

**NTP REPORT ON CARCINOGENS BACKGROUND
DOCUMENT for TAMOXIFEN**

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NTP Report on Carcinogens Listing for Tamoxifen

Carcinogenicity

Tamoxifen is *known to be a human carcinogen* based on studies in humans that indicate a causal relationship between exposure to tamoxifen and cancers of the uterine endometrium. However, there is also conclusive evidence that tamoxifen therapy reduces the risk of contralateral breast cancer in women with a previous diagnosis of breast cancer. IARC recently evaluated the carcinogenic risks of tamoxifen to humans and reached the same conclusion (IARC, V.66, 1996).

The potential effect of tamoxifen in increasing the risk of endometrial cancer has been reported in one adequate cohort study, four adequate case-control studies, and 14 randomized clinical trials.

The cohort study (Curtis et al., 1996) examined the effect of tamoxifen on risk of endometrial cancer in 87,323 women with breast cancer reported to the Surveillance, Epidemiology and End Results (SEER) program in the United States and found a statistically significant elevation of endometrial cancer in women who had received tamoxifen therapy. In two of the four case-control studies (Sasco et al., 1996; van Leeuwen et al., 1994), a nonsignificant elevation of risk for endometrial cancer was found, with a significant increase in risk with increasing duration of therapy in one of these studies (van Leeuwen et al., 1994). In the U.S. case-control study (Cook et al., 1995), no increase was seen, but a shorter duration of tamoxifen use was reported. In the fourth case-control study (Hardell, 1988b; cited by IARC V.66, 1996), increased risk of endometrial cancer for tamoxifen use was found, but confounding factors could not be eliminated.

In the two largest randomized clinical trials (Fisher et al., 1994; Rutqvist et al., 1995), there was a strong and statistically significant association between risk for endometrial cancer and use of tamoxifen. In the 12 other smaller trials, no statistically significant increases in endometrial cancer were seen, although 29 endometrial cancers were reported in tamoxifen-treated individuals and 14 in controls when these 12 studies were combined.

In 32 case studies, 102 cases of endometrial cancer were reported in women who received tamoxifen for breast cancer. One case series reported significantly more high-grade endometrial tumors in tamoxifen-treated breast cancer patients than in patients without tamoxifen use (Magriples et al., 1993); this difference, however, was not seen in six other studies.

MacMahon (1997) concluded that published results were suggestive of a causal association between tamoxifen use and endometrial cancer but were not conclusive because of confounding factors such as prior hysterectomy and/or hormone replacement therapy. Examining the same confounding factors, an IARC Working Group concluded that there is a positive association between tamoxifen use and endometrial cancer and cited several studies in support of this conclusion; the same potential confounders were considered unlikely to have a major effect on the reported relative risks (IARC, V.66, 1996).

Experimental animal studies also provide evidence of tamoxifen's carcinogenic effects. The IARC Working Group (IARC, V.66, 1996) reviewed experimental studies reported prior to 1996 and reached a similar conclusion. Tamoxifen, administered orally, was evaluated in one mouse study and eight rat studies. In mice, the incidences of benign ovarian and testicular tumors

were significantly increased after 3 months of treatment. In eight rat studies that varied in treatment lengths, tamoxifen induced preneoplastic liver lesions and benign or malignant liver tumors. One rat study reported a decrease in tumors in hormone-dependent tissues, but reduced weight gain may have been a contributing factor. In one additional study where tamoxifen was given by subcutaneous administration, mammary tumor development was inhibited in intact and ovariectomized mice (reviewed in IARC V.66, 1996).

Uterine abnormalities including endometrial carcinoma have also been reported in experimental animals exposed to tamoxifen. Rats receiving tamoxifen daily by oral gavage for 20 to 52 weeks were reported to have squamous cell metaplasia, dysplasia, and squamous cell carcinoma of the uterus while no comparable lesions were seen in controls (Mäntylä et al., 1996). Although not included in the IARC monograph, short-term developmental exposure to tamoxifen on days 1 to 5 of neonatal life has recently been reported to significantly increase the incidence of reproductive tract abnormalities in both female and male mice, including uterine carcinoma and seminal vesicle tumors (Newbold et al., 1996 abstr., 1997).

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Several studies reviewed by IARC (V.66, 1996) described tumor initiation/promotional and co-carcinogenicity attributes of tamoxifen. In mice, tamoxifen inhibited 3-methylcholanthrene-induced cervical cancer and virus-induced leukemia. In several studies with male and female rats, it enhanced liver tumors induced by *N*-nitrosodiethylamine. In one rat study, it enhanced the development of *N*-nitrosodiethylamine-induced kidney tumors; but in a number of other studies, it inhibited 7,12-dimethyl[*a*]benzanthracene-induced mammary tumors. In hamsters, two studies reported the inhibition of kidney and liver tumors induced by 17 β -estradiol.

Several reports in the literature (IARC V.21, 1979) demonstrate that women receiving estrogen replacement therapy unopposed by progesterone have a highly elevated risk for endometrial cancer. Because of these data, conjugated estrogens are considered known human carcinogens (IARC V.21, 1979; NTP, 1998 [*Report on Carcinogens, 8th ed.*]). Unlike the breast, where tamoxifen is an anti-estrogen (used to treat breast cancer because of this property), it acts as an estrogen agonist in the uterus. Therefore, tamoxifen would likely produce the same effects as conjugated estrogens in the uterus. Available data strongly indicate that endometrial cancer following exposure to estrogens is caused by estrogen receptor-mediated responses. DNA adducts have not been detected in human samples (IARC V.66, 1996) with one exception where low levels of DNA adducts were seen in leukocytes and endometrial tissue of breast cancer patients receiving tamoxifen (Hemminki et al., 1996, 1997).

