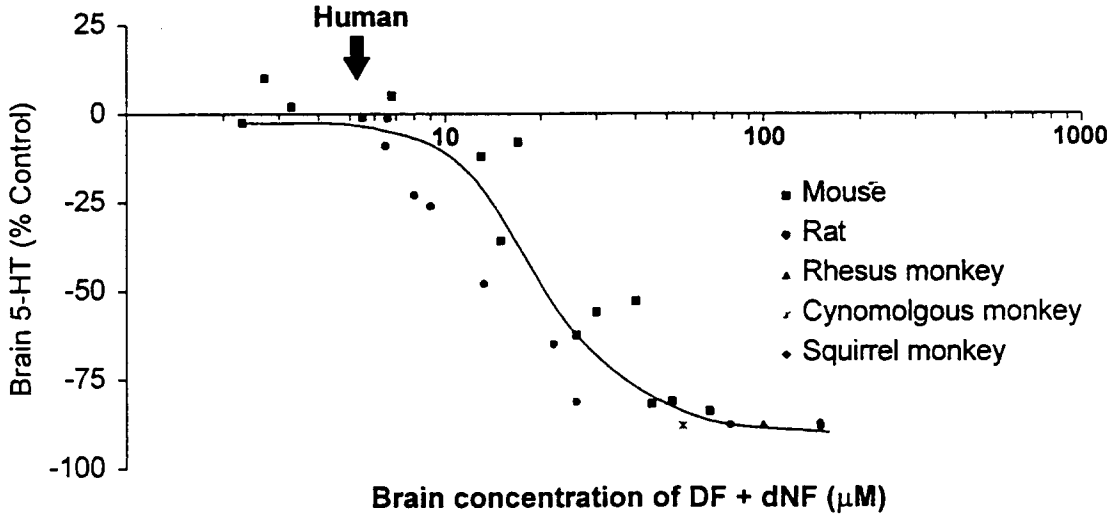
Glenn L. Cooper, M.D.

cc: Dr. James Bilstad, Director
Office of Drug Evaluation II

Joseph N. Bathish, Vice President
Worldwide Regulatory Affairs
Wyeth-Ayerst

investigators have concluded that this apparent greater sensitivity in monkeys is cause for concern about the use of dexfenfluramine in man (McCann et al., J Pharmacol Exp Ther 269: 792-798, 1994; Ricaurte et al., Lancet 338: 1487-1488, 1991). However, when the relationship between brain concentrations of dexfenfluramine and d-norfenfluramine to 5-HT or 5-HIAA concentrations is examined, it is apparent that these species differences are entirely pharmacokinetic in origin (i.e., at any given brain level of dexfenfluramine plus d-norfenfluramine, comparable decreases in brain 5-HT content are observed in all species studied). This relationship is shown graphically in Figure 1; regardless of the species, reductions in brain 5-HT content can be directly related to brain dexfenfluramine plus d-norfenfluramine concentration. Thus, the greater reductions in brain 5-HT content in non-human primates (rhesus, cynomolgous and squirrel monkeys) are a direct result of higher brain concentrations of dexfenfluramine plus d-norfenfluramine for a <u>given dose</u> of dexfenfluramine than those observed in rats or mice.

Figure 1. Relationship of brain dexfenfluramine (DF) + d-norfenfluramine (dNF) concentration to brain 5-HT concentration in different species



Viewed in another way, the brain levels of dexfenfluramine + d-norfenfluramine in various species following a very high pulsed dose regimen of dexfenfluramine administration (10 mg/kg/day, sc x 4 days) are presented in Table 3 below. Brain levels of dexfenfluramine + d-norfenfluramine in primates are significantly higher than those in rats or mice following this dosing regimen (and far above those observed in humans). It should be noted that the brain level of 16 μM dexfenfluramine + d-norfenfluramine produced by this dose regimen in mice results in acute reductions in brain 5-HT content of less than 20% (Fig. 1) that are <u>not</u> considered neurotoxic, even by Drs. Seiden and Molliver. Moreover, in contrast to statements by Dr. Molliver, acute reductions in brain 5-HT content in mice are observed when sufficient brain levels are reached (Fig. 1). For margin of exposure comparisons, the steady-state human brain concentration of

dexfenfluramine + d-norfenfluramine following therapeutic doses (15 mg bid) is 2.2-4.2 μM.

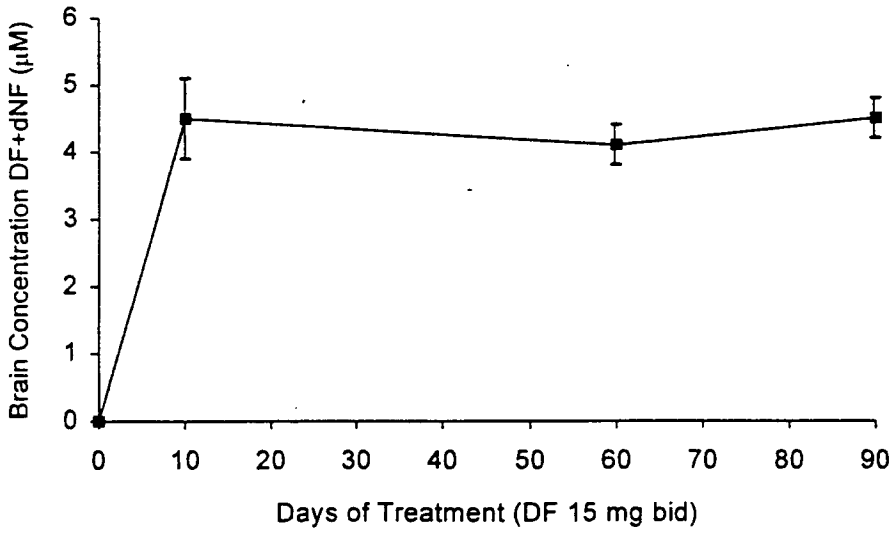
Table 3. Species differences in the brain dexfenfluramine (DF) + d-norfenfluramine (dNF) concentration produced by 10 mg/kg/day, sc x 4 days

Species	Dose	Brain DF + dNF Concentration (μM)
Mouse	10 mg/kg/day, sc x 4 days	16
Rat	10 mg/kg/day, sc x 4 days	61
Squirrel Monkey	10 mg/kg/day, sc x 4 days	147
Cynomolgous Monkey	10 mg/kg/day, sc x 4 days	57
Rhesus Monkey	10 mg/kg/day, sc x 4 days	101
Baboon	10 mg/kg/day, sc x 4 days	193
Human	15 mg bid/day, po x 90 days	4.2

Human brain concentrations are low

The human brain concentrations of dexfenfluramine and d-norfenfluramine were estimated recently by ^{19}F Magnetic Resonance Spectroscopy in 11 obese patients taking 15 mg of dexfenfluramine BID for 90 days (Figure 2). This technique permits the measurement of the combined brain concentration of dexfenfluramine and d-norfenfluramine. The results of this study indicate that steady-state levels are reached in brain (and plasma) by Day 10 of treatment. No accumulation is observed at 60 or 90 days of treatment compared to the values at Day 10. The mean steady-state brain concentration of dexfenfluramine plus d-norfenfluramine was 4.2 μM (99% confidence interval 3.4 μM to 4.9 μM). A parallel study in monkeys comparing ^{19}F Magnetic Resonance Spectroscopy and post-mortem gas chromatographic measurements of dexfenfluramine plus d-norfenfluramine suggest that the average human value may be as low as 2.2 μM with a brain/plasma ratio similar to previous values found in postmortem samples. In addition, this clinical study demonstrated that the brain/plasma ratios are inversely correlated to plasma concentration, suggesting that patients on the 15 mg BID regimen with higher plasma concentrations of dexfenfluramine and d-norfenfluramine do not achieve proportionally higher brain levels. The results of this study also suggest that increasing the dose of dexfenfluramine is unlikely to produce a linear increase in brain concentration.

Figure 2. Brain concentration of dexfenfluramine (DF) + d-norfenfluramine (dNF) in obese patients



Reductions in brain serotonin content are reversible

Similar to the effects of other serotonin reuptake inhibitor drugs (e.g., fluoxetine, sertraline, paroxetine), but in contrast to known neurotoxins (e.g., p-chloroamphetamine (PCA), 5,7-dihyroxytryptamine (5,7-DHT)), the effects of dexfenfluramine on brain indole concentrations are reversible over time. Table 4 shows interim data from an ongoing experiment in rats demonstrating the typical return of cortical 5-HT levels following various doses of dexfenfluramine. As discussed above, the short-term administration of dexfenfluramine produces an acute, pharmacologic, dose-related reduction in cortical 5-HT levels at one week post-dosing. Recovery of cortical 5-HT content is also dose- and time-related; the greater the initial suppression, the longer it takes to return to control levels. In this experiment, recovery of 5-HT content was achieved by 13 weeks in all but the highest dose group (16 mg/kg) which recovered by 26 weeks. Reversible reductions in brain indole content have also been demonstrated following high dose regimens of fenfluramine (e.g., 6.25-12.5 mg/kg/day, sc x 4 days; Schuster et al., Psychopharm Bull 22:148-151, 1986). The persistent reduction in brain serotonin content for 168 days following a single 7.5 mg/kg dose of dexfenfluramine cited by Dr. Contrera in his report was, in fact, significant at day 112, but no different from control at day 168 (Garattini et al., Appetite 7: 15-38, 1986). Moreover, the effects of fenfluramine on brain serotonin concentrations have been found to be reversible even after the administration of 10 mg/kg/day for 175 days (Duhault and Boulanger, Eur J Pharmacol 43: 203-205, 1977). The primary "evidence" cited for prolonged reductions in brain indole content comes from a study in squirrel monkeys by Ricaurte and colleagues (McCann et al., J Pharmacol Exp Ther 269: 792-798, 1994). However, it should be noted

that this "evidence" comes from immunohistochemistry in only one squirrel monkey (17 months post-dosing) and brain serotonin content in two squirrel monkeys (14 months post-dosing) administered a high dose of dexfenfluramine (10 mg/kg/day) for 4 days (producing brain levels at least 35 times that found in humans
Thus, the finding that the acute, dose-related reductions in cortical 5-HT levels recover to control levels following high doses of dexfenfluramine in animals is consistent with a reversible pharmacologic, rather than a permanent neurotoxic effect of the drug.

