

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

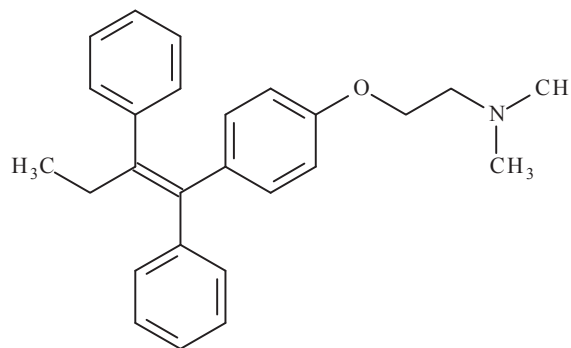
IUPAC Systematic Name: 2-[4-[(Z)-1,2-Diphenylbut-1-enyl]phenoxy]-N,N-dimethylethanamine

Synonyms: 1-*p*- β -

Dimethylaminoethoxyphenyl-*trans*-1,2-diphenylbut-1-ene; (Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethyl dimethylamine

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

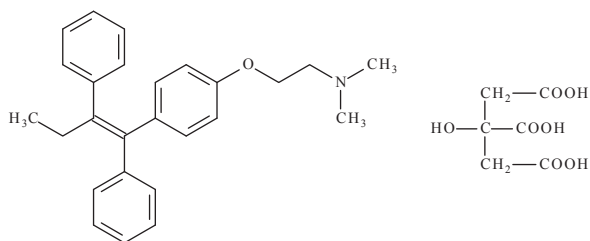
Chem. Abstr. Serv. Reg. No.: 54965-24-1

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-dimethylethanamine; 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*

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 ([Metcalf 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 (CrI: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) | <i>At 12 mo:</i> Liver (hepatocellular carcinomas)–0/5, 0/5, 3/5 (60%) <i>At 15 mo:</i> Liver (hepatocellular carcinomas)–0/8, 1/8 (12%), 6/6 (100%) | <i>P</i> = 0.003 for 45 mg/kg group ^a | Purity 99%; small number of animals at each time point |
| Rat, Sprague-Dawley (CrI: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) | <i>At 6 mo:</i> Liver (adenomas)–0/10, NR, 0/10, 5/7 (71%) Liver (carcinomas)–0/10, NR, 0/10, 2/7 (29%) <i>At 7 mo:</i> Liver (adenomas)–0/5, NR, 0/5, 3/4 (75%) Liver (carcinomas)–0/5, NR, 0/5, 3/4 (75%) <i>At 12 mo:</i> Liver (adenomas)–0/10, 0/10, 5/10 (50%), 2/4 (50%) Liver (carcinomas)–0/10, 0/10, 1/10 (10%), 3/4 (75%) <i>At 15 mo:</i> Liver (adenomas)–0/9, 0/22, 5/11 (45%), NR Liver (carcinomas)–0/9, 0/22, 5/11 (45%), NR | <i>P</i> = 0.003 for 45.2 mg/kg group ^a NS ^a <i>P</i> = 0.05 for 45.2 mg/kg group ^a <i>P</i> = 0.05 for 45.2 mg/kg group ^a <i>P</i> = 0.02 for 11.3 mg/kg group ^a <i>P</i> = 0.01 for 45.2 mg/kg group ^a <i>P</i> = 0.03 for 11.3 mg/kg group ^a <i>P</i> = 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 | <i>At 12 mo:</i> Liver (hepatocellular carcinomas)–0/5, 0/5, 4/5 (80%) <i>At 15 mo:</i> Liver (hepatocellular carcinomas)–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 | <i>At 6 mo:</i> Liver (tumours): Fischer–0/5, 0/5 Wistar–0/5, 3/5 (60%) Lewis–0/5, 1/5 (20%) <i>At 11 mo:</i> Liver (hepatocellular carcinomas): Wistar–10/10 (100%) Lewis–10/10 (100%) <i>At 20 mo:</i> 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 | <i>At 12 mo:</i> Liver (tumours)–0/6, 0/6 <i>At 20 mo:</i> 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 | <i>At 18 mo:</i> 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 | <i>At 3, 6, and 9 mo:</i> Uterus (tumours)–0/6, 0/6 <i>At 20 mo:</i> 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 [CrI: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 | <i>At 6 mo:</i> Liver (adenomas)–0/5, 1/5 (20%) <i>At 12 mo:</i> Liver (adenomas)–0/5, 1/5 (20%) <i>At 12 mo:</i> Liver (hepatocellular carcinomas)–0/5, 4/5 (80%) <i>At 15 mo:</i> 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) 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): 11/11 (100%) ≥ 10/11 (≥ 90%) 5/11 (45%) 5/11 (45%) Mammary gland (tumours): 30/30 (100%) 15/30 (50%) 5/30 (17%) 6/30 (20%) | $P = 0.006$ (intact tamoxifen vs intact control); $P \leq 0.03$ (ovariectomized tamoxifen vs ovariectomized control) $P < 0.003$ (intact and ovariectomized tamoxifen vs controls) | Purity NR; ovariectomy performed 1 wk after pups were weaned; no histopathology; only mammary gland and uterus examined Purity NR; ovariectomy performed at 2.5 mo of age; no histopathology; only mammary gland examined |

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 [Cri: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 | <i>P</i> = 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) of 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 <i>P</i> < 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 tricapyrylin, volume NR), Days 12–18 of gestation. Killed at 12, 24, 52 and 78 wk 10 (dams)/group | <i>At 12 wk:</i> Uterus (deciduomas)–0/10, 0/10, 1/10 (10%) <i>At 24 wk:</i> Uterus (deciduomas)–0/10, 0/10, 2/10 (20%) <i>At 52 wk:</i> Uterus (leiomyomas)–0/15, 0/15, 1/15 (67%) <i>At 53–78 wk:</i> Uterus (leiomyomas)–0/24, 1/23 (4%), 3/22 (14%) Uterus (leiomyosarcomas)–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 | <i>At 3, 6, 9, and 12 mo:</i> Reproductive tract (tumours)–0/6, 0/6 <i>At 24–35 mo:</i> 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%) | <i>P</i> = 0.01 <i>P</i> = 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 <i>N</i> -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. <i>N</i> -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[<i>a</i>]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 μ M) 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.

