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A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

TAMOXIFEN

Tamoxifen was considered by a previous IARC Working Group in 1996 (IARC, 1996). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

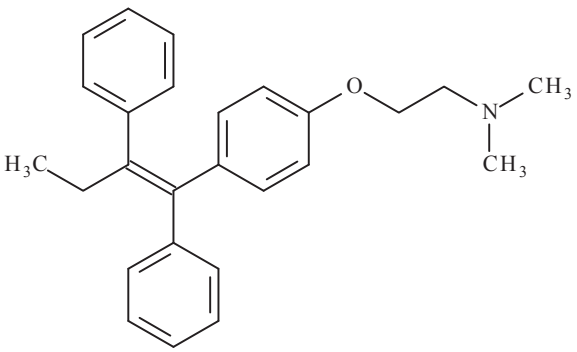
1. Exposure Data

1.1 Identification of the agent

1.1.1 Tamoxifen

Chem. Abstr. Serv. Reg. No.: 10540-29-1
Chem. Abstr. Name: (*Z*)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine
IUPAC Systematic Name: 2-[4-[(*Z*)-1,2-Diphenylbut-1-enyl]phenoxy]-*N*,*N*-dimethylethanamine
Synonyms: 1-*p*-*β*-Dimethylaminoethoxyphenyl-*trans*-1,2-diphenylbut-1-ene; (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethyldimethylamine
Description: Crystalline solid (O'Neil, 2006)

(a) Structural and molecular formulae, and relative molecular mass

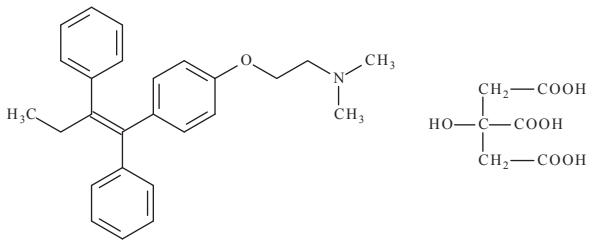


$C_{26}H_{29}NO$
Relative molecular mass: 371.52

1.1.2 Tamoxifen citrate

Chem. Abstr. Serv. Reg. No.: 54965-24-1
Chem. Abstr. Name: (*Z*)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)
IUPAC Systematic Name: 2-[4-[(*Z*)-1,2-Diphenylbut-1-enyl]phenoxy]-*N*,*N*-dimethylethylamine; 2-hydroxypropane-1,2,3-tricarboxylic acid
Synonyms: Kessar; Nolvadex; Soltamox; tamoxifen citrate; *Z*-tamoxifen citrate
Description: Fine, white, odourless crystalline powder (O'Neil, 2006)

(a) Structural and molecular formulae, and relative molecular mass



$C_{26}H_{29}NO.C_6H_8O_7$

Relative molecular mass: 563.64

1.2 Use of the agent

Information for Section 1.2 is taken from IARC (1996), AstraZeneca Pharmaceuticals LP (2005), Royal Pharmaceutical Society of Great Britain (2007), and Thomson Healthcare (2007).

1.2.1 Indications

Tamoxifen has been available since the early 1970s for the first-line treatment of metastatic breast cancer in postmenopausal women. Tamoxifen has also been used as adjuvant therapy for treatment of postmenopausal, node-positive women with positive estrogen-receptor or progesterone-receptor levels and, since the early 1990s, for the treatment of postmenopausal node-negative women with positive estrogen-receptor or progesterone-receptor levels. In the late 1980s and early 1990s, it was also widely used in treating postmenopausal receptor-negative women (IARC, 1996; Wolff & Abeloff, 2002; Albain, 2004).

In women with ductal carcinoma *in situ* following breast surgery and radiation therapy, tamoxifen is used to reduce the risk of invasive breast cancer.

Tamoxifen has been considered as a chemopreventive agent to reduce the incidence of breast cancer in women at high risk of breast cancer.

Tamoxifen has been used as the first-line therapy for hormone-responsive male breast cancer, and is also used as adjuvant therapy for estrogen receptor- or progesterone receptor-positive male breast cancer.

Tamoxifen has also been used for anovulatory infertility.

1.2.2 Dosage

Tamoxifen is available as 10 mg and 20 mg tablets (each tablet contains 15.2 mg and 30.4 mg, respectively, of tamoxifen citrate and as an oral solution (each 5 mL solution contains 15.2 mg tamoxifen citrate, equivalent to 10 mg tamoxifen).

(a) Cancer of the breast

(i) Metastatic breast cancer

For the treatment of metastatic breast cancer in women, the usual dosage of tamoxifen is 20–40 mg daily, typically starting with the 20 mg dose. Dosages exceeding 20 mg daily are typically given in divided doses (morning and evening). A 20 mg oral dose is administered as 10 mL of the oral solution.

(ii) Adjuvant therapy of breast cancer

When tamoxifen is used as an adjunct to surgery and radiation therapy in the treatment of breast cancer, the usual dosage of the drug is 20–40 mg daily. Dosages exceeding 20 mg daily typically are given in divided doses (morning and evening). The optimum duration of adjuvant tamoxifen therapy has not been established, but therapy for about 5 years has become the norm.

When tamoxifen is used in combination with chemotherapy as an adjunct to surgery in the treatment of breast cancer in postmenopausal women or in women 50 years of age or older who have positive axillary lymph nodes, the usual dosage of the drug is 10 mg twice daily. The optimum duration of adjuvant tamoxifen therapy has not been established.

(iii) Ductal carcinoma in situ (DCIS)

In women with DCIS following breast surgery and radiation therapy, tamoxifen is used for a recommended duration of 5 years to reduce the risk of invasive breast cancer.

(iv) Chemoprevention in women at high risk of breast cancer

For reduction in the incidence of breast cancer in women at high risk, the recommended dosage of tamoxifen is 20 mg daily given for 5 years.

(v) Male breast cancer

For the treatment of advanced (metastatic) breast cancer in men, the usual dosage of tamoxifen is 20–40 mg daily. Tamoxifen alone or in combination with radiation therapy was also used as an adjunct to surgery in the treatment of breast cancer in men at a dosage of 20 mg daily, usually for 1–2 years.

(b) Other uses

For anovulatory infertility, 20 mg of tamoxifen is administered daily on Days 2, 3, 4, and 5 of the cycle; if necessary, the daily dose may be increased to 40 mg and then 80 mg for subsequent courses; if menstrual cycles are irregular, the initial course may be started on any day, with subsequent course starting 45 days later or on Day 2 of cycle if menstruation occurs.

1.2.3 Trends in use

Although tamoxifen citrate is still available as a breast cancer treatment, it has largely been replaced by other treatments (AstraZeneca PLC, 2004, 2007).

2. Cancer in Humans

2.1 Cancer of the endometrium

In the sections that follow, the most important studies considered in the previous *IARC Monograph* (IARC, 1996) are included. Case reports are not considered.

Detection bias may pertain to both cohort studies and randomized clinical trials as tamoxifen is known to increase the frequency of symptoms such as vaginal bleeding or discharge, which may lead to gynaecological evaluation. In addition, tamoxifen is known to induce benign gynaecological changes such as endometrial hyperplasia and polyps. Other changes include poorly defined thickening of the endometrium that may be revealed by ultrasound examination.

The longer survival of tamoxifen-treated patients may lead to greater duration of follow-up in which second cancers may occur. The appropriate methods of statistical analysis in this context are life table analyses or analyses of rates based on person–years at risk.

2.1.1 Cohort studies

Of 11 cohort studies of women with breast cancer, three were based on data from the SEER (Surveillance, Epidemiology and End Results) Program in the United States of America (Curtis *et al.*, 1996, 2004; Newcomb *et al.*, 1999), all included substantial numbers of cases of endometrial cancer, and all found significant elevations of risk for endometrial cancer. Specific histological types were evaluated in the Curtis *et al.* (2004) study. The relative risk was higher for malignant mixed mullerian tumours (MMMTs) than for endometrial adenocarcinomas, although the excess absolute risk was smaller—an additional 1.4 versus 8.4 cancers per 10000 women per year, respectively. [The Working Group noted that the Curtis *et al.* (2004) study is an extension of Curtis *et al.* (1996). It is probable that there is some

overlap with the cohort reported by Newcomb *et al.* (1999), but the authors do not discuss this nor cite the paper by Newcomb *et al.* (1999). In these studies, the absence of hysterectomy could not be confirmed, nor were individual records of tamoxifen use available. Misclassification of hormonal treatment in the studies may have led to an underestimation of the difference in risk for cancer of the uterine corpus between the groups.]