Table 4: Duration of effect of 21 day (bid) oral dexfenfluramine treatment on cortical serotonin levels in rats

Daily Dose (mg/kg, po)	Time After Discontinuation of Dosing		
	1 Week	13 Weeks	26 Weeks
2.0	99 ± 11	105 ± 37	102 ± 42
4.0	83 ± 7*	78 ± 18	112 ± 31
8.0	57 ± 19*	87 ± 15	92 ± 34
16	35 ± 7*	49 ± 11*	82 ± 19

*$p<0.05$ vs. pair-fed control group; values shown as percent of pair-fed control

Lack of neurochemical effects with long-term (chronic) administration
Because dexfenfluramine will be administered chronically for the treatment of obesity, it is important to evaluate the neurochemical consequences of chronic treatment in animals. A long-term study has been conducted to address this issue. In $B_6C_3F_1$ mice treated with dexfenfluramine at a dose of 27 mg/kg/day for at least 106-108 weeks, no significant reduction in brain 5-HT, 5-HIAA or paroxetine binding sites were observed at sacrifice or 3 months after the last dose of dexfenfluramine (Table 5; NDA 20-344, vol 26, pp 246-255). At sacrifice, the brain levels of dexfenfluramine + d-norfenfluramine in mice receiving 27 mg/kg/day for over two years was 51 μM (a no effect concentration that is at least 12-times the human brain level). Acute reductions in brain 5-HT content of greater than 75% have been observed in mice at brain dexfenfluramine + d-norfenfluramine concentrations of approximately 51 μM (Fig. 1). Thus, any acute changes that might have existed in these parameters were not present following two years of drug administration at a dose producing at least 12-times the human brain level of dexfenfluramine + d-norfenfluramine. Again, these data demonstrate the reversible pharmacologic, rather than permanent neurotoxic nature of the effects of dexfenfluramine.

The data from the 2 year study in mice is also consistent with the lack of long-lasting reductions in brain serotonin content with escalating dose regimens of dexfenfluramine (e.g., Rose et al., Soc Neurosci Abstr 19: 1893, 1993; Caccia et al., Neuropharmacology 31: 875-879, 1992). As opposed to the typical pulsed high dose regimen (e.g., 5.0 mg/kg, ip x 4 days), the stepwise administration of escalating doses of dexfenfluramine (e.g., 0.5, 1, 1.5, 2.0, 3.0, 4.0 and 5.0 mg/kg, ip x 4 days at each dose) produced reductions in 5-HT levels in rats only at 1 day post-dosing and only in the cortex; no changes in brain 5-HT levels were observed at subsequent sacrifice intervals up to 30 days after the last dose of dexfenfluramine (Rose et al., 1992). Differences between the two dose regimens could not be accounted for by the small differences in brain levels of the drug (ibid). These data with escalating doses are consistent with an acute, pharmacologic desensitization to the 5-HT content reducing effects of high dose pulsed dose regimens. These studies also highlight the reason why Drs. Molliver, Seiden and Contrera use the high dose pulsed dose regimens of dexfenfluramine; they can't produce long-lasting reductions in brain 5-HT content without them.

Table 5. Lack of long-term effects of dexfenfluramine (27 mg/kg/day for over 2 years) on brain neurochemistry in mice

Parameter Measured (% control)	106-108 Weeks Dosing		118-120 Weeks Dosing
	Time After Last Dose		
	At Sacrifice	3 Months	At Sacrifice
5-HT	109 ± 6 (n=10)	100 ± 8 (n=9)	107 ± 11 (n=15)
5-HIAA	91 ± 3 (n=10)	109 ± 13 (n=9)	88 ± 7 (n=15)
Paroxetine Binding	95 ± 8 (n=5)	130 ± 5* (n=5)	Not tested

*$p \leq 0.05$ vs. control; values shown as percent of controls

Lack of effect on indices of neurotoxicity

Because the pharmacologic actions of high doses of dexfenfluramine predictably result in an acute reduction in brain 5-HT content, techniques that use a measure of 5-HT as their basis (e.g., 5-HT neurochemical assays of tissue homogenates or immunohistochemical techniques that depend upon 5-HT content) are not suitable indices of neurotoxicity. Any drug, including serotonin reuptake inhibitors like fluoxetine, that acutely reduce serotonin content would be classified as "neurotoxic" by these criteria. In addition, "limitations of

immunocytochemistry include the qualitative nature of data analysis and a steep sensitivity curve for antigen detection" (Axt et al., In: *Amphetamine and Its Analogs*, AK Cho and DS Segal (Eds), pp 315-367, 1994). Moreover, immunohistochemical techniques have limited sensitivity, particularly in forebrain areas where serotonin is distributed widely and extensively through fine axons, and are likely to underestimate 5-HT content. In fact, immunohistochemical techniques require the pretreatment of animals with a monoamine oxidase inhibitor or tryptophan prior to sacrifice (to increase synaptic serotonin levels) to sufficiently increase serotonin antigen levels for visualization (e.g., Appel et al., J Pharmacol Exp Ther 249: 928-943, 1989). Moreover, the abnormal axonal morphology sometimes reported using immunohistochemical techniques may be directly related to the increase in synaptic 5-HT produced by monoamine oxidase inhibitors, independent of the effects of test drug (George et al., Soc Neurosci Abst 19: 1170, 1993). Finally, and most importantly, reductions in serotonin content do not equal reductions in nerve terminal function or viability. Reductions in serotonin content reflect reductions in stored 5-HT. Like the dopaminergic neurotransmitter system, basal 5-HT release and the response to pharmacologic challenge are unaltered until 5-HT content is reduced by more than 80% (e.g., Kirby et al., Synapse 20: 99-105, 1995). Thus, even large reductions in 5-HT content (storage) do not impair normal neuronal function. These important limitations to the procedures which rely on serotonin content and the constraints which should be placed on the interpretation of results have not been presented to the Advisory Committee by the FDA (Dr. Contrera) or its invited speakers.

There are several techniques that are independent of neurotransmitter content that provide sensitive and specific measures of neurotoxicity. Silver stains react with degenerating axons and cell bodies to produce a dense, black reaction product. Early silver stains, such as Fink-Heimer, caused false positive results in control tissues. Using the Fink-Heimer stain, Harvey and McMaster (e.g., Psychopharmacol Commun 1: 217-228, 1975) reported apparent degenerative changes in the brain following fenfluramine administration. These findings could not be replicated and were considered to be artifactual by an FDA advisory panel in 1978. However, the recent development of more selective staining procedures (e.g., cupric silver stains) have made these techniques very useful in providing a positive image of degenerating neurons and their processes (Balaban et al., Neuroscience 26: 337-361, 1988). Using these newer staining procedures, known neurotoxins (e.g., parachloroamphetamine (PCA), 5,7-dihydroxytryptamine (5,7-DHT) and methylenedioxymethamphetamine (MDMA)), but not dexfenfluramine, produce an increase in silver staining (indicative of neuronal damage) (see Table 6).

Brain injury produces reactive gliosis and the activation of astrocytes. These cellular responses are characterized by the accumulation of glial filaments, such as glial fibrillary acidic protein (GFAP). Using a radioimmunoassay for GFAP, known neurotoxins (e.g., 5,7-DHT and MDMA), but not dexfenfluramine, produce an increase in brain GFAP

content (indicative of gliosis in response to neuronal damage) at doses of each that reduce brain 5-HT content by at least 50% (see O'Callaghan and Miller, NIDA Res Monograph 136: 188-212, 1993 and Table 6). Because astrocytes respond to injury by hypertrophy rather than hyperplasia (O'Callaghan, Ann NY Acad Sci 679: 195-210, 1993), less sensitive immunohistochemical measures of GFAP used by other investigators (e.g., Rowland et al., Brain Res 624: 35-43, 1993) have failed show an increase in GFAP immunoreactivity following 5,7-DHT administration, although there is a significant increase in GFAP concentration within these astrocytes. Dr. Contrera has failed to recognize the importance of these methodological differences and has therefore questioned the value of this procedure.
considered. the measurement of GFAP

Retrograde transport techniques have been used to demonstrate functionally important structural damage to neurons. Known neurotoxins (e.g., PCA and 5,7-DHT) reduce the retrograde axonal transport of specific markers from the nerve terminal region to their cell bodies (Table 6). In contrast, the retrograde transport of cholera toxin-horseradish peroxidase by serotonergic neurons from nerve terminals in the cerebral cortex to the cell bodies in the dorsal raphe nucleus was unaffected in rats treated with 8.0 or 16 mg/kg (po) of dexfenfluramine for 4 days or 8.0 mg/kg (po) of dexfenfluramine for 21 days (Kalia and O'Malley, NDA 20-344, Vol. 40, pp 199-215, 1993). No significant differences in cell body counts in the dorsal raphe nucleus between control and dexfenfluramine-treated animals were observed 18 hours or 21 days after drug treatment. The neurotoxin PCA (6 mg/kg/day, po x 2 days) produced significant reductions in retrograde transport using this same procedure. The effects of a single, high dose of dexfenfluramine (26.8 mg/kg, ip) on retrograde transport in rats was examined in another study (Lategan and Lehmann, NDA 20-344, Amendment 9). This dose produces a brain levels of dexfenfluramine plus d-norfenfluramine of at least 115 μM, which is at least 27-times human therapeutic brain levels. In this study, dexfenfluramine produced an 18% decrease in the transport of fast blue dye from the dorsal hippocampus to the median and dorsal raphe nuclei. However, the authors of this study concluded that "because of serious drawbacks in the experimental procedure and other relating factors, no conclusion can be drawn from the data." Dr. Contrera reports only the decrease in retrograde transport without even considering the authors conclusions; he further uses this inconclusive study to impune the integrity of the well-controlled study by Kalia and O'Malley. The results of this well-controlled study clearly demonstrate that dexfenfluramine has no effect on retrograde transport at doses that produce significant reductions in 5-HT content. Furthermore, this study demonstrates that the nerve terminals in this region of the brain are present and functional in the face of reductions of 5-HT content.

