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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

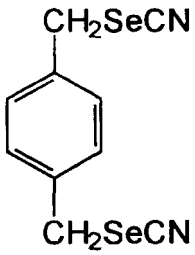
CLINICAL DEVELOPMENT PLAN: 1,4-PHENYLENEBIS(METHYLENE)SELENOCYANATE

DRUG IDENTIFICATION

CAS Registry No.: 85539-83-9

CAS Name (9CI): 1,4-Phenylenebis(methylene)selenocyanate
Synonyms: Xyleneselenocyanate
p-Xylylselenocyanate (*p*-XSC)
Related Compounds:
Benzyl Selenocyanate (BSC)
Potassium Selenocyanate (KSeCN)

Molecular Wt.: 314.1
Structure:



EXECUTIVE SUMMARY

Selenium (Se) is an essential nonmetallic trace element in mammals; the human nutritional intake of Se ranges from 50–350 μg/day (0.009–0.06 μmol/kg-bw/day). Low Se status has been linked epidemiologically to significantly increased incidence of cardiovascular disease [1] and significantly increased incidence or mortality from cancers such as bladder, breast, gastrointestinal (GI) tract, lung, and prostate, although these relationships are inconsistent between studies and geographical areas [2–9]. The most consistent inverse associations between cancer risk and Se in prospective studies have been observed in males (*e.g.*, GI tract, pancreas and all sites). High doses of both inorganic and organic Se compounds have inhibited chemically and virally induced tumors in animal mammary gland, colon, skin, lung, trachea, liver, stomach and pancreas models, as well as the development of transplanted tumors [10]. However, toxic effects in animals exposed to chronic high intakes of Se include body weight loss, liver damage, splenomegaly, pancreatic enlargement, anemia, hair loss, and abnormal nails. In humans, the estimated MTD is 819 g dietary organic Se/day (0.15 μmol/kg-bw/day) [11]; six times this dose of organoselenium compounds causes garlic breath, nausea, fatigue, irritability, dermatitis, hair loss, and nail changes or loss [12]. The lowest observed adverse effect level (LOAEL) of dietary Se is estimated to be 1540 μg/day.

Since the major source of Se in the human diet is in organic forms (primarily selenomethionine, Se-methyl-selenomethionine, selenocystine, and selenocysteine) contained in grains, meat, yeast, and certain vegetables [1], organic Se compounds would be the most appropriate forms to investigate for chemopre-

ventive potential. However, the effective and toxic doses are close, especially for Se compounds containing amino acids (*e.g.*, selenocysteine and selenomethionine) which are nonspecifically incorporated into proteins. Within the protein molecule, the Se-amino acid compounds are not free to release the monomethylated Se metabolites, replete GSH-Px, or participate in other reactions which have been associated with chemopreventive activity [12,13].

A search for more effective, less toxic organoselenium agents has led to the synthesis of 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) and benzylselenocyanate (BSC). *p*-XSC has a chemopreventive index (defined as the ratio of MTD to effective dose) of 6.5 in rats, compared with indices of 1, 1.3, 1.3, 2.0, and 2.5 for selenomethionine, sodium selenite, potassium selenocyanate, methylselenocyanate and BSC, respectively [14,15]. In contrast to *l*-selenomethionine, it avoids nonspecific incorporation into proteins. *p*-XSC toxicity appears to target the liver and hematopoietic system. Rats treated with doses above the MTD developed mild multifocal centrilobular hypertrophy of the liver, abnormally high plasma AST and ALT values, depressed triglycerides (females), and significantly lower hemoglobin and hematocrit values [14].

In published preclinical studies, *p*-XSC was effective in the AOM-induced rat colon carcinogenesis model, the DMBA-induced rat mammary model, and the NNK mouse lung model during initiation and/or postinitiation phases. In comparison, epidemiological studies have shown an inverse association of plasma Se levels with colon adenomas, a modest inverse association with breast cancer, and an inconsistent relationship with lung cancer [2, 6].