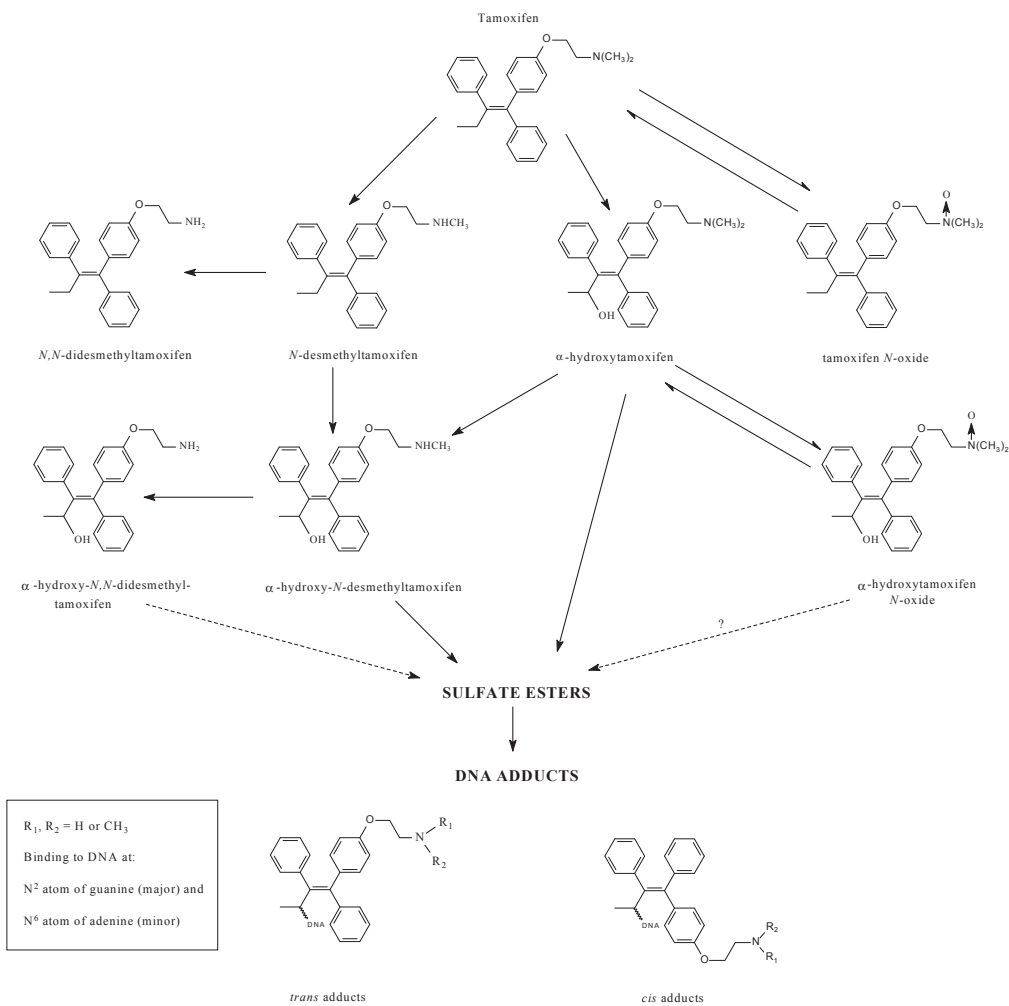
There are conflicting results on the formation of tamoxifen–DNA adducts in humans. The evidence for (Hemminki *et al.*, 1996; Shibutani *et al.*, 1999, 2000a; Martin *et al.*, 2003) and against (Carmichael *et al.*, 1996, 1999; Beland *et al.*, 2004a) such adducts in the human endometrium *in vivo* has 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.



the synthetic model esters, α -acetyltamoxifen 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 (Umamoto *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 [¹⁴C]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 skipings 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 -acetytamoxifen, 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).

References

- Ahotupa M, Hirsimäki P, Pärssinen R, Mäntylä E (1994). Alterations of drug metabolizing and antioxidant enzyme activities during tamoxifen-induced hepatocarcinogenesis in the rat. *Carcinogenesis*, 15: 863–868. doi:10.1093/carcin/15.5.863 PMID:8200088
- Albain KS (2004). Adjuvant chemotherapy for lymph node-negative, estrogen receptor-negative breast cancer: a tale of three trials. *J Natl Cancer Inst*, 96: 1801–1804. doi:10.1093/jnci/djh347 PMID:15601631
- Apak TI & Duffel MW (2004). Interactions of the stereoisomers of alpha-hydroxytamoxifen with human hydroxysteroid sulfotransferase SULT2A1 and rat hydroxysteroid sulfotransferase STa. *Drug Metab Dispos*, 32: 1501–1508. doi:10.1124/dmd.104.000919 PMID:15371299

