NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for TAMOXIFEN

FINAL
MARCH 1999

Prepared for

the October 30-31, 1997,
Meeting of the Report on Carcinogens Subcommittee
of the NTP Board of Scientific Counselors

Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

TABLE OF CONTENTS

NTP Report on Carcinogens Listing for Tamoxifen 1
Listing Criteria from the Report on Carcinogens, Eighth Edition 4

1.0 CHEMICAL PROPERTIES 5
1.1 Chemical Identification 5
1.1.1 Tamoxifen [10540-29-1] 5
1.1.2 Tamoxifen citrate [54965-24-1] 5
1.2 Physical-Chemical Properties 6
1.2.1 Tamoxifen 6
1.2.2 Tamoxifen Citrate 6

2.0 HUMAN EXPOSURE 7
2.1 Use 7
2.2 Production Process and Volume 7
2.3 Environmental Exposure 7
2.4 Occupational Exposure 8
2.5 Regulations and Criteria 8

3.0 HUMAN STUDIES 9
3.1 Human Studies Reviewed by IARC (1996) 9
3.2 Human Studies Published Post IARC (1996) 9
Table 3-1 Summary of Randomized Clinical Trial of Adjuvant Use of Tamoxifen (not reviewed by IARC, 1996): Endometrial Cancers in Patients Treated for Breast Cancer 10

4.0 EXPERIMENTAL CARCINOGENESIS 11

5.0 GENOTOXICITY 11
Table 5-1 Summary of Additional Genotoxicity Studies 13

6.0 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION 17
6.1 Absorption, Distribution, and Excretion 17
6.2 Metabolites 17
6.3 Structure-Activity Relationships 18

7.0 MECHANISMS 18
7.1 Genotoxicity 18
7.2 Tamoxifen-Estrogen Receptor Interactions 19

8.0 REFERENCES.....19

APPENDIX A - Excerpts from the IARC Monograph on the Evaluation Of the Carcinogenic Risks of Chemicals to Humans Volume 66 (Some Pharmaceutical Drugs) Tamoxifen, pp. 253-365, 1996.....A-1

APPENDIX B - Description of Online Searches for Tamoxifen.....B-1

APPENDIX C - Report on Carcinogens (RoC), 9th Edition Review Summary.....C-1

NTP Report on Carcinogens Listing for Tamoxifen

Carcinogenicity

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

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

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

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

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

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

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

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

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

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

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

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

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

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

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

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

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

Reasonably Anticipated To Be A Human Carcinogen:

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

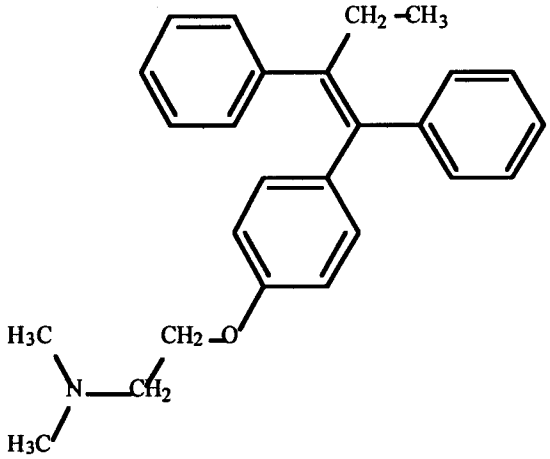
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to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

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

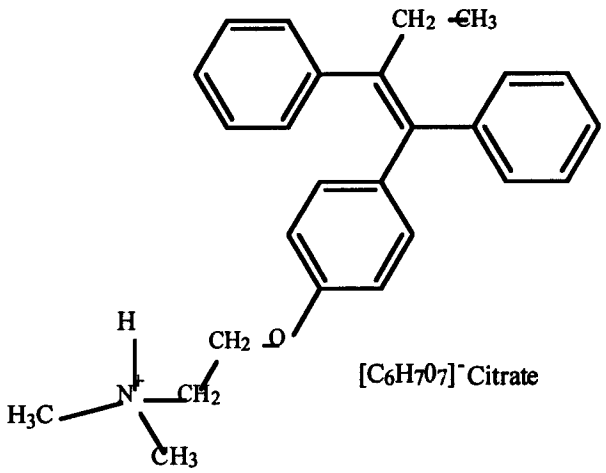
Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive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

1.0 CHEMICAL PROPERTIES

Tamoxifen
[10540-29-1]



Tamoxifen citrate
[54965-24-1]



1.1 Chemical Identification

1.1.1 Tamoxifen [10540-29-1]

Tamoxifen ($C_{26}H_{29}NO$, mol. wt. = 371.52) is also called:

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

1.1.2 Tamoxifen citrate [54965-24-1]

Tamoxifen citrate ($C_{32}H_{37}NO_8$, mol. wt. = 563.65) is also known as:

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

1.2 Physical-Chemical Properties

1.2.1 Tamoxifen

Property	Information	Reference
Color	White	HSDB (1997)
Physical State	Crystal from petroleum ether	HSDB (1997)
Melting Point, °C	96-98	HSDB (1997)
cis-Form base	72-74 from methanol	Budavari (1996)
cis-Form citrate $C_{26}H_{29}NO.C_6H_8O$	126-128	Budavari (1996)

1.2.2 Tamoxifen Citrate

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

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

2.0 HUMAN EXPOSURE

2.1 Use

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

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

2.2 Production Process and Volume

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

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

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

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

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

2.3 Environmental Exposure

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

2.4 Occupational Exposure

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

2.5 Regulations and Criteria

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

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

REGULATIONS

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

3.0 HUMAN STUDIES

3.1 Human Studies Reviewed by IARC (1996)

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

3.2 Human Studies Published Post IARC (1996)

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

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

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

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

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

4.0 EXPERIMENTAL CARCINOGENESIS

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

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

5.0 GENOTOXICITY

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

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

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

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

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

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

Table 5-1. Summary of Additional Tamoxifen Genotoxicity Studies

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

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

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Dose/Duration	Exogenous Response/+ Activation	Comments	Reference
Syrian hamster embryo (SHE) cells	Morphological transformation	n.a.	NG in source used	3, 10, and 20 µM for 48 h followed by replating and incubation for 7 d	Positive	Morphological transformation was significantly induced in cells treated at the top dose (20 µM) only compared to controls (0.36% vs. 0.03% transformation).	Tsutsui et al. (1997)
Sprague-Dawley rat uterine extracts	DNA (calf thymus) adducts from two tamoxifen metabolites (4-HO-TAM and *cis/trans* metabolite E)	Uterine extract (2 mg) with peroxidase activity and hydrogen peroxide (1 mM) separate experiment with the model peroxidase HRP (15 U) and hydrogen peroxide (1 mM)	NG in source used	Daily i.p. treatment of female rats with 5, 10, and 20 mg/kg tamoxifen for 7 d, then *in vitro* study with 100 µM metabolite	Positive (all doses) in a dose-dependent manner only when H_2O_2 in reaction mixture; no DNA adducts detected without the addition of H_2O_2	Uterine peroxidase activity increased 10-fold in 7 d. There was good correlation between uterine peroxidase activity levels and DNA adduct levels from the two metabolites. DNA adducts were also detected in the liver of rats treated with all doses and in the uterus of rats treated with a tamoxifen dose of 20 mg/kg. The adduct in uterine tissue was the same as the adduct produced by uterine extract activation of 4-HO-TAM	Pathak et al. (1996)
Mammalian Systems *in vivo*							
Female Big Blue® transgenic rats	*lacI* gene mutations in liver	n.a.	Tamoxifen citrate; NG in source used	20 mg/kg/d (36 µmol/kg/d) p.o. for 6 wk with sacrifice 2 wk after the last dose	Positive	A significant increase in mutant frequency (approximately 3-fold over controls) was observed at the lacI gene. G→C to T→A transversions were the most common point mutations.	Davies et al. (1997)

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

System	Biological Endpoint	S9/Other Metabolic Activation	Formulation/ Purity	Doses Used	Exogenous Response (+/-) Activation	Comments	Reference
Female Fischer rats	DNA adducts (^{32}P-postlabeling) in liver, kidney, and uterus	n.a.	NG in source used	Following 70% partial hepatectomy, 250 and 500 ppm (672 and 1350 µmol/kg) in diet for 18 months	Positive (liver), negative (kidney, uterus)	Six major adduct spots were detected in all exposed livers. A low (but not significantly different from control) level of DNA adducts was observed in kidney.	Li et al. (1997)
Swiss albino mice	Chromosomal aberrations in bone marrow	n.a.	Tamoxifen citrate, NG in source used	0.1, 0.2, 0.4, and 0.8 mg/kg (0.2, 0.4, 0.7, and 1 µmol/kg) p.o. daily for 10 d followed by sampling at 24, 48, 72, and 96 h after the last dose.	Positive	The maximum yield of aberrations was observed at 24 hours (LED = 0.1 mg/kg) although the yield of aberrations was still significant at 96 h.	Vijayalaxmi and Rai (1996)
Female Sprague-Dawley rats	Chromosomal aberrations in hepatocyes	n.a.	NG in source used	35 mg/kg (94 µmol/kg) p.o. (single dose) followed by hepatocyte isolation 24 h later and 50 h in culture	Positive	Treatment resulted in a variety of chromosomal changes including chromosome breaks, exchanges, endoreduplications, and spontaneous G-banding.	Sargent et al. (1996)
Female Sprague-Dawley rats	Aneuploidy in hepatocytes	n.a.	NG in source used	35 mg/kg (94 µmol/kg) p.o. (single dose) followed by hepatocyte isolation 24 h later and 50 h in culture	Positive	Treatment resulted in an 85±7% incidence of aneuploidy compared to 3.0±3% in solvent controls.	Sargent et al. (1996)

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

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Dose Used	Response +/- Activation	Comments	References
Swiss albino mice	Micronuclei induction in bone marrow	n.a.	Tamoxifen citrate, NG in source used	0.1, 0.2, 0.4, and 0.8 mg/kg (0.2, 0.4, 0.7, and 1 µmol/kg) p.o. daily for 10 d followed by sampling at 24, 48, 72, and 96 h after the last dose	Positive	A significant increase in micronuclei was observed at 24 and 48 h (LED = 0.1 mg/kg) post treatment.	Vijayalaxmi and Rai (1996)
Human Studies *in vivo*							
7 Female breast cancer patients (ages 45-90)	DNA adducts (^{32}P-postlabeling and HPLC) in leukocytes	n.a.	Tamoxifen citrate	40 mg/d (110 µmol/d) for 4-21 mo	Positive	Four of the six samples showed a positive increase in DNA adducts (radioactivity was over twice the background).	Hemminki et al. (1997)
6 Female breast cancer patients (mean age 67)	DNA adducts (^{32}P-postlabeling and HPLC) in endometrium	n.a.	Tamoxifen citrate	20-40 mg/d (54-110 µmol/d) for 3-60 mo	Positive	Five of the six samples showed a positive increase in DNA adducts (radioactivity was over twice the background).	Hemminki et al. (1996)

[a] For purposes of conversion, tamoxifen was assumed if tamoxifen citrate was not specified.

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

6.0 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

6.1 Absorption, Distribution, and Excretion

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

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

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

6.2 Metabolites

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

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

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

6.3 Structure-Activity Relationships

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

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

7.0 MECHANISMS

7.1 Genotoxicity

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

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

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

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

7.2 Tamoxifen-Estrogen Receptor Interactions

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

8.0 REFERENCES

Adam, H. K., J. S. Patterson, and J. V. Kemp. 1980. Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. Cancer Treatm. Rep. 64:761-764. (Cited by IARC, 1996)

Budavari, S., Ed. 1996. The Merck Index. 12th ed. Merck & Co, Inc., Whitehall, NJ.

Cook, L. S., N. S. Weiss, S. M. Schwartz, E. White, B. McKnight, D. E. Moore, and J. R. Daling. 1995. Population-based study of tamoxifen therapy and subsequent ovarian, endometrial, and breast cancers. J. Natl. Cancer Inst. 87:1359-1364. (Cited by IARC, 1996)

Cunningham, A., G. Klopman, and H. S. Rosenkranz. 1996. A study of the structural basis of the carcinogenicity of tamoxifen, toremifene and their metabolites. Mutat. Res. 349:85-94.

Curtis, R. E., J. D. Boice, Jr., D. A. Shriner, B. F. Hankey, and J. F. Fraumeni, Jr. 1996. Second cancers after adjuvant tamoxifen therapy for breast cancer. Brief communication. J. Natl. Cancer Inst. 88(12):832-834 (Cited by IARC, 1996 as "in press").

Davies, R., V. I. C. Oreffo, E. A. Martin, M. F. W. Festing, I. N. H. White, L. L. Smith, and J. A. Styles. 1997. Tamoxifen causes gene mutations in the livers of lambda/*lacI* transgenic rats. Cancer Res. 57:1288-1293.

Diogenes. 1997. DIOGENES FDA regulatory updates. DIALOG database file number 158. Last updated July 1997. Record numbers 903779, 907425, 908845, 911393, 912242, 4013929-4013931.

Fisher, B., J. P. Costantino, C. K. Redmond, E. R. Fisher, D. L. Wickerham, and W. M. Cronin. 1994. Endometrial cancer in tamoxifen-treated breast cancer patients: Findings from the National

Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J. Natl Cancer Inst. 86:527-537. (Cited by IARC, 1996)

Fromson, J. M., S. Pearson, and S. Bramah. 1973a. The metabolism of tamoxifen (ICI 46,474). Part II: In female patients. Xenobiotica 3:711-714. (Cited by IARC, 1996)

Furr, B. J. A. and V. C. Jordan. 1984. The pharmacology and clinical uses of tamoxifen. Pharmacol. Ther. 25:127-205. (Cited by IARC, 1996)

Gallo, M. A., and D. Kaufman. 1997. Antagonistic and agonistic effects of tamoxifen: Significance in human cancer. Semin. Oncol. 24:S1-71 to S1-80.

Gennaro, A. R., Ed. 1995. Remington: The Science and Practice of Pharmacy. 19th ed., vol. II. Mack Publishing Co., Easton, PA, p. 1094. (Cited by IARC, 1996)
Guelen, P. J. M., D. Stevenson, R. J. Briggs, and D. de Vos. 1987. The bioavailability of Tamoplex (tamoxifen). Part 2. A single dose cross-over study in healthy male volunteers. Methods Find. Exp. Clin. Pharmacol. 9:685-690. (Cited by IARC, 1996)

Hard, G. C., M. J. Iantropoulos, K. Jordan, L. Radi, O. P. Kaltenberger, A. R. Imondi, and G. M. Williams. 1993. Major differences in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats. Cancer Res. 53:4534-4541. (Cited by Kuramochi, 1996)

Hardell, L. 1988b. Pelvic irradiation and tamoxifen as risk factors for carcinoma of corpus uteri [letter]. Lancet ii:1432. (Cited by IARC, 1996)

Hemminki, K., H. Rajaniemi, B. Lindahl, and B. Moberger. 1996. Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients. Cancer Res. 56:4374-4377.

Hemminki, K., H. Rajaniemi, M. Koskinen, and J. Hansson. 1997. Tamoxifen-induced DNA adducts in leucocytes of breast cancer patients. Carcinogenesis (London) 18:9-13.

HSDB (Hazardous Substances Data Bank). 1997. Online database produced by the National Library of Medicine. Tamoxifen profile last updated May 9, 1997.

IARC (International Agency for Research on Cancer). 1979. Estrogens and progestins in relation to human cancer. IARC Monogr. Eval. Carcinog. Risks Chem. Hum. 21(Sex hormones [II]): 83-129.

IARC (International Agency for Research on Cancer). 1996. Tamoxifen. IARC Monogr. Eval. Carcinog. Risks Chem. Hum. 66(Some Pharmaceutical Drugs):253-365.

ICI (Imperial Chemical Industries). 1997. Bacterial mutagenicity study using selected strains of *Salmonella typhimurium*. Study no. TMV/227 3/HG/009756. (Cited by Tannenbaum, 1997)

Jordan, V. C., and G. Prestwich. 1977. Binding of [^{3}H]tamoxifen in rat uterine cytosols: A comparison of swinging bucket and vertical tube rotor sucrose density gradient analysis. Mol. Cell Endocrinol. 8:179-188. (Cited by IARC, 1996)

Kuramochi, H. 1996. Conformational studies and electronic structures of tamoxifen and toremifene and their allylic carbocations proposed as reactive intermediates leading to DNA adduct formation. J. Med. Chem. 39:2877-2886.

Lewis, R. J. 1992. Sax's Dangerous Properties of Industrial Materials. 8th ed., vol. III. Van Nostrand Reinhold, New York.

Li, D., Y. Dragan, V. C. Jordan, M. Wang, and H. C. Pitot. 1997. Effects of chronic administration of tamoxifen and toremifene on DNA adducts in rat liver, kidney, and uterus. Cancer Res. 57:1438-1441.

Lien, E. A., E. Solheim, Q. A. Lea, S. Lundgren, S. Kvinnsland, and P. M. Ueland. 1989. Distribution of 4-hydroxy-*N*-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res. 49:2175-2183. (Cited by IARC, 1996)

Lien, E. A., E. Solheim, and P. M. Ueland. 1991. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res. 51:4837-4844. (Cited by IARC, 1996)

Lien, E. A., G. Anker, and P. M. Ueland. 1995. Pharmacokinetics of tamoxifen in premenopausal and postmenopausal women with breast cancer. J. Steroid Biochem. Mol. Biol. 55:229-231. (Cited by IARC, 1996)

Lim, C. K., Z. Yuan, J. H. Lamb, I. N. H. White, F. De Matteis, and L. L. Smith. 1994. A comparative study of tamoxifen metabolism in female rat, mouse and human liver microsomes. Carcinogenesis (London) 15:589-593. (Cited by Smith and White, 1995)

Love, R. R., R. B. Mazess, H. S. Barden, S. Epstein, P. A. Newcombe, V. C. Jordan, P. P. Carborne, and D. L. DeMets. 1992b. Effect of tamoxifen on bone mineral density in post-menopausal women with breast cancer. N. Engl. J. Med. 326:852-856. (Cited by IARC, 1996)

Mack, T. 1995. Tamoxifen ruling in California [letter]. Science 270:1102-1103.

MacMahon, B. 1997. Overview of studies on endometrial cancer and other types of cancer in humans: Perspectives of an epidemiologist. Semin. Oncol. 24:S1-S139

Magriples, U., F. Naftolin, P. E. Schwartz, and M. l. Carcangiu. 1993. High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. J. Clin. Oncol. 11:485-490. (Cited by IARC, 1996)

Mani, C., and D. Kupfer. 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 mono-oxygenases in tamoxifen activation. Cancer Res. 51:6052-6058. (Cited by IARC, 1996)

Mäntylä, E. T. E., S. H. Karlsson, and L. S. Nieminen. 1996. Induction of endometrial cancer by tamoxifen in the rat. In: Hormonal Carcinogenesis. II. Proceedings of the Second International Symposium. Li, J. J., S. A. Li, J.-C. Gustafsson, S. Nandi, and L. I. Sekely, Eds. Springer Verlag, New York, pp. 442-445. (Cited by IARC, 1996)

McVie, J. G., G. P. C. Simonetti, D. Stevenson, R. J. Briggs, P. J. M. Guelen, and D. de Vos. 1986. The bioavailability of Tamoplex (tamoxifen). Part 1. A pilot study. Methods Find. Exp. Clin. Pharmacol. 8:505-512. (Cited by IARC, 1996)

Medical Economics. 1996. PDR® : Physicians' Desk Reference. 50th ed. Medical Economics Data Products Co., Montvale, NJ, pp. 2842-2844. (Cited by IARC, 1996)

Newbold, R. R., B. C. Bullock, W. N. Jefferson, and E. Padilla-Burgos. 1996. Effects of developmental exposure of mice to tamoxifen. Proc. Am. Assoc. Cancer Res. 37:109 (Abstract no. 753).

Newbold, R. R., W. N. Jefferson, E. Padilla-Burgos, and B. C. Bullock. 1997. Uterine carcinoma in mice treated neonatally with tamoxifen. Carcinogenesis (London) 18(12):2293-2298.

NTP (National Toxicology Program). 1998. Report on Carcinogens, 8th ed., National Toxicology Program, U.S. Department of Health and Human Services, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Otto, A. M., R. Paddenberg, S. Schubert, and H. G. Mannherz. 1996. Cell-cycle arrest, micronucleus formation, and cell death in growth inhibition of MCF-7 breast cancer cells by tamoxifen and cisplatin. J. Cancer Res. Clin. Oncol. 122:603-612.

Pasqualini, J. R., C. Sumida, N. A. Giambiagi, and B. L. Nguyen. 1987. The complexity of antioestrogen responses. J. Steroid. Biochem. 27:883-889. (Cited by IARC, 1996)

Pathak, D. N., K. Pongracz, and W. J. Bodell. 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.

PDR® 1995. Physicians' Desk Reference® 49th ed. Medical Economics Data Products. Montvale, NJ, pp. 2757-2759.

Poon, G. K., Y. C. Chui, R. McCague, P. E. Lønning, R. Feng, M. G. Rowlands, and M. Jarman. 1993. Analysis of Phase I and Phase II metabolites of tamoxifen in breast cancer patients. Drug Metab. Dispos. 21:1119-1124. (Cited by IARC, 1996)

Poon, G. K., B. Walter, P. E. Lønning, M. N. Horton, and R. McCague. 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. (Cited by IARC, 1996)

Potter, G. A., R. McCague, and M. Jarman. 1994. A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. Carcinogenesis (London) 15:439-442. (Cited by Kuramochi, 1996)

Rubagotti, A., A. Perrotta, C. Casella, and F. Boccardo. 1996. Risk of new primaries after chemotherapy and/or tamoxifen treatment for early breast cancer. Ann. Oncol. 7:239-244.

Rutqvist, L. E., H. Johansson, T. Signomklao, U. Johansson, T. Fornander, and N. Wilking, for the Stockholm Breast Cancer Study Group. 1995. Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies. J. Natl. Cancer Inst. 87:645-651. (Cited by IARC, 1996)

Sargent, L. M., Y. P. Dragon, C. Sattler, N. Bahnub, G. Sattler, P. Martin, A. Cisneros, J. Mann, S. Thorgeirsson, V. C. Jordan, and H. C. Pitot. 1996. Induction of hepatic aneuploidy *in vivo* by tamoxifen, toremifene and idoxifene in female Sprague-Dawley rats. Carcinogenesis (London) 17:1051-1056.

Sasco, A.J., G. Chaplain, E. Amoros, and S. Saez. 1996. Endometrial cancer following breast cancer: Effect of tamoxifen and castration by radiotherapy. Epidemiology 7:9-13.

Smith, L. L., and I. N. H. White. 1995. Chemoprevention of breast cancer by tamoxifen: Risks and opportunities. Toxicol. Lett. 82/83:181-186.

Strum, K., Ed. 1996. Tamoxifen citrate. In: Chemcyclopedia 97: The Manual of Commercially Available Chemicals, vol. 15, American Chemical Society, Washington, DC, p. 161.

Styles, J. A., A. Davies, R. Davies, I. N. White, and L. L. Smith. 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 (London) 18:303-13.

Tannenbaum, S. R. 1997. Comparative metabolism of tamoxifen and DNA adduct formation and *in vitro* studies on genotoxicity. Semin. Oncol. 24(Suppl 1):S1-81 to S1-86.

Tsutsui, T., S. Taguchi, Y. Tanaka, and J. C. Barrett. 1997. 17β-Estradiol, diethylstilbestrol, tamoxifen, toremifene and ICI 164, 384, induce morphological transformation and aneuploidy in cultured Syrian hamster embryo cells. Int. J. Cancer 70:188-193.

Vancutsem, P. M., P. Lazarus, and G. M. Williams. 1994. Frequent and specific mutations of the rat *p53* gene in hepatocarcinomas induced by tamoxifen. Cancer Res. 54:3864-3867. (Cited by IARC, 1996)

van Leeuwen, F. E., J. Benraadt, J. W. W. Coebergh, L. A. L. M. Kiemeney, C. H. F. Gimbrére, R. Otter, L. J. Schouten, R. A. M. Damhuis, M. Bontenbal, F. W. Diepenhorst, A. W. van den Belt-Dusebout, and H. van Tinteren. 1994. Risk of endometrial cancer after tamoxifen treatment of breast cancer. Lancet 343:448-452. (Cited by IARC, 1996)

Vijayalaxmi, K. K., and S. P. Rai. 1996. Studies on the genotoxicity of tamoxifen citrate in mouse bone marrow cells. Mutat. Res. 368:109-114.

White, I. N. H., F. De Matteis, A. Davies, L. L. Smith, C. Crofton-Sleigh, S. Venitt, A. Hewer, and D. H. Phillips. 1992. Genotoxic potential of tamoxifen and analogues in female Fischer F344/N rats, DBA/2 and C57B1/6 mice and in human MCL-5 cells. Carcinogenesis (London) 13:2197-2203. (Cited by Smith and White, 1995)

White, I. N. H., A. Davies, L. L. Smith, S. Dawson, and F. De Matteis. 1993. Induction of CYP2B1 and 3A1, and associated monooxygenase activities by tamoxifen and certain analogues in the livers of female rats and mice. Biochem. Pharmacol. 45:21-30. (Cited by Smith and White, 1995)

Wilson, A. S., M. D. Tingle, M. D. Kelly, and B. K. Park. 1995. Evaluation of the generation of genotoxic and cytotoxic metabolites of benzo[a]pyrene, aflatoxin B1, naphthalene and tamoxifen using human liver microsomes and human lymphocytes. Hum. Exp. Toxicol. 14:507-515.

APPENDIX A

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

TAMOXIFEN

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

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: (*Z*)-2-[*para*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine

Synonyms: 1-*para*-β-Dimethylaminoethoxyphenyl-*trans*-1,2-diphenylbut-1-ene; (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethyldimethylamine

Tamoxifen citrate

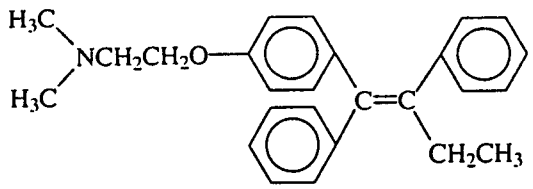
Chem. Abstr. Serv. Reg. No.: 54965-24-1

Chem. Abstr. Name: (*Z*)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine, 2-hydroxy-1,2,3-propanetricarboxylate (1:1)

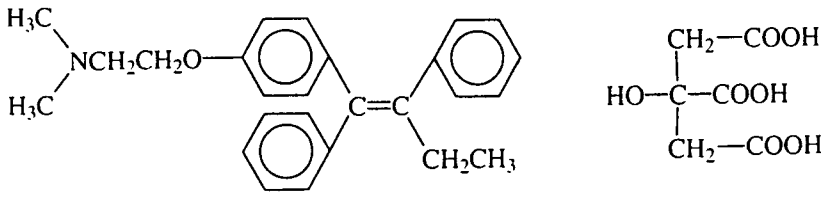
IUPAC Systematic Name: (*Z*)-2-[*para*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine citrate (1:1)

Synonyms: Tamoxifen citrate; *Z*-tamoxifen citrate

1.1.2 *Structural and molecular formulae and relative molecular mass*



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



$C_{26}H_{29}NO.C_6H_8O_7$ Relative molecular mass: 563.65

1.1.3 *Chemical and physical properties of the pure substances*

From Budavari (1995), unless otherwise specified

Tamoxifen

(*a*) *Description*: White crystals

(*b*) *Melting-point*: 96–98 °C

Tamoxifen citrate

(*a*) *Description*: Fine, white, odourless crystalline powder

(*b*) *Melting-point*: 140–142 °C

(*c*) *Spectroscopy data*: Infrared spectral data have been reported (British Pharmacopoeial Commission, 1993).

(*d*) *Solubility*: Slightly soluble in water; soluble in acetone, ethanol and methanol

(*e*) *Stability*: Hygroscopic at high relative humidities; sensitive to ultraviolet light

(*f*) *Dissociation constant*: $pK_a = 8.85$ (Medical Economics, 1996)

1.1.4 *Technical products and impurities*

Tamoxifen in pharmaceutical formulations is invariably present as its citrate salt. Tamoxifen citrate is available as 15.2-, 30.4- and 45.6-mg (equivalent to 10, 20 and 30 mg tamoxifen base) tablets which also may contain carboxymethylcellulose calcium, croscarmellose sodium (type A) [a polymer of carboxymethylcellulose sodium], gelatin, hydroxypropyl methylcellulose 2.910, lactose, Macrogel 300, magnesium stearate, mannitol, polyvinylpyrrolidone (povidone), sodium carboxymethylstarch, corn starch or titanium oxide (Thomas, 1991; Farmindustria, 1993; Reynolds, 1993; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1994; Medical Economics, 1996).

The impurities limited by the requirements of the European Pharmacopoeia include: (*E*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethyldimethylamine (the *E*-isomer of tamoxifen); 2-[4-(1-hydroxy-1,2-diphenylbutyl)phenoxy]ethyldimethylamine; 2-[4-(1,2-diphenylvinyl)phenoxy]ethyldimethylamine; 2-[4-(1,2-diphenylprop-1-enyl)phenoxy]dimethylamine; 2-[2-(1,2-diphenylbut-1-enyl)phenoxy]ethyldimethylamine; (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]ethylmethylamine; and 1-(4-dimethylaminoethoxyphenyl)-2-phenylbutan-1-one (Council of Europe, 1995). The United States of America and

British pharmacopoeias limit the *E*-isomer content to not more than 0.3% and 1%, respectively (British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994).

Trade names and designations for tamoxifen citrate and its pharmaceutical preparations include: Apo-Tamox; Citofen; Dignotamoxi; Duratamoxifen 5; Emblon; ICI-46 474; Jenoxifen; Kessar; Ledertam; Noltam; Nolvadex; Nourytam; Novofen; Oestrifen; Oncotam; Retaxim; Tafoxen; Tam; Tamaxin; Tamifen; Tamofen; Tamone; Tamoplex; Tamoxasta; Tamox-Gry; Tamoxigenat; Tamox-Puren; Taxfeno; Terimon; Valodex; Zemide; Zitazonium.

1.1.5 *Analysis*

Several international pharmacopoeias specify potentiometric titration with perchloric acid as the assay for purity of tamoxifen citrate, and liquid chromatography (LC) or gas chromatography with flame ionization detection for determining levels of the *E*-isomer and other impurities and decomposition products. The assays specified for tamoxifen citrate in tablets use LC and ultraviolet/visible absorption spectroscopy with standards. An assay for heavy metal impurities is also specified (British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994; Council of Europe, 1995).

Tamoxifen and its metabolites can be analysed in biological fluids and tissues by thin-layer chromatography (Furr & Jordan, 1984), gas chromatography–mass spectrometry (MS) (Furr & Jordan, 1984) and high-performance liquid chromatography with ultraviolet, fluorimetric or electrochemical detection (Chamart *et al.*, 1989; Berthou & Dréano, 1993; Lim *et al.*, 1993; Fried & Wainer, 1994).

1.2 Production and use

1.2.1 *Production*

Tamoxifen is prepared by reacting 4-β-dimethylaminoethoxy-α-ethyldesoxybenzoin with phenylmagnesium bromide or phenyl lithium to form 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbutanol, which on dehydration yields a mixture of tamoxifen and its *E*-isomer that may be separated with petroleum ether. Tamoxifen is converted to the 1:1 citrate for pharmaceutical use (Gennaro, 1995).

Worldwide production of tamoxifen citrate has increased from approximately 7.0 tonnes in 1989 to 8.5 tonnes in 1991, 10.1 tonnes in 1993 and 10.3 tonnes in 1995.

1.2.2 *Use*

Tamoxifen was first synthesized by Bedford and Richardson in the United Kingdom (Bedford & Richardson, 1966). It was shown to be an anti-fertility agent in rats (Harper & Walpole, 1967a,b), but was soon found to induce ovulation in women and to have either oestrogenic or antioestrogenic effects, depending on species specificity and tissue and receptor status (Harper & Walpole, 1966, 1967a). The earliest clinical studies were carried out in postmenopausal women with advanced breast cancer in Manchester,

United Kingdom (Cole *et al.*, 1971). It was soon appreciated that tamoxifen was a successful palliative therapy for advanced breast cancer, yielding response (see Glossary, p. 449) rates similar to those seen with other endocrine approaches while producing few side-effects (Ward 1973; O'Halloran & Maddock, 1974). It was approved for use as a pharmaceutical in the United Kingdom in 1973 (Jordan, 1988). By 1978, it was being widely adopted as first-line endocrine therapy for postmenopausal women with advanced disease, particularly after it was shown to be as effective as, and less toxic than, diethylstilboestrol, the previous standard in that setting (Ingle *et al.*, 1981). It was tested in premenopausal women for therapy of metastatic breast cancer from the late 1970s (Manni *et al.*, 1979; Pritchard *et al.*, 1980; Planting *et al.*, 1985; Buchanan *et al.*, 1986; Ingle *et al.*, 1986; Sawka *et al.*, 1986) and shown to be 20–30% effective, but it has probably been far less widely used in this younger group, with chemotherapy or ovarian ablation remaining the more usual approaches (Sunderland & Osborne, 1991; Early Breast Cancer Trialists' Collaboration Group, 1992).

Since the early 1980s, tamoxifen has been widely accepted as first-line endocrine therapy for metastatic disease in postmenopausal women (Ingle, 1984). Most postmenopausal women who develop metastatic disease will at some point undergo at least one attempt at endocrine therapy, either as a palliative treatment or as first-line therapy for metastatic disease. Even those women who respond to tamoxifen for metastatic disease generally receive it for, on average, only 9–12 months (Muss, 1992).

In the mid- to late 1970s, many investigators became interested in using tamoxifen as adjuvant therapy in women at high risk for recurrence of breast cancer following surgery, because of the equivalence of this approach to other endocrine therapies and the drug's extremely low short-term toxicity profile. Several large trials of adjuvant tamoxifen therapy in mostly postmenopausal, axillary node-positive women demonstrated a small but statistically significant improvement (about 25%) in both recurrence-free and overall survival in these patient groups (Nolvadex Adjuvant Trial Organisation, 1983; Ribeiro & Swindell, 1985). Although the relative merits of tamoxifen and chemotherapy for use in this setting were widely debated, particularly between the United Kingdom and the United States, the final results from the Oxford Overview (Early Breast Cancer Trialists' Collaborative Group, 1988) convinced clinicians on both sides of the Atlantic of the benefit of tamoxifen not only in this patient group but in others as well (Breast Cancer Chemotherapy Consensus Conference, 1985; Glick *et al.*, 1992).

From the early days of trials of adjuvant tamoxifen therapy, British clinicians in particular favoured the use of tamoxifen in node-negative (lower-risk) and premenopausal women as well as in postmenopausal, node-positive women. Several large trials soon supported the use of tamoxifen in this setting and showed that it improved overall and recurrence-free survival (Breast Cancer Trials Committee, 1987; Fisher *et al.*, 1989a,b).

From the early days of these trials, dose and dosage varied from one country to another. In the United States and the United Kingdom, 20 mg daily given for one to two years was the early norm (Nolvadex Adjuvant Trial Organisation, 1983; Fisher *et al.*, 1986; Nolvadex Adjuvant Trial Organisation, 1988), while in continental Europe doses

of 30–40 mg daily for one to two years were more usual (Fornander *et al.*, 1991; Mouridsen *et al.*, 1988; Rutqvist *et al.*, 1992; Rutqvist & Mattsson, 1993; Rutqvist *et al.*, 1995). The wide variety of dosing is clear from the summary tables of the Early Breast Cancer Trialists' Collaborative Group (1988, 1992). In addition, data from rat models suggested that longer tamoxifen treatment would be advantageous (Jordan, 1978; Jordan *et al.*, 1979, 1980) and trials examining two versus five years, five versus ten years and two or five years versus indefinite tamoxifen were undertaken. From the mid-1980s to the mid-1990s, tamoxifen therapy of five years or longer was used increasingly in many countries. Further, with reports of its effectiveness in node-negative women, the use of tamoxifen spread widely, to the point where it was sometimes used to treat even very small (< 1 cm) invasive carcinomas and carcinomas *in situ* for which it had never been tested in randomized trials. Trials of its use for very small invasive cancers and carcinoma *in situ* are now in progress: National Surgical Adjuvant Breast and Bowel Project, B21, B24.

Tamoxifen has been the adjuvant therapy of choice for postmenopausal, node-positive women and oestrogen receptor-positive or progesterone receptor-positive (see Glossary, p. 448) since the mid-1980s and for postmenopausal, node-negative and oestrogen receptor-positive or progesterone receptor-positive women since the early 1990s. It is also used in many cases in postmenopausal receptor-negative women and in premenopausal women with low-risk (node-negative) receptor-positive disease (Glick *et al.*, 1992; National Institutes of Health Consensus Development Panel, 1992; Goldhirsch *et al.*, 1995). In both pre- and postmenopausal women, it is also often given concurrently with or following chemotherapy as a type of adjuvant maintenance (Glick *et al.*, 1992; Tormey *et al.*, 1993; Goldhirsch *et al.*, 1995). Thus, a high proportion (40–60%) of all women who undergo potentially curative surgery for breast cancer now receive adjuvant tamoxifen therapy for a period of some two to five years.

Most breast cancer patients with metastatic disease receive, at some time in the course of their treatment, cytotoxic chemotherapy as well as tamoxifen and often other hormonal therapies. Similarly, many women, particularly in the postmenopausal, axillary node-positive subset, receive cytotoxic chemotherapy as well as tamoxifen as part of their adjuvant therapy. Again, the Early Breast Cancer Trialists' Collaborative Group (1988, 1992) summary tables document the use of these drugs, which are mainly cyclophosphamide, methotrexate and 5-fluorouracil-based combinations, but also include other cytotoxic agents such as melphalan and adriamycin, many of which are documented carcinogens in their own right (see IARC, 1987a). There are nevertheless many randomized trials of tamoxifen adjuvant therapy versus no therapy, particularly in postmenopausal axillary node-positive and pre- and postmenopausal node-negative subjects, from which information relating to tamoxifen exposure without accompanying cytotoxic agents can be obtained (Early Breast Cancer Trialists' Collaborative Group, 1992).

Tamoxifen has also been commonly used, without surgery, as a primary therapy for breast cancer in elderly women who are considered poor candidates for surgery (Akhtar *et al.*, 1991), although two randomized studies have suggested that surgical removal of the primary tumour plus tamoxifen treatment is preferable, leading to fewer problems with local recurrence (Bates *et al.*, 1991; Mustacchi *et al.*, 1994).

In the mid-1980s, because of its known cytostatic action and because of the reduction in new contralateral breast cancers observed in many clinical trials of tamoxifen (Breast Cancer Chemotherapy Consensus Conference, 1985; Nolvadex Adjuvant Trial Organisation, 1988; Early Breast Cancer Trialists' Collaborative Group, 1992), considerable interest arose in using tamoxifen as a preventive agent. At least three large trials are in progress in Italy (hysterectomized women), North America and the United Kingdom (Powles *et al.*, 1990; National Surgical Adjuvant Breast and Bowel Project, 1992; Redmond *et al.*, 1993; Vanchieri, 1993; Costa *et al.*, 1996). The subjects are women believed to be at high risk for developing breast cancer. These so-called high-risk women include those at a risk as low as that of the average 60-year-old in at least one trial (National Surgical Adjuvant Breast and Bowel Project, 1992). Although the practice is discouraged by most investigators, it is known that some women are currently prescribed tamoxifen as a preventive agent outside of study, in various high-risk situations (such as women with lobular carcinoma *in situ* or a family history of breast cancer).

Tamoxifen has been widely adopted as the appropriate first-line therapy for hormone-responsive male breast cancer and is also used frequently as adjuvant therapy for men with oestrogen receptor- or progesterone receptor-positive breast cancer, in spite of the lack of any large randomized trials in this relatively small group of patients (Jaiyesimi *et al.*, 1992).

Tamoxifen, at doses similar to those used in breast cancer treatment, has given response rates of 20–30% in several phase II trials in women with advanced endometrial cancer (Quinn & Campbell, 1989; Barakat & Hoskins, 1994; Lentz, 1994) and has been accepted as a second-line endocrine therapy for unresectable or recurrent endometrial cancer (Swenerton *et al.*, 1984).

There have also been studies, but probably not wide use, of tamoxifen treatment for a variety of other malignancies, including hepatocellular carcinoma (Martínez Cerezo *et al.*, 1994), carcinoma of the stomach (Harrison *et al.*, 1989), renal-cell carcinoma (Yagoda *et al.*, 1995), melanoma (McClay & McClay, 1994), adenocarcinoma of the pancreas (Taylor *et al.*, 1993; Wong & Chan, 1993), carcinoma of the cervix (Vargas Roig *et al.*, 1993), carcinoma of the ovary (Hatch *et al.*, 1991), glioblastoma multiforme (Baltuch *et al.*, 1993), carcinoma of the biliary tract (West *et al.*, 1990), desmoid tumours (Brooks *et al.*, 1992) and meningiomas (Goodwin *et al.*, 1993). In addition, tamoxifen has been suggested to be useful in treating certain non-malignant conditions including retroperitoneal fibrosis, the POEMS syndrome (a multi-system syndrome consisting of polyneuropathy, organomegaly, endocrinopathy, serum M-band and skin changes) (Enevoldson & Harding, 1992), oligoospermia in men with incomplete androgen sensitivity (Gooren, 1989), idiopathic oligozoospermia (Sterzik *et al.*, 1993), pulmonary lymphangioleiomyomatosis (Kitaichi *et al.*, 1995), rectal polyps in patients with Gardner's syndrome (Parry *et al.*, 1993), Peyronie's disease (Ralph *et al.*, 1992), autoimmune progesterone dermatitis (Stephens *et al.*, 1989), menstrual migraine (O'Dea & Davis, 1990) and painful idiopathic gynaecomastia (McDermott *et al.*, 1990). It has also been shown to have anti-candidal activity (Beggs, 1993).

Tamoxifen has also been suggested to be useful as an adjuvant to cancer chemotherapy of various types, in that it displays synergy with drugs such as cisplatin (McClay *et al.*, 1993). Tamoxifen has also been shown to cause apoptotic effects which appear to be independent of the oestrogen receptor status of the cells affected (Perry *et al.*, 1985).

Interest has focused more recently on positive effects of tamoxifen on cardiovascular lipid profiles (Bruning *et al.*, 1988; Love *et al.*, 1990, 1991; Shewmon *et al.*, 1994; Thangaraju *et al.*, 1994; Grey *et al.*, 1995a; Guetta *et al.*, 1995; Gylling *et al.*, 1995; Kenny *et al.*, 1995), on cardiovascular events (Rutqvist & Mattsson, 1993) and cardiovascular deaths (McDonald & Stewart, 1991) and on bone density (Love *et al.*, 1988; Wolter *et al.*, 1988; Kristensen *et al.*, 1994; Love *et al.*, 1994a; Wright *et al.*, 1994; Grey *et al.*, 1995b; Kenny *et al.*, 1995; Leslie *et al.*, 1995). These apparently positive effects have made the drug even more attractive for long-term use in early breast cancer and as a preventive agent. Some authors have even hypothesized that it may have more beneficial preventive effects on mortality and morbidity from causes other than breast cancer, at least for women over 60 (Gray, 1993).

Thus, tamoxifen has become very widely used around the world. It is estimated that there have been over 7 million patient-years of treatment with tamoxifen since it was first approved in 1973. Tamoxifen is now registered for use in 97 countries. Most women and men with metastatic breast cancer receive it at some time in their therapy, and 40–60% of women may receive it as adjuvant therapy, in some instances for five years or longer. It is being increasingly tested as a preventive agent against the development of breast cancer. Studies are in progress to examine potential effects of its long-term use on risks for cardiovascular disease and osteoporosis.

1.3 Occurrence

Tamoxifen is not known to occur as a natural product.

The National Occupational Exposure Survey conducted between 1981 and 1983 in the United States by the National Institute for Occupational Safety and Health indicated that approximately 350 and 2100 employees were potentially occupationally exposed to tamoxifen and tamoxifen citrate, respectively. The estimate was based on a survey of companies and did not involve measurements of actual exposure (United States National Library of Medicine, 1996).

1.4 Regulations and guidelines

Tamoxifen citrate is listed in the following pharmacopoeias: British, French, Greek and United States (Reynolds, 1993; Vidal, 1995).