Several mechanisms are believed to contribute to the chemopreventive effects of *p*-XSC. Investigators have shown that it inhibits oxidant stress and inflammation, conditions which can have both initiation and postinitiation effects [15–17]. *p*-XSC shares the ability to replete GSH-Px in Se-deficient animals with other antioxidant Se compounds [17], and shares inhibition of PGE_2 synthesis in the colon [17] with NSAID chemopreventive agents such as sulindac and aspirin. *p*-XSC also inhibits DMBA-DNA adduct and epoxide formation in mammary glands and NNK-induced O^6-methylguanine and N^7-methylguanine DNA adduct formation in liver and lung [14,18]. Besides these activities, *p*-XSC enhances UDP-glucuronyl transferase activity in the liver [19], inhibits thymidine kinase activity in mammary tumor cells, and stimulates apoptosis [20,21].

No preclinical efficacy studies of *p*-XSC have been completed by the Chemoprevention Branch; it is tentatively scheduled for testing in the estrogen/testosterone-induced Noble rat prostate cancer model for modulation of intermediate biomarkers. One published biomarker study showed efficacy in the AOM-induced rat aberrant crypt focus assay.

There is no published report of human exposure to *p*-XSC; therefore, human pharmacodynamics can only be extrapolated from accounts of exposure to dietary organic Se compounds. Based on a chemopreventive index of 6.5 in animals and an approximate MTD of 819 μg Se (0.15 μmol/kg-bw) qd in humans, the effective human dose is estimated to be 0.014 μmol Se/kg-bw qd as *p*-XSC (0.007 μmol *p*-XSC/kg-bw qd), or about 153.9 μg *p*-XSC (77 μg Se) qd. This is within the nutritional intake range of 50–350 μg Se/day.

A Chemoprevention Branch-sponsored 30-day toxicity study of *p*-XSC in dogs is in progress and a 90-day dog study is planned. A 90-day rat study may also be required, if the FDA will not accept data from the published non-GLP 90-day toxicity study. A supply of 2–300 grams of *p*-XSC is being synthesized for the Chemoprevention Branch by Dr. Karam El-Bayoumy of the American Health Foundation which is estimated to be adequate for the preclinical toxicity studies. All of these studies will be completed before starting a Phase I trial. The initiation of the Phase I trial will depend on the scheduling of GMP synthesis of *p*-XSC, formulation, and stability testing. Phase II trial(s) could be initiated in high-risk breast and colon cancer cohorts, or smokers at high risk for lung cancer, but dose levels would depend on the outcome of the Phase I trial since no other data on human exposure have been published.

PRECLINICAL EFFICACY STUDIES

Low Se status has been linked epidemiologically to increased risk for cancer of the bladder, breast, GI tract, lung, and prostate [2–9]; the most consistent association is with cancers of the stomach, pancreas and all sites in men [2]. Interestingly, in published studies, *p*-XSC has shown efficacy in rat colon and mammary gland, and mouse lung—organs which were also inversely associated with Se status in epidemiological studies. No Chemoprevention

Branch-sponsored preclinical efficacy studies with *p*-XSC have been completed. A Noble rat study is tentatively planned to measure the effect of *p*-XSC on prostatic intermediate biomarkers.

In the DMBA-induced rat mammary cancer model, *p*-XSC was effective when administered during the initiation phase at doses of 5, 10, 15, and 40 ppm Se as *p*-XSC (1.6, 3.2, 4.8, and 12.5 μmol *p*-XSC/kg-bw/day or 3.2, 6.4, 9.5, and 25.0 μmol Se/kg-bw/day) [14, 15,22]. In contrast, only doses of 10 and 15 ppm Se as *p*-XSC were effective in the post initiation phase [15]. This outcome was supported by studies showing early (at 24–48 hours) *p*-XSC inhibition of DMBA-diolepoxide binding to DNA in the rat mammary gland but not in the liver [14]. In this model, the relative potency of *p*-XSC was compared with sodium selenite: 3 ppm Se (1.9 μmol/kg-bw/day) as sodium selenite was as potent as 5 ppm Se as *p*-XSC (1.6 μmol *p*-XSC/kg-bw/day or 3.2 μmol Se/kg-bw/day) in the initiation phase and 15 ppm Se as *p*-XSC (4.8 μmol *p*-XSC/kg-bw/day or 9.5 μmol Se/kg-bw/day) in the postinitiation phase [15].