In animal and *in vitro* experiments, tamoxifen readily forms DNA adducts in several tissues and cells, and either these adducts or the estrogenic activity of tamoxifen could be responsible for liver cancer observed in rodents exposed to tamoxifen.

Although tamoxifen is not mutagenic in bacteria, it is positive for micronuclei formation in human cells *in vitro* (Otto et al., 1996). *In vivo*, it increases aneuploidy and chromosomal aberrations in the livers of female Sprague-Dawley rats (Sargent et al., 1996).

NTP Report on Carcinogens 1997 Background Document for Tamoxifen

Available data indicate that the receptor-mediated mechanisms involved in the carcinogenic actions of tamoxifen are operative in humans. Genotoxic mechanisms may also be operative in people, but preliminary studies suggest that they are quantitatively less than in rodents.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

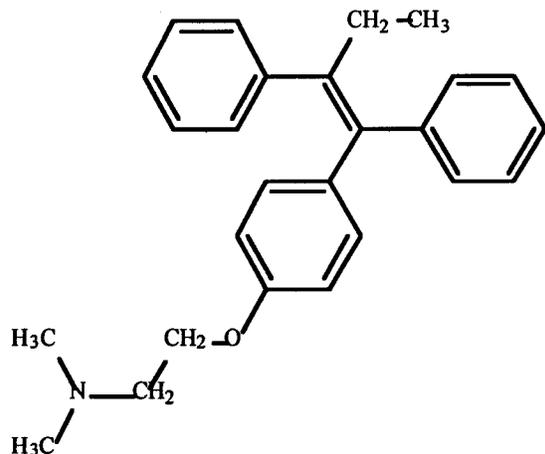
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

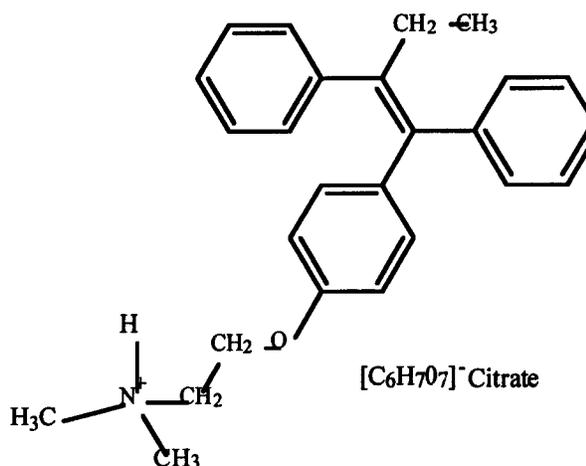
Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Tamoxifen
[10540-29-1]



Tamoxifen citrate
[54965-24-1]



1.1 Chemical Identification

1.1.1 Tamoxifen [10540-29-1]

Tamoxifen (C₂₆H₂₉NO, mol. wt. = 371.52) is also called:

1-*p*-β-Dimethylaminoethoxyphenyl-*trans*-1,2-diphenylbut-1-ene
cis-1-(*p*-2-(*N,N*-Dimethylamino)ethoxy)phenyl-1,2-diphenylbut-1-ene
 (*Z*)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethanamine
 (*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine
trans-Tamoxifen
Z-Tamoxifen

1.1.2 Tamoxifen citrate [54965-24-1]

Tamoxifen citrate (C₃₂H₃₇NO₈, mol. wt. = 563.65) is also known as:

(*Z*)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethanamine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)
 (*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine citrate (1:1)
Z-Tamoxifen citrate
 Nolvadex®
 Nolradex

1.2 Physical-Chemical Properties

1.2.1 Tamoxifen

Property	Information	Reference
Color	White	HSDB (1997)
Physical State	Crystal from petroleum ether	HSDB (1997)
Melting Point, °C	96-98	HSDB (1997)
<i>cis</i> -Form base	72-74 from methanol	Budavari (1996)
<i>cis</i> -Form citrate C ₂₆ H ₂₉ NO.C ₆ H ₈ O	126-128	Budavari (1996)

1.2.2 Tamoxifen Citrate

Property	Information	Reference
Color	White	HSDB (1997)
Physical State	Fine, crystalline powder	HSDB (1997)
Melting Point, °C	140-142	HSDB (1997)
Dissociation Constant at 25 °C (pKa)	8.85	Med. Econ. (1996; cited by IARC, 1996)
Odor	odorless	HSDB (1997)
Solubility:		
Water at 20 °C	Slightly soluble in water	HSDB (1997)
Organic Solvents	Soluble in ethanol, methanol, and acetone	HSDB (1997)
Equilibrium Solubility:		
Water 37 °C	0.5 mg/mL	PDR (1995)
0.02 N HCl 37 °C	0.2 mg/mL	PDR (1995)
Stability	Hygroscopic at high relative humidities, sensitive to ultraviolet light	IARC (1996)

When heated to decomposition, Nolvadex® (tamoxifen citrate) emits toxic fumes of NO_x (Lewis, 1992).