2. Studies of Cancer in Humans

Introduction

In this section, epidemiological evidence is considered with regard to the occurrence of second primary cancers after the use of tamoxifen (invariably as tamoxifen citrate) for the treatment of breast cancer. These data include descriptive studies of single cases and case series, case–control studies, cohort studies and randomized clinical trials. Data on the use of tamoxifen in healthy women are currently very limited.

2.1 Endometrial cancer

2.1.1 *Case reports*

Following an initial report by Killackey *et al.* (1985) of three cases of endometrial cancer in women who received tamoxifen for breast cancer, a large number of case reports have been published. Those available to the Working Group are summarized in Table 1. One hundred and two cases of endometrial cancer were reported in 32 case reports, ranging from isolated cases to series of up to 20 cases. The reports concern 72 adenocarcinomas, including 8 mucinous, 3 clear-cell adenocarcinomas and 1 serous papillary adenocarcinoma, 14 Müllerian mixed tumours, 1 stromal sarcoma and 13 carcinomas not otherwise specified. [The Working Group noted that rare histological types of endometrial cancer are over-represented in these reports.]

2.1.2 *Case series*

Two groups of case series were available to the Working Group. The first comprises series of cases of endometrial cancer following breast cancer, among whom tamoxifen use was assessed (Table 2). In one of these series, 15 of 53 cases had received tamoxifen, and more of these had high-grade tumours (poorly differentiated endometrial carcinomas) (67%) than in the group not treated with tamoxifen (24%; $p = 0.03$) (Magriples *et al.*, 1993). In another series of 73 cases, of whom 23 had received tamoxifen, the proportion of high-grade tumours was 23% in tamoxifen-treated patients and 19% in non-treated patients. The disease stage (according to the current International Federation of Gynecology and Obstetrics (FIGO) criteria) in this series did not differ between tamoxifen-treated and untreated cases (Barakat *et al.*, 1994). The second group comprises series of cases of breast cancer treated with tamoxifen, among whom the development of endometrial cancer was assessed (Table 3).

2.1.3 *Case–control studies*

The case–control studies considered by the Working Group were those which compared tamoxifen use in women with breast cancer who did (cases) or did not (controls) subsequently develop endometrial cancer. A fundamental requirement for these controls is that they, like the cases, were at risk for developing endometrial cancer (namely, that they had an intact uterus). The failure to consider the hysterectomy status

Table 1. Case reports of gynaecological cancers in patients receiving tamoxifen for breast cancer

Reference, country	No. of cases	Dose (mg/day)	Duration (years)	Latency (years)	Additional therapy[a]	Endometrial cancers[b]	Other cancers, when specified	Histological grade[c]	Tumour stage[d]
Killackey *et al.* (1985) United States	3 (1 pre-meno-pausal)	all, 20	all, < 2	all, < 2	2 cases, chemo	2 adenocarcinomas 1 carcinoma (NOS)	–	2G1, 1G2	all, I
Atlante *et al.* (1990) Italy	4	2 cases, 40 2 cases, 60	3 cases, 2–5 1 case, > 5	3 cases, 3–5 1 case, > 5	3 cases, chemo 1 case, radio	4 adenocarcinomas	–	1G1, 2G2, 1G3	all, I
Dauplat *et al.* (1990) France	2	1 case, 30 1 case, 20	1 case, < 2 1 case, 2	1 case, < 2 1 case, 2–5	NS	1 adenocarcinoma 1 adenoacanthoma	–	G1 NS	NS
Malfetano (1990) United States	7	all, 40	2 cases, < 2 5 cases, 2–4	1 case, < 2 5 cases, 2–5 1 case, > 5	3 cases, chemo	7 carcinomas (NOS)	–	2G1, 3G2, 2G3	6 cases, I 1 case, NS
Mathew *et al.* (1990) United States	5	3 cases, 20 2 cases, NG	2 cases, 2–5 3 cases, > 5	2 cases, 2–5 3 cases, > 5	NS	3 adenocarcinomas 2 carcinomas (NOS)	–	2G1 3NS	1 case, I 4 cases, NS
Rodier *et al.* (1990) France	1	40	< 2	< 2	Radio	Adenocarcinoma	–	G1	II
Lang-Avérous *et al.* (1991) Germany	3	10–30	2–5	2–5	NS	3 adenocarcinomas (2 mucinous)	–	2G1, 1G2	NS
Rasmussen & Nielsen (1991) Denmark	2	both, 30	both, > 5	both, > 5	both, radio	2 adenocarcinomas	–	1G2, 1G3	1 case, IV 1 case, II
Spinelli *et al.* (1991) Italy	3 (preme-nopausal)	all, 40	1 case, < 2 1 case, 2–5 1 case, > 5	1 case, < 2 1 case, 2–5 1 case, > 5	2 cases, chemo	3 adenocarcinomas	–	2G1, 1G3	all, I
Bocklage *et al.* (1992) United States	1	20	< 2	< 2	Radio and chemo	Müllerian mixed tumour	–	G3	I

Table 1 (contd)

Reference, country	No. of cases	Dose (mg/day)	Duration (years)	Latency (years)	Additional therapy[a]	Endometrial cancers[b]	Other cancers, when specified	Histological grade[c]	Tumour stage[d]
Deprest *et al.* (1992) Belgium	1	40	> 5	> 5	Chemo	Serous papillary adenocarcinoma	–	G3	III
Le Bouëdec & Dauplat (1992) France	4	2 cases, 20 2 cases, 30	2 cases, < 2 2 cases, 2–3	2 cases, < 2 2 cases, 2–5	1 radio, 1 chemo 2 radio + chemo	3 adenocarcinomas 1 adenoacanthoma	–	3G1 G2	All, I
Mignotte *et al.* (1992) France	20	all, 20	14 cases, 2–5 6 cases, > 5	1 case, 2–5 1 case, 2 2 cases, < 2	13 chemo	8 in situ adeno-carcinomas 10 invasive adeno-carcinomas 2 Müllerian mixed tumours	–	16 G1 2 G2 2 NS	8 cases, 0 10 cases, I 1 case, II 1 case, III
Segna *et al.* (1992) United States	11	2 cases, 10 9 cases, 20	2 cases, < 2 8 cases, 2–5 1 case, > 5	NG	–	11 adenocarcinomas	–	5G1 4G2 2G3	All, I
Altaras *et al.* (1993) Israël	1	20	> 5	> 5	Radio	Müllerian mixed tumour	–	G3	I
Clarke (1993) United States	1	20	> 5	> 5	0	Müllerian mixed tumour	–	G3	I
McAuliffe (1993) Ireland	5	NG	NG	NG	NG	3 carcinomas (NOS) 1 Müllerian mixed tumour 1 papillary adeno-carcinoma	–	3G3 2 NS –	NS
Palacios *et al.* (1993) Spain	1	20	> 5	> 5	Radio	Adenocarcinoma	–	G2	I

Table 1 (contd)

Reference, country	No. of cases	Dose (mg/day)	Duration (years)	Latency (years)	Additional therapy[a]	Endometrial cancers[b]	Other cancers, when specified	Histological grade[c]	Tumour stage[d]
Seoud *et al.* (1993) United States	5	20	2 cases, < 2 3 cases, > 2	NG	NS	3 adenocarcinomas		3G2	2 cases, I 1 case, III
						1 Müllerian mixed tumour		NS	III
							1 fallopian tube adeno-carcinoma		
Bardi *et al.* (1994) Italy	1	30	> 2	2–5	Chemo	None	Endometrioid carcinoma in pelvic endo-metriosis	G1	–
Cohen *et al.* (1994) Israël	1	20	1	< 2	None	None	Ovarian endometrioid carcinoma	G2	I
Gherman *et al.* (1994) United States	1	20	< 2	2	Radio	None	Ovarian granulosa cell tumour	NS	I
Krause & Gerber (1994) Germany	1	30	~ 2	~ 2	NS	Adenocarcinoma	–	G1	NS
Lanza *et al.* (1994) Italy	2	1 case, 20 1 case, 40	1 case, 5 1 case, > 5	1 case, 5 1 case, > 5	Chemo	2 adenocarcinomas	–	1G1, 1G3	1 case, II 1 case, III
Sonnendecker *et al.* (1994) South Africa	1	20	2	2	Radio	None	Fallopian tube adenocarci-noma *in situ*	G1	0
Beer *et al.* (1995) United Kingdom	1	20	5	5	None	1 adenocarcinoma + 1 stromal sarcoma in the same patient	–	G1 NS	Both, I

Table 1 (contd)

Reference, country	No. of cases	Dose (mg/day)	Duration (years)	Latency (years)	Additional therapy[a]	Endometrial cancers[b]	Other cancers, when specified	Histological grade[c]	Tumour stage[d]
Dallenbach-Hellweg & Hahn (1995) Germany	10	1 case, 10 2 cases, 20 7 cases, 30	2 cases, < 2 5 cases, 2–5 2 cases, > 5 1 case, NG	NS	NS	3 clear-cell adenocarcinomas 6 mucinous adenocarcinomas 1 papillary adenocarcinoma of endometrioid type	–	1G1, 9G2	All, I
Evans *et al.* (1995) United Kingdom	6	NG	1 case, 3 5 cases, > 5	NG	NS	6 Müllerian mixed tumours	–	NS	NS
Gillett (1995) Australia	1	20	4.5	> 5	NS	None	1 leiomyosarcoma of myometrium uteri	–	I
Jose *et al.* (1995) India	1	20	> 5	> 5	Radio	Adenocarcinoma	–	G1	I
LiVolsi *et al.* (1995) United States	1	20	1	1	Radio	–	Papillary mucinous endocervical adenocarcinoma	G1	I
Sasco *et al.* (1995) France	1	20	< 5	> 5	Radio	Müllerian mixed tumour	–	NS	II

[a] Chemo, chemotherapy; radio, radiotherapy
[b] NOS, not otherwise specified; NS, not specified; NG, not given
[c] Histological classification: G1, grade 1, well differentiated; G2, grade 2, moderately differentiated; G3, grade 3, poorly differentiated
[d] FIGO stages [International Federation of Gynecology and Obstetrics]: 0, carcinoma in situ; I, tumour confined to corpus; II, tumour invades cervix but does not enter beyond uterus; III, local and/or regional spread; IV, distant metastasis or/and tumour invades bladder mucosa and/or bowel mucosa.

Table 2. Use of tamoxifen among endometrial cancer case series following breast cancer

Reference	Country (period)	No. of endometrial cancer cases	No. (%) of tamoxifen users	Dose (mg/day)	Duration (years)[a]	Comments
Hardell (1988a)	Sweden (1959–88)	23	11 (48)	40	1–9	Series also included in the case–control study, p. 267. In tamoxifen users, 6 cases also had pelvic radiotherapy for ovarian ablation and 2 cases also had adjuvant chemotherapy; the tumours of the corpus uteri were: 9 carcinomas, 1 carcinosarcoma, 1 anaplastic cancer.
Magriples *et al.* (1993)	USA (1980–90)	53	15 (28) (5 deaths)[b]	40	0.2–10 (4.2)	Higher-grade tumours in tamoxifen users (67% versus 24%). The endometrial cancers were: 9 endometrioid carcinomas, 3 papillary serous carcinomas, 1 clear-cell carcinoma, 2 Müllerian mixed tumours; 3 cases had also adjuvant chemotherapy and 1 case pelvic radiotherapy for ovarian ablation. One premenopausal woman among the 53 cases.
Barakat *et al.* (1994)	USA (1980–92)	73	23 (32) (5 deaths)[b]	20	1–10.5 (4.5)	No difference in stage between tamoxifen-treated and untreated patients. In tamoxifen users, 17 had adenocarcinoma of the corpus uteri, 1 had a papillary serous carcinoma, 5 had Müllerian mixed tumours; 5 cases received adjuvant chemotherapy; some cases received radiotherapy.
Silva *et al.* (1994)	USA (NA)	72	15 (20) (1 death)[b]	20	0.2–5.5 (2)	In tamoxifen users, the tumours of the uterine corpus were: 3 endometrial carcinomas, 4 clear-cell carcinomas, 5 serous carcinomas, 1 Müllerian mixed tumour, 2 leiomyosarcomas. 2/15 also received premarin.

NA, not available
[a] Mean or median in parentheses
[b] Number of deaths from endometrial cancer

Table 3. Case series of endometrial cancer among breast cancer patients treated with tamoxifen

Reference	Country (period)	No. of tamoxifen users[a]	Dose (mg/day)	Duration (years)[b]	No. (%) of endometrial cancer cases	Comments
De Muylder *et al.* (1991)	Belgium (NA)	46 (23 LRT, 15 CT)	NA	0.5–3	2 (4)	Both adenocarcinomas were of grade 3 and stage I; 12 premenopausal women
Samelis *et al.* (1992)	Greece (NA)	243	20–40	2–13 (4)	1 (0.5)	Six other cancers found (2 ovarian); 64 pre-menopausal women
Lahti *et al.* (1993)	Finland (1991)	51 (1 CT, 48 LRT)	20–40	0.5–8 (2.5)	1 (2)	One adenocarcinoma of grade 1
Uziely *et al.* (1993)	Israel (1990–92)	95	20	1–7 (2)	3 (3)	All endometrial cancers have been found in women with > 1 year tamoxifen therapy.
Gibson *et al.* (1994)	USA (1986–93)	72	NA	2	6 (8)	Six adenocarcinomas

NA, not available

[a] Patients had also local radiotherapy (LRT) and chemotherapy (CT)

[b] Mean or median in parenthesis

of controls may invalidate a case–control study of endometrial cancer. Other issues to be considered include factors which either may be determinants of risk for endometrial cancer (age, nulliparity, obesity, diabetes, hypertension, age at menopause and use of unopposed oestrogen therapy) or may influence the likelihood of tamoxifen prescription (calendar year of breast cancer diagnosis, menopausal status, and stage and oestrogen receptor status of the breast cancer). 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 pertain. Finally, the possibility that endometrial cancer was diagnosed preferentially in women who had received tamoxifen constitutes a potential bias that is considered in greater detail in the introductory remarks to cohort studies and randomized trials (Sections 2.1.4 and 2.1.5).

Hardell (1988a) described a case series from a Swedish registry, covering 32 cases of endometrial cancer diagnosed at least one year after breast cancer. He then examined the same group in a case–control study (Hardell, 1988b), including 23 of these cases compared to 92 age- and breast cancer-matched controls. Eleven cases (48%) compared with 18 controls (20%) had received tamoxifen at 40 mg/day and nine (39%) cases compared with 10 (11%) controls had received pelvic irradiation. The odds ratio for patients receiving both treatments was 7.1 (95% confidence interval (CI), 2.3–22.1) compared to neither treatment, that for tamoxifen alone was 2.6 (95% CI, 0.7–9.6) and that for pelvic irradiation alone was 4.7 (95% CI, 0.8–27.3). [The Working Group noted that the presence of an intact uterus was not confirmed in the controls.]

van Leeuwen *et al.* (1994) conducted a large case–control study in the Netherlands in which 98 cases of endometrial cancer diagnosed at least three months following breast cancer were matched by age and date of diagnosis of breast cancer with 285 controls with breast cancer who survived with an intact uterus at least up to the time of diagnosis of endometrial cancer of the cases. Twenty-three cases and 58 controls had had treatment with tamoxifen. The relative risk associated with any use of tamoxifen was 1.3 (95% CI, 0.7–2.4). Statistically significant trends with duration and cumulative dose were found ($p < 0.05$ for both). The odds ratio for women who had taken tamoxifen for more than two years was 2.3 (95% CI, 0.9–5.9). Most women were treated with doses of 40 mg/day (59%), 17% received 30 mg/day and 23%, ≤ 20 mg/day. The duration–response trends were similar with daily doses of 40 mg or 30 mg and less. No difference in stage or histology between the exposed and unexposed cases was found. [The Working Group noted that the statistical power to detect differences in risk associated with different dose intensities of tamoxifen was low.]

Cook *et al.* (1995) conducted a case–control study involving women under the age of 85 years registered in the Washington State Cancer Registry with a diagnosis of breast cancer between 1978 and 1990, who subsequently developed endometrial cancer at least six months after breast cancer. These were matched with controls without second primary cancer by age, year of breast cancer diagnosis and stage of cancer. Controls had to have an intact uterus and to have survived at least up to the time of diagnosis of endometrial cancer of their matched cases. Thirty-four endometrial cancer patients and 64 matched controls were analysed. All but two cases were postmenopausal. Tamoxifen

use [mainly 20 mg/day] was more common in the controls (31% versus 26%). After adjustment for cytotoxic chemotherapy and duration of oestrogen replacement therapy, the matched odds ratio for any use was 0.6 (95% CI, 0.2–1.9). The mean duration of use was 14 months for cases and 21 months for controls. The odds ratio for more than one year's use was 0.2 (95% CI, 0.1–1.0) based on three cases and 16 controls. [The Working Group noted the inability of this study to address risks associated with long-term use of tamoxifen.]

Sasco *et al.* (1996) conducted a case–control study in Lyon and Dijon, France. Forty-three cases of endometrial cancer occurring at least one year after the diagnosis of breast cancer were matched with 177 controls for age, region, year of diagnosis of breast cancer, intact uterus and survival with breast cancer. The median dose was 20 mg/day; the median duration of treatment was greater in cases (63 months) than in controls (37 months); and tamoxifen was used in 67% of cases and 60% of controls (odds ratio, 1.4; 95% CI, 0.6–3.5). Information on duration of use was missing for 21% of exposed cases and 45% of exposed controls. The risk appeared to increase with duration of use, with a relative risk of 3.5 (95% CI, 0.9–12.7) for more than five years of use. [The Working Group noted that a difference in the percentages of cases and controls with unknown duration of treatment could have exaggerated the estimate of the effect of duration.]

2.1.4 *Cohort studies*

Detection bias may pertain to both cohort studies and randomized clinical trials, since 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. Growth of leiomyomata may occur and provide a further opportunity for the diagnosis of endometrial cancer.

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 analysis or analysis of rates based on person-years at risk.

In an abstract, Champion *et al.* (1991) reported a breast cancer registry-based series from northern Alberta, Canada. Between 1953 and 1988, a total of 1874 women had taken tamoxifen, 20 mg/day for 22, 36 and 39 months and 8201 had not. Thirty-one women developed uterine cancer, three (two sarcomas and one adenocarcinoma) in the tamoxifen group and 28 in the other group. [The Working Group noted that no adjustment for period of diagnosis was made in this study and the results therefore could not be interpreted.]

Robinson *et al.* (1995) reviewed 586 eligible breast cancer patients without a previous hysterectomy in a medical centre series from Texas, United States. Of 108 patients who received tamoxifen (20 mg/day for at least one year), four developed endometrial adenocarcinoma and, of 478 breast cancer patients who did not receive tamoxifen, four developed endometrial cancer [odds ratio, 4.6; 95% CI, 1.3–16.0]. After adjustment for

hypertension and diabetes mellitus, the odds ratio for development of endometrial cancer after tamoxifen use was 15.2 (95% CI, 2.8–84.4). [The Working Group noted the imprecision of the estimates and the difference between the crude and adjusted odds ratios, that the results were not adjusted for follow-up time and that the presence of an intact uterus during follow-up was not controlled for.]

Curtis *et al.* (1996) examined the effect of tamoxifen on risk for endometrial cancer in 87 323 women with breast cancer reported to the SEER (Surveillance, Epidemiology and End Results) Program in the United States. All women included in this study were diagnosed with early-stage (localized or regional) breast cancer between 1980 and 1992, were aged at least 50 years at diagnosis and had not been given chemotherapy as an initial treatment. For 14 358 women defined as the study group, the SEER database indicated that they had received hormonal therapy (which for over 90% was tamoxifen treatment). After a mean follow-up of [4.4] years, 73 cancers of the uterine corpus were observed, resulting in a standardized incidence ratio (SIR) (based on SEER incidence rates) of 2.0 (95% CI, 1.6–2.6). The SIR for women not known to have received hormones was significantly lower (1.2; 95% CI, 1.1–1.4). The differences in risk for endometrial cancer between hormone-treated women and women with no/unknown hormone treatment status were greater in five-year survivors (SIRs of 3.6 and 1.2, respectively). There was little difference in the severity of grade or stage of cancer of the uterine corpus according to initial therapy: in hormone-treated patients, 59% of the uterine cancers which developed were grade 1 or 2, 25% were grade 3 or 4 and for 16% the grade was unknown (versus 63%, 21% and 16% in no/unknown hormone treatment). The stage distribution was: localized, 78%; regional, 12%; distant, 4%; unknown, 6% (versus 76%, 11%, 8% and 5%, respectively). [The Working Group noted that no information on hysterectomy status was available and that misclassification of hormonal treatment in the study may have led to an underestimation of the difference in risk for cancer of the uterine corpus between the two groups.]

2.1.5 *Randomized clinical trials* (see Table 4)

The design of randomized trials is such that they allow both known and unknown confounding factors to be distributed randomly between treatment groups. Therefore, these studies offer the best evidence regarding tamoxifen and the occurrence of second cancers. However, assessment of the risk for endometrial cancer was not the principal aim of the trials considered by the Working Group. None of the trials included procedures which would assure complete reporting of second primary tumours. Even in the trials (such as those in Scandinavia) that ascertained second primary tumours after linkage of the trial data to a population-based cancer registry, complete ascertainment of such tumours cannot be assumed, as second primary tumours are not reported systematically to cancer registries. However, there is no reason to believe that the reporting of such tumours would be biased by being related to tamoxifen therapy. Other biases which are relevant to randomized clinical trials are the same as those already discussed for cohort studies.

Table 4. Endometrial cancers in patients treated for breast cancer: summary results of randomized clinical trials of adjuvant use of tamoxifen

Reference, country	No. of patients and treatment[a]		Dose of tamoxifen (mg/day)	Duration (years)	Median follow-up (years)	No. of endometrial cancers	Odds ratio (95% CI)
Pritchard *et al.* (1987) (updated by Nayfield *et al.*, 1991) Canada	198	Tamoxifen	20	2	5.8	0	–
	202	Observation				1	
NATO (1988) (updated by Nayfield *et al.*, 1991) United States	564	Tamoxifen	20	2	5.5	0	–
	567	Observation				0	
Palshof (1988) Denmark	164	Tamoxifen (52 postmenopausal)	30	2	9	2	–
	153	Placebo (52 postmenopausal)				0	
Castiglione *et al.* (1990) Several countries	167	Tamoxifen + prednisone	20	1	8	0	–
	153	Observation				0	
Andersson *et al.* (1992) Denmark	864	Tamoxifen + LRT	30	0.9	8	7	3.3 (0.6–31[b])
	846	LRT				2	
Ribeiro & Swindell (1992a) United Kingdom	199	Premenopausal, tamoxifen	20	1	13	0	–
	174	Premenopausal, pelvic irradiation for ovary ablation				0	
	282	Postmenopausal, tamoxifen	20	1	max. 13	1	[1.0 (0.1–16)]
	306	Postmenopausal, observation				1	
Rydén *et al.* (1992) South Sweden	244	Tamoxifen	30	1	9	5	[2.4 (0.5–12)] LRT-tamoxifen versus LRT
	239	Tamoxifen + LRT				4	
	236	LRT				2 (1 death[c])	
Stewart (1992) as updated in Nayfield *et al.* (1991) Scotland	661	Tamoxifen	20	≥ 5	4–10	4	[2.0 (0.4-11)]
	651	Observation				2	

Table 4 (contd)

Reference, country	No. of patients and treatment[a]		Dose of tamoxifen (mg/day)	Duration (years)	Median follow-up (years)	No. of endometrial cancers	Odds ratio (95% CI)
Cummings *et al.* (1993)	85	Tamoxifen	20	2	10	1	[1.0 (0.1–15)]
United States	83	Placebo				1	
Boccardo *et al.* (1994a)	168	Tamoxifen	30	5	≥ 5	0	–
Italy	171	Tamoxifen + Chemotherapy					
		77 premenopausal				0	–
		94 postmenopausal				1	
	165	Chemotherapy				0	
Fisher *et al.* (1994)[d]	1419	Tamoxifen	20	5	8	15	7.5 (1.7–32.7)
Canada , United States						(4 deaths[c,e])	
	1424	Placebo				2	
						(Both received tamoxifen)	
Kedar *et al.* (1994)	61	Tamoxifen	20	NG	2	0	–
United Kingdom	50	Placebo				0	
Rivkin *et al.* (1994)	295	Tamoxifen	20	1	6.5	2	–
United States	303	Tamoxifen + Chemotherapy				3	–
	300	Chemotherapy				0	
Rutqvist *et al.* (1995)	1372	Tamoxifen	40	2 or 5	9	23[f]	5.6 (1.9–16.2)
Stockholm, Sweden						(4 deaths[c])	
	1357	Observation				4	

NG, not given; LRT, local radiotherapy

[a] Observation: patients receiving no adjuvant therapy

[b] The upper 95% confidence limit reported by Andersson *et al.* (1992) is probably overestimated.

[c] Death from endometrial cancer

[d] Fisher *et al.* (1994) in a non-randomized population reported 8 cancers of the endometrium (7 endometrioid cancers) in 1220 registered breast cancer patients given tamoxifen for breast cancer

[e] One did not receive tamoxifen

In a trial in Canada (Pritchard *et al.*, 1987, updated by Nayfield *et al.*, 1991), no case of endometrial cancer was found among 198 breast cancer patients treated with 20 mg/day tamoxifen for two years, but one occurred among 202 patients receiving no adjuvant therapy for breast cancer.

In a trial in the United States of tamoxifen therapy (20 mg/day) for two years for treatment of early breast cancer in women ≤ 75 years old versus no treatment, the NATO (Nolvadex Adjuvant Trial Organization) (1988) reported no case of endometrial cancer in 564 tamoxifen patients or in 567 patients receiving no further treatment following mastectomy during 1977–81 and followed up for eight years.

In a trial carried out in Denmark during 1975–78 with follow-up until 1988 of tamoxifen therapy (30 mg/day) for two years in women who were admitted for breast tumour (stage I, II and III), Palshof (1988) reported two endometrial cancers in 52 tamoxifen-treated postmenopausal patients (< 70 years old) and none in 52 patients in the placebo group. In premenopausal women, no case was found in either 112 tamoxifen-treated patients or 101 placebo patients.

In the IBCSG (International Breast Cancer Study Group) trial of tamoxifen (20 mg/day) plus low-dose prednisone (7.5 mg/day) therapy for one year, no endometrial cancer was observed in either 167 treated patients aged 66–80 years with operable breast cancer or 153 women randomized to observation (Castiglione *et al.*, 1990). Subjects were entered into this trial during 1978–81 and were followed up for a median observation time of eight years.

A Danish study (Andersson *et al.*, 1992) reported seven cases of endometrial cancer among 864 postmenopausal patients receiving radiotherapy and 30 mg tamoxifen for 48 weeks compared with two cases among 846 patients receiving radiotherapy alone. Eleven cases were reported among a third group of 1828 untreated, 'low-risk' patients. SIRs for endometrial cancer were computed from the incidence rates of the female Danish population. [The most valid comparison was between women with and without tamoxifen treatment in the radiotherapy group.] The SIRs for endometrial cancer were 1.9 (95% CI, 0.8–3.9) for patients who received radiotherapy and tamoxifen and 0.6 (95% CI, 0.1–2.1) for patients who received radiotherapy without tamoxifen (ratio of the SIRs, 3.3 (95% CI, 0.6–31).

After a maximal follow-up of 13 years, Ribeiro and Swindell (1992) reported one case of endometrial cancer among 282 postmenopausal patients ≤ 70 years old treated with tamoxifen (20 mg/day) for one year and one endometrial cancer among 306 untreated patients. There was no case of endometrial cancer among 199 premenopausal patients randomized to tamoxifen treatment or 174 patients randomized to irradiation-induced menopause.

In a trial in postmenopausal women, < 71 years old, in Sweden, of radiotherapy alone (236 patients), radiotherapy plus tamoxifen (30 mg/day) for one year (239 patients) or the same tamoxifen regimen alone (244 patients), two, four and five cancers of the corpus uteri were reported, respectively (Rydén *et al.* 1992). One endometrial cancer death was reported in the group receiving radiotherapy only. The median follow-up period was nine years.

In a Scottish trial of tamoxifen treatment (20 mg/day) for five or more years, Stewart and Knight (1989) found three uterine sarcomas in 539 tamoxifen-treated postmenopausal patients and two endometrial cancers in 531 untreated patients. In a subsequent set of 374 adjuvant tamoxifen-treated patients and 373 untreated patients, one endometrial cancer was observed in each group (Stewart, 1992). In another report of the same trial (Nayfield *et al.*, 1991), four cases of endometrial cancer were reported among 661 tamoxifen-treated patients and two cases among 651 untreated patients.

The ECOG (Eastern Cooperation Oncology Group) in the United States open to accrual during 1978–82 (Cummings *et al.*, 1993) found one case of endometrial cancer among 85 treated patients 65–84 years old (20 mg/day tamoxifen for two years) and another case among 83 placebo patients.

In a trial of the Cooperative Group for Chemohormonal Therapy of Early Breast Cancer (GROCTA) in Italy comparing tamoxifen (30 mg/day) for five years with six cycles of cyclophosphamide + methotrexate + 5-fluorouracil followed by four cycles of epidoxorubicin or a combination of the two, no case of endometrial cancer was seen among the 168 patients randomized to tamoxifen alone or among the 165 patients randomized to chemotherapy. One case was observed among 94 postmenopausal women who received both treatments. Women were 35–65 years old (Boccardo *et al.*, 1994a).

In a large placebo-controlled trial, the National Surgical Adjuvant Breast and Bowel Project in Canada and the United States in 1982–88, Fisher *et al.* (1994) reported 15 cases of endometrial cancer among women with invasive breast cancer randomized to tamoxifen (20 mg/day) for five years compared with two cases among the placebo group. It was known at randomization that the proportion of women with hysterectomy was similar in the two groups. Both of the placebo patients with endometrial cancer had received tamoxifen for breast cancer relapse. One of the 15 patients who developed endometrial cancer was allocated to tamoxifen but never received it. One of the 15 endometrial cancers after review was found not to be a cancer. The annual hazard rate in the treated group was 1.6/1000 compared to 0.2/1000 in the placebo group. The latter rate was below that of the general population, from which about seven cancers would have been expected. The relative risk estimates were 7.5 (95% CI, 1.7–32.7) using the placebo control group and 2.2 [95% CI, 1.2–2.9] using population rates. The histological characteristics and grades of endometrial cancer were similar to those in patients who had not been treated with tamoxifen; 9/15 were grade 1 and 11 were stage I. Four patients allocated to tamoxifen died of endometrial cancer (among whom one never received the treatment). [The Working Group noted that the most valid comparison was between the group allocated to tamoxifen and the placebo control group. The rates used for calculating the expected numbers were from the United States SEER Program, whereas seven of the 12 major contributing centres were from Canada, where the rates for endometrial cancer are lower than in the United States.]

Kedar *et al.* (1994) in the United Kingdom recruited a randomized cohort of healthy postmenopausal women with a family history of breast cancer into groups given tamoxifen (20 mg/day) (61) or placebo (50) [duration of treatment not specified]. Fifty-five of the treated women had detectable serum levels of tamoxifen. No endometrial cancer was

found in either group (median follow-up period, two years). [The Working Group noted the small numbers and short follow-up period.]

The SWOG (Southwest Oncology Group) in the United States (Rivkin *et al.*, 1994) reported two cases of endometrial cancer among 295 postmenopausal patients treated with tamoxifen alone, three cases among 303 patients given tamoxifen and chemotherapy and none among 300 patients given chemotherapy alone during 1979–89.

Fornander *et al.* (1989) and Rutqvist *et al.* (1995) reported a study of endometrial cancer in the trial of treatment of postmenopausal women < 71 years old with tamoxifen (40 mg/day) for two or five years. 'Low-risk' patients (1774) and 'high-risk' patients (955) were randomized to tamoxifen or no tamoxifen; 678 of the 'high-risk' patients were also randomized to receive either radiotherapy or chemotherapy. Of the patients who received tamoxifen for two years, 809 were re-randomized to stop treatment or receive an additional three years of tamoxifen therapy. After a median follow-up time of nine years, 23 (one refused to take tamoxifen) endometrial cancers were found in 1372 patients randomized to tamoxifen versus four in 1357 untreated women (RR, 5.6; 95% CI, 1.9–16.2). Other corpus uteri cancers were reported: one in the tamoxifen-allocated group and three in the untreated group. A joint analysis of three Scandinavian trials (in Stockholm, Denmark (Andersson *et al.*, 1992) and the south Sweden (Rydén *et al.*, 1992)) presented in the same paper also showed a significant increase in the risk for endometrial cancer in tamoxifen-allocated patients (RR, 4.1; 95% CI, 1.9–8.9). Fornander *et al.* (1993) provided further details of 22 of the cases in the Stockholm trial, 17 of whom had actually received tamoxifen (out of 19 patients assigned to receive it). All cases were grade 1 or grade 2, and all but three were stage I. Endometrial cancer developed less than two years from the beginning of tamoxifen treatment in five of the 17 treated cases and after five years in four cases. Nine cases had received radiotherapy and two others had received chemotherapy. There were three deaths from endometrial cancer among the 17 cases receiving tamoxifen.

2.2 Breast cancer

2.2.1 *Case–control study*

Cook *et al.* (1995) conducted the only case–control study that considered contralateral breast cancer. A total of 188 (18 receiving tamoxifen) < 85-year-old cases in Washington State, United States, were matched to 328 (58 receiving tamoxifen) controls without second primary cancer as described in Section 2.1.3. A 50% reduction in new breast tumours was observed for any use of tamoxifen (matched odds ratio, 0.5; 95% CI, 0.3–0.9) and an increased protection in women who used tamoxifen for more than one year (matched odds ratio, 0.4; 95% CI, 0.2–0.9). Odd ratios for any use of tamoxifen were somewhat larger in premenopausal than in postmenopausal women (0.7 versus 0.4), but were similar in pre- and postmenopausal women who used tamoxifen for more than one year (0.3 versus 0.4).

2.2.2 *Cohort study*

In the study by Curtis *et al.* (1996) (described in Section 2.1.4) of 87 323 breast cancer patients reported to the United States SEER Program, tamoxifen-treated patients had an SIR for contralateral breast cancer of 1.1 (95% CI, 1.0–1.3), compared with an SIR of 1.6 (95% CI, 1.5–1.7) for patients not known to have received tamoxifen. This represents a significant reduction [of approximately 30%] in the risk for contralateral breast cancer in tamoxifen-treated patients.

2.2.3 *Randomized clinical trials*

Results of trials of contralateral breast cancer following tamoxifen treatment are summarized in Table 5.

In Canada, Pritchard *et al.* (1987) (updated by Nayfield *et al.*, 1991) found no difference in the risk of contralateral breast cancer: 3 cases in 198 tamoxifen-treated patients and 3 cases in 202 untreated patients.

In a trial of tamoxifen (20 mg/day) for two years versus no treatment, the NATO (Nolvadex Adjuvant Trial Organization) (1988) and Nayfield *et al.* (1991) reported 15 contralateral tumours in 564 tamoxifen-treated patients and 17 in 567 untreated patients.

A Danish study (Andersson *et al.*, 1992) reported 8 cases of contralateral breast cancer occurring at least one year after the first primary cancer among 864 patients receiving radiotherapy and tamoxifen (30 mg/day) for 48 weeks compared with 10 cases among 846 patients receiving radiotherapy alone. A third group of 1828 untreated, 'low-risk' patients [with longer survival] experienced 10 cases in this period.

Baum *et al.* (1992), of the Cancer Research Campaign Breast Cancer Trials Group in the United Kingdom, in a 2×2 trial of 20 mg/day tamoxifen or perioperative cyclophosphamide found no overall effect of tamoxifen on contralateral breast tumours (RR, 0.9; 95% CI, 0.5–1.5). However, a reduction was seen in postmenopausal women (RR, 0.5; 95% CI, 0.2–1.1), but not in premenopausal women (RR, 1.4; 95% CI, 0.6–3.3).

After a maximal 13 years of follow-up, Ribeiro and Swindell (1992) reported seven cases of contralateral breast cancer in 282 postmenopausal patients treated with tamoxifen (20 mg/day) for one year and nine in 306 untreated patients.

In a trial in southern Sweden of radiotherapy alone (236 patients), radiotherapy plus tamoxifen (30 mg/day) for one year (239 patients) or the same tamoxifen regimen alone (244 patients), 15, 11 and 9 contralateral breast cancers were reported, respectively (Rydén *et al.*, 1992).

In a Scottish trial of tamoxifen (20 mg/day) treatment for five or more years, Stewart (1992) found seven contralateral breast cancers in 374 tamoxifen-treated patients and 20 in 373 untreated patients.

The ECOG (Eastern Cooperative Oncology Group) group (Cummings *et al.*, 1993) found one case of contralateral breast cancer among 85 patients treated with tamoxifen (20 mg/day) for two years and five among 83 untreated patients.

In a trial of the Cooperative Group for Chemohormonal Therapy of Early Breast Cancer (GROCTA) in Italy comparing tamoxifen (30 mg/day) for five years against

Table 5. Contralateral breast cancers: summary results of randomized clinical trials of adjuvant use of tamoxifen

Reference, country	No. of patients and treatment		Dose of tamoxifen (mg/day)	Duration (years)	Median follow-up (years)	No. of contralateral breast cancers	Odds ratio (95% CI)
Pritchard *et al.* (1987) (updated by Nayfield, 1991) Canada	198 202	Tamoxifen Observation	20	2	5.8	3 3	[1.0 (0.2–5.0)]
NATO (1988) (updated by Nayfield *et al.*, 1991) United States	564 567	Tamoxifen Observation	20	2	5.5	15 17	[0.9 (0.4–1.8)]
Palshof (1988) Denmark	164 153	Tamoxifen Placebo	30	2	9	3 4	[0.7 (0.2–3.1)]
Castiglione *et al.* (1990) Several countries	167 153	Tamoxifen + prednisone Observation	20	1	8	1 4	[0.2 (0.03–2.0)]
Andersson *et al.* (1992) Denmark	864 846	Tamoxifen + LRT LRT	30	0.9	8	8 10	[0.8 (0.3–2.0)]
Ribeiro & Swindell (1992a) United Kingdom		Pre-menopausal					
	199 174	Tamoxifen Pelvic irradiation	20	1	Max. 13	3 2	[1.3 (0.2–7.6)]
		Post-menopausal					
	282 306	Tamoxifen Observation	20	1	Max. 13	7 9	[0.8 (0.3–2.2)]

Table 5 (contd)

Reference, country	No. of patients and treatment		Dose of tamoxifen (mg/day)	Duration (years)	Median follow-up (years)	No. of contralateral breast cancers	Odds ratio (95% CI)
Rydén *et al.* (1992) Southern Sweden	244	Tamoxifen	30	1	9	9	[0.6 (0.3–1.3)]
	239	Tamoxifen + LRT				11	[0.7 (0.3–1.5)]
	236	LRT				15	
Stewart (1992) Scotland	374	Tamoxifen	20	≥ 5	4–10	7	[0.3 (0.1–0.8)]
	373	Observation				20	
Cummings *et al.* (1993) United States	85	Tamoxifen	20	2	10	1	[0.2 (0.02–1.6)]
	83	Placebo				5	
Boccardo *et al.* (1994a) Italy	168	Tamoxifen	30	5	≥ 5	0	–
	171	Tamoxifen + Chemotherapy				1	
	165	Chemotherapy				4	[0.2 (0.03–2.1)]
Fisher *et al.* (1994) Canada, United States	1419	Tamoxifen	20	5	8	30	[0.6 (0.4–1.0)]
	1424	Placebo				49	
Rutqvist *et al.* (1995) Stockholm, Sweden	1372	Tamoxifen	40	2	9	40	0.6 (0.4–0.9)
	1357	Observation				66	

LRT, localized radiation therapy

chemotherapy or a combination of the two, four contralateral breast tumours were seen in the 165 patients randomized to chemotherapy, one contralateral tumour in the 171 patients who received both treatments and none in the 168 patients who received tamoxifen alone (Boccardo *et al.*, 1994a).

In a placebo-controlled trial of tamoxifen (20 mg/day) for five years, Fisher *et al.* (1994) found 30 primary contralateral tumours in 1419 patients who received tamoxifen in the first five years of follow-up compared with 49 cases among 1424 patients receiving placebo. The reduction in incidence rate was 42%.

In a Swedish trial of treatment with tamoxifen (40 mg/day) for two or five years, Rutqvist *et al.* (1995) reported 40 contralateral tumours in 1372 women in the tamoxifen group and 66 in 1357 untreated patients. The risk ratio was 0.6 (95% CI, 0.4–0.9).

All of the studies mentioned above, and many other randomized trials, were included in an overview of data available up to 1990 on the occurrence of contralateral breast cancer in women allocated to tamoxifen treatment in trials (EBCTCG (Early Breast Cancer Trialists' Collaborative Group), 1992). In contrast to the analyses in some of the original reports, the life table method of analysis was used in this overview. Based on the occurrence of 122 contralateral breast cancers in 9128 tamoxifen-allocated women and 184 breast cancers in 9135 control patients, a 39% reduction in risk for contralateral breast cancer was observed ($p < 0.00001$). The effects were greater in trials using longer duration of tamoxifen treatment (reductions of risk were 26% for less than two years of tamoxifen treatment, 37% for two years of treatment and 53% for more than two years of treatment), although this was not statistically significant.

2.3 Liver cancer

2.3.1 *Case report*

Johnstone *et al.* (1991) reported a single case of primary hepatocellular carcinoma in a woman who had received 20 mg tamoxifen daily and chemotherapy. The tumour developed six months after treatment began.

2.3.2 *Cohort studies*

There is probably some under-reporting of primary liver cancer in follow-up studies of breast cancer patients, because of confusion of these tumours with metastases from the breast.

Mühlemann *et al.* (1994) examined the incidence of hepatocellular cancer from 1974 to 1989 in white women aged 50 years or more with breast cancer in the United States and found no evidence of an increase after the introduction of tamoxifen in 1977.

Curtis *et al.* (1996) reported on liver cancer risk in 87 323 breast cancer patients reported to the United States SEER Program (see Section 2.1.4). In tamoxifen-treated patients, three cases of liver cancer were observed (SIR, 1.1; 95% CI, 0.2–3.2) and, in the larger group of patients not known to have received tamoxifen, eight cases were seen (SIR, 0.4; 95% CI, 0.2–0.7). [The Working Group noted that the low SIR for liver cancer

in patients not known to have received tamoxifen may indicate underascertainment of liver cancers following breast cancer.]

2.3.2 *Randomized clinical trials*

In the Swedish trial (see p. 274), Rutqvist *et al.* (1995) reported three cases of hepatobiliary (two liver) cancer among subjects randomized to tamoxifen (40 mg/day) for two or five years and one case among untreated patients. Two of the three cases had been treated for 20 and 46 months, respectively (Rutqvist, 1993). Verification of the exposure was not reported for the third case.

The Danish study by Andersson *et al.* (1991) (see p. 271) reported one case of hepatobiliary cancer among 864 treated breast cancer patients (30 mg/day tamoxifen for 48 weeks + radiotherapy) and two cases among 846 radiation-treated breast cancer patients.

A study from southern Sweden (Rydén *et al.* 1992) reported two cases of hepatobiliary cancer among 244 patients who had received tamoxifen (30 mg/day) for one year, no case among 239 patients who had received tamoxifen plus radiotherapy and no case among 236 patients treated with radiotherapy alone.

No case of liver cancer was reported among 1419 tamoxifen-treated (20 mg/day for 5 years) patients and 1424 controls in the NSABP (National Surgical Adjuvant Breast and Bowel Project) B-14 trial (Fisher *et al.*, 1994).

2.4 Other cancers

2.4.1 *Case reports*

Reports exist of one case of papillary mucinous endocervical adenocarcinoma, one in-situ and one invasive fallopian tube adenocarcinoma, one endometrioid carcinoma of the pelvic endometrium, one endometrioid carcinoma, one granulosa cell tumour of the ovary and one leiomyosarcoma of the myometrium uteri among women treated with tamoxifen (see Table 1).

2.4.2 *Case–control study*

Cook *et al.* (1995) reported on a population-based study of ovarian, endometrial and contralateral breast cancers following tamoxifen therapy of breast cancer patients. The results for endometrium and breast cancer have been reported above (pp. 267–268 and 274). For ovarian cancer, 34 cases (6 given tamoxifen) were compared with 89 controls who did not develop a second primary cancer, of whom 18 were given tamoxifen (matched odds ratio, 0.6; 95% CI, 0.2–1.8).