Thus, using sensitive and specific techniques that are not confounded by a dependence on 5-HT content, known neurotoxins (e.g., PCA, 5,7-DHT and MDMA), but not dexfenfluramine, produce an increase in silver staining (indicative of neuronal damage), an increase in glial fibrillary acidic protein (GFAP) content (indicative of gliosis in response to neuronal damage), and a decrease in retrograde axonal transport of specific markers from the cortex to 5-HT cell bodies (indicative of functionally important structural damage to the neuron) at doses of each that reduce 5-HT content by at least 50% (Table 6). Therefore, by these techniques, dexfenfluramine clearly differs from known neurotoxins. Morover, Table 6 clearly shows that a reduction in 5-HT content is not necessarily associated with neurotoxicity.

Table 6: Comparison of dexfenfluramine (DF) with PCA, 5,7-DHT and MDMA in animal models of neurotoxicity

Test	Compound			
	PCA	5,7-DHT	MDMA	DF
Increased Silver Staining	+[a,i]	+[e]	+[f,j]	-[j]
Increased GFAP	+[b]	+[g,h]	+[h,j]	-[h,j]
⇓ Retrograde Transport (5-HT Neurons)	+[c,d]	+[i]	NT	-[d,i]

[a]Harvey et al., 1975
[b]Axt et al., 1994
[c]Fritschy et al., 1988
[d]Kalia and O' Malley, 1993
[e]O'Callaghan (personal communication)
[f]Jensen et al., 1993
[g]Frankfurt et al, 1991
[h]O'Callaghan and Miller, 1993
[i]Commins et al., 1987
[j]O'Callaghan and Miller, 1994
NT = not tested

Large Margin of Exposure

As with any potential toxic effect, it is important to establish whether or not that effect is likely to occur at clinically relevant doses; margin of exposure calculations can help make that determination. Comparisons of brain concentrations of dexfenfluramine plus d-norfenfluramine in animals receiving doses that have no effect on specific markers of neurotoxicity (i.e., increased silver staining, increased GFAP content, or decreased retrograde axonal transport in 5-HT neurons) with those in obese patients receiving therapeutic doses (15 mg bid) reveal that the **margin of exposure is at least 16- to 25-fold** (Table 7). Thus, a large margin of exposure exists between human therapeutic doses and doses in animals that have no effect on specific markers of neurotoxicity. This

margin of exposure is similar to clinical margin of safety of 15 estimated by Dr. Contrera in his report of September 1, 1995, to the Committee.

Table 7. Margin of exposure for dexfenfluramine based on no effect levels in specific tests of neurotoxicity

Test	Dose (mg/kg)	Species	Outcome	Brain DF+dNF (μM)*	Exposure Margin
Altered Silver Staining	100	mouse	No Effect[a]	>100	>25
Gliosis (Increased GFAP)	12	rat	No Effect[b]	67	>16
⇓ Retrograde Transport (5-HT neurons)	16	rat	No Effect[c]	107	>25

*Estimated concentrations in separate pharmacokinetic experiments in the same species.
[a]O'Callaghan and Miller, J Pharmacol Exp Ther 270: 741-751, 1994
[b]Rowland et al., Soc Neurosci Abstr 18: 1605, 1992
[c]Kalia and O'Malley, NDA 20-344, Vol. 40, pp 199-215, 1993

Summary of neurochemical effects

In summary, several key points have been made in support of the safety of dexfenfluramine: 1) reductions in brain serotonin produced by high doses of dexfenfluramine in animals are pharmacologic in nature and common to other serotonin reuptake inhibitors (e.g., fluoxetine); 2) differences between species in the reduction in 5-HT content are pharmacokinetic in origin and directly related to the brain concentrations of dexfenfluramine and d-norfenfluramine achieved. Following therapeutic doses (15 mg bid), human brain concentrations of dexfenfluramine and d-norfenfluramine rapidly reach steady-state, are low (approximately 2.2-4.2 μM) and do not accumulate; 3) acute reductions in brain serotonin content are reversible; 4) long-term administration of high doses of dexfenfluramine produces no long-term reductions in brain serotonin content or paroxetine binding, suggesting that there is something unique to the acute, high dose pulsed dose regimen; 5) techniques that use a measure of 5-HT as their basis (e.g., 5-HT neurochemical assays of tissue homogenates or immunohistochemical techniques that depend upon 5-HT content) are not suitable indices of neurotoxicity of a serotonergic drug; 6) acute reductions in brain serotonin produced by high doses of dexfenfluramine in animals are not accompanied by specific markers of neurotoxicity (i.e., altered silver staining, gliosis, impaired retrograde axonal transport in 5-HT neurons), as is observed with known neurotoxins (e.g., 5,7-DHT); and finally, 7) comparisons of brain concentrations of dexfenfluramine plus d-norfenfluramine in animals receiving doses that

have no effect on specific markers of neurotoxicity with those in obese patients receiving therapeutic doses (15 mg bid) reveal that the **margin of exposure is at least 16- to 25-fold**. Taken together with the animal behavioral/functional data, clinical trial data assessing neuropsychometric parameters (summarized in the following sections) and extensive post-marketing experience over the last 10 years in an estimated 10 million patients (40 million patients when fenfluramine is considered), it can be concluded that there is no risk of neurotoxicity when dexfenfluramine is used for the treatment of obesity.

LACK OF ADVERSE BEHAVIORAL/FUNCTIONAL EFFECTS IN ANIMALS

Introduction

The acute administration of high doses of either dexfenfluramine or fenfluramine to animals has been reported to produce prolonged neurochemical changes (e.g., reduction in brain 5-HT and 5-HIAA content and reduced neuronal 5-HT immunoreactivity) that some investigators have interpreted as an indication of neurotoxicity. However, these neurochemical effects are reversible and are directly related to brain levels of dexfenfluramine/fenfluramine and their active metabolite(s) (d- and l-norfenfluramine). Moreover, these drugs, even at very high doses, do not produce any other histopathological signs (e.g., altered silver staining, reactive gliosis or decreased retrograde axonal transport) classically associated with neurotoxicity. Thus, an important question to answer is whether or not the reported neurochemical changes correlate with any adverse functional or behavioral effects in animals or in man.

Based on a review of the available literature, it can be concluded that the administration of either dexfenfluramine or fenfluramine does not produce any significant or persistent (permanent) adverse functional or behavioral effects in animals. Furthermore, these drugs do not produce neurotoxic effects according to functional/behavioral criteria (see Abou-Donia, In: *Principles and methods for evaluating neurotoxicity,* M.B. Abou-Donia (Ed), pp 509-525, 1992 and Tilson and Harry, ibid, pp 527-571). Presented below is an overview of the functional/behavioral effects of dexfenfluramine and fenfluramine in animals.

Locomotor/Exploratory Activity

Unlike the amphetamines (e.g., methamphetamine), the acute administration of fenfluramine has been reported by a number of investigators to decrease activity in rats and mice. For example, Lindquist and Gotestam (Psychopharmacology 55: 129-133, 1977) reported that the intravenous administration of 1.0 to 16 mg/kg of fenfluramine decreased ambulation, rearing and grooming of rats in an open field setting. Consistent with its mild sedative effect, fenfluramine has been found by EEG analysis to increase total and slow wave sleep time in cats (e.g., Funderburk et al., J Pharm Pharmacol 23: 509-513, 1971). However, rabbits showed little effect of fenfluramine on the EEG (ibid) and the spontaneous activity and sleep of rhesus monkeys have been shown to be unaffected by fenfluramine treatment at doses of 1.0 to 3.0 mg/kg (po) (Tang and Kirsch, Psychopharmacologia 21:139-146, 1971).

Critical to the issue of potential neurotoxicity is whether or not persistent behavioral changes can be observed once dosing has been discontinued. In this regard, doses of fenfluramine (5.0-20 mg/kg, sc, bid x 4 days) that have been reported to be "neurotoxic" in rats (i.e., doses that reduce brain 5-HT and 5-HIAA content and neuronal 5-HT immunoreactivity) have been demonstrated to have no effect on exploratory activity (12

min exposure to a novel open field/holeboard), motor coordination and stamina (14 min swim test) when measured either 2 or 8 weeks post-treatment (Lorens et al., NIDA Res Monogr 95:347, 1989). Known 5-HT neurotoxins, like 5,7-dihydroxytryptamine, reduce exploratory behavior and impair swimming ability in rats when examined under the same conditions (ibid).

