CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-290

PHARMACOLOGY REVIEW(S)

MEMORANDUM

AdreView Injection (Iobenguane I-123 injection)

Date: Sept 19, 2008
To: File for NDA #22-290
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and pharmacology/toxicology supporting review provided by Dr. Biade and concur with the conclusion that AdreView may be approved. No additional pharmacology/toxicology studies are necessary.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Leighton
9/19/2008 09:06:03 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-290**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**03/21/08**
PRODUCT:	**AdreView**
INTENDED CLINICAL POPULATION:	**Patients with suspected primary or metastatic pheochromocytomas and neuroblastomas**
SPONSOR:	**GE Healthcare**
DOCUMENTS REVIEWED:	**N/A (eCTD)**
REVIEW DIVISION:	**Division of Medical Imaging and Hematology Drug Products (HFD-160)**
PHARM/TOX REVIEWER:	**Siham Biade, Ph.D.**
PHARM/TOX SUPERVISOR:	**Adebayo Laniyonu, Ph.D.**
DIVISION DIRECTOR:	**R. Dwaine Rieves, MD**
PROJECT MANAGER:	**James Moore, Pharm D.**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: This NDA is recommended for approval from a Pharmacology/Toxicology perspective

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling:

The following modifications, addition (italics) and deletion (strike out), were made to the proposed label.

b(4)

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5 WARNINGS AND PRECAUTIONS

Benzyl alcohol has been associated with a fatal "Gasping Syndrome" in premature infants and infants of low birth weight. Exposure to excessive amounts of benzyl alcohol has been associated with toxicity (hypotension, metabolic acidosis), particularly in neonates, and an increased incidence of kernicterus, particularly in small preterm infants. There have been rare reports of deaths, primarily in preterm infants, associated with exposure to excessive amounts of benzyl alcohol.

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13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Iobenguane sulfate was *not mutagenic in vitro* in the Ames *bacterial mutation assay, and in the in vitro* mouse lymphoma and was negative in the *in vivo* micronucleus tests *in rats.* b(4)

Long-term animal studies have not been conducted to evaluate *AdreView's* carcinogenic potential or *potential* effects on fertility.

13.2 Animal Toxicology and or Pharmacology

Iobenguane sulfate testing in revealed electrocardiographic (ECG) changes after administration of 202 times the mg/m^2 conversion of the maximum human dose for a 60 kg adult. The no observable effect level (NOEL) was not *determined.* When *was* tested in a cell system stably expressing hERG-1 potassium channels, inhibition of potassium *channels* was not observed at *an* 80 µM *iobenguane concentration and* the IC50 *was* 487 µM. b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor relies on the Agency's previous findings of safety and efficacy for this 505(b)(2) application. The safety of *m*IBG was established in preclinical studies conducted by the innovator. The innovator's drug was approved as an adjunctive diagnostic agent in the localization of primary or metastatic pheochromocytomas and neuroblastomas. Based on the innovator's label, the Iobenguane I131 sulfate recommended dose in adults was 0.5 mCi to a maximum

of 1 mCi in obese patients (1.7 m^2 or 65 kg), and in children was 0.3 mCi/m^2 up to a maximum total dose of 0.5 mCi.

The innovator's drug was radiolabeled with ^{131}I, whereas the sponsor's drug is radiolabeled with ^{123}I. These isotopes are chemically identical and the only difference would result from the different type of radiation. To this end, the sponsor provided human radiation dosimetry data showing that the radiation absorbed dose estimates to different organs following AdreView administration. were comparable for the two isotopes.

The sponsor evaluated the effects of *m*IBG hemisulfate in a hERG assay and in a battery of genotoxicity assays. *m*IBG blocked the K channels in a dose-dependent manner, but at doses that were much higher than plasma levels achievable with the intended clinical dose and the IC_{50} was 487 μM. *m*IBG hemisulphate was negative in the Ames test, the in vitro mouse lymphoma assay, and the rat micronuclei assay.

B. Pharmacologic activity

*m*IBG, meta-iodobenzylguanidine, is structurally and pharmacologically similar to norepinephrine (NE). Similarly, it is taken up by the NE transporter on adrenergic nerve terminals and stored in the presynaptic storage vesicles, accumulating in adrenergically innervated tissues such as the adrenal medulla, salivary glands, heart, liver, spleen, and lungs. *m*IBG is not metabolized in the mitochondria by the monoamine oxidase (MAO). By labeling iobenguane with the isotope I-123, it is possible to obtain scintigraphic images of the desired organs and structures in which it accumulates.

C. Nonclinical safety issues relevant to clinical use

None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

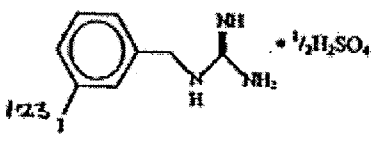
NDA number:	22,290
Review number:	000
Sequence number/date/type of submission:	N000/03-21-08/NDA
Information to sponsor:	Yes ( ) No (X)
Sponsor and/or agent:	GE Healthcare 101 Carnegie center, Princeton, New Jersey
Manufacturer for drug substance:	GE Healthcare, 3350 North Ridge Avenue, Arlington Heights, Illinois (Drug Establishment Number: 1417338)
Reviewer name:	Siham Biade, Ph.D.
Division name:	Medical Imaging & Hematology Products
HFD #:	160
Review completion date:	**9/7/08**

Drug:

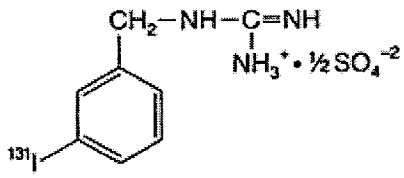
Trade name: AdreView (Iobenguane I 123 Injection)™
Generic name: Iobenguane I 123
Code name: N/A
Chemical names: 1-(3-[^{123}I]iodobenzyl)guanidine sulfate (2:1)
((*m*-Iodo-^{123}I)-benzyl)guanidine sulfate (2:1)
CAS registry number: 139755-80-9
Molecular formula/molecular weight: $C_8H_{10}IN_3$/271.9919
[mIBG is the sulfuric acid amine salt of the base meta-Iodobenzylguanidine (molar ratio mIBG/ sulfate = 2/1, CAS 87862-25-7)]

Structure:

AdreView



Iobenguane sulfate I 131



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Relevant INDs/NDAs/DMFs: INDs # 62,669, ——— NDA # 20-084 (CIS-US)

Drug class: Diagnostic radiopharmaceutical

Intended clinical population: Patients with suspected primary or metastatic pheochromocytomas and neuroblastomas

Clinical formulation:
GE Healthcare (Sponsor) formulation: AdreView is supplied in a 10 mL glass vial, containing a total volume of 5 mL with a total activity at calibration time of 2 mCi [^{123}I]*m*IBG per mL. The composition is provided in the table below b(4)

Ingredient	Amount/	Function	Standard
			Own monograph
[^{123}I]*m*IBG		Active ingredient	Own monograph
$NaH_2PO4 \cdot 2H_2O$			USP/NF
$Na_2HPO4 \cdot 2H_2O$			USP/NF
Benzyl alcohol			USP/NF
			USP/NF
Total weight			

*At calibration

b(4)

CIS-US (Innovator) formulation: Based on the package insert information, the innovator's CIS-US Iobenguane I131 sulfate injection is supplied in a 2 mL glass vial, containing a total volume of 0.5 mL with a total activity at calibration time of 42.6 MBq (1.15 mCi) Iobenguane Sulfate I 131 injection.

Ingredient	Amount/mL
Iobenguane Sulfate	0.69 mg
Iobenguane sulfate I 131	85.1 MBq (2.30 mCi)
Sodium acetate	0.36 mg
Acetic acid	0.27 mg
Sodium chloride	4.2 mg
Methyl paraben	0.56 mg
Propylparaben	0.056 mg
Benzyl alcohol	0.01 mL

Comparison of GEHC and CIS-US formulations

1. The GE formulation contains ^{123}I whereas the CIS-US formulation contains ^{131}I. ^{123}I has a shorter half-life (13.2 hours) and does not produce β-emission compared to ^{131}I (8.04 days). ^{123}I's primary energy consists in γ-emission which is more suited for scintigraphy. The two isotopes are chemically identical, and thus display the same pharmacokinetics and distribution. Therefore, the difference between the two isotopes is the type of radiation. To this end, the sponsor provided human radiation dosimetry data showing that the radiation absorbed dose estimates to different organs following AdreView administration. were comparable for the two isotopes.