In the NNK-induced mouse lung model, *p*-XSC administered during the initiation phase significantly decreased tumor multiplicity at doses of 5, 10, and 15 ppm Se as *p*-XSC (4.1, 8.2, and 12.2 μmol *p*-XSC/kg-bw/day or 8.2, 16.3, and 24.5 μmol Se/kg-bw/day) with a strong dose-response [23]. Selenite at 5 ppm Se (8.2 μmol/kg-bw/day) was ineffective in this model. An examination of the activity of *p*-XSC at a molecular level demonstrated a dose-dependent inhibition of NNK-induced O^6-methylguanine and N^7-methylguanine DNA adducts in mouse liver and lung [18]; a similar result was observed in rats [18,24].

In contrast, *p*-XSC was more effective in the postinitiation phase in the AOM-induced rat colon carcinogenesis model. At a dose of 40 ppm *p*-XSC (6.4 μmol *p*-XSC/kg-bw/day or 12.7 μmol Se/kg-bw/day), incidence and multiplicity were significantly inhibited in the initiation and postinitiation phases, while 20 ppm *p*-XSC (3.2 μmol *p*-XSC/kg-bw/day or 6.4 μmol Se/kg-bw/day) significantly inhibited multiplicity only in the postinitiation phase [16]. Confirmation of this effect was obtained in another study using the same model which found that *p*-XSC at 10 and 20 ppm Se (3.2 and 6.4 μmol *p*-XSC/kg-bw/day or 6.4 and 12.7 μmol Se/kg-bw/day) was ineffective during the initiation phase, but significantly inhibited colonic tumors in the postinitiation phase [17]. *p*-XSC failed to prevent AOM-induced DNA methylation in rat colon mucosa at comparable doses, illustrating the lack of antiinitiation effect. Inhibition in the postinitiation phase was attributed to enhancement of colonic mucosal Se-dependent GSH-Px activity and suppression of colonic prostaglandin E_2 levels [17]. The effect of *p*-XSC on AOM-induced colon carcinogenesis was also attributed to possible accelerated detoxification in the liver by enhanced UDP-glucuronyl transferase, which was observed after two weeks of treatment [19].

p-XSC has been shown to inhibit a histological intermediate biomarker in the rat colon. AOM-induced aberrant crypt foci were suppressed during initiation by 20 ppm *p*-XSC (3.2 mol *p*-XSC/kg-bw/day or 6.4 μmol Se/kg-bw/day); in comparison, *o*- and *m*-isomers were inactive [25]. The Chemoprevention Branch will investigate the effect of *p*-XSC on dysplasia in the Noble rat prostate.

PRECLINICAL SAFETY STUDIES

Se is an essential mineral which is acutely toxic in the form of inorganic salts. Chronic exposure of experimental animals to excessive selenite has resulted in growth failure and weight loss, as well as liver damage, splenomegaly, pancreatic enlargement, anemia, dermatitis, hair loss, and abnormal nails [reviewed in 26]. Organic selenium compounds appear to have relatively lower acute toxicity and comparable or enhanced chemopreventive activity.

