CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
21-248

PHARMACOLOGY/TOXICOLOGY REVIEW

MEMORANDUM

Date: June 9, 2000
From: Paul A. Andrews, Ph.D. PAA 6/9/2000
Supervisory Pharmacologist, HFD-150
To: Files for NDA# 21-248
Re: Approvability for Pharmacology and Toxicology

[redacted]

[redacted] or arsenolite, is an aqueous solution of arsenic trioxide. Cell Therapeutics, Inc. seeks approval of [redacted] for treatment of relapsed or refractory acute promyelocytic leukemia. For the pharmacology and toxicology section of this NDA, Cell Therapeutics submitted relevant articles from the extensive published literature on trivalent arsenic. Since all of the non-clinical data essential for regulatory purposes was available in the biomedical literature and is considered common knowledge, no further animal studies were requested to support this NDA. Although the toxicology literature regarding oral exposure is very large due to the concerns over environmental exposure to arsenic, the literature regarding the toxicologic effects of parenteral administration is much less extensive. Dr. John Leighton has focused his review on the data related to parenteral administration because this is the route relevant to [redacted] administration. Although arsenic bioavailability is near 100% following oral administration, there is significant first pass metabolism in the liver to methylated metabolites that have a different toxicologic profile than inorganic As^{+3}. The toxicity of oral arsenic thus differs from intravenous arsenic. The relevant parenteral literature has been thoroughly and thoughtfully reviewed by Dr. Leighton who considers the data adequate to support approval for the intended indication. I concur with his recommendation. The non-clinical data in the NDA covered the core expectations for cytotoxic drugs in HFD-150. The package included information from:

- acute studies of arsenite salts in mice, rats, and cats by various parenteral routes,
- an acute study of i.v. arsenic trioxide in rabbits,
- repeat dose studies of arsenic trioxide in rats using an i.p. weekly x 18 schedule and in rabbits using an i.v. 3x/week x 12 week schedule,
- several chronic studies of orally administered arsenic trioxide in mice and rats, and
- chronic studies of orally administered arsenite salts in mice and dogs.

Although conventional ICH Stage C-D developmental toxicity studies have not been reported using the intravenous route, studies in mice, rats, and hamsters demonstrate that a single parenteral dose of arsenic is sufficient to cause developmental mortality, dysmorphogenesis, and growth retardation. [redacted] should thus be labeled Pregnancy Category D as is the practice for cytotoxic oncology drugs.

Numerous genetic toxicity studies have been published over the years with trivalent arsenic. Mutagenicity has not been clearly demonstrated, but arsenic is clastogenic *in vitro*. Carcinogenicity studies are not necessary to support approval for the intended indication. In any case, significant data exists to indicate that oral arsenic is a human carcinogen and additional animal studies would be of little value for assessing carcinogenic risk.

A detailed labeling review was provided by Dr. Leighton and I agree with the requested changes.

Recommendations: The pharmacology and toxicology data supports approval of this NDA. There are no outstanding issues.

Original NDA
cc: Div File
HFD-150
/JLeighton
/DSpillman (in drs)
/SHirschfeld
/AIbrahim

Spillman

JUN 19 2000

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review #2

Keywords: label review

NDA: 21,248

Serial No. 000 **Type:** NDA **Letter Dated** 3/27/00 **Received by CDR:** 3/28/00
Information to be conveyed to the sponsor: YES
Reviewer: John K. Leighton, Ph.D.
Date Review Completed: 6/8/00
Sponsor: Cell Therapeutics, INC.
Manufacturer: [redacted]

Drug:

drug name:	arsenolite
Generic name:	arsenic trioxide
Trade name:	
Chemical name:	arsenic trioxide
CAS Registry no.	1327-53-3
Molecular formula/weight:	As_2O_3; 197.8
Structure:	

Related INDs, NDAs; DMFs: [redacted]

Drug Class: arsenical

Indication: "For the induction of remission and consolidation in patients with relapsed or refractory acute promyelocytic leukemia, characterized by the presence of the t(15; 17) translocation and/or the presence of the PML/RAR (alpha) gene who are refractory to, or have relapsed from, retinoid and anthracycline chemotherapy, or for whom anthracycline-based chemotherapy is contraindicated."

Clinical Formulation: 1 mg/mL in water, pH 7-9, diluted in 100-250 mL 5% dextrose
Route of administration and dosage form: intravenous solution infused over 1-2 hours
Proposed clinical protocol: 0.15 mg/kg/day for up to 60 days
Previous review: Dr. Clark's reviews for [redacted] Dr. Leighton review of NDA, 6/8/00

The sponsor conducted no original preclinical pharmacology or toxicology studies. The studies and reviews submitted for the NDA were a selection of the available pharmacology and toxicology literature of arsenic.

___1___ pages redacted from this section of the approval package consisted of draft labeling

Spillman

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review #1

JUN - 8 2000

Keywords: reproductive toxicology

NDA: 21-248

Serial No. 000 **Type:** NDA **Letter Dated** 3/27/00 **Received by CDR:** 3/28/00
Information to be conveyed to the sponsor: YES
Reviewer: John K. Leighton, Ph.D.
Date Review Completed: 6/8/00
Sponsor: Cell Therapeutics, INC.
Manufacturer:

Drug: drug name: arsenolite
Generic name: arsenic trioxide
Trade name:
Chemical name: arsenic trioxide
CAS Registry no. 1327-53-3
Molecular formula/weight: As_2O_3; 197.8
Structure:

Related INDs, NDAs; DMFs:

Drug Class: arsenical
Indication: "For the induction of remission and consolidation in patients with relapsed or refractory acute promyelocytic leukemia, characterized by the presence of the t(15; 17) translocation and/or the presence of the PML/RAR (alpha) gene who are refractory to, or have relapsed from, retinoid and anthracycline chemotherapy, or for whom anthracycline-based chemotherapy is contraindicated."

Clinical Formulation: 1 mg/mL in water, pH 7-9, diluted in 100-250 mL 5% dextrose
Route of administration and dosage form: intravenous solution infused over 1-2 hours
Proposed clinical protocol: 0.15 mg/kg/day for up to 60 days
Previous review: Dr. Clark's reviews for

The sponsor conducted no original preclinical pharmacology or toxicology studies. The following studies and reviews were submitted based on a selective literature review for information most relevant to the pharmacology and toxicology of intravenous use of arsenic trioxide. Studies investigating effects of pentavalent arsenic are only briefly discussed where appropriate.

Studies reviewed in this submission:

PHARMACOLOGY

	Title	Ref#	Vol.	Page
	Göyer RA: Toxic Effects of Metals, in CD Klaassen (ed): Casarett and Doulls Toxicology: The Basic Science of Poisons. New York, McGraw-Hill; 1996:691 -698.	135	2	100

	Lynn S, Lai HT, Gurr J-R, Jan KY: Arsenite retards DNA break rejoining by inhibiting DNA ligation. Mutagenesis 1997; 12:353-358.	153	3	123
	Wang ZG, Rivi R, Delva L, et al: Arsenic trioxide and melarsoprol induce programmed cell death in myeloid leukemia cell lines and function in a PML and PML-RARα independent manner. Blood 1998; 92:1497-1504.	46	4	318
	Raelson JV; Nervi C; Rosenauer A, et al: The PML/RARα protein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells. Blood 1996; 88:2826-2832.	108	3	149
	Dai J, Weinberg RS, Waxman S, Jing Y: Malignant cells can be sensitized to undergo growth inhibition and apoptosis by arsenic trioxide through modulation of the glutathione redox system. Blood 1999; 93:268-277.	32	1	156
	Chen GQ, Shi XG, Tang W, et al: Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): 1. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 1997; 89:3345-3353.	57	1	147

SAFETY PHARMACOLOGY

No studies were submitted.

PHARMACOKINETICS AND TOXICOKINETICS

Distribution

	Fielder RJ, Dale EA, Williams SD: Inorganic arsenic compounds. HSE Toxicity Review 1986; 16:1-66.	132	1	238
	DeSesso JM, Jacobson CR, Scialli AR, Farr CH, Holson JF: An assessment of the developmental toxicity of inorganic arsenic. Reproductive Toxicology 1998; 12:385-433.	128	1	166
	Goyer RA: Toxic Effects of Metals, in CD Klaassen (ed): Casarett and Doulls Toxicology: The Basic Science of Poisons. New York, McGraw-Hill; 1996: 691 -698.	135	2	100

Metabolism

	Aposhian HV: Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu Rev Pharmacol Toxicol 1997; 37:397-419.	121	1	70
	Fielder RJ, Dale EA, Williams SD: Inorganic arsenic compounds. HSE Toxicity Review 1986; 16:1-66.	132	1	238

Excretion

	Fielder RJ, Dale EA, Williams SD: Inorganic arsenic compounds. HSE Toxicity Review 1986; 16:1-66.	132	1	238

TOXICOLOGY

	Fielder RJ, Dale EA, Williams SD: Inorganic arsenic compounds. HSE Toxicity Review 1986; 16:1-66.	132	1	238
	United States Department of Health and Human Services. Agency for Toxic Substances and Disease Registry TP-92/02. 1993. Washington, D.C.: U.S. Department of Commerce, National Technical Information Service. Toxicological Profile for Arsenic (update).	164	4	1

CARCINOGENICITY

	IARC. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42., in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1987:100-106.	145	2	305
	IARC. Some Inorganic and Organometallic Compounds: Arsenic and Inorganic Arsenic Compounds. in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. 1972:48-73.	142	2	168

	USEPA: Evaluation of the potential carcinogenicity of arsenic and inorganic arsenic compounds.	165	4	204
	USEPA. EPA/625/3-87-013. 1988. Washington, D.C., U.S. Department of Commerce, National Technical Information Service. Special Report on Ingested Inorganic Arsenic. Skin cancer; Nutritional essentiality.	166	4	249

IMMUNOTOXICOLOGY

No studies were submitted.

REPRODUCTIVE TOXICOLOGY

	DeSesso JM, Jacobson CR, Scialli AR, Farr CH, Holson JF: An assessment of the developmental toxicity of inorganic arsenic. Reproductive Toxicology 1998; 12:385-433.	128	1	166
	Golub MS, Macintosh MS, Baumrind N: Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. J Toxicol Environ Health Part B 1998; 1:199-241.	134	2	57
	Ferm VH: Arsenic as a teratogenic agent. Environ Health Perspect 1977; 19:215-217.	131	1	235

GENETIC TOXICOLOGY

	Jacobson-Kram D, Montalbano D: The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environmental Mutagenesis 1985; 7:787-804.	148	3	1
	IARC. Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1987:71-76.	14	2	298
	Zhao CQ, Young MR, Diwan BA, Coogen TP, Waalkes MP: Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proc Natl Acad Sci USA 1997; 94:10907-10912.	170	5	49
	Eguchi N, Kuroda K, Endo G: Metabolites of arsenic induced tetraploids and mitotic arrest in cultured cells. Arch Environ Contam Toxicol 1997; 32:141-145.	130	1	230
	Kashiwada E, Kuroda K, Endo G: Aneuploidy induced by dimethylarsinic acid in mouse bone marrow cells. Mutation Research 1998; 413:33-38.	149	3	41
	Tice RR, Yager JW, Andrews P, Crecelius E: Effect of hepatic methyl donor status on urinary excretion and DNA damage in B6C3F1 mice treated with sodium arsenite. Mutation Res 1997; 386:315-334.	163	3	231

ADDITIONAL STUDIES REVIEWED

	Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. Am J Epidemol 1992; 135: 462-476	Carcinogenicity
	Stump DG, Holson JF, Fleeman TL, Nemec MD, Farr CH. Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 1999; 60:283-291.	Reproductive toxicology
	Wilhite CC. Arsenic-induced axial skeletal (dysraphic) disorders. Exp Molec Pathol 1981; 34:145-158.	Reproductive toxicology
	Machado AF, Hovland DN, Jr., Pilafas S, Collins MD. Teratogenic response to arsenite during neurulation: relative sensitivities of C57BL/6J and SWV/Fnn mice and impact of splotch allele. Toxicol Sci 1999; 51:98-107.	Reproductive toxicology
	Pollock JL, Westervelt P, Kurichety AK, Pelicci PG, Grisolano JL, Ley TJ. A bcr-3 isoform of RARα-PML potentiates the development of PML-RARα-driven acute promyelocytic leukemia. Proc Natl Acad Sci USA 1999; 96:15103-15108.	Pharmacology
	Lallemand-Breitenbach V, Guillemin M-C, Janin A, Daniel M-T, Degos L, Kogan SC, Bishop JM, de The H. Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. J Exp Med 1999; 189:1043-1052.	Pharmacology