Learning and Memory (Cognition)

A number of animal models have been developed to detect subtle changes in cognitive function; some of these procedures involve either active or passive avoidance of a noxious stimulus or spatial memory in a variety of mazes. Because cognitive changes are often subtle, caution must be exercised in interpreting the disruption of learning and memory processes by high dose drug treatment (administered immediately before testing) that also affects motor function. Such high dose impairment in cognitive testing has been reported with fenfluramine. For example, Srimal et al. (Arch Int Pharmacodyn 188: 320-331, 1970) found that acute high doses of fenfluramine (ED_{50} = 9.7 mg/kg) interfere with the acquisition of a one-way conditioned avoidance response (pole climbing) in rats. Similar results were obtained by McElroy et al. (Psychopharmacology 77: 356-359, 1982) using a two-way shuttlebox avoidance procedure in rats and by Southgate et al. (J Pharmac Pharmacol 23: 600-605, 1071) using a step-through passive avoidance procedure in mice at doses greater than 5.0 and 16.0 mg/kg (ip), respectively. High doses of fluoxetine (>2.5 mg/kg) in rats produced similar disruptions in two-way shuttlebox avoidance performance (McElroy et al., 1982) and one-trial appetitive learning (Lalonde and Vikis-Freibergs, Pharmacol Biochem Behav 22: 377-382, 1985). In contrast, Carli and Saminin (Psychopharmacology 106: 228-234, 1992) reported that while a low dose of dexfenfluramine (1.25 mg/kg, ip) increased the percentage of omitted responses and the latency to respond, it did <u>not</u> affect the accuracy of rats in a five-choice serial reaction time task. Consistent with its pharmacological effects, Rech et al. (Pharmacol Biochem Behav 20: 489-493, 1984) found that dexfenfluramine reduced the number of food pellets consumed without affecting the number of errors committed in a sequential X-maze task. Moreover, Cox and Maickel (J Pharmacol Exp Ther 181: 1-9, 1972) demonstrated that the doses of fenfluramine required to decrease continuous avoidance responding (ED_{50} = 4.0 mg/kg, ip) were significantly higher than those required to decrease food intake (ED_{50} = 2.0 mg/kg, ip); the opposite relationship was found for methamphetamine.

Important to the issue of neurotoxicity is whether or not behavioral deficits are observed with prolonged treatment. Lorens et al. (ISN conference, Nice, France, August 1993) have recently shown that subchronic treatment (30-38 days) of young (5-7 months of age) and old (19-21 months of age) male rats with dexfenfluramine
water) failed to affect the acquisition of a one-way (place) conditioned avoidance response or the acquisition of a two-way visually-discriminated conditioned avoidance response. In addition, the administration of dexfenfluramine (0.6 mg/kg in drinking

water) to old (19-21 months of age) rats for 8 months failed to affect spatial memory in a Morris water maze (Lorens, personal communication). These data suggest that long-term treatment with low doses of dexfenfluramine will not affect learning processes in either young or old patients.

As with locomotor/exploratory activity, the presence of persistent changes in learning and memory once dosing has been discontinued is critical to the issue of potential neurotoxicity. In this regard, very high doses of fenfluramine (sc, bid x 4 days) fail to affect either one-way or two-way conditioned avoidance responding in rats 2 and 8 weeks after the discontinuation of drug treatment (Lorens et al., ISN conference, Nice, France, August 1993). Similarly, spatial memory formation and its extinction in rats in an 8-arm radial maze are not affected 2 and 8 weeks after the termination of this dosing regimen of fenfluramine (Lorens et al., NIDA Res Monogr 95:347, 1989). Thus, no persistent effects on learning and memory are observed after exposure to very high doses of fenfluramine.

Aggression/Social Behavior

The behavioral effects of relatively high doses of fenfluramine have been examined in a variety of nonhuman primate species. For example, Bedford et al. (Pharmacol Biochem Behav 20: 317-321, 1984) have studied the effects of 1.0, 5.0 and 10 mg/kg (im) of fenfluramine on the social behavior of stumptailed monkeys (*Macaca arctoides*). The lowest dose increased vocalization, while the highest dose suppressed vocalization, social- and self-grooming behavior. These changes in social behavior were not observed on the days immediately preceding and following the fenfluramine injections. No change in aggression or "presenting" (a behavior ascribed to submissiveness) was observed in these animals. In male vervet monkeys (*Cercopithecus aethiops sabeus*), treatment with of fenfluramine for 70 days produced an increase in locomotor activity, hyperaggressivity and decreased social dominance in a competitive social environment (Brammer et al., Pharmacol Biochem Behav 40: 267-271, 1991). Importantly, these behavioral changes were rapidly reversed on discontinuation of drug treatment (Raleigh et al., Brain Res 559: 181-190, 1991). No behavioral abnormalities were observed in rhesus monkeys (*Macaca mullata*) receiving 10 mg/kg bid (sc) of fenfluramine for 14 days (Schuster et al., Psychopharmacol Bull 22: 148-151, 1986) or in squirrel monkeys (*Saimiri sciureus*) receiving bid (sc) dexfenfluramine for 4 days (Ricaurte et al., Lancet 338: 1487-1488, 1991).

Another test of aggressive behavior is to measure the defensive behavior of rodents in response to the introduction of an intruder animal into the home cage. The treatment of rats with very high doses of fenfluramine (sc, bid x 4 days) failed to alter defensive behavior 2 and 10 weeks after drug treatment (Lorens et al., ISN conference, Nice, France, August 1993). The known neurotoxin 5,7-dihydroxytryptamine produces an increase in aggressive behavior under the same conditions (ibid). Shock-induced

aggression in rats and centrally-induced hissing in cats have been reported to be decreased by 5.0 mg/kg of fenfluramine (Panksepp et al., Biol Psych 6: 181-186, 1973). An increase in tactile sensitivity/aggression was reported in long-term toxicology studies in rats and dogs at doses of dexfenfluramine greater than 10 mg/kg/day (NDA #20-344, vols 17 & 24). These changes in sensitivity to handling often occurred in conjunction with a decline in general condition. It is not hard to understand an increase in tactile sensitivity if the animals do not feel well. Similar effects are observed in rats and dogs with high daily doses of fluoxetine (Fluoxetine Investigators' Brochure) and with parachlorophenylalanine (a serotonin synthesis inhibitor) in rats (Lorens, personal communication). Depletion of serotonin in humans by parachlorophenylalanine has not been reported to induce hyperaggressivity (Cremata and Koe, Clin Pharmacol Ther 7: 768-776, 1966).

Dr. Contrera has suggested that Dr. Seiden's recent report (Richards et al., J Pharmacol Exp Ther 267: 1256-1263, 1993) of a disruptive effect of fenfluramine on differential reinforcement of low rate 72 second (DRL-72) schedule performance in rats may indicate a decrease in impulse control. It should be noted that fenfluramine had no effect on either response rate or in the number of reinforcers received during the initial dose-response determination, only subtle changes in the response distribution were observed. Furthermore, the reported decrease in reinforcement rate with 4.0 mg/kg of fenfluramine was only observed on redetermination of the dose-response curve. Thus, the reported effect of fenfluramine could not even be replicated within the same study. Therefore, there are no relevant animal data to suggest that fenfluramine has any effect on impulse control.

Taken together, the data above suggest that fenfluramine and dexfenfluramine, as well as other serotonin reuptake inhibitors, are unlikely to affect social behavior or to induce aggressive behavior in humans. The social effects of even high doses of fenfluramine appear to depend on the strain of monkey tested and to disappear rapidly on discontinuation of drug treatment. In addition, no aggressive behavior is observed in rats following discontinuation of very high doses of fenfluramine; thus, concerns over potential delayed aggressive behavior are unwarranted.

Endocrine Function

Serotonin plays a role in regulating the normal secretion of several hypophysial releasing factors and trophic hormones. It is well known that the acute administration of both direct and indirect serotonin agonists, including fenfluramine/dexfenfluramine, cause the release of these releasing factors and hormones (e.g., Van de Kar, Ann Rev Pharmacol Toxicol 31: 289-420, 1991). The repeated daily administration of dexfenfluramine leads to the rapid development of tolerance to these endocrinological effects (e.g., Serri and Rasio, Horm Res 31: 180-183, 1989). An extensive clinical literature supports these animal findings.

The effects of subchronic (30-38 days) and chronic (6 months-2 years) dexfenfluramine administration on endocrine function and histopathology have been evaluated in animals. The subchronic treatment of young (5-7 months of age) and old (19-21 months of age) rats of both sexes with dexfenfluramine (0.2-0.6 mg/kg/day, po, in drinking water) did not adversely affect basal prolactin, ACTH or corticosterone levels (Handa et al., Pharmacol Biochem Behav 46: 101-109, 1993; Lorens et al., ISN conference, Nice, France, August 1993; Handa et al., Pharmacol Biochem Behav, in press, 1995). In fact, subchronic dexfenfluramine treatment normalized the exaggerated ACTH/corticosterone response to novelty stress in old male animals and attenuated the enhanced prolactin response to stress in old female rats (Handa et al., Pharmacol Biochem Behav, in press, 1995). Similar findings were obtained by Storlien and Smythe (Brain Res 597: 60-65, 1992) in rats receiving 5.0 mg/kg/day (po) of dexfenfluramine for 8-10 days. Chronic toxicology (rats, dogs) and carcinogenicity (mice, rats) studies of dexfenfluramine ranging from 6 months to 2 years in duration and in doses from (po) failed to identify any histopathological evidence of an endocrine disorder (NDA #20-344). Extrapolation of these animal data to humans suggests that patients receiving therapeutic doses of dexfenfluramine should not experience any significant endocrinological side effects. Indeed, no adverse endocrinological syndrome has been reported in humans.