- AstraZeneca Pharmaceuticals LP (2005). *NOLVADEX[®] Tamoxifen Citrate*, package insert. Wilmington, DE, 41 pp.
- AstraZeneca PLC (2004). *Annual Report and Form 20-F Information 2003*. London, 144 pp.
- AstraZeneca PLC (2007). *Annual Report and Form 20-F Information 2006*. London, 184 pp.
- Bartsch H, Phillips DH, Nair J *et al.* (2000). Lack of evidence for tamoxifen- and toremifene-DNA adducts in lymphocytes of treated patients. *Carcinogenesis*, 21: 845–847. doi:10.1093/carcin/21.4.845 PMID:10753226
- Beiner ME, Finch A, Rosen B *et al.* Hereditary Ovarian Cancer Clinical Study Group. (2007). The risk of endometrial cancer in women with BRCA1 and BRCA2 mutations. A prospective study. *Gynecol Oncol*, 104: 7–10. doi:10.1016/j.ygyno.2006.08.004 PMID:16962648
- Beland FA, Churchwell MI, Doerge DR *et al.* (2004a). Electrospray ionization-tandem mass spectrometry and 32P-postlabeling analyses of tamoxifen-DNA adducts in humans. *J Natl Cancer Inst*, 96: 1099–1104. doi:10.1093/jnci/djh195 PMID:15265972
- Beland FA, Churchwell MI, Hewer A *et al.* (2004b). Analysis of tamoxifen-DNA adducts in endometrial explants by MS and 32P-postlabeling. *Biochem Biophys Res Commun*, 320: 297–302. doi:10.1016/j.bbrc.2004.05.168 PMID:15219826
- Beland FA, McDaniel LP, Marques MM (1999). Comparison of the DNA adducts formed by tamoxifen and 4-hydroxytamoxifen in vivo. *Carcinogenesis*, 20: 471–477. doi:10.1093/carcin/20.3.471 PMID:10190564
- Bergman L, Beelen ML, Gallee MP *et al.* (2000). Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of Liver and Endometrial cancer Risk following Tamoxifen. *Lancet*, 356: 881–887. doi:10.1016/S0140-6736(00)02677-5 PMID:11036892
- Boocock DJ, Maggs JL, Brown K *et al.* (2000). Major interspecies differences in the rates of O-sulphonation and O-glucuronylation of alpha-hydroxytamoxifen in vitro: a metabolic disparity protecting human liver from the formation of tamoxifen-DNA adducts. *Carcinogenesis*, 21: 1851–1858. doi:10.1093/carcin/21.10.1851 PMID:11023543
- Bouchardy C, Verkooijen HM, Fioretta G *et al.* (2002). Increased risk of malignant mullerian tumor of the uterus among women with breast cancer treated by tamoxifen. *J Clin Oncol*, 20: 4403 doi:10.1200/JCO.2002.99.174 PMID:12409344
- Braithwaite RS, Chlebowski RT, Lau J *et al.* (2003). Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J Gen Intern Med*, 18: 937–947. doi:10.1046/j.1525-1497.2003.20724.x PMID:14687281
- Brown K, Brown JE, Martin EA *et al.* (1998). Determination of DNA damage in F344 rats induced by geometric isomers of tamoxifen and analogues. *Chem Res Toxicol*, 11: 527–534. doi:10.1021/tx9702289 PMID:9585484
- Brown K, Heydon RT, Jukes R *et al.* (1999). Further characterization of the DNA adducts formed in rat liver after the administration of tamoxifen, N-desmethyltamoxifen or N, N-didesmethyltamoxifen. *Carcinogenesis*, 20: 2011–2016. doi:10.1093/carcin/20.10.2011 PMID:10506118
- Brown K, Tompkins EM, Boocock DJ *et al.* (2007). Tamoxifen forms DNA adducts in human colon after administration of a single [14C]-labeled therapeutic dose. *Cancer Res*, 67: 6995–7002. doi:10.1158/0008-5472.CAN-07-0913 PMID:17638912
- Buckley MM & Goa KL (1989). Tamoxifen. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs*, 37: 451–490. doi:10.2165/00003495-198937040-00004 PMID:2661195
- Carmichael PL, Sardar S, Crooks N *et al.* (1999). Lack of evidence from HPLC 32P-post-labelling for tamoxifen-DNA adducts in the human endometrium. *Carcinogenesis*, 20: 339–342. doi:10.1093/carcin/20.2.339 PMID:10069474
- Carmichael PL, Ugwumadu AH, Neven P *et al.* (1996). Lack of genotoxicity of tamoxifen in human endometrium. *Cancer Res*, 56: 1475–1479. PMID:8603387
- Carthew P, Edwards RE, Nolan BM *et al.* (1996a). Tamoxifen associated uterine pathology in rodents: relevance to women. *Carcinogenesis*, 17: 1577–1582. doi:10.1093/carcin/17.8.1577 PMID:8761412
- Carthew P, Edwards RE, Nolan BM *et al.* (2000). Tamoxifen induces endometrial and vaginal cancer in rats in the absence of endometrial hyperplasia. *Carcinogenesis*, 21: 793–797. doi:10.1093/carcin/21.4.793 PMID:10753217
- Carthew P, Lee PN, Edwards RE *et al.* (2001). Cumulative exposure to tamoxifen: DNA adducts and liver cancer in the rat. *Arch Toxicol*, 75: 375–380. doi:10.1007/s002040100244 PMID:11570696
- Carthew P, Martin EA, White IN *et al.* (1995b). Tamoxifen induces short-term cumulative DNA damage and liver tumors in rats: promotion by phenobarbital. *Cancer Res*, 55: 544–547. PMID:7834623
- Carthew P, Nolan BM, Edwards RE, Smith LL (1996b). The role of cell death and cell proliferation in the promotion of rat liver tumours by tamoxifen. *Cancer Lett*, 106: 163–169. doi:10.1016/0304-3835(96)04310-8 PMID:8844968
- Carthew P, Rich KJ, Martin EA *et al.* (1995a). DNA damage as assessed by 32P-postlabelling in three rat strains exposed to dietary tamoxifen: the relationship between cell proliferation and liver tumour formation. *Carcinogenesis*, 16: 1299–1304. doi:10.1093/carcin/16.6.1299 PMID:7788846
- Chandanós E, Lindblad M, Jia C *et al.* (2006). Tamoxifen exposure and risk of oesophageal and gastric adenocarcinoma: a population-based cohort study of breast cancer patients in Sweden. *Br J Cancer*, 95: 118–122. doi:10.1038/sj.bjc.6603214 PMID:16755290