2.4.3 *Cohort studies*

Curtis *et al.* (1996) reported the risks for various second cancers in 87 323 breast cancer patients reported to the United States SEER Program (see p. 269). Between patients who had initial tamoxifen treatment and those not known to have received such treatment, there was no difference in the risks for ovarian cancer, digestive tract cancers or cancers at various other sites (other than endometrial cancer and contralateral breast

cancer). The observed/expected ratios for all digestive system cancers combined were 1.0 (95% CI, 0.9–1.2) in tamoxifen-treated women and 0.9 (95% CI, 0.9–1.0) in women not known to have received tamoxifen treatment.

2.4.3 *Randomized clinical trials*

Two reports of randomized trials have been published which address the risks for second cancers other than those of the endometrium, breast and liver.

In a large trial (see p. 273), Fisher *et al.* (1994) found no significant difference between women allocated to tamoxifen and women allocated to placebo in the risk for second cancers of the colon, rectum, ovary or other sites.

In a joint analysis of three Scandinavian trials (see p. 274), Rutqvist *et al.* (1995) reported an excess risk for gastrointestinal cancer in tamoxifen-allocated women compared with those in untreated groups (RR, 1.9; 95% CI, 1.2–2.9). The relative risk for colorectal cancer was 1.9 (95% CI, 1.1–3.3) and that for stomach cancer was 3.2 (95% CI, 0.9–11.7). For other sites, no difference was observed between tamoxifen-allocated patients and untreated patients. [In view of the number of comparisons made, these results should be interpreted with caution.]

3. Studies of Cancer in Experimental Animals

In the studies reviewed here, the usual form of tamoxifen citrate will be referred to as tamoxifen.

3.1 Oral administration

3.1.1 *Mouse*

In a study reported in a monograph, groups of 25 male and 25 female Alderley Park Strain 1 mice [age unspecified] were given 0 (control), 5 or 50 mg/kg bw tamoxifen [purity not specified] per day by gastric instillation for three months. The mice were then maintained for 12 months on a diet containing tamoxifen at concentrations to provide 0, 5 or 50 mg/kg bw tamoxifen, after which the experiment was terminated because of skeletal abnormalities in many of the exposed mice. Numbers surviving at 15 months were 16/25 control, 11/25 low-dose and 17/25 high-dose males and 15/25 control, 17/25 low-dose and 12/25 high-dose females. In males, interstitial cell tumours of the testes were found in 0/25 control, 2/25 low-dose and 21/25 high-dose animals. In females, granulosa-cell adenomas of the ovary were found in 0/25 control, 9/25 low-dose and 9/25 high-dose animals. Two other studies at lower doses were briefly described (Tucker *et al.*, 1984). [The Working Group noted that the descriptions of these lower-dose studies did not provide sufficient information for evaluation.]

3.1.2 *Rat*

Groups of 51 male and 52 female Alderley Park Wistar-derived rats, five weeks of age, were given 5, 20 or 35 mg/kg bw tamoxifen [purity not specified] per day by gastric instillation in 0.5% hydroxypropyl methylcellulose for two years. A control group of 102 male and 104 female rats was given the vehicle alone. Moribund animals and those surviving to the end of the exposure period were killed and subjected to necropsy; all major tissues were examined histologically. Growth was reduced (by about 30% in females and 40% in males) in all tamoxifen-treated groups compared with controls. Survival was reduced in the groups given the two higher doses but increased in those given the lower dose. The reduced survival was attributed to early deaths from liver tumours and resulted in termination of the study at 87 weeks for the mid-dose group and at 71 weeks for the high-dose group. Hepatocellular adenomas occurred in 1/102 control, 8/51 low-dose, 11/51 mid-dose and 8/51 high-dose males and in 1/104 control, 2/52 low-dose, 6/52 mid-dose and 9/52 high-dose females ($p < 0.0001$ for trend). Hepatocellular carcinomas were found in 1/102 control, 8/51 low-dose, 34/51 mid-dose and 34/51 high-dose males and in 0/104 control, 6/52 low-dose, 37/52 mid-dose and 37/52 high-dose females [$p < 0.001$]. Hepato/cholangiocellular carcinomas were found in 0/102 control, 0/51 low-dose, 2/51 mid-dose and 5/51 high-dose males and in 0/104 control, 0/52 low-dose, 4/52 mid-dose and 5/52 high-dose females ($p < 0.0001$ for trend). No increase in the incidence of tumours was observed at any other site. Significant decreases in tumour incidence were observed in the pituitary and parathyroid glands of males and in the pituitary and mammary glands of females (see Table 6) (Greaves *et al.*, 1993). [The Working Group noted that part of the reduction in tumour rates may have been related to decreased body-weight gain.]

In a study that also evaluated toremifene, groups of 57, 84 and 75 female Sprague-Dawley [Crl:CD(BR)] rats, approximately six weeks of age, were given 0, 11.3 and 22.6 mg/kg bw tamoxifen (99% pure) per day by gastric instillation in 0.5% carboxymethylcellulose on seven days per week for up to 12 months followed by a three-month recovery period. Nine rats from each group were killed at three and six months. At 12 months, 18 control, 36 low-dose and 24 high-dose rats were killed. The experiment was terminated at 15 months. All rats, including those found dead or moribund, were subjected to necropsy; organs examined histopathologically included liver, ovaries, uterus, mammary gland, adrenal glands, kidneys, tail bone, sternum, brain and pituitary. Weight gain in both groups receiving tamoxifen was less than that in controls. In the group killed at 15 months, uterine weights were significantly reduced in both high- and low-dose treated groups. A significant increase in the incidence of hepatocellular carcinomas occurred in both treated groups, compared with controls. At 12 months, the incidence of hepatocellular carcinomas was 0/18 control, 16/36 low-dose and 24/24 high-dose animals ($p < 0.001$); that at 15 months was 0/13, 13/21 and 8/9, respectively ($p < 0.001$). The incidence of hyperplasia in the mammary gland and the incidence of pituitary adenomas were decreased in tamoxifen-treated animals (Hard *et al.*, 1993). [The Working Group noted the small numbers of animals, that the exposure was for one year and that the study was terminated at 15 months.]

Table 6. Incidence (%) of tumours in Alderley Park Wistar-derived rats exposed to tamoxifen

Tumour	Dose (mg/kg bw per day)			
	0	5	20	35
Females				
Mammary adenocarcinomas[a]	9	0	0	0
Pituitary adenomas[b]	73	0	0	0
Liver adenomas[b]	1	4	12	17
Liver carcinomas[b]	0	12	71	71
Males				
Pituitary adenomas[c]	14	2	0	0
Parathyroid gland adenomas[d]	10	0	0	0
Liver adenomas[b]	1	16	22	16
Liver carcinomas[b]	1	16	67	67

From Greaves *et al.* (1993)
[a] $p < 0.02$, trend test
[b] $p < 0.0001$, trend test
[c] $p = 0.009$, trend test
[d] $p = 0.003$, trend test

In a study that also included toremifene, groups of 20 female Sprague-Dawley rats, six weeks of age, were given 0, 11.3 or 45 (maximum tolerated dose, MTD) mg/kg bw tamoxifen (purity > 99%) in carboxymethylcellulose per day by gastric instillation on seven days per week for up to one year. Five animals from each group were killed after 26 weeks and 52 weeks of treatment; all surviving rats were killed 65 weeks after the beginning of treatment. Weight gain was reduced in both tamoxifen-treated groups. No tumour was found in animals killed at 26 weeks. At 52 weeks, the incidences of hepatocellular carcinomas were 0/5 control, 0/5 low-dose and 3/5 high-dose animals; those at 65 weeks were 0/8, 0/8 and 5/6, respectively. Histopathological findings in tissues other than the liver were not reported (Hirsimäki *et al.*, 1993). [The Working Group noted the small numbers of animals, that the exposure was for one year and that the study was terminated at 65 weeks.]

Groups of 55–57 female Sprague-Dawley [Crl:CD(BR) Charles River] rats, six weeks of age, were given 0, 2.8, 11.3 or 45.2 mg/kg bw tamoxifen [purity not specified] per day by gastric instillation in 0.5% carboxymethylcellulose on seven days per week for up to 12 months, followed by a three-month recovery period. Mortality was 5.3% in control, 9.0% in low-dose, 1.7% in mid-dose and 40% in high-dose animals. All tamoxifen-treated animals had weight gain depression and some developed alopecia. Among the seven high-dose rats examined at six months, five had adenomas and two had carcinomas. At 12 months of exposure, the incidences of hepatic adenomas were 5/10 mid-dose and 2/4 high-dose rats. The incidence of liver carcinomas in these groups was 1/10 and 3/4, respectively. During the three months of recovery, the occurrence of carcinomas

in the mid-dose group increased to 5/11. No liver tumour was seen in control or low-dose groups (Williams *et al.*, 1993). [The Working Group noted that exposure was for only one year and that the study was terminated at 15 months.]

In a study that included toremifene, groups of five female Sprague-Dawley rats, six weeks of age, were given 0, 11.3 or 45 (MTD) mg/kg bw tamoxifen (purity > 99%) per day by gastric instillation in 0.5% carboxymethylcellulose seven days per week for 12 months. Groups were killed at 12 months or after a further 13 weeks of recovery. The incidence of liver tumours (hepatocellular carcinomas) at 12 months was 0/5 control, 0/5 low-dose and 4/5 high-dose animals; that at 65 weeks was 0/5, 0/5 and 5/5, respectively (Ahotupa *et al.*, 1994). [The Working Group noted the small number of animals and that the exposure was for only one year.]

In a study that also included droloxifene, groups of 50 male and 50 female rats [strain and age not specified] were given to 0 (control), placebo [not specified] or 36 mg/kg bw tamoxifen [purity not specified] in the diet for 24 months. The incidences of hepatocellular carcinomas were: males — control, 0/50; placebo, 0/50; tamoxifen, 49/50; females — control, 0/50; placebo, 0/50; tamoxifen, 50/50 (Hasmann *et al.*, 1994). [The Working Group noted the lack of experimental detail.]

Groups of 10 female Fischer (344/Tox), Wistar (LAC-P) and Lewis (LEW Oka) rats, six weeks of age, were fed either basal diet or diets containing 420 mg/kg diet (ppm) tamoxifen (purity > 98%) until 50% mortality was reached, at which time all surviving animals were killed. In groups of five rats killed after 90 days of exposure, the incidence of hepatocellular altered foci was increased in the Wistar and Lewis animals (Table 7). In rats killed at 180 days, no liver tumour was present in controls, while, in treated rats, the incidence was 3/5 in Wistar, 1/5 in Lewis and 0/5 in Fischer rats. By 11 months, 50% of the tamoxifen-treated Wistar and Lewis rats developed palpable liver nodules or were in ill health and these animals were killed. In the Wistar and Lewis rats killed at or before 11 months, all 10 had multiple liver tumours of which one or more was a carcinoma. In Fischer rats, 50% mortality was reached at 20 months and the remaining animals were killed at this time. All 10 rats exhibited at least one hepatocellular carcinoma (Carthew *et al.*, 1995a).

In a compilation of three experiments also including toremifene, reported in the proceedings of a meeting, groups of 109, 25 or 104 female Sprague-Dawley rats [age not specified] were given tamoxifen (> 99% pure) at 0, 11.3 or 45.0 mg/kg bw per day 7 days per week, for 20, 26 or 52 weeks with recovery periods of 12 or 13 weeks. In the high-dose group, squamous-cell metaplasia of the endometrium was found in 10 rats, dysplasia with metaplasia in three and squamous-cell carcinoma in two. The carcinomas were found after 20 or 26 weeks of dosing and a recovery period of 12–13 weeks. Among control or low-dose animals, no lesion of the uterus was reported (Mäntylä *et al.*, 1995, 1996). [The Working Group noted the lack of study details.]

Table 7. Hepatocellular altered foci in tamoxifen-exposed rats at 90 days of treatment

Strain	Exposure	Foci per cm^2
Wistar	None	1.5 ± 0.4
	Tamoxifen	14.0 ± 2.2[a]
Lewis	None	0.9 ± 0.3
	Tamoxifen	3.8 ± 1.0[a]
Fischer	None	1.0 ± 0.6
	Tamoxifen	1.5 ± 0.6

From Carthew *et al.* (1995a)
[a] $p < 0.05$

As part of a tumour-promotion study in which toremifene was also included, groups of 14–22 female Sprague-Dawley rats, weighing 130 ± 10 g [age not specified], were subjected to partial hepatectomy and two weeks later were exposed to tamoxifen [purity not specified] in the diet at concentrations of 0 (control), 250 or 500 mg/kg diet (ppm) for up to 18 months. Both exposures to tamoxifen suppressed body-weight gain. In animals killed at six months, uterine weights were reduced. At this time, increases were found in both the number and volume of hepatocellular altered foci identified by staining for γ-glutamyl transpeptidase. Incidences of hepatic neoplastic nodules were not increased. In the remaining animals killed at 18 months, hepatocellular carcinomas were found in 0/22 controls, 1/15 low-dose and 8/15 high-dose rats (Dragan *et al.*, 1995).

3.2 Subcutaneous administration

3.2.1 *Mouse*

In a study using a strain of mouse with a high incidence of mammary tumours, groups of female C3H/OUJ mice, received a subcutaneous implantation of a silastic capsule containing 28 mg tamoxifen (release, 125 μg/day for at least six months) [purity not specified] on the back at two weeks (11 mice) or at five weeks (15 mice) after a pregnancy/weaning cycle (3.5 months of age). A control group of 11 mice received implantations of a placebo silastic capsule at the start of the experiment. After 15 months, the percentage of mice with mammary tumours was 100% in the controls, about 20% in the group exposed to tamoxifen at two weeks and about 50% in the group exposed to tamoxifen at five weeks (Jordan *et al.*, 1990). [The Working Group noted that only the uterus and mammary glands were examined and only macroscopically.]

In a study using a strain of mouse with a high incidence of mammary tumours, two groups of 30 female C3H/OUJ mice, 2.5 months of age, were ovariectomized and two further groups of 30 females were left intact. Two weeks later, one ovariectomized and one intact group received an implantation of a silastic capsule containing 28 mg tamoxifen (release, approximately 125 μg/day for six months) [purity not specified]. The

capsules were replaced every six months up to 17 months of treatment. Mice were observed up to 27 months of age. In the intact controls, the mammary tumour incidence was 100% by about 20 months of age. This was reduced to about 20% by tamoxifen. In ovariectomized mice, the incidence of mammary tumours was 50%, which was reduced to about 20% by tamoxifen. In a further experiment, four groups of 20 female C3H/OUJ mice, three months old, received a tamoxifen capsule implant for three, six or 12 months (capsule replaced at six months in this group). The incidence of mammary tumours in controls was 100% by 17 months, whereas all tamoxifen-treated groups had an incidence of about 25% (Jordan *et al.*, 1991). [The Working Group noted that only the mammary gland was examined and only macroscopically.]

3.2.2 *Rat*

As part of a tumour-promotion study, groups of 10 female Sprague-Dawley rats, weighing 125–175 g [age not specified], were subjected to partial hepatectomy and, one week later, received subcutaneous implants of time-release tablets providing 0 or 50 μg/day tamoxifen. At four months, the body weights of treated rats were significantly lower than those of controls. The livers were examined for γ-glutamyl transpeptidase-positive altered hepatocellular foci. Controls had 0.07 ± 0.08 foci/cm^2, whereas tamoxifen-exposed rats had 1.11 ± 0.25 foci/cm^2 (Yager *et al.*, 1986).

3.3 Administration with known carcinogens

3.3.1 *Mouse*

Groups of 25–70 female Swiss mice, six to eight weeks of age, were ovariectomized or left intact and were divided into seven groups, one of which was left untreated; the others received a beeswax-impregnated thread inserted into the canal of the uterine cervix; subcutaneous injection of olive oil thrice weekly; an intracervical insertion of thread impregnated with 3-methylcholanthrene (MCA); an MCA-impregnated thread plus subcutaneous injections of 50 μg/kg bw tamoxifen in olive oil three times per week; a beeswax thread plus tamoxifen; or tamoxifen alone. The experiment lasted for at least 393 days. Vaginocervical smears were taken to monitor cervical dysplasia and carcinoma. In intact mice, the incidence of cervical carcinoma in mice exposed to MCA was 60 ± 8.2%, while, in mice receiving MCA and tamoxifen, it was 30 ± 6.8%. No other intact mouse developed a carcinoma. In ovariectomized mice, the incidence of cervical carcinoma in the MCA-treated group was 62 ± 7.2% and that in the mice also receiving tamoxifen was 27 ± 6.7%. No other ovariectomized mouse developed a carcinoma (Sengupta *et al.*, 1991).

Groups of four to six male and female BALB/c/Bln mice, 8–12 weeks of age, were given intraperitoneal injections of diluted serum from leukaemic mice infected with the Rauscher murine leukaemia virus. Starting one day later, mice were given intraperitoneal injections of 0.2, 0.5 or 1 mg/animal tamoxifen [purity not specified] in dimethyl sulfoxide three times a week for three weeks (total doses, 1.6, 4 and 8 mg). At the end of treatment, the mice were killed and spleens were weighed as an index of leukaemic

disease. Compared to the controls, the spleen weights of the tamoxifen-treated animals were about 60%, 45% and 20% in the low-, mid- and high-dose groups, respectively, indicative of reduced leukaemic activity (Sydow & Wunderlich, 1994).

3.3.2 *Rat*

A group of 36 male Fischer rats, approximately three months of age, was given a single gastric instillation of 100 mg/kg bw *N*-nitrosodiethylamine (NDEA), then fed a diet containing 200 mg/kg diet (ppm) 2-acetylaminofluorene (2-AAF) [duration not specified] and underwent partial hepatectomy. Six weeks after cessation of 2-AAF treatment, one group of 17 rats was maintained with no further treatment. The remaining rats were divided into three groups of seven, five and five animals that were given subcutaneous injections of 0.25, 1.0 and 2.5 mg/animal tamoxifen [purity not specified] in peanut oil twice a week. The experiment was terminated at 10 months. From graphic presentations, the incidence of liver malignancy [not further specified] was about 75% in controls and 10% in the low-dose, 25% in the mid-dose and 35% in the high-dose tamoxifen-treated groups (Mishkin *et al.*, 1985).

Groups of 7–15 female Sprague-Dawley rats, 50 ± 2 days of age, were given 12 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) per animal as a single gastric instillation in sesame oil. After six weeks, when mammary tumours had reached about 1 cm in diameter, groups were treated with 0, 1.0, 3.0 or 7.5 mg/kg bw tamoxifen (> 98% pure) per day by gastric instillation for at least five weeks. The numbers of new tumours per animal were 3.0 ± 2.6 in controls and 2.0 ± 0.6 in low-dose, 2.1 ± 1.4 in mid-dose and 0.3 ± 0.5 in high-dose tamoxifen-treated groups (Kangas *et al.*, 1986) [The Working Group noted the small numbers of animals.]

Groups of 10 female Sprague-Dawley rats, weighing 125–175 g [age not specified], were given 25 mg/kg bw NDEA by intraperitoneal injection 24 h after partial hepatectomy (Yager & Shi, 1991). One week later, the animals received implants of a time-release tablet containing quantities of tamoxifen to give a daily release of 15 or 50 μg or no tamoxifen. At four months, liver samples were examined for γ-glutamyl transpeptidase-positive altered hepatocellular foci. The body weights of treated rats were substantially lower than those of controls. The incidence of altered foci per cm^2 was 0.9 ± 0.3 in controls, 7.1 ± 1.9 in low-dose and 4.9 ± 1.0 in high-dose tamoxifen-treated rats (Yager *et al.*, 1986).

Male Sprague-Dawley rats, eight weeks of age, were given 200 mg/kg bw NDEA by intraperitoneal injection and were allocated to four groups two weeks later: four rats were fed olive oil in the diet; 12 rats were fed 1 mg/animal tamoxifen (analytical grade) in the diet daily; eight rats were fed 0.5 mg/animal diethylstilboestrol (DES) in olive oil in the diet daily; and 11 rats were fed both DES and tamoxifen. At eight months, the incidence of altered foci in the liver was quantified using γ-glutamyl transpeptidase as a marker. The numbers of foci per cm^2 [derived from graphic presentations] were about 10 in the NDEA-treated rats, about 20 in the NDEA/tamoxifen-treated rats, about 16 in the NDEA/DES-treated rats and about 22 in the NDEA/DES/tamoxifen-treated rats. The lesions in the NDEA/tamoxifen-treated group were larger than those in the group given

NDEA alone, whereas, when tamoxifen was given with NDEA/DES, the lesions were smaller than with NDEA/DES alone (Kohigashi *et al.*, 1988).

Groups of 20 female Sprague-Dawley rats, 50 days of age, were given 20 mg/animal DMBA in peanut oil by gastric instillation. Twenty-eight days later, one group was treated with 200 µg/animal tamoxifen per day by gastric instillation, the other one with peanut oil. At 100 days after DMBA administration, 75% of the rats given DMBA alone had developed mammary tumours, whereas, in the group also receiving tamoxifen, less than 20% had tumours. Cessation of tamoxifen treatment after four months led to the development of mammary tumours, so that by 4.5 months about 70% of animals had tumours (Robinson *et al.*, 1988).

Groups of 12 female Sprague-Dawley rats, weighing 140–160 g [age not specified], and 12 female Fischer 344 rats, weighing 120–140 g [age not specified], were allocated to one of two protocols. To evaluate initiating activity, groups of Sprague-Dawley rats either remained untreated as controls or were fed diets containing 10 mg/kg diet (ppm) ethinyloestradiol, 400 ppm tamoxifen or ethinyloestradiol plus tamoxifen for six weeks. After seven days, all rats underwent partial hepatectomy and, on day 49 after a one-week recovery phase on basal diet, were fed a diet containing 200 ppm 2-AAF for two weeks; at the midpoint, they were given 1 mL [1.6 mg]/kg bw carbon tetrachloride by gastric instillation in corn oil. In order to evaluate promoting activity, groups of 12 Sprague-Dawley and 12 Fischer 344 rats were fed a diet containing 200 ppm 2-AAF for two weeks and, on day 7, were given 1 mL/kg bw carbon tetrachloride by gastric instillation in corn oil. On day 21, after a one-week recovery period, these groups were maintained as untreated controls or were fed diets containing 10 ppm ethinyloestradiol, 400 ppm tamoxifen or ethinyloestradiol plus tamoxifen for six weeks; on day 28 animals were subjected to partial hepatectomy. All treated rats showed reduced weight gain. Liver samples were examined for γ-glutamyl transpeptidase-positive altered hepatocellular foci. After the initiation protocol, the numbers of foci per cm^2 were 15.6 ± 6.5 in controls, 67.4 ± 10 in tamoxifen-treated rats, 68.2 ± 13.9 in ethinyloestradiol-treated rats and 140.7 ± 21.2 in ethinyloestradiol/tamoxifen-treated rats. In the promotion protocol, the numbers per cm^2 were 25.9 ± 6.9, 72.0 ± 13.0, 56 ± 10.4 and 98.5 ± 18.4 in the respective groups (Ghia & Mereto, 1989).

Thirty female Fischer 344 rats, weighing 130–200 g [age not specified], were subjected to partial hepatectomy and 24 h later were given 10 mg/kg bw NDEA by gastric instillation. After a two-week recovery period, nine rats were maintained on basal diet (controls), 11 rats were fed 250 mg/kg diet (ppm) tamoxifen [purity not specified] and 10 were fed 500 ppm tamoxifen in a semipurified diet for six months. At termination, livers were examined for altered hepatocellular foci with the help of a variety of histochemical markers. The numbers of foci per liver were 90 ± 30 in controls, and 5430 ± 310 in low-dose and 7280 ± 490 in high-dose tamoxifen-treated rats (Dragan *et al.*, 1991a).

Groups of 6–12 female Fischer 344 rats weighing 125–150 g [age not specified] were subjected to partial hepatectomy and 24 h later were given 40 mg/kg bw tamoxifen [purity not specified] by gastric instillation. The animals were then maintained on a basal

diet or a diet containing 500 mg/kg diet (ppm) phenobarbital. After six months, all rats were killed and slices of the three main lobes of the liver were used for enzymic histochemical demonstration of altered hepatocellular foci with the help of a variety of histochemical markers. The numbers of foci per liver were 100 ± 50 in controls, 130 ± 50 in those given tamoxifen alone and 370 ± 130 in those given tamoxifen followed by phenobarbital (Dragan *et al.*, 1991b).

Three groups of 10 female Sprague-Dawley rats, seven weeks of age, were given 20 mg DMBA by gastric instillation in sesame oil; starting 92 days later, two of the groups were given 1.0 or 10.0 mg/kg bw tamoxifen by gastric instillation in 0.5% methylcellulose for seven days, while the third group received no further treatment. At 20 weeks, mammary tumours were found in 8/9 controls, 5/10 low-dose tamoxifen-treated rats and 7/9 high-dose tamoxifen-treated rats (Kawamura *et al.*, 1991).

A group of 10 female Sprague-Dawley rats, weighing 200 g [age not specified], was given 0.5 mg/rat tamoxifen in peanut oil twice at a five-day interval by subcutaneous injection; one day after the last injection, the animals underwent partial hepatectomy and 24 h later received 10 mg/kg bw NDEA by gastric instillation. Another group of 10 rats was subjected to partial hepatectomy and NDEA treatment only (controls). At six weeks, the severity of altered hepatocellular foci [from graphic presentations] was graded as 1.8 in controls and 0 in tamoxifen-treated rats (Oredipe *et al.*, 1992). [The Working Group noted that the evaluation of foci was semi-quantitative.]

Groups of female Sprague-Dawley rats [numbers not specified], 59 days of age, were given 5 mg/rat DMBA weekly by gastric instillation for four weeks. One group was also simultaneously given 10 mg/kg bw tamoxifen weekly by subcutaneous injection for four weeks. After 14 weeks, the incidence of mammary tumours was 100% and the multiplicity was 6.7 ± 0.8 tumours per animal in the DMBA-treated group. In the DMBA/-tamoxifen-treated group, the incidence was 49.5% and the multiplicity was 1.4 ± 0.2 tumours per animal. In a second experiment, two groups of rats were treated with DMBA by gastric instillation for four weeks. Beginning at week 9, one group was given 1 mg/kg bw tamoxifen twice daily by subcutaneous injection for three weeks. At 12 weeks, all animals were ovariectomized. A large percentage of tumours regressed. In the control group, 1.3 new tumours per rat appeared, whereas in the tamoxifen-treated rats 0.3 new tumours per rat developed. All new tumours that appeared in tamoxifen-treated rats were hormone-independent, whereas in controls, only 13% continued to grow (Fendl & Zimniski, 1992). It was subsequently reported that, following the cessation of tamoxifen treatment, some tumours resumed growth (Zimniski & Warren, 1993).

A group of 20 female Sprague-Dawley rats, 50 days of age, was given 50 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) as a single intravenous injection. When at least one mammary tumour had reached a diameter of 10 mm, the animals were given 6 mg/kg bw tamoxifen by gastric instillation on five days a week for four weeks. A positive control group of 50 female rats received the treatment with MNU alone. At the cessation of treatment (207 days), the multiplicity of mammary tumours was 1.1 in controls (median size, 10.2 cm^3) and 1.25 in the tamoxifen-treated group (median size, 2.5 cm^3) (Winterfeld *et al.*, 1992).

Groups of female Sprague-Dawley rats, weighing 200–220 g [age not specified], received no treatment (control; 26 rats) or 1 mg/kg bw tamoxifen in propylene glycol by intraperitoneal injection 1 h (four rats) or 24 h (three rats) before a single intraperitoneal injection of 50 mg/kg bw NDEA. Other groups were subjected to partial hepatectomy (eight rats) or partial hepatectomy and 1 mg/kg bw tamoxifen (eight rats) 24 h before treatment with NDEA. Eight weeks after treatment, rats were killed and liver samples were examined for altered hepatocellular foci identified by glutathione *S*-transferase immunohistochemistry. Pretreatment for 1 h with tamoxifen increased the number of foci almost 2-fold and pretreatment for 24 h increased the number 3.4-fold (Servais & Galand, 1993).

Two groups of female OFA rats [number and age not specified], weighing 130–160 g, were given 20 mg/animal DMBA in sesame oil as a single gastric instillation. After seven weeks, one group was given 1 mg/kg bw tamoxifen in sesame oil or sesame oil alone by subcutaneous injection three times per week for six weeks. At 11 weeks, the mean number of mammary tumours in controls was 9.3 tumours per rat; in tamoxifen-treated rats, this was reduced by 17.2 ± 2.8% (Weckbecker *et al.*, 1994).

Groups of 34–35 male Sprague-Dawley rats, aged 25 days, were given 20 mg/kg bw 1,2-dimethylhydrazine weekly by subcutaneous injection for 20 weeks and were fed control diet or diets initially containing 4 mg/kg diet (ppm) tamoxifen [purity not specified], which was reduced to 2 ppm at 29 days, 1 ppm at 56 days and to 0.5 ppm at 65 days. Most animals were killed 65 days after the last injection of 1,2-dimethylhydrazine. Animals receiving tamoxifen displayed reduced body weights. Mortality was comparable in control and treated animals. The total number of colon adenocarcinomas and their distribution in the proximal and distal portions did not differ between the groups (Gershbein, 1994).

Groups of 16–20 female Fischer 344 rats, 130–150 g [age not specified], were subjected to partial hepatectomy and 24 h later were given 10 mg/kg bw NDEA in tricaprilyn or vehicle alone by gastric instillation. Two weeks later, groups of NDEA- or vehicle-exposed rats were fed 250 mg/kg diet (ppm) tamoxifen free base [purity not specified] in a semi-purified diet. Treatment with tamoxifen reduced body weights by 16–24%. Tamoxifen increased the incidence of liver tumours at 15 months in rats given NDEA (3/8 hepatocellular carcinomas versus 0/6 in rats given NDEA alone) (Dragan *et al.*, 1994).

In a study that also included toremifene, virgin Sprague-Dawley rats, aged 43 days, were randomized into groups of 20 and allocated to control diet or to diets containing 0.2 mg/kg diet (ppm) tamoxifen. Seven days later, groups were given either 50 mg/kg bw MNU or saline by intravenous injection. Animals were killed when moribund and the experiment was terminated at 180 days after MNU treatment. Tamoxifen did not affect the incidence, multiplicity or latency of mammary tumours compared with controls (Moon *et al.*, 1994). [The Working Group noted that the dose of MNU, producing 100% of tumours in the MNU- and MNU/tamoxifen-treated groups, may have been too high to allow the detection of a protective effect.]

In a tumour promotion study, in which toremifene was also studied, groups of 14–22 female Sprague-Dawley rats, weighing 130 ± 10 g [age not specified], were subjected to partial hepatectomy and 24 h later were given a single dose of 10 mg/kg bw NDEA in trioctanoin by gastric instillation. Two weeks later, the rats were given either basal diet or diet containing 250 or 500 mg/kg diet (ppm) tamoxifen for 18 months. All exposures to tamoxifen suppressed body-weight gain. Uterine weights were suppressed at six months. The number of altered hepatic foci identified by any of four histochemical markers as well as the volume of liver occupied by foci was increased by tamoxifen. At six months, the incidence of hepatic neoplastic nodules was: controls, 8/15; low-dose, 13/15; and high-dose, 11/15. At 18 months, the incidence of hepatocellular carcinomas in all groups approached 100% and the incidence of renal carcinomas was slightly increased by tamoxifen [p = 0.008, Cochran–Armitage trend test] (Table 8) (Dragan *et al.*, 1995).

Table 8. Incidence of tumours in female Sprague-Dawley rats exposed to tamoxifen after NDEA

Exposure	Renal cell adenomas	Renal cell carcinomas	Hepatocellular carcinomas
None	5/19	0/19	2/17
Tamoxifen 250 ppm	5/18	0/18	11/18
Tamoxifen 500 ppm	5/8	2/8[a]	8/8

From Dragan *et al.* (1995)

[a] [p = 0.008, Cochran-Armitage trend test]

3.3.3 *Hamster*

Groups of 7–12 male Syrian hamsters, four to six weeks of age, received subcutaneous implants of one pellet containing either 25 mg 17β-oestradiol or 25 mg tamoxifen, or one pellet of each. All animals were killed after seven months. The number of kidney tumour-bearing animals was 3/3 oestradiol-treated hamsters examined, 2/8 oestradiol/tamoxifen-treated hamsters and 0/8 tamoxifen-treated hamsters. The multiplicity of kidney tumours was reduced from 6.5 with oestradiol to 1.0 in oestradiol/-tamoxifen animals; tamoxifen alone produced no kidney tumour (Liehr *et al.*, 1988).

Two groups of 5–20 male and female Armenian hamsters (*Cricetulus migratorius*), two to three months of age, received subcutaneous implants of 36-mg pellets of zeranol on study days 0 and 94. One group was given 5 mg/animal of tamoxifen by subcutaneous injection twice a week up to day 32 and then once a week until day 202, when the experiment was terminated. Among 9 males and 7 females receiving zeranol only, the incidence of hepatocellular carcinomas was about 60%, whereas in four males and four females also receiving tamoxifen, the incidence was reduced to 1–2% (Coe *et al.*, 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

In the studies reviewed here, tamoxifen citrate, if used, will be referred to as tamoxifen.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The absorption, distribution, metabolism and excretion of tamoxifen have been reviewed extensively (Furr & Jordan, 1984; Buckley & Goa, 1989; Wiseman, 1994). All studies have involved oral administration of tamoxifen citrate unless stated otherwise.

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). Tamoxifen absorption shows wide interindividual variation, which is probably due to differences in liver metabolism and differences in absorption in the gastrointestinal tract. Administration of 40 mg/day for two months to patients with breast cancer produced steady-state mean plasma concentrations of tamoxifen of 186–214 ng/mL. The single maximum plasma concentrations in this study were about 70 ng/mL tamoxifen and about 20 or 40 ng/mL *N*-desmethyltamoxifen (the different values being with Tamoplex® and Nolvadex®, respectively) (McVie *et al.*, 1986). Administration of a single 20-mg dose of tamoxifen to six male volunteers resulted in peak plasma concentrations of 42 ng/mL tamoxifen and 12 ng/mL *N*-desmethyltamoxifen. Maximal levels were achieved approximately 5 h after administration and area under the curve (AUC) was 2606 ng × h/mL (Adam *et al.*, 1980). The distribution half-life (i.e. initial $t_{½}$) of tamoxifen is 7–14 h. The mean terminal half-life was 111 h, somewhat shorter than the previously reported seven days or more (Fromson *et al.*, 1973a). Steady-state concentrations of tamoxifen and of *N*-desmethyltamoxifen were reached after three to four weeks of 20 mg b.i.d. (40 mg/day) administration (McVie *et al.*, 1986) and after 4–8 weeks of 20 mg/day administration (Lien *et al.*, 1995).

The apparent volume of distribution for tamoxifen in humans is 50–60 L/kg (Lïen *et al.*, 1989), indicating that most of the drug (99.9%) is present in peripheral compartments, which is suggestive of extensive tissue binding (Lien *et al.*, 1991).

In healthy male volunteers given 40 mg tamoxifen, the plasma elimination half-life of tamoxifen during the first day was 10 h. However, after 34 h, appreciable levels of tamoxifen and *N*-desmethyltamoxifen were still present, suggesting a lengthening of half-life with increasing study duration or the existence of multiple half-lives (Guelen *et al.*, 1987). The pharmacokinetics of tamoxifen appear to be biphasic, with a distribution phase of 7–14 h and an elimination phase of about seven days (Fromson *et al.*, 1973a). The elimination half-life of *N*-desmethyltamoxifen is around seven days and 4-hydroxytamoxifen has a shorter half-life than tamoxifen (Buckley & Goa, 1989).

Tamoxifen and its metabolites are mostly excreted via bile into faeces as glucuronides and other conjugates. Urinary excretion is a very minor route of elimination (Furr & Jordan, 1984).

In seven premenopausal breast cancer patients and nine postmenopausal women with non-neoplastic diseases treated with tamoxifen for 56 days, the serum levels of *N*-didesmethyltamoxifen were higher in the postmenopausal women ($p < 0.02$). A similar trend was observed for *N*-desmethyltamoxifen ($p < 0.06$) (Lien *et al.*, 1995).

Administration of 40 mg/day tamoxifen (20 mg twice a day) to primary breast cancer patients for 15–940 days (Daniel *et al.*, 1981) resulted in plasma concentrations of 27–520 (mean, 300) ng/mL tamoxifen, 210–761 (mean, 462) ng/mL *N*-desmethyltamoxifen and 2.8–11.4 (mean, 6.7) ng/mL 4-hydroxytamoxifen. Concurrent tumour biopsy concentrations were 5.4–117 (mean, 25.1) ng tamoxifen/mg protein, 7.8–210 (mean, 52) ng *N*-desmethyltamoxifen/mg protein and 0.29–1.13 (mean, 0.53) ng 4-hydroxytamoxifen/mg protein.

After 14 daily doses of 40 mg tamoxifen, concentrations in plasma and in breast tumour cell nuclear and cytosolic fractions were measured in three patients (Murphy *et al.*, 1987). The results are shown in Table 9.

Table 9. Concentrations of tamoxifen and its metabolites in plasma and breast tumour cells of three patients receiving 40 mg/day for 14 days

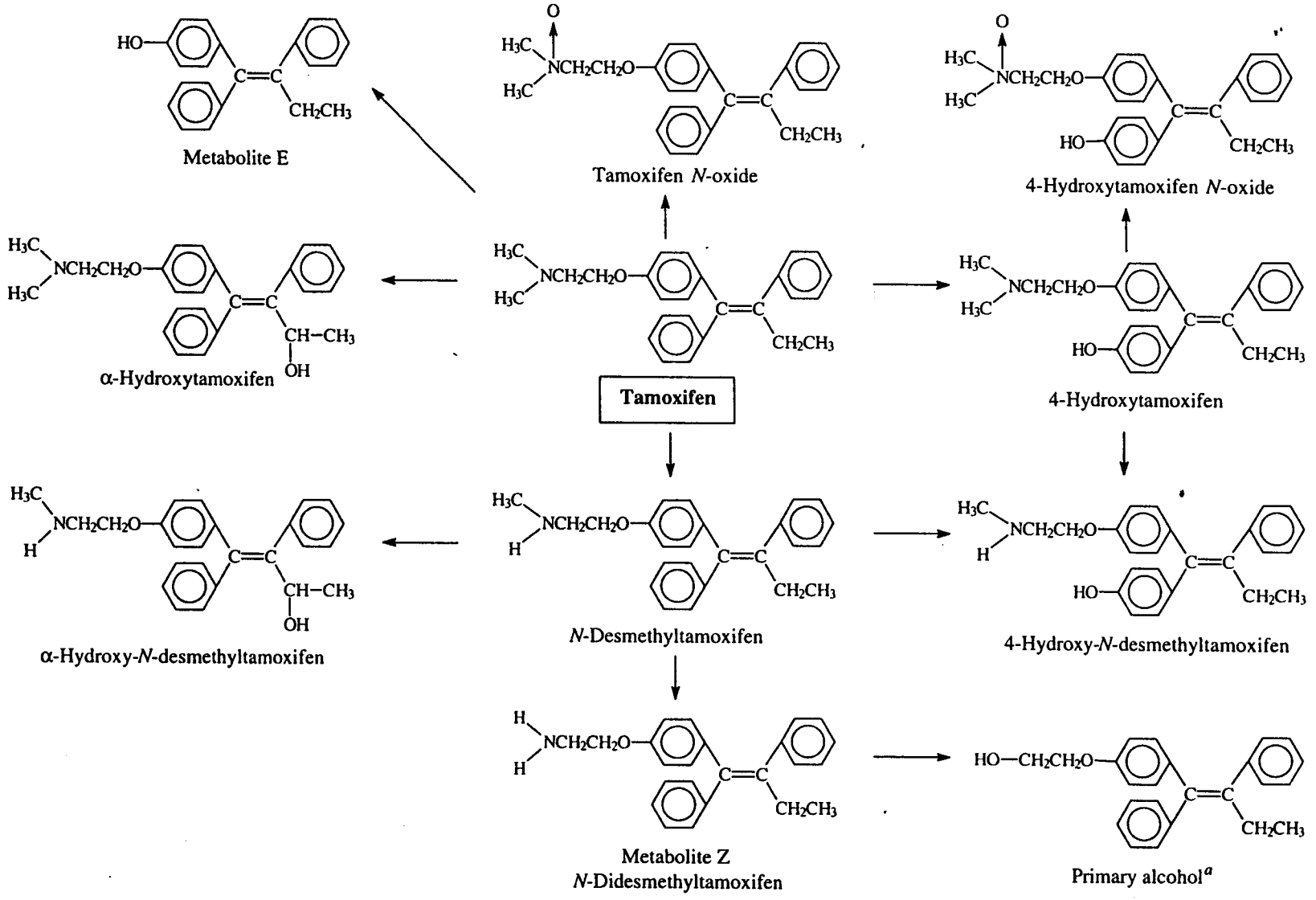
	Plasma (ng/mL)	Breast tumour cell fractions	
		Nucleus (ng/mg protein)	Cytosol (ng/mg protein)
Tamoxifen	363–745	8.0–11.1	8.1–18.5
N-Desmethyltamoxifen	185–422	3.6–7.9	6.6–26.8
4-Hydroxytamoxifen	1.4–3.1	0.16–0.26	0.02–0.36

From Murphy *et al.* (1987)

A number of metabolites (see Figure 1) have been identified in urine and plasma of human breast cancer patients by LC/MS/MS techniques. Plasma extracts contained tamoxifen, *N*-desmethyltamoxifen and tamoxifen-*N*-oxide (Poon *et al.*, 1993). Glucuronides of four hydroxylated metabolites (4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, dihydroxytamoxifen and another monohydroxy- (possibly α-hydroxy-) *N*-desmethyltamoxifen) were detected in the patients' urine. In a more recent 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 taken from 14 patients, 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, being

Figure 1. Postulated metabolic pathways of tamoxifen



Adapted from Ruenitz & Nanavati (1990), Lien *et al.* (1991), Pongracz *et al.* (1995) and Poon *et al.* (1995)
[a]May be oxidized to the oxyacetic acid of tamoxifen and to 4-hydroxytamoxifen oxyacetic acid.

particularly high in liver and lung. Nine of the patients were in steady-state (treatment for more than 35 days), three had received tamoxifen for 7–13 days and two had received tamoxifen for 3–3.5 years (but had been tamoxifen-free for 28 days and 14 months respectively, at the time of tissue sampling). Tissue samples from all other patients were obtained within 4–60 h. Tissues from the pancreas, pancreatic tumour, primary breast cancer and metastatic breast cancer in brain also retained large amounts of the drug. The amounts of N-demethylated and hydroxylated metabolites were high in most tissues except in fat, and tamoxifen and some of its metabolites were also present in specimens of skin and bone (Lien *et al.*, 1991). Post-mortem and biopsy analysis of liver from tamoxifen-treated patients showed the presence of tamoxifen (0.14–15 nmol/g), 4-hydroxytamoxifen and *N*-desmethyltamoxifen (Martin *et al.*, 1995).

4.1.2 *Experimental systems*

The kinetics, absorption, distribution, excretion and metabolism of tamoxifen in experimental animals have been reviewed (Furr & Jordan, 1984; Buckley & Goa, 1989; Wiseman, 1994).

In rats, mice, dogs and rhesus monkeys, tamoxifen is well absorbed following oral administration. Most of the dosed material appears in the faeces, but bile duct cannulation experiments with rats and dogs demonstrated that this was a result of biliary excretion (Fromson *et al.*, 1973b).

In order to achieve similar plasma levels, much higher oral doses of tamoxifen are required by rats and mice than by human breast cancer patients: doses in rats over seven days of 3.0 mg/kg bw gave < 1 ng/mL and 200 mg/kg bw gave 1000 ng/mL; doses in mice over 7–10 days of 2.5 mg/kg bw gave < 10 ng/mL and 200 mg/kg bw gave 300 ng/mL; doses in human patients over 10 days of 4.9 mg/kg bw gave 1300 ng/mL (Robinson *et al.*, 1991).