Of the remaining cohort studies of women with breast cancer, one was a nested case–control study (Cook *et al.*, 1995) in which tamoxifen use was more common in the controls (31% versus 26%). Another small cohort study (Katase *et al.*, 1998) found no increase in risk of endometrial cancer in those treated with tamoxifen. Three of the other cohort studies found a positive but non-significant elevation of the risk of endometrial cancer (Matsuyama *et al.*, 2000; Ursic-Vrscaj *et al.*, 2001; Yamazawa *et al.*, 2006), though the numbers of endometrial cancer cases in these studies were small. Of the two remaining cohort studies, one (Bouchardy *et al.*, 2002) found significantly elevated risks of uterine cancer with tamoxifen use, and the other (Lavie *et al.*, 2008), borderline increases in risk. There was one additional cohort study, based on women at high risk of breast cancer, with a significantly increased risk of endometrial cancer following tamoxifen use (Beiner *et al.*, 2007).

See Table 2.1 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.1.pdf.

2.1.2 Case–control studies

The case–control studies considered by the Working Group were those that compared tamoxifen use in women with breast cancer who did (cases) or did not (controls) subsequently develop endometrial cancer. A fundamental requirement for the controls is that they were at risk of developing endometrial cancer (i.e. they had an intact uterus). Determinants of risk for endometrial cancer are confounding factors in the studies discussed below only to the extent that they influence the likelihood of tamoxifen prescription. As in any case–control study, information and selection bias may also be present. Finally, the possibility that endometrial cancer was diagnosed preferentially in women who had received tamoxifen constitutes a potential bias that is considered in the introductory remarks to Section 2.1.

All of the seven case–control studies found elevations in risk of endometrial cancer following tamoxifen use. Three of the studies found significant elevations of risk following tamoxifen use (Mignotte *et al.*, 1998; Bergman *et al.*, 2000; Swerdlow & Jones, 2005). In two (Bergman *et al.*, 2000; Swerdlow & Jones, 2005), greater (significant) increases in risk followed durations of use of tamoxifen of 5 years or more. The study of Chu *et al.* (2007) was conducted to examine whether a genetic variant of the *CYP3A4* gene, *CYP3A4*1B*, influences endometrial cancer risk–alone or when associated with tamoxifen exposure, as tamoxifen is metabolized by various cytochrome P450 (CYP) enzymes, but predominantly by CYP3A4. This resulted in the finding of an increase in risk in women who carried the *CYP3A4*1B* allele following treatment with tamoxifen.

[Although several potential confounders were not systematically addressed in most of the case–control studies, the Working Group considered that these were unlikely to have had a major effect on the reported relative risks.]

See Table 2.2 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.2.pdf.

2.1.3 Randomized trials

In most of the randomized trials of breast cancer treatment, small numbers of endometrial cancers were reported. In two of the largest trials, however, there was a strong and statistically significant association between risk of

endometrial cancer and use of tamoxifen (Fisher *et al.*, 1996; Rutqvist & Johansson, 2007). Data from the large majority of the trials individually cited in IARC (1996) were included in the meta-analyses described below. Therefore, these trials are not further discussed here.

All of the reports show statistically significant elevations of the risk of endometrial cancer except in the trial of Delozier *et al.* (2000), in which all participants received tamoxifen for at least 2 years. The pooled analysis of The Early Breast Cancer Trialists' Collaborative Group (1998) was the largest of these analyses. Information was collected in 1995, which was analysed centrally. The incidence of endometrial cancer had approximately quadrupled in trials of 5 years of tamoxifen use (although the number of cases was small, and these ratios were not significantly different from the findings at 2 years). Mortality from endometrial cancer was also significantly increased in those who received tamoxifen, 27 versus 5 deaths in controls, for a 10-year risk per 1000 of 1.7 versus 0.4. Two of the reports (Braithwaite *et al.*, 2003; Cuzick *et al.*, 2003) included findings from chemoprevention trials using tamoxifen as well as treatment trials. [The Working Group noted that there is some overlap in these reports with some of the treatment trial data included in The Early Breast Cancer Trialists' Collaborative Group (1998) overview analysis, but the extent is unknown.] The 7-year follow-up data from the National Surgical Adjuvant Breast and Bowel Project chemoprevention trial confirmed the earlier reported excess of endometrial cancer in those who received tamoxifen (20 mg/day) (Fisher *et al.*, 2005).

See Table 2.3 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.3.pdf.

2.2 Contralateral breast cancer

Although for some of the smaller trials of breast cancer treatment there seemed to be little difference in the number of contralateral breast cancer in tamoxifen-treated women compared with controls, for the larger trials, there was a substantially and significantly reduced risk for contralateral breast cancer in tamoxifen-treated women compared with controls. In a combined analysis of nearly all trials published in 1992 with data available to 1990, there was a significant reduction of 39% in contralateral breast cancer in the tamoxifen-treated groups (IARC, 1996), confirmed by subsequent overview analyses. In the Cuzick *et al.* (2003) overview analysis of nine treatment trials, the benefit was restricted to reduction in estrogen-receptor-positive cancers.

See Table 2.4 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.4.pdf.

Four cohort studies reported on contralateral breast cancer. In three (Cook *et al.*, 1995; Curtis *et al.*, 1996; Newcomb *et al.*, 1999), the risk of contralateral breast cancer was reduced in the tamoxifen-treated women, compared with women with no reported tamoxifen use. However, Matsuyama *et al.* (2000) reported no risk reduction in their cohort study from Japan.

See Table 2.5 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100-A-08-Table2.5.pdf.

2.3 Chemoprevention of cancer of the breast

Four trials of chemoprevention have been conducted with one being stopped because the desired end-point was reached earlier than anticipated (Fisher *et al.*, 2005). The latest data available from this trial are summarized in Table 2.6 (available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.6.pdf), together with the results of a

meta-analysis combining earlier data from this trial with that available from the three other trials (Cuzick *et al.*, 2003). It is apparent that there is a significant reduction in the incidence of breast cancer in the women who received tamoxifen. As for contralateral breast cancer, the benefit was restricted to reduction in estrogen-receptor-positive cancers.

2.4 Gastrointestinal cancers

Although an excess of gastrointestinal cancer was reported following an early combined analysis of three Scandinavian trials, this was not confirmed by other studies reported to 1996 (IARC, 1996). Data on gastrointestinal cancer was reported in five cohort studies (Curtis *et al.*, 1996; Newcomb *et al.*, 1999; Matsuyama *et al.*, 2000; Srinivasan *et al.*, 2005; Chandanos *et al.*, 2006), with only one providing data on all gastrointestinal sites combined (Curtis *et al.*, 1996). Three studies provided data on oesophageal cancer, four on stomach cancer, and four on colon or colorectal cancer. None of the studies reported significant excess risks for oesophageal, stomach or colorectal cancer in comparisons between breast cancer patients treated with tamoxifen and those who did not receive tamoxifen. However, Newcomb *et al.* (1999) reported a borderline significant positive association between hormonal therapy use and colorectal cancer in the period 5 or more years after diagnosis, while Matsuyama *et al.* (2000) and Chandanos *et al.* (2006) reported significant excesses of stomach cancer in comparison to the general population. [The Working Group thought that such comparisons are biased, and although comparisons between subjects with breast cancer treated and not treated with tamoxifen may also not be entirely valid as discussed above, they are preferable to comparisons with the general population.]

In The Early Breast Cancer Trialists' Collaborative Group (1998) analysis, tamoxifen had no apparent effect on the incidence of colorectal cancer. However, in the Braithwaite *et al.* (2003) meta-analysis, tamoxifen was associated with significantly increased risks of gastrointestinal cancers (reported in 16 trials), with a relative risk of 1.31 (95% CI: 1.01–1.69).

See Table 2.7 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.7.pdf.

2.5 Cancer of the ovary

Five cohort studies (Cook *et al.*, 1995; Curtis *et al.*, 1996; Newcomb *et al.*, 1999; Matsuyama *et al.*, 2000; Ursic Vrscaj *et al.*, 2001), and two case–control studies (Metcalfe *et al.*, 2005; Swerdlow & Jones, 2007) evaluated the role of tamoxifen therapy in women with breast cancer in relation to the risk of subsequent ovarian cancer. No study showed any indications of increased risk, though the numbers of cases in some of the cohort studies were very small.

See Table 2.8 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.8.pdf and Table 2.9 available online at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-08-Table2.9.pdf.

2.6 Synthesis

In summary, the potential effect of tamoxifen in increasing the risk of endometrial cancer among women with breast cancer has been reported in nine cohort studies, four case–control studies, five randomized controlled treatment trials, and one major chemoprevention trial; the majority of these in meta-analyses, though there were four separate reports from individual trials. The data from the observational studies and randomized controlled trials are largely consistent in showing that tamoxifen, whether given as adjuvant therapy for women

with breast cancer or for chemoprevention in women at high risk for breast cancer, increases the risk for endometrial cancer. Also, there is evidence that the use of tamoxifen in the treatment of breast cancer significantly reduces the incidence of contralateral breast cancer. The use of tamoxifen, when given for chemoprevention, reduces the incidence of estrogen-receptor-positive breast cancers.

Finally, there is some indication that the risk of various forms of gastrointestinal cancer may be increased in tamoxifen-treated patients, however the data are not consistent.