It has been suggested that damage to serotonin pathways within the hypothalamic-pituitary-adrenal axis can be detected through disturbances in the homeostatic mechanisms of these hormones (Heninger et al., Arch Gen Psychiatry 41:398-402, 1984). Damage to serotonergic pathways might be reflected in a blunted (severe damage) or an exaggerated (partial damage) hormonal response to challenge with a serotonin agonist. For example, Battaglia et al. (Third IUPHAR satellite meeting on serotonin, Chicago, 1994, Abstr 105, pp. 109) have interpreted the attenuation of hormone (ACTH, corticosterone, prolactin) release on challenge with dexfenfluramine following treatment of rats with very high doses of fenfluramine (12 mg/kg, bid, sc x 4 days) as evidence of neurotoxicity. However, even Drs. McCann and Ricaurte (NIDA Res Monogr 136: 53-62, 1993) have stated that "like CSF measures [of 5-HIAA], neuroendocrine measures are indirect and nonspecific. Furthermore, because of the compensatory mechanisms, results of the neuroendocrine challenge may not be consistent over time. Therefore, although potentially lending support for the diagnosis of neurotoxicity, neuroendocrine responses are not definitive." Thus, not only are the results of neuroendocrine challenge studies "indirect, nonspecific and not definitive," but their interpretation as evidence of "neurotoxicity" ignores the simple, parsimonious explanation of tolerance or tachyphylaxis (an interpretation that would be applied to most other classes of drugs).

Immune Function

Serotonin (5-HT) appears to play a key role in the interactions between the central nervous system (CNS) and the immune system. Experimental observations indicate that

5-HT receptors are located on a variety of immunocompetent cells and that 5-HT participates in controlling functional activity within the immune system, in particular its natural killer (NK) arm (Clancy et al., In: Serotonin, ed by P.M. Vanhoutte et al., Kluwer Academic Publishers, Netherlands, pp. 353-357, 1993). Reciprocally, changes in immune responsivity are able to alter CNS 5-HT activity (ibid).

The administration of serotonergic agents (e.g., dexfenfluramine) have been shown to modulate various arms of the rodent immune system in a dose-, age- and sex-dependent fashion. For example, only young (5-6 months of age) male and old (21-23 months of age) female Fischer 344 rats demonstrated an elevation in *ex vivo* assessed basal- and IL-2-stimulated splenic NK cell activity, as well as concanavalin-A-induced T cell proliferation, after subchronic (30-44 days) administration of low doses (0.6-1.8 mg/kg/day, po) of dexfenfluramine (Clancy et al., Int J Immunopharmacol 13: 1203-1212, 1991). The administration of higher subchronic doses of dexfenfluramine (3.0-9.0 mg/kg/day, po) produced a dose-dependent decrease in *ex vivo* basal NK cell activity in young male rats which returned to control levels after overnight incubation with IL-2; only the 6.0 mg/kg/day dose caused T cells to be more responsive to concanavalin-A-induced proliferation (Clancy et al., Behav Brain Res., in press, 1995). These results suggest that small or moderate increases in 5-HT release and availability stimulate immune function, whereas high 5-HT levels are associated with no change or inhibition of immune function. These observations are in agreement with previous murine and human studies (Hellstrand and Hermodsson, Cell Immunol 127: 1999-209, 1990; Kut et al., Immunopharmacol & Immunotoxicol 14: 783-796, 1992). Additional evidence of the functional significance of dexfenfluramine administration on the immune system comes from a study in which C57bl/6 mice were inoculated intradermally with *Candida albicans* (Mathews et al., Behav Brain Res, in press, 1995). The results of this study demonstrated that dexfenfluramine (1.0 mg/kg/day, ip x 9 days) augmented the lymphocyte response to this pathogen by increasing the number of T lymphocytes ($CD3^+$, $CD8^+$ and $NK1.1^+$ cells) draining the site of infection and by increasing the biological activity of the lymphocytes at that site.

The chronic exposure (8 months) of aging male rats (beginning at 15 months of age) to 0.6 mg/kg/day (po) of dexfenfluramine caused their NK and concanavalin-A responsiveness to return to 7 month old control levels (Clancy et al., Behav Brain Res., in press, 1995). Importantly, the old dexfenfluramine-treated rats showed significantly less splenic hypertrophy or lesions compared to old control rats. Thus, long-term exposure of aging male rats to dexfenfluramine appears to maintain the NK and T cell arms of the immune system at "youthful" levels and prophylactically reduce the splenic pathology associated with advancing age. In summary, data from subchronic and chronic administration studies in animals suggest that therapeutic doses of dexfenfluramine are likely to have only positive effects on immune function in man.

Summary of behavioral/functional effects in animals

Based on this review of the effects of dexfenfluramine and fenfluramine on animal behavior (locomotor activity, learning and memory, social behavior/aggression), endocrine and immune function, it can be concluded that these drugs do not produce any significant or persistent adverse functional or behavioral effects. As an expected consequence of their acute pharmacology, high doses of dexfenfluramine/fenfluramine produce sedative effects in animals that have been reported to reduce locomotor activity, social behavior and acquisition of learning paradigms. These effects are not observed once high dose drug treatment is discontinued, and are thus not consistent with any interpretation of neurotoxicity. Similarly, as an expected consequence of their acute pharmacology, both direct and indirect serotonin agonists, including fenfluramine/dexfenfluramine, cause the release of several releasing factors and hormones. The repeated daily administration of dexfenfluramine leads to the rapid development of tolerance to these endocrinological effects and is certainly not an indicator of neurotoxicity.

Critical to the issue of neurotoxicity is whether or not behavioral or functional deficits are observed with prolonged treatment. In this regard, the subchronic (30-38 days) and chronic (>6 months) administration of dexfenfluramine/fenfluramine fail to produce any significant or persistent adverse functional or behavioral effects. Sensitive memory processes in aged rats, for example, are unaffected by subchronic and chronic dexfenfluramine administration. Furthermore, the subchronic and chronic administration of dexfenfluramine , po) has been found to actually restore the normal hormonal response to stress and to maintain the NK and T cell arms of the immune system of aged rats at youthful levels.

In summary, the reported neurochemical changes appear to have no significant or persistent adverse functional or behavioral correlates in animals. Thus, if the animals themselves do not experience the predicted adverse effects, then it is highly unlikely that humans will experience these effects, particularly at the low, clinically-effective doses of dexfenfluramine. Moreover, the animal data are consistent with the lack of significant clinical neuropsychological findings with dexfenfluramine in controlled trials (see next section) and in extensive post-marketing experience over the last 10 years in an estimated 10 million patients (40 million patients when fenfluramine is considered).

CALCIFICATION IN RODENTS

Introduction

Calcification was observed in several organs, including the brain, of rats and mice receiving dexfenfluramine in feed for at least two years. The question has been raised as to whether these findings in rodents, brain calcification in particular, are of clinical relevance. The results of the long-term rodent studies are summarized below. In addition, relevant discussion of calcification and its lack of clinical significance are provided.

Calcifications occur spontaneously in rodents; a high spontaneous incidence of calcifications has been documented for the $B_6C_3F_1$ strain of mice (e.g., Majeed, Arzheim Forsch Drug Res 41(II): 1189-1191, 1991; Yanai et al., Jpn J Vet Sci 46: 761-765, 1984). Spontaneous calcifications in rodents are caused by progressive nephropathy; the resulting disturbance in calcium homeostasis leads to secondary hyperparathyroidism and multiple systemic calcifications. Spontaneous calcifications occur in rodents with varying intensity and can be observed in animals as young as 3 months of age (Goldstein et al., FASEB J 2: 2241-2251, 1988). The prevalence of calcification is correlated with age. Male animals are affected more frequently than females. Brain (thalamic) calcifications in mice are extracellular and are often closely associated with blood vessels (not neural tissue) (e.g., Morgan et al., Acta Neuropathol 58: 120-124, 1985).

Mineralization similar to that observed in rodents has also been documented in monkeys and humans. For example, of cynomolgous monkeys (*Macaca fascicularis*) were found by Yanai and colleagues (Vet Pathol 31: 546-552, 1994) to exhibit some degree of calcification. As in rodents, extracellular deposits were found to be associated with the walls of small or medium arteries or arterioles. Importantly, no abnormalities in growth, weight gain or neurologic signs were associated with this mineralization. Similarly, a mild degree of mineralization without any clinical signs is common in elderly humans in and around the vessel wall of the globus pallidus (e.g., Adams and Graham, In: *An Introduction to Neuropathology*, pp 219-296, 1988; Hurst, J Pathol Bacteriol 29: 65-85, 1926; Oppenheimer, In: *Greenfield's Neuropathology, 4th ed*, pp 699-647, 1984). In fact, cerebral mineralization has been found in 80% of the brains of humans over the age of 70 (ibid). Thus, calcification in man, as in rodents, appears to be a normal consequence of aging.

Two-Year Mouse Study

Male and female $B_6C_3F_1$ mice were administered dexfenfluramine (0, 3, 9, 27 mg/kg, po) in feed for at least 107 weeks. Food and water consumption, body weight and general physical condition were monitored at regular intervals thoughout the study. Histologic examination of organs from all major body systems were evaluated at sacrifice.

Additional groups of animals were sacrificed at weeks 107 and 121 for specialized analysis of brain serotonergic (5-HT) neurochemical function (i.e., plasma and brain 5-HT and 5-HIAA levels and paroxetine binding).