2.0 HUMAN EXPOSURE

2.1 Use

Tamoxifen has proven to be a successful palliative therapy for advanced breast cancer yielding response rates similar to those seen with other endocrine treatments, but with few side effects. It has been commonly used as the citrate as a primary therapy for breast cancer in elderly women who are considered poor candidates for surgery. Tamoxifen has been the adjuvant therapy of choice for postmenopausal, node-positive, and estrogen or progesterone receptor-positive women since the mid-1980s, and for postmenopausal, node-negative, and estrogen or progesterone receptor-positive women since the early 1990s. It is also being used in many cases of node-negative and receptor-positive premenopausal women. A high proportion (40-60%) of all women who undergo potentially curative surgery for breast cancer now receive adjuvant tamoxifen therapy for a period of 2 to 5 years (IARC, 1996).

First approved for pharmaceutical use in the United Kingdom in 1973 and in the United States in 1977 (Diogenes, 1997), tamoxifen is presently registered in 97 countries. Tamoxifen use has been estimated at more than 7 million patient-years. The usual dose in the United States and the United Kingdom is 20 mg/day for 1 to 2 years whereas in continental Europe, usual doses are 30 to 40 mg/day (IARC, 1996).

2.2 Production Process and Volume

Tamoxifen is produced by treating 4- β -dimethylaminoethoxy- α -ethyl-desoxybenzoin with phenylmagnesium bromide or phenyllithium to form 1-(4- β -dimethylaminoethoxyphenyl)-1,2-diphenylbutanol. Dehydration of the product yields a mixture of tamoxifen and its *E*-isomer, (*E*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethyl-dimethylamine, which may be separated with petroleum ether. For pharmaceutical preparations, tamoxifen is converted to the 1:1 citrate (Gennaro, 1995; cited by IARC, 1996).

The U.S. and British pharmacopoeias limit the *E*-isomer to not more than 0.3% and 1%, respectively, in tamoxifen and tamoxifen citrate (IARC, 1996).

Tamoxifen in pharmaceutical formulations is present as its citrate salt. Tamoxifen citrate is available as 15.2-, 30.4-, and 45.6-mg tablets. These correspond to 10, 20, and 30 mg of tamoxifen (IARC, 1996).

Two suppliers of tamoxifen citrate are listed in the *Chemcyclopedia 1997* (Strum, 1996). The product Nolvadex[®] is marketed by Zeneca Pharmaceuticals (PDR, 1995).

Production of tamoxifen citrate worldwide increased from approximately 15,000 lb [7.0 metric tons (Mg)] in 1989 to 19,000 lb (8.5 Mg) in 1991, 22,300 lb (10.1 Mg) in 1993, and 22,700 lb (10.3 Mg) in 1995 (IARC, 1996).

2.3 Environmental Exposure

Tamoxifen is not known to occur as a natural product (IARC, 1996).

2.4 Occupational Exposure

A U.S. National Institute of Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES) for 1981-1983 indicated that 350 employees were potentially exposed to tamoxifen in the workplace. Additionally, 2100 employees were potentially exposed to tamoxifen citrate (IARC, 1996).

2.5 Regulations and Criteria

Tamoxifen citrate was first allowed on the U.S. market in 1977 (equivalent to 10 mg base). The June 1997 edition of the New Drug Application List (NDL) lists both 10-mg and 20-mg base forms with indications for the treatment of metastatic breast cancer in premenopausal women as an alternative to oophorectomy or ovarian irradiation and for the treatment of panic disorder, with or without agoraphobia. In 1986, it was allowed in postmenopausal women as a single agent to delay breast cancer recurrence following total mastectomy and axillary dissection. In 1989, it was allowed in premenopausal women as an alternative to oophorectomy or ovarian irradiation. In 1990, it was allowed in women with axillary node negative breast cancer. In 1993, tamoxifen was permitted to be used for the treatment of metastatic breast cancer in males. In 1994, the FDA established a new strength (20 mg) and dosage regimen (once or twice daily) (Diogenes, 1997).

California listed tamoxifen as a carcinogen in May 1995. The expert committee, established for Proposition 65, decided to let the public know that tamoxifen use is likely to cause endometrial cancer. Zeneca Pharmaceuticals, the supplier of Nolvadex[®], did not challenge these findings (Mack, 1995).

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998, 03/27/75. U.S. Code: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, and 379e.	The regulations govern the proper labeling procedures for a drug and drug product.
	21 CFR 310—PART 310—NEW DRUGS. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, and 263b-263n.	Regulations govern the administrative rulings and decisions on new drug status, new drugs exempted from prescription-dispensing requirements, records, reports, and requests for specific new drugs or devices.

3.0 HUMAN STUDIES

3.1 Human Studies Reviewed by IARC (1996)

IARC (1996, pp. 260-280; see Appendix A) reviewed descriptive studies of single cases and case series, case-control studies, cohort studies, and randomized clinical trials involving tamoxifen (invariably as tamoxifen citrate) reported prior to 1996. Based on 1 adequate cohort study, 4 adequate case-control studies, and 14 randomized clinical trials, IARC (1996) concluded that tamoxifen use increased the risk of endometrial cancer. The positive case-control studies were considered evidence of cancer because potential confounders, while generally acknowledged, were not regarded as important relative to the magnitude of reported relative risks. Two of the 14 randomized clinical trials were considered particularly important because of the strong and statistically significant association between the use of tamoxifen and risk of endometrial cancer. The relative risk (odds ratio) for endometrial cancer was 5.6 (95% confidence interval or CI = 1.9-16.2) in the randomized clinical trial reported by Rutqvist et al. (1995; cited by IARC, 1996), and 7.5 (95% CI = 1.7-32.7) in the randomized clinical trial reported Fisher et al. (1994; cited by IARC, 1996).