Safety: A Chemoprevention Branch-sponsored 30-day toxicity study in dogs with *p*-XSC is in progress. For purposes of comparison, the published LD_{50}s for *l*-selenomethionine, sodium selenite, benzyl selenocyanate and *p*-XSC in rats are 26, 39, 125, and >1,000 mg/kg-bw, respectively (0.13, 0.2, 0.6, and >3.2 mmol/kg-bw; 6.4 mmol Se/kg-bw for *p*-XSC) [27]. One 90-day subchronic toxicity study of *p*-XSC in F344 rats has been reported [27]. *p*-XSC doses of 37.7, 70, and 149.5 ppm Se (12.0, 22.2, and 47.5 μmol *p*-XSC/kg-bw/day or 23.9, 44.5, and 95.0 mol Se/kg-bw/day) in males, and doses of 60.8, 118.3, and 243.6 ppm Se as *p*-XSC (19.3, 37.6, 77.4 μmol *p*-XSC/kg-bw/day or 38.6, 75.2, and 154.8 μmol Se/kg-bw/day) in females, resulted in body weight depressions up to 10% and 4.5%, respectively, possibly related to reduced food consumption. Mean liver and kidney weights increased in a dose-dependent manner. Histopathologic evaluation demonstrated dose-related multifocal centrolobular hypertrophy with fatty changes in all dose groups; no

kidney pathology could be related to *p*-XSC treatment, although rats in the high-dose group drank less. Abnormally high plasma AST and ALT occurred in high-dose groups of both sexes, depressed hemoglobin was seen in all groups, and lower hematocrit values were reported in the high- and low-dose males and in all female dose groups. A dose-related increase in plasma Se was noted but specific values were omitted in the report [27]. In this study, MTDs of 32.5 ppm Se as *p*-XSC (10.3 μmol *p*-XSC/kg-bw/day or 20.6 μmol Se/kg-bw/day) (sex not specified) and 3 ppm Se as BSC (1.9 μmol/kg-bw/day) were estimated in comparison with reported MTD values of 2–3 ppm Se as sodium selenite (1.3–1.9 μmol/kg-bw/day). Another estimate of the MTD was 50 ppm *p*-XSC (8.0 μmol *p*-XSC/kg-bw/day or 16.0 μmol Se/kg-bw/day) based on an efficacy study in male rats using doses of 20–100 ppm *p*-XSC (3.2–15.9 μmol *p*-XSC/kg-bw/day or 6.4–31.8 μmol Se/kg-bw/day) [16]. It should be noted that certain dietary components, especially protein, methionine, and vitamin E have been shown to alter the toxicity of Se compounds [28,29].

ADME: No pharmacokinetic studies have been performed by the Chemoprevention Branch. The only published study investigated the urinary and fecal excretion of a single ig dose of 50 μmol *p*-XSC (*ca.* 166.7 μmol/kg-bw) in rats. After two days, 60% of the *p*-XSC had been excreted in the feces, suggesting that either *p*-XSC is not well absorbed from the GI tract, or it is efficiently metabolized and excreted in the bile [30]. When *p*-XSC was administered by different routes, the latter alternative was eliminated; thus, the lower toxicity of *p*-XSC compared with other Se compounds may be due to decreased bioavailability.

No other information was found specifically on *p*-XSC ADME that would explain the observed anticarcinogenic activities which are believed to depend on the generation of monomethylated metabolites [31]. However, the fate of selenocyanate ($SeCN^-$), a putative metabolite, has been explored. KSeCN fed to rats in the diet at doses ranging from 0.5– 10 ppm Se (0.3–6.4 μmol/kg-bw/day) showed dose-related accumulation in the liver and kidney; blood level increases were not proportional to dose [32]. $SeCN^-$ is converted to dimethylselenide and trimethylselenonium ion, suggesting possible further breakdown to hydrogen selenide, which is believed to be the precursor of selenocysteine in the active site of GSH-Px [32]. It is hypothesized that the conversion of $SeCN^-$ to selenide might be the result of interaction with GSH or a response to physiological conditions. The degree of methylation and the rate of generation and elimination of Se metabolites is believed to relate to chemopreventive activity [13,31,33]; methylselenol and dimethylselenide, $SeCN^-$ metabolites in rats, are reported to have chemopreventive activity [33,34].