Studies not reviewed within this submission:

PHARMACOLOGY

	Klevay LM: Pharmacology and toxicology of heavy metals: arsenic. Pharmac Ther A 1976; 1:189-210.	150	3	58
	Beliles RP: The Metals, in G.D.Clayton and F.E.Clayton (ed): Patty's Industrial Hygiene and Toxicology. New York, John Wiley & Sons, Inc; 1994:1913-1925.	125	1	106
	Klaassen CD: Heavy Metals and Heavy Metal Antagonists, in JG Hardman et al, ed.: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th Ed. New York, NY, MacMillan; 1985:1605-1627.	172	3	47
	IARC vol 23. Some Metals and Metallic Compounds, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. 1980:39-141.	143	2	195
	Warrell RP Jr.; de The H; Wang ZY; Degos L: Acute promyelocytic leukemia. N Engl J Med 1993; 329:177-189.	104	4	326
	de The H; Chomienne C; Lanotte M; Degos L; Dejean A: The t(15; 17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor α gene to a novel transcribed locus. Nature 1990; 347:558	105	1	215
	Kakizuka A; Miller WH, Jr.; Umesono K; et al: Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARα with a novel putative transcription factor, PML. Cell 1991; 66:663-674.	106	3	29
	Dyck JA; Maul GG; Miller WH, Jr; Chen JD; Kakizuka A; Evans RM: A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 1994; 76:333-343.	107	1	219
	Slack JL: Biology and treatment of acute progranulocytic leukemia. Curr Opin Hematol 1999; 6:236-240.	12	--	--
	Slack JL: The biology and treatment of acute progranulocytic leukemia. Curr Opin Oncol 1999; 11:9-13.	30	3	194
	He L-Z; Peruzzi D; Delva L; Wang Z-G; Warrell RP, Jr; Pandolfi PP: Therapeutic trials with retinoic acid, IFN, As_2O_3 and melarsoprol in transgenic models of APL. Anticancer Research 1997; 17:3927	139	2	142
	Look AT: Arsenic and apoptosis in the treatment of acute promyelocytic leukemia [editorial; comment]. J Natl Cancer Inst 1998; 90:86-88.	52	3	111
	Tallman MS: Therapy of acute promyelocytic leukemia: all-trans retinoic acid and beyond. Leukemia 1998;12 Suppl 1:S37-40.	40	3	219
	Fowler BA: Toxicology of Environmental Arsenic. Toxicology of Trace Elements, in RA Goyer and MA Mehlman. (ed): Advances in Modern Toxicology. Washington, D.C., Hemisphere Publishing Co; 1977:79-122.	133	2	2
	Chen GQ, Zhu J, Shi XG, et al: In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As203 induces N134 cell apoptosis with down-regulation of Bcl-2 expression and modulation of PML-RAR α/PML proteins. Blood 1996; 88:1052-1061.	63	1	137
	Soignet SL, Maslak P, Wang ZG, et al: Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide [see comments]. N Engl J Med 1998; 339:1341-1348.	39	3	199
	Andre C, Guillemin MC, Zhu J, et al: The PML and PML/RARα domains: from autoimmunity to molecular oncology and from retinoic acid to arsenic. Exp. Cell Res 1996; 229:253-260.	61	1	62
	Zhu J, Koken MH, Quignon F, et al: Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. Proc Natl Acad Sci U.S.A. 1997; 94:3978-3983.	58	5	62
	Rego E, He LZ, Wang Z-G, Peruzzi D, Warrell RP Jr., Pandolfi PP: Therapeutic trials with arsenic trioxide (As_2O_3) and retinoic acid in PML-RARα transgenic mice as models of APL. Blood 1998; 92:403A	156	3	157
	Lallemand-Breitenbach V, Guillemin MC, Janin A, et al: Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. J Exp Med 1999; 189:1043-1052.	23	3	101

	Gianni M, de The H: In acute promyelocytic leukemia N134 cells, the synthetic retinoid CD437 induces contemporaneously apoptosis, a caspase-3-mediated degradation of PML/RARα protein and the PML retargeting on PML-nuclear bodies. Leukemia 1999; 13:739-749.	17	2	46
	Jing Y, Dai J, Chalmers-Redman RM, Tatton WG, Waxman S: Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. Blood 1999; 94:2102-2111.	6	3	19
	Gurr JR, Bau DT, Liu F, Lynn S, Jan KY: Dithiothreitol enhances arsenic trioxideinduced apoptosis in NB4 cells. Mol Pharmacol 1999; 56:102-109.	136	2	118
	Huang XJ, Wiernik PH, Klein RS, Gallagher RE: Arsenic trioxide induces apoptosis of myeloid leukemia cells by activation of caspases. Med Oncol 1999; 16:58-64.	16	2	161
	Tang B, Bajenova O, Feinman-Siegel R, Childs B, Pearse R, Michaeli J: Arsenic compounds induce apoptosis in multiple myeloma (MM), activate pro-caspase-3 but do not affect Bcl_2 family members. Blood 1998; 92:638A	162	3	230
	Tallman MS: The thrombophilic state in acute promyelocytic leukemia. Semin Thromb Hemost 1999; 25:209-215.	20	3	223
	Zhu J, Guo WM, Yao YY, et al: Tissue factors on acute promyelocytic leukemia and endothelial cells are differently regulated by retinoic acid, arsenic trioxide and chemotherapeutic agents. Leukemia 1999; 13:1062-1070.	11	5	68
	Ishitsuka K, Hanada S, Suzuki S, et al.: Arsenic trioxide inhibits growth of human T-cell leukemia virus type I infected T-cell lines more effectively than retinoic acids. Br J Haematol 1998; 103:721-728.	147	2	327
	Bazarbachi A, El-Sabban ME, Nasr R, et al: Arsenic trioxide and interferon-α synergize to induce cell cycle arrest and apoptosis in human T-cell lymphotropic virus type I- transformed cells. Blood 1999; 93: 278-283.	123	1	93
	Zhu XH, Shen YL, Jing YK, et al: Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations [see comments]. J Natl Cancer Inst 1999; 91:772-778.	22	5	77
	Lu M, Levin J, Sulpice E, et al: Effect of arsenic trioxide on viability, proliferation, and apoptosis in human megakaryocytic leukemia cell lines. Exp Hematol 1999; 27:845-852.	21	3	115
	Rousselot P, Larghero J, Arnulf B, et al: Use of arsenic trioxide (As_2O_3) in the treatment of chronic myelogenous leukemia: in vitro and in vivo studies. Blood 99 A.D.;94: (Abstract)	85	--	--
	Konig A, Wrazel L, Warrell RP, Jr., et al: Comparative activity of melarsoprol and arsenic trioxide in chronic B-cell leukemia lines. Blood 1997; 90:562-570.	54	3	80
	Zheng J, Deng YP, Lin C, Fu M, Xiao PG, Wu M: Arsenic trioxide induces apoptosis of HPV16 DNA-immortalized human cervical epithelial cells and selectively inhibits viral gene expression. Int J Cancer 1999; 82:286-292.	171	5	55
	Shen ZY, Tan U, Cai WJ, et al: Arsenic trioxide induces apoptosis of oesophageal carcinoma in vitro. Int J Mol Med 1999; 4:33-37.	18	3	189
	Akao Y, Mizoguchi H, Kojima S, Naoe T, Ohishi N, Yagi K: Arsenic induces apoptosis in B-cell leukaemic cell lines in vitro: activation of caspases and down-regulation of Bcl-2 protein. Br J Haematol 1998; 102:1055-1060.	45	--	--
	Barchowsky A, Roussel RR, Klei LR, et al: Low levels of arsenic trioxide stimulate proliferative signals in primary vascular cells without activating stress effector pathways. Toxicol Appl Pharmacol 1999; 159:65-75.	122	1	82
	Beck LV: Action of adrenal hormones on lethal toxicities of certain organic compounds. Proc Soc for Exp Biol and Med 1951; 78:392-397.	124	1	99
	Abernathy CO, Liu YP, Longfellow D, et al. Arsenic: health effects, mechanisms of actions, and research issues. Environ. Health Perspect 1999; 107:593-599.	119	1	42
	Reichl FX, Kreppel H, Szinicz L, et al: Effect of chelating agents on biliary excretion of arsenic in perfused livers of guinea pigs pretreated with As_2O_3. Vet Hum Toxicol 1990; 32:223-226.	157	3	158
	Akao Y, Nakagawa Y, Akiyama K. Arsenic trioxide induces apoptosis in neuroblastoma cell lines through activation of caspase 3 in vitro. FEBS Lett 1999; 455:59-62.	9	1	047

	Documentation of the threshold limit values and biological exposure indices. Sixth Edition. 1991	120	1	51
	Andre C, et al. The PML and PML/RARα domains: from autoimmunity to molecular oncology and from retinoic acid to arsenic. Exp Cell Res 1996; 229:253-260.	61	1	62
	Rousselot P, Labaume S, Marolleau JP, et al. Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. Cancer Res 1999; 59:1041-1048.	27	3	162

Pharmacokinetics

	Brunet Cl, Luyckx M, Cazin M: Etude pharmacocinetique de l'anhydride arsenieux chez la souris. Toxicol Eur Res 1982; 4:175-179.	126	1	121
	Hall LL, George SE, Kohan MJ, Styblo, M, Thomas DJ: In vitro methylation of inorganic arsenic in mouse intestinal cecum. Toxicol Appl Pharmacol 1997; 147:101-109.	137	2	126
	Styblo M, Thomas DJ: Binding of arsenicals to proteins in an in vitro methylation system. Toxicol Appl Pharmacol 1997; 147:1-8.	161	3	210
	Yoshida K, Inoue K, Kuroda K: Urinary excretion of arsenic metabolites after long-term oral administration of various arsenic compounds to rats. J Toxicol Environ Health Part A 1998; 54:179-192.	169	5	35
	Yamauchi H, Yamamura Y: Metabolism and excretion of orally administrated arsenic trioxide in the hamster. Toxicology 1985; 34:113-12 1.	168	5	26
	Buchet JP, Lauwerys R. Study of inorganic arsenic methylation by rat liver in vitro: Relevance for the interpretation of observations in man. Arch Toxicol 1985; 57:125-129.	127	1	131
	Nixon DE, Moyer TP: Arsenic analysis II: rapid separation and quantification of inorganic arsenic plus metabolites and arsenobetaine from urine. Clin Chem 1992; 38:2479-2483.	155	3	144

Toxicology

	Harrisson JW, Packman EW, Abbott DD: Acute oral toxicity and chemical and physical properties of arsenic trioxides. AMA Arch Indust Health 1958; 17:118-123.	138	2	136
	Hilmy AM, El-Domiaty NA, Kamal MA, Mohamed MA, Abou Samra WE: Effect of some arsenic antagonists on the toxicity, distribution and excretion of arsenite and arsenate in rats. Comp Biochem Physiol 1991;99C:357-362.	140	2	144
	Ishinishi N, Tomita M, Hisanaga A: Study on chronic toxicity of arsenic trioxide in rats with special reference to the liver damages. Fukuoka Acta Med 1980; 71:2740.	146	2	313

Carcinogenicity

	Mass MJ, Wang L: Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutat Res 1997; 386:263-277.	154	3	129

Reproductive Toxicology

	Willhite CC, Ferm VH: Prenatal and developmental toxicology of arsenicals. Advances in Experimental Medicine and Biology 1984; 177:205-228.	167	5	1
	Holson JF, Stump DG, Ulrich CE, Farr CH: Absence of prenatal development toxicity from inhaled arsenic trioxide in rats. Toxicol Sci 1999; 51:87-97.	141	2	150
	Stump DG, Clevidence KJ, Knapp JF, Holson JF, Farr CH: Evaluation of the teratogenicity of sodium arsenate and arsenic trioxide following single oral and intraperitoneal administration in rats. Teratology 1998; 57:217 (Abstract; data now published)	159	3	209