3. Cancer in Experimental Animals

Tamoxifen has been tested for carcinogenicity by oral and subcutaneous administration to adult and infant mice and rats, and by transplacental exposure to mice.

3.1 Oral administration

3.1.1 Mouse

In mice treated orally, tamoxifen increased the incidence of benign ovarian and benign testicular tumours in one study (Tucker *et al.*, 1984), but did not increase the tumour incidence in two other studies (Carthew *et al.*, 1996a; Martin *et al.*, 1997).

3.1.2 Rat

Rats dosed orally with tamoxifen had an increased incidence of benign and malignant liver cell tumours in multiple studies (Greaves *et al.*, 1993; Hard *et al.*, 1993; Hirsimäki *et al.*, 1993; Williams *et al.*, 1993, 1997; Ahotupa *et al.*, 1994; Hasmann *et al.*, 1994; Carthew *et al.*, 1995b; Dragan *et al.*, 1995; Kärki *et al.*, 2000; Carthew *et al.*, 2001; Kasahara *et al.*, 2003). When given at a lower dose level, tamoxifen decreased the incidence of benign and malignant mammary gland tumours in female rats, and benign pituitary tumours in both sexes (Maltoni *et al.*, 1997).

See Table 3.1.

3.2 Subcutaneous administration

3.2.1 Mouse

In mice treated subcutaneously, tamoxifen decreased the incidence of mammary tumours in multiple studies (Jordan *et al.*, 1990, 1991). One study using a transgenic mouse model susceptible to spontaneous mammary tumours resulted in an increased incidence of malignant mammary tumours (Jones *et al.*, 2005).

3.2.2 Rat

Female rats administered the tamoxifen metabolite 4-hydroxytamoxifen subcutaneously had a decreased incidence of benign and malignant mammary tumours in one study (Sauvez *et al.*, 1999).

See Table 3.2.

3.3 Perinatal administration

3.3.1 Mouse

Tamoxifen given orally or subcutaneously to female neonatal mice increased the incidence of uterine tumours in one study (without an obvious dose–response) (Newbold *et al.*, 1997), but had no effect upon urogenital tumours in three other studies (Green *et al.*, 2005; Waalkes *et al.*, 2006a; Razvi *et al.*, 2007). Male mice exposed transplacentally to arsenite and treated neonatally with tamoxifen had a decreased incidence of benign and malignant lung tumours in one study (Waalkes *et al.*, 2006b). One study in which tamoxifen was administered transplacentally to mice was negative (Diwan *et al.*, 1997).

See Table 3.3.

Table 3.1 Studies of cancer in experimental animals exposed to tamoxifen (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Alderley Park Strain 1 (M, F) 15 mo Tucker et al. (1984)	0, 5, 50 mg/kg bw/d for 3 mo by gastric intubation, then in the diet for 12 mo 25/sex/group	Testis (benign interstitial cell tumours): 0/25, 2/25 (8%), 21/25 (84%) Ovary (granulosa cell adenomas): 0/25, 9/25 (36%), 9/25 (36%)	$P < 0.0001$ for 50 mg/kg dose[a] $P = 0.0008$ for 5 & 50 mg/kg doses[a]	Purity NR; age NR
Mouse, B6C3F1 (F) 24 mo Carthew et al. (1996a)	0 or 420 mg/kg diet for 8 wk, then 140 mg/kg diet for 22 mo 30/group	*At 3, 6, and 9 mo:* Uterus (tumours)–0/5, 0/5 *At 24 mo:* Uterus (tumours)–0/15, 0/15		Purity NR; only the uterus examined
Mouse, B6C3F1 (F) 24 mo Martin et al. (1997)	0 or 420 mg/kg diet for 8 wk, then 140 mg/kg diet for 22 mo 48/group	Liver (adenomas)–0/15, 2/15 (13%)	NS	Purity > 98%; complete histopathology
Rat, Alderley Wistar-derived (M, F) 107 wk Greaves et al. (1993)	0, 5, 20, 35 mg/kg bw/d for 2 yr by gastric intubation (suspension in 0.5% hydroxypropyl methyl cellulose in 0.1% polysorbate 80; 5 mL/kg) M: 102, 51, 51, 51 F: 104, 52, 52, 52	Liver (hepatocellular adenomas): M–1/102 (1%), 8/51 (16%), 11/51 (22%), 8/51 (16%) F–1/104 (1%), 2/52 (4%), 6/52 (12%), 9/52 (17%) Liver (hepatocellular carcinomas): M–1/102 (1%), 8/51 (17%), 34/51 (67%), 34/51 (67%) F–0/104, 6/52 (12%), 37/52 (71%), 37/52 (71%) Liver (hepato/cholangiocellular carcinomas): M–0/102, 0/51, 2/51 (4%), 5/51 (10%) F–0/104, 0/52, 4/52 (8%), 5/52 (10%)	$P < 0.0001$ for trend for each sex $P < 0.0001$ for trend for each sex $P < 0.0001$ for trend for each sex	Purity NR
Rat, Sprague-Dawley (Crl:CD(BR) (F) 15 mo Hard et al. (1993)	0, 11.3, 22.6 mg/kg bw/d for 12 mo by gastric intubation (suspension in 0.5% carboxymethyl cellulose; 5 mL/kg) 57, 84, 75/group	*At 12 mo:* Liver (adenomas)–0/18, 21/36 (58%), 24/24 (100%) Liver (carcinomas)–0/18, 16/36 (44%), 24/24 (100%) *At 15 mo:* Liver (adenomas)–0/13, 13/21 (62%), 9/9 (100%) Liver (carcinomas)–0/13, 13/21 (62%), 8/9 (89%)	$P < 0.001$ for 11.3, 22.6 mg/kg $P < 0.001$ for 11.3, 22.6 mg/kg $P < 0.001$ for 11.3, 22.6 mg/kg $P < 0.001$ for 11.3, 22.6 mg/kg	Purity = 99%

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) 15 mo Hirsimäki et al. (1993)	0, 11.3, 45 mg/kg bw/d for 12 mo by gastric intubation (suspension in 0.5% carboxymethyl cellulose, volume NR) 20/group (5 rats/group killed after 6 and 12 mo)	*At 12 mo:* Liver (hepatocellular carcinomas)–0/5, 0/5, 3/5 (60%) *At 15 mo:* Liver (hepatocellular carcinomas)–0/8, 1/8 (12%), 6/6 (100%)	 $P = 0.003$ for 45 mg/kg group[a]	Purity 99%; small number of animals at each time point
Rat, Sprague-Dawley (Crl:CD(BR) (F), age NR 15 mo Williams et al. (1993)	0, 2.8 (0.56 mg/mL), 11.3 (2.6 mg/mL), 45.2 (9.04 mg/mL) mg/kg bw/d for 12 mo by gastric intubation (suspension in 0.5% carboxymethyl cellulose) 55–57, 57 controls (10 rats/group killed after 6 or 12 mo; 5 rats/group killed after 7 mo)	*At 6 mo:* Liver (adenomas)–0/10, NR, 0/10, 5/7 (71%) Liver (carcinomas)–0/10, NR, 0/10, 2/7 (29%) *At 7 mo*: Liver (adenomas)–0/5, NR, 0/5, 3/4 (75%) Liver (carcinomas)–0/5, NR, 0/5, 3/4 (75%) *At 12 mo:* Liver (adenomas)–0/10, 0/10, 5/10 (50%), 2/4 (50%) Liver (carcinomas)–0/10, 0/10, 1/10 (10%), 3/4 (75%) *At 15 mo:* Liver (adenomas)–0/9, 0/22, 5/11 (45%), NR Liver (carcinomas)–0/9, 0/22, 5/11 (45%), NR	 $P = 0.003$ for 45.2 mg/kg group[a] NS[a] $P = 0.05$ for 45.2 mg/kg group[a] $P = 0.05$ for 45.2 mg/kg group[a] $P = 0.02$ for 11.3 mg/kg group[a] $P = 0.01$ for 45.2 mg/kg group[a] $P = 0.03$ for 11.3 mg/kg group[a] $P = 0.03$ for 11.3 mg/kg group[a]	Purity NR; small number of animals at each time point; age NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) 15 mo Ahotupa et al. (1994)	0, 11.3, 45 mg/kg bw/d for 12 mo by gastric intubation (suspension in 0.5% carboxymethyl cellulose, volume NR) 5/group	*At 12 mo:* Liver (hepatocellular caracinomas)–0/5, 0/5, 4/5 (80%) *At 15 mo:* Liver (hepatocellular caracinomas)–0/5, 0/5, 5/5 (100%)	$P = 0.02$ at 45 mg/kg dose[a] $P = 0.004$ at 45 mg/kg dose[a]	Purity > 99%; small number of animals at each time point
Rat, (Strain, NR) (M, F) 24 mo Hasmann et al. (1994)	0 (diet only), 36 mg/kg bw/d for 24 mo 50/group/sex, 0 (placebo)	Liver (hepatocellular adenomas): M–8/50 (16%), 7/49 (14%), 50/50 (100%) F–2/50 (4%), 1/50 (2%), 25/50 (50%) Liver (hepatocellular carcinomas): M–0/50, 0/49, 49/50 (98%) F–0/50, 0/50, 50/50 (100%) Liver (cholangiomas): M–0/50, 0/49, 8/50 (16%) F–0/50, 0/50, 17/50 (34%)	$P < 0.0001$ for both sexes vs both control groups[a] $P < 0.0001$ for both sexes vs both control groups[a] $P \leq 0.003$ for both sexes vs both control groups[a]	Purity NR; very few experimental details. Vehicle unspecified
Rat, F344/Tox; Wistar (LAC-P), LEW Ola (Lewis) (F) 20 mo Carthew et al. (1995a, 1996b)	0 or 420 ppm in diet for 180 d 11 mo (Wistar & Lewis) or 20 mo (Fischer)5–10/group	*At 6 mo:* Liver (tumours): Fischer–0/5, 0/5 Wistar–0/5, 3/5 (60%) Lewis–0/5, 1/5 (20%) *At 11 mo:* Liver (hepatocellular carcinomas): Wistar–10/10 (100%) Lewis–10/10 (100%) *At 20 mo:* Liver (hepatocellular carcinomas)–Fischer 10/10 (100%)	 NS[a] NS[a] NS[a]	Purity > 98%; incidence in control rats at 11 and 20 mo not indicated