3.2 Human Studies Published Post IARC (1996)

In a recent review on the association between tamoxifen use and increased risk of endometrial cancer, MacMahon (1997) concluded that the published results (including those reviewed by IARC, 1996), while suggestive of an association, were not conclusive. MacMahon (1997) based this conclusion on the fact that a positive association was not seen in all randomized clinical trials, that a deficit of endometrial cancer appears to have been present in the comparison groups of two of the most important studies, that none of the studies adequately addressed the problems of confounding by hormone replacement therapy and/or prior hysterectomy, and that none of the studies addressed the issue of detection bias. These same potential confounders were considered by the IARC Working Group and discounted as having a major effect on the reported relative risks (IARC, 1996).

A recent study (Rubagotti et al., 1996), which was not reviewed by IARC, does not show increased risk of endometrial or other cancer among breast cancer patients treated with tamoxifen (Table 3-1). Breast cancer patients (656) were treated with tamoxifen and followed up for 3 to 9 years; detailed information about secondary malignancies was available for all patients. Site-specific tumor incidence was compared to cancer incidence in the general population. A calculated risk ratio of 1.4 (95% CI = 0.2-5.1) was reported for secondary endometrial cancer among patients treated with 30 mg/day tamoxifen for 2 to 5 years; a risk ratio of 0.7 (95% CI = 0.0-3.9) was reported for the corresponding untreated group. The authors noted that the short follow-up times might explain the lower endometrial cancer incidence compared to other studies. The imprecise confidence intervals limit the usefulness of this study.

As concluded by IARC (1996) and by MacMahon (1997), a significant excess of any other cancer was not found in either the cohort study or the randomized clinical trials (a combined analysis of three Scandinavian clinical trials suggested an excess of gastrointestinal cancer; however, this has not yet been confirmed by other studies). A significantly reduced risk for contralateral breast cancer among women treated with tamoxifen was reported in several studies.

Table 3-1. Summary of Randomized Clinical Trial of Adjuvant Use of Tamoxifen (not reviewed by IARC, 1996): Endometrial Cancers in Patients Treated for Breast Cancer

Population Description	Exposed	Controls	Dose Duration Latency	Effects and Remarks	Reference
<p>Patients from the GROCTA trials and additional Italian clinic outpatients</p>	<p>656 tamoxifen (median age 59) and 220 tamoxifen + chemotherapy (median age 55)</p>	<p>410 no treatment (median age 60) and 410 chemotherapy only (median age 49)</p> <p>The number of observed cases of breast cancer was compared to the number of expected cases to give the Standardized Incidence Ratio and 95% confidence intervals (CI). The expected numbers of cases was derived from cancer incidence rates in the Lombardy Cancer Registry in 1983-1987 specified for age, sex, and cancer site.</p>	<p>30 mg/d</p> <p>2 to 5 yr</p> <p>median 40 mo (tamoxifen only) to 91 mo (tamoxifen + chemotherapy)</p>	<p>Risk ratio for endometrial cancer: 1.4 (95% CI = 0.2-5.1)</p> <p>The percent of premenopausal women was similar for the tamoxifen (22.7%) and no-treatment groups (22.9%). The authors noted that the short follow-up times might explain the lower endometrial cancer incidence compared to other studies.</p>	<p>Rubagotti et al. (1996)</p>

4.0 EXPERIMENTAL CARCINOGENESIS

Experimental carcinogenicity studies reported prior to 1996 are reviewed in IARC (1996, pp. 280-290, see Appendix A). IARC (1996) concluded that there was sufficient evidence for the carcinogenicity of tamoxifen in experimental animals. This conclusion is based on studies that demonstrated a significant increase in the incidence of benign ovarian and testicular tumors in mice (one study) and benign or malignant liver tumors in rats (eight studies) following oral exposure. More recent experimental carcinogenicity studies describe a significantly increased incidence of reproductive tract tumors in both female and male mice following short-term exposure to tamoxifen on days 1 to 5 of neonatal life (Newbold et al., 1996 abstr., 1997).

Also, in several studies, tamoxifen enhanced the hepatocarcinogenicity of previously administered *N*-nitrosodiethylamine in male and female rats, and the development of *N*-nitrosodiethylamine-induced kidney tumors in rats (one study). In contrast, treatment of rats with tamoxifen decreased the incidence of liver neoplasia induced by nitrosodiethylamine and 1-acetylaminofluorene, and of mammary tumors induced by 7,12-dimethyl[*a*]benzanthracene.

5.0 GENOTOXICITY

Genotoxicity studies are reviewed in IARC (1996, pp. 326-334; see Appendix A). Studies not considered in this review are integrated into the following summary and summarized in **Table 5-1**.