2. [redacted] However, the sponsor proposes a radioactive clinical dose of 10 mCi, which is 10 times higher than the approved dose; [redacted]

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[redacted]

b(4)

4. Benzyl alcohol is present at the same concentration (1%) in both formulations (GE and CIS-US); however, GE's drug is supplied in a 10 mL vial containing 5 mL of solution with a potential maximum human dose of 52.1 mg of benzyl alcohol, whereas the CIS-US drug is supplied in a 2 mL vial containing a volume of 0.5 mL, which would result in a maximum of 5.21 mg of benzyl alcohol. The safety implications of this difference is discussed in more detail at the end of this review (Benzyl alcohol risk assessment)

The potential maximum amounts of benzyl alcohol administered to patients were compared for the CIS-US formulation and the GEHC formulation (see table below).

Adult and pediatric weight	**GEHC (5mL) mg/kg Benzyl alcohol (max: 52.1mg)**	**CIS-US (0.5mL) mg/kg Benzyl alcohol (max: 5.21mg)**
60 kg adult	0.87	0.087
9 kg child	5.8	0.58
8 kg child	6.5	0.65
7 kg child	7.4	0.74
6 kg child	8.7	0.87
5 kg child	10.4	1.04
4 kg child	13.0	1.3
3 kg child	17.4	1.74
2 kg child	26.1	2.61
1 kg child	52.1	5.21

Note: The CIS-US label has no restrictions with regard to the amount of benzyl alcohol administered to pediatric patients.

Reviewer's comments:
The pharmacology/toxicology reviewer for NDA #20-084 evaluated the safety of CIS-US iobenguane assuming a minimum pediatric weight of 9 kg and a maximum dose of 69 µg/kg *m*IBG. Based on AdreView's proposed label [see § Pediatrics, in Dosage and Administration], pediatric subjects weighing 3 kg would receive the drug. Therefore, if an entire vial of AdreView were to be injected to a 3 kg child, the maximum injected dose would be 117 µg/kg. This is double the maximum dose of 69 µg/kg used in the safety evaluation of CIS-US Iobenguane I 131 solution. ..

Route of administration: Slow intravenous infusion over at least 1-2 minutes

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

[For (b)(2) applications:

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA # 22,290 are owned by GE Healthcare or are data for which GE Healthcare has obtained a written right of reference. Any information or data necessary for approval of NDA #22,290 that GE Healthcare does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that GE Healthcare does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA # 22,290.

Studies reviewed for IND # 62,669 and submitted for NDA # 22,290

Study 854001 (Vol4, p22): m-Iodobenzylguanidine hemisulphate (mIBG hemisulfate): effect on HERG-1 Tail currents recorded from stably transfected HEK 293 cells.

Study B122002 (Vol4, p181): m-Iodobenzylguanidine hemisulphate (mIBG): reverse mutation in five histidine-requiring strains of Salmonella thyphimurium.

Study B122003 (Vol4, p235): m-Iodobenzylguanidine hemisulphate (mIBG): mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the b(4)

Study B122004 (Vol4, p299): m-Iodobenzylguanidine hemisulphate (mIBG): induction of micronuclei in the bone marrow of treated rats.

Studies not reviewed within this submission:
None

2.6.2 PHARMACOLOGY

Meta-iodobenzylguanidine (*m*IBG, also known as iobenguane) is structurally and pharmacologically similar to guanethidine and norepinephrine (NE). *m*IBG is, therefore, largely subject to the same uptake and accumulation pathways as NE. Like NE, *m*IBG is taken up by the NE transporter in adrenergic nerve terminals and stored in the presynaptic storage vesicles. However, unlike NE, *m*IBG is not metabolized in the mitochondria by the MAO or produce the same pharmacodynamic effects. *m*IBG accumulates in adrenergically innervated tissues such as the adrenal medulla, salivary glands, heart, liver, spleen, and lungs. *m*IBG for clinical imaging purposes has been labeled with either ^{123}I or ^{131}I. [^{123}I]*m*IBG has been extensively used for more than 20 years in research and clinical imaging of tumors of neural crest and neuroendocrine origin, most often for assessment of patients with suspected neuroblastoma and suspected pheochromocytoma. Presence of tumor is indicated by increased uptake of *m*IBG compared with surrounding normal or uninvolved tissue. This NDA is for the use of AdreView, ^{123}I-mIBG, in subjects evaluated for known or suspected neuroblastoma or phaechromocytoma. ^{123}I has a shorter half-life (13.2 hours) compared to ^{131}I (8.04 days), and does not produce β-emission. Its primary energy consists in γ-emission which is more suited for scintigraphy. The two isotopes are chemically identical, and thus display the same pharmacokinetics and distribution.

2.6.2.4 Safety pharmacology

Cardiovascular effects:

Study 854001: m-Iodobenzylguanidine hemisulphate (mIBG hemisulfate): effect on HERG-1 Tail currents recorded from stably transfected HEK 293 cells (Study conducted [redacted] (Compliance with the Swiss ordinance relating to the GLP, based on the 1997 OECD Principles of GLP) b(4)

The purpose of this study was to evaluate the effects of mIBG hemisulfate on HERG-1 tail currents recorded from stably transfected HEK293 cells. This test is recommended for assessing the potential to induce delay ventricular repolarization (QT interval prolongation).

Key findings: mIBG exhibits a potential for QT prolongation at concentrations greater than plasma concentration achievable at the proposed clinical dose. The concentration-dependent reduction fitted curve indicated an IC_{50} of 487 μM, and a Hill coefficient (n_H) of 1.6.

Test article information

Abbreviated name	mIBG hemisulphate
Molecular formula	[*m*IBG]2H2SO4
Batch number	F051P2
Molecular weight	648.26 (salt)
	276.1 (free base)
Purity	100%

(Table prepared by reviewer)

Study design

Experiments were performed using the whole-cell patch-clamp method in HEK293 cells stably expressing the hERG-1 cDNA channel to study inhibition of the inward rectifier potassium current. The vehicle control (DMSO 0.5%), the reference item (100 nM ——— b(4), an antiarrhythmic drug known for its outward rectifier K^+ channels blocking activity) and mIBG hemisulphate at free-base final concentrations of 8, 80, 200, 400, and 800 µM were administered through a perfusion system to the cells. Each concentration level of mIBG and the reference item were tested in 4 different cells. The vehicle was tested in 6 cells. hERG-1 currents elicited by a voltage protocol were measured and all recordings were performed at room temperature. Briefly, the outward currents were measured upon depolarization of the cell membrane to 20 mV for 4 sec from a holding potential of -80 mV and upon subsequent repolarization to -50 mV for 5sec. This voltage protocol was run throughout the recording at intervals of 15 sec. The level of current inhibition was determined as a decrease in the tail current amplitude after the drug application compared to the control level of the same cell in the pretreatment phase (relative tail current). Percent inhibition was calculated from tail current amplitudes normalized by the vehicle results ($p<0.01$, Dunnett's test against the vehicle treated group).

Results

Treatment with 100 nM of reference item ——— produced a 90% inhibition of the tail current with a mean relative tail current of 10%, indicating that the system was sensitive. Treatment of HEK293 cells with nominal concentrations of 8, 80, 200, 400, and 800 µM of mIBG produced relative current amplitudes of 85.2, 78.3, 62.5, 46.9, and 20% respectively. After normalization by the vehicle effects, the proportion of current inhibited was 0, 7, 26, 45, and 77% respectively indicating a concentration dependent effect of mIBG on the hERG-mediated potassium current. The reversibility of this effect was not investigated by the sponsor. The concentration-dependent reduction fitted curve indicated an IC_{50} of 487 µM, and a Hill coefficient (n_H) of 1.6. The results are presented in the table below: b(4)

Table 1. Normalized tail current amplitudes (provided by sponsor)

Treatment	Mean relative tail current amplitudes	Normalized tail currents[1]	Percent inhibition[2]
Vehicle (0.5%DMSO)	85%	-	-

mIBG (8 μM)	85%	101%	-
mIBG (80 μM)	78%	93%	7%
mIBG (200 μM)	63%	74%	26%
mIBG (400 μM)	47%	55%	45%
mIBG (800 μM)	20%	23%	77%

[1]Percent current left at the end of the recording taking into account rundown from exposure to vehicle (0.5% DMSO)
[2]Normalized tail current reduced from 100%. Value showing the proportion of the current blocked by the test item

Reviewer's comments:
HPLC analysis of samples taken from the perfusion bath post recording showed that the actual concentrations of the test article were 73-86%, 100-117%, 94-115%, 104-112%, and 92-97% of the nominal concentrations of 8, 80, 200, 400, and 800 μM, respectively. This reviewer made dose adjustments accordingly, and adjusted results are presented in the following table.