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (Alderley Park; TOX-P) (F) 20 mo Carthew et al. (1995b)	0 or 420 ppm for 3 mo in the diet, 36/group	*At 12 mo:* Liver (tumours)–0/6, 0/6 *At 20 mo:* Liver (adenomas and carcinomas)–0/15, 5/15 (33%)	$P = 0.02$[a]	Purity NR; only liver examined
Rat, Fischer (F) 18 mo Dragan et al. (1995)	0, 250, 500 mg/kg diet 15, 22 controls	*At 18 mo*: Liver (hepatocellular carcinomas)–0/22, 1/15 (6.7%), 8/15 (53%)	$P = 0.0002$ for 500 mg/kg group[a]	Purity NR; rats received a 70% partial hepatectomy 2 wk before being placed on tamoxifen diet; age NR
Rat, Sprague-Dawley (F), age NR 104 wk Mäntylä et al. (1996)	0, 11.3, 45 mg/kg bw/d by gastric intubation (solvent and volume NR) for 13, 20, 26, or 52 wk 25–104, 109 controls	Endometrium (squamous cell carcinomas): 0/109, 0/25, 2/104 (2%)	NS[a]	Purity > 99%; there was a lack of study details; age NR
Rat, Wistar (Alderley Park; TOX-P) (F) 20 mo Carthew et al. (1996a)	0 or 420 mg/kg diet for 3 mo 48, 50 controls	*At 3, 6, and 9 mo:* Uterus (tumours)–0/6, 0/6 *At 20 mo:* Uterus (deciduomas)–0/26, 2/24 (8%) Uterus (haemangiomas)–0/26, 1/24 (4%) Uterus (leiomyomas)–0/26, 1/24 (4%)		Purity NR; only the uterus examined
Rat, Sprague-Dawley [Crl:CD(BR)] and F344 (F) 36 wk Williams et al. (1997)	0 or 40 mg/kg bw/day by gastric intubation (suspension in 0.5% carboxymethyl cellulose, 8 mg/mL) for 36 wk 26, 22 controls	Liver (hepatocellular adenomas): Sprague-Dawley–0/10, 3/4 (75%) Fischer–0/10, 1/10 (10%) Liver (hepatocellular carcinomas): Sprague-Dawley–0/10, 3/4 (75%) Fischer–0/10, 0/10	$P = 0.01$ for Sprague-Dawley[a] $P = 0.01$ for Sprague-Dawley[a]	Purity 99%; used tamoxifen citrate; small number of animals

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 159 wk Maltoni et al. (1997)	0 or 3.3 mg/kg bw/day by gastric intubation (suspension in water, volume NR), 6 d per wk for 159 wk 100/sex/group	Mammary gland (fibroadenomas): M–1/100, 6/100 F–37/100, 0/100	NS[a], $P < 0.0001$[a]	Purity NR
		Mammary gland (adenocarcinomas): M–0/100, 2/100 F–8/100, 0/100	NS[a], $P = 0.003$[a]	
		Pituitary gland (adenomas): M–11/100, 5/100 F–16/100, 2/100	NS[a], $P = 0.0004$[a]	
		Adrenal gland (medullary pheochromocytomas): M–19/100, 16/100 F–17/100, 12/100	NS[a], NS[a]	
		Liver (adenomas or carcinomas): M–0/100, 3/100 F–0/100, 4/100	NS[a], NS[a]	
		Pancreas (islet cell adenomas or adenocarcinomas): M–8/100, 1/100 F–1/100, 0/100	$P = 0.02$[a]	
		Testes (Leydig cell tumours): M–4/100, 0/100	NS[a]	
Rat, Sprague-Dawley (F) 154 wk Maltoni et al. (1997)	0 or 3.3 mg/kg bw/day by gastric intubation (suspension in water, volume NR) for 8 d (consecutive) every 8 wk 150/group	Mammary gland (fibroadenomas): 68/150 (28%), 42/150 (45%)	$P = 0.0009$[a]	Purity NR
		Mammary gland (adenocarcinomas): 15/150 (10%), 5/150 (3%)	NS[a]	
		Pituitary gland (adenomas): 45/150 (15%), 22/150 (30%)	$P = 0.001$[a]	
		Adrenal gland (medullary pheochromocytomafibromas): 18/150 (12%), 8/150 (5%)	$P = 0.03$[a]	
		Liver (carcinomas): 1/150 (1%), 0/15	NS[a]	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) 87 wk Maltoni et al. (1997)	0 or 3.3 mg/kg bw/day by gastric intubation (suspension in water, volume NR), 6 d per wk for 40 wk 139/group	Mammary gland (fibroadenomas): 65/139 (46%), 48/139 (35%) Mammary gland (adenocarcinomas): 12/139 (9%), 4/139 (3%) Pituitary gland (adenomas): 44/139 (32%), 27/139 (19%) Adrenal gland (medullary pheochromocytomafibromas): 15/139 (11%), 13/139 (9%)	$P = 0.03$[a] $P = 0.02$[a] $P = 0.01$[a] NS[a]	Purity NR; age, 56 wk
Rat, Sprague-Dawley (F) 15 mo Kärki et al. (2000)	0 or 45 mg/kg bw/day by gastric intubation (suspension in 0.5% carboxymethyl cellulose, volume NR) for 12 mo 15/group	*At 6 mo:* Liver (adenomas)–0/5, 1/5 (20%) *At 12 mo:* Liver (adenomas)–0/5, 1/5 (20%) *At 12 mo:* Liver (hepatocellular carcinomas)–0/5, 4/5 (80%) *At 15 mo:* Liver (hepatocellular carcinomas)–0/5, 5/5 (100%)	$P = 0.02$[a] $P = 0.004$[a]	Purity > 99%; used tamoxifen citrate; only the liver examined; small number of animals at each time point
Rat, Wistar (Han) (F) 34 mo Carthew et al. (2001)	420 ppm in diet for 0, 1, 4, 8, or 12 wk 36/group	Liver cancer: 0/36; 0/36; 2/36 (5%); 3/36 (8%); 11/36 (30%)	$P = 0.0002$ for 12-week exposure	Purity NR; only the liver examined; histopathology conducted, but tumour type NR
Rat, Sprague-Dawley (F) 52 wk Kasahara et al. (2003)	0 or 20 mg/kg bw/day by gastric intubation (suspension in 0.5% carboxymethyl cellulose, volume NR) for 52 wk 14/group	Liver (adenomas): 0/14, 2/14 (14%) Liver (carcinomas): 0/14, 11/14 (78%)	$P < 0.0001$[a]	Purity NR; used tamoxifen citrate; only liver examined

[a] Working Group analysis (1-tailed Fisher exact test)

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; vs, versus; wk, week or weeks; yr, year or years