	Stump DG, Clevidence KJ, Knapp JF, Holson JF, Farr CH: An oral developmental toxicity study of arsenic trioxide in rats. Teratology 1998; 57:216-217. (Abstract; data now published)	160	3	208

GENETIC TOXICOLOGY

	Rudel R, Slayton TM, Beck BD: Implications of arsenic genotoxicity for dose response of carcinogenic effects. Regul Toxicol Pharmacol 1996; 23:87-105.	158	3	170

SPECIAL TOXICOLOGY STUDIES

	Kreppel H, Reichl FX, Szinicz L, Fichtl B, Forth W: Efficacy of various dithiol compounds in acute As_2O_3 poisoning in mice. Arch Toxicol 1990; 64:387-392.	151	3	89
	Kreppel H, Paepcke U, Thiermann H, et al: Therapeutic efficacy of new dimercaptosuccinic acid (DMSA) analogues in acute arsenic trioxide poisoning in mice. Arch. Toxicol 1993; 67:580-585.	152	3	95

Studies previously reviewed: none

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION AND DRUG HISTORY

The toxicology of inorganic arsenic has been extensively studied due to human risk associated with potential environmental exposure through drinking water, several documented large-scale population exposures, exposure in certain occupations, and medical uses (e.g., Fowler's solution of potassium arsenite). Arsenic also may be an essential nutrient for humans. Although a minimum daily requirement has not been established, it is probably in the range of 12-50 μg. Arsenic has been linked to cancers of the skin, liver, bladder and kidney; cardiovascular disorders; and to skin lesions of the hands and feet (hyperpigmentation and hyperkeratosis). Sources of inorganic arsenic include primary copper, zinc and lead smelters, glass manufacturers that add arsenic to raw materials, and chemical manufacturers (Goyer, 1996). Most US drinking water contains a few micrograms per liter or less of arsenic but some people may be exposed to 50 ug/l. In certain parts of the world levels as high as 3.4 mg/L have been reported. Another source of arsenic is food. Total daily intake of arsenic is estimated at 0.3 mg/d.

Inorganic arsenic forms arsenate (As^{+5}) and arsenite (As^{+3}) ions in solution. Arsenic trioxide, along with sodium arsenite ($NaAsO_2$) and arsenic trichloride, are the most common environmental forms of trivalent arsenic compounds. The mechanisms of action of trivalent and pentavalent forms of arsenic are thought to be different. Trivalent arsenic is thought to bind electron-rich sulfhydryl groups on proteins, including structural proteins of the cytoskelton, and inhibit enzymes of the mitochondrial citric acid cycle by binding to thiol-containing active sites. In contrast, arsenate is thought to substitute for phosphate during glycolytic phosphorylation, thus inhibiting ATP formation. Additional arsenate toxicity may be mediated through metabolic reduction to trivalent compounds.

Most acute promyelocytic leukemia (APL) cases are associated with a specific t(15: 17) translocation in which the gene for the PML protein fuses with the gene for retinoic acid nuclear receptor, creating PML/RARα and RARαPML fusion genes. The t(15;17) translocation is detected in as many as 90% of APL patients and has become the definitive marker of the disease. Most patients with APL express the PML-RARα mRNA and 70-80% express RARα-PML mRNA. Expression of PML-RARα is sufficient for oncogenicity; however, potentiation of APL development by RARα-PML has been suggested (Pollock et al., 1999).

Normal PML protein is found in the nucleus localized on discrete subnuclear structures identified b, electron microscopy (PML nuclear bodies, NBs) that are part of the nuclear matrix. PML may serve as a growth suppressor. The gene product for the PML-RARα may displace the PML protein to a different ill defined subcellular structure, antagonizing normal PML function. Retinoic acid treatment of cultured cells and transgenic mice induces disappearance of the PML-RARα chimeric protein and restores normal PML-NBs.

PHARMACOLOGY

Sponsor's proposed mechanism

Apoptosis of leukemic cells has been identified as the most probable mechanisms through which arsenic trioxide induces remission in APL patients. Evidence for this includes morphological changes and DNA fragmentation in cultured NB4 cells, a cell line modeling APL. This apoptosis is not due to arsenic effects on the PML-RARα protein. Initially, there was some indication that arsenic trioxide-induced apoptosis involved down regulation of bcl-2 expression whereas other genes related to apoptosis were not affected. However, bcl-2 expression occurs only at non-pharmacological levels (10^{-5} M) and in some cells apoptosis is not related to changes in bcl-2 expression. Instead, the sponsor now suggests that induction of apoptosis by arsenic trioxide is related to enzymatic regulation of cellular H_2O_2 content. High levels of intracellular H_2O_2 result in mitochrondial membrane degradation, cytochrome c release, caspase activation, and DNA fragmentation. Sensitivity to arsenic trioxide-induced apoptosis may be modulated by intracellular glutathione levels, which may affect the redox potential of cells. Sensitivity is inversely proportional to GSH content. Arsenic trioxide also induces apoptosis in a number of non-APL cell lines.

ARSENIC EXERTS DUAL EFFECTS ON APL CELLS

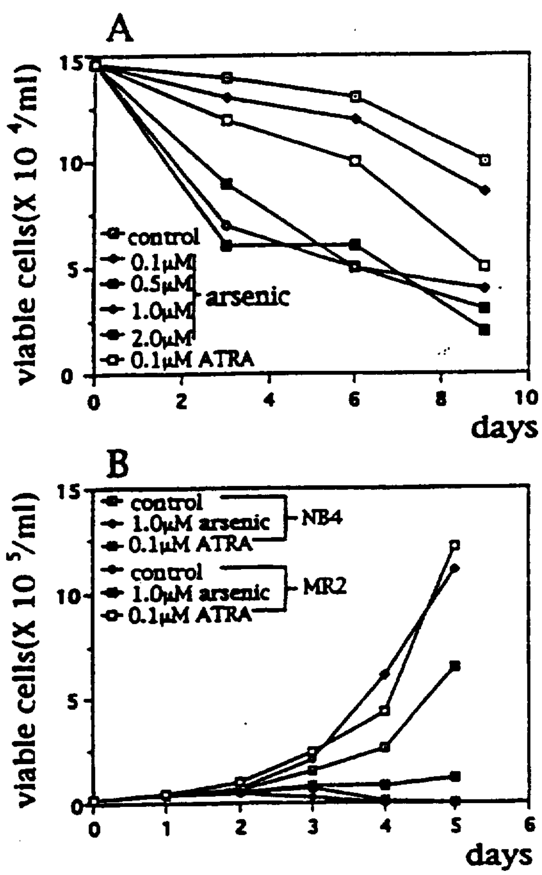

Fig 1. Effects of As_2O_3 on APL-cell growth. (A) Primary BM cells from one relapsed APL patient were incubated with As_2O_3 (0.1 to 2.0 μmol/L) and ATRA (0.1 μmol/L) for 9 days. Fresh APL cells from five other patients had similar results (not shown); (B) ATRA-susceptible NB4 cells and ATRA-resistant-MR2 cells were incubated with As_2O_3 (1 μmol/L) and ATRA (0.1 μmol/L) for 5 days. Viable cell numbers were calculated with trypan-blue exclusion. Every point represents the mean of triplicated samples.

The effect of arsenic trioxide on primary APL cells from bone marrow and the NB4 cell line is shown in the graphs.

Data from Chen et al., 1997.

Pharmacology Summary

Based on a review of the scientific literature, primarily in experiments using cultured cells, the sponsor proposed that inhibition of enzymatic regulation of peroxide formation by arsenic trioxide results in inducion of apoptosis. Early evidence for this pathway was the association of high peroxide levels with arsenic-induced apoptosis. More recent work extended this observation U937 monocytic leukemic cells, which have a lower peroxide concentration than NB4 cells. Use of catalase inhibitors renders U937 cells susceptible to arsenic trioxide-induced apoptosis. Arsenic trioxide (5 mg/kg ip for 8 days) has been shown to induce tumor regression and apoptosis in syngenic mice injected with leukemic blasts derived from PML/RARα transgenic mice (Lallemand-Breitenbach et al., 1999). The mechanism of apoptosis induction by arsenic *in vivo* has not been demonstrated. Arsenic-induced apoptosis may be multifactoral in origin and is the subject of current mechanistic investigations.

SAFETY PHARMACOLOGY

No studies reviewed.

PHARMACOKINETICS/TOXICOKINETICS

Distribution

The following information was obtained from Fielder et al., 1986.

Arsenic is widely distributed following administration. There does not appear to be any particular tissue or organ system susceptible to accumulation in any species examined, with the exception of the rat and marmoset. In these species arsenic tends to accumulate in the erythrocyte and liver, respectively. In the rat, administration of 250-300 pg arsenic/kg im resulted in 61% of the arsenic present in erythrocytes at 24 hr postdose and 44% present after 4 days. In another study, intravenous administration of 2-4 μg arsenic/kg arsenite or arsenate salt resulted in 10% of the administered material in blood at 5 minutes post dose. After 4 hours, 67% of arsenite but only 28% of arsenate remained in the blood. [The higher level at 4 hours compared to 5 minutes was not explained.] After 5 minutes, 16% and 11% of arsenite was found in the liver and kidneys, respectively, declining to 3% and 2% respectively, at 4 hours. In other species examined (mice, rabbits, guinea-pigs, dogs and primates), 1% or less of an administered dose (sc, im, iv) was found in the blood 1-2 days postdose. At this timepoint, liver, kidney, spleen, muscle, bone and lung contained 0.5-5% of an administered dose.

In mice following an intravenous dose of 0.4 mg arsenic/kg (arsenite or arsenate salt), the highest concentration post dose was found in the liver, kidney, bile, lung, spleen, epididymis and GI tract. At 72 hrs, arsenic was present in the skin, hair, epididymis, liver and stomach at the highest concentrations. Arsenic levels in tissues tended to be higher after arsenite administration, with the exception of the skeleton, where arsenate is thought to substitute for phosphate in bone crystals. In addition to the above tissues, autoradiographic studies indicated accumulation in the lens of the eye, thyroid and vas deferens in mice and Syrian hamsters.

Inorganic arsenicals are low molecular species poorly bound to plasma proteins and thus expected to cross the placenta (DeSesso et al.). Placental transfer has been demonstrated in hamsters, rats, mice and primates in early and late pregnancy. Arsenic in the fetus appears to generally distributed. ~~Arsenic~~ may also be transferred to newborns through milk. Human milk contains about 3 μg/liter of arsenic (Goyer, 1996).

Metabolism

The metabolism of inorganic arsenic has been investigated in detail in laboratory animals and in humans. In all species investigated monomethyl (methylarsonic acid, MMA) and dimethyl (dimethylarsenic acid, DMA) arsenic are sequentially formed. In addition, in some species a trimethylated metabolite is formed. This metabolite has not been detected in humans. Pentavalent arsenics are probably reduced to trivalent arsenic prior to methylation. Dimethylarsenic acid is the major form excreted by humans. Methyl derivatives are generally less toxic than inorganic arsenic.

Following oral exposure of 0.5 mg, 50% of the dosed material was excreted in the urine as dimethylarsenic acid, 25% as methylarsonic acid, and the remainder as inorganic arsenic. Dosing at 1 mg resulted in a slightly reduced proportion of dimethylarsonic acid (42%), an increase in methyl arsonic acid (32%), suggesting saturation of the metabolic process.

Guinea-pigs possess low levels methyltransferase, and new-world animals (e.g., marmoset monkeys) do not possess methyltransferases for detoxifying inorganic arsenics. In contrast, other primates (Cynomolgus and Rhesus monkeys) readily methylate inorganic arsenics. The primary site of methylation of inorganic arsenics is probably the liver; low enzymatic activity is also reported in erythrocytes, brain, lung, intestine, kidney homogenates. Arsenic methyltransferase is a found primarily in the cytosolic fraction; thus, addition of Aroclor-treated rat microsomal S9 fraction in genetic toxicology studies is of questionable value.