One publication reported data on the ADME of BSC. Although BSC has a similar structure to *p*-XSC (BSC has only one selenocyanate function attached to the benzene ring), its excretion pattern in rats is very different from *p*-XSC [30], suggesting that it may not be valid to extrapolate results of ADME studies on BSC to *p*-XSC.

CLINICAL SAFETY: PHASE I STUDIES

The Chemoprevention Branch is considering a Phase I trial of *p*-XSC if the animal toxicology results are acceptable.

Drug Effect Measurement: No human data have been published specifically on *p*-XSC. *p*-XSC has the ability to replete GSH-Px in Se-deficient rats and increase GSH-Px activity in the colonic mucosa of AOM-treated rats [16,22]. Based on experience with *l*-selenomethionine, the functional status of *p*-XSC could be determined by measuring GSH-Px activity in plasma, erythrocytes or platelets. GSH-Px activity in platelets can give functional information on short-term bioavailability, but a plateau is reached at concentrations of *ca.* 1.3 μM Se [35]. Plasma levels of Se can be used for short-term studies but might reach a plateau after a few weeks of exposure, as is the case for pharmacological doses of *l*-selenomethionine [36]; erythrocyte Se levels appear to be better indicators of Se levels for exposures lasting several weeks [36]. Urine levels of *p*-XSC or its metabolites may not be useful because urinary excretion is minimal [30]. The body burden of Se has been estimated from amounts found in hair or nails, but this may not be related to chemopreventive activity. For other organoselenium compounds, hair and toenail Se levels are considered the most reliable assessments of body burden for periods of one or more years [reviewed in 2], but even these are only moderately reproducible [37,38].

Safety: No specific reports of human toxicity from *p*-XSC were found in the literature. The recommended daily allowances for Se intake are 70 and 55

μg/day (*ca.* 0.01 mol/kg-bw/day) for males and females in the US, respectively [40]. The upper limit of daily human nutritional intake of Se was previously set at 500 μg (*ca.* 0.09 μmol/kg-bw/day) [39]; however, this was recently increased to 819 μg (*ca.* 0.15 μmol/kg-bw/day) [11]. Another source cautions that a maximal daily intake of 200 μg Se qd (*ca.* 0.04 μmol/kg- bw/day) should not be exceeded habitually [28]. According to an abstract describing preliminary results of the NCI-funded Phase III clinical trial (Dr. Larry Clark, University of Arizona) of 200 and 400 μg Se qd (*ca.* 0.04 and 0.07 μmol/kg-bw/day) as Se-enriched yeast, no hair and nail, or clinical chemistry changes indicating Se toxicity have been identified after 8,520 person-years of observation [41].

Adverse effects following ingestion of 5,000 μg Se/day (*ca.* 0.9 μmol/kg-bw/day) as organoselenium compounds in local produce of Enshi County, China, included garlic breath, nausea, fatigue, irritability, dermatitis, hair loss, dental defects, and nail changes and loss [12]. Based on studies in experimental animals, the difference between efficacy and toxicity doses is much wider for *p*-XSC than for other organic selenium compounds, even though the range of effective doses is higher. For example, *p*-XSC is effective in the DMBA-induced rat mammary model at 5 ppm Se (*ca.* 1.6 μmol *p*-XSC/kg-bw/day or 3.2 μmol Se/kg-bw/day) but has an estimated MTD in rats of 25–32 ppm Se as p-XSC (*ca.* 7.9–10.2 μmol *p*-XSC/kg-bw/day or 15.9–20.3 mol Se/kg-bw/day) [15,16,27]; in contrast, *d,l*-selenomethionine was effective in the same model at 3 ppm (*ca.* 1.9 μmol Se/kg-bw/day) [42], but the MTD was approximately the same dose [15].

