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COMMITTEE ON THE CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

UPDATE ON THE BREAST CANCER RISK FROM EXPOSURE TO ORGANOCHLORINE INSECTICIDES: CONSIDERATION OF THE EPIDEMIOLOGY DATA ON DIELDRIN, DDT AND CERTAIN HEXACHLOROCYCLOHEXANE ISOMERS

DISCUSSION PAPER

Introduction

1. The potential association between exposure to certain organochlorine insecticides and increased risk of breast cancer has been of interest to research organisations (e.g. US NIEHS) and interest groups (e.g. Friends of the Earth and the Women's Environmental Network). The COC completed a statement in 2000, which reviewed published epidemiology results up to 1999. There are a considerable number of published epidemiology studies since the previous COC statement and it is now timely to review the available information.
2. In 1995, the COC reviewed the available epidemiological studies on three chemicals (DDT and isomers/metabolites, and the hexachlorocyclohexane isomers γ -HCH (lindane) and β -HCH). The Committee agreed that the available evidence indicated no clear association. It was felt, however, that the matter should be kept under review. A number of conclusions were reached and published in the 1995 Annual Report. A further review was initiated in 1999 and a statement was published in 2000 (<http://www.doh.gov.uk/ocbreast.html>). An additional chemical was included in the 1999 review namely, dieldrin, for there was new epidemiological data available. All pesticidal uses of organochlorine insecticides (OCIs) considered in this review now have been phased out.
3. There have been a considerable number of published epidemiology studies since 1999 and these are considered below. Some background information on the COCs conclusions reached in 1999, regarding the hypothesis underlying the suggested association between organochlorine insecticides and breast cancer can be summarised as follows;
 - i) Many of the known or proposed risk factors for breast cancer are related to endogenous or exogenous hormones (in particular oestrogen). These factors include age at first birth, at menarche, and at menopause, and obesity, parity and use of oral contraceptives and hormone replacement,

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- ii) There is some evidence available to suggest that the OCIs under consideration may have weak estrogenic activity,
 - iii) These OCIs have been shown to induce tumours (predominantly of the liver) in experimental animals,
 - iv) These OCIs persist in the environment and exposure of the population has occurred mainly via the diet.
4. This paper does not review the most recent evidence supporting the mechanisms by which organochlorine insecticides could cause breast cancer. The main objective is to update members on the published epidemiology. It is proposed to submit a draft statement to the June meeting, along with any additional literature on the epidemiology and oestrogenicity of organochlorine insecticides and their interactions, necessary for the Committee to reach conclusions.

Additional Epidemiological studies

5. Epidemiological studies identified from the literature (1999-2003) have been summarised in table 1 below. Short summaries of all papers are presented in Annex 1. Full papers of epidemiological studies with positive findings are appended in Annex 2. Studies reporting negative findings have not been included at this juncture in order to keep the paper size more manageable, but can be provided to members for the June meeting, if required. A copy of the previous statement is presented in Annex 3.
6. Of the nineteen studies reported, thirteen report no significant effect of organochlorines on breast cancer risk. Twelve of these refer to p,p' DDE. Seven of the thirteen reports of negative findings also report no significant effect of p,p' DDT on breast cancer risk. Four of the reports detail that levels of beta-HCH were not associated with increased breast cancer risk, and one study (Gammon *et al.*, 2002) reported that there was no association between levels of dieldrin and breast cancer risk.
7. The remaining six studies report that some organochlorines can have an association with breast cancer risk. Of these reports, three describe positive results for dieldrin. Two of these studies (Hoyer *et al.*, 2000a; Hoyer *et al.*, 2001) present significant odds ratios when comparing stratified exposures. The third study (Hoyer *et al.*, 2002) presents a high odds ratio (3.53, 95% CI = 0.79-15.79) for levels of dieldrin in individuals with P53 mutations, however the test for trend was not significant (P = 0.12).
8. Two reports (Romieu *et al.*, 2000; Woolcott *et al.*, 2001) show a significant association between levels of p,p' DDE and breast cancer risk. A third report detailing a breast survival analysis study (Hoyer *et al.* 2000a) shows a statistically significant association between DDE and reduced survival, however this was no longer seen when adjustment for tumour

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characteristics (positive lymph nodes, tumour size and grade) was made. In contrast, three of the studies that found positive associations between organochlorines and breast cancer risk, showed no evidence of an association between p,p' DDE and breast cancer (Hoyer *et al.* 2000b; Hoyer *et al.* 2001; Hoyer *et al.*, 2002).