Elimination

The following table contains a summary of studies reviewed by Fielder et al., 1986. Except as noted, studies refer to single dose experiments. (DMA, dimethylarsenic acid; MMA, methylarsonic acid)

Species	Dose	Route	Urine	Fecal	Comments
Mice	Up to 4 mg arsenic/kg as arsenite or arsenate salts	Oral	70-90%	6-9%	Time period 48 hours postdose; multiphasic elimination pattern [initial phase t ½ 4 hrs; second phase t ½ 2 days; third phase t ½ 15 days]
Mice	0.04 mg arsenic/kg as arsenate or arsenite	iv	> 90%	2.3%	Time period 48 hr; 52% inorganic As; 40 % DMA
Mice	0.65 mg arsenic/kg as arsenic acid	iv	87%	2%	Time period 48 hr postdose; 55% of urine amount was inorgainc arsenic and 43% was DMA; feces contained equal amounts of inorgainc arsenic and DMA
Syrian hamsters	0.4 mg arsenic/kg as arsenite or arsenate	oral	NR	NR	In urine: Arsenite: 67% in rapid phase (t ½ 4 hours); followed by 29% (t ½ 1 day) and the remainder with a t ½ of 14 days; similar results for arsenate
hamster	65-106 ng arsenic/kg as arsenic acid (pentavalent)	iv	NR	NR	In urine: Biphasic elimination: 65% with t ½ of 10 hr; 35% with t ½ of 4.5 days
Hamster	0.65 mg arsenic/kg as arsenic acid	iv	84%	4%	Time period 48 hr postdose; ~ equal amounts of inorganic acid and DMA
Hamster	0.5 mg arsenic/kg as arsenite	ip	31%	NR	5% found in bile in cannulated animals

dogs	Dose not reporteu, form arsenic acid	Oral or iv	95%	4%	Time period 10 days post dosing; 85% excreted in rapid phase (t ½ 4 hr); remainder secreted with a t ½ of 2.5 days
rabbits	0.04 mg arsenic/kg as arsenate or arsenite	iv	50% arsenite 63% arsenate	NR	Time period 48 hr postdose; urinary arsenic was DMA for arsenite but ~ equal inorganic and DMA for arsenate
rats	0.4 mg arsenic/kg as arsenite or arsenate	Oral	20% arsenite 35% arsenate (initial phase)	NR	Initial phase t ½ 8 hr; ~ equal amounts inorganic arsenate and DMA following arsenate dosing; remainder of dosed arsenic eliminated with a t ½ of 65 days
rats	500 μg/kg as arsenate	iv	6%	11%	Time period 24 hours; bile cannulated animals
rats	0.65 mg arsenic/kg as arsenic acid	iv	50%	0.7%	Time period 24 hr, predominantly inorganic acid
rats	0.2-4.6 mg arsenic/kg as arsenic chloride	iv	8%	5%	Time period 24 hr; 25% of dosed material was found in bile in cannulated animals over a 2 hour time period; peak excretion in these animals was at 6 min
Marmoset	0.4 mg arsenic/kg as arsenite	ip	30%	4%	Time period 4 days; 8% excreted in urine in first 24 hr; no methylated derivatives found
Cynomolgus monkey	1 mg arsenic/kg as arsenic trioxide	oral	60%	NR	Time period 24 hr; 75% found in urine after 5 days
Human	3 mg/subject (~ 40 μg arsenic/kg as arsenite)	oral	48%	NR	Time period 5 days; overall t ½ 30 hr
Human	0.5 mg	oral	45%	NR	Time period 4 days; 50% DMA; 25% MAA and 25% inorganic acid
Human Repeat dose	1 mg (~ 15 μg arsenic/kg as arsenite or arsenic trioxide)	oral	60-70% (24 hour)	NR	Steady state urinary levels reached at 5 days; t ½ at 1 mg/d was 59 hr; at 125 μg/d was 39 hr
Human	Pentavalent arsenic radiolabel tracer dose	oral	40% - 2 days 60%- 1 week	4%-2D 6%-1wk	50% DMA; 25% MAA and 25% inorganic arsenic; whole body counting for 100 days indicates most radioactivity (66%) eliminated with t ½ of 2.1 days, followed by 30% with a t ½ of 9.5 days and the remainder with a t ½ of 38 days

Toxicokinetics

No studies are reviewed. No data are available to link any *in vivo* pharmacologic or toxicologic findings in preclinical pharmacology/toxicology studies to arsenic blood levels or AUC parameters. Lallemand-Breitenbach et al. (1999) report that 1 mg/kg ip was ineffective in mice whereas 10 mg/kg ip led to many early deaths (hepatic toxicity and pulmonary edema). A dose of 5 mg/kg, while much higher than the human dose, leads to blood levels in the range reported for arsenic-treated APL patients.

Pharmacokinetics summary

The principal route of arsenic elimination in animals and humans is renal. With the exception of the rat, human and animal elimination appears very similar. Significant amounts of arsenic are found in bile but very little is excreted in feces, indicating extensive absorption of secreted material. Elimination appears to be triphasic; the initial phase t ½ is about 1-2 days. The proportion of inorganic arsenic to methylated metabolites found in urine decreases as time post dose increases. Approximately 50% of the arsenic found in urine is dimethylarsenic acid and approximately equal amounts of methylarsonic acid and inorganic arsenic. In studies in mice, a higher proportion of inorganic arsenic compared to organic metabolites is found after iv dosing relative to oral dosing. The proportion of inorganic to organic arsenic in urine also increased with increasing dose.

TOXICOLOGY

Acute toxicology

Below are a summary of findings in acute toxicology studies as summarized by Fielder et al., 1986.

Species	Route	Compound	Dose	Comments
Mice	Iv	Sodium arsenite	11 mg/kg LD_{50}	None reported
Cats	Iv	Sodium arsenite	$\geq$ 0.5 mg/kg	Capillary dilation; hypotension
Rabbits	Iv	Trivalent salt		↑ vascular permeability
Rabbits	Iv	Arsenic trioxide	2 mg/kg (1.5 mg arseinc/kg)	Transient impairment of hepatic and renal function
Mice	ip	Sodium arsenite	LD50 1-20 mg/kg	Spasmodic contractions of the flanks, general distress, labored respiration and cyanosis (may in part refer to acute toxicity of sodium arsenate)
Rats	ip	Sodium arsenite	5 or 10 mg/kg	blood glucose significantly ↑ 1.5 and 3 hr post dosing; dose dependent; no effect in adrenalectomized animals
Mice	im	Sodium arsenite	8 mg/kg LD_{50}	None reported
Mice	sc	Sodium arsenite	17 mg/kg LD_{50}	None reported
Mice	sc	Potassium arsenite	10 mg/kg	None reported
Mice	sc	Sodium arsenite	15, 20, 25, 30 or 35 mg/kg	All doses lethal; survival times dose-dependent

Repeat dose studies – parenteral administration

Below are a summary of findings of repeat dose toxicology studies of arsenic administered parenterally as summarized by Fielder et al., 1986.

Species	Route	Compound	Dose	Schedule	Comments
Rats Sprague Dawley	ip	Arsenic trioxide	2, 5, 10 or 15 mg/kg 1.5, 3.8, 7.6 or 11.4 mg As/kg	Weekly x 18 mo	Study to investigate neuropathy HD died after 2nd injection: hemorrhage in GI and kidney HMD: (10 mg/kg) ↑ mortality (4/5 dead after 4 mo); bronchial pneumonia and hepatic congesion at autopsy LMD (5 mg/kg) no signs of toxicity; no pathology findings No evidence of neurotoxicity at any dose.
rabbits	iv	Arsenic trioxide	1 mg/kg (0.76 mg As/kg)	3x/wk x 12 weeks	Evidence of hepatic and renal impairment (↑ bromosulphthalein and phenolsulphonthalein retention time); no macro or microscopic pathology examination

Repeat dose studies – oral administration

By far most long-term toxicology studies were conducted by the oral route as this route is the most relevant to human risk assessment from environmental exposure. These studies have been reviewed in detail previously and are summarized below (Fielder et al., 1986; ATSDR, 1993).

Toxicological effect of repeat doses of arsenic trioxide in animal models.

Species	Dose	Schedule	Conclusion
Rat ♂ Wistar	0.125, 12.5 or 62.5 ppm in drinking water;	7 mo As + 4 mo water	↑ dose-related degenerative changes in liver and proliferation of bile duct with fibrosis; no other lesions in any organs.
Mouse ♂ hairless	5 or 50 ppm in drinking water	256 days	Histological evidence of dose dependent lesions in skin: kidneys, liver, spleen; testis effects HD only Skin: widespread inflammation and hemorrhage; granular appearance Kidney: distension of renal parenchyma and hyperemia of cortical interstitium and glomeruli; tubular deposits in HD Liver: vacuolation; deposits of Kupffer cells in dilated blood sinuses Spleen: ↑ white pulp, ↓ red pulp; necrotic foci in HD Testes: HD: detactment of germinal epithelium from basement membrane and dilation of interstitial vessels and edema Other tissues not examined
Rat Sprague Dawley	5, 7.5 or 10 mg/kg/d by gavage	D1-21 postpartum; observations to 30 wks of age	↑ mortality in weanlings dose dependent; deaths beginning D2, 5, 6 HD-LD, respectively
Rat ♂, ♀ Wistar-King	2, 10 or 20 mg/kg/d	LD: 6 d/wk for 40 doses; 7 d or 6 mo observation period following treatment MD: 7 d/wk for 40 doses; 0 day or 6 mo observation period HD: 1-2 doses	HD: lethal after 2 doses MD: impaired growth rate; skin: marked lesions include ulceration, hyperkeratosis; liver: cloudy swelling; spleen: follicular atrophy; adrenals: congestion; impaired performance in behavior tests LD: no overt toxicological signs or pathology findings; impaired performance in behavior tests
Rat Fischer F344 or Sprague Dawley	1.5, 3, 6 or 12 mg/kg/d	5 d/wk x 15 wk with 6 mo observation period	Behavioral study: ↓ undifferentiated motor activity after 3 weeks of treatment; ↓ weight gain in treated groups
rat	15 mg/kg/d gavage	5 d/wk 2-4 wk	Arteriolotoxicity study; ↓ vasoreactivity

Toxicological effects of repeat doses of arsenite salts in animal models.

Rat ♀ Wistar	5, 50 or 100 ppm as sodium arsenite in drinking water	7 weeks	No pathology: ↑ urinary uroporphyrins
Dog Beagle	5, 25, 50 or 125 ppm sodium arsenite in diet	2 years	HD: 5/6 animals died between 3-9 mo; 1/6 died after 19 mo; atrophy of spleen, bone marrow, testis and skeletal muscle NOEL was 50 ppm
Mouse ♂ ICR	50 ppm sodium arsenite drinking water (~ 6 mg/kg/d)	64 d	Liver study only: ultrastructural changes in hepatocytes
Mouse Non-inbred	5 or 50 ppm sodium arsenite (~ 0.6 or 6 mg As/kg/d)	1-64 d	HD: ↓ wt gain, relative wt of liver and spleen
mouse	5, 50 or 250 ppm sodium arsenite in drinking water	2, 4, 8, 16, 32 or 64 d	HD: ↑ group mean liver dehydrogenase through treatment; 44-70% of control
Dog ♀ Beagle	1, 2 or 4 mg/kg/d sodium arsenite in feed; ↑ to 2, 4 or 8 mg/kg/d on d 59-183	183 d	↓ body weight, feed consumption ≥ 4 mg/kg/d; ↑ AAT ≥4 mg/kg/d; ↑ AST ≥ 2 mg/kg/d; liver: no gross or microscopic findings; weight loss attributed to decreased feed consumption

Adverse effects of ingestion of inorganic arsenics have been observed in humans, including effects as shown below. The following data were summarized from ASTDR, 1993.

Toxic effects of arsenic in humans.