A number of studies have been conducted using ³²P-postlabeling to evaluate the ability of tamoxifen to induce DNA adducts *in vitro* in mammalian cells, in treated experimental animals, and in treated breast cancer patients. *In vitro*, tamoxifen was positive for the induction of adducts in DNA (in the presence of metabolic activation using liver microsomes from phenobarbital-induced rats), calf thymus DNA (with metabolic activation), and DNA of primary mouse and rat hepatocytes, and DNA of human lymphocytes (without metabolic activation). In addition, a study by Pathak et al. (1996) described the ability of rat uterine extracts with high peroxidase activity to further activate the tamoxifen metabolite, 4-HO-TAM, to form DNA adducts in rat uteri. However, tamoxifen was negative for DNA adduct formation in primary human hepatocytes and human endometrium (without metabolic activation). *In vivo* treatment with tamoxifen (by i.p. injection, gavage, or diet) induced DNA adducts in liver of male and female rats, mice, and Syrian hamsters. In addition, administration of tamoxifen by i.p. injection induced DNA adducts in the kidneys of female rats and lungs and kidneys of female mice. DNA adducts from HO-TAM were detected in uterine tissue of female rats following i.p. administration of tamoxifen at a dose of 20 mg/kg for 7 days but not at lower doses (Pathak et al., 1996). Three studies reviewed by IARC (1996) reported the lack of DNA adduct formation in liver, leukocytes, and endometrium of female breast cancer patients receiving tamoxifen daily for 2 to 108 months. However, Hemminki et al. (1996; 1997) later reported that DNA adducts could be detected in both leukocytes and endometrial tissue of breast cancer patients treated with tamoxifen if ³²P-postlabeling was based on high performance liquid chromatography (HPLC) rather than thin layer chromatography (TLC).

Tamoxifen is not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (with and without metabolic activation) (ICI, 1997; cited by Tannenbaum, 1997). *In vitro*, tamoxifen is positive for the induction of micronuclei in human

lymphoblastoid P450-expressing MCL-5 cells, human lymphoblastoid cells expressing CYP2E1 and CYP2A4 but not CYP1A1 or CYP1A2, and human breast cancer MCF-7 cells (Otto et al., 1996), all without metabolic activation; for apoptosis in human breast cancer MCF-7 cells (Otto et al., 1996), for chromosome aberrations in P450-expressing MCL-5 cells (Styles et al., 1997); and for aneuploidy and morphological transformation in Syrian hamster embryo cells. The positive response in Syrian hamster cells was replicated by Tsutsui et al. (1997).

Tamoxifen was negative *in vitro* for the induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes, for sister chromatid exchanges (SCE) in human lymphocytes, with and without metabolic activation (Wilson et al., 1995), for micronuclei in human AHH-1 cells (without metabolic activation), and for chromosome aberrations in Syrian hamster embryo cells (Tsutsui et al., 1997).

In vivo, tamoxifen was positive for the induction of *lacI* gene mutations (primarily G to T transversions) in the livers of female Big Blue[®] transgenic rats (Davies et al., 1997), for chromosomal aberrations and micronuclei in Swiss albino mouse bone marrow (Vijayalaxmi and Rai, 1996), and for chromosomal aberrations and aneuploidy in female Sprague-Dawley rat hepatocytes (Sargent et al., 1996).

Table 5-1. Summary of Additional Tamoxifen Genotoxicity Studies

System	Biological Endpoint	SCE/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response +/- Activation	Comments	Reference
Mammalian Systems <i>in vitro</i>							
Human lymphocytes	Sister chromatid exchanges (SCE)	+/-	NG in source used	50 µM for 2-h exposure (+S9) at 0 and 48 h after initiation of cultures or 30 µM for 72-h exposure (-S9) at 0 h to harvest at 72 h	Negative/Negative	No increase in SCE frequency was observed at any time point or exposure period.	Wilson et al. (1995)
Human breast cancer MCF-7 cells	MN induction and apoptosis	-	NG in source used	1 µM for 5-d incubation	Positive	Micronuclei were induced in treated cultures compared to controls (6.4±1.6 vs. 4.7±1.2%). The mitotic index was decreased (0.7±0.3 vs. 2.1±0.6%). The percentage of apoptotic cells was increased (6.12% vs. 0.88%).	Otto et al. (1996)
Human lymphoblastoid MCL-5 cells expressing CYP1A1, CYP1A2, CYP2E1, CYP3A4, and CYP2D6	Chromosomal aberrations and aneuploidy	-	NG in source used	0.25-10 µg/mL (0.67-27 µM) for 48 h	Positive	The incidence of cells with both structural and numerical aberrations was increased in treated cultures.	Styles et al. (1997)
Syrian hamster embryo (SHE) cells	Chromosomal aberrations and aneuploidy	n.a.	NG in source used	3, 10, and 30 µM for 24, 48 or 72 h	Negative (CA), positive (aneuploidy)	Dose-dependent increase in the percentage of aneuploid cells with a near diploid number of chromosomes.	Tsutsui et al. (1997)

Table 5-1. Summary of Additional Tamoxifen Genotoxicity Studies (Continued)