- Chen T, Gamboa da Costa G, Marques MM *et al.* (2002). Mutations induced by alpha-hydroxytamoxifen in the lacI and cII genes of Big Blue transgenic rats. *Carcinogenesis*, 23: 1751–1757. doi:10.1093/carcin/23.10.1751 PMID:12376486
- Chu W, Fyles A, Sellers EM *et al.* (2007). Association between CYP3A4 genotype and risk of endometrial cancer following tamoxifen use. *Carcinogenesis*, 28: 2139–2142. doi:10.1093/carcin/bgm087 PMID:17434921
- Cook LS, Weiss NS, Schwartz SM *et al.* (1995). Population-based study of tamoxifen therapy and subsequent ovarian, endometrial, and breast cancers. *J Natl Cancer Inst*, 87: 1359–1364. doi:10.1093/jnci/87.18.1359 PMID:7658496
- Curtis RE, Boice JD Jr, Shriner DA *et al.* (1996). Second cancers after adjuvant tamoxifen therapy for breast cancer. *J Natl Cancer Inst*, 88: 832–834. doi:10.1093/jnci/88.12.832 PMID:8637050
- Curtis RE, Freedman DM, Sherman ME, Fraumeni JF Jr (2004). Risk of malignant mixed müllerian tumors after tamoxifen therapy for breast cancer. *J Natl Cancer Inst*, 96: 70–74. doi:10.1093/jnci/djh007 PMID:14709741
- Cuzick J, Powles T, Veronesi U *et al.* (2003). Overview of the main outcomes in breast-cancer prevention trials. *Lancet*, 361: 296–300. doi:10.1016/S0140-6736(03)12342-2 PMID:12559863
- Dasaradhi L & Shibutani S (1997). Identification of tamoxifen-DNA adducts formed by alpha-sulfate tamoxifen and alpha-acetoxytamoxifen. *Chem Res Toxicol*, 10: 189–196. doi:10.1021/tx960114h PMID:9049430
- Davies AM, Martin EA, Jones RM *et al.* (1995). Peroxidase activation of tamoxifen and toremifene resulting in DNA damage and covalently bound protein adducts. *Carcinogenesis*, 16: 539–545. doi:10.1093/carcin/16.3.539 PMID:7697811
- Davies R, Gant TW, Smith LL, Styles JA (1999). Tamoxifen induces G:C→T:A mutations in the cII gene in the liver of lambda/lacI transgenic rats but not at 5'-CpG-3' dinucleotide sequences as found in the lacI transgene. *Carcinogenesis*, 20: 1351–1356. doi:10.1093/carcin/20.7.1351 PMID:10383911
- Davies R, Oreffo VI, Bayliss S *et al.* (1996). Mutational spectra of tamoxifen-induced mutations in the livers of lacI transgenic rats. *Environ Mol Mutagen*, 28: 430–433. doi:10.1002/(SICI)1098-2280(1996)28:4<430::AID-EM19>3.0.CO;2-G PMID:8991074
- Davies R, Oreffo VI, Martin EA *et al.* (1997). Tamoxifen causes gene mutations in the livers of lambda/lacI transgenic rats. *Cancer Res*, 57: 1288–1293. PMID:9102215
- Davis W, Hewer A, Rajkowski KM *et al.* (2000). Sex differences in the activation of tamoxifen to DNA binding species in rat liver in vivo and in rat hepatocytes in vitro: role of sulfotransferase induction. *Cancer Res*, 60: 2887–2891. PMID:10850433
- Davis W, Venitt S, Phillips DH (1998). The metabolic activation of tamoxifen and alpha-hydroxytamoxifen to DNA-binding species in rat hepatocytes proceeds via sulphation. *Carcinogenesis*, 19: 861–866. doi:10.1093/carcin/19.5.861 PMID:9635875
- DeLozier T, Spielmann M, Macé-Lesec'h J *et al.* Fédération Nationale des Centres de Lutte Contre le Cancer Breast Group. (2000). Tamoxifen adjuvant treatment duration in early breast cancer: initial results of a randomized study comparing short-term treatment with long-term treatment. *J Clin Oncol*, 18: 3507–3512. PMID:11032592
- Diwan BA, Anderson LM, Ward JM (1997). Proliferative lesions of oviduct and uterus in CD-1 mice exposed prenatally to tamoxifen. *Carcinogenesis*, 18: 2009–2014. doi:10.1093/carcin/18.10.2009 PMID:9364013
- Dragan VP, Vaughan J, Jordan VC, Pitot HC (1995). Comparison of the effects of tamoxifen and toremifene on liver and kidney tumor promotion in female rats. *Carcinogenesis*, 16: 2733–2741. doi:10.1093/carcin/16.11.2733 PMID:7586193
- Firozi PF, Vulimiri SV, Rajaniemi H *et al.* (2000). Characterization of the major DNA adducts in the liver of rats chronically exposed to tamoxifen for 18 months. *Chem Biol Interact*, 126: 33–43. doi:10.1016/S0009-2797(00)00151-4 PMID:10826652
- Fisher B, Costantino JP, Wickerham DL *et al.* (2005). Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst*, 97: 1652–1662. doi:10.1093/jnci/dji372 PMID:16288118
- Fisher B, Dignam J, Bryant J *et al.* (1996). Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *J Natl Cancer Inst*, 88: 1529–1542. doi:10.1093/jnci/88.21.1529 PMID:8901851
- Fromson JM, Pearson S, Bramah S (1973a). The metabolism of tamoxifen (I.C.I. 46,474). I. In laboratory animals. *Xenobiotica*, 3: 693–709. doi:10.3109/00498257309151594 PMID:4361333
- Fromson JM, Pearson S, Bramah S (1973b). The metabolism of tamoxifen (I.C.I. 46,474). II. In female patients. *Xenobiotica*, 3: 711–714. doi:10.3109/00498257309151595 PMID:4783632
- Gamboa da Costa G, Hamilton LP, Beland FA, Marques MM (2000). Characterization of the major DNA adduct formed by alpha-hydroxy-N-desmethyltamoxifen in vitro and in vivo. *Chem Res Toxicol*, 13: 200–207. doi:10.1021/tx990187b PMID:10725117
- Gamboa da Costa G, Manjanatha MG, Marques MM, Beland FA (2002). Induction of lacI mutations in Big Blue rats treated with tamoxifen and alpha-hydroxytamoxifen. *Cancer Lett*, 176: 37–45. doi:10.1016/S0304-3835(01)00741-8 PMID:11790452
- Gamboa da Costa G, Marques MM, Freeman JP, Beland FA (2003). Synthesis and investigation of alpha-hydroxy-N,N-didesmethyltamoxifen as a proximate carcinogen in the metabolic activation of tamoxifen. *Chem*

- Res Toxicol*, 16: 1090–1098. doi:10.1021/tx030010o PMID:12971796
- Gamboa da Costa G, McDaniel-Hamilton LP, Heflich RH *et al.* (2001). DNA adduct formation and mutant induction in Sprague-Dawley rats treated with tamoxifen and its derivatives. *Carcinogenesis*, 22: 1307–1315. doi:10.1093/carcin/22.8.1307 PMID:11470763
- Gamboa da Costa G, Pereira PC, Churchwell MI *et al.* (2007). DNA adduct formation in the livers of female Sprague-Dawley rats treated with toremifene or α -hydroxytoremifene. *Chem Res Toxicol*, 20: 300–310. doi:10.1021/tx600275d PMID:17261033
- Glatt H, Bartsch I, Christoph S *et al.* (1998b). Sulfotransferase-mediated activation of mutagens studied using heterologous expression systems. *Chem Biol Interact*, 109: 195–219. doi:10.1016/S0009-2797(97)00133-6 PMID:9566746
- Glatt H, Davis W, Meinel W *et al.* (1998a). Rat, but not human, sulfotransferase activates a tamoxifen metabolite to produce DNA adducts and gene mutations in bacteria and mammalian cells in culture. *Carcinogenesis*, 19: 1709–1713. doi:10.1093/carcin/19.10.1709 PMID:9806149
- Greaves P, Goonetilleke R, Nunn G *et al.* (1993). Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Res*, 53: 3919–3924. PMID:8358718
- Green AR, Styles JA, Parrott EL *et al.* (2005). Neonatal tamoxifen treatment of mice leads to adenomyosis but not uterine cancer. *Exp Toxicol Pathol*, 56: 255–263. doi:10.1016/j.etp.2004.10.001 PMID:15816354
- Hachisuga T, Tsujioka H, Horiuchi S *et al.* (2005). K-ras mutation in the endometrium of tamoxifen-treated breast cancer patients, with a comparison of tamoxifen and toremifene. *Br J Cancer*, 92: 1098–1103. doi:10.1038/sj.bjc.6602456 PMID:15756272
- Halakivi-Clarke L, Cho E, Onojafe I *et al.* (2000). Maternal exposure to tamoxifen during pregnancy increases carcinogen-induced mammary tumorigenesis among female rat offspring. *Clin Cancer Res*, 6: 305–308. PMID:10656462
- Hard GC, Iatropoulos MJ, Jordan K *et al.* (1993). Major difference in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats. *Cancer Res*, 53: 4534–4541. PMID:8402624
- Hardcastle IR, Horton MN, Osborne MR *et al.* (1998). Synthesis and DNA reactivity of alpha-hydroxylated metabolites of nonsteroidal antiestrogens. *Chem Res Toxicol*, 11: 369–374. doi:10.1021/tx970198+ PMID:9548808
- Hasmann M, Rattel B, Löser R (1994). Preclinical data for Droloxifene. *Cancer Lett*, 84: 101–116. doi:10.1016/0304-3835(94)90364-6 PMID:8076367
- Hemminki K, Rajaniemi H, Koskinen M, Hansson J (1997). Tamoxifen-induced DNA adducts in leucocytes of breast cancer patients. *Carcinogenesis*, 18: 9–13. doi:10.1093/carcin/18.1.9 PMID:9054583
- Hemminki K, Rajaniemi H, Lindahl B, Moberger B (1996). Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients. *Cancer Res*, 56: 4374–4377. PMID:8813128
- Hirsimäki P, Hirsimäki Y, Nieminen L, Payne BJ (1993). Tamoxifen induces hepatocellular carcinoma in rat liver: a 1-year study with two antiestrogens. *Arch Toxicol*, 67: 49–54. doi:10.1007/BF02072035 PMID:8452480
- IARC (1996). Some pharmaceutical drugs. *IARC Monogr Eval Carcinog Risks Hum*, 66: 1–514.
- Jones LP, Li M, Halama ED *et al.* (2005). Promotion of mammary cancer development by tamoxifen in a mouse model of Brca1-mutation-related breast cancer. *Oncogene*, 24: 3554–3562. doi:10.1038/sj.onc.1208426 PMID:15750629
- Jordan VC, Lababidi MK, Langan-Fahey S (1991). Suppression of mouse mammary tumorigenesis by long-term tamoxifen therapy. *J Natl Cancer Inst*, 83: 492–496. doi:10.1093/jnci/83.7.492 PMID:2005632
- Jordan VC, Lababidi MK, Mirecki DM (1990). Anti-oestrogenic and anti-tumour properties of prolonged tamoxifen therapy in C3H/OUJ mice. *Eur J Cancer*, 26: 718–721. doi:10.1016/0277-5379(90)90125-D PMID:2144160
- Karim BO, Landolfi JA, Christian A *et al.* (2003). Estrous cycle and ovarian changes in a rat mammary carcinogenesis model after irradiation, tamoxifen chemoprevention, and aging. *Comp Med*, 53: 532–538. PMID:14655997
- Kärki A, Mäntylä E, Hirsimäki Y *et al.* (2000). Comparison of the effects of tamoxifen and toremifene on rat hepatocarcinogenesis. *Arch Toxicol*, 74: 249–256. doi:10.1007/s002040000116 PMID:10959800
- Karlsson S (2006). Histopathology and histomorphometry of the urogenital tract in 15-month old male and female rats treated neonatally with SERMs and estrogens. *Exp Toxicol Pathol*, 58: 1–12. doi:10.1016/j.etp.2006.03.011 PMID:16709447
- Kasahara T, Kuwayama C, Hashiba M *et al.* (2003). The gene expression of hepatic proteins responsible for DNA repair and cell proliferation in tamoxifen-induced hepatocarcinogenesis. *Cancer Sci*, 94: 582–588. doi:10.1111/j.1349-7006.2003.tb01486.x PMID:12841865
- Katase K, Sugiyama Y, Hasumi K *et al.* (1998). The incidence of subsequent endometrial carcinoma with tamoxifen use in patients with primary breast carcinoma. *Cancer*, 82: 1698–1703. doi:10.1002/(SICI)1097-0142(19980501)82:9<1698::AID-CNCR16>3.0.CO;2-# PMID:9576291
- Kim SY, Laxmi YR, Suzuki N *et al.* (2005). Formation of tamoxifen-DNA adducts via O-sulfonation, not O-acetylation, of alpha-hydroxytamoxifen in rat and