System affected	Human finding
Cardiovascular	Altered myocardial depolarization (prolonged Q-T interval, nonspecific S-T segment changes); cardiac arrhythmias; progressive loss of circulation in hands and feet leading to necrosis and gangrene; marked thickening of small and medium sized arteries in tissues, especially heart
Gastrointestinal	Nausea, vomiting, diarrhea and abdominal pain
Hematology	Anemia and leukopenia
Hepatic	Elevated enzymes; portal tract fibrosis
Kidney	↑ serum creatinine and bilirubin; renal failure rare, attributed to fluid imbalance or vascular injury; generally unremarkable
Dermal	Generalized hyperkeratosis and formation of hyperkeratotic warts or corns on palms and soles; hyperpigmentation interspersed with small areas of hypopigmentation on the face, neck and back; Blackfoot disease
Neurological	Acute high dose effects include encephalopathy, headache, lethargy, mental confusion, hallucination, seizures, and coma; chronic exposure effects include peripheral neuropathy

Toxicology Summary

The principal effects in humans from environmental exposure of arsenic include dermal, cardiovascular, GI, liver, and neurological effects. Dermal effects from low-level chronic exposure are considered the most sensitive toxicological endpoint. The relationship between arsenic trioxide exposure to these findings is uncertain; environmental exposure is often from arsenic mixtures (e.g., arsenate and arsenite salts and oxides), of unknown duration, and quantitatively suspect. Repeat oral dose animal studies indicate that arsenic trioxide and trivalent salts of arsenic may be responsible for some, if not all the toxic effects reported in humans. This is particularly true for hepatic and skin effects. Toxic effects of trivalent arsenic in animals on the cardiovascular system or neurologic damage are much weaker and not supported by histologic observations. The effect of trivalent arsenics in animal models on hematologic parameters has not been fully investigated. However, arsenic trioxide is clastogenic in mouse bone marrow assays. Repeat dose studies of arsenic by parenteral administration are inadequate for any conclusions to be drawn.

CARCINOGENICITY

No preclinical studies were submitted. For unknown reasons, arsenic is the only known human carcinogen that is not a carcinogen in animals, except perhaps by intratracheal instillation. Through epidemiology studies arsenic has been associated with cancers of the skin (basal cell carcinoma, squamous cell carcinoma and carcinoma *in situ*); lung; liver (hepatic angiosarcoma); bladder; kidney; and possibly other internal sites. The evidence that inorganic arsenic when ingested causes skin cancer is clear; the relationship between arsenic and internal cancers is a matter of some debate (Bates et al., 1992).

IARC (1987) lists arsenic as a Group I carcinogen (sufficient evidence for carcinogenicity to humans). The USEPA (1988) has listed arsenic and inorganic arsenic compounds as human carcinogens, classified as weight-of-evidence Group A compounds. Evidence of potential carcinogenicity from human studies was "Sufficient".

IMMUNOTOXICOLOGY

No studies were submitted or reviewed

REPRODUCTIVE TOXICOLOGY

Background note: Extensive reviews of reproductive and developmental toxicity of arsenic have recently been published in peer-reviewed literature. These reviews have focused on human risk from environmental sources of arsenic. Studies involving parenteral administration were considered irrelevant for this risk assessment. In contrast to the paucity of studies available examining general toxicology by the parenteral route, several GLP studies have been conducted to assess developmental toxicity of arsenic by parenteral administration. Several factors account for this difference in approach. First, parenteral administration of aqueous forms of arsenic is common in the hamster because of poor gastrointestinal absorption (~40%) in this animal compared to extensive absorption in humans and most experimental animals (> 90%). Humans appear more sensitive to arsenic toxicity than animals, and as early studies identified the hamster conceptus as especially sensitive to arsenite, many non-oral studies were conducted with the model. Second, several epidemology studies suggested that environmental exposure to arsenic is related to an adverse reproductive outcome, primarily spontaneous abortion and stillbirths. These studies were confounded by exposure of subjects to multiple chemicals and difficulty in relating time and dose exposure to a toxic endpoint. Developmental toxicology findings were also apparent in early animal studies, supporting a possible human risk. However, these studies were mechanistic in nature and designed to deliver a maximum dose, meaning via injection to bypass intestinal and hepatic metabolism, at a specific time in gestation, and often at maternally toxic doses. More recent studies were designed to compare effects after oral and parenteral exposure to arsenic and to assess dose-response relationships. The association between prenatal exposure to inorganic arsenic in laboratory animals and human risk was determined. Relevant studies and reviews are discussed in detail.

Wilhite CC. Arsenic-induced axial skeletal (dysraphic) disorders. Exp Molec Pathol 1981; 34: 145-158. The manuscript was not submitted as part of the NDA. Non GLP. Conducted by the author at the University of Arizona Health Sciences Center, Tucson, AZ.

Conclusion: A single intravenous dose of sodium arsenite at 2, 5 or 10 mg/kg produced neural tube defects in hamsters when administered on gestation day (GD) 7. Embryotoxicity as determined by % resorptions was dose-dependent. Litter incidences were not reported. Intravenous injection of 20, 50 or 100 mg/kg methylarsonic acid or dimethylarsinic acid on GD 7 failed to produce a significant teratogenic response. No sign of overt maternal toxicity was observed.

Species: golden hamsters
Drug: sodium arsenite ($NaAsO_2$)
Route intravenous
Treatment: single injection, gestation day 7, sacrifice GD 13
Maternal toxicity: none by clinical signs

Dose (mg/kg)	# litters	Implantation sites	Resorptions # (%)	Living fetuses				
						Abnormalities		
				Normal # (%)	Abnormal # (%)	Cranioschisis[a]	ribs	Kidney[c]
2[b]	5	68	3 (4)	58 (91)	6 (9)	4	0	2
5	5	68	14 (21)	33 (61)	21 (39)	19	5	2
10	5	64	58 (90)	2 (33)	4 (66)	4	1	1

[a] animals exhibited crainoschisis aperta and exencephaly; [b]No findings were observed in the water control group; [c] findings not detailed.

Stump DG, Holson JF, Fleeman TL, Nemec MD, Farr CH: Comparative effects of single intraperitoneal and oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 1999; 60: 283-291. No study number provided. Conducted according to GLPs by WIL Research, Ashland, OH. No QA/QC statement or signed GLP statement was included. The study was conducted to investigate the association between the route of exposure and maternal/development effects and to define the dose-response from exposure to environmental sources of arsenic.

Note: Arsenic trioxide was administered by gavage and by i.p. injection and sodium arsenate was administered by i.p. injection. Only the arsenic trioxide i.p. injection data are summarized.

Toxicokinetics: not examined
Male fertility: not examined
Fetal development: not examined
Prenatal and postnatal development: not examined

Conclusion: The authors conclude: "IP administration of inorganic arsenic on GD 9 increased the incidence of fetal malformations, especially exencephaly, microphthalmia/anophthalmia, and other craniofacial defects." The small number of surviving dams in the 15 mg/kg group precludes drawing any firm conclusions in this dose group. The incidence of localized edema reported in this study, while not statistically significant, exceeded historical controls and was located in the head/neck/thoracic regions. This finding was considered drug related. The occurrence of macroglossia and cephalocele ~~were~~ also considered drug related as neither abnormality has been observed in control animals in the authors' laboratory.

Reviewer's comment: The decreased body weight and food consumption in dams treated with 10 mg/kg is suggestive of maternal toxicity at this dose. The reduced weight of fetuses of dams treated at this dose may be a consequence of this maternal toxicity. However, in data not summarized, dams treated with 5-30 mg/kg arsenic trioxide p.o. showed reduced food consumption and body weight loss; no effect on fetal body weight or dysmorphogenesis was observed but an increase in resorptions was observed at 30 mg/kg. These data suggest a complex relationship between in maternal and fetal arsenic toxicity.

	NOAEL	LOAEL
Maternal	5 mg/kg	10 mg/kg
Developmental	5 mg/kg	10 mg/kg

Species: Crl:CD(SD)BR rats
Age/weight: 70 days old females
Number: 25/group
Drug: sodium arsenate (98.4% purity)
Arsenic trioxide (99% purity)

Compound	Dose (mg/kg)	As equivalents (mg/kg)
Arsenic trioxide (i.p.)	0, 1, 5, 10 or 15	0, 0.8, 3.8, 7.6 or 11.4

Lot no.: not provided
Schedule: single dose on gestation day 9 (GD 0 = presence of copulatory plug or sperm in vaginal smear); dams sacrificed on GD 20
Route ip arsenic trioxide
Volume: 2 mL/kg
Vehicle: water

	Observations
Mortality	2x/day
Sacrifice – planned	Day 20
Clinical signs	1 hr post dose
Body weights	GD 0, 6, 9, 10, 12, 15, 18 and 20
Food consumption	GD 0, 6, 9, 10, 12, 15, 18 and 20
In life observations Mating index Libido Assessment of fertility	Due to timing of dose, evaluating these parameters was not relevant.

Effects of arsenic trioxide (ip injection) on dams.

Dose (mg/kg)	0	1	5	10	15
# ♀	25	25	25	25	25
Maternal mortality (GD 10)	0	0	0	1	19
Nongravid				1	2
Gravid					17
# examined at termination	25	25	25	24	6
# pregnant at termination	19	19	17	22	4
Total # gravid	19	19	17	22	21
Maternal food consumption (g/animal/d)					
GD 9-10	22	22	20	6[a]	13[a]
GD 9-20	26	25	26	21[a]	26
GD 0-20	24	24	24	22[a]	23
Maternal bw GD 20 (g)	416	420	421	350[a]	420
Maternal bw change (g)					
GD 9-10	4	3	4	-15[a]	-1
GD 9-20	120	117	118	46[a]	115
GD 0-20	168	167	168	95[a]	166
Net maternal bw change (g)	81.0	81.7	78.8	63.2[a]	78.3

Clinical signs: 4 animals in the 10 mg/kg group exhibited rales or peri-orbital and/or buccal red exudates on GD 10.

[a] statistically significant from control ($p < 0.01$)

Gestational data for arsenic trioxide (ip injection) effects.

Dose (mg/kg)	0	1	5	10	15
No gravid ♀ examined	19	19	17	22	4
No corpora lutea per litter	17.2	18.3	17.4	16.9	18.5
No. implantation sites per litter	16.2	16.2	16.8	15.5	17.0
No live fetuses per litter	15.2	14.9	15.7	5.7[a]	15.8
Preimplantation loss per litter					
No.	1.0	2.1	0.6	1.4	1.5
%	5.5	10.7	3.2	7.8	8.1
Resorptions (%/litter)					
Early	5.8	7.9	6.7	64.8[a]	7.6
Late	0.3	0.4	0.0	0.3	0.0
Total	6.1	8.2	6.7	65.1[a]	7.6
No litters totally resorbed (%)	0 (0)	0 (0)	0 (0)	9 (40.9)	0 (0)
Postimplantation loss per litter					
No.	1.0	1.3	1.1	9.9	1.3
%	6.1	8.2	6.7	65.1[a]	7.6
Fetal weight (g)					
♂	3.8	3.9	3.9	3.3[a]	3.7
♀	3.7	3.7	3.7	3.3[b]	3.6
combined	3.7	3.8	3.8	3.3[a]	3.7
% ♂/litter	49.5	46.0	45.2	56.2	46.6

[a] statistically significant from control ($p < 0.01$)
[b] statistically significant from control ($p < 0.05$)

External malformations and developmental variations in rats following maternal exposure to arsenic trioxide (i.p. injection).

Dose (mg/kg)	0	1	5	10	15
No examined (fetuses/litter)	289/19	283/19	267/17	125/13	63/4
Exencephaly				6/3	
Cephalocele				1/1	
Micro/anophthalmia				8/3	
Macroglossia				2/1	
Omphalocele					1/1
Anury				1/1	
Localized edema	1/1			3/3	
Number with malformations	1/1	0/0	0/0	11/4	1/1
% per litter with malformations	0.3	0.0	0.0	23.6[b]	1.6
No with variations	0/0	0/0	0/0	0/0	0/0

[b] statistically significant from control ($p < 0.05$)

Machado AF, Hovland DN, Pilafas S, Collins MD. Teratogenic response to arsenite during neurulation: Relative sensitivities of C57BL/6J and SWV/Fnn mice and impact of the splotch allele. Toxicol Sci 1999; 51: 98-107. Non GLP study. The authors are from the Department of Environmental Health Sciences, UCLA. Data for the SWV/Fnn mice are not summarized.

Conclusion: Arsenite at 10 mg/kg ip was teratogenic. The spectrum of malformations depended upon the gestational time point of exposure. No differences were seen at the 5 mg/kg dose compared to controls. Mutation in the splotch gene increased sensitivity to arsenic-induced malformations.