Table 3.2 Studies of cancer in experimental animals exposed to tamoxifen (subcutaneous administration)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C3H/OUJ (F) 15 mo Jordan et al. (1990)	Silastic implant of tamoxifen (0 or 28 mg; 125 µg/d drug released over 6 mo) - Intact mice: 11–15; 11 controls. Implanted 2 or 5 wk after pregnancy/weaning cycle. Killed after 1, 2, 3, 4, 8 or 16 wk - Ovariectomized mice: 11–15, 22 controls. Implanted 1 or 4 wk after ovariectomy. Killed after 1, 2, 3, 4, 8, 16 or 24 wk	Mammary gland (tumours): Intact controls–11/11 (100%) Ovariectomized controls–12/22 (55%) Intact mice + tamoxifen–3/11 (27%); 7/15 (46%) Ovariectomized mice+ tamoxifen–5/11 (45%); 3/7 (43%)	$P < 0.001$ vs intact control for each of the tamoxifen groups	Purity NR; ovariectomy performed 1 wk after pups were weaned; no histopathology
Mouse, CH3/OUJ (F) 24 mo Jordan et al. (1991)	Experiment 1: 11/group Group 1: intact control Group 2: ovariectomized control Group 3: intact tamoxifen Group 4: ovariectomized tamoxifen (0 or ~28 mg; 2 or 5 wk after pregnancy/weaning cycle over 9 mo)	Mammary gland (tumours): 11/11 (100%) ≥ 10/11 (≥ 90%) 5/11 (45%) 5/11 (45%)	$P = 0.006$ (intact tamoxifen vs intact control); $P \leq 0.03$ (ovariectomized tamoxifen vs ovariectomized control)	Purity NR; ovariectomy performed 1 wk after pups were weaned; no histopathology; only mammary gland and uterus examined
	Experiment 2: 30/group Group 1: intact control Group 2: ovariectomized control Group 3: intact tamoxifen Group 4: ovariectomized tamoxifen (0 or ~56 mg at 3 and 9 mo of age, over 17 mo)	Mammary gland (tumours): 30/30 (100%) 15/30 (50%) 5/30 (17%) 6/30 (20%)	$P < 0.003$ (intact and ovariectomized tamoxifen vs controls)	Purity NR; ovariectomy performed at 2.5 mo of age; no histopathology; only mammary gland examined

Table 3.2 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Jordan et al. (1991) (contd)	Experiment 3: 11/group	Mammary gland (tumours):		Purity NR; no histopathology; only mammary gland examined
	Group 1: intact control	~10/11 (91%)		
	Group 2: intact tamoxifen (0 or ~42 mg at 3 and 9 mo of age, over 12 mo)	~4/11 (36%)	$P < 0.02$	
	Experiment 4: 20/group	Mammary gland (tumours):	$P < 0.001$ (for all tamoxifen treatments vs control)	Purity NR; no histopathology; only mammary gland examined
	Group 1: control	20/20 (100%)		
	Group 2: ~14 mg over 3 mo	~4/20 (~20%)		
	Group 3: ~28 mg over 6 mo	~4/20 (~20%)		
	Group 4: ~56 mg over 12 mo (implant received at 3 and 9 mo of age, replaced every 6 mo) Duration of Experiment 4, 16 mo	~5/20 (~25%)		
Mouse, Brca1$^{Co/Co}$MMTV-Cre/p53± (F) 8 mo Jones et al. (2005)	0 or 25 mg tamoxifen, over 6 mo 31, 28 controls	Mammary gland (adenocarcinomas): 8/22 (36%), 16/25 (64%)	$P < 0.05$ (incidence); $P = 0.039$ (time-to-tumour)	Purity NR
Rat, Sprague-Dawley (F) 22 mo Karim et al. (2003)	0 or 200 µg/d tamoxifen for 60 d; then 20 µg/d for the remainder of the experiment 6, 10 controls	Ovary (tumours): at 12 mo, 0/1, 0/2; at 17 mo, 0/3, 0/3; at 22 mo, 0/4, 0/1		Purity NR; histopathology on genital tract and ovaries only; control rats did not appear to receive implants; small number of animals at each time point
4-Hydroxytamoxifen				
Rat, Sprague-Dawley (F) 101 wk Sauvez et al. (1999)	0, 0, 20, 140, 1 000 µg/kg/day 4-hydroxytamoxifen (in ethanol and water (65:35), volume NR) 50/group	Liver (hepatocellular adenomas): 0/50, 0/50, 0/50, 3/50 (6%) 1/50 (2%)	NS	Purity NR; complete histopathology
		Mammary gland (benign and malignant): 31/50; 21/49; 11/50, 0/50, 0/49	$P < 0.05$ vs control (for all treated groups)[a]	

[a] Working Group analysis (1-tailed Fisher Exact test)

bw, body weight; d, day or days; F, female; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

Table 3.3 Studies of cancer in experimental animals exposed to tamoxifen (perinatal exposure)

Species, strain (sex), age Duration Reference	Dosing regimen Route Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CD-1 [Crl:CD-1(ICR) BR] (F), Day 1 of life 17 mo Newbold et al. (1997)	0, 1, 2, 5, 10, 25, 50 µg/pup/d on Days 1–5 of life by subcutaneous injection (in corn oil, volume NR) Initial number of mice NR	Uterus (adenocarcinomas): 0/12, 4/21 (19%), 3/16 (19%), 0/11, 7/14 (50%), 1/11 (9%), 0/11	$P = 0.005$ for 10 µg dose group[a]	Purity NR; only reproductive tract examined
Mouse, CD-1 (F), Day 2 of life 36 mo Green et al. (2005)	0, 1 mg/kg bw/day on Days 2–5 of life by gastric intubation (mixture of peanut oil, lecithin, condensed milk (2:0.2:3); 5 µL/g bw) 88, 97 controls	Uterus (tumours) at interim sacrifices at 1.5, 3, 6, 9, and 12 mo: 0/4, 0/4 Uterus (tumours) at 36 mo: 0/77, 0/68		Purity NR
Mouse, CD-1 (F), Day 1 of life 90 wk Waalkes et al. (2006a)	Transplacental exposure to arsenite (85 ppm from 8–18 of gestation) of 0, 10 µg/pup/day on Days 1–5 of life by subcutaneous injection (in corn oil, volume NR) 35/group (female offspring)	Urogenital (adenomas or carcinomas): 0/33, 4/35 (11%)	NS	Purity > 99%
Mouse, CD-1 (F), Day 1 of life 18 mo Razvi et al. (2007)	Experiment 1: 0, 10 µg/pup/d on Days 1–5 of life by subcutaneous injection (in 5 µl peanut oil). Killed at 1.5, 3, 6, 12 and 18 mo 30/group Experiment 2: 5, 10, 25 or 50 µg/pup/d on Days 1–5 of life by subcutaneous injection (in 5 µl peanut oil) 35/group. Killed at 3, 6, 12 and 18 mo	Experiment 1 Uterus (adenocarcinomas) at 1.5, 3, 6 and 12 mo: 0/4, 0/4 Experiment 2 Uterus (adenocarcinomas) at 3, 6, and 12 mo: 0/4, 0/4 Experiments 1 and 2 Uterus (adenocarcinomas) at 18 mo: 0/20, 0/15, 0/20, 0/17, 0/16		Purity 99%; complete necropsy, histopathology on reproductive tract only
Mouse, CD-1 (M), Day 1 of life 90 wk Waalkes et al. (2006b)	Transplacental exposure to arsenite (85 ppm from Days 8–18 of gestation) 0, 10 µg/pup/day on Days 1–5 of life by subcutaneous injection (in corn oil, volume NR) 35/group (male offspring)	Liver (adenoma or carcinomas): 2/35 (6%), 0/30 Lung (adenomas or adenocarcinomas): 14/35 (40%), 2/30 (7%)	NS $P < 0.05$	Purity ≥ 99%

Table 3.3 (continued)

Species, strain (sex), age Duration Reference	Dosing regimen Route Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CD-1 (F), 8–10 wk 78 wk Diwan et al. (1997)	0, 5 or 7.5 mg/kg bw by gastric intubation (in tricaprylin, volume NR), Days 12–18 of gestation. Killed at 12, 24, 52 and 78 wk 10 (dams)/group	*At 12 wk:* Uterus (deciduomas)–0/10, 0/10, 1/10 (10%) *At 24 wk:* Uterus (deciduomas)–0/10, 0/10, 2/10 (20%) *At 52 wk:* Uterus (leimyomas)–0/15, 0/15, 1/15 (67%) *At 53–78 wk*: Uterus (leimyomas)–0/24, 1/23 (4%), 3/22 (14%) Uterus (leimyosarcomas)–0/24, 0/23, 1/22 (4%) Ovary (granulosa tumours)–0/24, 0/23, 3/22 (17%)		Purity NR
Rat, Wistar (Han) (F), Day 2 of life 35 mo Carthew et al. (2000)	0, 1 mg/kg bw/d by gastric intubation (in a mixture of peanut oil, lecithin, and condensed milk (2:0.2:3)) on Days 2–5 after birth 78, 72 controls	*At 3, 6, 9, and 12 mo:* Reproductive tract (tumours)–0/6, 0/6 *At 24–35 mo:* Endometrium (adenocarcinomas)–3/48 (6%),13/54 (24%) Uterus (adenosquamous carcinomas)–0/48, 1/54 (2%) Vagina/cervix (squamous cell carcinomas)–0/48, 5/54 (9%)	$P = 0.01$ $P = 0.04$	Purity NR; only reproductive tract examined
Rat, Sprague-Dawley (M, F), Day 1 of life 15 mo Karlsson (2006)	0, 14 mg/kg bw/d by subcutaneous injection (in 4 µL/g bw of an aqueous mixture of 133 mM NaCl, 2.59% polyethylene glycol 3 000, 0.173% polysorbate 80, 0.99 mM propyl parahydroxybenzoate, 10.3 mM methyl parahydroxybenzoate, and 7% ethanol) on Days 1–5 of life M: 6, 8 controls F: 15, 6 controls	Reproductive tract tumours: M–0/8, 0/5 F–0/6, 0/15		Purity > 99.5%; small number of animals

[a] Working Group analysis (1-tailed Fisher exact test)

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

3.3.2 Rat

The administration of tamoxifen to female neonatal rats caused an increase in reproductive tract tumours in one study (Carthew *et al.*, 2000), but no effect in another study of shorter duration with fewer animals (Karlsson, 2006).