In rats given tamoxifen orally at 1 mg/kg bw per day for 3 or 14 days, essentially the same amounts of drug were found after three days and 14 days treatment (except for fat), suggesting that steady-state is obtained within three days. Concentrations of tamoxifen and its metabolites (*N*-desmethyl, *N*-didesmethyl, 4-hydroxy and 4-hydroxy-*N*-desmethyl) were 8–70-fold higher in tissues (brain, adipose, liver, heart, lung, kidney, uterus, testis) than in serum. The highest levels were found in lung and liver, but substantial amounts were also found in kidney and adipose tissue. Within one dosing interval (24 h), marked fluctuations in the tissue concentrations were observed in rats receiving the steady-state treatment, with maximum/minimum concentration (C_{max}/C_{min}) ratios for tamoxifen found in female rat lung and liver being 6.3 and 4.1, respectively (Lien *et al.*, 1991).

Groups of female Fischer 344 rats were treated with a non-necrotic, subcarcinogenic dose of *N*-nitrosodiethylamine (10 mg/kg orally) and were given tamoxifen at 250 mg/kg of AIN-76A diet for 6 or 15 months (Dragan *et al.*, 1994). Treatment with tamoxifen resulted in a decrease in body weight of 16–24% at serum levels comparable to the therapeutic level in humans. In serum, the ratio of tamoxifen/4-hydroxytamoxifen/*N*-desmethyltamoxifen was 1/0.1/0.5–1. Rat livers had 20–30 times more tamoxifen and

4-hydroxytamoxifen and at least 100 times more *N*-desmethyltamoxifen than the serum at both 6 and 15 months. The ratio in the liver after 6 or 15 months of continuous administration was 1/0.1/1.3–2.3.

Administration of [^{14}C]tamoxifen to dogs, rats, mice and rhesus monkeys has shown that tamoxifen has a long half-life in all of these species: in rats, the distribution half-life in blood was 53 h but the elimination half-life was 10 days. The route of excretion in rats, mice, rhesus monkeys and dogs is predominantly faecal, with virtually none of the recovered material being unchanged tamoxifen (Fromson *et al.*, 1973b).

In rats, tamoxifen is eliminated in urine to a significant extent as the acidic metabolites. In one study, in the period 0–24 h after dosing with [^{14}C]tamoxifen, the radioactive components recovered (expressed as percentages of the administered dose) were: total radioactivity, 8.7%; tamoxifen acid, 1.02%; and 4-hydroxytamoxifen acid, 1.81%. In faeces, the corresponding values were 30.5%, 0.5% and 2.40%. In contrast to other tamoxifen metabolites, neither of these metabolites is excreted as glucuronic acid or glycine conjugates (Ruenitz & Nanavati, 1990).

Tamoxifen can be metabolized *in vitro* by both microsomal cytochrome P450 and flavin monooxygenase pathways to intermediates that bind irreversibly to microsomal proteins (Mani & Kupfer, 1991). Incubation of tamoxifen with rat liver microsomes yielded three major polar metabolites identified as the *N*-oxide, *N*-desmethyl and 4-hydroxy derivatives. Formation of the *N*-oxide was catalysed by flavin monooxygenase, while that of the *N*-desmethyl and 4-hydroxy metabolites was mediated by cytochrome P450. Tamoxifen N-demethylation appears to be catalysed in rats by CYP1A, CYP2C and CYP3A enzymes, while in man the evidence points to the CYP3A enzyme. However, these enzymes are not major contributors to the 4-hydroxylation of tamoxifen (Mani *et al.*, 1993, 1994). Peroxidases may also metabolize tamoxifen to a reactive intermediate that binds covalently with protein (Davies *et al.*, 1995) and DNA (Pathak & Bodell, 1994; Pathak *et al.*, 1995).

Lim *et al.* (1994) compared the metabolism of tamoxifen in microsomes from female human, rat and mouse liver. The major metabolites formed by rat liver microsomes were 4-hydroxytamoxifen, 4′-hydroxytamoxifen, *N*-desmethyltamoxifen and tamoxifen *N*-oxide. In addition, it was suggested that two previously unreported epoxide metabolites, 3,4-epoxytamoxifen and 3′,4′-epoxytamoxifen, and their hydrolysed derivatives, 3,4-dihydroxytamoxifen and 3′,4′-dihydroxytamoxifen, had been identified, but these conclusions were based only upon mass spectral data; no synthetic standards were available. Jarman *et al.* (1995) were unable to confirm the existence of these dihydroxy compounds in microsomal incubates containing tamoxifen or deuterated analogues of tamoxifen. Using tamoxifen and [*ethyl*-D_5]tamoxifen, they showed a large isotope effect in the formation of α-hydroxytamoxifen (see Section 4.4). They confirmed the presence of α-hydroxytamoxifen-*N*-oxide and identified a new metabolite, α-hydroxy-*N*-desmethyltamoxifen.

Metabolites of tamoxifen were examined in human liver homogenate and a human hepatic G2 cell line treated with a mixture of tamoxifen and its deuterated analogues (Poon *et al.*, 1995). In both the hepatic G2 cell line and the liver homogenate, α-hydroxy-

tamoxifen, 4-hydroxytamoxifen, *N*-desmethyltamoxifen and tamoxifen *N*-oxide were detected. In the liver homogenate, *N*-didesmethyltamoxifen was also detected.

When primary cultures of human, rat and mouse hepatocytes were incubated with tamoxifen (10 μM) for 18–24 h, the concentration of α-hydroxytamoxifen in the medium was 50-fold lower in the human cultures (0.41 ± 0.55 ng/mL, two determinations) than in the rat (26.8 ± 10.1 ng/mL, three determinations) and mouse (18.9 ± 13.5 ng/mL, four determinations) cultures (Phillips *et al.*, 1996a).

4.2 Toxic effects

4.2.1 *Humans*

The most reliable data regarding the association between tamoxifen and gynaecological symptoms come from randomized trials of tamoxifen versus placebo, in which some of these clinical data were collected prospectively.

The potential long-term toxicity of tamoxifen therapy has been reviewed. In large adjuvant trials, about 4% of recipients stop therapy because of side-effects (Love, 1989; Jaiyesimi *et al.*, 1995). The most commonly reported side-effects of tamoxifen therapy are vasomotor symptoms, such as hot flushes and tachycardia, nausea and vomiting. A reduction over time in the vasomotor symptoms reported by patients receiving tamoxifen was observed in a randomized, double-blind, placebo-controlled clinical trial (Love & Feyzi, 1993). Atrophy is a common uterine response. Gynaecological adverse effects such as changes in vaginal discharge, bleeding, vaginal/external genitalia irritation, endometrial hyperplasia, polyps of the endometrium and, in premenopausal women, menstrual irregularities are summarized in Table 10.

Some evidence exists that tamoxifen may be associated with thromboembolic events in patients with advanced breast cancer. Table 11 summarizes studies regarding the effects of tamoxifen on blood coagulation. In general, the effects of tamoxifen on clotting factors are not clinically significant and probably do not persist during chronic (more than six months) administration or after cessation of therapy.

In a randomized trial of tamoxifen (20 mg/day for five years) versus placebo in pre- and postmenopausal women with breast cancer, thromboembolic disease was reported in 0.2% of women who received placebo versus 0.9% of those who received tamoxifen (Fisher *et al.*, 1989a).

In a randomized controlled study, morbidity due to cardiac and thromboembolic disease was assessed in 2365 postmenopausal breast cancer patients with (40 mg/day, two or five years) or without tamoxifen therapy. The median follow-up period was six years. Tamoxifen therapy was associated with a statistically significantly reduced incidence of hospital admissions due to any cardiac disease, with a relative risk of 0.7 for tamoxifen for two and five years versus control (95% CI, 0.5–1.0; $p = 0.03$). In the randomized comparison of five versus two years of tamoxifen treatment, there was a statistically significant reduction in risk with longer treatment (relative risk, 0.4; 95% CI, 0.2–0.9; $p = 0.03$). Although the trend of reduced risk in the tamoxifen group was also evident in the analysis of specific subgroups of cardiac diseases such as myocardial

infarct and ischaemic heart disease, the results failed to reach significance. There was no association between tamoxifen treatment and relative risk for admission to hospital due to thromboembolic disease (1.1; 95% CI, 0.7–1.6) (Rutqvist *et al.*, 1993). In another study, a total of 1312 women who had undergone mastectomy for breast cancer were randomized to receive either adjuvant treatment with tamoxifen (20 mg/day) or a placebo, with tamoxifen given only on first recurrence of disease. The maximal duration of tamoxifen treatment was 14 years. Use of tamoxifen was associated with lower rates of myocardial infarction, the relative risk in the control group being 1.9 (95% CI, 1.0–3.7) compared with women allocated to tamoxifen treatment (McDonald *et al.*, 1995).

Studies of beneficial effects of tamoxifen upon blood cholesterol levels, and increased levels of sex hormone-binding globulin and thyroid-binding globulin (Dewar *et al.*, 1992; Love *et al.*, 1994a) are summarized in Table 12. Tamoxifen and 4-hydroxytamoxifen have also been shown to protect human low-density lipoproteins *in vitro* against copper-ion dependent lipid peroxidation, a model system that is relevant to events occurring within atherosclerotic lesions (Wiseman *et al.*, 1993; Wiseman, 1994). Tamoxifen also lowers the levels of atherogenic amino acid homocysteine in humans (see Table 12) (Anker *et al.*, 1995).

Some studies of the effects of tamoxifen on bone mineral density suggest that tamoxifen acts as an oestrogen agonist to preserve bone density in postmenopausal breast cancer patients; however, the effects are weak and not consistent among the different studies (Table 13).

Over 20 years of use have made tamoxifen one of the most studied anti-cancer drugs (Jordan, 1993), and it is associated with less toxicity than other current endocrine treatments for breast cancer (Muss, 1992). While the potential additional benefits of treatment in breast cancer patients in terms of blood lipid and cholesterol levels and bone mineral density remain to be fully established, it is worthy of note that two well-designed randomized trials of tamoxifen versus no adjuvant endocrine therapy (described above) have shown significantly reduced numbers of hospital admissions for cardiac disease and no difference in deaths due to cardiac or thromboembolic disease (Rutqvist *et al.*, 1993) and significantly reduced risks of myocardial infarction and cardiac deaths (McDonald *et al.*, 1995) in women receiving tamoxifen.

Tamoxifen has been associated in case reports with changes in liver enzyme levels in the serum (Hayes *et al.*, 1995) and on rare occasions a spectrum of more severe events including fatty liver (Noguchi *et al.*, 1987), cholestasis and hepatitis (Cortez Pinto *et al.*, 1995) and a fatal case of hepatocellular damage and agranulocytosis (Ching *et al.*, 1992).

Several in-vitro studies have demonstrated the antioxidant action of tamoxifen on microsomal and liposomal lipid peroxidation. The effects of tamoxifen on serum malondialdehyde and several antioxidant components were evaluated in 64 postmenopausal breast cancer patients after three and six months of treatment with 20 mg/day tamoxifen (Thangaraju *et al.*, 1994). Serum malondialdehyde levels decreased significantly from 7.64 ± 1.2 nmol/dL before treatment initiation to 6.04 ± 0.95 nmol/dL ($p < 0.001$) after three months to 5.83 ± 0.91 nmol/dL ($p < 0.001$) after six months of tamoxifen administration. The levels of blood glutathione slightly increased from 2.61 ± 0.50 μmol/mL red

Table 10. Tamoxifen-associated side-effects in the reproductive tract

Reference	Study groups and methods	Main results
Breast cancer patients		
Ferrazzi *et al.* (1977)	Karyopycnotic index in vaginal smear cells in 35 post-menopausal patients with advanced breast cancer before and after treatment with 30–40 mg/day tamoxifen for 30–45 days	Increase of the karyopycnotic index to 10–30%; in 4 cases, the index reached ≥ 50% and in 1 case 80%. Two months after cessation of therapy, karyopycnotic index had returned to atrophic pattern.
Boccardo *et al.* (1981)	Karyopycnotic index in vaginal smear cells in 28 post-menopausal patients with breast cancer before and after treatment with 20 mg/day tamoxifen at 4 and 8 weeks	Increase of karyopycnotic index in 68% of the study group. Mean values at 0, 4 and 8 weeks were 1, 5 and 10%. Large variation
Burke *et al.* (1987)	Comparative sonographic examination of the uterus in 30 postmenopausal women receiving 20–30 mg/day tamoxifen and 15 postmenopausal controls	Increased uterine volume in 26.6%; hyperechogenicity in 46.6% of the tamoxifen group compared to none in the controls
Ford *et al.* (1988)	Case report, 1 postmenopausal patient receiving tamoxifen for recurrent benign breast disease	After 5 months of treatment, stage IV endometriosis
Pons & Rigonnot (1988)	45 postmenopausal patients receiving tamoxifen for 12–90 months; cytology and clinical examination of vagina, cervix and uterus	Oestrogenization of cervix and vagina in 23 patients; endometrial hyperplasia in 11; polyploid hyperplasia in 5; glandular cystic hyperplasia in 4; polyps in 2; cervical early adenocarcinoma in 1 and endometrial early adenocarcinoma in 1; proliferative stimulation of pre-existing leiomyoma in 2
Cano *et al.* (1989)	Case report, one 33-year-old patient receiving 20 mg/day tamoxifen for two years after mastectomy	Endometrioma of the left ovary with multiple adherence to the uterus; grade IV endometriosis
Fisher *et al.* (1989a)	1326 post-operative breast cancer patients receiving tamoxifen (20 mg/day) for 5 years and 1318 post-operative breast cancer placebo controls	Hot flushes in 40% of controls versus 57% of tamoxifen; vaginal discharge in 12% of controls versus 23% of tamoxifen; irregular menses in 15% of controls versus 19% of tamoxifen
Nuovo *et al.* (1989)	Case report: 3 postmenopausal patients (one also with chronic lymphocytic leukaemia) with metastatic breast cancer receiving 20 mg/day tamoxifen; 2 cases for 6 years, 1 case for 2 years	Endometrial polyps (one patient with leiomyomata)

Table 10 (contd)

Reference	Study groups and methods	Main results
Neven *et al.* (1989)	14 breast cancer patients receiving tamoxifen (20 mg/day) examined for postmenopausal bleeding [duration of treatment not given]; 42 breast cancer patients with postmenopausal bleeding without tamoxifen treatment	Increased incidence of endometrial hyperplasia in the tamoxifen group (RR, 5.2; 95% CI, 2–13.9; $p < 0.05$) and increased frequency of endometrial polyps (RR, 3.5; 95% CI, 2–6.2; $p < 0.05$)
Neven *et al.* (1989)	30 breast cancer patients receiving 20 mg/day tamoxifen; 29 breast cancer patients without tamoxifen. Hysteroscopic findings	Higher relative risks for polyps for the tamoxifen group (RR, 6.7; 95% CI, 1.3–35.7; $p < 0.05$) and for proliferative uterine mucosa (RR, 2.9; 95% CI, 1.2–7.3; $p < 0.05$)
Neven *et al.* (1990)	16 breast cancer patients receiving 20 mg/day tamoxifen for 6–36 months; 10 postmenopausal, 4 with induced amenorrhoea, 2 premenopausal. Mean age, 55.8 ± 10.2; mean parity, 1.6 ± 1.1	Endometrial changes: mild proliferation of the mucosa (7 cases), polyps (4 cases), adenocarcinoma (1 case)
Le Bouëdec *et al.* (1990)	22 breast cancer patients with oestrogen receptor-positive tumour; 3 premenopausal, 19 postmenopausal; mean age, 62.5 years; treatment duration, 26 months; cumulative dose, 5.4–43.2 g	Examination for uterine bleeding revealed 12 cases of endometrial hyperplasia; 6 cases of endometrial polyps; 6 cases of uterine myomas; 1 case with adenocarcinoma of the uterus; 1 case with acanthoma; 3 cases with endometrial atrophy
Cross & Ismail (1990)	Case report: 54-year-old woman mastectomized for breast carcinoma 18 years earlier followed 9 years later by bilateral oophorectomy because of axillary metastatic node. Tamoxifen introduced at that time at 40 mg/day for 6 months and then reduced to 20 mg/day until this report	Uterine bleeding leading to hysterectomy; endometrial hyperplasia and no residual ovarian tissue identified; polyp; a few intramural tumours
Buckley (1990)	Case report: 44-year old breast cancer patient receiving 20 mg/day tamoxifen for 3 years	Severe simple endometrial hyperplasia
Le Bouëdec *et al.* (1991)	Case report: 69-year old breast cancer patient receiving 20 mg/day tamoxifen for 7 years	Severe endometriosis, large uterine adenomyoma
De Muylder *et al.* (1991)	46 breast cancer patients with hormone-receptor positive tumour receiving tamoxifen for 6–36 months; 34 post-menopausal; 12 premenopausal; tamoxifen dose-rate not indicated	13 cases with endometrial polyps, 8 with endometrial hyperplasia, 2 with uterine adenocarcinoma; the rate of endometrial hyperplasia correlated with the cumulative dose of tamoxifen

Table 10 (contd)

Reference	Study groups and methods	Main results
Lang-Avenous *et al.* (1991)	28 patients receiving tamoxifen [dose not stated] after diagnosis of breast cancer	11 cases with atrophic endometrium; 5 with atrophic endometrium associated with polyps; 3 with cystic glandular polyps; 2 with regressive glandular cystic hyperplasia
Corley *et al.* (1992)	Case reports: 3 postmenopausal breast cancer patients (aged 77, 72 and 58) and one premenopausal breast cancer patient (age 45) receiving 20 mg/day tamoxifen for 3, 6 and 10 years (postmenopausal patient) or for 2 years (premenopausal patient)	Endometrial polyps, in one case metastatic breast carcinoma was present in the polyp
Dilts *et al.* (1992)	49-year-old woman, gravida 3, para 1, bilaterally mastectomized for metachronous breast cancer at 1 year interval. Tamoxifen started after 2nd mastectomy for 3 months (20 mg/day)	Echographic diagnosis of leiomyoma and an ovarian cyst. Exploratory laparotomy revealed a marked oestrogen-stimulated pelvis similar to that seen with a term pregnancy.
Hulka & Hall (1993)	14 postmenopausal breast cancer patients receiving tamoxifen: duration and dose not stated; no control group; pelvic sonograms and endometrial biopsy	11 patients with abnormal (> 7 mm) endometrial thickening and abnormalities in the sonogram (hyperechoic and cystic zones); 9 cases of uterine polyps, 4 cases of endometrial hyperplasia, 2 cases of endometritis, 1 proliferative endometrium, 1 inactive endometrium, 1 endometrial carcinoma
Rayter *et al.* (1993)	49 breast cancer patients receiving tamoxifen (20 mg/day) for an average of 47.5 months (37% premenopausal; 63 post-menopausal; average age 54.5 years); 45 breast cancer patients without tamoxifen (42% premenopausal, 51% post-menopausal; average age, 54 years)	Clinical enlargement of the uterus in 8 tamoxifen patients versus 0 control ($r = 0.006$). Endometrial thickness seemed greater in the tamoxifen group but not so great as the effect of menopause (pre-menopausal, 9.2 mm; postmenopausal, 6.4 mm); more endometrial nuclear hyperplasia ($p = 0.047$).

Table 10 (contd)

Reference	Study groups and methods	Main results
Lahti *et al.* (1993)	51 postmenopausal breast cancer patients receiving 20–40 mg/day tamoxifen for an average of 30 months; 52 postmenopausal breast cancer patients without tamoxifen; groups matched for age, parity, age at menopause and body mass index	Thicker endometrium in the tamoxifen group (10.4 ± 5 versus 4.2 ± 2.7 mm, $p = 0.0001$) by transvaginal sonography; larger uterine volume in the tamoxifen group (45 ± 27 versus 25 ± 11 cm^3; $p = 0.001$) by transvaginal sonography; endometrial polyps more frequent in the tamoxifen group (36% versus 10%; $p = 0.004$). 1 atypical hyperplasia, 1 adenomatous hyperplasia and 1 endometrial adenocarcinoma in the tamoxifen group; two endometrial adenocarcinomas in the control group
Seoud *et al.* (1993)	Six postmenopausal breast cancer patients received tamoxifen (20 mg/day), 2 for < 2 years, 4 for > 2 years.	Three with endometrial adenocarcinoma; 1 with homologous mixed Müllerian sarcoma; 1 with primary fallopian tube carcinoma; 1 with endometrial polyps and glandular hyperplasia
Ugwumadu *et al.* (1993)	Case report: 58-year old breast cancer patient treated 13 years earlier by bilateral oophorectomy; 40 mg/day tamoxifen for 8 years	Postmenopausal bleeding, myometrial adenomyosis, cystic atrophy of the endometrium and endometrial polyp
Uzily *et al.* (1993)	95 breast cancer patients; mean age, 58 years, receiving 20 mg/day tamoxifen for median time of 24 (1–84) months: vaginal ultrasonography and endometrial biopsy. No control group	89% of tamoxifen users > 12 months had endometrial thickness of > 0.5 cm versus 71% of < 12 months therapy. Four cases with endometrial hyperplasia; 4 with benign endometrial polyp, 3 showed dysplasia and 3 with endometrial cancer; except for one patient with endometrial hyperplasia, all had received tamoxifen for more than 12 months.
Ismail (1994)	19 breast cancer patients receiving tamoxifen (either 20 or 40 mg/day) (2 patients died of other than gynaecological disease and necropsy was carried out; the remaining 17 tamoxifen-treated patients were examined because of gynaecological symptoms) and 15 patients with gynaecological symptoms without tamoxifen; matched for age and presentation	Tamoxifen versus control group; endometrial hyperplasia 11 versus 4; endometrial polyps: 11 versus 1; primary endometrial malignancies: 2 versus 4; endometrial polyp cancers: 4 versus 0; all carcinomas in the tamoxifen group were observed in cases with > 35 g cumulative doses.

Table 10 (contd)

Reference	Study groups and methods	Main results
Leo *et al.* (1994)	2 case reports: 36-year-old woman with mastectomy and axillary node dissection followed by chemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil) cycles and tamoxifen 30 mg/day as adjuvant therapy	After 4 years, sudden increase of uterine volume and leiomyoma of the corpus uteri
	50-year-old woman, mastectomized for breast cancer followed by chemotherapy and tamoxifen 30 mg/day	After 4 years, admitted for severe abdominal pain; vaginal serography showed uterine fibroid of the corpus uteri and increased endometrial thickness.
Krause & Gerber (1994)	8 patients treated with tamoxifen following breast cancer	6 endometrial polyps, 1 adenosis uteri, 1 endometrial cancer
Ugwumadu & Harding (1994)	56-year-old woman underwent mastectomy and lymph node biopsy for invasive duct carcinoma, then received radiotherapy and tamoxifen 20 mg/day.	After 2 years of treatment, uterus was about the size of a 6–8-week gestation on physical examination and four years later the size of a 20-week gestation. Laparotomy revealed enlarged uterus with thickened endometrium, polypoid in places and multiple benign leiomyomata was confirmed.
	62-year-old patient treated by lumpectomy and radiotherapy for a poorly differentiated duct carcinoma. General and pelvic examinations were normal and tamoxifen was started at 20 mg/day.	2.5 years later, a uterus equivalent to a 14-week gestation was found, with a rather well oestrogenized vagina.
Healthy women		
Kedar *et al.* (1994)	111 postmenopausal healthy women (46–71 years) from the Pilot Breast Cancer Prevention Trial: 61 receiving 20 mg/day tamoxifen (average age, 56), 50 placebo controls (average age, 58 years)	The tamoxifen group had a larger uterus (34 versus 22.2 mL; $p < 0.001$), and increased endometrial thickness (9.1 versus 4.8 mm; $p < 0.001$); in addition 10 cases (16%) of atypical hyperplasia versus none in the placebo group and 5 cases (8%) of endometrial polyps in the tamoxifen group versus one in the placebo group.

Table 11. Tamoxifen-associated side-effects on blood coagulation

Reference	Study groups and methods	Main results
Breast cancer patients		
Nevasaari *et al.* (1978)	Case report: 4 patients (43, 57, 60 and 68 years old) with metastatic breast cancer receiving 20-40 mg tamoxifen for 2 weeks to 3½ months	Deep vein thrombosis
Lipton *et al.* (1984)	220 patients with metastatic breast cancer started on tamoxifen	7 venous thromoses within 6 months; 4 cases with phlebitis, 2 with phlebitis and pulmonary embolism, 1 with phlebitis
Enck & Rios (1984)	39 postmenopausal patients (average age: 64 years) with metastatic breast cancer: 24 receiving 20 mg/day tamoxifen for mean of 36 (2–87) weeks; 4 receiving 15 mg/day diethyl-stilboestrol; 11 controls	Lower concentrations of antithrombin-III in 42% (10/24) of the tamoxifen-treated (mean duration, 36 weeks) cases compared with 9% (1/11) in the control group
Hendrick & Subramanian (1980)	Case report: 72-year-old breast cancer patient with lung and bone metastasis receiving 20 mg/day tamoxifen	Death 4 weeks after start of tamoxifen treatment due to thrombosis of the superior mesenteric artery with no evidence of local metastasis in the artery and no sign of atheroma
Jordan *et al.* (1987)	25 premenopausal and 22 postmenopausal breast cancer patients receiving 20 mg/day tamoxifen for between 434 and 2592 days and between 91 and 1560 days respectively. 95 premenopausal and 8 postmenopausal breast cancer patients receiving only combination chemotherapy served as controls.	Antithrombin-III levels decreased compared to only chemotherapy controls ($p < 0.001$). However, in no case were the antithrombin-III values decreased by > 30%, which is considered the level of clinical significance.
Bertelli *et al.* (1988)	55 breast cancer patients receiving 20 mg/day tamoxifen for ≥ 3 months and 36 breast cancer patients without any treatment after mastectomy as controls	Decreased antithrombin-III levels in the tamoxifen group (26.6 ± 1 versus 30.2 ± 1.2 mg/dL; $p = 0.03$)
Love *et al.* (1992a)	140 women with axillary node-negative breast cancer, 70 receiving 20 mg/day tamoxifen, 70 placebo controls	Fibrinogen levels decreased by 15% in the tamoxifen group by 6 months ($p < 0.001$); antithrombin-III concentrations decreased significantly in the tamoxifen group but not to clinically significant levels. Decrease in platelet counts of 7–9%.

Table 11 (contd)

Reference	Study groups and methods	Main results
Cuzick *et al.* (1993)	153 breast cancer patients, 20 current tamoxifen users (mean duration of treatment 72 months), 73 ex-users (mean duration of treatment: 24 months, median time after cessation of tamoxifen administration: 58 months) and 60 breast cancer controls, who had never used tamoxifen.	No differences between controls and ex-users; in current users kaolin cephalin clotting times were marginally shorter, fibrinogen and fast alpha-2 antiplasmin levels were lower ($p = 0.03$, $p = 0.0001$ and $p = 0.009$ respectively) and plasminogen levels were higher ($p = 0.02$).
Love *et al.* (1994a)	30 breast cancer patients receiving 20 mg/day tamoxifen for 5 years and 32 breast cancer placebo control patients	After 5 years fibrinogen levels were 17% lower in the tamoxifen-treated group compared with base-line ($t = 0$) compared with the change in the placebo group ($p = 0.08$).
Healthy women		
Jones *et al.* (1992)	515 normal healthy premenopausal and postmenopausal women with a history of breast cancer, receiving tamoxifen (20 mg/day) as a chemopreventive agent; follow-up for 36 months every 6 months by determination of fibrinogen, antithrombin-III, protein C and protein S	Marginal reduction of antithrombin-III (postmenopause only) and protein S (a natural coagulation inhibitor) after 6 months, which was no longer observed after 12 months. No increase in the incidence of thromboembolic events

Table 12. Tamoxifen-associated side-effects on blood lipids, steroid hormone-binding globulin and thyroid function-associated parameters

Reference	Study groups and methods	Main results[a]
Gordon *et al.* (1986)	50 postmenopausal breast cancer patients receiving 20 mg/day tamoxifen (average treatment duration, 11 months; range, 1–42 months); 50 healthy (not breast cancer) postmenopausal women as control	Serum thyroxin levels were elevated in 10/50 tamoxifen cases versus 1/50 control cases ($p < 0.04$). No significant difference in triiodothyronine levels. Thyroid-binding globulin levels increased in 6/6 (> 30 mg/L) tamoxifen cases who had elevated serum thyroxin levels.
Bertelli *et al.* (1988)	55 breast cancer patients receiving 20 mg/d tamoxifen for ≥ 3 months and 36 breast cancer patients without any treatment after mastectomy as controls	Total cholesterol and LDL-cholesterol were significantly ($p < 0.05$) lower in the tamoxifen group. Total cholesterol: 212.6 ± 6 versus 254.3 ± 8 mg/dL and LDL-cholesterol 126.7 ± 62 versus 175.9 ± 9.6 mg/dL.
Bruning *et al.* (1988)	8 premenopausal (mean age, 43.3 years) and 46 postmenopausal (mean age, 63.2 years) receiving 20 mg/day tamoxifen for 6 months	No change in total cholesterol; significant ($p < 0.05$) increase in HDL and decrease ($p < 0.05$) in LDL. Significant increase in sex hormone binding globulin (SHBG) ($p < 0.001$)
Bagdade *et al.* (1990)	8 postmenopausal women receiving 20 mg/day tamoxifen for 3 months	No significant changes in total cholesterol or triglyceride levels; significant decrease in non-esterified free cholesterol levels ($p < 0.05$) and in LDL fraction; concentrations of cholesterol, free cholesterol and free cholesterol/lecithin ratio fell ($p < 0.025$; $p < 0.05$; $p < 0.025$). Significant increase in SHBG ($p < 0.005$)

Table 12 (contd)

Reference	Study groups and methods	Main results[a]
Cuzick *et al.* (1993)	153 breast cancer patients (116 postmenopausal); 20 (14) current tamoxifen users (mean duration of treatment, 72 months); 73 (55) ex-users (mean duration of treatment, 24 months; median time after cessation of tamoxifen administration, 58 months) and 60 (47) breast cancer controls who had never used tamoxifen	In the premenopausal groups, no difference between controls and ex-users. In the postmenopausal groups: Ex-users / Controls / Current users TC: 7.00 / 6.63 / 5.81; $0.01 < p < 0.05$ LDL-C: 5.20 / 4.82 / 3.75; $0.01 < p < 0.05$ TG: 1.31 / 1.12 / 1.33; $0.01 < p < 0.05$ SHBG: 62 / 55 / 105; $p < 0.001$ T3: 1.81 / 1.81 / 2.15; $0.001 < p \leq 0.01$ T4: 96.9 / 97.6 / 126.9; $0.001 < p \leq 0.01$
Dewar *et al.* (1992)	44 postmenopausal women (mean age, 59; range, 45–76) with breast cancer; 24 receiving 20 mg/day tamoxifen for 5 years and 20 placebo controls; after five years, 18 treated patients were randomly selected either to continue ($n = 10$) or stop ($n = 8$) treatment and followed for additional 3 years	Tamoxifen treatment consistently lowered total cholesterol levels but the effect ended after cessation of treatment: Year: 1 / 2 / 3 / 4 / 5 Change (mmol/L): –0.52 / –0.96 / –0.96 / –0.88 / –1.02 p: 0.013 / 0.003 / 0.022 / 0.005 / 0.001 Apparently no change in HDL

Table 12 (contd)

Reference	Study groups and methods	Main results[a]
Dnistrian *et al.* (1993)	24 breast cancer patients (11 premenopausal, 13 postmenopausal), mostly hormone receptor-positive, receiving 20 mg/day tamoxifen for 4–8 weeks (only 4 received tamoxifen alone, 20 also received chemotherapy.)	Tamoxifen induced 17% decrease in total cholesterol levels and 27% decrease in LDL-cholesterol. No clear change in HDL-cholesterol levels; significant decrease (33%) in LDL/HDL ratio.
Love *et al.* (1994a)	30 breast cancer patients receiving 20 mg/day tamoxifen for 5 years and 32 breast cancer placebo control patients	At baseline ($t = 0$) no differences between the two groups. Total cholesterol and LDL-cholesterol levels were significantly decreased in the tamoxifen-treated group at 5 years ($p = 0.001$) and the changes were significantly greater than in the placebo control ($p = 0.01$ versus $p = 0.001$).
Anker *et al.* (1995)	31 postmenopausal breast cancer patients (mean age, 65 years) receiving 20–30 mg tamoxifen for 1–> 19 months. Levels of plasma homocysteine (a risk factor for atherosclerotic disease) and serum cholesterol were determined at various time points.	Plasma homocysteine was suppressed by a mean value of 29.8% after 9–12 months and by 24.5% after 13–18 months of treatment. Cholesterol levels decreased by mean values varying between 7.2% and 17.6% after 5–9 months of treatment.
Grey *et al.* (1995a)	23 healthy postmenopausal women randomly assigned to receive tamoxifen (20 mg/day) for 2 years (mean age, 58 ± 6 years) compared with 23 similar women receiving placebo (mean age, 60 ± 5 years)	Tamoxifen lowered serum cholesterol by 12 ± 2% and LDL-cholesterol by 19 ± 3%; HDL-cholesterol not altered.
Mamby *et al.* (1995)	14 postmenopausal breast cancer patients participating in a longitudinal, double-blind, randomized placebo-controlled study of tamoxifen (20 mg/day) and 14 placebo controls.	Significant increases in the tamoxifen group after 3 months in thyroid-binding globulin levels (from 21.26 ± 1.06 to 26.94 ± 1.81), thyroxine (T4) levels (from 7.02 ± 0.3 to 8.39 ± 0.46) and thyroxine uptake (from 127.71 ± 5.61 to 142.43 ± 6.82). No change in thyroid-stimulating hormone levels or free thyroxine index

LDL, low-density lipoprotein; HDL, high-density lipoprotein; SHBG, sex hormone binding globulin (nmol/L); TC, total cholesterol (mmol/L); LDL-C, low-density lipoprotein-cholesterol (mmol/L); TG, triglycerides (mmol/L); T3, triiodothyronine (nmol/L); T4, thyroxine (nmol/L)

Table 13. Effects of tamoxifen on bone mineralization

Reference	Study groups and methods	Main results
Love *et al.* (1988)	48 women with breast cancer treated with tamoxifen for at least 2 years and 37 women not treated with tamoxifen	No difference in bone mineral density (BMD) between the two groups
Fentiman *et al.* (1989)	Premenopausal women taking tamoxifen (20 mg/day for 36 months) for mastalgia, compared with placebo group (50 mg/day vitamin C)	No change from baseline levels in either treated or placebo group after 3 months treatment, and no change in tamoxifen group after 6 months
Fornander *et al.* (1990)	75 recurrence-free postmenopausal breast cancer patients taking tamoxifen (40 mg/day for 2 or 5 years) compared with control patients taking no adjuvant endocrine therapy	BMD similar in treated and control groups, measured about 7 years after initial randomization. Cortical bone: 1.03 g/cm^2 in tamoxifen versus 1.03 g/cm^2 in controls; trabecular bone: 0.74 g/cm^2 versus 0.73 g/cm^2
Love *et al.* (1994b)	Two-year, randomized, double-blind trial of tamoxifen (20 mg/day) in 70 treated women with breast cancer compared with 70 placebo controls	Mean BMD of lumbar spine increased by 0.6% per year in treated group and decreased by 1.00% per year in placebo group ($p < 0.001$). Radial BMD decreased to same extent in both groups.
Ward *et al.* (1993)	15 early postmenopausal women with stage I or II breast cancer taking tamoxifen (20 mg/day) and 21 healthy postmenopausal controls. Serum sex hormone-binding globulin and antithrombin III levels measured, and BMD at various sites measured	Tamoxifen prevented bone loss at femoral neck (+ 1.4% gain in BMD/year versus –1.8% in control group; $p = 0.03$) and lumbar spine (+0.09%/year versus –2.3%; $p = 0.04$), and reduced bone turnover. Sex hormone binding globulin was increased and antithrombin III reduced in treated group.
Kristensen *et al.* (1994)	20 women receiving tamoxifen (30 mg/day) for 2 years and 23 untreated controls. All patients postmenopausal with primary breast cancer, classified as low risk after surgery	Lumbar BMD increased by about 3% in first year in tamoxifen group and then stabilized. Lumbar BMD decreased by about 2.5% in control group in the first year ($p = 0.00074$), continued to decrease to about 4.5% after two years. BMD at forearms stable in tamoxifen but declined in control group ($p = 0.024$).
Wright *et al.* (1994)	41 women with breast cancer, 22 treated with tamoxifen for $\geq$ 15 months (mean, 33 months) and 19 untreated. Transiliac crest bone biopsies analysed by histomorphometry	No statistically significant difference between treated and control groups in bone area, osteoid perimeter and area, or osteoid width. Tissue-based bone formation significantly lower ($p = 0.05$) and remodelling period significantly longer ($p < 0.05$) in treated group
Grey *et al.* (1995b)	23 healthy postmenopausal women randomly assigned to receive tamoxifen (20 mg/day) for 2 years (mean age, 58 $\pm$ 6 years) compared with 23 similar women receiving placebo (mean age, 60 $\pm$ 5 years)	1.4% increase in lumbar spine BMD in the tamoxifen group versus 0.7% decline in the placebo group ($p < 0.01$). This small protective effect was comparable in magnitude to calcium supplementation and less than that of either oestrogen or bisphosphonates.

cells to 2.86 ± 0.43 μmol/mL ($p < 0.01$) to 2.91 ± 0.47 μmol/mL ($p < 0.01$) and slight increases were also observed in serum levels of ceruloplasmin, uric acid, vitamins A, C and E and selenium. [Except for concentrations of malondialdehyde, the changes in all parameters were ≤ 10% of the initial value.]

Ocular toxicity has been reported following tamoxifen treatment, the first published reports of retinopathy and corneal changes being associated with particularly high doses of tamoxifen of at least 240 mg/day (Kaiser-Kupfer & Lippman, 1978). It is characterized by white refractile intraretinal deposits distributed mainly at the posterior pole. Ocular toxicity also appears to be an infrequent but serious complication of tamoxifen therapy at doses of 20–40 mg/day (Griffiths, 1987; De Jong-Busnac,1989). In the study by Pavlidis *et al.* (1992), four of 63 patients administered 20 mg tamoxifen per day displayed retinopathy and/or keratopathy 10, 27, 31 and 35 months, respectively, after the start of therapy. However, in another controlled study, no ocular toxicity was found in 79 breast cancer patients taking tamoxifen in conventional doses (10–20 mg two or three times a day) for an average of two years and three months (Longstaff *et al.*, 1989). Ocular toxicity has not been reported in any of the major adjuvant breast cancer studies involving tamoxifen (Fisher *et al.*, 1989a; Ribeiro & Swindell, 1992; Fornander *et al.*, 1991). Where retinopathy was reported in patients, it was reversible on cessation of tamoxifen treatment if the condition was detected at an early stage (Ashford *et al.*, 1988; Chang *et al.*, 1992).

Leukopenia and neutropenia (Glick *et al.*, 1981; Boccardo *et al.*, 1994b; Miké *et al.*, 1994) have been reported on rare occasions following the administration of tamoxifen.

4.2.2 *Experimental systems*

In single-dose toxicity studies, the oral LD_{50} of tamoxifen was approximately 3 g/kg for mice and 2.5 g/kg for rats (Furr & Jordan, 1984). Tamoxifen is well tolerated upon chronic administration to mice, rats and dogs at large multiples of the pharmacologically active dose (approximately 0.1 mg/kg), the pharmacological properties of tamoxifen (behaving as an oestrogen in some species and tissues and as an antioestrogen in others) accounting for many of the effects described in toxicology studies (Tucker *et al.*, 1984). Tamoxifen is an antioestrogen with complex pharmacology encompassing variable species-, tissue-, cell-, gene- and duration of administration-specific effects from oestrogen-like agonist actions to complete blockage of oestrogenic action (Jordan & Robinson, 1987). In short-term laboratory assays, tamoxifen is usually classified as oestrogenic in mice but as a partial agonist/antagonist in rats. The concept that tamoxifen is oestrogenic in mice may not provide a complete description of the effects in this species, as prolonged administration to ovariectomized mice results in the uterus becoming refractory to oestrogen administration (Jordan *et al.*, 1990). Whilst the initial response of the uterus to tamoxifen is oestrogen-like, as administration continues, uterine weight returns to initial values. In immature rats, administration of tamoxifen increases uterine weight in a dose-dependent manner without attaining the same maximal effect as obtained with 17β-oestradiol; administration of tamoxifen together with oestradiol provides a partial but incomplete antagonism of the uterotrophic action of 17β-oestradiol (Harper &

Walpole, 1967a,b). The increase in uterine weight is largely due to hypertrophy of the luminal epithelium, with little change in the myometrium and stroma. This hypertrophic effect was not associated with any change in thymidine incorporation or cell division typical of uterine response to 17β-oestradiol treatment (Jordan *et al.*, 1980). More recent studies have confirmed the hypertrophic effect of tamoxifen and other antioestrogens on the luminal and glandular epithelium of the rat uterus and contrasted this effect with that of oestrogens (Branham *et al.*, 1993).

In mice, repeated administration of doses up to 50 mg/kg for 13–15 months caused atrophy of gonads and accessory sex organs, with cystic endometrial hyperplasia in the uterus, elongation of the vertebrae and a marked increase in bone density with resorption and new bone formation in irregular patterns. These changes are consistent with the pharmacological action in this species. In the liver, there were fatty changes and a swelling of the parenchymal cells (Tucker *et al.*, 1984).

In rats, administration of tamoxifen for 6–24 months at doses between 35 and 100 mg/kg per day caused atrophy of the gonads and accessory sex organs, whilst, at lower doses (2 mg/kg per day), the uterine endometrium showed an absence of glands, flattening of the epithelium and occasional squamous metaplasia (Tucker *et al.*, 1984; Greaves *et al.*, 1993). After six months' treatment with 35 mg/kg per day, there was nodular hyperplasia in the liver (Greaves *et al.*, 1993). The lysosomal lipidosis seen in a number of tissues, including retina and cornea, after chronic administration of 100–130 mg/kg per day is consistent with the cationic amphiphilic structure of tamoxifen (Lüllmann & Lüllmann-Rauch, 1981); the higher incidence of cataracts may be related to changes in sex hormone status (Greaves *et al.*, 1993).

In dogs, repeated administration of up to 75 mg/kg tamoxifen per day for three months resulted in cessation of ovulation and hyperplasia of the germinal epithelium of the ovary and severe endometritis with squamous metaplasia, as well as biliary stasis with no other morphological change in the liver at the highest-dose level only (Tucker *et al.*, 1984).

Six months' administration of tamoxifen at doses up to 8 mg/kg per day to marmosets produced a slight increase in ovarian follicular cyst weight and number, probably as a result of its antioestrogenic effect in this species (Furr *et al.*, 1979; Tucker *et al.*, 1984).

Rat, chicken and human microsomes exhibited low tamoxifen-binding activity compared with hamster and mouse microsomes (Mani *et al.*, 1994). In another study (White *et al.*, 1995), covalent binding of tamoxifen to microsomal proteins was observed with human, rat and mouse microsomes; the activity of mouse microsomes was highest (17-fold higher than human microsomes), while rat microsomes had intermediate activity (3.8-fold higher than human microsomes).

White *et al.* (1993) showed that, while the total hepatic microsomal cytochrome P450 content of rats given tamoxifen intraperitoneally (0.12 mmol/kg or 45 mg/kg per day for four days) was not increased (and, indeed, was transiently decreased), there were 30–60-fold increases in the metabolism of benzyloxy- and pentoxyresorufin; the metabolism of ethoxyresorufin was only slightly increased. Immunoblotting experiments revealed two- to three-fold increases in CYP2B1, CYP2B2 and CYP3A1 proteins. Induction of these

proteins was centrilobular. None of these monooxygenase activities was induced in C57Bl/6 mice and only small increases in benzyloxy- and pentoxyresorufin metabolism were seen in DBA/2 mice. There has been independent confirmation of the induction of CYP2B1, CYP2B2 and CYP3A in the liver of Fischer 344 rats (particularly females) administered tamoxifen orally for seven days. In addition, microsomal epoxide hydrolase was induced. These were selective inductions, there being no increased expression of CYP1A1, CYP1A2 or γ-glutamyl transpeptidase (Nuwaysir *et al.*, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Tamoxifen is contraindicated during pregnancy (Vidal, 1995; Medical Economics, 1996).

Cullins *et al.* (1994) reported the birth of an infant with Goldenhar's syndrome (oculoauriculovertebral dysplasia) delivered by Caesarian section at 26 weeks to a 35-year-old woman with breast cancer who had received 20 mg/day tamoxifen throughout pregnancy. They noted that 50 pregnancies reported to the manufacturer had been associated with tamoxifen administration. There were 19 normal births, 8 terminations, 13 unknown outcomes and 10 associated with a fetal or neonatal disorder. Two infants had congenital craniofacial defects. [The Working Group noted that details of these 50 cases were not available in this secondary reference.]