- human livers. *Drug Metab Dispos*, 33: 1673–1678. doi:10.1124/dmd.105.005330 PMID:16099924
- Kim SY, Suzuki N, Laxmi YR *et al.* (2006a). Antiestrogens and the formation of DNA damage in rats: a comparison. *Chem Res Toxicol*, 19: 852–858. doi:10.1021/tx060052n PMID:16780365
- Kim SY, Suzuki N, Laxmi YR, Shibutani S (2006b). Inefficient repair of tamoxifen-DNA adducts in rats and mice. *Drug Metab Dispos*, 34: 311–317. doi:10.1124/dmd.105.007013 PMID:16299164
- Kim SY, Suzuki N, Santosh Laxmi YR *et al.* (2003). Alpha-hydroxylation of tamoxifen and toremifene by human and rat cytochrome P450 3A subfamily enzymes. *Chem Res Toxicol*, 16: 1138–1144. doi:10.1021/tx0300131 PMID:12971802
- Kitagawa M, Ravindernath A, Suzuki N *et al.* (2000). Identification of tamoxifen-DNA adducts induced by alpha-acetoxy-N-desmethyltamoxifen. *Chem Res Toxicol*, 13: 761–769. doi:10.1021/tx000074o PMID:10956064
- Lavie O, Barnett-Griness O, Narod SA, Rennert G (2008). The risk of developing uterine sarcoma after tamoxifen use. *Int J Gynecol Cancer*, 18: 352–356. doi:10.1111/j.1525-1438.2007.01025.x PMID:18334013
- Lecce G, Meduri G, Ancelin M *et al.* (2001). Presence of estrogen receptor β in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells. *J Clin Endocrinol Metab*, 86: 1379–1386. doi:10.1210/jc.86.3.1379 PMID:11238535
- Li D, Dragan Y, Jordan VC *et al.* (1997). Effects of chronic administration of tamoxifen and toremifene on DNA adducts in rat liver, kidney, and uterus. *Cancer Res*, 57: 1438–1441. PMID:9108442
- Lien EA, Solheim E, Lea OA *et al.* (1989). Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res*, 49: 2175–2183. PMID:2702659
- Lien EA, Solheim E, Ueland PM (1991). Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res*, 51: 4837–4844. PMID:1893376
- Maltoni C, Minardi F, Pinto C *et al.* (1997). Results of three life-span experimental carcinogenicity and anticarcinogenicity studies on tamoxifen in rats. *Ann NY Acad Sci*, 837: 469–512. doi:10.1111/j.1749-6632.1997.tb56895.x PMID:9472359
- Mani C, Gelboin HV, Park SS *et al.* (1993). Metabolism of the antimammary cancer antiestrogenic agent tamoxifen. I. Cytochrome P-450-catalyzed N-demethylation and 4-hydroxylation. *Drug Metab Dispos*, 21: 645–656. PMID:8104124
- Mani C & Kupfer D (1991). Cytochrome P-450-mediated activation and irreversible binding of the antiestrogen tamoxifen to proteins in rat and human liver: possible involvement of flavin-containing monooxygenases in tamoxifen activation. *Cancer Res*, 51: 6052–6058. PMID:1933868
- Mani C, Pearce R, Parkinson A, Kupfer D (1994). Involvement of cytochrome P4503A in catalysis of tamoxifen activation and covalent binding to rat and human liver microsomes. *Carcinogenesis*, 15: 2715–2720. doi:10.1093/carcin/15.12.2715 PMID:8001226
- Mäntylä ETE, Karlsson SH, Nieminen LS (1996). *Induction of Endometrial Cancer by Tamoxifen in the rat*. In: *Hormonal Carcinogenesis II Proceedings of the 2nd International Symposium on Hormonal Carcinogenesis*. Li JJ, Li SA, Gustafsson JA *et al.*, editors. New York: Springer Verlag, pp. 442–445.
- Marques MM & Beland FA (1997). Identification of tamoxifen-DNA adducts formed by 4-hydroxytamoxifen quinone methide. *Carcinogenesis*, 18: 1949–1954. doi:10.1093/carcin/18.10.1949 PMID:9364005
- Martin EA, Brown K, Gaskell M *et al.* (2003). Tamoxifen DNA damage detected in human endometrium using accelerator mass spectrometry. *Cancer Res*, 63: 8461–8465. PMID:14679010
- Martin EA, Carthew P, White IN *et al.* (1997). Investigation of the formation and accumulation of liver DNA adducts in mice chronically exposed to tamoxifen. *Carcinogenesis*, 18: 2209–2215. doi:10.1093/carcin/18.11.2209 PMID:9395223
- Martin EA, Heydon RT, Brown K *et al.* (1998). Evaluation of tamoxifen and alpha-hydroxytamoxifen 32P-post-labelled DNA adducts by the development of a novel automated on-line solid-phase extraction HPLC method. *Carcinogenesis*, 19: 1061–1069. doi:10.1093/carcin/19.6.1061 PMID:9667745
- Martin EA, Rich KJ, White IN *et al.* (1995). 32P-postlabelled DNA adducts in liver obtained from women treated with tamoxifen. *Carcinogenesis*, 16: 1651–1654. doi:10.1093/carcin/16.7.1651 PMID:7614701
- Matsuyama Y, Tominaga T, Nomura Y *et al.* (2000). Second cancers after adjuvant tamoxifen therapy for breast cancer in Japan. *Ann Oncol*, 11: 1537–1543. doi:10.1023/A:1008383804811 PMID:11205460
- McLuckie KI, Crookston RJ, Gaskell M *et al.* (2005). Mutation spectra induced by alpha-acetoxytamoxifen-DNA adducts in human DNA repair proficient and deficient (xeroderma pigmentosum complementation group A) cells. *Biochemistry*, 44: 8198–8205. doi:10.1021/bi047399e PMID:15924439
- McLuckie KI, Routledge MN, Brown K *et al.* (2002). DNA adducts formed from 4-hydroxytamoxifen are more mutagenic than those formed by alpha-acetoxytamoxifen in a shuttle vector target gene replicated in human Ad293 cells. *Biochemistry*, 41: 8899–8906. doi:10.1021/bi025575i PMID:12102632
- Metcalf KA, Lynch HT, Ghadirian P *et al.* (2005). The risk of ovarian cancer after breast cancer in BRCA1 and BRCA2 carriers. *Gynecol Oncol*, 96: 222–226. doi:10.1016/j.ygyno.2004.09.039 PMID:15589605