Nominal concentrations (μM)	Actual concentrations (μM)	Human plasma level multiples (50 kg adult)	Inhibition (%)
8	5.8-6.9	14-17 X	0
80	80-93.6	194-226 X	7
200	188-230	455-557 X	26
400	416-448	1007-1085 X	45
800	736-776	1782-1879 X	77
IC_{50}	487	1180 X	50

(Table prepared by reviewer)

This study demonstrated that mIBG (final concentrations were free base) inhibits the hERG potassium channel in a dose-dependent manner. No effect was observed at approximately 15 times the human plasma concentration achievable with an estimated calculated maximum clinical dose of 0.4 mg of mIBG. At approximately 200 times the equivalent clinical concentration, a slight percent inhibition (7%) was observed whereas significant potassium channel inhibition was observed at concentrations higher than 450 X human plasma level. These findings indicate that although mIBG exhibits a potential for QT prolongation, this effect is observed at high plasma concentration that is not achievable at the clinical proposed dose. Because the positive findings were observed at high concentrations and the IC_{50} value is >10 μM, they are not considered to be biologically important.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Because the two products are iodine isotopes, their pharmacokinetics would be similar. The only difference due to the isotope would be the absorbed doses to organs since their radiation spectra are different; therefore, the PK and biodistribution of ^{131}I could be applied to ^{123}I.

Comparison of radiation absorbed doses for Iobenguane I-131 (CIS-US) and Adreview (GE Healthcare)

Table 1. Estimated absorbed doses for Iobenguane I-131 (data from CIS-US label)

Table 4: Estimated Absorbed Radiation Doses*: Iobenguane Sulfate I-131

Organ	Adult		15 Years		10 years		5 years		1 years		Newborn	
	mGy/ 37 MBq	rads/ 1mCi	mGy/ 18.5 MBq	rads/ 0.5 mCi	mGy/ 18.5 MBq	rads/ 0.5 mCi	mGy/ 18.5 MBq	rads/ 0.5 mCi	mGy/ 18.5 MBq	rads/ 0.5 mCi	mGy/ 18.5 MBq	rads/ 0.5mCi
Urinary Bladder Wall	28.0	2.8	18.5	1.9	28.0	2.8	43.5	4.4	85.0	8.5	215.0	21.5
Liver	29.0	2.9	19.0	1.9	29.5	3.0	43.5	4.4	85.0	8.5	190.0	19.0
Spleen	22.0	2.2	15.5	1.6	24.5	2.5	38.5	3.9	70.0	7.0	195.0	19.5
Heart Wall	2.9	0.3	1.9	0.2	2.9	0.3	4.5	0.5	8.5	0.9	19.5	2.0
Adrenals	7.5	0.8	5.5	0.6	8.0	0.8	10.5	1.1	16.5	1.7	16.5	1.7
Gallbladder Wall	5.1	0.5	3.0	0.3	4.4	0.4	7.0	0.7	12.5	1.3	28.0	2.8
Pancreas	3.8	0.4	2.4	0.2	3.8	0.4	6.0	0.6	10.5	1.1	23.5	2.4
Thyroid	3.3	0.3	2.6	0.3	4.0	0.40	8.5	0.9	16.5	1.7	24.0	2.4
Kidneys	3.2	0.3	2.0	0.2	3.1	0.3	4.9	0.5	8.5	0.9	20.0	2.0
Uterus	3.3	0.3	2.1	0.2	3.3	0.3	5.0	0.5	9.5	1.0	22.0	2.2
Ovaries	2.7	0.3	1.8	0.2	2.8	0.3	4.4	0.4	8.5	0.9	19.5	2.0
Testes	2.2	0.2	1.4	0.1	2.3	0.2	3.7	0.4	7.0	.7	17.5	1.8
Brain	1.7	0.2	1.1	0.1	1.9	0.2	3.1	0.3	6.0	0.6	15.0	1.5
Effective Dose Equivalent (rem)		0.7		0.5		0.8		1.2		2.2		5.0

* Based on data gathered in patients — Jacobsson et al, 4th International Radiopharmaceutical Dosimetry Symposium, CONF—851113, pp. 389–398.

Estimate calculated using phantoms of Christy & Eckerman (Report ORNL/TM–8381/V1 & V7).

The effective dose equivalent is a quantity which may be suitable for comparing risks of different procedures in nuclear medicine, radiology, and other applications involving ionizing radiation, but should not be construed to give information about risks to individual patients and should not be applied to situations involving radiation therapy.

Table 2: radiation dose estimates: AdreView

Appears This Way
On Original

ORGAN / TISSUE		ABSORBED DOSE PER UNIT ADMINISTERED ACTIVITY											
		ADULT		15-YEAR OLD		10-YEAR OLD		5-YEAR OLD		1-YEAR OLD		NEONATES	
		µGy/ MBq	rad/mCi	µGy/ MBq	rad/mCi	µGy/ MBq	rad/mCi	µGy/ MBq	rad/mCi	µGy/ MBq	rad/mCi	µGy/ MBq	rad/mCi
Adrenals		16	0.059	21	0.078	31	0.115	42	0.155	67	0.248	111	0.411
Brain		3.9	0.014	4.9	0.018	8.1	0.030	13	0.048	24	0.089	55.9	0.207
Breast		4.7	0.017	5.9	0.022	9.4	0.035	15	0.056	28	0.104	65.3	0.242
Gallbladder		20	0.074	24	0.089	34	0.126	51	0.189	95	0.352	200	0.740
GI Tract	Stomach Wall	7.6	0.028	10	0.037	17	0.063	27	0.100	51	0.189	114	0.422
	Small Intestine Wall	7.7	0.028	9.8	0.036	16	0.059	25	0.093	46	0.170	104	0.385
	Colon Wall	8.1	0.030	10	0.037	16	0.059	26	0.096	46	0.170	104.3	0.386
	Upper Large Intestine Wall	8.4	0.031	11	0.041	18	0.067	30	0.111	53	0.196	119	0.440
	Lower Large Intestine Wall	7.7	0.028	9.6	0.036	15	0.056	21	0.078	38	0.141	84.9	0.314
Heart Wall		18	0.067	23	0.085	35	0.130	53	0.196	94	0.348	182	0.673
Kidneys		13	0.048	16	0.059	24	0.089	35	0.130	59	0.218	132	0.488
Liver		67	0.248	87	0.322	130	0.481	180	0.666	330	1.221	720	2.664
Lungs		16	0.059	23	0.085	32	0.118	48	0.178	89	0.329	215	0.796
Muscles		6	0.022	7.6	0.028	12	0.044	17	0.063	33	0.122	75.1	0.278
Esophagus		6	0.022	7.6	0.028	11	0.041	18	0.067	32	0.118	72.2	0.267
Osteogenic Cells		16	0.059	21	0.078	31	0.115	47	0.174	100	0.370	254	0.940
Ovaries		7.9	0.029	10	0.037	15	0.056	22	0.081	41	0.152	92.3	0.342
Pancreas		12	0.044	15	0.056	25	0.093	39	0.144	68	0.252	143	0.529
Red marrow		5.6	0.021	6.8	0.025	10	0.037	15	0.056	30	0.111	89.5	0.331
Skin		3.7	0.014	4.4	0.016	7.1	0.026	11	0.041	21	0.078	53.1	0.196

2.6.6 TOXICOLOGY

2.6.6.4 Genetic toxicology

Summary of genetic toxicology results:

The sponsor evaluated *m*IBG genotoxic potential in 1) a reverse mutation assay in five histidine-requiring strains of *Salmonella thyphimurium*, 2) a mutation assay at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells, and 3) a micronuclei assay in the bone marrow of treated rats.

- *Salmonella thyphimurium* assay: Sporadic increases of less than 1.6 fold in the mutation frequency were observed in the reverse mutation assay, with values falling within the range of historical controls. Therefore, *m*IBG was considered not mutagenic in this system.