Zemlickis *et al.* (1992) reported on three women with breast cancer who received treatment during the first trimester of pregnancy. One woman received tamoxifen with cyclophosphamide, methotrexate, fluorouracil and vincristine sulfate; the pregnancy ended in live birth, and the baby was alive and well at the time of follow-up. The other two women did not receive tamoxifen and the pregnancy ended in miscarriage. Two further women with breast cancer received chemotherapy during the third trimester. One received tamoxifen with fluorouracil, doxorubicin and cyclophosphamide, and a live-born infant was delivered who was alive and well at the time of follow-up. The other woman did not receive tamoxifen but also delivered a live birth with some intrauterine growth retardation; this child was well at the time of the follow-up.

Lai *et al.* (1994) reported the birth of a normal male infant with a birthweight of 3340 g at term following six months' daily therapy with 30 mg tamoxifen and 160 mg megestrol acetate for adenocarcinoma of the endometrium and three months' treatment with a combined oestrogen/gestagen oral contraceptive pill.

Two studies have been made of the effect of tamoxifen on reproductive parameters in healthy female volunteers. In the first, 16 women with regular menstrual cycles were followed during one control cycle, one treatment cycle during which women received 20 mg tamoxifen twice daily from cycle day 18 until day 30 or onset of menstruation, whichever came first, and one follow-up cycle (Swahn *et al.*, 1989). The length of the treatment cycle (28.5 days ± 2.0 days) was significantly longer than that of the control (27.2 ± 2.0 days) or follow-up (27.5 ± 1.9 days) cycles, owing to a prolonged luteal phase. Levels of follicle-stimulating hormone (FSH), progesterone, 17-hydroxyproges-

terone, 20-dihydroprogesterone, oestrone, oestrone sulfate and 17β-oestradiol were significantly elevated during the treatment cycle compared with the control cycle. Levels of pregnanediol glucuronide remained unchanged during the treatment cycle, whereas the concentrations during the follow-up cycle were approximately double those of the control cycle. Prolactin levels decreased slightly during tamoxifen treatment, but the effect was not statistically significant. There was a positive correlation between plasma levels of tamoxifen and 17-hydroxyprogesterone ($r = 0.72$; $p < 0.05$), but not with plasma levels of the other hormones during treatment or follow-up cycles. The treatment did not cause any major disturbance of the bleeding pattern.

In the second study, Mäentausta *et al.* (1993) studied the effects of tamoxifen on endometrial 17β-hydroxysteroid dehydrogenase and progesterone and oestrogen receptors during the luteal phase of the menstrual cycle in 11 healthy female volunteers. The study included one control and two treatment cycles. During the first treatment cycle, the subjects received 200 mg mifepristone two days after the peak serum luteinizing hormone (LH) concentration. During the second treatment cycle, the subjects received 40 mg tamoxifen on the second and third days after the peak serum LH concentration. The time interval between these treatments was about a month, and the authors state that this ensured that the effects of each treatment modality had disappeared before the next treatment. 17β-Hydroxysteroid dehydrogenase and progesterone and oestrogen receptors were examined immunohistochemically in endometrial tissue specimens taken on the sixth to eighth day after the peak serum LH concentration. Tamoxifen did not have any significant effect on staining of 17β-hydroxysteroid dehydrogenase or the abundance of receptors. The authors state that serum concentrations of 17β-oestradiol, progesterone and LH were not significantly affected by the administration of tamoxifen.

Six women were treated for uterine fibroids for at least three months with 10 mg tamoxifen twice daily starting between days 1 and 3 of the menstrual period. Increased variability in the length of the menstrual and ovarian cycle was associated with significant lengthening of the luteal phase from 12.5 ± 1.5 days to 16.9 ± 3.5 days ($p < 0.02$; Lumsden *et al.*, 1989). A significant increase in the excretion of oestrone and pregnanediol glucuronide in the urine was associated with increased concentrations of 17β-oestradiol and progesterone in plasma, reflecting multiple follicular development and ovulation. A significant rise in the concentration of FSH occurred during the luteal phase of the cycle.

A number of studies of endocrine parameters have been carried out in pre- and postmenopausal women receiving tamoxifen therapy (for review, see Sunderland & Osborne, 1991). Most studies report that in postmenopausal women levels of gonadotrophins, FSH and LH decrease with tamoxifen therapy, although remaining within the normal postmenopausal range. 17β-Oestradiol and progesterone levels do not change in postmenopausal women receiving tamoxifen. In contrast, in premenopausal women receiving tamoxifen, 17β-oestradiol and progesterone levels show a striking elevation, often to two or three times the normal level. The elevated hormone levels follow a pattern consistent with the normal menstrual cycle. Despite these supraphysiological levels of 17β-oestradiol, FSH and LH levels remain unchanged or only slightly increased. Many premenopausal women receiving long-term tamoxifen therapy continue to have regular ovulation

and menstrual cycles, although as many as one third may develop temporary amenorrhoea or oligomenorrhoea.

In a study of the pregnancy outcome in the partners of men who had been treated with tamoxifen (n = 22) or clomiphene (n = 12) for oligozoospermia, no important difference in the course of pregnancy was found between the groups (Salata *et al.*, 1993).

Gooren (1989) reported a patient with incomplete androgen insensitivity syndrome (hypospadias, unilateral cryptorchidism and pubertal gynaecomastia, all surgically corrected) and plasma FSH levels below the reference range for adult men. After treatment with 10 mg tamoxifen twice daily, his plasma FSH level rose, spermatogenesis improved and his wife conceived three times within a period of five years.

In a number of uncontrolled studies, treatment with tamoxifen has been reported to be associated with an increase in sperm count of men with idiopathic oligozoospermia. However, in addition to the fact that these studies were not randomized controlled trials, a limitation of the studies was that patients who would be considered fertile were included (Kotoulas *et al.*, 1994). Krause *et al.* (1992) reported a randomized trial of tamoxifen in the treatment of idiopathic oligozoospermia. The sperm output and pregnancy rate was somewhat higher in the group of 39 patients who received 30 mg tamoxifen daily than in the 37 patients who received the placebo. [The numbers of subjects assigned to each group of the trial in the reporting of the pregnancy rates conflict with the numbers assigned to therapy recorded earlier in the paper.] Kotoulas *et al.* (1994) reported that 122 men randomly assigned to treatment with 10 mg tamoxifen twice daily for a period of three months had improved sperm density and number of live spermatozoa compared with 117 men who received the placebo therapy for the same period of time. The improvement in sperm density was more marked in the subgroups who were oligozoospermic than in normozoospermic men. In addition, there was a statistically significant decrease in the number of abnormal sperm after tamoxifen treatment, but the authors comment that this decrease was observed in only 12 patients in the tamoxifen group compared with eight patients in the placebo group.

4.3.2 *Experimental systems*

(*a*) *Effects on the fetus*

Cunha *et al.* (1987) assessed the potential oestrogenicity and teratogenicity of tamoxifen in 54 genital tracts isolated from 4–19-week-old human female fetuses and grown from one to two months in untreated athymic nude mice. After grafting of the human fetal genital tracts, the hosts were given subcutaneous implants of 20-mg pellets of tamoxifen, clomiphene or diethylstilboestrol, or were sham operated. In all mice that received tamoxifen, the vaginal epithelia of the hosts were thickened and cornified, and the uteri exhibited cystic hyperplasia. In specimens of human genital tract grown to a gestational age equivalent of 15 weeks or less, the vagina and urogenital sinus were lined with an immature squamous epithelium, which was similar in drug-treated and untreated specimens. The authors noted that the absence of oestrogenic response in these specimens correlated with the apparent absence of oestrogen receptors. Two of the four tamoxifen-treated specimens grown to a gestational age equivalent of 16 weeks or more

exhibited epithelial hyperplasia and maturation. Untreated specimens were unstimulated. Formation of endometrial and cervical glands proceeded in 13/15 (87%) control specimens grown to a gestational age equivalent to 13 weeks or more in untreated hosts. The results from tamoxifen-treated specimens were similar to those obtained with clomiphene-treated specimens, and therefore were pooled. Glands were present in only 6/13 (46%) age-matched specimens treated with tamoxifen or clomiphene. In the developing uterine corpus of untreated controls, the uterine mesenchyme segregated into inner (endometrial stroma) and outer (myometrial) layers, whereas, in specimens treated with tamoxifen, condensation and segregation of the mesenchyme were greatly impaired. The epithelium of the fallopian tubes of specimens treated with tamoxifen was hyperplastic and disorganized, and the complex mucosal plications characteristic of the fallopian tube were also distorted.

As tamoxifen is an antifertility agent in rats, it has proved difficult to conduct studies of possible teratogenic effects in this species (Furr & Jordan, 1984). Tucker *et al.* (1984) reported that 0.025 mg/kg bw tamoxifen was the highest dose that could be given throughout pregnancy in Alpk/AP rats without completely preventing implantation. At this dose, about 50% of matings gave rise to successful pregnancies. These authors also stated that in several teratogenic studies in rats, all doses above 2 mg/kg bw produced an incidence of irregular ossification of ribs in the fetus. They considered that this was secondary to a tamoxifen-induced reduction in the size of the uterus of the dam and noted that the effect disappeared in the early neonatal period. In rabbits, administration of 0.1 and 0.2 mg/kg bw tamoxifen throughout pregnancy did not produce any effect on implantation or on the fetus. In marmosets, doses of up to 10 mg/kg bw tamoxifen from days 25 to 35 of pregnancy did not produce any effect in the fetus. [The Working Group noted that few details of these studies were reported.]

Twenty adult female cynomolgus monkeys (*Macaca fascicularis*) with two spontaneous menstrual cycles of normal duration during an initial two- to three-month period of observation were randomly assigned to receive a low dose of tamoxifen (0.5 mg/kg bw per day; $n = 6$), a high dose of tamoxifen (3.0 mg/kg bw per day; $n = 7$) or lactose ($n = 7$) by gastric instillation daily for 12 days, starting four days after the mid-cycle 17β-oestradiol peak (Olive *et al.*, 1990). The luteal phase of the menstrual cycle was prolonged in the groups receiving tamoxifen compared with the control group, but no difference between the groups receiving low-dose and high-dose tamoxifen was observed. No noteworthy difference in hormonal characteristics between the groups was observed. The authors concluded that administration of tamoxifen during the luteal phase did not alter pituitary gonadotropin secretion or corpus luteum function. They suggested that the prolongation by tamoxifen of the length of the luteal phase in a subset of monkeys was perhaps due to a direct effect on the endometrium. In a study of 26 female *Macaca fascicularis* with proven fertility and normal menstrual cycles, 13 were treated with a single oral dose of 5 mg/kg bw tamoxifen and 13 with vehicle only on postovulation day 4 (Tarantal *et al.*, 1993). Serum progesterone and tamoxifen concentrations were evaluated on postovulation days 4, 8, 12, 16 and 18. There was no effect of tamoxifen on serum progesterone levels or on the fertility rate — 6/13 (46%) treated females and 4/13 controls (31%) became pregnant. In the centre in which the study was carried

out, the conception rate for the *Macaca fascicularis* colony was approximately 50% per mated cycle. Among the six pregnant animals treated with tamoxifen, one aborted spontaneously on gestational day 40 (after detection of severe growth retardation and embryonic death on gestational day 38) and five delivered live births naturally during gestational days 160–163. Among the control females who became pregnant, one had an early embryonic loss (at or before gestational day 18), one had a still birth at gestional day 162 and two delivered live births. None of the live births exhibited any abnormality. Neither tamoxifen nor any of its metabolites was detected in maternal serum and urine. The authors postulated that either absorption of tamoxifen was negligible or its metabolism and excretion occurred at an extremely rapid rate.

Beyer *et al.* (1989) assessed the embryotoxic potential of tamoxifen by studying its effect on cultured whole rat embryos in the presence and absence of a NADPH-supplemented post-mitochondrial supernatant fraction from Aroclor 1254-induced male rat liver (S9). Embryos were obtained from pregnant animals on gestational day 10. Only viable embryos, as determined by the presence of visible heart beat and active vitelline circulation, were evaluated. At the time of explantation, conceptuses were up to 10 ± 2 somite stage and were exposed to tamoxifen added directly to the culture medium at the beginning of the 24-h culture period. The results are presented in Table 14. In order to explore further the possible role of oestrogenicity, the interactive effects of tamoxifen with diethylstilboestrol and 17β-oestradiol were investigated. At 0.19 mM diethylstilboestrol or 0.1 mM 17β-oestradiol, in the presence of S9, a series of four tamoxifen concentrations ranging from 0.05 to 0.19 mM were added to the culture medium at the onset of the culture period. In all cases, the effect of tamoxifen appeared to be additive rather than antagonistic. Thus, the effect of tamoxifen was to exacerbate the embryotoxic/dysmorphogenic effects of both diethylstilboestrol and 17β-oestradiol.

Table 14. Embryotoxicity of tamoxifen to rat embryos

Tamoxifen concentration	Exogenous metabolic activation system (S9)	No. of embryos tested	Embryo-lethality (%)	Rotation defects (%)	Neural tube defects (%)	All other defects combined[a] (%)
0.19 mM	+	20	20	27.5	6.3	93.8
0.19 mM	–	17	64.7	43.4	0	100
0	+	261	1.9	8.4	0.7	6.1
0	–	111	2.6	5.4	0.9	2.7

From Beyer *et al.* (1989)
[a]Relatively inconsistent and so combined for analysis

In a preliminary experiment, groups of two or three rabbits were given 0.25, 0.5, 1.0, 2.0 or 4.0 mg/kg bw tamoxifen daily by gastric instillation from day 6 to day 18 of gestation (Esaki & Sakai, 1980). Abortions were observed in both rabbits treated with 4 mg/kg bw and one of the three rabbits treated with 2 mg/kg died. In a following experiment, groups of 10–14 rabbits were given 0.125, 0.5 or 2.0 mg/kg bw tamoxifen

daily by gastric instillation from day 6 to day 18 of gestation. In the groups receiving 0.5 and 2.0 mg/kg doses, body-weight gain in the dams was suppressed. Abortions occurred in one animal in each of the lower-dose groups and in five animals in the group receiving 2.0 mg/kg bw. Fetal death rates, defined as the total number of resorption sites for dead embryos divided by the total number of implantations, were 11.6% in the control group, 16.7% in the low-dose group, 39.4% in the mid-dose group and 32.7% in the high-dose group. No effect of tamoxifen on the body weight of live fetuses was observed. On external observation of the live-born fetuses, one exencephaly was observed in the control group and one brain hernia [*sic*] in the group receiving 0.125 mg/kg and one abdominal hernia with brain hernia [*sic*] in the group receiving 2.0 mg/kg. On internal examination, four anomalies were observed in the control group, one in the group receiving the lowest dose of tamoxifen and two in each of the other groups. There were 11 instances of minor malformations of the skull, sternum, caudal vertebrae or ribs in the control group, 12 in the group receiving 0.125 mg/kg tamoxifen and 9 in each of the other two groups.

Groups of four pregnant rabbits were given 2 mg/kg bw tamoxifen per day orally, beginning on gestational day 10 or 20, or vehicle only (Furr *et al.*, 1976). Administration of the drug from gestational day 10 resulted in considerable embryonic loss; only two rabbits gave birth and the average number of young born was 1.75 ± 1.2 compared with 5.8 ± 1.6 for vehicle-treated controls. Since the number of implantation sites was similar, a major effect of the drug was to induce fetal resorption. Administration of the drug from day 20 caused premature parturition and abortion: the length of gestation in the group receiving tamoxifen was 26.8 ± 1.3 days compared with 32.5 ± 0.3 days in controls, and the percentage of young born alive was 65% as compared to 96%. Both of these effects were associated with a significant reduction in plasma progesterone concentration. The differences in plasma progesterone concentrations were not due to differences in the numbers of corpora lutea in the different groups.

Pregnant guinea-pigs (n = 6) of the Hartley albino strain were given 2 mg/kg bw tamoxifen by subcutaneous injection for three or six days (Gulino *et al.*, 1984). Uteri of the fetuses (n = 10) were weighed 24 h after the last administration. Compared with control fetuses, a dose-related increase in uterine wet weight was observed, such that the uterine weight of fetuses of the dam that had received injections for a six-day period was 2.5 times that of controls. This uterotropic effect was associated with a significant increase in uterine DNA content. Tamoxifen also induced an increase in the size of the uterine stroma and myometrium in the fetus. Luminal epithelial cell height was increased by 67 ± 2% after six days of treatment of the dam and luminal epithelial cell number was increased by 160 ± 20%. Uterine epithelial downgrowths invading the stroma were observed in 26 ± 4% of tamoxifen-treated fetuses. In a subsequent study from the same laboratory, groups of 6–10 pregnant guinea-pigs of the Hartley albino strain were given 5 mg/kg bw tamoxifen per day by subcutaneous injection for 12 days or the vehicle alone (Pasqualini & Lecerf, 1986). The uterine epithelial cells of the fetal guinea-pigs were examined by transmission electron microscopy for ultrastructural changes. The fetuses were at 60–64 days of gestation at the time of necropsy. A moderate increase in the height of the fetal uterine epithelial cells and moderate effects on the Golgi system, the

rough endoplasmic reticulum and mitochondria were observed. No change in the number of microvilli or cell degeneration was apparent. When the same dose of tamoxifen was administered in combination with 1 mg/kg bw 17β-oestradiol per day for 12 days, the effects on mitochondria were greater than when either compound was given on its own, while the increase in number of microvilli apparent when 17β-oestradiol was given on its own was reduced to the basal level. Therefore, although tamoxifen has antioestrogenic properties, in this species it acted as an agonist in the uterus during fetal development.

Pregnant pigs (sows) were fed diets containing 0 or 10 mg/kg diet (ppm) tamoxifen from gestational day 30 until weaning (Yang *et al.*, 1995). No significant differences in sow body weight, litter size, live births per litter, piglet mortality, piglet sex ratio or piglet birth or weaning weight were observed between the groups. At the age of 21 days, female piglets exposed to tamoxifen *in utero* and during lactation had smaller ovaries and enlarged uteri compared with controls, but no histological abnormality. Corresponding male piglets had testes 15% lighter than those produced by sows fed the control diet; no consistent histological difference was observed between the groups. Subsequent breeding performance was not affected.

In two trials, approximately 500 eggs from single comb White Leghorn hens were injected on the day of set with either 100 μL tamoxifen (2 mg/μL) or vehicle (corn oil) into the albumen (Coco *et al.*, 1992). Based on phenotypic sexing, the sex ratio of male to female chicks was 76 : 24 for those exposed to tamoxifen compared with 47 : 53 in those treated with vehicle only. When a subset of these chicks was sexed at three weeks of age by identification of gonadal type at necropsy, these ratios were corrected to 46 : 54 and 52 : 48, respectively. In the second trial, the sex ratio based on phenotypic sexing was 62 : 38 for tamoxifen-exposed chicks and 45 : 55 for vehicle-exposed chicks. Gonadal sexing corrected these ratios to 44 : 56 and 45 : 55, respectively. Thus, the genital sexing errors were 27% in the first trial and 18% in the second for tamoxifen-exposed chicks, significantly higher than those treated with vehicle (2 and 0.6%, respectively). Therefore, phenotypic genital sexual differentiation was altered by administration of tamoxifen.

(*b*) *Effects on the dam of gestational exposure to tamoxifen*

O'Grady *et al.* (1974) investigated the effect of doses of tamoxifen known to delay implantation (0.1 mg/kg bw) or to inhibit implantation (0.2 mg/kg bw) on mitosis in the uterus. Tamoxifen was administered to rats on the morning of gestational day 2 and mitosis of the luminal and glandular epithelial and subepithelial stroma was assessed on days 2–5 of the pre-implantation period. The authors considered the delay in implantation induced by the lower dose of tamoxifen to be mediated by an observed delay in oestrogen-supported stromal mitosis. In a study from the same laboratory, Watson *et al.* (1975) showed that the lower dose of 0.1 mg/kg bw tamoxifen in rats delayed the increase in plasma 17β-oestradiol level by 20 h, a time interval identical to the delay in implantation. While the absolute concentration of 17β-oestradiol reached was lower than that in control animals, the rate of decline was slower and therefore the exposure of the uterus to an increased peak of 17β-oestradiol was more prolonged. A dose of 0.2 mg/kg bw, which prevented implantation, completely eliminated the increase in plasma 17β-

oestradiol level and caused a decrease in pituitary LH and a marked rise in plasma LH levels. Neither dose of tamoxifen affected levels of progesterone.

Pugh and Sumano (1979) investigated the effect of tamoxifen on the occurrence of the trophoblastic surface coat change which is associated with implantation in mouse embryos. Groups of 19–26 embryos were cultured in a collagen-containing culture system. Tamoxifen (2.8×10^{-10} M) totally prevented the surface coat change and implantation. When this level of tamoxifen was added to a culture containing 10^{-8} M 17β-oestradiol, the percentage of blastocysts which became attached to collagen decreased from 90.5% to 39.1%.

Gupta and Roy (1987) assessed the effects of tamoxifen on concentrations of cytosolic oestrogen receptor in different parts of the fallopian tube and uterus during ovum transplant in New Zealand albino rabbits. Half of the animals were given 0.03 mg/kg bw tamoxifen orally and the other half were not treated. Groups of animals were killed at normal oestrus stage and at 14, 24, 34, 48, 72, 144 or 168 h *post coitum*. In the tamoxifen-treated animals, the concentration of cytosol receptor was reduced in the ampulla and ampullary isthmic junction but not in the isthmus or uterine isthmic junction, compared with untreated animals. In the treated animals, the ampullary concentration of cytosol receptor increased during 14–34 h post coitum, and suddenly decreased at 48 h post coitum, whereas, from 72 h to 144 h post coitum, it increased gradually. The authors concluded that tamoxifen modulates tubal cytosolic oestrogen receptors during egg transport. [Details of the timing of tamoxifen administration are unclear.]

Treatment of pregnant pigs with 0.7 (n = 4) or 7.0 (n = 2) mg/kg bw tamoxifen per day did not affect the development of mammary structures or the ability to lactate at parturition (Lin & Buttle, 1991a). Sows were fed diets containing 0 or 10 mg/kg (ppm) tamoxifen from gestational day 30 until weaning (Yang *et al.*, 1995). No significant difference was observed between sows exposed to tamoxifen and unexposed sows in ovarian or uterine weights 21 days after lactation, although there was a trend towards ovarian atrophy and uterine enlargement in exposed sows. The ovaries of exposed sows contained predominantly small and degenerated follicles, whereas numerous large follicles were observed in the ovaries of control sows. The histological appearance of the uteri and ovaries of the treated sows was similar to controls.

(c) *Effects of exposure of neonates and immature animals to tamoxifen on development of the reproductive system*

Uterotropic effects of tamoxifen have been observed in mice, rats and guinea-pigs (Table 15). In rats, these effects are seen in neonatal rats only after short periods of treatment, prolonged treatment appearing to inhibit uterine development. In guinea-pigs, these effects are much stronger when the dose is administered to neonates rather than to immature animals (Gulino *et al.*, 1984).

Irisawa and Iguchi (1990) reported that neonatal treatment of mice with tamoxifen initially caused an increase in uterine weight and height of the uterine epithelium as well as an increase in the thickness of the vaginal epithelium. Tamoxifen treatment also

Table 15. Effect of administration of tamoxifen to neonatal or immature animals on subsequent development of the uterus

Species	Controls	Tamoxifen dose	Age (or weight) at administration	Age when outcome assessed	Effects on uterus		Reference
					Uterine weight relative to body weight	Other effects	
Rat	Vehicle	100 µg s.c.	Day 5	Day 9	Increased 1.5-fold	Epithelial hypertrophy	Clark *et al.* (1981)
	Vehicle	5 µg s.c.	Days 1, 3 and 5	ca. Day 120	All uteri were atrophic.	90% replacement of luminal lining and glands by squamous metaplasia	Chamness *et al.* (1979)
	Saline	0.01, 0.1, 1.0, 10.0 mg/kg bw, orally or s.c. for 3 days	35–45 g	Days 4 or 7	Increased with increasing dose; more marked change with oral dose; continuing treatment for a further 3 days reduced uterine weight		Wakeling *et al.* (1983)
	Untreated	10 µg/day for 5 days s.c.	Days 1–5	Day 26	Significantly decreased compared with controls[a]	Cross-sectional areas of uterine glands, luminal epithelium and endometrial stroma were substantially reduced. Little change in cell density in any cell population, except for a marked reduction in the cell density of the luminal epithelium.	Branham *et al.* (1988)
	Vehicle	100 or 200 µg for 5 days s.c.	Days 1–5	Day 60	Significantly decreased	Uterine lumen of the greater part of the horns was narrow and lined with cuboidal epithelial cells. In about half the rats given the larger doses, some parts of the luminal epithelium disappeared.	Ohta *et al.* (1989)
Mouse	Saline vehicle	2, 20 or 100 µg for 5 days s.c.	Days 1–5	Days 35 or 150	Decreased at day 35 in mice receiving the 2 or 20 µg dose; increased in mice receiving the 100 µg dose. At day 150, all treated groups had lower uterine weights than controls.	In 40%, 90% and 100% of the groups receiving 2, 20 or 100 µg, respectively, the circular musculature of the myometrium exhibited involution. The number of uterine glands per section was significantly reduced.	Iguchi *et al.* (1986)

Table 15 (contd)

Species	Controls	Tamoxifen dose	Age (or weight) at administration	Age when outcome assessed	Effects on uterus		Reference
					Uterine weight relative to body weight	Other effects	
	Saline	100 µg for 5 days s.c.	Days 1–5	Days 5, 10, 15, 20, 30 and 60	Increased at days 5, 15 and 20 — lighter at day 60; increased weight resulted from oedematous change in stromal tissue	Cell heights of luminal epithelium greater at days 5–30 than controls; shorter at day 60	Irisawa & Iguchi (1990)
Guinea-pig	Vehicle	0.6 µg/g bw for 3 or 6 days s.c.	Days 6 or 27	24 h after last injection	Increased with administration at day 6 but only weakly with administration at day 27[a]	Increased uterine DNA content, luminal epithelial cell height. Luminal epithelial cell number increased with administration at day 6 but only slightly with administration at day 27.	Gulino *et al.* (1984)
	Vehicle	100 µg for 2 or 12 days s.c.	Newborn (2–15 days old)	24 h after last injection	–	Electron microscopy showed moderate effects on epithelial cell height, microvilli number, Golgi system, rough endoplasmic reticulum and mitochondria. The longer period of dosage produced a 'very intensive effect' on mitochondria.	Pasqualini & Lecerf (1986)
		100 µg for 2 or 12 days s.c.	Day 2		Increased[a]	Cell heights increased 2–3 fold.	Pasqualini *et al.* (1986)
Pig	Vehicle	0.1 or 1 mg/kg bw per day for 7 days i.m.	6 weeks old	24 h after last injection	Dose-related increase in wet weight: 6, 12, 28 g	DNA expressed as mg/g tissue decreased ca. 50%; RNA and protein stable	Lin & Buttle (1991)

s.c., subcutaneous injection; i.m., intramuscular injection
[a]Absolute weight

caused poor formation of the uterine gland and development of the mesenchymal stroma. However, by 60 days of age, the weights of the uteri of the tamoxifen-treated mice were lower and the cell heights of the uterine luminal epithelium were smaller than those of controls. The number of uterine glands and the number of mice having a well developed tunica muscularis remained smaller than those in controls of the same age. Thus, neonatal treatment with tamoxifen resulted in a permanent alteration in both the uterine epithelial and stromal compartments. The critical period for induction of these uterine abnormalities was determined to be within three to seven days after birth.

The genital organs of female C57Bl/Tw mice given five daily injections of 100 μg tamoxifen from the day of birth were examined at 5, 10, 15, 20, 30 and 60 days of age (Irisawa & Iguchi, 1990). Adenosis-like lesions were found in the vaginae of 5–30-day-old mice exposed to tamoxifen. These lesions were not detected at 60 days of age. The number of polyovular follicles containing two to four oocytes per follicle markedly increased from 10 to 15 days of age in mice exposed to tamoxifen, and the incidence was twice as high as that in age-matched controls. Corpora lutea were found in the ovaries of 60-day-old controls, whereas no corpora lutea were found in age-matched mice exposed to tamoxifen. In order to determine the critical period of induction by tamoxifen of abnormalities of the female genital organs, other groups of mice were also given five daily injections of 100 μg tamoxifen or of vehicle alone starting on the day of birth, or 3, 5, 7 and 10 days after birth. Tamoxifen injections starting within five days of birth caused a high incidence of polyovular follicles in the ovary and of aplasia of tunica muscularis in the uterus. Atrophy of the uterine luminal epithelium was also induced when the treatment was started within seven days of birth. However, in mice given tamoxifen from day 10, uterine weights were greater than in those given tamoxifen from zero to seven days, and the authors concluded that the postnatal limit of the critical period for the female genital organs lies within seven days of birth.

In female C57Bl/Tw mice given five daily injections of 2, 20 or 100 μg tamoxifen starting on the day of birth, uterine hypoplasia, myometrial involution and suppression of uterine-gland genesis were found at 35 and 150 days of age (Iguchi *et al.*, 1986). About half of the mice killed when 150 days old were ovariectomized at 90 days. Vaginal hypoplasia and hypospadia were observed in most treated animals at 150 days of age. Vaginal adenosis was found in 40% of the 35-day-old mice treated with 2 μg tamoxifen, 70% of those treated with 20 μg and 100% of those treated with 100 μg, whereas none of the controls showed these lesions. Adenosis was not observed in any of the 150-day-old mice. In both age groups, the weight of ovaries was significantly lower following injections of 20 or 100 μg tamoxifen than in controls. At 150 days of age, hernia of the urinary bladder, located under or below the symphysis pubis, was found in 56% and 100% of non-ovariectomized mice in the mid- and high-dose groups. The urinary bladders of the group receiving the lowest dose and the control group were situated normally in the abdominal cavity. The authors suggested that the tamoxifen treatment caused a long-lasting suppression of the development of the pubic bone and ligament, resulting in looseness of the symphysis pubis which allowed the bladder to descend through the extended subpubic space.

In NMRI mice treated neonatally with tamoxifen, adenosis-like lesions observed in the vagina and cervix were similar to those induced by neonatal treatment with diethylstilboestrol, but differed in that the tamoxifen-induced lesions regressed with time (Forsberg, 1985).

Fifteen male NMRI/Tg mouse pups were given 20 μg tamoxifen by subcutaneous injection daily for three days, starting approximately 24 h after birth. Ten control pups were given injections of saline. At three months of age, all mice were housed with normal females for two weeks to investigate reproductive capacities. All tamoxifen-exposed males were sterile and did not impregnate any female during the mating period, whereas all control males were able to impregnate one or both females with which they were housed. When killed at eight months of age, the exposed male pups had multiple reproductive tract lesions including testicular hypoplasia, undescended testes, epididymal cysts and squamous metaplasia of the seminal vesicle; two of the exposed mice had squamous metaplasia of the median prostate (Taguchi, 1987).

In addition to inhibiting uterine development, administration of 5 μg tamoxifen by subcutaneous injection to newborn female Sprague-Dawley rats on days 1, 3 and 5 of age produced early vaginal opening and absent cycles (Chamness *et al.*, 1979). At four months of age, all ovaries were atrophic and the oviducts showed severe squamous metaplasia with abscess formation. Corporea lutea were uniformly absent except for a few that were found in one animal. The vaginal observations were stated to be unremarkable, except that one animal had a vaginal adenosis suggestive of that observed in women whose mothers received diethylstilboestrol during pregnancy.

Branham *et al.* (1993) gave 20–24-day-old rats five daily subcutaneous injections of tamoxifen (0.01–100 μg/rat/day) and then examined the uteri 2 h after the last dose. Uterine weights increased only slightly, whereas luminal epithelium hypertrophy increased 3-fold at 10 μg/rat and glandular epithelium hypertrophy increased 2-fold at the same dose. In contrast, oestrogens (17β-oestradiol, ethinyloestradiol, diethylstilboestrol) tested over the same dose range produced substantial increases in uterine weight and no glandular epithelium hypertrophy; the luminal epithelium response to 17β-oestradiol was similar to that of tamoxifen, while the other oestrogens required a 100-fold lower dose to elicit the same response. The greater hypertrophic response in luminal epithelium to both oestrogens and antioestrogens does not correlate with the oestrogen receptor content, which is greatest in the endometrial stroma and glandular epithelium in mouse, rat and macaque (Martin, 1980; Korach *et al.*, 1988; Tse & Goldfarb, 1988; McClellan *et al.*, 1984). It was suggested by Branham *et al.* (1993) that the high concentrations of tamoxifen (and other antioestrogens) required to elicit glandular epithelial hypertrophy is consistent with either a nonoestrogen-receptor-mediated response or a stromal mediation of epithelial responses.

Female rats of the T strain given single daily injections of 100 or 200 μg tamoxifen for five days beginning on the day of birth exhibited continued vaginal dioestrus when sacrificed on day 60, whereas vehicle-treated controls showed regular oestrus cycles (Ohta *et al.*, 1989). Ovaries from the tamoxifen-treated rats were polyfollicular without corpora lutea, whereas ovaries from controls contained both follicles and corpora lutea.

The vaginae of tamoxifen-treated rats ovariectomized on days 10 or 60 failed to respond to a three-day priming with 0.1 μg 17β-oestradiol, showing no oestrus smears. The authors concluded, therefore, that continued vaginal dioestrus in tamoxifen-treated rats may be accounted for by changed sensitivity of the ovary to gonadotropin and/or of the vagina to sex hormones. Lack of cyclicity and corpora lutea in the ovaries in rats was also reported by Irisawa and Iguchi (1990) and in NMRI mice by Forsberg (1985). Thus, tamoxifen appears to impair the female genital organs directly and/or indirectly through the hypothalamus.

In a study of the regulation of reproductive behaviour in rats (Ulibarri & Micevych, 1993), male rat pups were castrated, given sham surgeries or implanted with tamoxifen [dose not specified] within 3 h of birth. Female animals were implanted with a similar dose of tamoxifen or with empty capsules. All capsules were removed on postnatal day 10. Tamoxifen treatment was toxic to pups and only nine males and two females survived to surgery on day 90. Tamoxifen-treated females had large green ovarian growths and smaller growths throughout their peritonea. Tamoxifen-treated males had significantly smaller testes at adult surgery than sham-treated males. Tamoxifen-treated males and sham-operated males did not show appreciable lordosis behaviour. The authors noted that this finding differs from results with other oestrogen antagonists. The authors speculated that the antioestrogenic *trans*-isomer of tamoxifen may have been converted to the oestrogenic *cis*-isomer or that the *trans*-isomer of tamoxifen may have acted oestrogenically.

Pasqualini *et al.* (1986) reported that 100 μg tamoxifen administered subcutaneously to two-day-old guinea-pigs for two or 12 days substantially increased the weight and the protein and DNA content of the uterus and vagina; the weight increase was 3–4-fold in the group receiving prolonged treatment. Progesterone did not block this action. Tamoxifen caused a strong increase in the content of progesterone receptor in cytosol and nuclei of the cells of the vagina after prolonged treatment.

In immature, six-week-old pigs given 0.1 or 1.0 mg/kg bw tamoxifen per day for seven days, significant dose-related increases were seen in uterine weight, in the total content of uterine DNA, RNA and protein and in levels of progesterone receptor per milligram DNA (Lin & Buttle, 1991). The total duct area in the mammary glands increased about three-fold in the groups treated with either dose of tamoxifen, compared with vehicle-treated controls. The concentration of progesterone receptors in cytosol extracts of mammary tissue was very heterogeneous and independent of treatment with tamoxifen. Concurrent administration of tamoxifen with oestradiol benzoate induced significant increases in total uterine protein and in the concentration of progesterone receptors compared with treatment with oestradiol benzoate alone. However, concurrent administration partially inhibited the effect of oestradiol benzoate in stimulating an increase in mammary duct area. Thus, tamoxifen acts as an oestrogen agonist in the uterus of immature pigs, but as an antagonist in the mammary gland.

In rabbits, tamoxifen appears to act as an oestrogen antagonist when administered to sexually immature animals (Foster *et al.*, 1993). Female New Zealand white rabbits were given 10 mg/kg bw tamoxifen per day by subcutaneous injection from day 22 of age for

a total of 108 days, while control rabbits were given the vehicle only. Tamoxifen treatment impaired sexual development profoundly, as assessed by ovarian weight, diameter of growing follicles, diameter of anthral follicles and number of such follicles. There was profound suppression of pituitary gonadotropin levels in the group receiving tamoxifen, but no difference in the circulating levels of plasma 17β-oestradiol between control and tamoxifen-treated rabbits. These differences were associated with suppression of the developmental shift from smooth to rough gonadotropin-releasing hormone cell types in the hypothalamus.

(*d*) *Effects of exposure of neonates and immature animals to tamoxifen on bone development*

Neonatal treatment of female mice with tamoxifen induces permanent chondrification of the pubic bones and, in consequence, expansion of the pubic ligament, leading to urinary bladder hernia [exstrophy] with or without caecum hernia [dilatated] (see above) (Iguchi *et al.*, 1986). Subsequently, Iguchi *et al.* (1988) examined sequential changes in the pelvic bone of male and female C57Bl/TW mice following neonatal treatment with tamoxifen and sought to determine a critical period for the induction by tamoxifen of bladder hernia with or without caecum hernia. Mice were given five daily subcutaneous injections of 100 μg tamoxifen or of the vehicle alone, starting on the day of birth or at 3, 5, 7 or 10 days of age. Untreated mice showed completely calcified pelvic bone after 30 days of age, whereas, in age-matched tamoxifen-treated mice, the greater part of the junctional regions in the pelvis remained cartilaginous. Treatment with tamoxifen starting within five days of age caused bladder hernia with or without caecum hernia. The pubic ligament in tamoxifen-treated mice aged 30–540 days was markedly expanded as compared with age-matched controls. Permanent chondrification in the pelvis was found in all mice given tamoxifen starting before 10 days of age. Neonatal treatment of mice with similar doses of clomiphene and nafoxidine did not induce permanent chondrification of the pelvis, expansion of the pubic ligaments or hernia. Therefore, the authors conclude that tamoxifen has a specific effect on the pubic symphysis and on some junctional regions of the developing pelvis in mice when given neonatally. In a study from the same laboratory, Uesugi *et al.* (1993) carried out a morphometric analysis of the pelvis of C57Bl/TW mice given five daily injections of 100 μg tamoxifen or saline alone starting at birth, and killed at 120 days of age. The total areas of the pelvis, ilium, ischium, and pubis were significantly smaller in tamoxifen-treated mice than in the controls. Differences were also observed in the shape of the pelvis, reflected by the lengths of the ilium and pubis, and the widths of the ilium, pubis and ischium were smaller in tamoxifen-treated mice than in controls. The numbers of osteoblasts and osteoclasts per 200 μm trabecular surface length and per 10 000 μm^2 subperiosteal area of pubic bone section were smaller in tamoxifen-treated females than in control females. The authors concluded that neonatal administration of tamoxifen retards the growth of the ilium and pubis in mice by changing the activities of osteoclasts and osteoblasts and that the drug acts directly on the pubis of the neonatal mouse to inhibit its ossification.

Tamoxifen has been reported to have oestrogen agonist effects, namely effects on rat bone in immature or ovariectomized animals, and antagonistic effects in intact mature

animals. As the different effects may be due to differences in ovarian status, oestrogen levels and tamoxifen dose, Moon *et al.* (1991) investigated the effects of different doses of tamoxifen on the long bones of intact and ovariectomized female rats, with or without oestrogen treatment. Pellets containing 0, 1.5, 3, 5, 15 or 30 mg tamoxifen were implanted subcutaneously into intact, young adult female Sprague-Dawley rats. An untreated baseline control group was available. Tamoxifen treatment resulted in a dose-dependent decrease in overall growth rate and a dose-dependent increase in the periosteal bone formation rate in both ovariectomized and intact rats. It also prevented a decrease in cancellous bone balance after ovariectomy, although the highest dose of tamoxifen caused a small decrease in the cancellous bone balance in intact female rats. In order to determine whether tamoxifen alters the skeletal response of ovariectomized rats to oestrogen, rats were implanted with pellets containing 5 mg tamoxifen or 0.1 mg 17β-oestradiol, or both drugs or none. Tamoxifen treatment did not alter the effects of 17β-oestradiol on the periosteal bone formation rate in ovariectomized rats, but reduced the increase in cancellous bone balance to values similar to those in intact rats. The authors concluded that tamoxifen behaves as a partial oestrogen agonist on rat bone.

(*e*) *Effects of exposure of adult non-pregnant animals to tamoxifen on reproductive parameters*

In mature, virgin female Spague-Dawley rats which had been bilaterally ovariectomized, a single subcutaneous injection of 1 mg/kg bw tamoxifen increased uterine wet weight and blood flow (Marshall & Senior, 1987). The uterotrophic response lasted for between 35 and 42 days. Twenty-four hours after tamoxifen treatment, cytosolic oestrogen receptor levels were markedly reduced compared with control values and remained depressed until 21 days after injection, when they began to increase towards control values. The concentration of nuclear receptors reached a maximum at 27 h after injection, then declined to a plateau by 21 days after injection, but this was still above control values. Weights of uteri and vaginae in ovariectomized adult female C57Bl/Tw mice given three daily subcutaneous injections of 100 μg tamoxifen were significantly greater than those in ovariectomized untreated mice (Chou *et al.*, 1992). The uterine weight increase was associated with increased DNA and protein contents of the uterus, and in the cell heights of uterine luminal epithelial cells.

Gill-Sharma *et al.* (1993) investigated the effects of oral administration of doses of 40, 200 or 400 μg/kg bw per day tamoxifen for 60, 70, 80 or 90 days on the circulating concentrations of plasma hormones, tissue weights and reproductive performance of adult male Holtzman rats. Tamoxifen produced a dose-related reduction in testosterone concentration. Concentrations of FSH, prolactin and 17β-oestradiol were unaffected. The plasma level of LH was significantly lower after treatment with 200 and 400 μg/kg bw tamoxifen per day than in controls, but the 40 μg/kg dose had no effect. Tamoxifen given at 40 μg/kg bw per day for 90 days did not affect the weights of testes, seminal vesicles, epididymes, ventral prostate glands or the pituitary glands. At higher doses, the weights of the ventral prostate glands, seminal vesicles and epididymes were significantly lower than those of controls. Histological examination of testes from tamoxifen-exposed animals showed marked disorganization of the cytoarchitecture of the tubule and

obliteration of the lumen. The potency, fecundity, number of implantation sites, fertility index and litter size of male rats treated with tamoxifen were significantly lower than those of controls, and these effects were more marked for the highest dose than for the lowest dose. Reversibility studies were performed with 200 µg/kg bw tamoxifen per day. All the effects of tamoxifen on weights of seminal vesicles, ventral prostate glands, epididymes, concentrations of LH and testosterone in plasma, potency, fecundity, fertility index and litter size were reversed 90 days after drug withdrawal.

In female guinea-pigs given 10 mg tamoxifen by subcutaneous injection daily from days 11 to 14 of the cycle, uterine output of prostaglandin F_2 was not inhibited and luteal regression was not delayed (Poyser, 1993). The uteri were removed on day 15. In homogenates of endometrium and myometrium, prostaglandin synthesis was redirected in the uteri from tamoxifen-treated animals from prostaglandin I_2 to F_2, showing tamoxifen to be an oestrogen agonist in guinea-pigs.

Groups of 12 female mink were fed diets containing 0 or 10 mg/kg (ppm) tamoxifen for two months before breeding (Yang *et al.*, 1995). Treated females were placed with untreated males. All the exposed females rejected the advances of the males and often attacked them. The females were provided with an opportunity to mate at four-day intervals during a 20-day period, but none mated. Upon necropsy four days later, the uteri showed various degrees of pyometra in one or both horns. Histological examination of two females from each group showed ovarian follicular atrophy and degeneration, mild to severe uterine atrophy, pyometra and endometritis in tamoxifen-exposed animals, whereas the reproductive tracts from the control females appeared normal.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 16 for references and Appendices 1 and 2)

Mutation and allied effects

Reports available to the Working Group for a lack of tamoxifen activity in a *Salmonella typhimurium* mutation test and a rat dominant lethal assay were not suitable for evaluation.