ADME: There is no record of human exposure to *p*-XSC. The limited information from animal exposure suggests that experience with inorganic and amino acid Se compounds cannot be extrapolated to *p*-XSC. Sodium selenite is well absorbed (84% of a 200 μg Se dose) with 65% remaining in the body after 12 hours. It is metabolized to selenodiglutathione (GSSeSG) before being incorporated into proteins or methylated for excretion. In contrast, oral consumption of selenomethionine generally produces higher tissue levels of Se than selenite because it can be incorporated directly into proteins in place of methionine, resulting in a $t_{1/2}$ which is reported to be 70 days in humans [43,44]. Even the ADME of the closely related compounds *p*-XSC and BSC cannot be compared, since the amount excreted by rats in the feces two days after a 50 μmol Se dose was 60% and 5%, respectively [30].

CLINICAL EFFICACY: PHASE II/III STUDIES

No Chemoprevention Branch-funded Phase II or III clinical trials with *p*-XSC are planned or in progress. Based on preclinical *p*-XSC and epidemiological Se data, a breast cohort would be a priority for future trials. High-risk colon and lung (smokers) cohorts would also be appropriate choices.

PHARMACODYNAMICS

A chemopreventive index, defined as the ratio of the MTD to the effective dose which causes *ca.* 50% inhibition of total DMBA-induced mammary tumor yield in rats, has been calculated for *p*-XSC, BSC, potassium selenocyanate (KSeCN), and sodium selenite [45]. The index for *p*-XSC was 6.5 compared with 2.5 for BSC, and 1.3 for KSeCN and sodium selenite. Assuming that the 6.5 index can be applied to humans, and the human dietary MTD for organic Se is 819 μg Se (0.15 μmol/kg-bw) qd [15,28], then the effective human chemopreventive dose would be 0.023 μmol Se/kg-bw as *p*-XSC or 0.012 μmol *p*-XSC/kg-bw (127.1 μg Se or 263.9 μg *p*-XSC) qd, which is slightly above the RDAs for men and women in North America (70 and 55 μg Se/day, respectively) [40]. Based on preliminary results from the Phase III study (Dr. Clark), which showed that chronic doses of 200 μg Se qd (0.04 μmol/kg-bw) as Se-enriched brewer's yeast over a period of two years are nontoxic, chemopreventive doses up to 200 μg Se qd (0.04 μmol Se/kg-bw qd or 0.02 μmol *p*-XSC/kg-bw qd) of less toxic *p*-XSC could be administered.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

There are no human studies of *p*-XSC described in the literature. Se status is estimated for other Se compounds by calculating dietary intakes, determining Se levels in tissues and excreta, or measuring GSH-Px in different blood components. No assessment of Se intake used in studies of other Se compounds is appropriate under all circumstances, particularly at high doses. GSH-Px activity is a functional assay which plateaus at high Se intakes; GSH-Px activity in the colon mucosa of rats treated with 20 or 40 ppm *p*-XSC (3.2 or 6.4 μmol *p*-XSC/kg-

bw/day or 6.4 or 12.7 μmol Se/kg-bw/day) reached a plateau below 20 ppm *p*-XSC [16]. Based on two human studies of chronic selenomethionine administration at 200 g Se qd (0.04 μmol/kg-bw), plasma Se levels plateaued at *ca* 2.2 μM between weeks 11 and 16 [36,46] and erythrocyte Se plateaued at 28 weeks. In epidemiological and long-term studies, the Se content of toenails has been recommended as a measure of intake, although the correlation coefficient (r value relating intake with toenail levels) is only 0.48 [38]. Since none of these parameters appears to provide accurate information reflecting chemopreventive activity, it might be worthwhile investigating blood levels of monomethyl selenonium, which may be an active chemopreventive metabolite [13].

Safety Issues

There have been no completed Chemoprevention Branch-sponsored preclinical toxicity studies, only one subchronic toxicity study in rats is reported in the literature, and no human studies; since *p*-XSC is a synthetic compound, no epidemiological data are available. A comparison of *p*-XSC with other Se compounds showed that the subchronic MTD dose of *p*-XSC in rats is ten-fold higher than BSC and sodium selenite and four-fold higher than selenomethionine [27].