- *Mouse lymphoma assay*: At doses up to or approaching toxic levels, *m*IBG showed evidence of an equivocal increase in mutant frequency at the *tk* locus of L5178Y mouse lymphoma cells in a 3 hour experiment in the absence of S9 (1.3 fold and 1.49 fold for doses of 145 and 160 µg/mL, respectively). In addition, a weak linear trend was observed at the highest dose in the same experiment. However, the magnitude of this increase was such that it was considered to be of no biological relevance (Email consult to OND Associate Director of Pharmacology/Toxicology, who determined the result not significant).

- Micronucleus assay: *m*IBG did not induce an increase in the micronuclei frequency in the polychromatic erythrocytes of the bone marrow of rats treated up to 10 mg/kg/day considered to be the MTD in this study.

Study 2255/19: m-Iodobenzylguanidine hemisulphate (mIBG): reverse mutation in five histidine-requiring strains of Salmonella thyphimurium (Vol4, p181).

The purpose of this study was to assay mIBG hemisulphate for mutation in five histidine requiring strains (TA98, TA100, TA1535, TA1537, and TA102) of *Salmonella typhimurium* both in the presence and in the absence of an exogenous metabolic activation system by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9), in two separate experiments.

Key findings: Under the conditions of this study, mIBG hemisulphate did not cause a significant positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of a rat liver metabolic activation system.

Volume #, and page #:	4, 181
Conducting laboratory and location:	[redacted] b(4)
Date of study initiation:	03/12/2004
GLP compliance:	OECD
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	mIBG hemisulphate, F053P1, 99.4-100% Vehicle control, DMSO

Methods: Plate incorporation and pre-incubation methods.

Strains/species/cell line: *Salmonella typhimurium* tester strains, TA98, TA100, TA1535, TA1537, and TA102.

Doses used in definitive study: The mutagenicity test was performed in two separate experiments (1 and 2), using 1.6, 8, 40, 200, 1000, and 5000 µg per plate (n=3) in presence and in absence of S9mix fraction in Experiment 1. In Experiment 2, due to solubility limitations in the pre-incubation step, the concentration of DMSO in this step was decreased. However, the plates' final concentrations were subsequently adjusted as follows: 20.48, 51.2, 128, 320, 800, 2000, 4000 µg/plate for all strains. A concentration of 5000 was added for TA1535.

Basis of dose selection:
The initial toxicity range finding assay was performed on TA100 strain, using final mIBG concentrations of 1.6, 8, 40, 200, 1000, and 5000 µg per plate, and negative and positive controls in presence and in absence of S9mix fraction (three plates per concentration, and

5 plates for each control). Cytotoxicity, assessed by examining bacterial lawn density and numbers of spontaneous revertants per plate, was observed at 1000 µg/plate and above in the absence of S9 and at 5000 µg/plate in the presence of S9. Due to unacceptable solvent and positive control data as determined by sponsor (values outside of control historical ranges), these treatments were not acceptable for mutation assessment.

Metabolic activation system: S-9 fraction was prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. The S-9 percentage was 10%, which is within the acceptable range.

Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Positive controls are listed in the table below:

Table Positive controls

Tester strain	S9 mix	Positive controls	Conc/plate (µg)
TA98	+	Benzo(a)pyrene	10
TA98	-	2-nitrofluorene	5.0
TA100	+	2-aminoanthracene	5.0
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	5.0
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	5.0
TA1537	-	9-aminoacridine	50.0
TA102	+	2-aminoanthracene	20.0
TA102	-	Glutaraldehyde	25.0

Incubation and sampling times: The tester strains were cultured for 10 hrs at 37°C then exposed to the test article, via the plate incorporation methodology, for 3 days in the dark at 37 ± 1°C. All experimentations commenced within 2 hours of the end of the incubation period. Following this incubation, revertant colonies were counted electronically or manually.

Criteria for assay validity: The assay was considered valid by the sponsor if 1) the mean negative control counts fell within the normal ranges as defined in sponsor's Appendix 4, 2) The positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains and an active S9 preparation, and 3) Not more than 5% of the plates were lost through contamination or some other unforeseen event.

Criteria for positive results: The test article was considered by the sponsor to be mutagenic if 1) The assay was valid, 2) Dunnett's test gave a significant response ($p<0.01$) and the data set(s) showed a significant dose correlation and 3) The positive responses described were reproducible.

Results

Study validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). Dose selection for the plate incorporation method and the pre incubation step was adequate based upon use of the limit dose level (i.e., 5000 µg/plate). Although many controls were either lower than or exceeded the historical ranges, these deviations were considered marginal, and the data were adequately interpreted.

SUMMARY RESULTS

Experiment 1. Summary of mean revertant colonies (- S9)

Substance	Dose level (µg/plate)	TA98 Mean±SD	TA100 Mean±SD	TA1535 Mean±SD	TA1537 Mean±SD	TA102 Mean±SD
DMSO	100 µl	23±3	169±8	16±4	17±1	315±25
mIBG	1.6	24±2	146±6	13±1	14±2 (M)	290±37
	8	26±5	136±14	14±4	14±4	297±19
	40	26±6	154±8	12±4	18±6	302±15
	200	19±3	136±10	19±4	18±4	289±13
	1000	22±5	151±4	13±2	17±3	205±17
	5000	8±2 (M+S)	47±5 (S)	14±2	4±1(V+M)	- (T)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean±SD	914±21	597±18	317±5	207±40	441±15

Experiment 1. Summary of mean revertant colonies (+ S9)

Substance	Dose level (µg/plate)	TA98 Mean±SD	TA100 Mean±SD	TA1535 Mean±SD	TA1537 Mean±SD	TA102 Mean±SD
DMSO	100 µl	35±4	157±13	16±4	19±2	218±27
mIBG	1.6	35±3	118±13	22±2	28±5	178±20
	8	35±3	145±7	18±5	24±3	229±6
	40	35±6	143±8	19±4	23±5	231±18
	200	39±3	140±2	15±4	17±2	203±7
	1000	35±2	114±22	16±5	14±10	148±38
	5000	20±6	71±3	10±4	9±2	-(T)
Positive controls	Compound	B(a)P	AAN	AAN	AAN	AAN
	Dose level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean±SD	385±12	1348±97	242±20	353±11	1175±117

Experiment 2. Summary of mean revertant colonies (- S9)

Substance	Dose level (µg/plate)	TA98 Mean±SD	TA100 Mean±SD	TA1535 Mean±SD	TA1537 Mean±SD	TA102 Mean±SD
DMSO	100 µl	31±8	139±15	17±4	9±3	307±59
mIBG	8.192	NT	NT	NT	NT	335±25
	20.48	34±13	111±8	17±7	8±3	262±134
	51.2	25±8	115±12	14±4	10±1	344±15
	128	21±3	120±12	13±5	8±3	432±118
	320	41±3	139±38	18±4	7±2	332±6
	800	22±5	129±6	13±4	9±5	267±16
	2000	29±6	121±10	13±4	10±5	105±46 (S)
	4000	16±2	89±14 (S)	NT	9±1 (S)	NT
	5000	NT	NT	12±2	NT	NT
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean±SD	1334±55	851±56	591±19	80±12	544±50

Experiment 2. Summary of mean revertant colonies (+ S9)

Substance	Dose level (µg/plate)	TA98 Mean±SD	TA100 Mean±SD	TA1535 Mean±SD	TA1537 Mean±SD	TA102 Mean±SD
DMSO	**100 µl**	**39±3**	**135±11**	**12±2**	**11±3**	**274±43**
mIBG	8.192	NT	NT	NT	NT	241±24
	20.48	26±5	127±15	12±6	11±6	289±29
	51.2	36±8	116±18	13±6	11±4	262±22
	128	39±8	110±21	14±5	10±4	269±11
	320	27±5	115±6	18±2	13±4	258±20
	800	40±5	99±5	18±4	15±10	258±33 (S)
	2000	26±2	78±19 (S)	19±3	11±3 (S)	154±21
	4000	17±10 (S)	81±7 (S)	NT	7±2 (S)	NT
	5000	NT	NT	15±4 (S)	NT	NT
Positive controls	Compound	B(a)P	AAN	AAN	AAN	AAN
	Dose level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean±SD	380±35	946±45	126±16	120±25	1689±372

Postfixes for all summary tables
S, slight thinning of background bacterial lawn
V, very thin background bacterial lawn
T, toxic, no revertant colonies
M, plate counted manually
NT, dose level not treated

Individual plate counts were averaged to give mean values. When the data were analyzed at the 1% level using Dunnett's test, the sponsor found no statistically significant increase in revertant numbers in any of the strain tested in the absence or in the presence of S9 mix.