Species:	C57BL/6J ♀ mice x C57BJ/6J ♂ mice
	C57BL/6J ♀ mice x C57BJ/6J *Sp/+* ♂ mice (see below for description of *Splotch* lesion)
Drug:	sodium arsenite
Doses:	5, 10, 15 or 20 mg/kg
Route:	intraperitoneal
Schedule:	injection on GD 8.0 (5, 10, 15 or 20 mg/kg groups)
	injection on GDs 6.5, 7.0, 7.5, 8.0, 8.5 or 9.0 (10 mg/kg group only)
	GD 0 = presence of vaginal plug; dams sacrificed on GD 18
Volume:	10 µL/g bw

Effect of sodium arsenite on administered by ip injection on GD 8 in C57BL/6J mice.

Dose (mg/kg)	Dams treated	Maternal lethality	Embryonic or fetal resorptions	Malformed fetuses	Fetal weight
Control	12	0	11.1%	3.4%	1.05
5	15	0	5.5%	9.3%	1.14[a]
10	23	0	36.2%[a]	45.0%[a]	1.01
15	4	0	100%[a]	--	
20	5	100%	--	--	

[a] significantly higher than control ($p \leq 0.05$)

Effect of Na arsenite (10 mg/kg, i.p.) on reproductive parameters and exencephaly in C57BL/6J mice.

Treatment day	# litters	Total implantations	Resorption %	Total fetuses	Avg fetal wt (g)	% exencephaly	Mean litter % exencephaly
Control	12	99	11.1	89	1.05	0	0
6.5	13	95	26.4[a]	69	0.88[a]	0	0
7.0	19	162	19.1	131	0.94[a]	15.4	10.9[a]
7.5	19	156	32.7[a]	105	0.95[a]	17.1	17.4[a]
8.0	23	221	36.2[a]	141	1.01	17.0	15.7[a]
8.5	13	110	35.5[a]	71	1.02	2.8	12.1
9.0	13	106	50.0[a]	53	1.01	0	0

[a] significantly different from controls ($p \leq 0.05$)

Mean litter % of selected fetal malformations and variations induced by Na arsenite (10 mg/kg, i.p.) administered C57BL/6J mice.

Treatment day	control	6.5	7.0	7.5	8.0	8.5	9.0
# fetuses examined externally	89	69	131	105	141	71	53
Wilson sections	46	34	63	49	70	34	27
Skeletal examinations	43	35	68	56	70	35	25
Malformations							
Exencephaly	0	0	10.9[b]	17.4[b]	15.7[b]	12.1	0
Omphalocele	0	3.5	6.0[b]	1.3	1.4	4.3	0
Hydrocephaly[a]	0	28.2[b]	81.6[b]	66.7[b]	56.7[b]	78.7[b]	42.5[b]
Enlarged bladder	0	12.2	15.1[b]	40.6[b]	29.1[b]	60.0[b]	15.3
Cardiovascular	0	3.8	35.2[b]	15.1[b]	4.4	0	2.5
Vertebral fusions	4.5	12.2	59.6[b]	48.0[b]	17.9	16.9	30.6[b]
Malformed vertebrae	2.3	23.7[b]	47.0[b]	70.4[b]	30.6[b]	29.8[b]	76.9[b]
Rib malformations	0	3.8	13.8[b]	21.9[b]	10.8[b]	26.3[b]	32.8[b]
Variations							
Subcutaneous hemorrhage	0	26.2[b]	6.6[b]	0.7	1.9	3.0	1.7
Cervical ribs	0	3.8	21.8[b]	35.4[b]	4.5	26.3[b]	0
Lumbar ribs	0	0	3.1	8.3	4.3	6.3	2.8

[a] excludes fetuses with exencephaly; [b] significantly different from control ($p \leq 0.05$)

Anophthalmia and microphthalmia due to arsenic treatment were discussed in the text but were not detailed in the data table.

Pax3 is a transcription factor expressed in the developing neural tube and crest cells and in limb buds. Splotch (*Sp*) is a *Pax3* mutant that demonstrates neural tube defects that are fatal in homozygous mice between gestation days 13 and 14. A statistically significant increase in mean litter % of fetal malformations (exencephaly, spina bifida aperta, an/microphthalmia, omphalocele, veterbral fusions and cervical ribs) was observed when *Sp/+* heterozygous ♂ mice were mated to +/+ ♀ mice compared to fetuses of +/+ x +/+ crosses. Genetic fingerprinting of fetuses indicated that the incidence of exencephaly and spina bifida aperta were statistically significantly increased in *Sp/+* heterozygote fetuses compared to +/+ fetuses. The incidence of exencephaly (%) was higher in female C57BL/6J fetuses compared to males (+/+ x +/+, 20% and 39% for ♂ and ♀; +/+ x Sp/+, 26% and 43% for ♂ and ♀). In contrast, spina bifida aperta is higher in male compared to female fetuses (8% vs. 4% in +/+ x Sp/+).

The following two manuscripts are recent reviews of reproductive and developmental toxicity submitted by the sponsor. The conclusions of the authors are presented.

DeSesso JM, Jacobson CR, Scialli AR, Farr CH, Holson JF: An assessment of the developmental toxicity of inorganic arsenic. Reproductive Toxicology 1998; 12: 385-433. The authors review available studies for oral, inhalation, intraperitoneal and intravenous administration of arsenic. The focus of the review was risk assessment for reproductive toxicology from environmental exposure to arsenic. The authors note in their introduction that few of the studies reviewed meet modern standards for safety assessment. Among the defects cited are low animal numbers used in most studies; lack of dose-response study design; and poor description of materials and methods.

Authors' Conclusion: "Inorganic arsenic induces neural tube defects and other malformations in rodents only when administered early in gestation (Gestational Days 7 [hamster, mouse], 8 [mouse] and 9 [rat]) and under the conditions of extreme dosages attainable exclusively by means of i.v. or i.p. injections. High-dose exposures by oral, inhalational, or dermal routes have not produced dose-related increases in neural tube or other defects. In GLP studies, repeated oral doses to rats, mice, and rabbits have also failed to cause a dose-related increase in malformations. Epidemology studies have not demonstrated a credible association between environmental exposure to inorganic arsenic and neural tube defects or other adverse pregnancy outcomes. Thus, it can be concluded that, under realistic human exposure scenarios, inorganic arsenic is unlikely to pose a threat to pregnant humans and their offspring."

Golub MS, Macintosh MS, Baumrind N: Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. J Toxicol Environ Health Part B 1998; 1: 199-241.

This review summarizes the developmental and reproductive toxicity of inorganic arsenic. The author reaches similar conclusions to that of DeSesso et al. In addition, the author reviews studies in which postnatal endpoints were evaluated. The following is slightly modified from the review (references omitted).

In an abstract report dated 1981, mice treated with sodium arsenite by gavage (5 mg/kg) or ip injection (2 or 4 mg/kg) on gestation day 1-17 were evaluated postnatally. No effect on postnatal weight or maturational indices were reported, but survival through 30 d of age was reduced. Forty-day-old mice from the 5 mg/kg oral group made more errors on the initial day of testing in a Lashley III maze. In an animal study of postnatal exposure, Nagaraja and Desiraju administered arsentae (5 mg/kg/d by gavage) to rats from 2-60 d of age and found changes in both behavior (operant learning and extinction) and regional neurotransmitter concentrations (cholinergic and adrenergic) 100 d after discontinuation of treatment.

Summary of reproductive toxicology

Reproductive and developmental toxicity studies for arsenic were not conducted by standard ICH design. Effects of arsenic on female fertility and fecundity, toxicity to the fetus (including effects on parturition), and lactation have not been assessed. Effects of arsenic on male fertility have not been thoroughly examined. Treatment of male mice by a single intraperitoneal injection of 4-12 mg/kg arsenic trioxide did not produce adverse effects on spermatogonial chromosomes. Reports of chronic oral dosing in general studies did not suggest any adverse effect on reproductive organs in either sex of mice, rats, hamster or dogs. Studies using radiolabeled arsenic indicate that the metal crosses the placenta and fetal exposure occurs.

A single intraperitoneal or intravenous injection of trivalent arsenic (arsenic trioxide, sodium arsenite) early in gestation produced neural tube defects in hamsters, mice and rats. In a recent rat GLP study, clear signs of toxicity to the embryo were observed at 10 mg/kg ip. Maternal toxicity at this dose included 1 death and decreased food consumption. No maternal toxicity or developmental effects were observed at 5 mg/kg ip. Similar findings occurred in mice; embryotoxicity was observed at 10 mg/kg but not at 5 mg/kg ip. No deaths or maternal toxicity was reported at the 10 mg/kg dose, but food consumption, considered a sensitive endpoint of maternal toxicity of arsenic, was not reported in this study. Common toxicity effects reported in both studies include increased resorptions, cranio-facial and neural tube defects, and omphalocele. Cardiovascular, bladder and skeletal defects were also observed in the mouse study. Studies in hamsters indicated that sodium arsenite from 2-10 mg/kg iv resulted in a dose dependent increase in resorptions, neural tube defects, and renal abnormalities.

Integrative Assessment of Concern for Human Reproductive and Developmental Toxicity based on practicing the approach described in the draft guidance.

Developmental toxicity studies were conducted with arsenic trioxide and sodium arsenite from 2-20 mg/kg. Single doses were administered by the iv or ip routes. Oral studies assessing reproductive risk from environmental exposure of arsenic are not considered relevant for assessing risk from parenteral administration because first pass metabolism limits exposure of the fetus. For example, administering pregnant mice 40 mg/kg sodium arsenate orally on day 17 of gestation resulted in a C_{max} for arsenic that was 30% of that obtained with a 20 mg/kg ip injection (6.93 and 2.05 µg As/mL, respectively). The t_{max} was reached at 2 hr and 10 min, respectively (reviewed in DeSesso et al., 1998).

The following is a practice of the reproductive risk analysis according to "Considerations in the Integration of Study Results for the Assessment of Concern for Human Reproductive and Developmental Toxicities", Final dated 2/28/2000. Insufficient information was available to assess reproductive toxicities, including fertility, parturition and lactation. Insufficient information was also not available to assess functional toxicities, the role of biomarkers, or relative exposures. Except as noted, the discussion below applies to dysmorphogenesis; little information is available regarding alterations to growth and mortality. The studies conducted with arsenic were not designed to be a complete assessment of developmental toxicity of arsenic but rather focused specifically on the restricted area of organogenesis.

Summary of concern for developmental toxicity

Assessment Factor	Developmental Toxicities		
	Mortality	Dysmorphogenesis	Alterations to Growth
Signal Strength I	+1	+1	+1
Cross-species concordance	↑	↑	↑
Multiplicity of effects	↑	↑	↑
Adverse effects as a function of time	-	-	-
Signal Strength II	+1	+1	-1
Maternal toxicity	↑	↑	↓
Dose response	↑	↑	-
Rare events	-	↑	-
Pharmacodynamics	+1	+1	+1
Therapeutic index	↑	↑	↑
Comparison of Biomarker Benchmarks	-	-	-
Similarity of pharmacological and toxic mechanisms	↑	↑	↑
Concordance to Humans	+1	+1	+1
Metabolism	↑	↑	↑
General toxicity profiles	-	-	-
Biomarker Profiles	-	-	-
Relative Exposure	NE	NE	NE
Class Alerts	NE	NE	NE
Total Score	+4	+4	+2

NE , not evaluated; ↑, enhanced concern; ↓, diminished concern; -, concern unchanged

A. Signal strength, part I:

Cross-species concordance observed in neural tube and craino-facial defects, increased early resorptions, skeletal defects and fetal weight loss enhanced concern. Implantation loss, late resorption, and other signs of mortality were not fully evaluated by these single injection studies. Crown-rump length and ano-genitial distance were not reported. Studies are inadequate to evaluate adverse effect as a function of time as studies entailed a single injection around GD 7-8. Developmental toxicity at earlier or later time points were not well studied. Since positive signals were seen in multiple developmental endpoints, concern is enhanced for each positive finding.