System	Biological Endpoints	S/DNA Adducts Activation	Reagent purity	Dose/Route	Metabolic Response/Activation	Comments	Reference
Syrian hamster embryo (SHE) cells	Morphological transformation	n.a.	NG in source used	3, 10, and 20 µM for 48 h followed by replating and incubation for 7 d	Positive	Morphological transformation was significantly induced in cells treated at the top dose (20 µM) only compared to controls (0.36% vs. 0.03% transformation).	Tsutsui et al. (1997)
Sprague-Dawley rat uterine extracts	DNA (calf thymus) adducts from two tamoxifen metabolites (4-HO-TAM and <i>cis/trans</i> metabolite E)	Uterine extract (2 mg) with peroxidase activity and hydrogen peroxide (1 mM) separate experiment with the model peroxidase HRP (15 U) and hydrogen peroxide (1 mM)	NG in source used	Daily i.p. treatment of female rats with 5, 10, and 20 mg/kg tamoxifen for 7 d, then <i>in vitro</i> study with 100 µM metabolite	Positive (all doses) in a dose-dependent manner only when H ₂ O ₂ in reaction mixture; no DNA adducts detected without the addition of H ₂ O ₂	Uterine peroxidase activity increased 10-fold in 7 d. There was good correlation between uterine peroxidase activity levels and DNA adduct levels from the two metabolites. DNA adducts were also detected in the liver of rats treated with all doses and in the uterus of rats treated with a tamoxifen dose of 20 mg/kg. The adduct in uterine tissue was the same as the adduct produced by uterine extract activation of 4-HO-TAM	Pathak et al. (1996)
Mammalian Systems <i>in vivo</i>							
Female Big Blue [®] transgenic rats	<i>lacI</i> gene mutations in liver	n.a.	Tamoxifen citrate; NG in source used	20 mg/kg/d (36 µmol/kg/d) p.o. for 6 wk with sacrifice 2 wk after the last dose	Positive	A significant increase in mutant frequency (approximately 3-fold over controls) was observed at the <i>lacI</i> gene. G→C to T→A transversions were the most common point mutations.	Davies et al. (1997)

Table 5-1. Summary of Additional Tamoxifen Genotoxicity Studies (Continued)

Species	Biological Endpoint	in Other Metabolic Activation	Form and Purity	Dose Used	End Point Positive / Activation	Comments	Reference
Female Fischer rats	DNA adducts (³² P-postlabeling) in liver, kidney, and uterus	n.a.	NG in source used	Following 70% partial hepatectomy, 250 and 500 ppm (672 and 1350 µmol/kg) in diet for 18 months	Positive (liver), negative (kidney, uterus)	Six major adduct spots were detected in all exposed livers. A low (but not significantly different from control) level of DNA adducts was observed in kidney.	Li et al. (1997)
Swiss albino mice	Chromosomal aberrations in bone marrow	n.a.	Tamoxifen citrate, NG in source used	0.1, 0.2, 0.4, and 0.8 mg/kg (0.2, 0.4, 0.7, and 1 µmol/kg) p.o. daily for 10 d followed by sampling at 24, 48, 72, and 96 h after the last dose.	Positive	The maximum yield of aberrations was observed at 24 hours (LED = 0.1 mg/kg) although the yield of aberrations was still significant at 96 h.	Vijayalaxmi and Rai (1996)
Female Sprague-Dawley rats	Chromosomal aberrations in hepatocytes	n.a.	NG in source used	35 mg/kg (94 µmol/kg) p.o. (single dose) followed by hepatocyte isolation 24 h later and 50 h in culture	Positive	Treatment resulted in a variety of chromosomal changes including chromosome breaks, exchanges, endoreduplications, and spontaneous G-banding.	Sargent et al. (1996)
Female Sprague-Dawley rats	Aneuploidy in hepatocytes	n.a.	NG in source used	35 mg/kg (94 µmol/kg) p.o. (single dose) followed by hepatocyte isolation 24 h later and 50 h in culture	Positive	Treatment resulted in an 85±7% incidence of aneuploidy compared to 3.0±3% in solvent controls.	Sargent et al. (1996)

Table 5-1. Summary of Additional Tamoxifen Genotoxicity Studies (Continued)

System	Biological Endpoint	Metabolic Activation	Form and Purity	Doses Used	Endpoint Response / Activation	Comments	Reference
Swiss albino mice	Micronuclei induction in bone marrow	n.a.	Tamoxifen citrate, NG in source used	0.1, 0.2, 0.4, and 0.8 mg/kg (0.2, 0.4, 0.7, and 1 µmol/kg) p.o. daily for 10 d followed by sampling at 24, 48, 72, and 96 h after the last dose	Positive	A significant increase in micronuclei was observed at 24 and 48 h (LED = 0.1 mg/kg) post treatment.	Vijayalaxmi and Rai (1996)
Human Studies <i>in vivo</i>							
7 Female breast cancer patients (ages 45-90)	DNA adducts (³² P-postlabeling and HPLC) in leukocytes	n.a.	Tamoxifen citrate	40 mg/d (110 µmol/d) for 4-21 mo	Positive	Four of the six samples showed a positive increase in DNA adducts (radioactivity was over twice the background).	Hemminki et al. (1997)
6 Female breast cancer patients (mean age 67)	DNA adducts (³² P-postlabeling and HPLC) in endometrium	n.a.	Tamoxifen citrate	20-40 mg/d (54-110 µmol/d) for 3-60 mo	Positive	Five of the six samples showed a positive increase in DNA adducts (radioactivity was over twice the background).	Hemminki et al. (1996)

* For purposes of conversion, tamoxifen was assumed if tamoxifen citrate was not specified.

Abbreviations: LED = lowest effective dose; n.a. = not applicable; NG = not given

6.0 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

6.1 Absorption, Distribution, and Excretion

In humans, tamoxifen is absorbed after oral administration and is readily bound (> 99%) by plasma proteins (Lien et al., 1989; cited by IARC, 1996). Patients with breast cancer showed steady-state mean plasma concentrations of 186-214 ng/mL after administration of 40 mg/day for 2 months (McVie et al., 1986; cited by IARC, 1996). Male volunteers showed peak plasma concentrations of 42 ng/mL tamoxifen and 12 ng/mL *N*-desmethyltamoxifen, following administration of a single 20-mg dose (Adam et al., 1980; cited by IARC, 1996). Steady-state concentrations of tamoxifen and *N*-desmethyltamoxifen were reached after 3 to 4 weeks of 40 mg/day administration (McVie et al., 1986; cited by IARC, 1996) and after 4 to 8 weeks of 20 mg/day administration (Lien et al., 1995; cited by IARC, 1996).