Reviewer's comments
In the absence of S9 mix, a slight increase of 1.3 and 1.4 fold was observed in the revertant numbers respectively for strain TA98 treated with 320 µg and strain TA102 treated with 128 µg. These changes were limited to one experiment, and no dose response can be established (see summary tables).

In the presence of S9 mix, a 1.4 fold increase in the revertant number was found in strain TA1535 at the lowest dose of 1.6 µg. No significant effect was noted at higher doses. In the repeat experiment with the same strain (TA1535), there was a somewhat dose dependent increase of 1.16, 1.50, 1.50, and 1.58 fold in the revertant numbers at 128, 320, 600, and 2000 µg/plate respectively. However, all the numbers were within the historical ranges, and the extent of the increase is likely biologically irrelevant.

In conclusion, all the increases observed in the revertant numbers were less than 1.5 fold compared to vehicle controls; most were isolated and not reproducible. The only dose response observed involved numbers of revertants within the range of historical controls; therefore, in these conditions, mIBG was considered to be not mutagenic in the Ames test.

Study B122003: m-Iodobenzylguanidine hemisulphate (mIBG): mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the

b(4)

Key findings: At doses up to or approaching toxic levels, *m*IBG showed evidence of an equivocal increase in mutant frequency at the *tk* locus of L5178Y mouse lymphoma cells in a 3 hour experiment in the absence of S9 (1.3 fold and 1.49 fold for doses of 145 and 160 µg/mL, respectively). In addition, a weak linear trend was observed at the highest dose in the same experiment. However, the magnitude of this increase was such that it was considered to be of no biological relevance.

Study no:	2255/20
Study type:	In vitro Mutagenesis and Clastogenesis
Volume #, and page #:	4, 235
Conducting laboratory and location:	b(4)
Date of study initiation:	03/12/2004
GLP compliance:	OECD
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	mIBG hemisulphate, F053P1, 99.4-100%

Methodology: liquid media and microwell plates. b(4)
Cell line: L5178Y/TK+/- Mouse Lymphoma

Dose selection criteria:

Preliminary dose range finding assays were performed for 3 and 24 hours to select doses for the definitive assay. The following deviation to the protocol was reported by the sponsor: In the 3 hour assay, cell counts were performed immediately following treatment and cells plated at this stage and scored for Relative Survival (%RS). In the 24 hour assay, cells were plated after the 24 hour incubation and Relative Total Growth (RTG) was determined. Although the sponsor claims that this deviation did not affect the assay, the cytotoxicity may be overestimated because the cells were not centrifuged at the end of expression period. This step usually rids the cell preparation from cells that have lost membrane integrity. However, in the definitive assay, the cytotoxicity endpoint was the Relative Total Growth (RTG), as recommended by OECD guidelines.

In the 3 hour toxicity assay: the concentrations tested ranged from 67.19 to 2150 µg/ml. Precipitation was observed at 2150 µg/ml, and at concentrations ≥ 537.5 µg/ml only in the presence of S9. Relative Survival (%RS) at 134.4 µg/ml was 3.89 and 0.21 in the absence and in the presence of S9 respectively. At the lowest dose tested, RS was ~ 30% both with and without metabolic activation. Accordingly, the highest dose level analyzed in the absence and presence of S9 in the definitive assay (160 and 130 µg/ml) yielded 15% and 14% RTG respectively.

In the 24 hour toxicity assay: the concentrations tested only in the absence of S9, ranged from 0.625 to 320 µg/ml. At 20 and 40 µg/ml, extreme toxicity was observed with %RTG of 8 and 6 respectively. Accordingly, doses ranging from 2.5 to 30 µg/mL were selected for the definitive assay, to allow determination of viability and TFT resistance 2 days after treatment. The highest dose level of 30 µg/mL yielded a 34% RTG.

Osmolality: was not measured because the highest dose of *m*IBG tested was less than 10 mM.

Test agent solubility: mIBG showed solubility in DMSO up to at least 253 mg/mL and the solubility limit in tissue culture medium was close to 2010 µg/mL.

Metabolic activation system: S-9 fraction was prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. The S-9 percentage was 10%, which is within the acceptable range.

Controls:

Vehicle: DMSO
Negative controls: DMSO
Positive controls: 4-nitroquinoline 1-oxide (NQO) and Benzo(a)pyrene (BP)
Historical controls: not provided

Exposure conditions:

Cell cultures (2/concentration) in suspension were exposed to *m*IBG at determined concentrations in the presence and absence of S-9 for 3 hours, and in the absence of S9 for 24 hours. Following treatment, cells were washed and cultured in suspension for a 48

hour expression period. Following the expression period, cells were plated in micro wells with and without the selective agent, trifluorothymidine (TFT) for 11-14 days.

Analysis:
Wells containing clones were identified and counted. In addition, the number of wells containing large colonies and the number containing small colonies were scored for the negative and positive controls and doses of test article where a statistically significant increase in mutant frequency was observed (Dunnett's test for multiple comparisons) Data were also checked for a linear trend in mutant frequency with treatment concentration using weighted regression.

Criteria for positive results:
The test article was considered positive by the sponsor if 1) the assay was valid, 2) the mutant frequency at one or more doses was significantly greater than that of the negative control ($p<0.05$), and 3) there was a significant dose relationship as indicated by the linear trend analysis ($p<0.05$).

Summary of individual study findings:

Study validity:
Vehicle control cultures exhibited a mean cloning efficiency of 50% or greater; vehicle control cultures gave a mean mutant frequency of 50 to 170 x 10^{-6} (defined by sponsor as validity criterion) with a marginal mean value 170.59 in the 24 hour assay. Positive controls exhibited appropriate responses, although the mutant frequency exceeded the historical data in most cases; 80% reduction in RTG was achieved in the 3 hour assays but not in the definitive 24 hour assay. For these reasons, the study failed to meet all the validity criteria. It was however considered acceptable by the sponsor.

Study outcome:

Result summary tables:

Table1. Effect of 3 hour mIBG hemisulphate treatment on Relative Total Growth (RTG) and Mutant frequency (MF)

	Experiment 1 (-S9)		Experiment 2 (-S9)			Experiment 1 (+S9)		Experiment 2 (+S9)	
Treatment (µg/mL)	RTG Duplicate (mean)	MF Duplicate (mean)	RTG Duplicate (mean	MF Duplicate (mean)	Treatment (µg/mL)	RTG Duplicate (mean)	MF Duplicate (mean)	RTG Duplicate (mean	MF Duplicate (mean)
0	1.09	115.60	1.03	170.63	0	0.97	122.85	0.94	115.56
	0.92	92.40	0.97	129.64		1.03	73.37	1.07	89.67
	(1.00)	(105.14)	(1.00)	(149.75)		(1.00)	(95.38)	(1.00)	(101.93)
25	0.97	100.76			25	0.98	96.87		
	1.07	81.41				0.87	117.88		
	(1.02)	(90.68)				(0.92)	(107.04)		
40	0.99	107.96			40	0.85	115.68	0.93	120.74
	0.86	102.08				1.00	85.06	0.95	80.95
	(0.92)	(106.00)				(0.92)	(99.36)	(0.94)	(100.55)
55	0.82	116.83	0.79	184.20	55	0.86	90.03	0.92	80.08
	0.80	109.22	0.66	182.69		1.16	107.98	0.89	75.40
	(0.81)	(112.96)	(0.72)	(185.43)		(0.99)	(100.87)	(0.91)	(77.73)
70	0.82	208.52	0.62	150.12	70	0.87	116.69	0.60	125.67
	0.83	89.36	0.56	131.68		0.87	69.02	0.70	105.93
	(0.83)	(144.36)	(0.59)	(145.31)		(0.87)	(91.17)	(0.65)	(115.69)
85	0.73	89.49	0.44	177.12	85	0.88	88.47	0.57	138.99
	0.87	73.82	0.50	160.01		0.70	116.26	0.67	110.74
	(0.79)	(81.48)	(0.47)	(168.39)		(0.78)	(102.38)	(0.62)	(124.33)
100	0.62	90.36	0.61	165.00	100	0.65	77.35	0.56	98.73
	0.62	75.45	0.52	163.60		0.59	86.94	0.52	84.73
	(0.62)	(83.93)	(0.57)	(164.43)		(0.62)	(82.06)	(0.55)	(91.54)
115	0.42	99.69	0.34	185.52	115	0.42	94.92	0.50	56.59
	0.51	94.32	0.41	163.76		0.41	72.43	0.41	81.47
	(0.46)	(98.47)	(0.37)	(174.93)		(0.42)	(83.05)	(0.45)	(68.38)
130	0.35	118.98	0.35	151.39	130	0.29	87.26	0.15	101.87
	0.29	103.21	0.30	137.85		0.12	180.96	0.13	89.64
	(0.32)	(111.10)	(0.32)	(145.76)		(0.19)	(128.56)	(0.14)	(96.23)
145			0.21	224.69					
			0.22	168.14					
			(0.22)	(195.56)					
160			0.12	243.84					
			0.19	205.11					
			(0.15)	(223.68)					
0.15 NQO	0.72	606.07	0.54	611.77	2 BP	0.56	751.03	0.38	1356.13
0.20 NQO	0.53	758.02	0.40	908.01	3BP	0.13	1650.91	0.22	1193.49