Liver appears to be the primary target of *p*-XSC toxicity. Hepatomegaly and centrolobular hypertrophy with fatty changes were seen at all doses in male rats treated with 37.7, 70, and 149.5 ppm Se as *p*-XSC (12.0, 22.2, and 47.5 μmol *p*-XSC/kg-bw/day or 23.9, 44.5, and 95.0 μmol Se/kg-bw/day) and female rats treated with 60.8, 118.3, and 243.6 ppm Se as *p*-XSC (19.3, 37.6, 77.4 μmol *p*-XSC/kg-bw/day or 38.6, 75.2, and 154.8 μmol Se/kg-bw/day) in NIH-07 diet; plasma levels of AST and ALT were significantly increased in the high dose group [27]. Hemoglobin was also reduced in all groups. In clinical trials, liver enzymes should be evaluated as a toxicity indicator.

Pharmacodynamics Issues

A chemopreventive index (MTD/effective dose) of 6.5 has been calculated for *p*-XSC in rats; if the MTD for organic Se in humans is 819 μg/day (0.15 μmol/kg-bw/day), an appropriate dose for humans would be 0.012 μmol/kg-bw *p*-XSC qd (0.23 μmol/kg-bw Se qd). This is equivalent to 263.9 μg *p*-XSC or 127.1 μg Se qd; the latter is slightly higher than the RDA for North American men and women. However, based on Dr. Clark's Phase III study, doses up to 200 μg Se (0.02 μmol *p*-XSC/kg-bw or 0.04 μmol Se/kg-bw) could probably be administered chronically to humans. Although a dose-response is seen in Se-replete rats treated with *p*-XSC in the DMBA-induced rat mammary carcinogenesis model [15], efficacy in a Se-replete human population is still unproven. This unknown could be resolved by stratifying subjects according to entering Se status in Phase II and III clinical trials or by investigating this issue in animals exposed to graded dietary levels of Se.

Regulatory Issues

To obtain regulatory permission to evaluate *p*-XSC in a Phase I clinical trial, the 30-day toxicity study in dogs must be completed and followed by a 90-day toxicity study in dogs. The published 90-day rat study may not be acceptable to the FDA because it was not performed under GLP regulations and a technical error resulted in a two-fold difference between male and female doses; therefore, it might have to be repeated. In addition, a pharmacokinetic study in dogs or non-human primates may be needed to estimate dose levels and predict the fate of *p*-XSC in humans.

Intermediate Biomarker Issues

The Chemoprevention Branch is planning to evaluate the ability of *p*-XSC to modulate intermediate biomarkers of prostatic cancer in the Noble rat model, including dysplastic lesion grade and number, nuclear and nucleolar morphometry (measured by computer-assisted image analysis), DNA ploidy, and proliferative biomarkers (PCNA and BrdU labelling). *p*-XSC has already shown significant activity against the aberrant crypt foci, a premalignant lesion in the colon. Other markers for future consideration include apoptosis and thymidine kinase in the breast, and prostaglandin E_2 in the colon.

Supply and Formulation Issues

Dr. Karam El-Bayoumy of the American Health Foundation has agreed to synthesize and supply the Chemoprevention Branch with up to 300 grams of *p*-XSC for preclinical toxicity studies. The Chemoprevention Branch has verified that *p*-XSC is not patented. Clinical supplies of *p*-XSC need to be synthesized by the American Health Foundation according to GMP regulations, formulations will have

to be developed, and stability tests must be completed before clinical trials can be initiated.

Clinical Studies Issues

A Phase I trial of *p*-XSC will be considered by the NCI, Chemoprevention Branch if results from the rat and dog toxicity studies are acceptable. Depending on the results of the Phase I trial, selected cohorts could be targeted for short-term Phase II trials (*e.g.*, breast cancer patients between diagnostic biopsy and surgery, smokers for a lung trial, and patients with previous colon adenomas or cancers) with modulation of selected intermediate biomarkers as endpoints.

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p-XSC DEVELOPMENT STATUS

Task Name	1995	1996
PRECLINICAL TOXICOLOGY		