The results of this long-term study in mice relative to calcification are summarized in Table 8 below. From the table it is apparent that mineralization occurred in several organs, including prostate and brain. Dexfenfluramine produced a significant increase in the number of male animals displaying brain calcification relative to the male control groups (C0 and D0). However, it should be noted that there was a significant difference between the two male control groups (n=50 each) in the number of animals with brain calcifications; 16 animals in one control group displayed brain calcification and 27 showed this effect in the other. This finding highlights the variability in the spontaneous brain calcification reported in this strain of mouse (e.g., Majeed, 1991). Brain calcification in male mice was not dose-dependent. No significant increases in brain calcification were observed in female mice with any dose of dexfenfluramine.

Table 8. Mineralizations observed in $B_6C_3F_1$ mice at the end of a 2 year study

Dose (mg/kg/day, po)	Sex	n	Number of Animals with Mineralizations		
			All Locations	Prostate	Brain
0 (C0)	M	50	41	30	16
0 (D0)	M	50	39	21	27*
3	M	50	44	12^+	39*
9	M	50	43	5^+	41*
27	M	50	42	23	38*
0 (C0)	F	50	24	-	24
0 (D0)	F	50	26	-	25
3	F	50	29	-	29
9	F	50	31	-	30
27	F	50	24	-	24

$^+$ partial histological examination
* $p<0.01$ vs. 0 (C0)

Histological examination of the brain revealed that the focal, extracellular deposits were

not different in appearance in control or drug-treated animals. This examination also revealed that the mineralization was not associated with neural tissue. Furthermore, the neighboring neuronal parenchyma was normal, without cell necrosis, gliosis or inflammatory infiltrate. Thus, the observed calcification does not appear to be of neural origin or to adversely affect adjacent neurons.

Further evidence of the functional integrity of the brain in the animals in this study was collected from separate groups of animals receiving 27 mg/kg/day of dexfenfluramine and sacrificed at week 107 (after 106 weeks of treatment) and at week 121 (3 months after the cessation of dosing). Relative to control animals, high dose dexfenfluramine treatment for over 2 years did not produce any alterations in brain 5-HT or 5-HIAA (its metabolite), nor did it alter paroxetine binding (an independent measure of the integrity of the serotonin neuronal plexus). This lack of effect after prolonged daily administration is in contrast to the reductions in these measures seen after acute dexfenfluramine administration. It should be noted that this lack of long-term effects occurred at brain concentrations (51 μM) of dexfenfluramine plus d-norfenfluramine at least 12-times that observed in obese patients receiving the recommended therapeutic dose (15 mg bid).

Two-Year Rat Study

Male and female Fischer 344 rats were administered dexfenfluramine (0, 2, 6, 18 (then 12) mg/kg, po) in feed for at least 118 weeks. Food and water consumption, body weight and general physical condition were monitored at regular intervals throughout the study. Histologic examination of organs from all major body systems were evaluated at sacrifice. After 55 weeks (females) and 58 weeks (males), the high dose of 18 mg/kg/day was reduced to 12 mg/kg/day due to convulsions, significant reductions in body weight, food consumption and general condition (piloerection, spontaneous limb movements) relative to control groups, particularly in female rats. This reduction in dose enabled these animals to survive until sacrifice at week 118. Behavioral testing (grip strength, air-righting reflex and open field activity) was performed in some groups after at 54-58 weeks of dosing.

Table 9. Mineralizations observed in Fischer 344 rats at the end of a 2 year study

Dose (mg/kg/day, po)	Sex	n	Number of Animals with Mineralizations				
			All Locations	Cornea	Lung	Tubuli seminiferi	Brain
0 (C0)	M	50	34	25	7	6	1
0 (D0)	M	50	34	24	6	17	1
2	M	50	37	11+	11	23	6*
6	M	50	42	16+	19*	26	3
18 (12)	M	50	47*	35*	13*	35*	5*
0 (C0)	F	50	24	17	7	-	1
0 (D0)	F	50	24	18	9	-	0
2	F	50	18	10+	6	-	3
6	F	50	24	8+	14	-	7*
18 (12)	F	50	35*	27*	14	-	9*

+ partial histological examination
* $p<0.01$ vs. 0 (C0)

The results of the long-term study in rats relative to calcification are summarized in Table 9 above. As in mice, mineralization occurred in a number of organs, including cornea, lung, tubuli seminiferi and brain. Similar organ mineralization was observed in a long-term carcinogenicity study in Fischer 344 rats treated with fluoxetine (Fluoxetine Investigators' Brochure). Dexfenfluramine produced a significant increase in the number of animals displaying brain calcification relative to the control groups. As in the mouse, the observed focal, extracellular calcification did not appear to be of neural origin or to adversely affect adjacent neurons. Mineralization was not strictly dose-dependent in all organs. In general, the incidence of brain mineralization in Fischer 344 rats was less than that observed in $B_6C_3F_1$ mice. Furthermore, because the control incidence of brain calcifications was so low in this study (1 animal in 50), even small increases in the number of animals with brain calcifications were statistically significant.

No significant alteration in behavior or physical appearance was observed in animals receiving 2.0 mg/kg/day of dexfenfluramine. From week 32 (females) and 39 (males), 18 mg/kg/day produced a gradual decline in general condition (i.e., reduced body weight,

arched back, piloerection, encrusted eyes, etc.); impairment was delayed and less noticeable with 6 mg/kg/day. Several behavioral tests (grip strength, air-righting reflex and open field activity) were performed in some groups at 54-58 weeks. In these tests, dexfenfluramine produced a trend towards an increase in muscle tone (grip strength) and exploratory activity. The effects of dexfenfluramine on behavior may, in part, reflect changes in general condition produced by the higher doses.

Discussion of calcification

Dr. Martin R. Berger has put forth the following hypothesis to explain the apparent increase in ectopic calcifications in male $B_6C_3F_1$ mice and male and female Fischer 344 rats treated with dexfenfluramine. Dr. Berger, a specialist in pharmacology and toxicology contracted by the German Federal Health Agency to review the long-term studies, has suggested that the administration of dexfenfluramine may have accelerated the normal, progressive nephropathy in rodents through the initial reduction in water intake. The resultant early disturbance in calcium homeostasis may have caused a secondary hyperparathyroidism (further aggravated by reduced food intake). Acceleration of these normal effects in rodents may have led to the more frequent observation of spontaneous calcifications in the typical target organs (rat - cornea, testes, lung, brain; mouse - prostate, brain).

The localization of the focal, extracellular calcium deposits in brain is an important consideration in the consideration of their clinical relevance. Histological examination of the brain revealed that these inert deposits were <u>not</u> associated with neural tissue. Furthermore, the neighboring neuronal parenchyma was normal, without cell necrosis, gliosis or inflammatory infiltrate. Thus, the observed calcification does <u>not</u> appear to be of neural origin or to adversely affect adjacent neurons. Moreover, neurochemical indices of serotonergic function directly related to the pharmacologic actions of dexfenfluramine were unaffected by long-term, high-dose drug treatment. Therefore, the clinical relevance of the observed calcification is questionable.

Summary of calcification in long-term rodent studies

Care must be taken in the extrapolation of any findings in animals to human clinical use. This is particularly true in the case of calcification; there are a number of factors which must be seriously considered. These factors include: 1) small, <u>extracellular</u> calcifications were observed in brain, as well as <u>other organs</u> (e.g., cornea , prostate), of <u>control</u> and dexfenfluramine-treated animals; 2) the apparent increase in the number of calcifications in dexfenfluramine-treated animals was <u>not</u> dose-dependent; 3) the spontaneous incidence of these extracellular calcifications varies widely; spontaneous calcifications in $B_6C_3F_1$ mice have been reported to range from 4) the observed calcification was <u>not</u> of neural origin and did <u>not</u> appear to adversely affect adjacent neurons (i.e., the neighboring neuronal parenchyma was normal, without cell necrosis, gliosis or inflammatory infiltrate); 5) neurochemical indices of serotonergic function directly related to the

pharmacologic actions of dexfenfluramine were unaffected by this long-term, high-dose drug treatment in mice (at brain levels at least 12-times the human therapeutic levels); 6) mineralization similar to that observed in rodents is common and well-documented in monkeys and humans (without functional consequences); and, 7) neither disturbed calcium homeostasis (e.g., tetany) nor secondary hyperparathyroidism has been reported in man following the administration of dexfenfluramine. In summary, the observed increase in brain calcium deposits in rodents appears to have no effects on neuronal structure or function, certainly none that can be extrapolated to humans. Attempts to relate the present findings to human brain function or pathology are unwarranted and scientifically invalid.

Dexfenfluramine HCl
NDA 20-344

CLINICAL EXPERT REVIEW LETTERS

SUPPLEMENT TO SPONSOR'S PACKAGE

November 6, 1995

Please insert in yellow binder behind tab "Neuro Issue - Human"

Interneuron Pharmaceuticals Incorporated

One Ledgemont Center
99 Hayden Avenue, Suite 340
Lexington, MA 02173
Tel: (617) 861-8444
Fax: (617) 861-3830

November 6, 1995

Kathleen Reedy
Executive Secretariat
Endocrinologic and Metabolic Drugs Advisory Committee
Food and Drug Administration, HFD-009
1901 Chapman Avenue, Room 200
Rockville, MD 20857

Reference: Dexfenfluramine HCl capsules, NDA 20-344

Dear Kathleen:

Enclosed are additional "expert reviews" which we have obtained regarding the clinical neurocognitive/behavioral effects of dexfenfluramine. For the committee's convenience, I have included all the reviews received to-date, including those which were contained in the yellow background packet. I have also included brief biographical sketches of each expert.