RTG, relative total growth
MF, mutant frequency
NQO, 4-nitroquinoline 1-oxide
BP, benzo(a)pyrene

Table2. Effect of 24 hour mIBG hemisulphate treatment on Relative Total Growth (RTG) and Mutant frequency (MF) in absence of S9

Treatment (µg/mL)	RTG Duplicate (mean)	MF Duplicate (mean)
0	1.01	191.02
	0.99	151.79
	(1.00)	(170.59)
2.5	1.18	127.64
	1.19	163.55
	(1.19)	(145.20)
5	0.98	139.11
	1.34	105.16
	(1.13)	(123.47)
7.5	1.08	132.52
	1.19	103.24
	(1.14)	(117.44)
10	1.09	119.28
	1.36	127.26
	(1.22)	(123.70)
12.5	1.10	95.44
	0.92	121.69
	(1.00)	(108.12)
15	0.94	144.21
	0.98	110.34
	(0.96)	(126.57)
17.5	0.89	134.30
	0.86	134.18
	(0.87)	(134.28)
20	0.81	124.67
	0.91	117.52
	(0.86)	(121.18)
22.5	0.94	110.97
	0.76	106.09
	(0.85)	(108.71)
25	0.48	146.56
	0.46	136.36
	(0.48)	(141.49)
27.5	0.39	116.55
	0.35	160.17
	(0.37)	(137.58)
30	0.35	109.47
	0.32	146.22
	(0.34)	(126.87)
0.05 NQO	0.70	912.73
0.1 NQO	0.49	1020.92

RTG, relative total growth
MF, mutant frequency
NQO, 4-nitroquinoline 1-oxide

In the absence of S9: in a first 3 hour assay, the maximum dose tested of 130 µg/mL yielded a 32% RTG with no increase in mutant frequency. The negative and

positive controls were comparable to historical controls. The experiment was repeated with doses of 145 and 160 µg/mL, which yielded a 12 to 22 % RTG, and respectively a 1.3 and 1.49 fold increases in the mutant frequency. The increase at the highest dose and a weak linear trend were found to be statistically significant by the sponsor.
This result could not be confirmed in the 24 hour experiment in the absence of S9, because the optimal toxicity level of 10-20% could not be achieved even though these same doses elicited such toxicity levels in the preliminary cytotoxicity study.

In the presence of S9: no statistical increase in mutation frequency could be observed in the two 3 hour assays.

Sponsor's conclusion: At doses up to or approaching toxic levels, mIBG showed evidence of an equivocal increase in mutant frequency at the *tk* locus of L5178Y mouse lymphoma cells in a single experiment in the absence of S9 when utilizing a 3 hour treatment period. Furthermore, a weak linear trend was observed at the highest dose in the same experiment. It was concluded that, under the conditions employed in this study, the magnitude of this increase was such that it was considered to be of no biological relevance. Although optimal toxicity level of 10-20% could not be achieved in the 24 hour experiment even though these same doses elicited such toxicity levels in the preliminary cytotoxicity study.

Reviewer's comments: I agree with the sponsor's conclusion on the biological relevance, although based on the positive criteria set by the contract lab, the result could be interpreted as positive, since the mutant frequency was significantly greater than that of the negative control in two instances, and there was a significant dose relationship as indicated by the linear trend analysis. Moreover, the 24 hr repeat experiment conducted to confirm the negative or equivocal result of the 3 hour experiment was not valid in view that optimal toxicity was not achieved at doses tested. However, based on currently accepted guidelines it has been the Agency's practice to consider an experiment positive when the response in mutant frequency, in at least one of the three highest concentrations, is at least 2-fold higher than the mean control value and is associated with an upward trend in the remaining doses. In the present study, the increase in mutant frequency was 1.3 fold and 1.49 fold for doses of 145 and 160 µg/mL, respectively, which is less than 2 fold. In addition, a dose of 130 µg/ml (~1000 X the calculated maximum clinical plasma concentration for a 60 kg adult) did not produce an increase in mutant frequency. Thus, at the doses at which it occurs, the observed increase does not provide strong evidence of a mutagenic effect (Email consult to OND Associate Director of Pharmacology/Toxicology, who determined the result not significant)

Study B122004: m-Iodobenzylguanidine hemisulphate (mIBG): induction of micronuclei in the bone marrow of treated rats.

The objective of this study was to evaluate the clastogenic/aneugenicity of *m*IBG hemisulphate in vivo by examining micronuclei in the PCE of rat bone marrow.

Key findings: *m*IBG hemisulphate did not significantly induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 10 mg/kg/day considered to be the MTD in this study.

Study no:	2255/21
Volume #, and page #:	4, 299
Conducting laboratory and location:	[redacted] b(4)
Date of study initiation:	03/22/2004
GLP compliance:	OECD
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	mIBG hemisulphate, F052P1, 100%

Methods: Strain/species: Sprague-Dawley/rats (6F/dose group)

Dose selection criteria: A range finding study was performed: Rats were dosed once daily for two consecutive days with the test article and observations made over a 2 day period following the second administration.

The range-finding experiment demonstrated that an IV dose of 25.6 mg/kg was lethal in the 1 male and the 1 female used in this dose group. A 10 mg/kg dose was lethal in the only male (1/1) treated at this dose level and was not in females (n=4). Therefore, 10 mg/kg was determined as the MTD. Lethargy and bradypnea were observed immediately post-dosing on day 1 and day 2 in all animals treated at 5 and 10 mg/kg. No substantial difference was observed between male and female animals; therefore, only female animals were selected for testing.

Controls: Vehicle: 20% (v/v) Polyethylene Glycol 400 (PEG400) prepared in PBS, pH 7.4
Negative controls: 20% (v/v) PEG400 prepared in PBS, pH 7.4
Positive controls: Cyclophosphamide (CPA), 20 mg/kg

Exposure conditions:
Incubation and sampling times: Bone marrow sampling took place 24 hours after final dose administration.
Doses used in definitive study: 0, 2.5, 5.0, and 10.0 mg/kg.

Study design: Rats were dosed once daily for two consecutive days with the test article. The positive control was given as a single administration by IV bolus injection into the tail vein at 20 mg/kg, on the second day of the dosing.

Analysis:
No. of replicates: Not applicable
Counting method: The bone marrow from a single femur was flushed and smears were taken on slides. Two thousand polychromatic erythrocytes (PCE) per animal were then

evaluated for incidence of micronuclei and 1000 red blood cells per animal were counted for determination of the ratio of polychromatic to all erythrocytes.

Criteria for positive results: A test article was considered positive by the sponsor if 1) a statistically significant increase in the frequency of micronucleated PCE occurred at least at one dose and 2) the frequency and distribution of micronucleated PCE within the group at such a point exceeded the historical vehicle control data. Statistical evaluation was performed using the χ^2 test, non parametric analysis or Wilcoxon rank sum test.

Summary of individual study findings:
Study validity:
The study was deemed valid for the following reasons: 1) the incidence of micronucleated PCE in the vehicle control group was consistent with the historical vehicle control group data as given in an appendix 6, 2) At least five animals out of each group were available for analysis, and 3) the positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE.