9. Three of the six studies (Hoyer *et al.* 2000a; Hoyer *et al.* 2000b; Woolcott *et al.*, 2001) report no statistically significant odds ratios for levels for beta HCH and breast cancer risk. Five of the six studies reported on the possible association between breast cancer risk and levels of DDT. Four of these present results indicating that levels of DDT were not associated with breast cancer risk. One study (Hoyer *et al.*, 2000b) showed a significant positive association between breast cancer risk and levels of DDT (Odds ratio = 3.6, p for trend = 0.02).
10. Five of the nineteen studies evaluated the risk of breast cancer according to levels of organochlorines, taking into account the hormone receptor status of breast tumour. Of these, only two studies report any effect of hormone receptor status. Hoyer *et al.* (2001) report that high dieldrin levels were positively associated with increased risk in developing estrogen negative breast cancer. On the other hand, Woolcott *et al.* (2001) present evidence that p,p' DDE is associated with increased breast cancer risk in estrogen negative breast tumour cases. No excess risk of developing estrogen receptor positive breast cancer associated with exposure to organochlorines was demonstrated in any study.

Table 1: Summary of epidemiology studies (1999-2003)

(odd ratios or relative risk values are presented as upper stratification compared to the lowest and statistical assessment of trend given where reported)

Study/population	Exposure	Results	Conclusions reached
NEGATIVE FINDINGS			
Aronson <i>et al.</i> (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 55-63 Hospital-based case-control study. 217 cases, 213 controls	Geometric means (breast adipose tissue) p,p' DDE 693µg/kg lipid (cases) 596µg/kg lipid (controls) p,p' DDT 22.0µg/kg lipid (cases) 19.3µg/kg lipid (controls) b-HCH 43.1µg/kg lipid (cases) 41.5µg/kg lipid (controls)	Adjusted Odds Ratio (OR) p,p' DDE OR = 1.62 (95% CI 0.84-3.11) p,p' DDT OR = 1.18 (95% CI 0.61-2.29) b-HCH OR = 0.69 (95% CI 0.34-1.40) No measure of trend Increased risk in pre-menopausal women (adjusted OR = 1.52), but	Results suggestive of an association between p,p' DDE and increased breast cancer risk, however statistical significance of the results not determined