Having support of these experts, several of whom are past or current members of the neuropharm advisory committee is heartening. We are now, more than ever, confident of our position that dexfenfluramine has no detrimental effects in these spheres.

Regards,

APPEARS THIS WAY
ON ORIGINAL

Sonja Barton Loar, Pharm.D.
Executive Director, Regulatory & Scientific Affairs

Enclosure(s)

SBL:lsw

Dr. Emil Coccaro

Dr. Coccaro received his M.D. degree from the New York University School of Medicine. He completed his residency training in Psychiatry at the Mt. Sinai Medical Center in New York City. Dr. Coccaro joined the faculty there and began his research work into the biology and treatment of human aggression.

He received the AE Bennett Neuropsychiatric Research Foundation Award from the Society of Biological Psychiatry for this work in 1989. Since then, he has been an Associate Professor of Psychiatry and the Director of the Clinical Neuroscience Research Unit in the Dept of Psychiatry at the Medical College of Pennsylvania and Hahnemann University.

Dr. Coccaro continues his research under NIMH grants in the area of human aggression. He currently sits on the FDA Advisory Committee on Psychopharmacologic Agents. He is on the Editorial Boards of: *Aggression and Violent Behavior*, and *International Clinical Psychopharmacology.* He is a reviewer for *Psychiatry Research* and *Biological Psychiatry.*

Dr. Coccaro has published over 70 scientific articles and book chapters on the biology and treatment of both mood and personality disorders. In addition he has co-edited a book entitled Serotonin in Major Psychiatric Disorders, published by American Psychiatric Press in 1990.

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Department of Psychiatry
Division of Clinical Neuroscience Research
Emil F. Coccaro, M.D.
Director

Eastern Pennsylvania Psychiatric Institute

3200 Henry Avenue
Philadelphia, PA 19129
Telephone (215) 842-4208

November 7, 1995

Richard Gammons, Ph.D.
Vice President, Clinical Research
Interneuron Pharmaceuticals, Inc.
99 Hayden Avenue, Suite 340
Lexington, MA 02173

Dear Dr. Gammons;

I have completed my review of your report entitled: "Evaluation of the Clinical Data That Pertain to the Human Risk for Adverse Neurologic, Psychiatric, Behavioral, and Cognitive Effects of Dexfenfluramine".

While the issue of the putative neurotoxicity of dexfenfluramine has been controversial, it is clear that methodological differences among studies are critical to understanding the interaction of dexfenfluramine with the central 5-HT system. First, evidence of neurotoxicity is not seen with dexfenfluramine when 5-HT-independent measures of neuronal damage are employed. Second, only pulsed high-dosing, but not chronic dosing, of dexfenfluramine are associated with changes in immunocytochemical studies; data which is actually consistent with a pharmacological effect on 5-HT shared by other 5-HT agents such as SSRI's which have already been approved by the FDA. Third, brain concentrations of dexfenfluramine at the recommended doses for treatment of obesity in humans are low and no different than brain concentrations which are clearly no associated with any evidence of neurotoxicity. It is noteworthy, that brain concentrations of dexfenfluramine in the non-human primates in which issues of "neurotoxicity" have been raised, are much higher than those which exist in humans under standard therapeutic conditions.

Most importantly, there is no evidence that treatment with, or withdrawal from, dexfenfluramine is associated with any significant effect on behavioral or cognitive functions in humans. Such effects would be expected if treatment with dexfenfluramine were to lead to neurotoxic damage to central 5-HT neurons. Our own research shows, in fact, that repeated single-dosing of dexfenfluramine (0.5 mg/kg once/week for four weeks), in healthy human volunteers, yields similarly robust prolactin (PRL) to each acute dexfenfluramine challenge indicating a physiologic stability in 5-HT function inconsistent with neurotoxic damage to 5-HT neurons.

In conclusion, I believe that dexfenfluramine, in the recommended doses, is safe for use in human subjects. There is no evidence of long-term neurotoxicity or impairment in behavioral or cognitive parameters in human subjects. Finally, given the world-wide exposure of dexfenfluramine, I believe that its safety profile is perhaps better established than most other psychoactive agents that are approved by the FDA for use in human subjects.

Very truly yours,

Emil F. Coccaro, M.D.
Professor and Director,
Clinical Neuroscience Research Unit
Department of Psychiatry
Medical College of Pennsylvania and Hahnemann University

Dr. Malcolm Lader

Dr. Lader is Professor of Clinical Psychopharmacology, Institute of Psychiatry, University of London; Member of the External Scientific Staff, Medical Research Council; and an Honorary Consultant in Psychiatry to the Bethlem Royal and Maudsley Hospital. His qualifications include: Doctor of Medicine (Psychiatry); Doctor of Philosophy (Pharmacology); Doctor of Science (Pharmacological Research); Fellow of the Royal College of Psychiatrists and Diploma in Psychological Medicine.

He was a member of the Committee on the Review of Medicines from 1978-1989. He is currently a member of the Advisory Council on the Misuse of Drugs; Trustee of the Mental Health Foundation; and a member of other national and regional advisory committees.

He was an adviser to the World Health Organization, and was vice-president of the International College of Psychopharmacology. He was also President of the Society for the Study of Addiction and President of the British Association for Psychopharmacology.

He is on the advisory boards of over 15 international scientific journals.

He has been engaged in medical research for over 30 years, with primary research interest in the drugs used in psychiatry, in particular, their side effects. His research has resulted in the publication of 12 books and about 550 scientific articles.

He conducts and supervises clinics at the Bethlem Royal and Maudsley Hospital (a Post-graduate Teaching Hospital) dealing with anxiety, sleep and depressive disorders and drug treatment problems.

UNIVERSITY OF LONDON
THE BETHLEM ROYAL HOSPITAL
AND
THE MAUDSLEY HOSPITAL

Clinical Psychopharmacology (MRC)
Institute Of Psychiatry

De Crespigny Park
Denmark Hill
London SE5 8AF
Tel: 0171-703 5411 Ext.3372
Fax: 0171-252 5437

Dr R E Gammans
Interneuron Pharmaceuticals Inc
99 Hayden Avenue, Suite 340
Lexington
MA 02173
USA

25 October 1995

Dear Dr Gammans

You have asked me to give my opinion on the document entitled *Evaluation of Clinical Data that pertain to the Human Risk for Adverse Neurologic, Psychiatric, Behavioral and Cognitive Effects of Dexfenfluramine*. I have examined the document in detail and note that it includes more than 10 years extensive post-marketing reporting of experiences with the drug. This, in my opinion, is a more than adequate database upon which to base an assessment of the risk of rare adverse events.

The possible effects of dexfenfluramine are addressed under several headings and in each case there are both controlled data and post-marketing data upon which to base an evaluation. I can see no evidence for any adverse effects on brain function as monitored by neurologic, psychiatric, behavioral and cognitive examinations.

In my opinion, dexfenfluramine is safe and well tolerated and the risk of any delayed adverse effects must be regarded as extremely remote.

Yours sincerely,

Malcolm Lader, D.Sc.,Ph.D., M.D.,F.R.C.Psych.
Professor of Clinical Psychopharmacology
Institute of Psychiatry
University of London
London SE5 8AF

Dr. J. John Mann

Dr. Mann is a Professor of Psychiatry, College of Physicians and Surgeons at Columbia University, New York, New York. Prior to this, he was a professor at Cornell University and the University of Pittsburgh. He obtained his MBBS in medicine at Melbourne University, Australia. He did his internal medicine training at Royal Australasian College of Physicians, in Australia. He received his DPM in Psychiatry and his MD in Neurobiology from Melbourne University. He has worked in the field of Psychiatry for twenty years.

Dr. Mann has published extensively in the area of suicide and depression. He has published papers regarding the ermergence of major depression during pharmacotherapy. He has studied and reported on the brain's serotoninergic response in major depression and suicidality. His research includes autoradiographic findings of serotonin and beta receptor binding in the brains of suicide victims.

College of Physicians & Surgeons of Columbia University | *New York, N.Y. 10032*

DEPARTMENT OF PSYCHIATRY

722 West 168th Street

Telephone: (212) 960-5571
FAX: (212) 795-8659

October 31, 1995

Dr. Richard Gammans
Vice President
Clinical Research
Interneuron
99 Hayden Avenue, Suite 3401
Lexington, MA 02173

Dear Richard,

I reviewed the extensive materials that you provided regarding D-fenfluramine. It does appear that the case for neurotoxicity does not appear to have been established on the basis of the literature that was published. It is of particular note that long-term treatment studies do not appear to demonstrate a persistent reduction in serotonin or 5-HIAA levels in animals. Similar studies in humans would be useful to confirm this observation. It is believed that it is increased serotonergic activity that suppresses appetite. D-fenfluramine has an anorectic effect that lasts, in controlled clinical trials, for at least several months. If there were a substantial degree of damage to the serotonergic system, one would expect a loss of the anorectic effect of this drug.

With regard to the safety margin in humans, pharmacokinetic differences exist between species, resulting in lower brain levels in human primates compared to non-human primates and rodents. Supersensitivity to the serotonin-depleting effects of fenfluramine has not been ruled out entirely in non-human primate studies because most of those studies generated high brain levels of fenfluramine. One would have to repeat those experiments using much lower drug levels and confirm that there is less serotonin depletion when lower brain levels are present, in order to rule out a sensitivity to the serotonin depleting effects of the drug. Nevertheless, it would be reasonable to assume that the substantial difference in brain levels of fenfluramine in human primates, compared to non-human primates and rodents, is a major factor in the relative safety of the drug in humans.