Study outcome:
Table 1. Results summary on the effect of mIBG hemisulphate on %micronuclei in female Sprague-Dawley rats.

Treatment (mg/kg/day)	% PCE	MN PCE	% MN PCE
Vehicle	68.4	1	0.05
	61.6	0	0.00
	59.1	1	0.05
	61.3	1	0.05
	57.0	1	0.05
***m*IBG (2.5)**	62.7	3	0.15
	76.5	0	0.00
	55.6	1	0.05
	67.5	2	0.10
	62.7	2	0.10
	67.0	1	0.05
***m*IBG (5.0)**	59.0	0	0.00
	71.4	4	0.20
	55.9	1	0.05
	47.9	3	0.15
	63.2	2	0.10
	68.5	2	0.10
***m*IBG (10)**	70.3	0	0.00
	76.2	1	0.05
	48.4	1	0.05
	68.7	0	0.00
	73.2	3	0.15
	63.8	2	0.10
CPA (20)	36.8	16	0.80
	40.1	15	0.75
	40.0	20	1.00
	39.2	28	1.40
	53.3	15	0.75
	58.8	18	0.90

CPA, cyclophosphamide
PCE, polychromatic erythrocytes
MN PCE, micronucleated PCE

Table of historical vehicle control data (for 34 females on 5 studies)

	Individual PCE %	Individual frequency of MN PCE (%)	% animals with 0, 1 (or more) MN for 2000 PCE				
			1	2	3	4	5+
Mean	53	1.47	17.6	38.2	26.5	14.7	2.9
SD	8.6	(0.074%)					
Median	55						
Observed range	31-69						

Groups of rats treated with mIBG exhibited %PCE values that were similar to vehicle controls. The group mean %PCE for all groups fell within the range of the contract laboratory's historical vehicle control data. Frequencies of micronucleated PCE were also similar to those seen in the vehicle control group and were consistent with the historical control data. The frequencies of micronucleated PCE observed in test article treated groups were not significantly different from those observed in the vehicle control group following χ^2 analysis.

It was concluded that mIBG hemisulphate did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated with up to 10 mg/kg/day considered to be the MTD in this study.

Reviewer's comments:
Agree with sponsor's conclusion.

2.6.6.5 Carcinogenicity

Not performed

2.6.6.6 Reproductive and developmental toxicology

Not performed. The sponsor has requested a waiver for reproductive toxicology studies.

2.6.6.7 Local tolerance

Not performed

2.6.6.8 Special toxicology studies

Study # A122001 (Amersham Health, April 2004): Risk Assessment of Benzyl Alcohol in the mIBG Drug Product for Injection

The clinical formulation of *m*IBG contains 1% benzyl alcohol, which is used as a [redacted] Benzyl alcohol was not part of the formulation used in the animal safety studies performed by the innovator, although the innovator's formulation contains 1% benzyl alcohol as well. Benzyl alcohol, used as

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a preservative in solutions which were previously used to flush umbilical catheters, has been linked to the gasping syndrome in premature human infants. The purpose of this study was to perform a literature survey to evaluate the risk of benzyl alcohol in the *m*IBG product for injection.

Human data

The sponsor's literature survey on benzyl alcohol revealed fatal evidence of toxic reactions in premature infants. According to the sponsor, the doses reported to have produced lethality in neonates ranged from 100 to 400 mg/kg/day; however, no "gasping respiratory syndromes" were reported for intakes of 27 to 99 mg/kg/day over similar periods of time [Gershanik et al., 1982], although there is one report of 32 to 105 mg/kg/day for 7 days causing breathing difficulty [Anderson et al., 1984]. "Gasping", the most striking feature of the benzyl alcohol syndrome, may be caused by benzyl alcohol-induced injury to the apneustic center of the brain stem [Gershanik et al., 1982].

According to a publication by The European Agency for the Evaluation of Medicinal Products (1997), the infants affected by benzyl alcohol developed severe metabolic acidosis, gasping respiration, and blood abnormalities. Some infants had severe neurological deterioration and hepatic and renal failure resulting in death. Survivors had a significantly increased incidence of cerebral palsy. It was estimated that the infants had received approximately 99-234 mg/kg/day of benzyl alcohol. Metabolically, benzyl alcohol is oxidized to benzoic acid, conjugated with glycine in the liver, and excreted as hippuric acid. The high concentrations of unmetabolized benzyl alcohol and relatively small amounts of hippuric acid in the urine suggested that the immaturity of the metabolic detoxification process in premature neonates made them more sensitive to the toxic effects.

Animal data

a. General systemic effects [single exposure: intravenous]

According to the report, the rat LD_{50} is 64 mg/kg. Transient respiratory arrest but no deaths occurred in 10 mice injected with 480 mg/kg as a 0.9% solution of benzyl alcohol in saline, while the same dose given as a 94% solution killed all animals. The sponsor states that in rats and dogs given 50 mg/kg or more, various effects including breathing difficulties, cardiac arrest, CNS effects and death were seen, with increased severity when the dose is injected more rapidly or at higher concentration. It was stated that no changes in blood pressure, heart rate, respiration or blood chemistry were seen in seven monkeys injected with approximately 9 mg/kg of 0.9% benzyl alcohol in saline.

b. Reproductive toxicity

Reduced birth weight and decreased growth were seen in the offspring of mice given 750 mg/kg/day by stomach tube on days 6 to 13 of pregnancy.

c. Mutation data

The literature survey conducted by the sponsor revealed the following results:

- Benzyl alcohol was not mutagenic to Salmonella typhimurium or Escherichia coli but it gave indirect evidence of being able to induce DNA damage in Bacillus subtilis.
- In cytogenetic assays with Chinese hamster ovary (CHO) cells, chromosomal damage was induced in the presence but not in the absence of a liver metabolic activation system, whilst a weakly positive result was found for the induction of sister chromatid exchanges both in the presence and absence of the activation system.
- An equivocal response was observed in an in vitro mouse L5178Y/TK+/- lymphoma assay in the absence of a liver metabolic activation system. In the presence of an activation system, no mutations were induced and the effect was associated with toxicity.
- Benzyl alcohol induced DNA double strand breaks in primary rat hepatocytes at 10 mmol/L but not at 1 or 3 mmol/L.

d. Carcinogenicity

According to the sponsor, no evidence of carcinogenicity was seen in rats given 200 or 400 mg/kg/day, by stomach tube, 5 days/week for up to 2 years, or in similarly sized groups of mice given 100 or 200 mg/kg/day by the same regimen.

Report's conclusions:

The sponsor was not able to make a conclusive determination on the potential for adverse effects in neonates or individuals with insufficient metabolizing capacity because the NOAEL for this group has not been determined.

For adults, the risk characterization was based on a maximum human exposure of 104.1 mg benzyl alcohol (10 mL), equivalent to 1.49 mg/kg benzyl alcohol for a 70 kg person. According to the sponsor, the single parenteral dose of 0.9% benzyl alcohol considered safe for healthy human adults was 30 ml, ~4 mg/kg benzyl alcohol. According to the FAO/WHO (1980), the acceptable daily intake of benzyl alcohol is estimated to 5 mg/kg. Therefore the sponsor determined the latter value to be about 3 times above the amount of benzyl alcohol in one vial of *m*IBG for Injection.

Reviewer's comments

Although the report concludes on the safety of benzyl alcohol in adults at the dose in which it will be administered, the threshold of toxicity of the compound in neonates remains unknown.

Benzyl alcohol is present at the same concentration (1%) in both formulations (GE and CIS-US); however, the sponsor's drug is supplied in a 10 mL vial containing 5 mL of solution resulting in a maximum human dose of 52.1 mg of benzyl alcohol, whereas the

CIS-US drug is supplied in a 2 mL vial containing a volume of 0.5 mL, which would result in a maximum of 5.21 mg of benzyl alcohol.

The maximum amount of benzyl alcohol for both the CIS-US formulation and the sponsor's formulation is presented in the following table for 60 kg human adults and pediatric patients 10 kg and under. Doses are provided on a mg/kg basis and on a body surface area comparison, using a maximum possible dose of 52.1 mg benzyl alcohol (GEHC) and 5.21 mg benzyl alcohol (CIS-US).