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		no measure of significance	
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Study/population	Exposure	Results	Conclusions reached
<p>Demers <i>et al.</i>, (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 161-166</p> <p>Hospital-based case-control study</p> <p>315 cases, 219 hospital controls (HC), 307 population controls (PC)</p>	<p>Mean plasma levels</p> <p>β-HCH 21.1 $\mu\text{g}/\text{kg}$ lipid (cases) 19.4 $\mu\text{g}/\text{kg}$ lipid (HC) 17.5 $\mu\text{g}/\text{kg}$ lipid (PC)</p> <p>p,p' DDE 508.9 $\mu\text{g}/\text{kg}$ lipid (cases) 462.7 $\mu\text{g}/\text{kg}$ lipid (HC) 480.4 $\mu\text{g}/\text{kg}$ lipid (PC)</p> <p>p,p' DDT 12.7 $\mu\text{g}/\text{kg}$ lipid (cases) 12.5 $\mu\text{g}/\text{kg}$ lipid (HC) 11.0 $\mu\text{g}/\text{kg}$ lipid (PC)</p>	<p>Relative Risk (RR)</p> <p>β-HCH RR=0.83 (95% CI = 0.43-1.61, compared to HC) RR=0.80 (95% CI = 0.47-1.35, compared to PC)</p> <p>p,p' DDE RR=1.36 (95% CI = 0.71-2.63, compared to HC) RR=1.00 (95% CI = 0.60-1.67, compared to PC)</p> <p>p,p' DDT RR=1.37 (95% CI = 0.73-2.56, compared to HC) RR=0.81 (95% CI = 0.48-1.37, compared to PC)</p> <p>Significance of trend not measured</p> <p>High β-HCH plasma levels associated with increased risk of large tumour (RR=2.25, 95% CI = 1.12-4.51)</p>	<p>High concentrations of organochlorines were not related to increased breast cancer risk, although high β-HCH levels were suggested to be associated with risk of large tumours.</p> <p>There was no evidence of an interaction between organochlorine exposure and hormonal status of the tumour</p>
<p>Gammon <i>et al.</i> (2002) Cancer Epidemiology, Biomarkers & Prevention, 11, 686-697</p> <p>Population-based case-control study</p> <p>646 cases, 428 controls</p>	<p>Geometric means (plasma)</p> <p>DDE 671.96 ng/g lipid 645.74 ng/g lipid</p> <p>DDT 68.98 ng/g lipid 69.32 ng/g lipid.</p> <p>Dieldrin 20.40 ng/g lipid 21.29 ng/g lipid</p>	<p>Multivariate-adjusted odds ratio (OR)</p> <p>DDE OR = 1.20 (95% CI = 0.76-1.90)</p> <p>DDT OR = 1.15 (95% CI = 0.74-1.79)</p> <p>Dieldrin OR=1.37 (95% CI = 0.69-2.72)</p> <p>Test of trend > 0.05 in all cases</p> <p>No increase in risk due to Breastfeeding status, weight, postmenopausal status, invasive/<i>in situ</i> disease, hormone receptor positive tumour</p>	<p>No evidence that organochlorines (Dieldrin, DDE or DDT) increase breast cancer risk.</p>
<p>Helzlsouer <i>et al.</i> (1999) Cancer Epidemiology, Biomarkers & Prevention, 8, 525-532</p> <p>Nested case-control study Samples taken in 1974 and 1989</p> <p>346 cases, 346 controls</p>	<p>Median levels (serum)</p> <p>p,p' DDE</p> <p>1974 1699 ng/g lipid (cases) 1920 ng/g lipid (controls)</p> <p>1989 1312 ng/g lipid (cases) 1586 ng/g lipid (controls)</p>	<p>Unadjusted OR</p> <p>p,p' DDE</p> <p>1974 OR = 0.73 (95% CI = 0.4-1.32), P for trend = 0.13</p> <p>1989 OR = 0.58 (95% CI = 0.29-1.17), P for trend = 0.15</p>	<p>Evidence that p,p' DDE levels not associated with increased risk of breast cancer. Suggestion that decreased risk associated with high levels of p,p' DDE</p> <p>No evidence that the polymorphisms of GSTM1, GSTT1, GSTP1 and COMT influenced the susceptibility to organochlorine compound effects</p>