- Mignotte H, Lasset C, Bonadona V *et al.* Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC). (1998). Iatrogenic risks of endometrial carcinoma after treatment for breast cancer in a large French case-control study. *Int J Cancer*, 76: 325–330. doi:10.1002/(SICI)1097-0215(19980504)76:3<325::AID-IJC7>3.0.CO;2-X PMID:9579567
- Mourits MJ, Hollema H, De Vries EG *et al.* (2002). Apoptosis and apoptosis-associated parameters in relation to tamoxifen exposure in postmenopausal endometrium. *Hum Pathol*, 33: 341–346. doi:10.1053/hupa.2002.32226 PMID:11979376
- Newbold RR, Jefferson WN, Padilla-Burgos E, Bullock BC (1997). Uterine carcinoma in mice treated neonatally with tamoxifen. *Carcinogenesis*, 18: 2293–2298. doi:10.1093/carcin/18.12.2293 PMID:9450472
- Newcomb PA, Solomon C, White E (1999). Tamoxifen and risk of large bowel cancer in women with breast cancer. *Breast Cancer Res Treat*, 53: 271–277. doi:10.1023/A:1006117220284 PMID:10369073
- Notley LM, Crewe KH, Taylor PJ *et al.* (2005). Characterization of the human cytochrome P450 forms involved in metabolism of tamoxifen to its alpha-hydroxy and alpha,4-dihydroxy derivatives. *Chem Res Toxicol*, 18: 1611–1618. doi:10.1021/tx050140s PMID:16533026
- O’Neil MJ, editor (2006). *The Merck Index*, 14th ed. Whitehouse Station, NJ: Merck & Co., Inc., p. 1554.
- Osborne MR, Davis W, Hewer AJ *et al.* (1999). 4-Hydroxytamoxifen gives DNA adducts by chemical activation, but not in rat liver cells. *Chem Res Toxicol*, 12: 151–158. doi:10.1021/tx980187w PMID:10027792
- Osborne MR, Hardcastle IR, Phillips DH (1997). Minor products of reaction of DNA with alpha-acetoxytamoxifen. *Carcinogenesis*, 18: 539–543. doi:10.1093/carcin/18.3.539 PMID:9067554
- Osborne MR, Hewer A, Hardcastle IR *et al.* (1996). Identification of the major tamoxifen-deoxyguanosine adduct formed in the liver DNA of rats treated with tamoxifen. *Cancer Res*, 56: 66–71. PMID:8548777
- Osborne MR, Hewer A, Phillips DH (2001). Resolution of alpha-hydroxytamoxifen; R-isomer forms more DNA adducts in rat liver cells. *Chem Res Toxicol*, 14: 888–893. doi:10.1021/tx010027b PMID:11453736
- Osborne MR, Hewer A, Phillips DH (2004). Stereoselective metabolic activation of alpha-hydroxy-N-desmethyltamoxifen: the R-isomer forms more DNA adducts in rat liver cells. *Chem Res Toxicol*, 17: 697–701. doi:10.1021/tx049957w PMID:15144227
- Pathak DN, Pongracz K, Bodell WJ (1995). Microsomal and peroxidase activation of 4-hydroxy-tamoxifen to form DNA adducts: comparison with DNA adducts formed in Sprague-Dawley rats treated with tamoxifen. *Carcinogenesis*, 16: 11–15. doi:10.1093/carcin/16.1.11 PMID:7834794
- Pathak DN, Pongracz K, Bodell WJ (1996). Activation of 4-hydroxytamoxifen and the tamoxifen derivative metabolite E by uterine peroxidase to form DNA adducts: comparison with DNA adducts formed in the uterus of Sprague-Dawley rats treated with tamoxifen. *Carcinogenesis*, 17: 1785–1790. doi:10.1093/carcin/17.9.1785 PMID:8824496
- Phillips DH, Carmichael PL, Hewer A *et al.* (1996a). Activation of tamoxifen and its metabolite alpha-hydroxytamoxifen to DNA-binding products: comparisons between human, rat and mouse hepatocytes. *Carcinogenesis*, 17: 89–94. doi:10.1093/carcin/17.1.89 PMID:8565142
- Phillips DH, Hewer A, Grover PL *et al.* (1996b). Tamoxifen does not form detectable DNA adducts in white blood cells of breast cancer patients. *Carcinogenesis*, 17: 1149–1152. doi:10.1093/carcin/17.5.1149 PMID:8640926
- Phillips DH, Hewer A, Horton MN *et al.* (1999). N-demethylation accompanies alpha-hydroxylation in the metabolic activation of tamoxifen in rat liver cells. *Carcinogenesis*, 20: 2003–2009. doi:10.1093/carcin/20.10.2003 PMID:10506117
- Phillips DH, Hewer A, Osborne MR *et al.* (2005). Organ specificity of DNA adduct formation by tamoxifen and alpha-hydroxytamoxifen in the rat: implications for understanding the mechanism(s) of tamoxifen carcinogenicity and for human risk assessment. *Mutagenesis*, 20: 297–303. doi:10.1093/mutage/gei038 PMID:15928012
- Pole J, Carmichael P, Griffin J (2004). Identification of transcriptional biomarkers induced by SERMS in human endometrial cells using multivariate analysis of DNA microarrays. *Biomarkers*, 9: 447–460. doi:10.1080/13547500400022192 PMID:15849065
- Pole JC, Gold LI, Orton T *et al.* (2005). Gene expression changes induced by estrogen and selective estrogen receptor modulators in primary-cultured human endometrial cells: signals that distinguish the human carcinogen tamoxifen. *Toxicology*, 206: 91–109. doi:10.1016/j.tox.2004.07.005 PMID:15590111
- Poon GK, Chui YC, McCague R *et al.* (1993). Analysis of phase I and phase II metabolites of tamoxifen in breast cancer patients. *Drug Metab Dispos*, 21: 1119–1124. PMID:7905393
- Poon GK, Walter B, Lønning PE *et al.* (1995). Identification of tamoxifen metabolites in human Hep G2 cell line, human liver homogenate, and patients on long-term therapy for breast cancer. *Drug Metab Dispos*, 23: 377–382. PMID:7628304
- Rajaniemi H, Koskinen M, Mäntylä E, Hemminki K (1998). DNA binding of tamoxifen and its analogues: identification of the tamoxifen-DNA adducts in rat liver. *Toxicol Lett*, 102-103: 453–457. doi:10.1016/S0378-4274(98)00338-5 PMID:10022295
- Rajaniemi H, Rasanen I, Koivisto P *et al.* (1999). Identification of the major tamoxifen-DNA adducts