Unscheduled DNA synthesis was induced in primary cultures of rat hepatocytes treated with tamoxifen, but only in cells isolated from animals that had been pretreated with three daily doses of tamoxifen itself (45 mg/kg orally).

Tamoxifen induced significant morphological transformation of Syrian hamster embryo cells.

Tamoxifen induced micronucleus formation in a number of in-vitro studies with MCL-5 cells, a genetically-engineered human lymphoblastoid cell line that expresses five human cytochrome P450s (CYP1A1, CYP1A2, CYP2A6, CYP2E1 and CYP3A4) and epoxide hydrolase. Kinetochore staining (Crofton-Sleigh *et al.*, 1993) showed that, at

Table 16. Genetic and related effects of tamoxifen

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–[c]	NT	1.86	White *et al.* (1992)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	0.037	Metzler & Schiffmann (1991)
MIH, Micronucleus test, human lymphoblastoid MCL-5 cells *in vitro*	+[d]	NT	0.74	White *et al.* (1992)
MIH, Micronucleus test, human lymphoblastoid MCL-5 cells *in vitro*	+[d]	NT	2	Crofton-Sleigh *et al.* (1993)
MIH, Micronucleus test, AHH-1 cells *in vitro*	–	NT	8	Crofton-Sleigh *et al.* (1993)
MIH, Micronucleus test, human lymphoblastoid MCL-5 cells *in vitro*	+[d]	NT	1	Phillips *et al.* (1994b)
MIH, Micronucleus test, human lymphoblastoid MCL-5 cells *in vitro*	+[d]	NT	0.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h1A1 cells expressing CYP1A1 *in vitro*	–	NT	1.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h1A2 cells expressing CYP1A2 *in vitro*	–	NT	1.5	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h2E1 cells expressing CYP2E1 *in vitro*	+	NT	0.125	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h3A4 cells expressing CYP3A4 *in vitro*	+	NT	0.25	Styles *et al.* (1994)
MIH, Micronucleus test, human lymphoblastoid h2D6 cells expressing CYP2D6 *in vitro*	(+)	NT	3	Styles *et al.* (1994)
CVA, Chromosomal aberrations, rat hepatocytes *in vivo*	+		0.3 po × 1	Sargent *et al.* (1994)
AVA, Aneuploidy, rat hepatocytes *in vivo*	+		0.3 po × 1	Sargent *et al.* (1994)
BID, Binding (covalent) to DNA, primary rat hepatocytes *in vitro* (^{32}P-postlabelling)	+	NT	0.37	Phillips *et al.* (1994a)
BID, Binding (covalent) to DNA, human lymphocytes *in vitro*	+	NT	10	Hemminki *et al.* (1995)
BID, Binding (covalent) to DNA, phenobarbital-treated rat liver microsomes *in vitro* (^{32}P-postlabelling)	NT	+	37	Pathak *et al.* (1995)

Table 16 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to DNA, calf thymus *in vitro*	NT	+	37	Moorthy *et al.* (1996)
BID, Binding (covalent) to DNA, primary rat hepatocytes *in vitro* (^{32}P-postlabelling)	+		0.37	Phillips *et al.* (1996a)
BID, Binding (covalent) to DNA, primary mouse hepatocytes *in vitro* (^{32}P-postlabelling)	+		3.7	Phillips *et al.* (1996a)
BID, Binding (covalent) to DNA, primary human hepatocytes *in vitro* (^{32}P-postlabelling)	–		3.7	Phillips *et al.* (1996a)
BID, Binding (covalent) to DNA, human endometrium *in vitro*	–		186	Carmichael *et al.* (1996)
BVD, Binding (covalent) to DNA, male and female SD rat liver *in vivo* (^{32}P-postlabelling)	+		20 ip × 1	Han & Liehr (1992)
BVD, Binding (covalent) to DNA, female SD rat kidney *in vivo* (^{32}P-postlabelling)	(+)[c]		20 ip × 6	Han & Liehr (1992)
BVD, Binding (covalent) to DNA, Syrian hamster liver *in vivo* (^{32}P-postlabelling)	+		5 ip × 1	Han & Liehr (1992)
BVD, Binding (covalent) to DNA, Fischer 344/N rat liver *in vivo* (^{32}P-postlabelling)	+[f]		5 po × 7	White *et al.* (1992)
BVD, Binding (covalent) to DNA, female Sprague-Dawley rat liver *in vivo* (^{32}P-postlabelling)	+		3.7 × 10	Montandon & Williams (1994)
BVD, Binding (covalent) to DNA, female Syrian hamster liver *in vivo* (^{32}P-postlabelling)	+		6.9 × 7	Montandon & Williams (1994)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver *in vivo*	+		22 po × 1	Phillips *et al.* (1994b)
BVD, Binding (covalent) to DNA, female ICR mice liver *in vivo* (^{32}P-postlabelling)	+		45 po × 1	Randerath *et al.* (1994a)
BVD, Binding (covalent) to DNA, female ICR mice liver, lung and kidney *in vivo* (^{32}P-postabelling)	+		45 ip × 4	Randerath *et al.* (1994b)
BVD, Binding (covalent) to DNA, female Sprague-Dawley rat liver *in vivo* (^{32}P-postlabelling)	+		45 ip × 4	Randerath *et al.* (1994b)
BVD, Binding (covalent) to DNA, female Wistar rat liver *in vivo* (^{32}P-postlabelling)	+		20 diet × 3 mo	Carthew *et al.* (1995a)

Table 16 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA, female Wistar, Fischer 344 and Lewis LEW rat liver *in vivo*	+		20 diet × 1 mo	Carthew *et al.* (1995b)
BVD, Binding (covalent) to DNA, female Sprague-Dawley rat liver *in vivo* (^{32}P-postlabelling)	+		20 ip × 7	Pathak *et al.* (1995)
BVD, Binding (covalent) to DNA, female ICR mice liver *in vivo* (^{32}P-postlabelling)	+		45 ip × 4	Moorthy *et al.* (1996)
BVD, Binding (covalent) to DNA, DBA/2 and C57Bl/6 mouse liver *in vivo* (^{32}P-postlabelling)	+		45 po × 4	White *et al.* (1992)
BVD, Binding (covalent) to DNA, female SD rat liver *in vivo* (^{32}P-postlabelling)	+		45 po × 7	Hard *et al.* (1993)
BHD, Binding (covalent) to DNA, female human breast cancer patient liver (^{32}P-postlabelling)	–		0.5 daily 2–39 mo	Martin *et al.* (1995)
BHD, Binding (covalent) to DNA, female human breast cancer patients, white blood cells (^{32}P-postlabelling)	–		0.36 daily 3–72 mo	Phillips *et al.* (1996b)
BHD, Binding (covalent) to DNA, female human breast cancer patients endometrium (^{32}P-postlabelling)	–		0.73 daily 3–108 mo	Carmichael *et al.* (1996)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day

[c] Positive when rats were pretreated orally with tamoxifen (45 mg/kg bw/day × 3); hepatocyte cultures established 24 h after the last dose and then exposed to tamoxifen

[d] Expressing native CYP1A1 and transfected CYPs 1A2, 2A6, 3A4 and 2E1 and microsomal epoxide hydrolase; cytochalasin B-arrested

[e] No adducts detected in uterine tissue

[f] No adduct formation in duodenum, kidney, lung, spleen, uterus or peripheral lymphocyte DNA at 45 mg/kg bw/day po × 7

every dose tested, an excess of micronuclei without kinetochores was induced, a result indicating that the micronuclei were probably induced by clastogenic events. Tamoxifen has also been tested in cell lines expressing each of the isozymes singly. The frequencies of micronucleus occurrence fell in the order MCL-5 > CYP2E1 > CYP3A4 > CYP2D6; micronuclei were not significantly induced in cells expressing only CYP1A1 or CYP1A2 (Styles *et al.*, 1994).

In hepatocytes isolated from female Sprague-Dawley rats given a single dose of tamoxifen by gavage, aneuploidy was observed in 70% of the cells, even at the lowest dose level of 0.3 mg/kg bw (Sargent *et al.*, 1994). Premature condensation (2–10%) and endoreduplication (5–10%) were also observed in the hepatocytes from tamoxifen-treated rats. Chromosome exchanges as well as breakages were observed and examination of the cells by electron microscopy revealed both unipolar and incompletely elongated spindles.

p53 Mutations

DNA from hepatocarcinomas of female Sprague-Dawley rats treated with tamoxifen was examined for the presence of mutations in exons 5–9 of the *p53* gene (Vancutsem *et al.*, 1994). Mutations were found in 12 out of 24 tumours; a total of 13 mutations were clustered at two specific sites, codons 231 (exon 6–7) and 294 (exon 8). Nine were A → G transitions at the second base of codon 231 (CAC), which resulted in a histidine to arginine substitution. Four tumours contained a silent C → T transition in the third base of codon 294 (TGC); one tumour contained both mutations.

DNA adducts

Several studies have demonstrated that tamoxifen forms DNA adducts when incubated with DNA in the presence of microsomal systems, in cells in culture and in rodents, but not in humans. In all of the studies, ^{32}P-postlabelling analysis was the detection method used.

Incubation of tamoxifen with DNA in the presence of rat or human liver microsomes resulted in the formation of DNA adducts, with the pattern of adducts depending on whether cumene hydroperoxide or nicotinamide-adenine dinucleotide phosphate (NADPH) was included in the incubation as the cofactor for peroxidase or cytochrome P450 enzyme-mediated reactions, respectively (Pathak & Bodell, 1994). With tamoxifen at a concentration of 100 μM, total adduct levels of 2.97 adducts per 10^8 nucleotides were obtained with human microsomes when NADPH was the cofactor and 11.1 adducts per 10^8 nucleotides when cumene hydroperoxide was used. With uninduced rat liver microsomes, the corresponding adduct levels were 0.86 and 6.5 adducts per 10^8 nucleotides, respectively.

In another study, somewhat lower levels of microsome-mediated DNA modification by tamoxifen at a concentration of 1 mM were achieved (Hemminki *et al.*, 1995). In the presence of NADP and glucose-6-phosphate, the adduct levels were 0.24 adducts per 10^8 nucleotides with rat microsomes and 0.043 adducts per 10^8 nucleotides with human microsomes.

Covalent binding of tamoxifen to DNA can also be mediated by horseradish peroxidase/hydrogen peroxide (Davies *et al.*, 1995).

In primary cultures of hepatocytes from uninduced female Fischer 344 rats treated with 1 μM or 10 μM [0.37 or 3.7 μg/mL] tamoxifen for 18 h, the levels of DNA adducts were 18.7 ± 7.5 and 115.1 ± 16.4 per 10^8 nucleotides, respectively (Phillips *et al.*, 1994a).

The metabolic activation of tamoxifen in primary cultures of rat, mouse and human hepatocytes has been compared (Phillips *et al.*, 1996a). DNA adducts were readily detected in rat hepatocytes treated with 1 μM or 10 μM tamoxifen (mean levels, 18.2 and 89.8 adducts per 10^8 nucleotides, respectively) and mouse hepatocytes (15.0 ± 1.8 adducts per 10^8 nucleotides) treated with 10 μM tamoxifen. However, DNA adducts were not detected in tamoxifen-treated human hepatocytes with a detection limit for the assay of 0.04 adducts per 10^8 nucleotides.

Low levels of DNA adducts (up to 0.16 adducts per 10^8 nucleotides) were detected in cultured human lymphocytes treated with 10–100 μg/mL tamoxifen but not at lower (2.5 and 5 μg/mL) doses (Hemminki *et al.*, 1995).

Han and Liehr (1992) demonstrated the formation of DNA adducts in the livers of Sprague-Dawley rats given 20 mg/kg bw tamoxifen by intraperitoneal injection daily for one, three or six days. Lower levels of adducts were detected in the kidneys of both sexes. No adducts were detected in uterine tissue. Adducts were also detected in the liver DNA of female hamsters treated with single intraperitoneal injections of 5 or 10 mg/kg bw tamoxifen.

Liver DNA adducts were also detected in Fischer 344 rats treated orally with tamoxifen (White *et al.*, 1992). The DNA adducts in rats reached levels of ≥ 100/10^8 nucleotides and were dose-related in the range 5–45 mg/kg bw tamoxifen per day for seven days. DNA adducts were not detected in the duodenum, kidney, lung, spleen, uterus or peripheral lymphocytes of treated rats.

C57Bl/6 and DBA/2 mice were given 45 mg/kg bw tamoxifen per day orally for four days. Tamoxifen–DNA adduct levels in the liver were 17 ± 5.7 and 28 ± 6.8 adducts per 10^8, respectively, compared with 116 ± 29 adducts per 10^8 in rats treated at the same dose. Dietary exposure of C57Bl/6 mice to tamoxifen (450 mg/kg of diet (ppm)) for 30 days resulted in about one third of the level of total adducts (69 ± 21 adducts per 10^8 nucleotides) seen in correspondingly treated Fischer rats (approximately 200 adducts per 10^8 nucleotides) (White *et al.*, 1992).

DNA adduct formation in rat liver was also demonstrated, but not quantified, in female Crl:CD(BR) rats given 45 mg/kg bw tamoxifen orally for seven days (Hard *et al.*, 1993). In a subsequent study (Montandon & Williams, 1994), Sprague-Dawley rats were given ten daily doses of 10, 30 and 90 μmol/kg (6, 17 and 51 mg/kg bw per day) tamoxifen and 2, 30 and 40.9 adducts per 10^8 nucleotides were measured [these values are the means of two different methods used by the authors to quantitate adducts]. Parallel studies in Syrian hamsters given 17, 53 and 160 μmol/kg bw/day (10, 30 and 90 mg/kg per day, respectively) for seven days produced adduct levels of 1.8, 3.5 and 13.6 adducts per 10^8 nucleotides [mean values of two quantitation methods used].

In three female Wistar (Alderley Park) rats given tamoxifen in the diet at 420 mg/kg (ppm) for three months, the level of liver adducts was 721 ± 420 per 10^8 nucleotides, which fell to 443 ± 38 per 10^8 nucleotides after a further three months on basal diet. In control rats, the background adduct levels ranged from 75 to 80 per 10^8 nucleotides (Carthew *et al.*, 1995a). In three other strains of rats (Fischer 344, Wistar LAC-P and Lewis LEW) fed diets containing 420 mg/kg diet (ppm) tamoxifen for up to 180 days, the adducts levels were about 500 per 10^8 nucleotides after 30 days, rising to about 3000 per 10^8 nucleotides after 180 days (Carthew *et al.*, 1995b).

In a pilot study, DNA adducts levels were compared in liver samples from seven women (37–91 years of age) who had been treated with tamoxifen (at either 20 mg or 2 × 20 mg/day) up to the time of sampling for 2–39 months, with those of seven women (42–74 years of age) not receiving tamoxifen (Martin *et al.*, 1995). Mean DNA adduct levels were 18–60 per 10^8 nucleotides in the tamoxifen-treated women and 38–80 per 10^8 nucleotides in the samples from women who had not received the drug. The adduct patterns observed did not show the characteristics of those seen with DNA from the livers of tamoxifen-treated rats.

In another small study, DNA from white blood cells of seven women receiving tamoxifen (serum concentrations, 34–178 ng/mL) as adjuvant therapy for breast cancer and of three women who served as healthy controls was analysed by ^{32}P-postlabelling (Phillips *et al.*, 1996b). With a limit of detection of 0.08 adducts per 10^8 nucleotides, adducts having the chromatographic properties of tamoxifen–DNA adducts formed in rodent liver cells were not detected in any of the individuals and no difference between the chromatograms of samples from the exposed and control women was observed.

Carmichael *et al.* (1996) analysed endometrial DNA from 18 patients receiving daily treatment with 10–40 mg tamoxifen for three months to nine years. Although all chromatograms of ^{32}P-labelled DNA digests displayed a background of low-level DNA damage, no evidence for the formation of tamoxifen–DNA adducts was found, and the adduct patterns were indistinguishable from those of endometrial DNA from unexposed controls.

The potential of tamoxifen to form DNA adducts in human endometrium *in vitro* has also been investigated using explant cultures incubated with 20–200 μM tamoxifen (Carmichael *et al.*, 1996). The viability of the metabolizing enzyme systems of the endometrial samples was demonstrated by the detection of expected DNA adducts after incubation with benzo[*a*]pyrene; however, no adducts were seen after incubation with tamoxifen, in spite of the generation of a metabolite with LC–MS analysis characteristics of α-hydroxytamoxifen.

4.4.3 *Metabolites of tamoxifen*

4-Hydroxytamoxifen caused morphological transformation of Syrian hamster embryo (SHE) cells. Treatment of SHE cells with 10 μM 4-hydroxytamoxifen for 48 h resulted in the emergence of immortalized cells in 3 out of 5 flasks; these cells were able to form fibrosarcomas when injected into thymus-aplastic mice (Metzler & Schiffmann, 1991).

Treatment of rat primary hepatocyte cultures with α-hydroxytamoxifen resulted in 15- to 63-fold higher levels of adducts (and the same pattern of adducts detected by ^{32}P-postlabelling) than with comparable concentrations of tamoxifen (Phillips *et al.*, 1994a; 1996a). A similar level of adducts (173.9 ± 4.1 adducts per 10^8 nucleotides) was seen in mouse hepatocytes treated with α-hydroxytamoxifen at a concentration of 1 μM. Treatment of human cells with α-hydroxytamoxifen resulted in DNA adduct formation at levels (1.94 ± 0.89 and 18.9 ± 17.9 adducts per 10^8 nucleotides at 1 μM and 10 μM, respectively) about 300-fold lower than those in rat hepatocytes. Concentrations of α-hydroxytamoxifen in the culture medium of cells incubated with tamoxifen were approximately 50-fold lower in experiments with human hepatocytes than in those with rat or mouse hepatocytes (Phillips *et al.*, 1996a).

The hypothesis has been advanced that tamoxifen is activated to a DNA-binding species through oxidation at the α-position of the ethyl group (Potter *et al.*, 1994). This proposal is supported by studies with the deuterated compound [*ethyl*-D_5]tamoxifen, which showed lower DNA-binding activity in rat liver *in vivo* than the non-deuterated compound (Phillips *et al.*, 1994b). It also had a lower ability to induce micronucleus formation in MCL-5 cells. The magnitude of the reduction in genotoxicity (two- to three-fold) correlated well with the comparative rates of metabolism of the deuterated and non-deuterated compounds in rat liver microsomal incubations (Jarman *et al.*, 1995). Thus, the reduced genotoxicity of [*ethyl*-D_5]tamoxifen is the result of its lower rate of oxidation due to the greater bond energy of the C–D bond compared with the C–H bond, implying that metabolic activation involves metabolism at the ethyl group of the molecule. This is supported by the observation of higher DNA-binding activity of α-hydroxytamoxifen in primary cultures of hepatocytes, in which DNA adducts were formed at between 25 and 49 times the level seen with equimolar concentrations of tamoxifen and which gave the same pattern of major adducts by ^{32}P-postlabelling (Phillips *et al.*, 1994a).

Studies on the nature of the DNA adducts formed from tamoxifen *in vivo* and *in vitro* provide evidence for this and other pathways of activation of tamoxifen to form DNA-binding products. The demonstration that tamoxifen–DNA adduct formation in mice was altered by pretreatment with pentachlorophenol, a sulfotransferase inhibitor, suggested the existence of two pathways of activation (Randerath *et al.*, 1994a,b). The levels of some of the major adducts were unaffected by pentachlorophenol, but levels of one major and several minor ones were increased 13–17-fold (Randerath *et al.*, 1994a). However, another sulfotransferase inhibitor, 2,6-dichloro-4-nitrophenol, did not enhance the levels of these adducts (Randerath *et al.*, 1994b). The metabolite 4-hydroxytamoxifen was found to give rise to adducts of the pentachlorophenol-inducible type (Randerath *et al.*, 1994b; Moorthy *et al.*, 1996).

α-Hydroxytamoxifen has low chemical reactivity towards DNA, but the synthetic compound α-acetoxytamoxifen is much more reactive. The DNA adducts formed with α-acetoxytamoxifen showed the same pattern on ^{32}P-postlabelling analysis as those from DNA treated with α-hydroxytamoxifen and those found in the DNA of rat hepatocytes treated with tamoxifen or of the livers of rats treated with tamoxifen *in vivo*. The major α-acetoxytamoxifen–DNA adduct was also isolated as a nucleoside and characterized by ultraviolet, mass and proton magnetic resonance spectroscopy and assigned the structure

(*E*)-α-(N^2-deoxyguanosinyl)tamoxifen, in which the α-ethyl position of tamoxifen is linked covalently to the exocyclic amino group of deoxyguanosine (Osborne *et al.*, 1996).

When incubated with rat liver microsomal fractions or with peroxidase enzymes in the presence of DNA, the metabolite 4-hydroxytamoxifen forms DNA adducts. The principal adduct formed in each case co-migrated on thin-layer chromatography with a minor adduct formed in the liver of tamoxifen-treated rats (Pathak *et al.*, 1995). In another study, two minor DNA adducts formed by rat microsomal activation of tamoxifen in the presence of DNA co-migrated in several thin-layer chromatography systems with the products of the reaction of (*Z*)-1,2-diphenyl-1-(4-hydroxyphenyl)but-1-ene ('metabolite E', see Figure 1) with DNA in the presence of silver(I) oxide (Pongracz *et al.*, 1995).

4.5 Mechanistic considerations

4.5.1 *Genotoxicity*

The ability of tamoxifen to form DNA adducts in rodent liver cells *in vivo* and *in vitro* suggests a genotoxic mechanism for carcinogenicity in rat liver. This hypothesis is supported by the dose–response relationship for both tumour formation (in both male and female rats) and adduct formation. Although adducts are also formed in mouse and hamster liver, the levels are lower and these species have not been tested adequately for carcinogenesis by tamoxifen. Tamoxifen also possesses tumour-promoting activity in rat liver. An unusual aspect of tamoxifen–DNA adduct formation is that, in the rat, little or no adduct formation occurs in other tissues following oral administration. Although some information is available on the nature of the reactive intermediates of tamoxifen, the enzymes involved in their formation have not been clearly identified. The possibility that tamoxifen is inactive in most assays for mutagenic activity (gene mutations) because of failures of in-vitro metabolizing systems and/or transport of the reactive intermediate to the target site has not been excluded.

Studies on human hepatocytes indicate that they have a much lower ability to activate tamoxifen to DNA-binding products than those of rodents. In addition, limited information indicates that tamoxifen–DNA adducts are not formed in either the liver or endometrium of women taking tamoxifen as adjuvant therapy for breast cancer. These findings suggest that humans are less susceptible to the genotoxicity of tamoxifen than rodents.

4.5.2 *Tamoxifen–oestrogen receptor interactions*

Tamoxifen acts as an oestrogen agonist and/or antagonist by binding directly to the oestrogen receptor. In some tissues, including breast, tamoxifen exerts antioestrogenic effects by binding to the oestrogen receptor with high affinity. The tamoxifen–oestrogen receptor complex is incapable of binding to DNA-responsive elements, and therefore fails to induce normal transcriptional activity (Pasqualini *et al.*, 1987). In other tissues, such as bone (Love *et al.*, 1992b), uterus (Jordan & Prestwich, 1977) and liver, tamoxifen acts as a partial agonist, possibly because cells from those tissues contain a

different array of DNA-binding sites, thereby leading to typical oestrogen-mediated changes in gene expression and subsequent biological effects on growth and differentiation.

Uterus

Several studies have demonstrated that tamoxifen exhibits agonistic properties in the uterus. Sustained occupancy of receptor–oestrogen complexes in the nucleus appears to be required for oestrogen-induced stimulation of DNA replication, an early event in cell division. For example, nuclear occupancy of the oestrogen receptor for 10–15 h is required to stimulate uterine cell division (Korach *et al.*, 1985). Assuming that tamoxifen–oestrogen receptor complexes are capable of interactions with oestrogen-response elements on DNA, then tamoxifen could enhance mitotic activity leading to biological changes which increase cancer risk. However, little is known about the sequence of events proceeding from interactions of DNA with the oestrogen receptor to biological changes.

Alternatively, tamoxifen could produce a partially antagonistic response by blocking oestrogen access to the receptor in tissues where there are appropriate DNA-responsive elements for the tamoxifen–oestrogen receptor complex. Because tamoxifen is not as potent as 17β-oestradiol, there is a diminished response in oestrogen-deficient tissue. This is likely to occur in tissues that are oestrogen receptor-positive and have oestrogen levels high enough to elicit a response, but low enough for tamoxifen to block oestrogen access to a sufficient number of receptors, thereby decreasing normal oestrogen responsiveness (Jordan & Prestwich, 1977). In any oestrogen receptor-positive tissues with very low levels of oestrogen, tamoxifen would act as an agonist, because there would be no tissue-specific oestrogen response to diminish.

Breast

Neoplasia of the breast appears to progress from a relatively differentiated state which is dependent on steroid hormones for growth to an undifferentiated state which is hormone-independent. This progression is reflected by the presence of receptors for oestrogen and progesterone in the dependent state, whereas few or no receptors are present in the independent state. Several effects of tamoxifen in mammary cells have been documented. Consistent with antioestrogenic activity, it decreased c-*myc* expression in the VHB_1 cell line derived from an infiltrating duct-cell carcinoma (Collyn-d'Hooghe *et al.*, 1991). A decrease in c-*myc* and c-*erb*B-2 mRNA levels has also been observed in breast tumour cells of patients treated with tamoxifen (Le Roy *et al.*, 1991). Another effect of tamoxifen is the induction of the autocrine secretion of transforming growth factor β (TGF-β) detected in human mammary ductal carcinoma biopsies. TGF-β is localized between and around stromal fibroblasts (Butta *et al.*, 1992). This was observed with both oestrogen receptor-positive and -negative tumours, suggesting an oestrogen-receptor independent mechanism of action for tamoxifen. TGF-β acts as a growth inhibitor in human breast cancer cells (Knabbe *et al.*, 1987), hence, its induction may be a

mechanism of growth inhibition by tamoxifen that takes account of its efficacy against both oestrogen receptor-positive and -negative tumours.

Liver

Increased incidence of liver adenomas, total nodular hyperplasia and hepatocellular carcinomas is observed in women following prolonged use of oral contraceptives, as used in the 1960s (IARC, 1987b). It is generally considered that ethinyloestradiol, the oestrogenic component of oral contraceptives, is responsible for their hepatocarcinogenic activity and that the hepatic oestrogen receptor is involved (Goldfarb, 1976; Mastri *et al.*, 1985).

Several studies have shown that the liver contains significant quantities of oestrogen receptors in hepatocytes, Kupffer cells and endothelial cells. Since tamoxifen possesses at least partial agonist activity in liver, tamoxifen-mediated increases in liver tumour incidences in rodents may reflect, in part, oestrogen-receptor-dependent responses. Occupancy of hepatic oestrogen receptors is associated with stimulation of growth factors involved in hepatocyte mitogenesis (Vickers & Lucier, 1991). The human hepatic oestrogen receptor appears to be quantitatively and qualitatively similar to that of rodents.

Thus, tamoxifen-mediated increases in liver tumour incidence in rodents may involve both DNA damage leading to increased numbers of initiated cells and oestrogen-receptor-mediated clonal expansion of those initiated cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Tamoxifen has been available since the early 1970s for the first-line treatment of metastatic breast cancer in postmenopausal women. Since the 1980s, it has become the therapy of choice for this condition. Tamoxifen has also become the adjuvant therapy of choice for treatment of postmenopausal, node-positive women with positive oestrogen-receptor or progesterone-receptor levels and, since the early 1990s, for the treatment of postmenopausal, node-negative women with positive oestrogen-receptor or progesterone-receptor levels. It is also widely used in treating postmenopausal receptor-negative women and premenopausal women with node-negative, receptor-positive disease. When used as adjuvant therapy, tamoxifen reduces the annual rates of both death from and recurrence of breast cancer by about 25%. Tamoxifen is commonly given at doses of 20 mg daily for periods of two to five years in the adjuvant setting, although doses of up to 40 mg daily have been used in the past. Several clinical trials are in progress to study the efficacy of tamoxifen in preventing breast cancer in healthy women believed to be at high risk of developing the disease.

Tamoxifen has been widely adopted as the first-line therapy of choice for hormone-responsive male breast cancer and is frequently used as adjuvant therapy for oestrogen receptor- or progesterone receptor-positive male breast cancer.

Tamoxifen is registered for use in nearly 100 countries and cumulative use since 1973 is estimated at 7 million patient-years.

5.2 Human carcinogenicity data

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

In the cohort study, based on follow-up of registered cases of breast cancer in the population-based Surveillance, Epidemiology and End Results (SEER) database in the United States, the only available data on therapy were those reported at the time of initial registration. Both groups of women with reported tamoxifen use and those with no such reported use had elevated rates of endometrial cancer compared with the rates expected from the SEER database as a whole. The risk was significantly greater for women with reported tamoxifen use. The similar stage distribution in the two groups suggests a lack of serious detection bias in this study. The absence of hysterectomies could not be confirmed in this study.

The case–control studies were based on the identification of a series of women with breast cancer who had subsequently been diagnosed with endometrial cancer, with tamoxifen exposure assessed in comparison with breast cancer patients who had not developed endometrial cancer. In two of these, case and control selection was based on the records of population-based cancer registries, and two used the same source as well as hospital-based cancer registries. For the Swedish study, although an increased risk of endometrial cancer for tamoxifen use was found, the only information on treatment was that recorded in the cancer registry. Further, the absence of hysterectomy in the control series could not be confirmed. For the remaining three case–control studies, more detailed data on treatment and on hysterectomies were obtained from medical records. In the studies in France and the Netherlands, a nonsignificant elevation of risk for endometrial cancer with use of tamoxifen was found, with a significant increase in risk with increasing duration of therapy in one. In the United States study, which reported on shorter duration of use, the point estimate of risk was less than unity.

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

In most of the randomized trials, small numbers of endometrial cancers were reported, and for many the data were not reported in a way that corrected for the greater survival time in most trials of the tamoxifen-treated patients compared to the control series. In two of the largest trials, however, there was a strong and statistically significant association between risk for endometrial cancer and use of tamoxifen. Although there may have been a tendency for publication bias and there is some possibility of a detection bias as a result of investigations in women with side-effects from tamoxifen, the magnitude of

the risk found in the two large trials is unlikely to be explained by such biases. Further, for the trials that reported deaths in women with endometrial cancer, to date there have been eight deaths in women allocated to tamoxifen treatment groups and one in those not allocated to tamoxifen.

One case series reported significantly more high-grade endometrial tumours in tamoxifen-treated cancer patients than in patients without prior tamoxifen use. However, in at least six other studies, this difference was not found.

The SEER-based cohort study found a significantly reduced risk for contralateral breast cancers in the tamoxifen-treated women, compared with women with no reported tamoxifen use. The case–control study from the United States also reported a significant reduction of risk for contralateral cancers of the breast following tamoxifen use.

Although for some small trials there seemed to be little difference in the numbers of contralateral breast cancers in tamoxifen-treated women compared with controls, for the large trials, there was a substantially and significantly reduced risk for contralateral breast cancer in tamoxifen-treated women compared with controls. Further, in an overview analysis of nearly all trials published in 1992 with data available to 1990, there was a significant reduction of 39% in contralateral breast cancers in the tamoxifen-treated groups.

For all other cancer sites, no significant excess of any cancer has been found in either the cohort study or the trials. Although an excess of gastrointestinal cancer was reported following a combined analysis of three Scandinavian trials, this has not yet been confirmed by other studies.

5.3 Animal carcinogenicity data

Tamoxifen was tested for carcinogenicity by oral administration in one study in mice and in eight studies in rats, only one of which was a formal two-year study. In mice, the incidences of benign ovarian and testicular tumours were increased. In rats, tamoxifen induced preneoplastic liver lesions and benign or malignant liver tumours. In one study, the incidence of some tumours in hormone-dependent tissues was decreased, including in the mammary gland, although reduced weight gain may have been a contributing factor. In two studies in which tamoxifen was tested by subcutaneous implantation in intact or ovariectomized female mice, it inhibited mammary tumour development in both.

In mice, tamoxifen was reported to inhibit 3-methylcholanthrene-induced cervical cancer and virus-induced leukaemia. In several studies in both male and female rats, tamoxifen enhanced the hepatocarcinogenicity of previously administered *N*-nitrosodiethylamine. In one study in rats, tamoxifen enhanced the development of *N*-nitrosodiethylamine-induced kidney tumours. In a number of studies in rats, tamoxifen inhibited 7,12-dimethylbenz[*a*]anthracene-induced mammary tumour development. In two studies in hamsters, tamoxifen inhibited hormonal carcinogenesis induced by 17β-oestradiol in the kidney and zeranol in the liver.

5.4 Other relevant data

Orally administered tamoxifen is well absorbed and maximum plasma levels are reached in about 5 h. Steady-state concentrations of tamoxifen in humans are reached in 3–4 weeks and those of the primary metabolite, *N*-desmethyltamoxifen, in about eight weeks. Tissue concentrations tend to be higher than plasma concentrations. Metabolism involves phenyl hydroxylation, alkyl hydroxylation, demethylation and *N*-oxide formation. Metabolism results in more products in man and rats than in mice. Much higher oral doses of tamoxifen are required for rats or mice to achieve plasma concentrations similar to human levels.

Tamoxifen is an antioestrogen with complex pharmacology encompassing variable species-, tissue-, cell-, gene-, age- and duration of administration-specific effects from oestrogen-like agonist actions to complete blockade of oestrogen action. This complexity is consistent with the various, and sometimes paradoxical, effects that have been associated with tamoxifen administration in animals and humans

The most frequent side-effects of tamoxifen administration are hot flushes and vaginal discharge. Tamoxifen has effects on the human uterus, inducing atrophy, hyperplasia and, less frequently, polyps. Randomized placebo-controlled trials revealed a slight increase of thromboembolic events, but also a protective effect regarding myocardial diseases, according to hospital admission rates and deaths. Tamoxifen administration has been shown to decrease blood total cholesterol and low-density lipoprotein-cholesterol concentrations in a number of studies. Several preliminary trials have suggested mildly positive effects of tamoxifen in preserving bone mineral density in postmenopausal women, but much longer follow-up is required to confirm this potentially beneficial effect.

The acute toxicity of tamoxifen in experimental animals is low. In repeated-dose studies in rats, tamoxifen induced hypertrophy, but not cell proliferation, in the endometrial epithelium; endometrial hyperplasia was, however, reported in mice. Furthermore squamous metaplasia and atrophy of the uterine epithelium was observed in chronic studies in rats. Induction of cytochrome P450s and preneoplastic lesions have been detected in the livers of rats.

Ocular toxicity, including lipidosis of the retina and cornea and increased incidence of cataracts, was reported in studies in rats of chronic exposure to tamoxifen.

In the presence of human, mouse, rat and hamster microsomes, tamoxifen binds covalently to protein.

Tamoxifen has oestrogenic effects on human fetal genital tracts grown in athymic mice. In rats, doses above 2 mg/kg body weight produce irregular ossification of ribs in the fetus, which is thought to be secondary to reduction of the size of the uterus of the dam. No effects on the fetus have been reported in rabbits, marmosets or cynomolgus monkeys.

There is no direct evidence that tamoxifen is active in tests for gene mutation. Evidence for the genotoxic potential of tamoxifen is supported by data obtained on DNA adduct formation in rodent liver cells *in vitro* and *in vivo*, and in rodent and human liver

microsomal systems; on unscheduled DNA synthesis in rat hepatocytes *in vitro*; and on the induction of clastogenic events both *in vitro*, in genetically-engineered human cells, and *in vivo* in rat liver.

There is evidence from ^{32}P-postlabelling studies that three metabolites, α-hydroxytamoxifen, 4-hydroxytamoxifen and (*Z*)-1,2-diphenyl-1-(4-hydroxyphenyl)but-1-ene (metabolite E) can be further metabolized to products that react with DNA. The major DNA adduct formed in rodent liver cells has been identified as (*E*)-α-(*N*^{2}-deoxyguanosinyl)tamoxifen. Human hepatocytes do not form detectable DNA adducts when treated *in vitro* with tamoxifen; they form 300-fold lower levels of adducts than rat and mouse hepatocytes when treated with α-hydroxytamoxifen.

Preliminary studies indicate that tamoxifen does not give rise to detectable levels of DNA adducts in human liver *in vivo* or in human endometrium *in vitro* and *in vivo*.

Mechanistic considerations

Tamoxifen increases liver tumour incidence in rats, which may involve both DNA damage leading to increased numbers of initiated cells and oestrogen receptor-mediated clonal expansion of those initiated cells.

The available evidence suggests that tamoxifen is carcinogenic in rat liver by a genotoxic mechanism. Preliminary information from studies of human tissues suggests that humans are less susceptible to the genotoxicity of tamoxifen. Tamoxifen also possesses tumour-promoting activity in the rat liver.

Several studies have shown that the liver contains significant quantities of oestrogen receptor in hepatocytes, Kupffer cells and endothelial cells.

Tamoxifen acts as an oestrogen agonist and/or antagonist by binding directly to the oestrogen receptor. In some tissues, such as breast, tamoxifen exhibits antioestrogenic properties by binding to the oestrogen receptor with high affinity. The tamoxifen–oestrogen receptor complex is incapable of binding to DNA-responsive elements. Thus, oestrogen receptor binding does not result in normal transcriptional activity. In other tissues, such as bone and liver, tamoxifen acts as a partial agonist, possibly because cells from those tissues contain a different array of DNA binding sites, thereby leading to typical oestrogen-mediated changes in gene expression and subsequent biological effects on growth and differentiation. Therefore, tissue-specific effects of tamoxifen–oestrogen receptor on gene expression may be involved in the ability of tamoxifen to increase or decrease tumour risk.

5.5 Evaluation[1,2]

There is *sufficient evidence* in humans for the carcinogenicity of tamoxifen in increasing the risk for endometrial cancer and there is conclusive evidence that tamo-

---

[1]Dr Cuzick dissociated himself from the evaluation process because he considered that the range of evaluation statements available within the framework of the *Monographs* was not suitable for this agent.
[2]For definition of the italicized terms, see Preamble, pp. 22–25.

xifen reduces the risk for contralateral breast cancer in women with a previous diagnosis of breast cancer.

There is *inadequate evidence* in humans for the carcinogenicity of tamoxifen in other organs.

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

Overall evaluation

Tamoxifen is *carcinogenic to humans (Group 1)* and there is conclusive evidence that tamoxifen reduces the risk of contralateral breast cancer.

6. References

Adam, H.K., Patterson, J.S. & Kemp, J.V. (1980) Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. *Cancer Treatment Rep.*, **64**, 761–764

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

Akhtar, S.S., Allan, S.G., Rodger, A., Chetty, U.D.I., Smyth, J.F. & Leonard, R.C.F. (1991) A 10-year experience of tamoxifen as primary treatment of breast cancer in 100 elderly and frail patients. *Eur. J. surg. Oncol.*, **17**, 30–35

Altaras, M.M., Aviram, R.,. Cohen, I., Cordoba, M., Yarkoni, S. & Beyth, Y. (1993) Role of prolonged stimulation of tamoxifen therapy in the etiology of endometrial sarcomas. *Gynecol. Oncol.*, **49**, 255–258

Andersson, M., Storm, H.H. & Mouridsen, H.T. (1991) Incidence of new primary cancers after adjuvant tamoxifen therapy and radiotherapy for early breast cancer. *J. natl Cancer Inst.*, **83**, 1013–1017

Andersson, M., Storm, H.H. & Mouridsen, H.T. (1992) Carcinogenic effects of adjuvant tamoxifen treatment and radiotherapy for early breast cancer. *Acta oncol.*, **31**, 259–263

Anker, G., Lønning, P.E., Ueland, P.M., Refsum, H. & Lien, E.A. (1995) Plasma levels of the antherogenic amino acid homocysteine in post-menopausal women treated with tamoxifen. *Int. J. Cancer*, **60**, 365–368

Ashford, A.R., Donev, I., Tiwari, R.P. & Garrett, T.J. (1988) Reversible ocular toxicity related to tamoxifen therapy. *Cancer*, **61**, 33–35

Atlante, G., Pozzi, M., Vincenzoni, C. & Vocaturo, G. (1990) Four case reports presenting new acquisitions on the association between breast and endometrial carcinoma. *Gynecol.Oncol.*, **37**, 378–380

Bagdade, J.D., Wolter, J., Subbaiah, P.V. & Ryan, W. (1990) Effects of tamoxifen treatment on plasma lipids and lipoprotein lipid composition. *J. clin. Endocrinol. Metab.*, **70**, 1132–1135

Baltuch, G., Shenouda, G., Langleben, A. & Villemure, J.-G. (1993) High dose tamoxifen in the treatment of recurrent high grade glioma: a report of clinical stabilization and tumour regression. *Can. J. neurol. Sci.*, **20**, 168–170

Barakat, R.R. & Hoskins, W.J. (1994) Current management of endometrial cancer. *Contemporary Ob./Gyn.*, **39**, 13–34

Barakat, R.R., Wong, G., Curtin, J.P., Vlamis, V. & Hoskins, W.J. (1994) Tamoxifen use in breast cancer patients who subsequently develop corpus cancer is not associated with a higher incidence of adverse histologic features. *Gynecol. Oncol.*, **55**, 164–168

Bardi, M., Arnoldi, E., Pizzocchero, G., Pezzica, E., Mattioni, D. & Perotti, M. (1994) Endometrioid carcinoma in pelvic endometriosis in a postmenopausal woman with tamoxifen adjuvant therapy for breast cancer. A case report. *Eur. J. Gynaec. Oncol.*, **15**, 393–395

Bates, T., Riley, D.L., Houghton, J., Fallowfield, L. & Baum, M. (on behalf of the Elderly Breast Cancer Working Party) (1991) Breast Cancer in elderly women: a Cancer Research Campaign trial comparing treatment with tamoxifen and optimal surgery with tamoxifen alone. *Br. J. Surg.*, **78**, 591–594

Baum, M., Houghton, J. & Riley, D. (1992) Results of the Cancer Research Campaign Adjuvant Trial for Perioperative Cyclophosphamide and Long-Term Tamoxifen in Early Breast Cancer reported at the tenth year of follow-up. *Acta oncol.*, **31**, 251–257

Bedford, G.R. & Richardson, D.N. (1966) Preparation and identification of *cis* and *trans* isomers of a substituted triarylethylene. *Nature*, **212**, 733–734

Beer, T.W., Buchanan, R. & Buckley, C.H. (1995) Uterine stromal sarcoma following tamoxifen treatment (Letter to the Editor). *J. clin. Pathol.*, **48**, 596

Beggs, W.H. (1993) Anti-candida activity of the anti-cancer drug tamoxifen. *Res. Comm. chem. Pathol. Pharmacol.*, **80**, 125–128

Bertelli, G., Pronzato, P., Amoroso, D., Cusimano, M.P., Conte, P.F., Montagna, G., Bertolini, S. & Rosso, F. (1988) Adjuvant tamoxifen in primary breast cancer: influence on plasma lipids and antithrombin III levels. *Breast Cancer Res. Treat.*, **12**, 307–310

Berthou, F. & Dréano, Y. (1993) High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites. *J. Chromatogr.*, **616**, 117–127

Beyer, B.K., Stark, K.L., Fantel, A.G. & Juchau, M.R. (1989) Biotransformation, estrogenicity and steroid structure as determinants of dysmorphogenic and generalized embryotoxic effects of steroidal and nonsteroidal estrogens. *Toxicol. appl. Pharmacol.*, **98**, 113–127

Boccardo, F., Bruzzi, P., Rubagotti, A., Nicolò, G. & Rosso, R. (1981) Estrogen-like action of tamoxifen on vaginal epithelium in breast cancer patients. *Oncology*, **38**, 281–285

Boccardo, F., Amoroso, D., Rubagotti, A., Capellini, M., Pacini, P., Castagnetta, L., Traina, A., Farris, A., Iacobelli, S., Mustacchi, G., Nenci, I., Piffanelli, A., Sismondi, P., De Sanctis, C., Mesiti, M., Gallo, L., Villa, E. & Schieppati, G. (1994a) Prolonged tamoxifen treatment of early breast cancer: the experience of the Italian Cooperative Group for chemohormonal therapy of early breast cancer. In: Jordan, V.C., ed., *Long-Term Tamoxifen Treatment for Breast Cancer*, Madison, WI, The University of Wisconsin Press

Boccardo, F., Rubagotti, A., Perrotta, A., Amoroso, D., Balestrero, M., De Matteis, A., Zola, P., Sismondi, P., Francini, G., Petrioli, R., Sassi, M., Pacini, P. & Galligioni, E. (1994b) Ovarian ablation versus goserelin with or without tamoxifen in pre-perimenopausal patients with advanced breast cancer: results of a multicentric Italian study. *Ann. Oncol.*, **5**, 337–342

Bocklage, T., Lee, K.R. & Belinson, J.L. (1992) Uterine Müllerian adenosarcoma following adenomyoma in a woman on tamoxifen therapy. *Gynecol. Oncol.*, **44**, 104–109

Branham, W.S., Zehr, D.R., Chen, J.J. & Sheehan, D.M. (1988) Alterations in developing rat uterine cell populations after neonatal exposure to estrogens and antiestrogens. *Teratology*, **38**, 271–279

Branham, W.S., Zehr, D.R. & Sheehan, D.M. (1993) Differential sensitivity of rat uterine growth and epithelium hypertrophy to estrogens and antiestrogens. *Proc. Soc. exp. Biol. Med.*, **203**, 297–303

Breast Cancer Chemotherapy Consensus Conference (1985) Adjuvant chemotherapy for breast cancer. *J. Am. med. Assoc.*, **254**, 3461–3463

Breast Cancer Trials Committee (1987) Adjuvant tamoxifen in the management of operable breast cancer: the Scottish Trial. *Lancet*, **ii**, 171–175

British Medical Association/Royal Pharmaceutical Society of Great Britain (1994) *British National Formulary Number 27 (March 1994)*, London, p. 329

British Pharmacopoeial Commission (1993) *British Pharmacopoeia* 1993, Vols. I & II, London, Her Majesty's Stationery Office, pp. 650–651, 1119–1120

Brooks, M.D., Ebbs, S.R., Colletta, A.A. & Baum, M. (1992) Desmoid tumours treated with triphenylethylenes. *Eur. J. Cancer*, **28A**, 1014–1018

Bruning, P.F., Bonfrer, J.M.G., Hart, A.A.M., de Jong-Bakker, M., Linders, D., van Loon, J. & Nooyen, W.J. (1988) Tamoxifen, serum lipoproteins and cardiovascular risk. *Br. J. Cancer*, **58**, 497–499

Buchanan, R.B., Blamey, R.W., Durrant, K.R., Howell, A., Paterson, A.G., Preece, P.E., Smith, D.C., Williams, C.J. & Wilson, R.G. (1986) A randomized comparison of tamoxifen with surgical oophorectomy in premenopausal patients with advanced breast cancer. *J. clin. Oncol.*, **4**, 1326–1330

Buckley, C.H. (1990) Tamoxifen and endometriosis. Case report. *Br. J. Obstet. Gynaecol.*, **97**, 645–646

Buckley, M.M.-T. & Goa, K.L. (1989) Tamoxifen: a reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs*, **37**, 451–490

Budavari, S., ed. (1995) *The Merck Index*, 12th Ed., Rahway, NJ, Merck & Co.