The distribution half-life (initial $t_{1/2}$) of tamoxifen is 7 to 14 hours (Adam et al., 1980; McVie et al., 1986; both cited by IARC, 1996). The apparent volume of distribution in humans is 50 to 60 L/kg (Lien et al., 1989; cited by IARC, 1996), suggesting extensive tissue binding (Lien et al., 1991; cited by IARC, 1996).

The plasma elimination half-life was 10 hours during the first day in male volunteers given 40 mg tamoxifen (Guelen et al., 1987; cited by IARC, 1996). However, significant levels of tamoxifen and *N*-desmethyltamoxifen were present after 34 hours, indicative of a lengthening of half-life with increasing study duration or the existence of biphasic half-lives. One study suggests biphasic pharmacokinetics with a terminal elimination phase of about 7 days (Fromson et al., 1973a; cited by IARC, 1996). Tamoxifen and metabolites are excreted as glucuronides and other conjugates (Furr and Jordan, 1984; cited by IARC, 1996).

6.2 Metabolites

Metabolites were identified in urine and plasma of breast cancer patients (Poon et al., 1993, 1995; cited by IARC, 1996). *N*-Desmethyltamoxifen, tamoxifen *N*-oxide, and six other metabolites were detected in plasma, while glucuronides of four hydroxylated metabolites (4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, dihydroxytamoxifen, and possibly a hydroxy-*N*-desmethyltamoxifen) were found in urine. In biopsy and autopsy tissue samples (liver, lung, pancreas, brain, adipose), tamoxifen metabolites (*N*-desmethyl-, *N*-didesmethyl-, 4-hydroxy-, and 4-hydroxy-*N*-desmethyl) were 10- to 60-fold higher than in serum, especially in liver and lung (Lien et al., 1991; cited by IARC, 1996).

Tamoxifen can be metabolized *in vitro* by microsomal cytochrome P450 and flavin monooxygenase pathways to metabolites that irreversibly bind to microsomal proteins (Mani and Kupfer, 1991; cited by IARC, 1996). In a human microsomal preparation, CYP3A4 and CYP2B6 were identified as active in tamoxifen catalysis to metabolites that bind to protein (White et al., 1993; cited by Smith and White, 1995). In human liver homogenate and a human hepatic G2 cell line treated with a mixture of tamoxifen and its deuterated analogs, several metabolites were detected, including α -hydroxytamoxifen, 4-hydroxytamoxifen, *N*-desmethyltamoxifen, and tamoxifen *N*-oxide (Poon et al., 1995; cited by IARC, 1996). *In vitro* studies show that tamoxifen-protein binding is greater in liver microsomes of rat (3.8-fold) and mice (17-fold) than of humans (White et al., 1993; cited by Smith and White, 1995).

Postulated metabolic pathways of tamoxifen are presented schematically by IARC (1996, page 293).

6.3 Structure-Activity Relationships

Toremifene is structurally similar to tamoxifen; toremifene has a chlorine instead of hydrogen atom in the ethyl group (Kuramochi, 1996). Both compounds have anti-estrogenic effects as demonstrated by their ability to compete with estrogen; they also have similar effects on estrogen-dependent breast cancer cell lines *in vitro* and *in vivo*, and have the same binding constant. However, tamoxifen is a heptocarcinogen in the rat while toremifene has not been demonstrated to induce rat liver tumors. Furthermore, in rat liver, DNA adducts are readily detected after treatment with tamoxifen, whereas toremifene has been associated with the induction of very few DNA adducts (Hard et al., 1993; cited by Kuramochi, 1996).

A study that examined the relationship between DNA-adduct forming ability and physicochemical properties of the two analogs strongly suggests that the stability of the carbocation intermediate arising from tamoxifen is greater than that for the carbocation intermediate arising from toremifene (Kuramochi, 1996). Two other tamoxifen derivatives, 4-iodotamoxifen and droxifene, which demonstrate no DNA-adduct-forming ability, are also expected to have less stable carbocation intermediates than tamoxifen.

7.0 MECHANISMS

7.1 Genotoxicity

A possible mechanism by which tamoxifen is carcinogenic is via the formation of DNA adducts induced by one or more genotoxic metabolite(s) (see section 5). Genotoxic metabolite(s) may be formed by the oxidation of tamoxifen to a DNA-reactive carbocation (Potter et al., 1994; cited by Kuramochi, 1996), or by the metabolism of tamoxifen to a DNA-reactive hydroxylamine intermediate (Cunningham et al., 1996). Epoxide metabolites that are potentially genotoxic were produced in rat, mouse, and human liver microsomal preparations (Lim et al., 1994; cited by Smith and White, 1995). Genotoxic metabolites, as judged by the induction of micronuclei in a human cell line, are produced by human cytochrome P450s (White et al., 1992; cited by Smith and White, 1995).