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Study/population	Exposure	Results	Conclusions reached
Laden et al., (2001) Int. J. Cancer 91:568-574 Nested case-control study 372 cases, 372 controls	Median levels (plasma) p,p' DDE 0.768µg/g lipid (cases) 0.817µg/g lipid (controls)	Adjusted multivariate Risk Ratio p,p' DDE RR = 0.82 (95% CI = 0.49-1.37) P for trend = 0.15	No evidence of an increased risk of breast cancer among women with relatively high levels of plasma DDE
Lopez-Carillo et al. (2002) European J. Cancer Prevention, 11, 129-135 Hospital-based case-control study 95 cases, 95 controls	Median levels (serum) β-HCH 104.16ng/g lipid (cases) 92.98ng/g lipid (controls)	Adjusted odds ratio β-HCH OR = 1.05 (95% CI = 0.46-2.40), P for trend = 0.80	No evidence of a positive association between risk of breast cancer among women with high serum levels of β-HCH.
Mendonca et al., (1999) Int. J. Cancer, 83, 596-600 Case control study 177 cases, 350 controls	Mean levels (serum) DDE 4.8ng/ml (controls) 5.1ng/ml (cases)	Adjusted odds ratio DDE OR = 0.9 (95% CI = 0.47-1.73) P for trend = 0.78	No evidence of increased risk of breast cancer associated with serum concentration of p,p' DDE.
Schrecker et al. (1997) Arch. Environ. Contam. Toxicology, 33, 453-456 Case control study 21 cases, 21 controls	Mean levels (serum) p,p' DDE 16.67ng/ml (controls) 12.17ng/ml (cases) p,p' DDT 2.37ng/ml (controls) 2.33ng/ml (cases)	Unadjusted odds ratio p,p' DDE OR = 1.14 (95% CI = 0.23-5.98) p,p' DDT OR = 1.21 (95% CI 0.23-5.68)	Results do not indicate an association between p,p' DDE or p,p' DDT and increased breast cancer risk.
Stellman et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 1241-1249 Hospital-based case-control study 232 cases, 323 controls	Median levels (adipose tissue) p,p' DDE 419.2ng/g (cases) 374.1ng/g (controls) p,p' DDT 12.3ng/g (cases) 12.1ng/g (controls) β-HCH 19.8ng/g (cases) 15.8ng/g (controls)	Adjusted odds ratio p,p' DDE OR = 0.74 (95% CI 0.44-1.25), P for trend = 0.3 DDT and β-HCH Odds ratios were not reported, although the authors stated that no associations were found with breast cancer risk	There were no significant odds ratios or trends when the authors considered associations between breast cancer risk and body burden of p,p' DDE, p,p' DDT and β-HCH.
Ward et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 1357-1367 Nested Hospital-based case-control study 150 cases, 150 controls	Mean serum levels p,p' DDE 1260ng/g lipid (controls) 1230ng/g lipid (cases) p,p' DDT 137.7ng/g lipid (controls) 119.5ng/g lipid (cases) β-HCH 63.4ng/g lipid (controls) 60.0ng/g lipid (cases)	Odds ratios p,p' DDE OR = 1.2 (95% CI not quoted) p,p' DDT OR = 0.3 (95% CI not quoted) β-HCH OR = 0.7 (95% CI not quoted) Significance of trend not determined	Results suggestive of an association between p,p' DDE and increased breast cancer risk, however statistical significance of the results not determined There was no positive association between β-HCH or p,p' DDT and breast cancer risk

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Study/population	Exposure	Results	Conclusions reached
<p>Wolff <i>et al.</i> (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 271-277</p> <p>Nested case control study</p> <p>148 cases, 295 controls</p>	<p>Geometric mean serum levels</p> <p>p,p' DDE</p> <p>977ng/g lipid (cases) 1097ng/g lipid (controls)</p>	<p>Adjusted odds ratios</p> <p>p,p' DDE</p> <p>OR = 1.30 (95% CI 0.51-3.35), P for trend = 0.99</p> <p>Levels of DDE were higher in ER negative cases than in their controls, but these differences were not statistically significant</p>	<p>No evidence of a positive association between DDE serum levels and risk of breast cancer</p> <p>No evidence of differences in OC levels in cases and controls with respect to tumour ER status</p>
<p>Wolff <i>et al.</i> (2000) Environmental Research, 84, 151-161</p> <p>Hospital-based case-control study</p> <p>175 cases, 355 controls</p>	<p>Geometric mean serum levels</p> <p>p,p' DDE</p> <p>0.61µg/g lipid (cases) 0.66µg/g lipid (controls)</p> <p>p,p' DDT</p> <p>0.030µg/g lipid (cases) 0.028µg/g lipid (controls)</p>	<p>Adjusted Odds Ratio</p> <p>p,p' DDE</p> <p>OR = 0.93 (95% CI 0.56-1.5), p for trend = 0.499</p> <p>p,p' DDT</p> <p>OR = 1.34 (95% CI 0.82-2.2), P for trend = 0.241</p> <p>DDE and DDT were higher in women with ER-positive tumours than in those with ER-negative tumours, however the differences were not significant after adjusting for age, BMI, menopausal status and race</p>	<p>No evidence of an association between DDE or DDT serum levels and increased risk of breast cancer</p> <p>No evidence of differences in OC levels in cases and controls with respect to tumour ER status</p>
<p>Zheng <i>et al.</i> (2000) Cancer Epidemiology, Biomarkers & Prevention, 9, 167-174</p> <p>Case-control study</p> <p>475 cases and 502 controls</p>	<p>Age and lipid adjusted geometric mean (serum) levels</p> <p>DDE</p> <p>460.1ppb (cases) 456.2ppb (controls)</p>	<p>Adjusted odds ratio</p> <p>DDE</p> <p>OR = 0.96 (95% CI. 0.67-1.36), p for trend = 0.58</p> <p>Further stratification by parity, lactation, and menopausal and estrogen receptor status also showed no significant association with serum levels of DDE.</p>	<p>No evidence of an association between DDE levels and breast cancer risk</p>