3.4 Administration with known carcinogens

In several studies in both male and female rats, tamoxifen enhanced the hepatocarcinogenicity of previously administered *N*,*N*-diethylnitrosamine (IARC, 1996). In one study in rats, tamoxifen enhanced the development of *N*-nitrosodiethylamine-induced kidney tumours (Dragan *et al.*, 1995). In another study, the administration of tamoxifen to pregnant rats increased mammary gland tumours in offspring subsequently treated with 7,12-dimethylbenz[*a*]anthracene (Halakivi-Clarke *et al.*, 2000).

See Table 3.4.

3.5 Synthesis

Oral administration of tamoxifen increased the incidence of testicular tumours in one study in mice and malignant liver cell tumours in multiple studies in rats. At lower dose level, tamoxifen decreased the incidence of benign and malignant mammary gland tumours in female rats, and pituitary tumours in both sexes.

Subcutaneous administration of tamoxifen decreased the incidence of mammary tumours in multiple studies in mice. One study using a transgenic mouse model showed an increased incidence in mammary tumours.

Perinatal exposure to tamoxifen increased the incidence of reproductive tract tumours in mice and rats.

4. Other Relevant Data

In the previous *IARC Monograph* (IARC, 1996), tamoxifen was found to increase liver tumour incidence in rats. The available evidence indicated that tamoxifen is both a genotoxic carcinogen and a tumour promoter in rat liver, and that humans are likely to be less susceptible to the genotoxicity of the drug. It was suggested that tissue-specific effects of tamoxifen binding to the estrogen receptor on gene expression might be involved in the ability of tamoxifen to increase or decrease tumour risk. The pertinent mechanistic data that appeared since this review are summarized below.

4.1 Absorption, distribution, metabolism, and excretion

(a) Humans

Tamoxifen is well absorbed after oral administration, and appears to be more than 99% bound to plasma proteins (mostly to albumin) (Lien *et al.*, 1989). The absorption of tamoxifen shows a wide interindividual variation, which is probably due to differences in liver metabolism and differences in absorption in the gastrointestinal tract. In rats, mice, dogs and rhesus monkeys, most of the dosed material appears in the faeces, but bile-duct cannulation experiments with dogs and rats showed also a biliary excretion (Fromson *et al.*, 1973a).

The pharmacokinetics of tamoxifen appear to be biphasic, with a distribution phase of 7–14 hours, and an elimination phase of about 7 days (Fromson *et al.*, 1973b). The elimination half-life of *N*-desmethyltamoxifen is around 7 days, and 4-hydroxytamoxifen has a shorter half-life than tamoxifen (Buckley & Goa, 1989).

Several metabolites have been identified in the urine and plasma of human breast cancer patients (IARC, 1996). Metabolites detected in plasma

Table 3.4 Studies of cancer in experimental animals exposed to tamoxifen and known carcinogens

Species, strain (sex), age Duration Reference	Dosing regimen Route Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Fischer (F) 18 mo Dragan et al. (1995)	0, 250, 500 mg/kg diet by oral administration (following a single dose of 10 mg *N*-nitrosodiethylamine/kg body weight in trioctanoin (route and volume NR)). Killed at 6 or 18 mo 8–18; 19 controls	Liver (hepatocellular carcinomas): 2/17 (12%), 11/18 (61%), 8/8 (100%) Kidney (renal cell carcinomas): 0/19, 0/18, 2/8 (25%)	$P \leq 0.003$ for each group[a]; $P = 0.008$ for trend	Purity NR; rats received a 70% partial hepatectomy 2 wk before being placed on tamoxifen diet. *N*-nitrosodiethylamine administered 24 h after partial hepatectomy; age, NR
Rat, Sprague-Dawley (F) 18 wk Halakivi-Clarke et al. (2000)	0 or 20 µg tamoxifen by subcutaneous injection (in 50 µl of 2% dimethylsulfoxide in peanut oil) to pregnant rats on gestation Days 15–20. Female offspring treated at 45 days of age by gavage with 10 mg 7,12-dimethylbenz[*a*] anthracene in 1 mL peanut oil 22, 24 controls (offspring)	Mammary gland (adenocarcinomas): 50%, 95%	$P < 0.001$	Purity NR; histopathology limited to mammary gland on representative animals

[a] Working Group analysis (1-tailed Fisher Exact test)

bw, body weight; mo, month or months; NR, not reported; wk, week or weeks

include tamoxifen, *N*-desmethyltamoxifen, and tamoxifen-*N*-oxide; and in urine, glucuronides of four hydroxylated metabolites (4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, dihydroxytamoxifen and another monohydroxy- (possibly α-hydroxy) *N*-desmethyltamoxifen) (Poon *et al.*, 1993). In another study, seven metabolites were identified in plasma (*N*-didesmethyltamoxifen, α-hydroxytamoxifen, 4-hydroxytamoxifen, tamoxifen-*N*-oxide, α-hydroxy-*N*-desmethyltamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, and 4-hydroxytamoxifen-*N*-oxide) (Poon *et al.*, 1995).

In biopsy and autopsy samples obtained from patients with breast cancer treated with tamoxifen, levels of tamoxifen and its metabolites (*N*-desmethyl-, *N*-didesmethyl-, 4-hydroxy- and 4-hydroxy-*N*-desmethyl-) were 10- to 60-fold higher in tissues (liver, lung, pancreas, brain, adipose) than in serum, with particularly high levels in liver and lung tissues. Similarly, pancreatic tumours, and brain metastases from breast cancer were found to contain high levels of the drug. Specimen of skin and bone tissue also contained tamoxifen and some metabolites (Lien *et al.*, 1991). Furthermore, tamoxifen, 4-hydroxytamoxifen and *N*-desmethyltamoxifen were also found in postmortem and biopsy analyses of liver from tamoxifen-treated patients (Martin *et al.*, 1995).

CYP3A4 and, to a lesser extent, isoforms 2D6, 2B6, 3A5, 2C9, and 2C19 mediate the conversion of tamoxifen to α-hydroxytamoxifen (Notley *et al.*, 2005), whereas hydroxysteroid sulfotransferase 2A1 (SULT2A1) catalyses the formation of sulfate ester from α-hydroxytamoxifen (Apak & Duffel, 2004). A recent study (Singh *et al.*, 2008) demonstrated the expression of genes encoding the enzymes CYP3A4 and SULT2A1 involved in the bioactivation of tamoxifen in the human endometrium. It has been reported that women carrying CYP3A4*1B, a variant of CYP3A4, are at increased risk for tamoxifen-induced endometrial cancer (Chu *et al.*, 2007).

(b) Experimental systems

In experimental animals, concentrations of tamoxifen and its metabolites are 8- to 70-fold higher in tissues (brain, adipose, liver, heart, lung, kidney, uterus, testis) than in serum. The highest levels are found in lung and liver tissue, with substantial amounts found in kidney and adipose tissue (Lien *et al.*, 1991).

Tamoxifen can be metabolized *in vitro* by both microsomal cytochrome P450 and flavin mono-oxygenase pathways to intermediates that bind irreversibly to microsomal proteins (Mani & Kupfer, 1991). Incubation of tamoxifen with rat liver microsomes results in the formation of three major polar metabolites (*N*-oxide, *N*-desmethyl and 4-hydroxy derivatives) (Mani *et al.*, 1993, 1994). Peroxidases may also metabolize tamoxifen to a reactive intermediate that binds covalently to protein (Davies *et al.*, 1995), and to DNA (Pathak *et al.*, 1995).

In both human liver homogenate and human hepatic G2 cell line treated with a mixture of tamoxifen and its deuterated analogues, the following metabolites were detected: α-hydroxytamoxifen, 4-hydroxytamoxifen, *N*-desmethyltamoxifen, and tamoxifen *N*-oxide. In the liver homogenate, *N*-didesmethyltamoxifen was also detected (Poon *et al.*, 1995).

When primary cultures of human, rat and mouse hepatocytes were incubated with tamoxifen (10 μM) for 18–24 hours, the concentration of α-hydroxytamoxifen in the medium was 50-fold lower in the human cultures than in the rat and mouse cultures (Phillips *et al.*, 1996a).