Burke, P., Fasciano, F., Frigerio, A., Temporelli, A. & Fortunati, N. (1987) Ultrasonography study of postmenopausal uterus in patients treated with tamoxifen. *Min. gin.*, **39**, 453–457 (in Italian)

Butta, A., MacLennan, K., Flanders, K.C., Sacks, N.P.M., Smith, I., McKinna, A., Dowsett, M., Wakefield, L. M., Sporn, M.B., Baum, M. & Colletta, A.A. (1992) Induction of transforming growth factor β1 in human breast cancer *in vivo* following tamoxifen treatment. *Cancer Res.*, **52**, 4261–4264

Cano, A., Matallin, P., Legua, V., Tortajada, M. & Bonilla-Musoles, F. (1989) Tamoxifen and endometriosis (Letter to the Editor). *Lancet*, **i**, 376

Carmichael, P.L., Ugwumadu, A.H.N., Neven, P., Hewer, A.J., Poon, G.K. & Phillips, D.H. (1996) Lack of genotoxicity of tamoxifen in human endometrium. *Cancer Res.*, **56**, 1475–1479

Carthew, P., Martin, E.A., White, I.N.H., De Matteis, F., Edwards, R.E., Dorman, B.M., Heydon, R.T. & Smith, L.L. (1995a) Tamoxifen induces short-term cumulative DNA damage and liver tumors in rats: promotion by phenobarbital. *Cancer Res.*, **55**, 544–547

Carthew, P., Rich, K.J., Martin, E.A., De Matteis, F., Lim, C.-K., Manson, M.M., Festing, M.F.W., White, I.N.H. & Smith, L.L. (1995b) DNA damage as assessed by ^{32}P-postlabelling in three rat strains exposed to dietary tamoxifen: the relationship between cell proliferation and liver tumour formation. *Carcinogenesis*, **16**, 1299–1304

Castiglione, M., Gelber, R.D. & Goldhirsch, A. (1990) Adjuvant systemic therapy for breast cancer in the elderly: competing causes of mortality. *J. clin. Oncol.*, **8**, 519–526

Chamart, S., Hanocq, M., Helson, M., Devleeschouwer, N. & Leclercq, G. (1989) Determination of 2-methyl derivatives of tamoxifen in cell culture medium using high-performance liquid chromatography and electrochemical detection. *J. Chromatogr.*, **496**, 365–375

Chamness, G.C., Bannayan, G.A., Landry, L.A., Jr, Sheridan, P.J. & McGuire, W.L. (1979) Abnormal reproductive development in rats after neonatally administered antioestrogen (tamoxifen). *Biol. Reprod.*, **21**, 1087–1090

Champion, P.E., Nabholtz, M., Jenkins, H., MacLean, G., Allen, S. & Lees, A. (1991) Is tamoxifen increasing the risk of uterine malignancy in women treated for breast cancer ? (Abstract). *Breast Cancer Res. Treat.*, **19**, 195

Chang, T., Gonder, J.R. & Ventresca, M.R. (1992) A case-report. Low-dose tamoxifen retinopathy. Can. J. Ophthalmol., 27, 148–149

Ching, C.K., Smith, P.G. & Long, R.G. (1992) Tamoxifen-associated hepatocellular damage and agranulocytosis (Letter to the Editor). *The Lancet*, **339**, 940

Chou, Y.-C., Iguchi, T. & Bern, H.A. (1992) Effects of antiestrogens on adult and neonatal mouse reproductive organs. *Reprod. Toxicol.*, **6**, 439–446

Clark, J.H., Guthrie, S.C. & McCormack, S.A. (1981) Neonatal stimulation of the uterus by clomiphene, tamoxifen and nafoxidine: relationship to the development of reproductive tract abnormalities. *Adv. exp. Med. Biol.*, **138**, 87–98

Clarke, M.R. (1993) Uterine malignant mixed Müllerian tumor in a patient on long-term tamoxifen therapy for breast cancer. *Gynecol. Oncol.*, **51**, 411–415

Coço, C.M., Hargis, B.M. & Hargis, P.S. (1992) Effect of in ovo 17β-estradiol or tamoxifen administration on sexual differentiation of the external genitalia. *Poultry Sci.*, **71**, 1947–1951

Coe, J.E., Ishak, K.G., Ward, J.M. & Ross, M.J. (1992) Tamoxifen prevents induction of hepatic neoplasia by zeranol, an estrogenic food contaminant. *Proc. natl Acad. Sci. USA*, **89**, 1085–1089

Cohen, I., Altaras, M.M., Lew, S., Tepper, R., Beyth, Y. & Ben-Baruch, G. (1994) Case Report. Ovarian endometrioid carcinoma and endometriosis developing in a postmenopausal breast cancer patient during tamoxifen therapy: a case report and review of the literature. *Gynecol. Oncol.*, **55**, 443–447

Cole, M.P., Jones, C.T.A. & Todd, I.D.H. (1971) A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI 46 474. *Br. J. Cancer*, **25**, 270–275

Collyn-d'Hooghe, M., Vandewalle, B., Hornez, L., Lantoine, D., Revillion, F., Lefebvre, J. & Kerckaert, J. P. (1991) c-*myc* Overexpression, c-*mil*, c-*myb* expression in a breast tumor cell line. Effects of estrogen and antiestrogen. *Anticancer Res.*, **11**, 2175–2180

Cook, L.S., Weiss, N.S., Schwartz, S.M., White, E., McKnight, B., Moore, D.E. & Daling, J.R. (1995) Population-based study of tamoxifen therapy and subsequent ovarian, endometrial, and breast cancers. *J. natl Cancer Inst.*, **87**, 1359–1364

Corley, D., Rowe, J., Curtis, M.T., Hogan, W.M., Noumoff, J.S. & LiVolsi, V.A. (1992) Post-menopausal bleeding from unusual endometrial polyps in women on chronic tamoxifen therapy. *Obstet. Gynecol.*, **79**, 111–116

Cortez Pinto, H., Baptista, A., Ermelinda Camilo, M., Bruno de Costa, E., Valente, A. & Carneiro de Moura, M. (1995) Tamoxifen-associated steatohepatitis — report of three cases. *J. Hepatol.*, **23**, 95–97

Costa, A., Sacchini, V., Boranni, B., Luini, A., Boyle, P., Rofmensz, N., Veronesi, U. & the Italian Tamoxifen Trial Units (1996) Breast cancer chemoprevention with tamoxifen. The Italian trial (Abstract). *Breast Cancer Res. Treat.*, **37**, 348

Council of Europe (1995) *European Pharmacopoeia*, 2nd Ed., Part II, 19th fasc., Sainte-Ruffine, France, Maisonneuve S.A., pp. 1046-1–1046-4

Crofton-Sleigh, C., Doherty, A., Ellard, S., Parry, E.M. & Venitt, S. (1993) Micronucleus assays using cytochalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase. *Mutagenesis*, **8**, 363–372

Cross, S.S. & Ismail, S.M. (1990) Endometrial hyperplasia in an oophorectomized women receiving tamoxifen therapy. Case report. *Br. J. Obstet. Gynaecol.*, **97**, 190–192

Cullins, S.L., Pridjian, G. & Sutherland, C.M. (1994) Goldenhar's syndrome associated with tamoxifen given to the mother during gestation. *J. Am. med. Assoc.*, **271**, 1905–1906

Cummings, F.J., Gray, R., Tormey, D.C., Davis, T.E., Volk, H., Harris, J., Falkson, G. & Bennett, J.M. (1993) Adjuvant tamoxifen versus placebo in elderly women with node-positive breast cancer: long-term follow-up and causes of death. *J. clin. Oncol.*, **11**, 29–35

Cunha, G.R., Taguchi, O., Namikawa, R., Nishizuka, Y. & Robboy, S.J. (1987) Teratogenic effects of clomiphene, tamoxifen, and diethylstilbestrol on the developing human female genital tract. *Hum. Pathol.*, **18**, 1132–1143

Curtis, R.E., Boice, J.D., Jr, Shriner, D.A., Hankey, B.F. & Fraumeni, J.F., Jr (1996) Second cancers after adjuvant tamoxifen therapy for breast cancer. Brief communication. *J. natl Cancer Inst.* (in press)

Cuzick, J., Allen, D., Baum, M., Barrett, J., Clark, G., Kakkar, V., Melissari, E., Moniz, C., Moore, J., Parsons, V., Pemberton, K., Pitt, P., Richmond, W., Houghton, J. & Riley, D. (1993) Long term effects of tamoxifen. Biological Effects of Tamoxifen Working Party. *Eur. J. Cancer*, **29A**, 15–21

Dallenbach-Hellweg, G. & Hahn, U. (1995) Mucinous and clear cell adenocarcinomas of the endometrium in patients receiving antiestrogens (tamoxifen) and gestagens. *Int. J. gynecol. Pathol.*, **14**, 7–15

Daniel, P., Gaskell, S.J., Bishop, H., Campbell, C. & Nicholson, R.I. (1981) Determination of tamoxifen and biologically active metabolites in human breast tumour and plasma. *Eur. J. Cancer clin. Oncol.*, **17**, 1183–1189

Dauplat, J., Le Bouëdec, G. & Achard, J.L. (1990) Adenocarcinoma of the endometrium in two patients treated with tamoxifen. *Presse méd.*, **19**, 380–381 (in French)

Davies, A.M., Martin, E.A., Jones, R.M., Lim, C.K., Smith, L.L. & White, I.N.H. (1995) Peroxidase activation of tamoxifen and toremifene resulting in DNA damage and covalently bound protein adducts. *Carcinogenesis*, **16**, 539–545

De Jong-Busnac, M. (1989) Ophthamological complications of low-dose tamoxifen in the treatment of breast cancer. *Ned. Tijdschr. Geneesk.*, **133**, 514–516 (in Dutch)

De Muylder, X., Neven, P., De Somer, M., Van Belle, Y., Vanderick, G. & De Muylder, E. (1991) Endometrial lesions in patients undergoing tamoxifen therapy. *Int. J. gynecol. Obstet.*, **36**, 127–130

Deprest, J., Neven, P. & Ide, P. (1992) An unusual type of endometrial cancer, related to tamoxifen? *Eur. J. Obstet. Gynecol. reprod. Biol.*, **46**, 147–150

Dewar, J.A., Horobin, J.M., Preece, P.E., Tavendale, R., Tunstall-Pedoe, H. & Wood, R.A.B. (1992) Long term effects of tamoxifen on blood lipid values in breast cancer. *Br. med. J.*, **305**, 225–226

Dilts, P.V., Jr, Hopkins, M.P., Chang, A.E. & Cody, R.L. (1992) Rapid growth of leiomyoma in patient receiving tamoxifen. *Am. J. Obstet. Gynecol.*, **166**, 167–168

Dnistrian, A.M., Schwartz, M.K., Greenberg, E.J,. Smith, C.A. & Schwartz, D.C. (1993) Effect of tamoxifen on serum cholesterol and lipoproteins during chemohormonal therapy. *Clin. chim. Acta*, **223**, 43–52

Dragan, Y.P., Rizvi, T., Xu, Y.-H., Hully, J.R., Bawa, N., Campbell, H.A., Maronpot, R.R. & Pitot, H.C. (1991a) An initiation-promotion assay in rat liver as a potential complement to the 2-year carcinogenesis bioassay. *Fundam. appl. Toxicol.*, **16**, 525–547

Dragan, Y.P., Xu, Y.-D. & Pitot, H.C. (1991b) Tumor promotion as a target for estrogen/antiestrogen effects in rat hepatocarcinogenesis. *Prev. Med.*, **20**, 15–26

Dragan, Y.P., Fahey, S., Street, K., Vaughan, J., Jordan, V.C. & Pitot, H.C. (1994) Studies of tamoxifen as a promoter of hepatocarcinogenesis in female Fischer F344 rats. *Breast Cancer Res. Treat.*, **31**, 11–25

Dragan, Y.P., Vaughan, J., Jordan, V.C. & Pitot, H.C. (1995) Comparison of the effects of tamoxifen and toremifene on liver and kidney tumor promotion in female rats. *Carcinogenesis*, **16**, 2733–2741

Early Breast Cancer Trialists' Collaborative Group (1988) Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. *New Engl. J. Med.*, **319**, 1681–1692

Early Breast Cancer Trialists' Collaborative Group (1992) Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomized trials involving 31 000 recurrences and 24 000 deaths among 75 000 women. *Lancet*, **339**, 1–15, 71–85

Enck, R.E. & Rios, C.N. (1984) Tamoxifen treatment of metastatic breast cancer and antithrombin III levels. *Cancer*, **53**, 2607–2609

Enevoldson, T.P. & Harding, A.E. (1992) Improvement in the POEMS syndrome after administration of tamoxifen (Letter to the Editor). *J. Neurol. Neurosurg. Psychiat.*, **55**, 71–72

Esaki, K. & Sakai, Y. (1980) Tamoxifen; influence of oral administration of tamoxifen on the rabbit fetus. *Jitchukan Zenrinsho Kenkyuho*, **6**, 217–231 (in Japanese)

Evans, M.J., Langlois, N.E.I., Kitchener, H.C. & Miller, I.D. (1995) Is there an association between long-term tamoxifen treatment and the development of carcinosarcoma (malignant mixed Müllerian tumor) of the uterus? *Int. J. Gynecol. Cancer*, **5**, 310–313

Farmindustria (1993) *Repertorio Farmaceutico Italiano* (Italian Pharmaceutical Directory), 7th Ed., Milan, Associazione Nazionale dell'Industria Farmaceutica, CEDOF, S.P.A., pp. A-794–A795; A-1101–A-1102

Fendl, K.C. & Zimniski, S.J. (1992) Role of tamoxifen in the induction of hormone-independent rat mammary tumours. *Cancer Res.*, **52**, 235–237

Fentiman, I.S., Caleffi, M., Rodin, A., Murby, B. & Fogelman, I. (1989) Bone mineral content of women receiving tamoxifen for mastalgia. *Br. J. Cancer*, **60**, 262–264

Ferrazzi, E., Cartei, G., Mattarazzo, R. & Fiorentino, M. (1977) Oestrogen-like effect of tamoxifen on vaginal epithelium (Letter to the Editor). *Br. med. J.*, **i**, 1351–1352

Fisher, B., Redmond, C., Brown, A., Fisher, E.R., Wolmark, N., Bowman, D., Plotkin, D., Wolter, J., Bornstein, R., Legault-Poisson, S., Saffer, E.A. & Other NSABP Investigators (1986) Adjuvant chemotherapy with and without tamoxifen in the treatment of primary breast cancer: 5-year results from the National Surgical Adjuvant Breast and Bowel Project Trial. *J. clin. Oncol.*, **4**, 459–471

Fisher, B., Costantino, J., Redmond, C., Poisson, R., Bowman, D., Couture, J., Dimitrov, N.V., Wolmark, N., Wickerham, D.L., Fisher, E.R., Margolese, R., Robidoux, A., Shibata, H., Terz, J., Paterson, A.H.G., Feldman, M.I., Fararr, W., Evans, J., Lickley, H.L. & Ketner, R.N. (1989a) A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. *New Engl. J. Med.*, **320**, 479–484

Fisher, B., Redmond, C., Wickerham, L., Wolmark, N., Bowman, D., Couture, J., Dimitrov, N.V., Margolese, R., Legault-Poisson, S. & Robidoux, A. (1989b) Systemic therapy in patients with node-negative breast cancer. A commentary based on two National Surgical Adjuvant Breast and Bowel Project (NSABP) clinical trials. *Ann. intern. Med.*, **111**, 703–712

Fisher, B., Costantino, J.P., Redmond, C.K., Fisher, E.R., Wickerham, D.L. & Cronin, W.M. (1994) Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J. natl Cancer Inst.*, **86**, 527–537

Folk, J.J., Mazur, M.T., Eddy, G.L. & Musa, A.G. (1995) Secretory endometrial adenocarcinoma in a patient on tamoxifen for breast cancer: a report of a case. *Gynecol. Oncol.*, **58**, 133–135

Ford, M.R., Turner, M.J,. Wood, C. & Soutter, W.P. (1988) Endometriosis developing during tamoxifen therapy. *Am. J. Obstet. Gynecol.*, **158**, 119

Fornander, T., Rutqvist, L.E., Cedermark, B., Glas, U., Mattsson, A., Silfverswärd, C., Skoog, L., Somell, A., Theve, T., Wilking, N., Askergren, J. & Hjalmar, M.-L. (1989) Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet*, **i**, 117–120

Fornander, T., Rutqvist, L.E., Sjöberg, H.E., Blomqvist, L., Mattsson, A. & Glas, U. (1990) Long-term adjuvant tamoxifen in early breast cancer: effect on bone mineral density in postmenopausal women. *J. clin. Oncol.*, **8**, 1019–1024

Fornander, T., Rutqvist, L.E., Cedermark, B., Glas, U., Mattsson, A., Skoog, L., Somell, A., Theve, T., Wilking, N., Askergren, J., Rotstein, S., Hjalmar, M.-L. & Perbeck, L. (1991) Adjuvant tamoxifen in early-stage breast cancer: effects on intercurrent morbidity and mortality. *J. clin. Oncol.*, **9**, 1740–1748

Fornander, T., Hellström, A.-C. & Moberger, B. (1993) Descriptive clinicopathologic study of 17 patients with endometrial cancer during or after adjuvant tamoxifen in early breast cancer. *J. natl Cancer Inst.*, **85**, 1850–1855

Forsberg, J.G. (1985) Treatment with different antioestrogens in the neonatal period and effects in the cervicovaginal epithelium and ovaries of adult mice: a comparison to estrogen-induced changes. *Biol. Reprod.*, **32**, 427–441

Foster, W.G., Jarrell, J.F. & YoungLai, E.V. (1993) Developmental changes in the gonadotropin releasing hormone neuron of the female rabbit: effects of tamoxifen citrate and pregnant mare serum gonadotropin. *Can. J. Physiol. Pharmacol.*, **71**, 761–767

Fried, K.M. & Wainer, I.W. (1994) Direct determination of tamoxifen and its four major metabolites in plasma using coupled column high-performance liquid chromatography. *J. Chromatogr. B*, **655**, 261–268

Fromson, J.M., Pearson, S. & Bramah, S. (1973a) The metabolism of tamoxifen (ICI 46,474). Part II: In female patients. *Xenobiotica*, **3**, 711–714

Fromson, J.M., Pearson, S. & Bramah, S. (1973b) The metabolism of tamoxifen (ICI 46,474). Part I: In laboratory animals. *Xenobiotica*, **3**, 693–709

Furr B.J.A. & Jordan, V.C. (1984) The pharmacology and clinical uses of tamoxifen. *Pharmacol. Ther.*, **25**, 127–205

Furr, B.J.A., Valcaccia, B. & Challis, J.R.G. (1976) The effects of nolvadex (tamoxifen citrate; ICI 46,474) on pregnancy in rabbits. *J. Reprod. Fert.*, **48**, 367–369

Furr, B.J.A., Patterson, J.S., Richardson, D.N., Slater, S.R. & Wakeling, A.E. (1979) Tamoxifen. In: Goldberg, M.E., ed., *Pharmacological and Biochemical Properties of Drug Substances*, Washington, American Pharmacological Association, Vol. II, pp. 355–399

Gennaro, A.R., ed. (1995) *Remington: The Science and Practice of Pharmacy*, 19th Ed., Vol. II, Easton, PA, Mack Publishing Co., p. 1094

Gershbein, L.L. (1994) Induction of colon adenocarcinomas in rats fed trypsin and tamoxifen diets by parenteral and intragastric 1,2-dimethylhydrazine. *Res. Comm. mol. Pathol. Pharmacol.*, **85**, 347–350

Gherman, R.B., Parker, M.F. & Macri, C.I. (1994) Granulosa cell tumor of the ovary associated with antecedent tamoxifen use. *Obstetr. Gynecol.*, **84**, 717–719

Ghia, M. & Mereto, E. (1989) Induction and promotion of γ-glutamyltranspeptidase-positive foci in the liver of female rats treated with ethinyl estradiol, clomiphene, tamoxifen and their associations. *Cancer Lett.*, **46**, 195–202

Gibson, L.E., Barakat, R.R., Curtin, J.P., Jones, W.B., Lewis, J.L., Jr & Hoskins, W.J. (1994) Tamoxifen effects on the endometrium: a retrospective comparative study (Abstract no. 837). *Proc. ASCO*, **13**, 263

Gill-Sharma, M.K., Gopalkrishnan, K., Balasinor, N., Parte, P., Jayaraman, S. & Juneja, H.S. (1993) Effects of tamoxifen on the fertility of male rats. *J. Reprod. Fert.*, **99**, 395–402

Gillett, D. (1995) Leiomyosarcoma of the uterus in a woman taking adjuvant tamoxifen therapy (Letter to the Editor). *Med. J. Aust.*, **163**, 160–161

Glick, J.H., Creech, R.H., Torri, S., Holdroyde, C., Brodovsky, H., Catalano, R.B. & Varano, M. (1981) Randomized clinical trial of tamoxifen plus sequential CMF chemotherapy versus tamoxifen alone in postmenopausal women with advanced breast cancer. *Breast Cancer Res. Treat.*, **1**, 59–68

Glick, J.H., Gelber, R.D., Goldhirsch, A. & Senn, H.-J. (1992) Meeting highlights: adjuvant therapy for primary breast cancer. *J. natl Cancer Inst.*, **84**, 1479–1485

Goldfarb, S. (1976) Sex hormones and hepatic neoplasia. *Cancer Res.*, **36**, 2584–2588

Goldhirsch, A., Wood, W.C., Senn, H.-J., Glick, J.H. & Gelber, R.D. (1995) Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J. natl Cancer Inst.*, **87**, 1441–1445

Goodwin, J.W., Crowley, J., Eyre, H.J., Stafford, B., Jaeckle, K.A. & Townsend, J.J. (1993) A phase II evaluation of tamoxifen in unresectable or refractory meningiomas: a southwest oncology group study. *J. Neuro-Oncol.*, **15**, 75–77

Gooren, L. (1989) Improvement of spermatogenesis after treatment with the antiestrogen tamoxifen in a man with the incomplete androgen insensitivity syndrome. *J. clin. Endocrinol. Metab.*, **68**, 1207–1210

Gordon, D., Beastall, G.H., McArdle, C.S. & Thomson, J.A. (1986) The effect of tamoxifen therapy on thyroid function tests. *Cancer*, **58**, 1422–1425

Gray, R. (1993) Tamoxifen: how boldly to go where no women have gone before. *J. natl Cancer Inst.*, **85**, 1358–1360

Greaves, P., Goonetilleke, R., Nunn, G., Topham, J. & Orton, T. (1993) Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Res.*, **53**, 3919–3924

Grey, A.B., Stapleton, J.P., Evans, M.C. & Reid, I.R. (1995a) The effects of the antiestrogen tamoxifen on cardiovascular risk factors in normal postmenopausal women. *J. clin. Endocrinol. Metabol.*, **80**, 3191–3195

Grey, A.B., Stapleton, J.P., Evans, M.C., Tatnell, M.A., Ames, R.W. & Reid, I.R. (1995b) The effects of the antiestrogen tamoxifen on bone mineral density in normal late postmenopausal women. *Am. J. Med.*, **99**, 636–641

Griffiths, M.F.P. (1987) Tamoxifen retinopathy at low dosage (Letter to the Editor). *Am. J. Ophthalmol.*, **104**, 185–186

Guelen, P.J.M., Stevenson, D., Briggs, R.J. & de Vos, D. (1987) The bioavailability of Tamoplex (tamoxifen). Part 2. A single dose cross-over study in healthy male volunteers. *Meth. Find. exp. clin. Pharmacol.*, **9**, 685–690

Guetta, V., Lush, R.M., Figg, W.D., Waclawiw, M.A. & Cannon, R.O., III (1995) Effects of the antiestrogen tamoxifen on low-density lipoprotein concentrations and oxidation in postmenopausal women. *Amer. J. Cardiol.*, **76**, 1072–1073

Gulino, A., Screpanti, I. & Pasqualini, J.R. (1984) Differential estrogen and antiestrogen responsiveness of the uterus during development in the fetal, neonatal and immature guinea pig. *Biol. Reprod.*, **31**, 371–381

Gupta, J.S. & Roy, S.K. (1987) Effect of tamoxifen on cytosolic estrogen receptor in the different parts of fallopian tube and uterus during ovum transport. *Exp. clin. Endocrinol.*, **90**, 293–300

Gylling, H., Pyrhönen, S., Mäntylä, E., Mäenpää, H., Kangas, L. & Miettinen, T.A. (1995) Tamoxifen and toremifene lower serum cholesterol by inhibition of Δ^8-cholesterol conversion to lathosterol in women with breast cancer. *J. clin. Oncol.*, **13**, 2900–2905

Han, X. & Liehr, J.G. (1992) Induction of covalent DNA adducts in rodents by tamoxifen. *Cancer Res.*, **52**, 1360–1363

Hard, G.C., Iatropoulos, M.J., Jordan, K., Radi, L., Kaltenberg, O.P., Imondi, A.R. & Williams, G.M. (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

Hardell, L. (1988a) Tamoxifen as risk factor for carcinoma of corpus uteri (Letter to the Editor). *Lancet*, **ii**, 563

Hardell, L. (1988b) Pelvic irradiation and tamoxifen as risk factors for carcinoma of corpus uteri (Letter to the Editor). *Lancet*, **ii**, 1432

Harper, M.J.K. & Walpole, A.L. (1966) Contrasting endocrine activities of *cis* and *trans* isomers in a series of substituted triphenylethylenes. *Nature,* **212**, 87

Harper, M.J.K. & Walpole, A.L. (1967a) A new derivative of triphenylethylene: effect on implantation and mode of action in rats. *J. Reprod. Fertil.,* **13**, 101–119

Harper, M.J.K. & Walpole, A.L. (1967b) Mode of action of ICI 46,474 in preventing implantation in rats. *J. Endocrinol.,* **39**, 83–92

Harrison, J.D., Morris, D.L., Ellis, I.O., Jones, J.A. & Jackson, I. (1989) The effect of tamoxifen and estrogen receptor status on survival in gastric carcinoma. *Cancer,* **64**, 1007–1010

Hasmann, M., Rattel, B. & Löser, R. (1994) Preclinical data for droloxifene. *Cancer Lett.*, **84**, 101–116

Hatch, K.D., Beecham, J.B., Blessing, J.A. & Creasman, W.T. (1991) Responsiveness of patients with advanced ovarian carcinoma to tamoxifen. A gynecologic oncology group study of second-line therapy in 105 patients. *Cancer,* **68**, 269–271

Hayes, D.F., Van Zyl, J.A., Hacking, A., Goedhals, L., Bezwoda, W.R., Mailliard, J.A., Jones, S.E., Vogel, C.L., Berris, R.F., Shemano, I. & Schoenfelder, J. (1995) Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer. *J. clin. Oncol.*, **13**, 2556–2566

Hemminki, K., Widlak, P. & Hou, S.-M. (1995) DNA adducts caused by tamoxifen and toremifene in human microsomal system and lymphocytes *in vitro*. *Carcinogenesis*, **16**, 1661–1664

Hendrick, A. & Subramanian, V.P. (1980) Tamoxifen and thromboembolism (Letter to the Editor). *J. Am. med. Assoc.*, **243**, 514–515

Hirsimäki, P., Hirsimäki, Y., Nieminen, L. & Payne, B.J. (1993) Tamoxifen induces hepatocellular carcinoma in rat liver: a 1-year study with two antiestrogens. *Arch. Toxicol.*, **67**, 49–54

Hulka, C.A. & Hall, D.A. (1993) Endometrial abnormalities associated with tamoxifen therapy for breast cancer: sonographic and pathologic correlation. *Am. J. Roentgentol.*, **160**, 809–812

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Update of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 82–83, 182–184, 210–211, 239–240, 241–242

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Update of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 272–310

Iguchi, T., Hirokawa, M. & Takasugi, N. (1986) Occurrence of genital tract abnormalities and bladder hernia in female mice exposed neonatally to tamoxifen. *Toxicology*, **42**, 1–11

Iguchi, T., Irisawa, S., Uchima, F.-D.A. & Takasugi, N. (1988) Permanent chondrification in the pelvis and occurrence of hernias in mice treated neonatally with tamoxifen. *Reprod. Toxicol.*, **2**, 127–134

Ingle, J.N. (1984) Additive hormonal therapy in women with advanced breast cancer. *Cancer,* **53**, 766–777

Ingle, J.N., Ahmann, D.L., Green, S.J., Edmonson, J.H., Bisel, H.F., Kvols, L.K., Nichols, W.C., Creagan, E.T., Hahn, R.G., Rubin, J. & Frytak, S. (1981) Randomized clinical trial of diethylstilbestrol versus tamoxifen in postmenopausal women with advanced breast cancer. *New Engl. J. Med.,* **304**, 16–21

Ingle, J.N., Krook, J.E., Green, S.J., Kubista, T.P., Everson, L.K., Ahman, D.L., Chang, M.N., Bisel, H.F., Windschitl, H.E., Twito, D.I. & Pfeifle, D.M. (1986) Randomized trial of bilaterial oophorectomy versus tamoxifen in premenopausal women with metastatic breast cancer. *J. clin. Oncol.*, **4**, 178–185

Irisawa, S. & Iguchi, T. (1990) Critical period of induction by tamoxifen of genital organ abnormalities in female mice. *In Vivo*, **4**, 175–180

Ismail, S.M. (1994) Pathology of endometrium treated with tamoxifen. *J. clin. Pathol.*, **47**, 827–833

Jaiyesimi, I.A., Buzdar, A.U., Sahin, A.A. & Ross, M.A. (1992) Carcinoma of the male breast. *Ann. intern. Med.*, **117**, 771–777

Jaiyesimi, I.A., Buzdar, A.U., Decker, D.A. & Hortobagyi, G.N. (1995) Use of tamoxifen for breast cancer : 28 years later. *J. clin. Oncol.*, **13**, 513–529

Jarman, M., Poon, G.K., Rowlands, M.G., Grimshaw, R.M., Horton, M.N., Potter, G.A. & McCague, R. (1995) The deuterium isotope effect for the α-hydroxylation of tamoxifen by rat liver microsomes accounts for the reduced genotoxicity of [D_5-ethyl]tamoxifen. *Carcinogenesis*, **16**, 683–688

Johnstone, A.J., Sarkar, T.K. & Hussey, J.K. (1991) Primary hepatocellular carcinoma in a patient with breast carcinoma. *Clin. Oncol.*, **3**, 180–181

Jones, A.L., Powles, T.J., Treleaven, J.G., Burman, J.F., Nicolson, M.C., Chung, H.-I. & Ashley, S.E. (1992) Haemostatic changes and thromboembolic risk during tamoxifen therapy in normal women. *Br. J. Cancer*, **66**, 744–747

Jordan, V.C. (1978) Use of the DMBA-induced rat mammary carcinoma system for the evaluation of tamoxifen as a potential adjuvant therapy. *Rev. Endocrinol. rel. Cancer (October Supplement)*, 49–55

Jordan, V.C. (1988) The development of tamoxifen for breast cancer therapy: a tribute to the late Arthur L. Walpole. *Breast Cancer Res. Treat.*, **11**, 197–209

Jordan, V.C. (1993) A current view of tamoxifen for the treatment and prevention of breast cancer. *Br. J. Pharmacol.*, **110**, 507–517

Jordan, V.C. & Prestwich, G. (1977) Binding of [^{3}H]tamoxifen in rat uterine cytosols: a comparison of swinging bucket and vertical tube rotor sucrose density gradient analysis. *Mol. Cell Endocrinol.*, **8**, 179–188

Jordan, V.C. & Robinson, S.P. (1987) Species-specific pharmacology of antiestrogens: role of metabolism. *Fed. Proc.*, **46**, 1870–1874

Jordan, V.C., Dix, C.J. & Allen, K.E. (1979) The effectiveness of long-term treatment in a laboratory model for adjuvant hormone therapy of breast cancer. In: Salmon, S.E. & Jones, S.E., eds, *Adjuvant Therapy of Cancer*, Vol. 2, New York, Grune and Stratton, pp. 19–26

Jordan, V.C., Allen, K.E. & Dix, C.J. (1980) Pharmacology of tamoxifen in laboratory animals. *Cancer Treat. Rep.*, **64**, 745–759

Jordan, V.C., Fritz, N.F. & Tormey, D.C. (1987) Long-term adjuvant therapy with tamoxifen: effects on sex hormone binding globulin and antithrombin III. *Cancer Res.*, **57**, 4517–4519

Jordan, V.C., Lababidi, M.K. & Mirecki, D.M. (1990) Anti-oestrogenic and anti-tumour properties of prolonged tamoxifen therapy in C3H/OUJ mice. *Eur. J. Cancer*, **26**, 718–721

Jordan, V.C., Lababidi, M.K. & Langan-Fahey, S. (1991) Suppression of mouse mammary tumorigenesis by long-term tamoxifen therapy. *J. natl Cancer Inst.*, **83**, 492–496

Jose, R., Kekre, A.N., George, S.S. & Seshadri, L. (1995) Endometrial carcinoma in a tamoxifen-treated breast cancer patient. *Aust. N.Z. J. Obstet. Gynaecol.*, **35**, 201

Kaiser-Kupfer, M.I. & Lippman, M.E. (1978) Tamoxifen retinopathy. *Cancer Treat. Rep.*, **62**, 315–320

Kangas, L., Nieminen, A.-L., Blanco, G., Grönroos, M., Kallio, S., Karjalainen, A., Perilä, M., Södervall, M. & Toivola, R. (1986) A new triphenylethylene compound, Fc-1157a. II. Anti-tumor effects. *Cancer Chemother. Pharmacol.*, **17**, 109–113

Kawamura, I., Mizota, T., Kondo, N., Shimomura, K. & Kohsaka, M. (1991) Antitumor effects of droloxifene, a new antiestrogen drug, against 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats. *Jpn. J. Pharmacol.*, **57**, 215–224

Kedar, R.P., Bourne, T.H., Powles, T.J., Collins, W.P., Ashley, S.E., Cosgrove, D.O. & Campbell, S. (1994) Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. *Lancet*, **343**, 1318–1321

Kenny, A.M., Prestwood, K.M., Pilbeam, C.C. & Raisz, L.G. (1995) The short term effects of tamoxifen on bone turnover in older women. *J. clin. Endocrinol. Metabol.*, **80**, 3287–3291

Killackey, M.A., Hakes, T.B. & Pierce, V.K. (1985) Endometrial adenocarcinoma in breast cancer patients receiving antiestrogens. *Cancer Treat. Rep.*, **69**, 237–238

Kitaichi, M., Nishimura, K., Itoh, H. & Izumi, T. (1995) Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. *Am. J. respir. crit. Care Med.*, **151**, 527–533

Knabbe, C., Lippman, M.E., Wakefield, L.M., Flanders, K.C., Kasid, A., Derynck, R. & Dickson, R.B. (1987) Evidence that TGF-β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell*, **48**, 417–428

Kohigashi, K., Fukuda, Y. & Imura, H. (1988) Inhibitory effect of tamoxifen on diethylstilbestrol-promoted hepatic tumorigenesis in male rats and its possible mechanism of action. *Jpn. J. Cancer Res. (Gann)*, **79**, 1335–1339

Korach, K.S., Fox-Davies, C., Quarmby, V.E. & Swaisgood, M.H. (1985) Diethylstilbestrol metabolites and analogs. Biochemical probes for differential stimulation of uterine estrogen responses. *J. biol. Chem.*, **260**, 15420–15426

Korach, K.S., Horigome, T., Tomooka, Y., Yamashita, S., Newbold, R.R. & McLachlan, J.A. (1988) Immunodetection of estrogen receptor in epithelial and stromal tissues of neonatal mouse uterus. *Proc. natl Acad. Sci.*, **85**, 3334–3337

Kotoulas, I.-G., Mitropoulos, D., Cardamakis, E., Dounis, A. & Michopoulos, J. (1994) Tamoxifen treatment in male infertility. I. Effect on spermatozoa. *Fert. Ster.*, **61**, 911–914

Krause, A. & Gerber, B. (1994) Postmenopausal hemorrhage and endometrial carcinoma during tamoxifen therapy. *Zbl. Gynakol.*, **116**, 44–47 (in German)

Krause, W., Holland-Moritz, H. & Schramm, P. (1992) Treatment of idiopathic oligozoospermia with tamoxifen — a randomized controlled study. *Int. J. Androl.*, **15**, 14–18

Kristensen, B., Ejlersten, B., Dalgaard, P., Larsen, L., Nistrup Holmegaard, S., Transbøl, I. & Mouridsen, H.T. (1994) Tamoxifen and bone metabolism in postmenopausal low-risk breast cancer patients: a randomized study. *J. clin. Oncol.*, **12**, 992–997

Lahti, E., Blanco, G., Kauppila, A., Apaja-Sarkkinen, M., Taskinen, P.J. & Laatikainen, T. (1993) Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. *Obstet. Gynecol.*, **81**, 660–664

Lai, C.-H., Hsueh, S., Chao, A.-S. & Soong, Y.-K. (1994) Successful pregnancy after tamoxifen and megestrol acetate therapy for endometrial carcinoma. *Br. J. Obstet. Gynaecol.*, **101**, 547–549

Lang-Avérous, G., Rupp, K., Wehner, H. & Dallenbach-Hellweg, G. (1991) The role of steroid hormones and related biological substances in the etiology of endometrial carcinoma. *Verh. Dtsch. ges. Pathol.*, **75**, 366–369 (in German)

Lanza, A., Alba, E., Re, A., Tessarolo, M., Leo, Bellino, R., Lauricella, A. & Wierdis, T. (1994) Endometrial carcinoma in breast cancer patients treated with tamoxifen. Two case reports and review of the literature. *Eur. J. Gynaec. Oncol.*, **15**, 455–459

Le Bouëdec, G. & Dauplat, J. (1992) Cancer of the endometrium caused by antiestrogens. *Rev. Fr. Gynécol. Obstét.*, **87**, 345–348 (in French)

Le Bouëdec, G., De Latour, M., Feillel, V. & Dauplat, J. (1990) Metrorrhagia and tamoxifen. 22 patients treated for breast cancer. *J. Gynécol. Obstét. Biol. Reprod.*, **19**, 889–894 (in French)

Le Bouëdec, G., Kauffmann, P., De Latour, M., Fondrinier, E., Curé, H. & Dauplat, J. (1991) Metastases of cancer of the breast in the uterus. 8 case histories. *J. Gynécol. Obstét. Biol. Reprod.*, **20**, 349–354 (in French)

van Leeuwen, F.E., Benraadt, J., Coebergh, J.W.W., Kiemeney, L.A.L.M., Gimbrère, C.H.F., Otter, R., Schouten, L.J., Damhuis, R.A.M., Bontenbal, M., Diepenhorst, F.W., van den Belt-Dusebout, A.W. & van Tinteren, H. (1994) Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet*, **343**, 448–452

Le Roy, X., Escot, C., Brouillet, J.-P., Theillet, C., Maudelonde, T., Simony-Lafontaine, J., Pujol, H. & Rochefort, H. (1991) Decrease of c-*erb*B-2 and c-*myc* RNA levels in tamoxifen-treated breast cancer. *Oncogene*, **6**, 431–437

Lentz, S.S. (1994) Advanced and recurrent endometrial carcinoma: hormonal therapy. *Sem. Oncol.*, **21**, 100–106

Leo, L., Lanza, A., Re, A., Tessarolo, M., Bellino, R., Lauricella, A. & Wierdis, T. (1994) Leiomyomas in patients receiving Tamoxifen. *Clin. exp. Obstet. Gynecol.*, **21**, 94–98

Leslie, W.D., Cowden, E.A. & MacLean, J.P. (1995) Oestrogen and bone density: a comparison of tamoxifen and hypo-oestrogenaemia. *Nuclear Med. Commun.*, **16**, 698–702

Liehr, J.G., Sirbasku, D.A., Jurka, E., Randerath, K. & Randerath, E. (1988) Inhibition of estrogen-induced renal carcinogenesis in male Syrian hamsters by tamoxifen without decrease in DNA adduct levels. *Cancer Res.*, **48**, 779–783

Lien, E.A., Solheim, E., Lea, O.A., Lundgren, S., Kvinnsland, S. & Ueland P.M. (1989) Distribution of 4-hydroxy-*N*-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res.*, **49**, 2175–2183

Lien, E.A., Solheim, E. & Ueland, P.M. (1991) Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res.*, **51**, 4837–4844

Lien, E.A., Anker, G. & Ueland, P.M. (1995) Pharmacokinetics of tamoxifen in premenopausal and postmenopausal women with breast cancer. *J. Steroid Biochem. mol. Biol.*, **55**, 229–231

Lim, C.K., Chow, L.C.L., Yuan, Z.-X. & Smith, L.L. (1993) High performance liquid chromatography of tamoxifen and metabolites in plasma and tissues. *Biomed. Chromatogr.*, **7**, 311–314

Lim, C.K., Yuan, Z.-X., Lamb, J.H., White, I.N.H., De Matteis, F. & Smith, L.L. (1994) A comparative study of tamoxifen metabolism in female rat, mouse and human liver microsomes. *Carcinogenesis*, **15**, 589–593

Lin, C.L. & Buttle, H.L. (1990) Progesterone receptor in the mammary tissue of pregnant and lactating gilts and the effect of tamoxifen treatment during late gestation. *J. Endocrinol.*, **130**, 251–257

Lin, C.L. & Buttle, H.L. (1991) Effect of oestradiol benzoate and tamoxifen on the growth of and induction of progesterone receptors in the uterus and mammary gland of immature pigs. *J. Endocrinol.*, **130**, 259–265

Lipton, A., Harvey, H.A. & Hamilton, R.W. (1984) Venous thrombosis as a side effect of tamoxifen treatment. *Cancer Treat. Rep.*, **68**, 887–889