Weight		***GEHC mIBG (5mL)***		***CIS-US mIBG (0.5mL)***	
		52.1 mg Benzyl alcohol		5.21 mg Benzyl alcohol	
		mg/kg	mg/m^{2*}	mg/kg	mg/m^2
Adult	60 kg	0.87	32.2	0.087	3.2
Children	10 kg	5.21	130.3	0.52	13.02
	8 kg	6.5	162.5	0.65	16.3
	6 kg	8.7	217.5	0.87	21.75
	4 kg	13.0	325	1.3	32.5
	3 kg	17.4	435	1.74	43.5
	2 kg	26.1	652.5	2.61	65.25
	1 kg	52.1	1302.5	5.21	130.25

*Conversion from mg/kg to mg/m^2 using a conversion factor (*km*) of 37 for adults and 25 for children.

Note: Although the benzyl alcohol evaluation was performed for a minimum pediatric weight of 9 kg in NDA #20-084, the CIS-US label has no restrictions for benzyl alcohol amounts for the use in pediatric patients.

Gershanick's group (article included in the NDA package) reported clinical data on a total of 18 premature infants of gestational age 3.7-4.8 weeks, weighing 620-2126 g. Ten out of the 18 infants received 99 to 234 mg/kg benzyl alcohol and developed neurological deterioration, gasping syndrome, severe metabolic acidosis, hematologic abnormalities, skin breakdown, hepatic and renal failure, hypotension and cardiac collapse, resulting in eventual death from benzyl alcohol poisoning, whereas the matched control group (n=8) received 27 to 99 mg/kg over the same period, without developing the gasping syndrome. The blood levels of benzyl alcohol ranged from 0.610 to 1.378 mmol/L in 6/10 infants with the gasping syndrome. There are no blood data for the control infants. It should be noted that Gerhanick's group states in their article that *"These [control] infants did not have the striking features of the gasping syndrome [but] it is possible that they did have less severe forms of benzyl alcohol toxicity that were not recognized."* Thus, no threshold for toxic effects can be established from this report with regard to benzyl alcohol use in neonates.

In addition, the Registry of Toxic Effects of Chemical Substances of the U.S. National Institute of Occupational Science and Health (NIOSH) lists the following data for benzyl alcohol:

Route/Organism	Dose	Effect
Intravenous dog	Lowest published dose: 50 mg/kg (1000 mg/m^2)	Ataxia, dyspnea, GI hypermotility, diarrhea
Intravenous mouse	LD_{50}: 324 mg/kg (972 mg/m^2)	
Intravenous rat	LD_{50}: 53 mg/kg (318 mg/m^2)	Dyspnea
Parenteral dog	Lowest published dose: 9 mg/kg (180 mg/m^2)	Tremor, respiratory changes

The sponsor states that in rats and dogs given 50 mg/kg (respectively 300 and 1000 mg/m^2) or more, breathing difficulties, cardiac arrest, CNS effects and death were seen, with increased severity when the dose was injected more rapidly or at higher concentration. Children 3 to 10 kg could receive up to 435 and 130 mg/m^2 of benzyl alcohol with the GEHC formulation, a range which comprises the LD_{50} in rats (318 mg/m^2).

Conclusion:

Like CIS-US ^{131}I *m*IBG, AdreView contains benzyl alcohol. The potential maximum amount of benzyl alcohol injected for an entire vial of drug is 10 times higher with AdreView. Benzyl alcohol has been associated with a fatal gasping syndrome mainly in premature infants. Infants affected developed severe metabolic acidosis, gasping respiration, and blood abnormalities. Some infants had severe neurological deterioration and hepatic and renal failure resulting in death and survivors had a significantly increased incidence of cerebral palsy. It was estimated that the infants had received approximately 99-234 mg/kg/day of benzyl alcohol. In this report, benzyl alcohol risk assessment based on analysis of both animal and human data, demonstrated that the amount of benzyl alcohol at which toxicity may occur is not known, suggesting that premature and/or low-birth-weight infants, and patients receiving high dosages of benzyl alcohol may be more likely to develop toxicity.

Although benzyl alcohol is present in several medications, a quick search in the Drugs@FDA database revealed that the potential maximum amounts administered to adults and children were usually lower compared to the amount that would be received from AdreView administration (17.4 mg/kg for a 3 kg infant). For several (although not all) FDA-approved drugs containing benzyl alcohol (e.g. AMICAR®, KENALOG®, LINCOCIN, etc…), the label contains warnings and precautions for pediatric use with regard to benzyl alcohol. There is no basis from a pharmacology/toxicology perspective to determine the safety of the use of benzyl alcohol in neonates. In case AdreView is approved for use in neonates by the clinical reviewer, we recommend including appropriate warning and precautions in the label.

Study# VLD-05-006: Toxicological Testing of [redacted] Closure (Feb 05)

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UV-HPLC analysis of the drug product revealed an unknown peak (retention time of [redacted] which is a leachate produced during the [redacted] for the drug product. The impurity is not, according to the sponsor, a result of a

b(4)

drug/stopper interaction since it can also be generated from ——— stopper interaction. The stopper leachate was produced and analyzed to ensure its presence at a concentration equivalent to that detected in the drug product, and its toxicological testing was conducted per USP <87> and <88>. b(4)

Report's results and conclusions: All requirements for USP <87> and <88>, Biologic Reactivity Tests, In Vitro and In Vivo were met (cytotoxicity by elution, acute systemic toxicity and intracutaneous toxicity) using an extract with the unknown leachate present at the highest theoretical concentration detected in drug product. The results of the toxicology testing support the safe use of Iobenguane I 123 Injection.

Reviewer's comments:

UV-HPLC analysis of the drug product revealed an unknown peak with a retention time of approximately ——— It is a leachate produced from the closure material's ——————————— According to the sponsor, the unknown impurity is ——————————— and is present at a concentration of ——————————— with a maximum of ——— per administration. The impurity exhibits a structural alert for genotoxic and carcinogenic potential. All the preclinical data used for this submission were obtained using a formulation that did not contain this impurity. Moreover, this unknown impurity is present in the drug product manufactured in Arlington facility, but not in the drug manufactured by GE Healthcare in Europe (Eindhoven), for which the stopper generating the impurity is not used. The sponsor states that the use of this closure system allows——————— and is more appropriate for reducing occupational radiation. b(4)

The sponsor was requested to provide the toxicity profile of the compound. In response to this request, the sponsor submitted <USP> studies, which are not adequate for nonclinical safety evaluation of drug impurities. The sponsor states that the same stopper is used for 3 FDA-approved products marketed by GE Healthcare. However, no evidence that the same impurity is similarly produced in those products at the ——— is provided. According to Guidelines on the limits of genotoxic impurities (EMEA, Committee for Medicinal Products for Human Use, CHMP, June 2006), "in the absence of data providing evidence for a threshold mechanism of genotoxicity, implementation of a generally applicable approach as defined by the Threshold of Toxicological Concern (TTC) is proposed. A TTC value of 1.5 µg/day intake of a genotoxic impurity is considered to be associated with an acceptable risk (excess cancer risk of <1 in 100,000 over a lifetime) for most pharmaceuticals. Higher limits may be justified under certain conditions such as short-term exposure periods", which is the case in the present NDA, with a maximum impurity level of——————————In addition, the OND Associate Director of Pharmacology/Toxicology was consulted by email on this issue, and his recommendation was that the presence of the unknown impurity at ——— was acceptable, and that no further evaluation/studies are needed. b(4)

OVERALL CONCLUSIONS AND RECOMMENDATIONS

IOBENGUANE SULFATE I 131 was approved by the FDA in 1994, and the clinical experience with *m*IBG is extensive. *m*IBG safety was established in clinical and preclinical studies (previous NDA # 20-084). The toxicological risk of benzyl alcohol exposure resulting from administration of 5 ml of *m*IBG was evaluated. Benzyl alcohol has been linked to fatal toxicity in premature infants and other type of toxicity in surviving infants. A threshold for toxic effects was not determined based on a literature survey. It is recommended that the label include warning and precaution for pediatric use.

Conclusions: There are no unresolved issues that would necessitate additional nonclinical studies.

Recommendations: This NDA is recommended for approval from a nonclinical perspective

Suggested labeling: See Executive summary section

Signatures (optional):

Reviewer Signature _____Siham Biade, Ph.D._____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Siham Biade
9/16/2008 11:46:48 AM
PHARMACOLOGIST

Adebayo Laniyonu
9/16/2008 04:59:58 PM
PHARMACOLOGIST