In support of this mode of action, a number of studies have demonstrated the ability of tamoxifen to induce DNA adducts *in vitro* in cultured mammalian cells (either after metabolic activation or using metabolically competent cells), and *in vivo* in multiple tissues of rats, mice, and Syrian hamsters. In breast cancer patients treated with tamoxifen, several investigators have reported the lack of DNA adduct formation in liver, leukocytes, and endometrium. However, Hemminki et al. (1996; 1997) using a modified ³²P-postlabeling technique, based on HPLC rather than TLC, reported the detection of DNA adducts in both leukocytes and endometrial tissue of breast cancer patients treated with tamoxifen.

Other studies support a causal relationship between *in vivo* genotoxicity and tumor response. Fifty percent of 24 hepatocarcinomas sampled from tamoxifen-treated female rats contained mutations in exons 5 to 9 of the *p53* gene (Vancutsem et al., 1994; cited by IARC,

1996). Nine of the 13 mutations detected involved A→G transitions in codon 231, while the other four mutations involved a silent C →T transition in codon 294.

7.2 Tamoxifen-Estrogen Receptor Interactions

As reviewed by IARC (1996, pp. 334-336; see Appendix A) and more recently by Gallo and Kaufman (1997), tamoxifen is an estrogen antagonist and/or agonist by binding directly to the estrogen receptor. In breast tissue, tamoxifen exerts antiestrogenic activity by binding with high affinity to the estrogen receptor, preventing normal estrogen-induced transcriptional activity (Pasqualini et al., 1987; cited by IARC, 1996). In other tissues, such as bone, uterus, and liver, tamoxifen acts as a partial agonist, thereby inducing typical estrogen-mediated alterations in gene expression and on cell growth and differentiation (Love et al., 1992b, Jordan and Prestwich, 1977; both cited by IARC, 1996). These tissue-specific effects may be involved in the ability of tamoxifen to decrease or increase cancer risk, respectively.

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APPENDIX A

**Excerpts from the IARC Monograph on the
Evaluation of the Carcinogenic Risks of Chemicals to Humans
Volume 66 (Some Pharmaceutical Drugs)
Tamoxifen, pp. 253-365, 1996**

APPENDIX B

Description of Online Searches for Tamoxifen

DESCRIPTION OF ONLINE SEARCHES FOR TAMOXIFEN

Searches were limited to 1995 [the year before the IARC Monograph (1996), which has an extensive literature review] through September 1997. ▼

Online searches for tamoxifen [CASRN 10540-29-1] were performed in databases on the systems of STN International, DIALOG, NLM's TOXNET. Toxicology information was sought in EMIC, EMICBACK, RTECS, and TOXLINE (specifically human and animal studies, focusing on carcinogenicity and the MESH heading for all neoplasms). Occupational safety and health information was obtained from HSDB. STN Registry file and SANSS provided chemical identification information.

The citrate structural analog [54965-24-1], was also searched in TOXLINE (emphasis on reviews and the MESH heading for all neoplasms).

Regulatory information was obtained from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to the CFR titles 21 (FDA), 29 (OSHA), and 40 (EPA) and from the DIALOG database DIOGENES.

Also, the review of 1200 life sciences journals was accomplished using Current Contents on Diskette® (and cumulative issues on CD-ROM).

APPENDIX C

Report on Carcinogens (RoC), 9th Edition Review Summary

**Report on Carcinogens (RoC), 9th Edition
Review Summary**

Tamoxifen

NOMINATION

Review based on the recent IARC classification of Tamoxifen as a known human carcinogen (IARC Vol. 66, 1996).

DISCUSSION

Tamoxifen is approved by the FDA for use as an anti-estrogen drug in the palliative treatment of breast cancer and to reduce the incidence of breast cancer in women at high risk of this disease. While effective in reducing breast cancer risk, there is a statistically significant association between tamoxifen use and increased risk for developing endometrial cancer of the uterus. This increased risk of endometrial cancer has been reported in one adequate cohort study, four adequate case-control studies, and fourteen randomized clinical trials. A recent report not reviewed during the listing deliberations indicates increased risk of endometrial cancer in women at risk of breast cancer and given tamoxifen in a successful breast cancer prevention trial (National Surgical Adjuvant Breast and Bowel Project P-1 Study). A clinical trial comparing the effectiveness of Tamoxifen with Raloxifene, which may have fewer side effects, is scheduled to begin later this year. Experimental animal studies provide evidence of tamoxifen's carcinogenic effects. In mice, benign ovarian and testicular tumors were increased after 3 months of oral treatment. In short-term developmental studies, exposure to tamoxifen on days 1-5 of life has been reported to significantly increase the incidence of reproductive tract abnormalities in both female and male mice, including uterine carcinoma and seminal vesicle tumors. In rats, tamoxifen has been reported to cause squamous cell carcinoma of the uterus and benign or malignant liver tumors. The recommendations from the three NTP reviews of this nomination are as follows:

<u>Review Committee</u>	<u>Recommendation</u>	<u>Vote</u>
NIEHS (RG1)	list as known human carcinogen*	10 yes/0 no
NTP EC Working Group (RG2)	list as known human carcinogen*	7 yes/0 no/1a**
NTP Board RoC Subcommittee	list as known human carcinogen*	6 yes/0 no

*Also recommended that the profile for Tamoxifen should emphasize there is conclusive evidence that tamoxifen therapy reduces the risk of contralateral breast cancer in women with a previous diagnosis of breast cancer.

**a-abstentions

Public Comments Received

A total of 9 public comments were received:

- 2 against listing in the Roc
- 3 on behalf of persons benefiting from Tamoxifen use
- 4 providing comments on the content of the background document prepared for the review of this nomination