4.2 Genetic and related effects

4.2.1 Direct genotoxicity

(a) DNA adducts

(i) Humans

Tamoxifen–DNA adducts have not been detected in human liver *in vivo* (IARC, 1996), and the low level of DNA covalent-binding by α-hydroxytamoxifen in cultured human hepatocytes (Phillips *et al.*, 1996a) probably reflects the intrinsic chemical reactivity of α-hydroxytamoxifen rather than enzymatic activation, as this metabolite is a poor substrate for human sulfotransferases (Glatt *et al.*, 1998a; Shibutani *et al.*, 1998a). Moreover, the glucuronidation pathway predominates in incubations of α-hydroxytamoxifen with human liver microsomes (Boocock *et al.*, 2000), which presumably leads to detoxification.

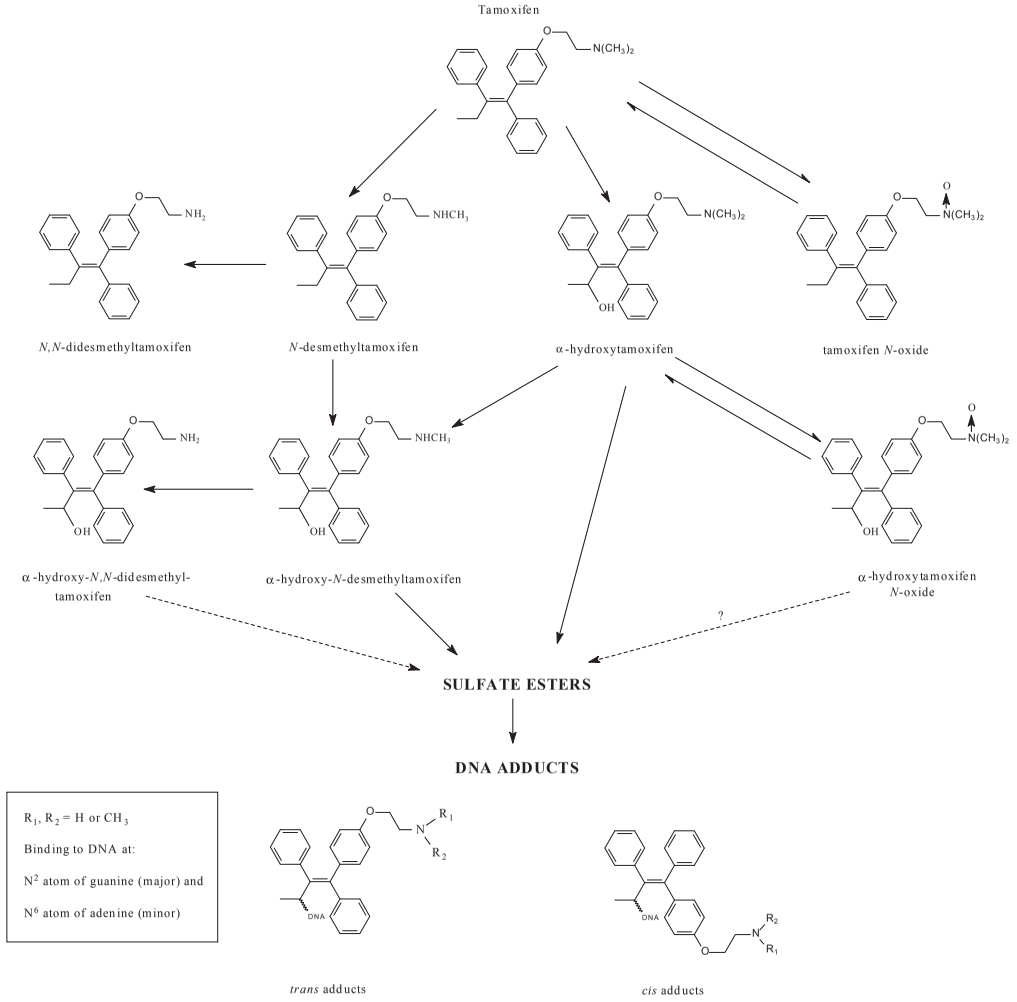
There are conflicting results on the formation of tamoxifen–DNA adducts in humans. The evidence for (Hemminki *et al.*, 1996; Shibutani *et al.*, 1999, 2000a; Martin *et al.*, 2003) and against (Carmichael *et al.*, 1996, 1999; Beland *et al.*, 2004a) such adducts in the human endometrium *in vivo* has been reported by several groups. This is also the case in studies on the formation of tamoxifen–DNA adducts from incubation of tamoxifen with human endometrium explants, with positive (Sharma *et al.*, 2003) and negative (Beland *et al.*, 2004b) findings being reported in samples from the same origin. Some studies reported the presence of such adducts in white blood cells from tamoxifen-treated patients (Hemminki *et al.*, 1997; Umemoto *et al.*, 2004), while others reported negative results (Phillips *et al.*, 1996b; Bartsch *et al.*, 2000). With the exception of the study by Martin *et al.* (2003), who used accelerator mass spectrometry, most studies used ^{32}P-postlabelling for adduct detection. Beland *et al.* (2004a, b) used HPLC coupled with tandem mass spectrometry, which can provide unequivocal structural characterization.

A recent study has reported the presence of (*E*)-α-(deoxyguanosin-N^2-yl)tamoxifen (dG-Tam) at levels of 1–7 adducts/10^9 nucleotides in enzymatically hydrolysed colorectal DNA from 3/10 women administered a single dose of 20 mg ^{14}C-labelled tamoxifen approximately 18 hours before colon resection surgery. The detection methodology involved HPLC coupled with accelerator mass spectrometry, and the identification was based upon comparison with an authentic adduct standard. All colon samples had detectable levels of CYP3A4 (Brown *et al.*, 2007).

(ii) Experimental systems

DNA adducts have been detected at dose-dependent levels in rat liver following administration of tamoxifen (IARC, 1996), and some of its metabolites, such as *N*-desmethyltamoxifen, α-hydroxytamoxifen, and α-hydroxy-*N*-desmethyltamoxifen (Brown *et al.*, 1998, 1999; Martin *et al.*, 1998; Phillips *et al.*, 1999, 2005; Gamboa da Costa *et al.*, 2000, 2001; White *et al.*, 2001). A quantitatively minor phase I pathway that leads to the metabolic activation of tamoxifen to DNA-binding electrophiles in rat liver is catalysed by CYP3A enzymes. This involves hydroxylation at the allylic (α) carbon of tamoxifen (Kim *et al.*, 2003) and *N*-desmethyltamoxifen, which is then followed by phase II conjugation. Although acetyltransferases have been proposed as mediators in the activation of α-hydroxylated tamoxifen metabolites, the most convincing evidence indicates that activation occurs through sulfotransferase-mediated sulfation, specifically by the STA2 isoform of hydroxysteroid sulfotransferase (Davis *et al.*, 1998, 2000; Glatt *et al.*, 1998a, b; Shibutani *et al.*, 1998a, b; Kim *et al.*, 2005; Phillips *et al.*, 2005). In addition, a parallel adduct formation pathway involving *N*-demethylation, as well as α-hydroxylation and *O*-sulfonation occurs (Fig. 4.1). The *N*-demethylation of tamoxifen is also mediated by the CYP3A subfamily (IARC, 1996). In-vitro reactions conducted with either

Figure 4.1 Proposed pathways of activation of tamoxifen in rat liver.



Compiled by the Working Group

the synthetic model esters, α-acetoxytamoxifen and α-acetoxy-*N*-desmethyltamoxifen (Osborne *et al.*, 1996; Dasaradhi & Shibutani, 1997; Kitagawa *et al.*, 2000) or the corresponding synthetic sulfates (Dasaradhi & Shibutani, 1997; Gamboa da Costa *et al.*, 2000) have led to the identification of the major DNA adducts as (*E*)-α-(deoxyguanosin-N^2-yl)tamoxifen (dG-Tam) and (*E*)-α-(deoxyguanosin-N^2-yl)-*N*-desmethyltamoxifen (dG-desMe-Tam), which exist as mixtures of epimers at the allylic carbon. Minor adducts from these reactions include the *Z* diastereomers from dG-Tam and dG-desMe-Tam (Dasaradhi & Shibutani, 1997; Osborne *et al.*, 1997; Kitagawa *et al.*, 2000), and a deoxyadenosine-tamoxifen adduct, linked through the amino group of adenine (Osborne *et al.*, 1997). Comparison with characterized synthetic standards has confirmed that dG-Tam and dG-desMeTam are the major adducts formed in rat liver following treatment with tamoxifen regardless of the rat strain, the route of administration, or the length of exposure (Osborne *et al.*, 1996; Rajaniemi *et al.*, 1998, 1999; Brown *et al.*, 1999; Phillips *et al.*, 1999; Firozi *et al.*, 2000; Gamboa da Costa *et al.*, 2000). Interestingly, the *R* enantiomers of α-hydroxytamoxifen (Osborne *et al.*, 2001) and α-hydroxy-*N*-desmethyltamoxifen (Osborne *et al.*, 2004) have much higher binding affinity in rat hepatocytes than the corresponding *S* isomers, presumably as a result of better affinity of the *R* enantiomers for the sulfotransferases.