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Study/population	Exposure	Results	Conclusions reached
POSITIVE FINDINGS			
<p>Høyer et al. (2000a) Journal of Clinical Epidemiology, 53, 323-330</p> <p>Breast survival analysis (cohort of 7712 women)</p> <p>1st examination (1976-1978), 195 women diagnosed</p> <p>2nd examination (1981-1983), 155 women diagnosed</p> <p>[Analysis of dieldrin, beta-HCH, HCB, op DDT, op DDE, pp DDT, pp DDE, pp DDD and 27 PCB congeners + total PCB]</p>	<p>Mean serum levels</p> <p>p,p' DDE 1379.29ng/g lipid (1st) 1368.26ng/g lipid (2nd)</p> <p>p,p' DDT 166.19ng/g lipid (1st) 119.84ng/g lipid (2nd)</p> <p>β-HCH 284.31ng/g lipid (1st) 271.21ng/g lipid (2nd)</p> <p>Dieldrin 38.41ng/g lipid (1st) 27.78ng/g lipid (2nd)</p>	<p>Relative Risk (RR)</p> <p>p,p' DDE (n=65 for cases, n=65 for controls) (1st) RR=1.08 (95% CI=0.65-1.80) (2nd) RR=2.21 (95% CI 1.07-4.58) p for trend = 0.02 (not seen when adjusted for tumour characteristics)</p> <p>p,p' DDT (n=35 for cases, n=31 for controls) (1st) RR=1.56, 95% CI=0.92-2.64), trend not significant (not shown) (2nd) RR=1.18 (0.39-3.62) trend not significant (not shown)</p> <p>β-HCH (n=65 for cases, n=65 for controls) (1st) RR=1.19 (95% CI=0.7-2.02), trend not significant (not shown) (2nd) RR=1.31 (95% CI=0.61-2.83) trend not significant (not shown)</p> <p>Dieldrin (n=65 for cases, n=65 for controls) (1st) RR=2.71 (95% CI 1.54-4.77), p for trend < 0.01 (2nd) RR=4.55 (95% CI=1.80-11.47), p for trend < 0.01</p>	<p>Evidence to suggest that higher levels of dieldrin may decrease survival rates in women diagnosed with breast cancer.</p> <p>Some evident to support that levels of DDE may be associated with breast cancer risk.</p> <p>No evidence that beta-HCH or DDT may be associated with breast cancer risk.</p>
<p>Høyer et al. (2000b) Cancer Causes and Control, 11, 177-184</p> <p>Nested case-control study</p> <p>155 cases, 274 controls</p> <p>[More limited details of analysis compared to Hoyer et al. 2000a]</p>	<p>Median serum levels (all subjects)</p> <p>p,p' DDT 144.2ng/g lipid 45.7ng/g lipid</p> <p>β-HCH 119.0ng/g lipid 60ng/g lipid</p> <p>p,p' DDE 1196.6ng/g lipid 1168.0ng/g lipid</p>	<p>Adjusted odds ratio (details of numbers not reported)</p> <p>p,p' DDT OR=3.6 (95% CI 1.1-12.2) p for trend = 0.02</p> <p>β-HCH OR=1.2 (95% CI = 0.5-3.0) p for trend not significant (not reported)</p> <p>p,p' DDE OR=1.4 (95% CI = 0.7 - 2.8) p for trend not significant (not reported)</p>	<p>A significant positive association between the average serum concentration of p,p' DDT and breast cancer risk was observed</p> <p>No evidence of an association between p,p' DDE or β-HCH and breast cancer risk</p>