LiVolsi, V.A., Salhany, K.E. & Dowdy, Y.G. (1995) Endocervical adenocarcinoma in tamoxifen-treated patient (Letter to the Editor). *Am. J. Obstet. Gynecol.*, **172**, 1065

Longstaff, S., Sigurdsson, H., O'Keefe, M., Ogston, S. & Preece, P. (1989) A controlled study of the ocular effects of tamoxifen in conventional dosage in the treatment of breast carcinoma. *Eur. J. Cancer clin. Oncol.*, **25**, 1805–1808

Love, R. (1989) Tamoxifen therapy in primary breast cancer: biology, efficacy and side effects. *J. clin. Oncol.*, **7**, 803–815

Love, R.R. & Feyzi, J.M. (1993) Reduction in vasomotor symptoms from tamoxifen over time. *J. natl Cancer Inst.*, **85**, 673–674

Love, R.R., Mazess, R.B., Tormey, D.C., Barden, H.S., Newcomb, P.A. & Jordan, V.C. (1988) Bone mineral density in women with breast cancer treated with adjuvant tamoxifen for at least two years. *Breast Cancer Res. Treat.*, **12**, 297–302

Love, R.R., Newcomb, P.A., Wiebe, D.A., Surawicz, T.S., Jordan, V.C., Carbone, P.P. & DeMets, D.L. (1990) Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *J. natl Cancer Inst.*, **82**, 1327–1332

Love, R.R., Wiebe, D.A., Newcomb, P.A., Cameron, L., Leventhal, H., Jordan, V.C., Feyzi, J. & DeMets, D.L. (1991) Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. *Ann. intern. Med.*, **115**, 860–864

Love, R.R., Surawicz, T.S. & Williams, E.C. (1992a) Antithrombin III level, fibrinogen level, and platelet count changes with adjuvant tamoxifen therapy. *Arch. intern. Med.*, **152**, 317–320

Love, R.R., Mazess, R.B., Barden, H.S., Epstein, S., Newcombe, P.A., Jordan, V.C., Carbone, P.P. & DeMets, D.L. (1992b) Effect of tamoxifen on bone mineral density in post-menopausal women with breast cancer. *New Engl. J. Med.*, **326**, 852–856

Love, R.R., Wiebe, D.A., Feyzi, J.M., Newcombe, P.A. & Chappell, R.J. (1994a) Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J. natl Cancer Inst.*, **86**, 1534–1539

Love, R.R., Barden, H.S., Mazess, R.B., Epstein, S. & Chappell, R.J. (1994b) Effect of tamoxifen on lumbar spine bone mineral density in postmenopausal women after 5 years. *Arch. intern. Med.*, **154**, 2585–2588

Lüllmann, H. & Lüllmann-Rauch, R. (1981) Tamoxifen-induced generalised lipidosis in rats subchronically treated with high doses. *Toxicol. appl. Pharmacol.*, **61**, 138–146

Lumsden, M.A., West, C.P. & Baird, D.T. (1989) Tamoxifen prolongs luteal phase in premenopausal women but has no effect on the size of uterine fibroids. *Clin. Endocrinol.*, **31**, 335–343

Mäentausta, O., Svalander, P., Danielsson, K.G., Bygdeman, M. & Vihko, R. (1993) The effects of an antiprogestin, mifepristone, and an antiestrogen, tamoxifen, on endometrial 17β-hydroxysteroid dehydrogenase and progestin and estrogen receptors during the luteal phase of the menstrual cycle: an immunohistochemical study. *J. clin. Endocrinol. Metab.*, **77**, 913–918

Magriples, U., Naftolin, F., Schwartz, P.E. & Carcangiu, M.L. (1993) High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. *J. clin. Oncol.*, **11**, 485–490

Malfetano, J.H. (1990) Tamoxifen-associated endometrial carcinoma in postmenopausal breast cancer patients. *Gynecol. Oncol.*, **39**, 82–84

Mamby, C.C., Love, R.R. & Lee, K.E. (1995) Thyroid function test changes with adjuvant tamoxifen therapy in postmenopausal women with breast cancer. *J. clin. Oncol.*, **13**, 854–857

Mani, C. & Kupfer, D. (1991) Cytochrome P-450-mediated activation and irreversible binding of the antioestrogen tamoxifen to proteins in rat and human liver: possible involvement of flavin-containing mono-oxygenases in tamoxifen activation. *Cancer Res.*, **51**, 6052–6058

Mani, C., Gelboin, H.V., Park, S.S., Pearce, R., Parkinson, A. & Kupfer D. (1993) Metabolism of the antimammary cancer antioestrogenic agent tamoxifen 1. Cytochrome P-450-catalyzed N-demethylation and 4-hydroxylation. *Drug Metab. Dispos.*, **21**, 645–656

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

Manni, A., Trujillo, J.E., Marshall, J.S., Brodkey, J. & Pearson, O.H. (1979) Antihormone treatment of stage IV breast cancer. *Cancer*, **43**, 444–450

Mäntylä, E.T.E., Nieminen, L.S. & Karlsson, S.H. (1995) Endometrial cancer induction by tamoxifen in the rat (Abstract no. 61). *Eur. J. Cancer*, **31A** (Suppl. 5), S14

Mäntylä, E.T.E., Karlsson, S.H. & Nieminen, L.S. (1996) Induction of endometrial cancer by tamoxifen in the rat. In: Li, J.J., Li, S.A., Gustafsson, J.-Å., Nandi, S. & Sekely, L.I., eds., *Hormonal Carcinogenesis. II. Proceedings of the Second International Symposium*, New York, Springer Verlag, pp. 442–445

Marshall, K. & Senior, J. (1987) A study on the effect of a single dose of tamoxifen on uterine hyperaemia and growth in the rat. *Br. J. Pharmacol.*, **92**, 429–435

Martin, L. (1980) Estrogens, anti-estrogens and the regulation of cell proliferation in the female reproductive tract *in vivo*. In: McLachlan, J.A., Korach, K. & Lamb, J.C., eds, *Estrogens in the Environment*, New York, Elsevier/North Holland, pp. 103–129

Martin, E.A., Rich, K.J., White, I.N.H., Woods, K.L., Powles, T.J. & Smith, L.L. (1995) ^{32}P-Postlabelled DNA adducts in liver obtained from women treated with tamoxifen. *Carcinogenesis*, **16**, 1651–1654

Martínez Cerezo, F.J., Tomás, A., Donoso, L., Enríquez, J., Guarner, C., Balanzó, J., Martínez Nogueras, A. & Vilardell, F. (1994) Controlled trial of tamoxifen in patients with advanced hepatocellular carcinoma. *J. Hepatol.*, **20**, 702–706

Mastri, C., Mistry, P. & Lucier, G.W. (1985) In vivo oestrogenicity and binding characteristics of alpha-zearalanol (P-1496) to different classes of oestrogen binding proteins in rat liver. *J. Steroid Biochem.*, **23**, 279–289

Mathew, A., Chabon, A.B., Kabakow, B., Drucker, M. & Hirschman, R.J. (1990) Endometrial carcinoma in five patients with breast cancer on tamoxifen therapy. *N.Y. State J. Med.*, **90**, 207–208

McAuliffe, F.M. & Foley, M.E. (1993) Tamoxifen and endometrial lesions (Letter to the Editor). *Lancet*, **342**, 1124

McClay, E.F. & McClay, M.E.T. (1994) Tamoxifen: is it useful in the treatment of patients with metastatic melanoma ? *J. clin. Oncol.*, **12**, 617–626

McClay, E.F., Albright, K.D., Jones, J.A., Christen, R.D. & Howell, S.B. (1993) Tamoxifen modulation of cisplatin cytotoxicity in human malignancies. *Int. J. Cancer*, **55**, 1018–1022

McClellan, M.C., West, N.B., Tacha, D.E., Greene, G.L. & Brenner, R.M. (1984) Immunocytochemical localization of estrogen receptors in the macaque reproductive tract with monoclonal antiestrophilins. *Endocrinol.*, **114**, 2002–2014

McDermott, M.T., Hofeldt, F.D. & Kidd, G.S. (1990) Tamoxifen therapy for painful idiopathic gynecomastia. *South. med. J.*, **83**, 1283–1285

McDonald, C.C. & Stewart, H.J. (1991) Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial. *Br. med. J.*, **303**, 435–437

McDonald, C.C., Alexander, F.E., Whyte, B.W., Forrest, A.P. & Stewart, H.J. (1995) Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. *Br. med. J.*, **311**, 977–980

McVie, J.G., Simonetti, G.P.C., Stevenson, D., Briggs, R.J., Guelen, P.J.M. & de Vos, D. (1986) The bioavailability of Tamoplex (tamoxifen). Part 1. A pilot study. *Meth. Find. exp. clin. Pharmacol.*, **8**, 505–512

Medical Economics (1996) *PDR®: Physicians' Desk Reference*, 50th Ed., Montvale, NJ, Medical Economics Data Production Co., pp. 2842–2844

Metzler, M. & Schiffmann, D. (1991) Structural requirements for the in vitro transformation of Syrian hamster embryo cells by stilbene estrogens and triphenylethylene-type antiestrogens. *Am. J. clin. Oncol.*, **14** (Suppl. 2), S30–S35

Mignotte, H., Sasco, A.J., Lasset, C., Saez, S. & Rivoire, M. (1992) Adjuvant therapy of breast cancer with tamoxifen and endometrial carcinoma. *Bull. Cancer*, **79**, 969–977 (in French)

Miké, V., Currie, V.E. & Gee, T.S. (1994) Fatal neutropenia associated with long-term tamoxifen therapy. *Lancet*, **344**, 541–542

Mishkin, S.Y., Farber, E., Ho, R., Mulay, S. & Mishkin, S. (1985) Tamoxifen alone or in combination with estradiol-17β inhibits the growth and malignant transformation of hepatic hyperplastic nodules. *Eur. J. Cancer clin. Oncol.*, **21**, 615–623

Montandon, F. & Williams, G.M. (1994) Comparison of DNA reactivity of the polyphenylethylene hormonal agents diethylstilbestrol, tamoxifen and toremifene in rat and hamster liver. *Arch. Toxicol.*, **68**, 272–275

Moon, L.Y., Wakley, G.K. & Turner, R.T. (1991) Dose-dependent effects of tamoxifen on long bones in growing rats: influence of ovarian status. *Endocrinology*, **129**, 1568–1574

Moon, R.C., Steele, V.E., Kelloff, G.J., Thomas, C.F., Detrisac, C.J., Mehta, R.G. & Lubet, R.A. (1994) Chemoprevention of MNU-induced mammary tumorigenesis by hormone response modifiers: toremifene, RU 16117, tamoxifen, aminoglutethimide and progesterone. *Anticancer Res.*, **14**, 889–894

Moorthy, B., Sriram, P., Pathak, D.N., Bodell, W.J. & Randerath, K. (1996) Tamoxifen metabolic activation: comparison of DNA adducts formed by microsomal and chemical activation of tamoxifen and 4-hydroxytamoxifen with DNA adducts formed *in vivo*. *Cancer Res.*, **56**, 53–57

Mouridsen, H.T., Rose, C., Overgaard, M., Dombernowsky, P., Panduro, J., Thorpe, S., Rasmussen, B.B., Blichert-Toft, M. & Andersen, K.W. (1988) Adjuvant treatment of postmenopausal patients with high risk primary breast cancer. *Acta oncol.*, **27**, 699–705

Mühlemann, K., Cook, L.S. & Weiss, N.S. (1994) The incidence of hepatocellular carcinoma in US white women with breast cancer after the introduction of tamoxifen in 1977. *Breast Cancer Res. Treat.*, **30**, 201–204

Murphy, C., Fotsis, T., Pantzer, P., Aldercreutz, H. & Martin, F. (1987) Analysis of tamoxifen, *N*-desmethyltamoxifen and 4-hydroxytamoxifen levels in cytosol and KCl-nuclear extracts of breast tumours from tamoxifen treated patients by gas chromatography-mass spectometry (GC-MS) using selected ion monitoring (SIM). *J. Steroid Biochem.*, **28**, 609–618

Muss, H.B. (1992) Endocrine therapy for advanced breast cancer: a review. *Breast Cancer Res. Treat.*, **21**, 15–26

Mustacchi, G., Milani, S., Pluchinotta, A., De Matteis, A., Rubagotti, A. & Perrota, A. (1994) Tamoxifen or surgery plus tamoxifen as primary treatment for elderly patients with operable breast cancer. The G.R.E.T.A. (Group for Research on Endocrine Therapy in the Elderly) Trial. *Anticancer Res.*, *14*, 2197–2200

National Institutes of Health Consensus Development Panel (1992) Consensus statement: treatment of early-stage breast cancer. *J. natl Cancer Inst.*, **11**, 1–5

National Surgical Adjuvant Breast and Bowel Project (1992) *NSABP Protocol P-1: A Clinical Trial to Determine the Worth of Tamoxifen for Preventing Breast Cancer,* Pittsburgh, PA, University of Pittsburgh, January 24

Nayfield, S.G., Karp, J.E., Ford, L.G., Dorr, F.A. & Kramer, B.S. (1991) Potential role of tamoxifen in prevention of breast cancer. *J. natl Cancer Inst.*, **83,** 1450–1459

Nevasaari, K., Heikkinen, M. & Taskinen, P.J. (1978) Tamoxifen and thrombosis. *Lancet.*, **ii**, 946–947

Neven, P., De Muylder, X., Van Belle, Y., Vanderick, G. & De Muylder, E. (1989) Tamoxifen and the uterus and endometrium (Letter to the Editor). *Lancet*, **i**, 375

Neven, P., De Muylder, X., Van Belle, Y., Vanderick, G. & De Muylder, E. (1990) Hysteroscopic follow-up during tamoxifen treatment. *Eur. J. Obstet. Gynecol. reprod. Biol.*, **35**, 235–238

Noguchi, M., Taniya, T., Tarjiri, K., Miwa, K., Miyazaki, I., Koshino, H., Mabuchi, H. & Nomomura, A. (1987) Fatal hyperlipaemia in a case of metastatic breast cancer treated by tamoxifen. *Br. J. Surg.*, **74**, 586–587

Nolvadex Adjuvant Trial Organisation (NATO) (1983) Controlled trial of tamoxifen as adjuvant agent in the management of early breast cancer. Interim analysis at four years. *Lancet*, **i**, 257–261

Nolvadex Adjuvant Trial Organisation (NATO) (1988) Controlled trial of tamoxifen as a single adjuvant agent in the management of early breast cancer. *Br. J. Cancer*, **57**, 608–611

Nuovo, M.A., Nuovo, G.J., McCaffrey, R.M., Levine, R.U., Barron, B. & Winkler, B. (1989) Endometrial polyps in postmenopausal patients receiving tamoxifen. *Int. J. gynecol. Pathol.*, **8**, 125–131

Nuwaysir, E.F., Dragan, Y.P., Jefcoate, C.R,. Jordan, V.C. & Pitot, H.C. (1995) Effects of tamoxifen administration on the expression of xenobiotic metabolising enzymes in rat liver. *Cancer Res.*, **55**, 1780–1786

O'Dea, J.P.K. & Davis, E.H. (1990) Tamoxifen in the treatment of menstrual migraine. *Neurology*, **40**, 1470–1471

O'Grady, J.E., Armstrong, E.M., Moore, I.A.R. & Vass, M.A. (1974) Effect of tamoxifen (ICI-46,474) on mitosis in the uterus of the rat during the early stages of pregnancy. *J. Endocrinol.*, **63**, 19P

O'Halloran, M.J. & Maddock, P.G. (1974) ICI 46,474 in breast cancer. *J. Irish med. Assoc.*, **67**, 38–39

Ohta, Y., Iguchi, T. & Takasugi, N. (1989) Deciduoma formation in rats treated neonatally with the anti-estrogens, tamoxifen and MER-25. *Reprod. Toxicol.*, **3**, 207–212

Olive, D.L., Groff, T.R., Schultz, N., Schenken, R.S. & Riehl, R.M. (1990) Effects of tamoxifen on corpus luteum function and luteal phase length in cynomolgus monkeys. *Fert. Ster.*, **54**, 333–338

O'Neill, E. & Rodríguez Mojica, W. (1992) Asymptomatic carcinoma of the endometrium in a patient on adjunctive tamoxifen therapy for carcinoma of the breast. *Bol. Asoc. Med P.R.*, **84**, 74–77

Oredipe, O.A., Barth, R.F., Dwivedi, C. & Webb, T.E. (1992) Dietary glucarate-mediated inhibition of initiation of diethylnitrosamine-induced hepatocarcinogenesis. *Toxicology*, **74**, 209–222

Osborne, M.R., Hewer, A., Hardcastle, I.R., Carmichael, P.L. & Phillips, D.H. (1996) Identification of the major tamoxifen-deoxyguanosine adduct formed in the liver DNA of rats treated with tamoxifen. *Cancer Res.*, **56**, 66–71

Palacios, A., Pertusa, S., Montoya, A. & Martínez San Pedro, R. (1993) Breast cancer, tamoxifen, and uterus (Letter to the Editor). *Med. clin. Barc.*, **100**, 479 (in Spanish)

Palshof, T. (1988) *Adjuvant Endocrine Therapy in Premenopausal and Postmenopausal Women with Breast Cancer. Report of the Copenhagen Breast Cancer Trials 1975–1987*, Thesis, Copenhagen, Medi-book

Parry, B.R., Hung, N.A., McCall, J.L. & Phipps, R.F. (1993) Apparent regression of rectal polyps in a patient with Gardner's syndrome receiving concomitant tamoxifen therapy. *Aust. N.Z. J. Surg.*, **63**, 578–579

Pasqualini, J.R. & Lecerf, F. (1986) Ultrastructural modifications provoked by tamoxifen either alone or combined with oestradiol in the uteri of fetal or newborn guinea-pigs. *J. Endocrinol.*, **110**, 197–202

Pasqualini, J.R., Nguyen, B.-L., Sumida, C., Giambiagi, N. & Mayrand, C. (1986) Tamoxifen and progesterone effects in target tissues during the perinatal period. *J. Steroid Biochem.*, **25**, 853–857

Pasqualini, J.R., Sumida, C., Giambiagi, N.A. & Nguyen, B.L. (1987) The complexity of anti-oestrogen responses. *J. Steroid. Biochem.*, **27**, 883–889

Pathak, D.N. & Bodell, W.J. (1994) DNA adduct formation by tamoxifen with rat and human liver microsomal activation systems. *Carcinogenesis*, **15**, 529–532

Pathak, D.N., Pongracz, K. & Bodell, W.J. (1995) Microsomal and peroxidase activation of 4-hydroxytamoxifen to form DNA adducts: comparison with DNA adducts formed in Sprague-Dawley rats treated with tamoxifen. *Carcinogenesis*, **16**, 11–15

Pavlidis, N., Petris, C., Briassoulis, E., Klouvas, G., Psilas, C., Rempapis, J. & Petroutsos, G. (1992) Clear evidence that long-term, low dose tamoxifen treatment can induce ocular toxicity. *Cancer*, **69**, 2961–2964

Perry, R.R., Kang, Y. & Greaves, B. (1985) Effects of tamoxifen on growth and apoptosis of estrogen-dependent and -independent human breast cancer cells. *Ann. surg. Oncol.*, **2**, 238–245

Phillips, D.H., Carmichael, P.L., Hewer, A., Cole, K.J. & Poon, G.K. (1994a) α-Hydroxytamoxifen, a metabolite of tamoxifen with exceptionally high DNA-binding activity in rat hepatocytes. *Cancer Res.*, **54**, 5518–5522

Phillips, D.H., Potter, G.A., Horton, M.N., Hewer, A., Crofton-Sleigh, C., Jarman, M. & Venitt, S. (1994b) Reduced genotoxicity of [D_5-ethyl]tamoxifen implicates α-hydroxylation of the ethyl group as a major pathway of tamoxifen activation to a liver carcinogen. *Carcinogenesis*, **15**, 1487–1492

Phillips, D.H., Carmichael, P.L., Hewer, A., Cole, K.J., Hardcastle, I.R., Poon, G.K., Keogh, A. & Strain, A.J. (1996a) Activation of tamoxifen and its metabolite α-hydroxytamoxifen to DNA-binding products: comparisons between human, rat and mouse hepatocytes. *Carcinogenesis*, **17**, 89–94

Phillips, D.H., Hewer, A., Grover, P.L., Poon, G.K. & Carmichael, P.L. (1996b) Tamoxifen does not form detectable DNA adducts in white blood cells of breast cancer patients. *Carcinogenesis*, **17**, 1149–1152

Planting, A.S.T., Alexieva-Figusch, J., Blonk-v.d.Wijst, J. & van Putten, W.L.J. (1985) Tamoxifen therapy in premenopausal women with metastatic breast cancer. *Cancer Treat. Rep.*, **69**, 363–368

Pongracz, K., Pathak, D.N., Nakamura, T., Burlingame, A.L. & Bodell, W.J. (1995) Activation of the tamoxifen derivative metabolite E to form DNA adducts: comparison with the adducts formed by microsomal activation of tamoxifen. *Cancer Res.*, **55**, 3012–3015

Pons, J.Y. & Rigonnot, L. (1988) Hyperplasia of the endometrium in menopausal patients treated with tamoxifen for a breast cancer. *J. Gynecol. Obstet. Biol. reprod.*, **17**, 11–20 (in French)

Poon, G.K., Chui, Y.C., McCague, R., Lønning, P.E., Feng, R., Rowlands, M.G. & Jarman, M. (1993) Analysis of Phase I and Phase II metabolites of tamoxifen in breast cancer patients. *Drug Metab. Disp.*, **21**, 1119–1124

Poon, G.K., Walter, B., Lønning, P.E., Horton, M.N. & McCague, R. (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. Disp.*, **23**, 377–382

Potter, G.A., McCague, R. & Jarman, M. (1994) A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. *Carcinogenesis*, **15**, 439–442

Powles, T.J., Tillyer, C.R., Jones, A.L., Ashley, S.E., Treleaven, J., Davey, J.B. & McKinna, J.A. (1990) Prevention of breast cancer with tamoxifen — an update on the Royal Marsden Hospital pilot programme. *Eur. J. Cancer*, **26**, 680–684

Poyser, N.L. (1993) Effects of onapristone, tamoxifen and ICI 182780 on uterine prostaglandin production and luteal function in nonpregnant guinea-pigs. *J. Reprod. Fert.*, **98**, 307–312

Pritchard, K.I., Thomson, D.B., Myers, R.E., Sutherland, D.J.A., Mobbs, B.G. & Meakin, J.W. (1980) Tamoxifen therapy in premenopausal patients with metastatic breast cancer. *Cancer Treat. Rep.*, **64**, 787–796

Pritchard, K.I., Meakin, J.W., Boyd, N.F., DeBoer, G., Paterson, A.H.G., Ambus, U., Dembo, A.J., Sutherland, D.J.A., Wilkinson, R.H., Bassett, A.A., Evans, W.K., Beale, F.A., Clark, R.M. & Keane, T.J. (1987) Adjuvant tamoxifen in postmenopausal women with axillary node positive breast cancer: an update. In: Salmon, S.E. & Jones, S.E., eds, *Adjuvant Therapy of Cancer*, New York, Grune & Stratton, pp. 391–400

Pugh, D.M. & Sumano, H.S. (1979) The effects of oestradiol-17β and tamoxifen on the development of mouse embryos cultured over collagen. *Br. J. Pharmocol.*, **67**, 458

Qinn, M.A. & Campbell, J.J. (1989) Tamoxifen therapy in advanced/recurrent endometrial carcinoma. *Gynecol. Oncol.*, **32**, 1–3

Ralph, D.J., Brooks, M.D., Bottazzo, G.F. & Pryor, J.P. (1992) The treatment of Peyronie's disease with tamoxifen. *Br. J. Urol.*, **70**, 648–651

Randerath, K., Bi, J., Mabon, N., Sriram, P. & Moorthy, B. (1994a) Strong intensification of mouse hepatic tamoxifen DNA adduct formation by pretreatment with the sulfotransferase inhibitor and ubiquitous environmental pollutant pentachlorophenol. *Carcinogenesis*, **15**, 797–800

Randerath, K., Moorthy, B., Mabon, N. & Sriram, P. (1994b) Tamoxifen: evidence by ^{32}P-post-labeling and use of metabolic inhibitors for two distinct pathways leading to mouse hepatic DNA adduct formation and identification of 4-hydroxytamoxifen as a proximate metabolite. *Carcinogenesis*, **15**, 2087–2094

Rasmussen, K.L. & Nielsen, K.M. (1991) Development of endometrial cancer during tamoxifen therapy. *Ugeskr. Laeger.*, **153**, 2638 (in Danish)

Rayter, Z., Shepherd, J., Gazet, J.-C., Trott, P., Svensson, W. & A'Hern, R. (1993) Tamoxifen and endometrial lesions. *Lancet.*, **342**, 1124

Redmond, C.K., Wickerham, D.L., Cronin, W., Fisher, B., Costantino, J. & NSABP Participants (1993) The NSABP Breast Cancer Prevention Trial (BCPT): a progress report (Abstract no. 78). *Proc. Am. Soc. clin. Oncol.*, **12**, 69

Reynolds, J.E.F., ed. (1993) *Martindale: The Extra Pharmacopoeia*, 30th Ed., London, The Pharmaceutical Press, pp. 500–502

Ribeiro, G. & Swindell, R. (1985) The Christie Hospital tamoxifen (Nolvadex) adjuvant trial for operable breast carcinoma: 7-yr results. *Eur. J. Cancer clin. Oncol.*, **21**, 897–900

Ribeiro, G. & Swindell, R. (1992) The Christie Hospital adjuvant tamoxifen trial. *J. natl Cancer Inst. Monogr.*, **11**, 121–125

Rivkin, S.E., Green, S., Metch, B., Cruz, A.B., Abeloff, M.D., Jewell, W.R., Costanzi, J.J., Farrar, W.B., Minton, J.P. & Osborn, C. K. (1994) Adjuvant CMFVP versus tamoxifen versus concurrent CMFVP and tamoxifen for postmenopausal, node-positive, and estrogen receptor-positive breast cancer patients: a Southwest Oncology Group study. *J. clin. Oncol.*, **12**, 2078–2085

Robinson, S.P., Mauel, D.A. & Jordan, V.C. (1988) Antitumor actions of toremifene in the 7,12-dimethylbenzanthracene (DMBA)-induced rat mammary tumor model. *Eur. J. Cancer clin. Oncol.*, **24**, 1817–1821

Robinson, S.P., Langan-Fahey, S.M., Johnson, D.A. & Jordan, V.C. (1991) Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. *Drug Metab. Dispos.*, **19**, 36–43

Robinson, D.C., Bloss, J.D. & Schiano, M.A. (1995) A retrospective study of tamoxifen and endometrial cancer in breast cancer patients. *Gynecol. Oncol.*, **59**, 186–190

Rodier, J.F., Camus, E., Janser, J.C., Renaud, R. & Rodier, D. (1990) Tamoxifen and endometrial adenocarcinoma. *Bull. Cancer,* **77,** 1207–1210 (in French)

Ruenitz, P.C. & Nanavati, N.T. (1990) Identification and distribution in the rat of acidic metabolites of tamoxifen. *Drug Metab. Dispos.*, **18**, 645–648

Rutqvist, L.E. (1993) Long-term toxicity of tamoxifen. *Recent Results Cancer Res.*, **127**, 257–266

Rutqvist, L.E. & Mattsson (1993) Cardiac and thromboembolic morbidity among postmenopausal women with early-stage breast cancer in a randomized trial of adjuvant tamoxifen. *J. natl Cancer Inst.*, **85**, 1398–1406

Rutqvist, L.E., Cedermark, B., Glas, U., Johansson, H., Rotstein, S., Skoog, L., Somell, A., Theve, T., Wilking, N., Askergren, J., Hjalmar, M.-L., Ringborg, U. & Participating Members of the Stockholm Breast Cancer Study Group (1992) Randomized trial of adjuvant tamoxifen in node negative postmenopausal breast cancer. *Acta oncol.*, **31**, 265–270

Rutqvist, L.E., Johansson, H., Signomklao, T., Johansson, U., Fornander, T. & Wilking, N., for the Stockholm Breast Cancer Study Group (1995) Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies. *J. natl Cancer Inst.*, **87**, 645–651

Rydén, S., Fernö, M., Möller, T., Aspegren, K., Bergljung, L., Killander, D. & Landberg, T. (1992) Long-term effects of adjuvant tamoxifen and/or radiotherapy. The South Sweden Breast Cancer Trial. *Acta oncol.*, **31,** 271–274

Salata, I., Kowalski, J., Czechowski, B., Ochedalski, T., Karowicz, A. & Kowalska-Koprek, U. (1993) Does treatment with antioestrogens in men with oligozoospermia influence the pregnancy course in women after artificial insemination by husband. *Gin. Pol.*, **64**, 493–497 (in Polish)

Samelis, S.F., Stathopoulos, G.P., Malamos, N.A., Kondili, E., Moschopoulos, N.P. & Papacostas, P. (1992) Toxicity of long-term treatment with tamoxifen in breast cancer patients (Abstract no. 300). *Ann. Oncol.*, **3** (Suppl. 5), 78

Sargent, L.M., Dragan, Y.P., Bahnub, N., Wiley, J.E., Sattler, C.A., Schroeder, P., Sattler, G. L., Jordan, V.C. & Pitot, H.C. (1994) Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single in vivo administration to female Sprague-Dawley rats. *Cancer Res.*, **54**, 3357–3360

Sasco, A.J., Raffi, F., Satgé, D., Goburdhun, J., Fallouh, B. & Leduc, B. (1995) Endometrial Müllerian carcinosarcoma after cessation of tamoxifen therapy for breast cancer. *Int. J. gynecol. Obstet.*, **48,** 307–310

Sasco, A.J., Chaplain, G., Amoros, E. & Saez, S. (1996) Endometrial cancer following breast cancer: effect of tamoxifen and castration by radiotherapy. *Epidemiology*, **7**, 9–13

Sawka, C.A., Pritchard, K.I., Paterson, A.H.G., Sutherland, D.J.A., Thomson, D.B., Shelley, W.E., Myers, R.E., Mobbs, B.G., Malkin, A. & Meakin, J.W. (1986) Role and mechanism of action of tamoxifen in premenopausal women with metastatic breast carcinoma. *Cancer Res.*, **46**, 3152–3156

Segna, R.A., Dottino, P.R., Deligdisch, L. & Cohen, C.J. (1992) Tamoxifen and endometrial cancer. *Mt Sinai. J. Med.*, **59,** 416–418

Sengupta, A., Dutta, S. & Mallick, R. (1991) Modulation of cervical carcinogenesis by tamoxifen in a mouse model system. *Oncology*, **48**, 258–261

Seoud, M.A.-F., Johnson, J. & Weed, J.C., Jr (1993) Gynecologic tumors in tamoxifen-treated women with breast cancer. *Obstet. Gynecol.*, **82**, 165–169

Servais, P. & Galand, P. (1993) Increased yield in GST-P-positive liver pre-neoplastic foci induced by DENA or ENU in rats pre-treated with estradiol or tamoxifen. *Int. J. Cancer*, **54**, 996–1001

Shewmon, D.A., Stock, J.L., Abusamra, L.C., Kristan, M.A., Baker, S. & Heiniluoma, K.M. (1994) Tamoxifen decreases lipoprotein (a) in patients with breast cancer. *Metabolism*, **43**, 531–532

Silva, E.G., Tornos, C.S. & Follen-Mitchell, M. (1994) Malignant neoplasms of the uterine corpus in patients treated for breast carcinoma: the effects of tamoxifen. *Int. J. gynecol. Pathol.*, **13**, 248–258

Sonnendecker, H.E.M., Cooper, K. & Kalian, K.N. (1994) Case report. Primary Fallopian tube adenocarcinoma *in situ* associated with adjuvant tamoxifen therapy for breast carcinoma. *Gynecol. Oncol.*, **52**, 402–407

Spinelli, G., Bardazzi, N., Citernesi, A., Fontanarosa, M. & Curiel, P. (1991) Endometrial carcinoma in tamoxifen-treated breast cancer patients. *J. Chemother.*, **3**, 267–270

Stephens, C.J.M., Wojnarowska, F.T. & Wilkinson, J.D. (1989) Autoimmune progesterone dermatitis responding to tamoxifen. *Br. J. Dermatol.*, **121**, 135–137

Sterzik, K., Rosenbusch, B., Mogck, J., Heyden, M. & Lichtenberger, K. (1993) Tamoxifen treatment of oligozoospermia: a reevaluation of its effects including additional sperm function tests. *Arch. Gynecol. Obstet.*, **252**, 143–147

Stewart, H.J. (for the Scottish Cancer Trials Breast Group) (1992) The Scottish trial of adjuvant tamoxifen in node-negative breast cancer. *Monogr. natl Cancer Inst.*, **11**, 117–120

Stewart, H.J. & Knight, G.M. (1989) Tamoxifen and the uterus and endometrium (Letter to the Editor). *Lancet*, **i**, 375–376

Styles, J.A., Davies, A., Lim, C.K., De Matteis, F., Stanley, L.A., White, I.N.H., Yuan, Z.-X. & Smith, L.L. (1994) Genotoxicity of tamoxifen, tamoxifen epoxide and toremifene in human lymphoblastoid cells containing human cytochrome P450s. *Carcinogenesis*, **15**, 5–9

Sunderland, M.C. & Osborne, C.K. (1991) Tamoxifen in premenopausal patients with metastatic breast cancer: a review. *J. clin. Oncol.*, **9**, 1283–1297

Swahn, M.I., Bygdeman, M., Matlin, S.A. & Wu, Z.Y. (1989) The effect of tamoxifen on the function and lifespan of the corpus luteum and on subsequent ovarian function. *Acta endocrinol.*, **121**, 417–425

Swenerton, K.D., Chrumka, K., Paterson, A.H.G. & Jackson, G.C. (1984) Efficacy of tamoxifen in endometrial cancer. In: Bresciani, ed., *Progress in Cancer Research and Therapy*, Vol. 31, New York, Raven, pp. 417–424

Sydow, G. & Wunderlich, V. (1994) Effects of tamoxifen, droloxifene and 17β-estradiol on Rauscher mouse leukemogenesis. *Cancer Lett.*, **82**, 89–94

Taguchi, O. (1987) Reproductive tract lesions in male mice treated neonatally with tamoxifen. *Biol. Reprod.*, **37**, 113–116

Tarantal, A.F., Hendrickx, A.G., Matlin, S.A., Lasley, B.L., Gu, Q.-Q., Thomas, C.A.A., Vince, P.M. & van Look, P.F.A. (1993) Tamoxifen as an antifertility agent in the long-tailed macaque. *Contraception*, **47**, 307–316

Taylor, O.M., Benson, E.A., McMahon, M.J. & the Yorkshire Gastrointestinal Tumour Group (1993) Clinical trial of tamoxifen in patients with irresectable pancreatic adenocarcinoma. *Br. J. Surg.*, **80**, 384–386

Thangaraju, M., Kumar, K., Gandhirajan, R. & Sachdanandam, P. (1994) Effect of tamoxifen on plasma lipids and lipoproteins in postmenopausal women with breast cancer. *Cancer*, **73**, 659–663

Thomas, J., ed. (1991) *Prescription Products Guide 1991*, 20th Ed., Victoria, Australian Pharmaceutical Publishing Co. Ltd., pp. 1218–1220

Tormey, D.C., Gray, R., Falkson, H.C., Gilchrist, K., Abeloff, M.D., Falkson, G. & Participating Investigators (1993) Maintenance tamoxifen after induction postoperative chemotherapy in node-positive breast cancer patients: the Eastern Cooperative Oncology Group Trials. *Recent Results Cancer Res.*, **127**, 185–196

Tse, J. & Goldfarb, S. (1988) Immunohistochemical demonstration of estrophilin in mouse tissues using a biotinylated monoclonal antibody. *J. Histochem. Cytochem.*, **36**, 1527–1531

Tucker, M.J., Adam, H.K. & Patterson, J.S. (1984) Tamoxifen. In: Laurence, D.R., McLean, A.E.M. & Weatherall, M., eds, *Safety Testing of New Drugs*, New York, Academic Press, pp. 125–162

Uesugi, Y., Sato, T. & Iguchi, T. (1993) Morphometric analysis of the pelvis in mice treated neonatally with tamoxifen. *Anat. Rec.*, **235**, 126–130

Ugwumadu, A.H.N. & Harding, K. (1994) Uterine leimyomata and endometrial proliferation in postmenopausal women teated with the anti-oestrogen tamoxifen. *Eur. J. Obstet. Gynecol.*, **54**, 153–156

Ugwumadu, A.H.N., Bower, D. & Ho, P.K.-H. (1993) Tamoxifen induced adenomyosis and adenomyomatous endometrial polyp. *Br. J. Obstet. Gynaecol.*, **100**, 386–392

Ulibarri, C. & Micevych, P.E. (1993) Role of perinatal estrogens in sexual differentiation of the inhibition of lordosis by exogenous cholecystokinin. *Physiol. Behav.*, **54**, 95–100

United States Library of Medicine (1996) *RTECS Database*, Bethesda, MD

United States Pharmacopeial Convention (1994) *The 1995 US Pharmacopeia*, 23rd Rev./*The National Formulary*, 18th Rev., Rockville, MD, pp. 1477–1479

Uziely, B., Lewin, A., Brufman, G., Dorembus, D. & Mor-Yosef, S. (1993) The effect of tamoxifen on the endometrium. *Breast Cancer Res. Treat.*, **26**, 101–105

Vanchieri, C. (1993) European tamoxifen studies moving ahead. *J. natl Cancer Inst.*, **85**, 1450–1451

Vancutsem, P.M., Lazarus, P. & Williams, G.M. (1994) Frequent and specific mutations of the rat *p53* gene in hepatocarcinomas induced by tamoxifen. *Cancer Res.*, **54**, 3864–3867

Vargas Roig, L.M., Lotfi, H., Olcese, J.E., Lo Castro, G. & Ciocca, D.R. (1993) Effects of short-term tamoxifen administration in patients with invasive cervical carcinoma. *Anticancer Res.*, **13**, 2457–2464

Vickers, A.E. & Lucier, G.W. (1991) Estrogen receptor, epidermal growth factor and cellular ploidy in elutriated subpopulations of hepatocytes during liver tumor promotion by 17 alpha-ethinylestradiol in rats. *Carcinogenesis*, **12**, 391–399

Vidal (1995) *Dictionnaire Vidal*, 71st Ed., Paris, Editions du Vidal, p. 144

de Vos, D., Guelen, P.J.M. & Stevenson, D. (1989) The bioavailability of Tamoplex (tamoxifen). Part 4. A parallel study comparing Tamoplex and four batches of Nolvadex in healthy male volunteers. *Meth. Find. exp. clin. Pharmacol.*, **11**, 647–655

Wakeling, A.E., O'Connor, K.M. & Newboult, E. (1983) Comparison of the biological effects of tamoxifen and a new antioestrogen (LY 117018) on the immature rat uterus. *J. Endocrinol.*, **99**, 447–453

Ward, H.W.C. (1973) Anti-oestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br. med. J.*, **i**, 13–14

Ward, R.L., Morgan, G., Dalley, D. & Kelly, P.J. (1993) Tamoxifen reduces bone turnover and prevents lumbar spine and proximal femoral bone loss in early postmenopausal women. *Bone Min.*, **22**, 87–94

Watson, J., Anderson, F.B., Alam, M., O'Grady, J.E. & Heald, P.J. (1975) Plasma hormones and pituitary luteinizing hormone in the rat during the early stages of pregnancy and after post-coital treatment with tamoxifen (ICI 46,474). *J. Endocrinol.*, **65**, 7–17

Weckbecker, G., Tolcsvai, L., Stolz, B., Pollak, M. & Bruns, C. (1994) Somatostatin analogue octreotide enhances the antineoplastic effects of tamoxifen and ovariectomy on 7,12-dimethylbenz(*a*)anthracene-induced rat mammary carcinomas. *Cancer Res.*, **54**, 6334–6337

West, C.M.L., Reeves, S.J. & Brough, W. (1990) Additive interaction between tamoxifen and rifampicin in human biliary tract carcinoma cells. *Cancer Lett.*, **55**, 159–163

White, I.N.H., De Matteis, F., Davies, A., Smith, L.L., Crofton-Sleigh, C., Venitt, S., Hewer, A. & Phillips, D.H. (1992) Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57Bl/6 mice and in human MCL-5 cells. *Carcinogenesis*, **13**, 2197–2203

White, I.N.H., Davies, A., Smith, L.L., Dawson, S. & De Matteis, F. (1993) Induction of CYP2B1 and 3A1, and associated monooxygenase activities by tamoxifen and certain analogues in the livers of female rats and mice. *Biochem. Pharmacol.*, **45**, 21–30

White, I.N.H., de Matteis, F., Gibbs, A.H., Lim, C.K., Wolf, C.R., Henderson, C. & Smith, L.L. (1995) Species differences in the covalent binding of [^{14}C]tamoxifen to liver microsomes and the forms of cytochrome P450 involved. *Biochem. Pharmacol.*, **49**, 1035–1042

Williams, G.M., Iatropoulos, M.J., Djordjevic, M.V. & Kaltenberg, O.P. (1993) The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis*, **14**, 315–317

Winterfeld, G., Hauff, P., Görlich, M., Arnold, W., Fichtner, I. & Staab, H.J. (1992) Investigations of droloxifene and other hormone manipulations on *N*-nitrosomethylurea-induced rat mammary tumours. I. Influence on tumour growth. *J. Cancer Res. clin. Oncol.*, **119**, 91–96

Wiseman, H. (1994) *Tamoxifen: Molecular Basis of Use in Cancer Treatment and Prevention*, Chichester, John Wiley

Wiseman, H., Paganga, G., Rice-Evans, C. & Halliwell, B. (1993) Protective actions of tamoxifen and 4-hydroxytamoxifen against oxidative damage to human low-density lipoproteins: a mechanism accounting for the cardioprotective action of tamoxifen. *Biochem. J.*, **292**, 635–638

Wolter, J., Ryan, W.G., Subbaiah, P.V. & Bagdade, J.D. (1988) Apparent beneficial effects of tamoxifen on serum lipoprotein subfractions and bone mineral content in patients with breast cancer (Abstract no. 34). *Proc. Am. Soc. clin. Oncol.*, **7**, 10

Wong, A. & Chan, A. (1993) Survival benefit of tamoxifen therapy in adenocarcinoma of pancreas. A case–control study. *Cancer*, **71**, 2200–2203

Wright, C.D.P., Garrahan, N.J., Stanton, M., Gazet, J.-C., Mansell, R.E. & Compston, J.E. (1994) Effect of long-term tamoxifen therapy on cancellous bone remodeling and structure in women with breast cancer. *J. Bone min. Res.*, **9**, 153–159

Yager, J.D. & Shi, Y.E. (1991) Synthetic estrogens and tamoxifen as promoters of hepatocarcinogenesis. *Prev. Med.*, **20**, 27–37

Yager, J.D., Roebuck, B.D., Paluszcyk, T.L. & Memoli, V.A. (1986) Effects of ethinyl estradiol and tamoxifen on liver DNA turnover and new synthesis and appearance of gamma glutamyl transpeptidase-positive foci in female rats. *Carcinogenesis*, **7**, 2007–2014

Yagoda, A., Abi-Rached, B. & Petrylak, D. (1995) Chemotherapy for advanced renal-cell carcinoma: 1983-1993. *Sem. Oncol.*, **22**, 42–60

Yang, H.-H., Aulerich, R.J., Helferich, W., Yamini, B., Chou, K.C., Miller, E.R. & Bursian, S.J. (1995) Effects of zearalenone and/or tamoxifen on swine and mink reproduction. *J. appl. Toxicol.*, **15**, 223–232

Zemlickis, D., Lishner, M., Degendorfer, P., Panzarella, T., Sutcliffe, S.B. & Koren, G. (1992) Fetal outcome after in utero exposure to cancer chemotherapy. *Arch. intern. Med.*, **152**, 573–576

Zimniski, S.J. & Warren, R.C. (1993) Induction of tamoxifen-dependent rat mammary tumors. *Cancer Res.*, **53**, 2937–2939

APPENDIX B

Description of Online Searches for Tamoxifen

DESCRIPTION OF ONLINE SEARCHES FOR TAMOXIFEN

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

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

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

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

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

APPENDIX C

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

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

Tamoxifen

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

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

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

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

Public Comments Received
A total of 9 public comments were received:

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