Although a significant level of the didesmethylated analogue of dG-Tam and dG-desMeTam was detected in rat liver following administration of the putative metabolite, α-hydroxy-*N*,*N*-didesmethyltamoxifen, the low extent of binding obtained upon dosage with *N*,*N*-didesmethyltamoxifen indicates that metabolic activation to α-hydroxy-*N*,*N*-didesmethyltamoxifen is a minor pathway in the rat (Gamboa da Costa *et al.*, 2003). Likewise, metabolism via 4-hydroxytamoxifen does not seem to be a significant pathway to DNA-adduct formation in the rat (Beland *et al.*, 1999; Osborne *et al.*, 1999; Kim *et al.*, 2006a), despite the fact that the metabolite can be activated enzymatically to products covalently bound to DNA in cell-free or subcellular systems (Pathak *et al.*, 1995, 1996), and both its oxidation products, 4-hydroxytamoxifen quinone methide (Marques & Beland, 1997) and α-4-dihydroxytamoxifen (Hardcastle *et al.*, 1998) give DNA adducts upon reaction with DNA *in vitro*. An additional minor pathway to DNA adduct formation in rat liver has been reported to proceed via α-hydroxytamoxifen *N*-oxide, again involving binding at the α carbon through the exocyclic nitrogen of deoxyguanosine (Umemoto *et al.*, 1999, 2001).

While tamoxifen–DNA adducts are consistently detected in rat liver, most studies have not detected DNA adducts in the uterus and other extrahepatic tissues from rats administered tamoxifen or tamoxifen metabolites (Li *et al.*, 1997; Brown *et al.*, 1998; Beland *et al.*, 1999; Carthew *et al.*, 2000; Gamboa da Costa *et al.*, 2001; Phillips *et al.*, 2005). However, one study, which involved the use of accelerator mass spectrometry, reported that [^{14}C]tamoxifen binds to DNA in the liver, intestine, reproductive tract, spleen, lung, and kidney of rats dosed orally (White *et al.*, 1997). However, this methodology does not provide any structural information. [The Working Group noted that it was not clear whether the measured radioactivity corresponded exclusively to tamoxifen covalently bound to DNA.]

Tamoxifen also forms DNA adducts in mouse liver, though the levels are typically lower than in the rat (IARC, 1996). In addition, chronic exposure does not lead to accumulation of DNA adducts, which, combined with the absence of tamoxifen-induced cell proliferation, may account for the lack of hepatic carcinogenicity in the mouse, as opposed to the rat (Martin *et al.*, 1997). Similarly to what is found in the rat, the major DNA adducts in mouse liver are dG-Tam and dG-desMeTam; although

still minor, the adduct diastereomers derived from α-hydroxytamoxifen *N*-oxide make up a higher proportion in the mouse than in the rat (Umemoto *et al.*, 2000, 2001). The presence of DNA adducts in mouse extrahepatic tissues, including the uterus, has not been investigated.

Low levels of combined dG-Tam and dG-desMeTam were detected by different methods in the liver, brain cortex, kidney, ovary, and uterus of a group of three female cynomolgus monkeys dosed with a daily regimen of 2 mg tamoxifen/kg body weight for 30 days (Schild *et al.*, 2003; Shibutani *et al.*, 2003). These studies have shown that tamoxifen DNA adducts can be formed in extrahepatic tissues of non-human primates.

(b) Additional genotoxic effects

Tamoxifen induces micronuclei in metabolically proficient human cells and causes aneuploidy and chromosomal aberrations in rat liver (IARC, 1996; Styles *et al.*, 1997). Moreover, both tamoxifen and α-hydroxytamoxifen cause mutations in the *lacI* reporter gene and the *cII* gene in the livers of Big Blue transgenic rats (Davies *et al.*, 1996, 1997, 1999; Styles *et al.*, 2001; Chen *et al.*, 2002; Gamboa da Costa *et al.*, 2002) although α-hydroxytamoxifen causes significantly higher mutant frequencies than does tamoxifen, the mutational spectra induced by the two compounds are very similar in both genes, with the predominant mutations being G→T transversions. Mutations are not observed in extrahepatic tissues, including the uterus, which is in agreement with the general lack of detection of DNA adducts in rat tissues other than the liver. Consistent with the mutation profile in rat liver, when single-stranded shuttle vectors containing each of the four dG-Tam diastereomers were transfected into simian kidney (COS7) cells, the prevalent mutations were, in all instances, G→T transversions (Terashima *et al.*, 1999). Likewise, when α-hydroxytamoxifen was tested in V79-rHSTa cells, a mammalian cell line with stable expression of rat hydroxysteroid sulfotransferase A (STA2), mutations at the *Hprt* gene were mainly GC→TA transversions, although single G:C base-pair deletions and partial/complete exon skippings were also observed, almost exclusively at guanines on the non-transcribed strand (Yadollahi-Farsani *et al.*, 2002). Additionally, both 4-hydroxytamoxifen quinone methide and the model ester, α-acetoxytamoxifen, are promutagenic using adducted pSP189 plasmid DNA containing the *supF* gene transfected into cultured human fibroblasts and kidney cells (McLuckie *et al.*, 2002, 2005). Experiments involving the use of site-specific modified oligonucleotides as templates in primer extension reactions with several mammalian DNA polymerases indicate that all four dG-Tam diastereomers have high miscoding potential (G→T mutation) (Shibutani & Dasaradhi, 1997; Yasui *et al.*, 2006). These adducts undergo nucleotide excision repair *in vitro* (Shibutani *et al.*, 2000b). A comparative study in excision-repair-deficient (XPC knockout) and wild-type mice indicated that they have similar removal rates in both strains, which indicates that hepatic tamoxifen DNA-adducts are not efficiently repaired by this pathway (Kim *et al.*, 2006b).

A study of the DNA-damaging potential of tamoxifen in normal human peripheral blood lymphocytes and MCF-7 breast cancer cells using the comet assay reported evidence for the presence of free radicals, which might account, in part, for the genotoxicity of tamoxifen under the experimental conditions presumably due to incomplete repair of double-strand breaks (Wozniak *et al.*, 2007).

Both tamoxifen and its β-chlorinated analogue toremifene, which has a much lower potential for DNA-adduct formation (Gamboa da Costa *et al.*, 2007), are associated with endometrial K-*ras* codon 12 mutations (Wallén *et al.*, 2005), although a different study concluded that toremifene has a much lower potential than tamoxifen for K-*ras* mutation induction in the

human endometrium (Hachisuga *et al.*, 2005). [The Working Group noted that mutations in *TP53* and *K-RAS* are low-frequency lesions in the common form of endometrial cancer and even those mutations appear late in the course of tumour development (Sherman, 2000).]

4.2.2 Estrogen-receptor-mediated mechanism

Experimental evidence increasingly supports the importance of estrogen-receptor-mediated gene regulation as the mechanism responsible for the differential action of tamoxifen in distinct tissues (Wu *et al.*, 2005). Selective estrogen-receptor modulators such as tamoxifen have tissue-specific estrogenic activity. Tamoxifen is an estrogen-receptor antagonist in the breast but an estrogen-receptor agonist in the bone and uterus. The two main forms of the estrogen receptor, estrogen receptor-α and estrogen receptor-β, have different tissue expression profiles. The uterus predominantly expresses estrogen receptor-α but the observation of increased cell proliferation and excessive response to estrogen in estrogen-receptor-β-knockout mice has suggested that estrogen receptor-β could modulate estrogen receptor-α in the uterus, and have an antiproliferative role (Lecce *et al.*, 2001). Tamoxifen stimulates proliferation of the human endometrial epithelium (Mourits *et al.*, 2002). Tamoxifen-liganded estrogen receptors associate with multiple co-activator proteins, which together determine tamoxifen binding and transactivation activity (Shang, 2006). Tamoxifen regulates gene transcription in epithelial cells from type I endometrial carcinomas (Wu *et al.*, 2005), and transcriptional responses have been identified in epithelial cells but not in stromal cells (Pole *et al.*, 2004). There is also evidence that the genes targeted by tamoxifen differ from those targeted by estrogen (Pole *et al.*, 2005).

4.3 Synthesis

There is strong evidence that in rat liver, tamoxifen is a genotoxic carcinogen through a pathway involving α-hydroxylation, sulfation of the α-hydroxy metabolite, and subsequent DNA-adduct formation.

Evidence for the role of this pathway in induction of human endometrial tumours is less compelling; rather, the data suggest that the carcinogenicity of tamoxifen is associated with an estrogen-receptor-dependent pathway.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of tamoxifen. Tamoxifen causes cancer of the endometrium.

For cancer of the female breast, there is *evidence suggesting lack of carcinogenicity*. An inverse relationship has been established between exposure to tamoxifen and cancer of the female breast.

There is *sufficient evidence* in experimental animals for the carcinogenicity of tamoxifen.

Tamoxifen is *carcinogenic to humans (Group 1)*.

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