- in rat liver by mass spectroscopy. *Carcinogenesis*, 20: 305–309. doi:10.1093/carcin/20.2.305 PMID:10069469
- Razvi N, Greaves P, Styles J *et al.* (2007). Absence of uterine tumours in CD-1 mice treated neonatally with subcutaneous tamoxifen or 4-hydroxyoestradiol. *Exp Toxicol Pathol*, 59: 177–185. doi:10.1016/j.etp.2007.06.002 PMID:17825543
- Royal Pharmaceutical Society of Great Britain (2007). *British National Formulary*, No. 54. London: BMJ Publishing Group Ltd./RPS Publishing.
- Rutqvist LE & Johansson H Stockholm Breast Cancer Study Group. (2007). Long-term follow-up of the randomized Stockholm trial on adjuvant tamoxifen among postmenopausal patients with early stage breast cancer. *Acta Oncol*, 46: 133–145. doi:10.1080/02841860601034834 PMID:17453361
- Sauvez F, Drouin DS, Attia M *et al.* (1999). Cutaneously applied 4-hydroxytamoxifen is not carcinogenic in female rats. *Carcinogenesis*, 20: 843–850. doi:10.1093/carcin/20.5.843 PMID:10334202
- Schild LJ, Divi RL, Beland FA *et al.* (2003). Formation of tamoxifen-DNA adducts in multiple organs of adult female cynomolgus monkeys dosed with tamoxifen for 30 days. *Cancer Res*, 63: 5999–6003. PMID:14522927
- Shang Y (2006). Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. *Nat Rev Cancer*, 6: 360–368. doi:10.1038/nrc1879 PMID:16633364
- Sharma M, Shubert DE, Sharma M *et al.* (2003). Antioxidant inhibits tamoxifen-DNA adducts in endometrial explant culture. *Biochem Biophys Res Commun*, 307: 157–164. doi:10.1016/S0006-291X(03)01134-3 PMID:12849995
- Sherman ME (2000). Theories of endometrial carcinogenesis: a multidisciplinary approach. *Mod Pathol*, 13: 295–308. doi:10.1038/modpathol.3880051 PMID:10757340
- Shibutani S & Dasaradhi L (1997). Miscoding potential of tamoxifen-derived DNA adducts: alpha-(N2-deoxyguanosinyl)tamoxifen. *Biochemistry*, 36: 13010–13017. doi:10.1021/bi970243c PMID:9335562
- Shibutani S, Dasaradhi L, Terashima I *et al.* (1998b). Alpha-hydroxytamoxifen is a substrate of hydroxysteroid (alcohol) sulfotransferase, resulting in tamoxifen DNA adducts. *Cancer Res*, 58: 647–653. PMID:9485016
- Shibutani S, Ravindernath A, Suzuki N *et al.* (2000a). Identification of tamoxifen-DNA adducts in the endometrium of women treated with tamoxifen. *Carcinogenesis*, 21: 1461–1467. doi:10.1093/carcin/21.8.1461 PMID:10910945
- Shibutani S, Reardon JT, Suzuki N, Sancar A (2000b). Excision of tamoxifen-DNA adducts by the human nucleotide excision repair system. *Cancer Res*, 60: 2607–2610. PMID:10825130
- Shibutani S, Shaw PM, Suzuki N *et al.* (1998a). Sulfation of alpha-hydroxytamoxifen catalyzed by human hydroxysteroid sulfotransferase results in tamoxifen-DNA adducts. *Carcinogenesis*, 19: 2007–2011. doi:10.1093/carcin/19.11.2007 PMID:9855017
- Shibutani S, Suzuki N, Laxmi YR *et al.* (2003). Identification of tamoxifen-DNA adducts in monkeys treated with tamoxifen. *Cancer Res*, 63: 4402–4406. PMID:12907611
- Shibutani S, Suzuki N, Terashima I *et al.* (1999). Tamoxifen-DNA adducts detected in the endometrium of women treated with tamoxifen. *Chem Res Toxicol*, 12: 646–653. doi:10.1021/tx990033w PMID:10409405
- Singh MN, Stringfellow HF, Walsh MJ *et al.* (2008). Quantifiable mRNA transcripts for tamoxifen-metabolising enzymes in human endometrium. *Toxicology*, 249: 85–90. doi:10.1016/j.tox.2008.04.009 PMID:18502016
- Srinivasan R, Yang YX, Rubin SC *et al.* (2005). Women with a prior diagnosis of breast cancer are not at an increased risk for subsequent colorectal cancer. *Am J Gastroenterol*, 100: 2759–2764. doi:10.1111/j.1572-0241.2005.00316.x PMID:16393232
- Styles JA, Davies A, Davies R *et al.* (1997). Clastogenic and aneugenic effects of tamoxifen and some of its analogues in hepatocytes from dosed rats and in human lymphoblastoid cells transfected with human P450 cDNAs (MCL-5 cells). *Carcinogenesis*, 18: 303–313. doi:10.1093/carcin/18.2.303 PMID:9054622
- Styles JA, Davies R, Fenwick S *et al.* (2001). Tamoxifen mutagenesis and carcinogenesis in livers of lambda/lacI transgenic rats: selective influence of phenobarbital promotion. *Cancer Lett*, 162: 117–122. doi:10.1016/S0304-3835(00)00627-3 PMID:11121869
- Swerdlow AJ & Jones ME (2007). Ovarian cancer risk in premenopausal and perimenopausal women treated with Tamoxifen: a case-control study. *Br J Cancer*, 96: 850–855. doi:10.1038/sj.bjc.6603605 PMID:17285129
- Swerdlow AJ & Jones ME British Tamoxifen Second Cancer Study Group. (2005). Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-control study. *J Natl Cancer Inst*, 97: 375–384. doi:10.1093/jnci/dji057 PMID:15741574
- Terashima I, Suzuki N, Shibutani S (1999). Mutagenic potential of alpha-(N2-deoxyguanosinyl)tamoxifen lesions, the major DNA adducts detected in endometrial tissues of patients treated with tamoxifen. *Cancer Res*, 59: 2091–2095. PMID:10232593
- The Early Breast Cancer Trialists' Collaborative Group. (1998). Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet*, 351: 1451–1467. doi:10.1016/S0140-6736(97)11423-4 PMID:9605801
- Thomson Healthcare (2007). *Physicians' Desk Reference*, 61st ed. Montvale, NJ: Thomson, pp. 2149, 3527–3533.
- Tucker MJ, Adam HK, Patterson JS (1984). *Tamoxifen*. In: *Safety Testing of New Drugs*. Laurence DR, McLean AEM, Weatherall M, editors. New York: Academic Press, pp. 125–162.