Morphological studies should distinguish between actual toxic effects (that would result in permanent or long-term damage), and pharmacological effects, such as transient depletion

of serotonin. As you correctly point out, immunocytochemical studies depending on the concentration of serotonin do not provide a reliable guide as to neuronal damage. I suggest a more specific discussion of the papers by Molivar would strengthen your findings. For example, they have reported swelling and varicosities in serotonin fibers that may be an effect independent of serotonin depletion.

Neuropsychological tests that have been carried out with fenfluramine do not have a great deal of sensitivity to the kinds of abnormalities that one might predict. For example, the Mini Mental Status Examination is a rather insensitive test. Relatively few sophisticated memory tests and tests of disinhibition or impulsivity have been carried out. So that although there is a vast clinical experience suggesting the drug does not produce detectable abnormalities, the neurocognitive testing that has been employed has been of rather poor quality, at this stage, a point that has been made by Dr. Seiden and others at the last FDA hearing.

With regard to suicide risk, I would agree that there is no evidence that there is increased rate of suicide or suicide attempts in the large number of individuals who have taken fenfluramine. Given that there probably is a significant under-reporting of such events, if one were to assume very conservatively that only one percent of the events involving suicide or suicide attempts were reported, fenfluramine use would still be associated with a rate of suicide and suicide attempt below that predicted for the general population. This very strongly suggests that this drug does not result in increased suicide risk.

The data available for the assessment of the safety of fenfluramine with regard to neurotoxicity are considerable and the evidence available indicates that this drug is safe. Studies, using more sophisticated neuropsychological testing and functional brain imaging techniques, such as PET, can further establish the safety of the drug.

Sincerely,

J. John Mann, M.D.
Professor of Psychiatry

APPEARS THIS WAY
ON ORIGINAL

jjm/id

Dr. M. -Marsel Mesulam

Dr. Mesulam is the Ruth and Ellen Dunbar Professor of Neurology and Psychiatry at Northwestern University Medical School in Chicago, Illinois. He is Professor and Director of the Center for Behavioral and Cognitive Neurology and the Alzheimer Program there. He received his BA at Harvard College in Psychology and Social Relations. He received his MD at Harvard Medical School. He did his residency and Fellowship in Neurology at the Harvard Medical School and Beth Israel Hospital in Boston.

Dr. Mesulam is renowned for his expertise in the area of neuropsychological testing. He has done research in the areas of cognition, cholinergic innervation, and Alzheimer's disease. He has done research in the toxin-induced lesions of various brain areas and their effects. Among his many publications, include a chapter entitled "Four Neuropsychological Profiles in Dementia" for the Handbook of Neuropsychology

Northwestern University Medical School

Center for Behavioral and
Cognitive Neurology and
The Alzheimer Program
Departments of Neurology and
Psychiatry and Behavioral Sciences

320 East Superior Street
Room 11-450
Chicago, Illinois 60611-3010
Fax (312) 908-8789

M.-Marsel Mesulam, MD
Professor and Director
(312) 908-9339

NEUROLOGY
Darren Gitelman, MD
Assistant Professor
(312) 908-8614

NEUROPSYCHOLOGY
Sandra Weintraub, PhD
Associate Professor
Head of Neuropsychology
(312) 908-9013

COGNITIVE
NEUROSCIENCE
Anna Nobre, PhD
Visiting Scholar
(312) 908-9339

BASIC NEUROSCIENCE
John Smiley, PhD
Assistant Professor
(312) 908-8571

FELLOWS
Zoran Grujic, MD
Neurology
(312) 908-9681

Nancy Johnson, PhD
Neuropsychology
(312) 908-9432

October 27, 1995

Bobby W. Sandage, Jr., Ph.D.
Senior Vice President
Research and Development
Interneuron Pharmaceuticals, Inc.
One Ledgemont Center
99 Hayden Avenue, Suite 340
Lexington, Massachusetts 02173

Dear Dr. Sandage:

Thank you for sending me the material on dexfenfluramine. The studies which you cite show that dexfenfluramine, in the doses that are recommended for clinical usage, does not give rise to suicidality, depression, sleep disturbances, or other psychiatric manifestations noted in Table 3. None of the studies that you cite indicate any adverse effects of dexfenfluramine on indices of attention, concentration and information processing speed that were tested. Still other studies in your report indicate that discontinuation of dexfenfluramine (after a dosage of up to 30 mg bid for three months) does not induce a rebound craving for carbohydrates.

In sum, I am impressed by the number of patients who have taken this substance without obvious adverse effects on the parameters that you list.

With best wishes.

APPEARS THIS WAY
ON ORIGINAL

M.-Marsel Mesulam, M.D.
Ruth and Evelyn Dunbar Professor of
Neurology and Psychiatry

MMM:ekm
cc: Richard J. Wurtman, M.D.

Dr. Ira Shoulson

Dr. Shoulson is currently the Louis C. Lasagna Professor of Experimental Therapeutics and Professor of Neurology, Pharmacology and Medicine at the University of Rochester School of Medicine. He is also a Neurologist at Strong Memorial Hospital, Rochester, New York. Dr. Shoulson has a B.A. in Psychology, with honors, from the University of Pennsylvania; he received his M.D. degree from the University of Rochester School of Medicine. Following this, he was a clinical associate at the NIMH and then NINDS branches of the NIH.

He is active in neurological research, with special interests in movement disorders, attention deficit disorder, and Alzheimer's disease. He is currently serving his second term as a member of the FDA Peripheral and Central Nervous System Advisory Committee.

He is on the Editorial Boards of: *Clinical Neuropharmacology, Archives of Neurology, Experimental Neurology, Acta Neurologica Scandinavia* and *CNS Drugs*. He is a Councilor for the American Neurological Association; Chair, of the Performance and Safety Monitoing Board of the NINDS. He is a Fellow of: the American Adademy of Neurology, American College of Physicians, American Society for Neural Transplantation, and a member of the New York Adademy of Sciences, as well as other societies.

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EXPERIMENTAL THERAPEUTICS

Ira Shoulson, M.D.
Professor of Neurology, Pharmacology, and Medicine
Louis C. Lasagna Professor of Experimental Therapeutics

November 1, 1995

CONFIDENTIAL

Richard E. Gammans, PhD
Vice President, Clinical Research
Interneuron Pharmaceuticals Incorporated
One Ledgemont Center
99 Hayden Avenue, Suite 340
Lexington, MA 02173

Re:**"Neurotoxic" Potential of Dexfenfluramine**

Dear Dr. Gammans:

I write to summarize my review of the dexfenfluramine material, particularly your report *Evaluation of Clinical Data That Pertains to the Human Risk for Adverse Neurologic, Psychiatric, Behavioral and Cognitive Effects of Dexfenfluramine*. I also read most of the primary references pertaining to the potential "neurotoxic" effects of dexfenfluramine.

In my view, dexfenfluramine hydrochloride 30 mg per day is safe in terms of its potential to induce adverse neuropsychological outcomes or irreversible "neurotoxic" sequelae in adult humans. My conclusion is based on the cited placebo-controlled studies and the 10 years of post-marketing surveillance. The safety margin of dexfenfluramine is particularly reassuring for individuals who are normal neuropsychologically. While a variety of symptoms referable to the nervous system may occur rarely in dexfenfluramine-treated persons, these symptoms are reversible and should not be misconstrued as evidence of long-term or permanent "neurotoxicity."

I also note that your phase IV

Data from these studies should further address any lingering concerns regarding the "neurotoxic" potential of dexfenfluramine. I am confident that such studies can proceed in a post-marketing setting.

I trust this information will be of assistance to you and your reviewers.

Sincerely yours,

Ira Shoulson, MD

IS:rn

601 Elmwood Avenue, Box 673
Rochester, New York 14642
Telephone: (716) 275-5130 Fax: (716) 473-9745

Dr. Paul A. Spiers

Dr. Spiers is a Clinical Psychologist/Neuropsychologist. He has a bachelor of arts degree (summa cum laude) from McGill University in Psychology, with honors in the area of abnormal psychology. He has a masters degree from Clark University in Clinical Psychology and a Ph.D. from the same institution in Clinical Psychology/Neuro-psychology. His formal training included training under internationally recognized experts including: Edith Kaplan, Harold Goodglass, and Norman Geschwind. He did a fellowship at the University of Paris, France, studying acalculia with Dr. Henry Hecaen. He completed his internship at the Neurobehavior Service of the V.A. Medical Center in Boston and practiced at the Harvard Medical School Behavioral Neurology Unit. Dr. Spiers has been involved with several clinical/research consultation and teaching activities at such institutions as the National Institute of Mental Health, Bethesda, M.D.; Minister of Health and Welfare, Government of Canada, Ottowa Canada; Department of Mental Health and Retardation, Commonwealth of Massachusetts. He is now a visiting professor at MIT currently studying changes in memory and cognitive function as a result of normal aging and the efficacy of new drugs which may enhance memory performance in older adults.

Dr. Spiers has published in peer-reviewed journals and authored chapters on topics ranging from drug abuse and behavioral changes associated with epilepsy, to methodology in neuropsychological testing and acalculia.

Dr. Spiers has been admitted as a qualified expert in courts, including the U.S. Supreme Court, the Federal District Court in Massachusetts and Connecticut, and other courts. He has worked as a forensic consultant for various state divisions, including, the Office of the Attorney General, in California and Kentucky; the Federal Public Defender in Pennsylvania; the Department of Justice, Criminal Division, War Crimes Bureau in Washington D.C., etc.