B. Signal strength, part II:

A reasonably clear association between maternal and developmental toxicity was observed in one GLP rat study at 10 mg/kg ip. In a hamster study, developmental toxicity (dose dependent ↑ resorptions, neural tube defects) similar to that found in rats was observed at the lowest dose tested, 2 mg/kg iv, in the absence of overt maternal toxicity. Toxic endpoints examined in this study were not reported. After a review of the literature, Golub et al. concluded, "It is not possible to determine whether fetal toxicity occurred in the absence of any maternal toxicity; however, it is possible to state that fetal toxicity occurred in the absence of severe maternal toxicity (>10% mortality)."

Similar to findings in rats and hamsters, injection of arsenic in mice at 10 mg/kg ip resulted in developmental toxicity similar to the other species. While not maternally lethal in the strain used (C57BL/6J) until 20 mg/kg, other potential toxicities (e.g., food consumption) were not discussed. In another study, heterozygous fetuses (*Sp*/+) had significantly increased incidences of exencephaly and spina bifida aperta compared to wild type fetuses (+/+). Together these data indicate that ~~maternal~~ toxicity may have contributed but was not alone sufficient to cause neural tube defects. While it is unknown if the Splotch animal model is inappropriately sensitive to developmental toxicants to assess risk to humans, it is recognized that animal models as measured by some toxic endpoints (e.g., carcinogenicity) are less sensitive to arsenic than humans.

Dose-response relationships: This element enhances concern for mortality and dysmorphogenesis, but not alterations to growth. A dose-response relationship (increased resorptions, neural tube defects) was observed in hamsters from 2-10 mg/kg iv. In addition, a time-effect relationship in dysmorphogenesis was seen in C57BL/6J mice injected with 10 mg/kg ip during GD 6.5-9.0

Rare events: This element enhances concern for the dysmorphogenesis endpoint. The finding of macroglossia and cephalocele were findings rare in treated rats and not observed in control animals in the authors' laboratory.

C. Pharmacodynamics:

Therapeutic index; concern is enhanced for dysmorphogenesis

Estimation basis	Calculation	TI
Dose of hamster dysmorphogenesis (LOAEL)/ human dose	$8.1\ mg/m^2/5.6\ mg/m^2$	1.4 enhanced concern
Dose of rat dysmorphogenesis (NOEL)/ human dose	$30\ mg/m^2/5.6\ mg/m^2$	5.3 unchanged concern
Dose of mouse dysmorphogenesis (NOEL)/ human dose	$15\ mg/m^2/5.6\ mg/m^2$	2.7 – enhanced concern

The therapeutic index for mortality and growth alterations are similar to calculations for dysmorphogenesis.

While the exact mechanism of arsenic trioxide action is unknown, it is likely that the pharmacodynamic activity and dysmorphogenesis are due to related mechanisms (e.g., apoptosis), enhancing concern.

D. Concordance between test species and humans:

Metabolic and drug distribution profiles: Profiles are similar among test species and humans. Concern is enhanced for all positive endpoints.

General toxicology profile: Similar profiles in general toxicology were observed in animal studies and in humans in oral dosing. Principal toxicity is to skin and liver; cardiovascular toxicity was not well investigated in animal models, particularly EKG parameters. Carcinogenesis animal models do not mimic human risk. Developmental toxicity studies based on epidemology are equivocal. Concern is unchanged.

E. Relative exposures: Not evaluated. Studies are inadequate to assess human and animal toxicokinetic parameters.

F. Class Alerts: Not evaluated. Studies involving both trivalent arsenic salts and oxides were considered in assessing risk of reproduction and development rather than a specific focus on arsenic trioxide as the metal is considered the principal toxic component. The human data for reproductive toxicity of arsenical drug products or other forms of arsenic are equivocal.

Summary/Integration of Positive Findings

Arsenic trioxide receives a range of scores from +2 to +4 for endpoints of fetal mortality, dysmorphogenesis and alterations to growth, indicating enhanced concern for humans. The quality and nature of the data (single dose ip or iv) relative to intended therapeutic application (repeat iv) prevents a full evaluation of the potential risk to reproductive and developmental toxicity of arsenic trioxide.

GENETIC TOXICOLOGY

Fielder RJ, Dale EA, Williams SD: Inorganic arsenic compounds. HSE Toxicity Review 1986; 16:1-66.

Mutagenicity assessment of arsenic compounds in microorganisms.

Compound	Amount	Activation	Strains	Results
Sodium arsenite	Concentrations toxic to bacteria	± S9	TA 1535, 1537, 1538, 98, 100	Negative
Arsenic trioxide	No details	No details	TA 1535, 1537, 1538, 98, 100 WP2, WP2 B/V	Negative
Sodium arsenite	0.4-2 mM	None	WP2	Negative
Arsenite salt	25 mM	None	Plate assay, WP44 S-NF	Negative
Sodium arsenite	0.8 mM 0.2 mM	None	WP2 WP2 *uvrA*	No conclusion
Sodium arsenite	Not provided	None	*Saccharomyces cerevisiae* D7	Weak pos

Authors' conclusions: "In summary, the ability of arsenic compounds to produce point mutations in micro-organisms has not been investigated in detail. There are two reports of negative results being obtained with both tri-and pentavalent arsenic compounds using *Salmonella typhimurium*, but insufficient details were given to enable an assessment of this work to be made. Negative or equivocal results were obtained in three studies using *Escherichia coli*. A weak positive result was observed in a single study using the yeast *Saccharomyces cerevisiae*, but in view of the negative results in the gene conversion assay, it is doubtful if the small increase in reverse mutations reported was of biological significance.

Thus there is no definite evidence from any study to indicate that arsenic compounds produce point mutations in microorganisms."

Effect of arsenic compounds in mammalian cell mutation assays.

Assay	Compound	Amount	Activation	Results
L5178Y mouse lymphoma cells	Sodium arsenite	Dose related increase (1.7-3.2 fold) from 0.5-2.0 µg/mL; no information regarding + S9; one time point (48 hrs)	± S9	Positive; no independent confirmation of results
Chinese hamster V79 cells	Sodium arsenite	0.5 µM for 2 days or 5 µM for 1 hr (ouabain resistance); 20 or 100 µM for 1.5 h (thioguanine resistance)		Negative

Authors' conclusion: "In summary, there is no clear evidence to indicate that arsenite or arsenate salts produce point mutations in mammalian cells, but this aspect has not been adequately investigated."

Chromosomal effects of arsenic compounds *in vitro*.

Assay	Compound	Amount	Result	Comment
Human fibroblasts MRC-5 and WI 38 cell lines	Sodium arsenite	MRC-5: 1.2-5.8 x 10^{-8}M WI 38: 2.9-58 x 10^{-9}M	+	dose dependent ↑ chromatid breaks
Chinese hamster lung fibroblast cells	Sodium arsenite	1 x 10^{-9} to 5 x 10^{-6} M	+	dose related ↑ chromosome aberrations excluding gaps
Chinese hamster ovary cells	Sodium arsenite	1-10 x 10^{-6} M	+	dose related ↑ chromosome aberrations excluding gaps
Syrian hamster embryo cells	Sodium arsenite	7.7 x 10^{-6} M	+	↑ chromosome aberrations excluding gaps
Human peripheral lymphocytes	Arsenic salt	0.5-5.0 x 10^{-6} M 48 hr incubation	+	dose related ↑ chromosome aberrations excluding gaps
Human peripheral lymphocytes	Arsenic salt	6.7-72 x 10^{-7} M 24 hr incubation	+	dose related ↑ chromosome aberrations excluding gaps
Human peripheral lymphocytes	Arsenic trioxide	3-36 x 10^{-7} M	+	dose related ↑ chromatid breaks
Human peripheral lymphocytes	Arsenic trichloride	6-72 x 10^{-7} M	+	dose related ↑ chromatid breaks
Human peripheral lymphocytes	Sodium arsenite	7.7-31 x 10^{-7} M 48 hr incubation	+	dose related ↑ chromatid breaks

Authors' conclusion: "Sodium arsenite has been shown to consistently produce chromosome aberrations in a number of different established cell lines. The effects were observed at concentrations down to about 1 x 10^{-9} M. The principal types of aberrations produced were chromatid breaks and exchanges."

"The cytogenic effects of arsenite salts have been extensively investigated by metaphase analysis of subcultures of PHA (phytohaemagglutin) stimulated human peripheral lymphocytes. An increased incidence of chromosome aberations was consistently obtained, the principal effect noted being chromatid breaks. Effects were noted at concentrations of the order of 5 x 10^{-7}M and above. An increase incidence of exchanges was also frequently observed. Arsenite salts were clastogenic (producing chromosome aberrations) in this system.

Chromosomal effects of arsenic compounds *in vivo*.

			Route/schedule	Comments
♀ mice	Sodium arsenite	10 or 100 ppm	Daily via drinking water for 8 weeks	Chromosome breaks and gaps observed; 1.6% at HD, 5.2% at LD; 1% in controls; no conclusion drawn
mice	Arsenic trioxide	4, 8 or 12 mg As/kg	Ip single dose	Negative; ♂ mice also negative for adverse effects on spermatogonial chromosomes

Authors' conclusions: "There is no evidence from these *in vivo* studies to indicate that arsenic compounds produced any significant clastogenic effects. However these studies were limited and this aspect has not been adequately investigated."

Jacobson-Kram D, Montalbano D: The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environmental Mutagenesis 1985; 7:787-804.

The authors reviewed the available data and concluded the following:

1) Arsenic is either inactive or extremely weak for the induction of gene mutations in vitro.

2) Arsenic is clastogenic and induces sister chromatid exchanges in a variety of cell types, including human cells, in vitro; trivalent arsenic is approximately an order of magnitude more potent than pentavalent arsenic.

3) Arsenic does not appear to induce chromosome aberrations in vivo in experimental animals.

4) Several studies suggest that human beings exposed to arsenic demonstrate higher frequencies of SCE and chromosomal aberrations in peripheral lymphocytes.

5) Arsenic may affect DNA by inhibition of DNA repair processes or by its occasional substitution for phosphorus in the DNA backbone.

IARC. Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42, in IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1987:71-76.

"Trivalent arsenic did not induce dominant lethal mutations in mice, but it produced a small increase in the incidence of chromosomal aberrations and micronuclei in bone marrow cells of mice treated *in vivo*. It induced chromosomal aberrations and sister chromatid exchanges in human and rodent cells *in vitro*, and transformation of Syrian hamster embryo cells; it did not induce mutation in rodent cells *in vitro*. It induced gene conversion in yeast but did not cause mutation or induce prophage in bacteria."

Tice RR, Yager JW, Andrews P, Crecelius E: Effect of hepatic methyl donor status on urinary excretion and DNA damage in B6C3F1 mice treated with sodium arsenite. Mutation Res 1997; 386:315-334. Methyl donor status was altered through dietary choline restriction (i.e., choline restriction reduces hepatic methyl donor activity, potentially decreasing arsenic metabolism).

Conclusion. Oral sodium arsenite increased the frequency of micronucleated PCE in hepatic methyl donor deficient (HMDD) and sufficient (HMDS) mouse bone marrow when administered by repeat but not as a single dose.

Species:	B6C3F1 male mice
Number:	4/group
Age:	8-9 weeks old
Route:	oral gavage
Dose:	2.5, 5 or 10 mg/kg
Purity:	not reported
Pos. control:	acrylamide at 100 mg/kg (single dose) or 50 mg/kg (repeat dose)
Schedule:	single or d x 4
Sacrifice:	24 (single and repeat doses) and 48 (single dose only) hrs following treatment
Volume:	10 mg/kg
Diet:	choline deficient for 2 weeks prior to arsenic treatment or standard diet

Effect of a single dose of arsenic on mouse bone marrow.

	Dose	HMDS mice				HMDD mice			
	(mg/kg)	24 hr sample		48 hr sample		24 hr sample		48 hr sample	
Sodium		MN-PCE*	% PCE	MN-PCE*	% PCE	MN-PCE*	% PCE	MN-PCE*	% PCE
arsenite	0.0	2.25	62.1	2.50	58.4	3.38	64.5	2.50	56.3
	2.5	3.88	52.5	1.75	65.1	2.38	62.9	2.75	51.2
	5.0	2.75	54.0	2.38	54.4	3.14	56.9	2.88	60.6
	10.0	2.50	65.0	2.25	46.0	3.63	54.0	2.38	45.6
acrylamide	100	6.13	60.1	3.75	55.8	5.50	60.6	5.13	45.6

* per 1000 PCE

Effect of repeat dose of arsenic on mouse bone marrow.