- Umemoto A, Komaki K, Monden Y *et al.* (2001). Identification and quantification of tamoxifen-DNA adducts in the liver of rats and mice. *Chem Res Toxicol*, 14: 1006–1013. doi:10.1021/tx010012d PMID:11511174
- Umemoto A, Monden Y, Komaki K *et al.* (1999). Tamoxifen-DNA adducts formed by alpha-acetoxytamoxifen N-oxide. *Chem Res Toxicol*, 12: 1083–1089. doi:10.1021/tx990132+ PMID:10563834
- Umemoto A, Monden Y, Lin CX *et al.* (2004). Determination of tamoxifen-DNA adducts in leukocytes from breast cancer patients treated with tamoxifen. *Chem Res Toxicol*, 17: 1577–1583. doi:10.1021/tx049930c PMID:15606132
- Umemoto A, Monden Y, Suwa M *et al.* (2000). Identification of hepatic tamoxifen-DNA adducts in mice: alpha-(N(2)-deoxyguanosinyl)tamoxifen and alpha-(N(2)-deoxyguanosinyl)tamoxifen N-oxide. *Carcinogenesis*, 21: 1737–1744. doi:10.1093/carcin/21.9.1737 PMID:10964106
- Ursic Vrscaj M, Kovacic J, Bebar S *et al.* (2001). Endometrial and other primary cancers after tamoxifen treatment of breast cancer – results of retrospective cohort study. *Eur J Obstet Gynecol Reprod Biol*, 95: 105–110. doi:10.1016/S0301-2115(00)00376-6 PMID:11267730
- Waalkes MP, Liu J, Ward JM *et al.* (2006a). Urogenital carcinogenesis in female CD1 mice induced by in utero arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res*, 66: 1337–1345. doi:10.1158/0008-5472.CAN-05-3530 PMID:16452187
- Waalkes MP, Liu J, Ward JM, Diwan BA (2006b). Enhanced urinary bladder and liver carcinogenesis in male CD1 mice exposed to transplacental inorganic arsenic and postnatal diethylstilbestrol or tamoxifen. *Toxicol Appl Pharmacol*, 215: 295–305. doi:10.1016/j.taap.2006.03.010 PMID:16712894
- Wallén M, Tomás E, Visakorpi T *et al.* (2005). Endometrial K-ras mutations in postmenopausal breast cancer patients treated with adjuvant tamoxifen or toremifene. *Cancer Chemother Pharmacol*, 55: 343–346. doi:10.1007/s00280-004-0923-x PMID:15592834
- White IN, Carthew P, Davies R *et al.* (2001). Short-term dosing of alpha-hydroxytamoxifen results in DNA damage but does not lead to liver tumours in female Wistar/Han rats. *Carcinogenesis*, 22: 553–557. doi:10.1093/carcin/22.4.553 PMID:11285188
- White IN, Martin EA, Mauthe RJ *et al.* (1997). Comparisons of the binding of [¹⁴C]radiolabelled tamoxifen or toremifene to rat DNA using accelerator mass spectrometry. *Chem Biol Interact*, 106: 149–160. doi:10.1016/S0009-2797(97)00063-X PMID:9366900
- Williams GM, Iatropoulos MJ, Djordjevic MV, Kaltenberg OP (1993). The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis*, 14: 315–317. doi:10.1093/carcin/14.2.315 PMID:8435874
- Williams GM, Iatropoulos MJ, Karlsson S (1997). Initiating activity of the anti-estrogen tamoxifen, but not toremifene in rat liver. *Carcinogenesis*, 18: 2247–2253. doi:10.1093/carcin/18.11.2247 PMID:9395228
- Wolff AC & Abeloff MD (2002). Adjuvant chemotherapy for postmenopausal lymph node-negative breast cancer: it ain't necessarily so. *J Natl Cancer Inst*, 94: 1041–1043. PMID:12122089
- Wozniak K, Kolacinska A, Blasinska-Morawiec M *et al.* (2007). The DNA-damaging potential of tamoxifen in breast cancer and normal cells. *Arch Toxicol*, 81: 519–527. doi:10.1007/s00204-007-0188-3 PMID:17593413
- Wu H, Chen Y, Liang J *et al.* (2005). Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. *Nature*, 438: 981–987. doi:10.1038/nature04225 PMID:16355216
- Yadollahi-Farsani M, Davies DS, Boobis AR (2002). The mutational signature of alpha-hydroxytamoxifen at Hprt locus in Chinese hamster cells. *Carcinogenesis*, 23: 1947–1952. doi:10.1093/carcin/23.11.1947 PMID:12419845
- Yamazawa K, Miyazawa Y, Suzuki M *et al.* (2006). Tamoxifen and the risk of endometrial cancer in Japanese women with breast cancer. *Surg Today*, 36: 41–46. doi:10.1007/s00595-004-3126-5 PMID:16378192
- Yasui M, Suzuki N, Laxmi YR, Shibutani S (2006). Translesion synthesis past tamoxifen-derived DNA adducts by human DNA polymerases ϵ and κ . *Biochemistry*, 45: 12167–12174. doi:10.1021/bi0608461 PMID:17002316