	Dose	HMDS mice				HMDD mice			
	(mg/kg)	Exp 1		Exp 2		Exp 1		Exp 2	
		MN-PCE*	% PCE	MN-PCE*	% PCE	MN-PCE*	% PCE	MN-PCE*	% PCE
Sodium	0.0	0.88	77.3	1.50	65.3	1.63	72.9	1.63	46.9
arsenite	2.5	1.75	70.8	2.13	68.1	1.38	68.1	0.75	53.6
	5.0	2.50[a]	75.5	3.00[a]	64.8	1.00	67.8	1.75	56.8
	10.0	5.00[a]	65.9[a]	3.88[a]	49.9[a]	3.25[a]	30.8[a]	3.88[a]	42.8
acrylamide	50	3.13[a]	69.3[a]	4.38[a]	59.9	3.13	68.9	6.50[a]	62.5

[a]significantly different from control.

Eguchi N, Kuroda K, Endo G: Metabolites of arsenic induced tetraploids and mitotic arrest in cultured cells. Arch Environ Contam Toxicol 1997; 32:141-145. DMA, MMA and TMAO are methylated metabolites of ingested arsenic. The purpose of the study was to investigate possible mechanisms of arsenic carcinogenicity.

Conclusion: Methylated metabolites of inorganic arsenics inhibited cell growth of cultured V79 cells to a lesser extent than parent compound. Methylated metabolites induced mitotic arrest; tetraploidy production was also observed in cells exposed to DMA and TMAO. The authors suggest that MMA, DMA and TMAO exert an incomplete mitotic arrest in metaphase, thus leading to tetraploidy formation.

Drugs:
- arsenate (As V)
- Arsenite (As III)
- Monomethylarsonic
- Dimethylarsinic acid
- Trimethylarsine oxide

Concentrations tested: 0.01 to 10 mg/mL
Purity: > 99% for all chemicals tested
Methods: Chinese hamster V79 lung cells were grown in Eagle's MEM supplemented with fetal bovine serum, penicillin, streptomycin, and BrdU. Colcemid was added for the last 2 hours of incubation. Cells were harvested, fixed to glass slides, and differentially stained. Metaphase figures with 40-50 chromosomes per cell were considered tetraploids.

Compound	IC_{50} Mg/mL	Mitotic index	Tetraploid index[a]
None	NR	3.6%	0%
Water	NR	4.3%	3.5%
Na arsenite	0.0008	1.7%	0%
Na arsenate	0.04	2.0%	0%

MMA	0.7	6.2%[b]	0%
DMA	3.3	11.4%[b]	47.3%
TMAO	> 10	5.1%	22.5%

[a](number of tetraploids/number of twice divided metaphase score)
[b] significantly different from group without arsenic ($p < 0.05$)
NR – not reported

In a separate experiment (data not summarized in this review), TMAO induced a statistically significant increase in the mitotic index from 4 mg/mL and the tetraploid index from 1 mg/mL. The magnitude of the increase in mitotic index and tetraploid index was concentration dependent for MMA, DMA and TMAO.

Kashiwada E, Kuroda K, Endo G: Aneuploidy induced by dimethylarsinic acid in mouse bone marrow cells. Mutation Research 1998; 413:33-38. Dimethyl arsenic is the principal metabolite of arsenic.

Conclusion: DMA induced aneuploidy in mouse bone marrow cells.

Species: male CD-1 mice
Number: 3/timepoint
Age: 6 week old
Drug: dimethylarsenic
Dose: 300 mg/kg bw
Route: ip
Purity of compound: not reported

Frequency of chromosome number of M_2 metaphases in mouse bone marrow cells 24 h after i.p. injection of DMA or saline.

	Number of cells with a given chromosome # (% of total counted)						
# of chromosomes	39	40*	41	42	50	>70	Total cells counted
DMA	2 (2.9)	38 (55.1)	18 (26.1)	8 (11.6)	1 (1.4)	2 (2.9)	69 (100)
Saline	1 (2.0)	47 (94.0)	2 (4.0)	--	--	--	50 (100)

*euploid

Summary of Genetic Toxicology

There is little evidence that arsenic is mutagenic in bacteria. Several reviews have concluded that trivalent arsenic is clastogenic *in vivo* and *in vitro*. In addition, several studies suggest that methylated metabolites of arsenic may be clastogenic.

SPECIAL TOXICOLOGY STUDIES

No studies were reviewed.

OVERALL SUMMARY AND EVALUATION

Arsenic trioxide is proposed for the treatment of patients with relapsed or refractory acute promyelocytic leukemia characteized by the presence of the t(15: 17) translocation . The dose proposed for this indication is 0.15 mg/kg/d for up to 60 days (5.6 mg/m^2/d). Most acute APL cases are associated with this translocation in which the gene for the PML protein fuses with the gene for retinoic acid nuclear receptor, creating PML/RARα and RARαPML fusion genes. Expression of PML-RARα is sufficient for oncogenicity; however, potentiation of APL development by RARα-PML has been suggested. Normal PML protein is found in the nucleus localized on discrete subnuclear structures identified by electron microscopy (PML nuclear bodies, NBs) that are part of the nuclear matrix. PML-RARα may displace the PML protein to a different ill defined subcellular structure, antagonizing normal PML function.

The sponsor did not conduct any preclinical toxicology studies. Below is a summary of information available in the scientific literature relating to arsenic, with particular emphasis on arsenic trioxide and other trivalent forms of arsenic.

Pharmacology

Arsenic induces apoptosis *in vitro* and *in vivo*. The exact mechanism through which apoptosis is induced is under current investigation. Experiments suggest that regulation of high intercellular peroxide levels and/or low levels of peroxide-regulatory enzymes may be essential for generation of reactive oxygen species and apoptosis. The pathway of apoptosis induction by arsenic *in vivo* or *in vitro* has not been convincingly demonstrated. In addition, a role for arsenic in restoring normal PML and RARα function and degradation of the chimeric PML-RARα protein has not been eliminated.

Pharmacokinetics

Distribution

Arsenic is widely distributed following administration. In humans, steady state urinary levels are reached after 5 days of oral dosing. There does not appear to be any particular tissue or organ system susceptible to accumulation, with the exception of the rat erythrocyte and marmoset liver. In the rat, intravenous administration of arsenite salt resulted in 10% of the administered material in blood at 5 minutes post dose. After 4 hours, 67% of arsenite remained in the blood. [The higher level at 4 hours compared to 5 minutes was not explained.] After 5 minutes, 16% and 11% of arsenite was found in the liver and kidneys, respectively, declining to 3% and 2% respectively, at 4 hours. In other species examined (mice, rabbits, guinea-pigs, dogs and primates), 1% or less of an administered dose was found in the blood 1-2 days postdose. At this timepoint, liver, kidney, spleen, muscle, bone and lung contained 0.5-5% of an administered dose.

In mice following an intravenous dose, the highest concentration post dose was found in the liver, kidney, bile, lung, spleen, epididymis and GI tract. At 72 hrs, arsenic was present in the skin, hair, epididymis, liver and stomach at the highest concentrations. Autoradiographic studies indicated accumulation in the lens of the eye, thyroid and vas deferens in mice and Syrian hamsters.

Inorganic arsenicals are low molecular species poorly bound to plasma proteins and thus expected to cross the placenta. Placental transfer has been demonstrated in hamsters, rats, mice and primates in early and late pregnancy. Arsenic in the fetus appears to generally distributed. Arsenic is transferred to newborns through milk.

Metabolism and Elimination

Inorganic arsenic is metabolized to mono and dimethyl arsenics in most species. The principal site of metabolism is probably the liver. Methylation appears to be a detoxification mechanism. Most elimination occurs by the kidney but some fecal elimination, usually less than 5%, occurs in the feces. Bile cannulated animals indicate that a substantial amount of arsenic is secreted within the first several hours in bile. Most of this material is reabsorbed. Elimination appears to be triphasic; the initial phase $t_{1/2}$ is about 1-2 days. The proportion of inorganic arsenic to methylated metabolites found in urine decreases as time post dose increases. Approximately 50% of the arsenic found in urine is dimethylarsenic acid and approximately equal amounts of methylarsonic acid and inorganic arsenic. In studies in mice, a higher proportion of inorganic arsenic compared to organic metabolites is found after iv dosing relative to oral dosing. The proportion of inorganic to organic arsenic in urine also increased with increasing dose.

Toxicology

The principal effects in humans from environmental exposure of arsenic include dermal, cardiovascular, GI, liver, and neurological effects. Dermal effects from low-level chronic exposure are considered the most sensitive toxicological endpoint. The relationship between arsenic trioxide exposure to these findings is uncertain; environmental exposure is often from arsenic mixtures (e.g., arsenate and arsenite salts and oxides), of unknown duration, and quantitatively suspect. Repeat oral dose animal studies indicate that arsenic trioxide and trivalent salts of arsenic may be responsible for most, if not all the toxic effects reported in humans. This is particularly true for hepatic and skin effects. Toxic effects of trivalent arsenic in animals on the cardiovascular system or neurologic damage are much weaker and not supported by histologic observations. The effect of trivalent arsenics in animal models on hematologic parameters has not been fully investigated. However, arsenic trioxide is clastogenic in mouse bone marrow assays. Repeat dose studies of arsenic by parenteral administration are inadequate for any conclusions to be drawn. Toxicokinetic evaluation of arsenic is not available.

Reproductive toxicology

Reproductive and developmental toxicity studies for arsenic were not conducted by standard ICH design. Effects of arsenic on female fertility and fecundity, including effects on parturition and lactation, have not been adequately assessed. Effects of arsenic on male fertility have not been thoroughly examined. Treatment of male mice by a single intraperitoneal injection of 4-12 mg/kg arsenic trioxide did not produce adverse effects on spermatogonial chromosomes. Reports of chronic oral dosing in general studies did not suggest any adverse effect on reproductive organs in either sex of mice, rats, hamster or dogs. Studies using radiolabeled arsenic indicate that the metal crosses the placenta and fetal exposure occurs.

A single intraperitoneal or intravenous injection of trivalent arsenic early in gestation produced neural tube defects in hamsters, mice and rats. Other findings include increased resorptions, cranio-facial and and omphalocele. Cardiovascular, bladder and skeletal defects were also observed in the mouse study. Studies in hamsters indicated that animals treated with sodium arsenite showed a dose dependent increase in resorptions, neural tube defects, and renal abnormalities. Differential embryofetal sensitivity of males and females to arsenic toxicity has not been examined in detail but studies in mice suggest that a sex difference may exist.

Studies in rats indicate that no developmental toxicity was observed after a single 5 mg/kg (30 mg/m^2) ip dose of arsenic trioxide. Studies in mice also indicated that no developmental toxicity was observed after a single ip injection of sodium arsenite at 5 mg/kg (15 mg/m^2). These levels are approximately 5.3 and 2.7 times the projected human daily iv dose on a body surface area basis. Studies in hamsters demonstrated developmental toxicity after a single dose of sodium arsenite at 2 mg/kg iv (8.2 mg/m^2). This was the lowest dose tested in this study. This is approximately 1.5 times the projected daily human dose on a body surface area basis. The reason for the increased sensitivity to developmental toxicity in studies involving hamsters compared to other rodents is unclear, but may include differences in route of administration or genetic sensitivity of the experimental animal.

Carcinogenicity

Arsenic is a known human carcinogen. Epidemiology studies have linked arsenic to cancers of the skin (basal cell carcinoma, squamous cell carcinoma and carcinoma *in situ*); lung; liver (hepatic angiosarcoma); bladder; kidney; and possibly other internal sites.

Genetic toxicology

There is little evidence that arsenic is mutagenic in bacteria. Several reviews have concluded that trivalent arsenic is clastogenic *in vivo* and *in vitro*.

RECOMMENDATION: The pharmacology/toxicology data supports approval of arsenic trioxide for the treatment of induction of remission and consolidation in patients with relapsed or refractory acute promyelocytic leukemia.

Label comments to follow.

/S/ 6/8/00

John K. Leighton, Ph.D., DABT Date
Biologist

/S/ 6/8/00

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cc:
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