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Study/population	Exposure	Results	Conclusions reached
<p>Høyer et al. (2001) BMC Cancer, 1, 8</p> <p>Nested Case control study</p> <p>161 cases, 161 controls</p> <p>[Details of analysis similar to Hoyer <i>et al.</i>, 2000a]</p>	<p>Median serum level</p> <p>Dieldrin (among breast cancer cases) = 28.3ppb</p> <p>p,p' DDE (among breast cancer cases) = 1,129.75ppb</p>	<p>Odds ratio (OR)</p> <p>Dieldrin OR=7.6 (95% CI=1.3-46.1), p for trend = 0.01 (Estrogen negative tumour, n=45) OR = 1.4 (95% CI=0.8-2.5), p for trend > 0.2 (Estrogen positive tumour, n=116)</p> <p>p,p' DDE OR = 0.9 (95% CI=0.6-1.5), p for trend > 0.2 (estrogen receptor positive, n=45) OR = 0.6 (95% CI=0.2-1.7), p for trend >0.2 (estrogen receptor negative, n=116)</p>	<p>High dieldrin levels were positively associated with increased risk in developing estrogen receptor negative breast cancer.</p> <p>No evidence that levels of DDE associated with breast cancer risk.</p>
<p>Høyer et al. (2002) Breast Cancer Research and Treatment, 71, 59-65</p> <p>Nested case control study</p> <p>240 cases (162 for p53 mutation analysis), 477controls</p> <p>[Limited details of chemicals analysed]</p>	<p>Serum levels of organochlorines were not reported</p>	<p>Dieldrin</p> <p>OR=3.53 (95% CI 0.79-15.79), p for trend=0.12 (P53 mutation, n=36 for cases, n=72 for controls)</p> <p>OR=1.20 (95% CI=0.56-2.58), p for trend=0.60 (Wild type p53, n=123 for cases, n=244 for controls)</p> <p>p,p' DDT OR=0.95 (95% CI=0.30-2.98), p for trend=0.98 (P53 mutation, n=36 for cases, n=72 for controls)</p> <p>OR=1.32 (95% CI=0.68-2.59), p for trend=0.85 (Wild type p53, n=123 for cases, n=244 for controls)</p> <p>p,p' DDE OR=0.81 (95% CI=0.23-2.84), p for trend=0.61 (P53 mutation, n=36 for cases, n=72 for controls)</p> <p>OR=0.96 (95% CI=0.50-1.83), p for trend=0.38 (Wild type p53, n=123 for cases, n=244 for controls)</p> <p>total DDT OR=0.88 (95% CI=0.19-4.17), p for trend=0.78 (P53 mutation, n=28 for cases, n=56 for controls)</p> <p>OR=0.70 (95% CI=0.32-1.55), p for trend=0.98 (Wild type p53, n=86 for cases, n=171 for controls)</p>	<p>A three-fold increase (not significant) in the risk of breast cancer associated with the highest exposure levels of dieldrin among cases with p53 mutations</p> <p>For p,p' DDT, p,p'DDE and total DDT there was no difference in breast cancer risk between cases with or without mutant p53</p>

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Study/population	Exposure	Results	Conclusions reached
<p>Romieu <i>et al.</i> (2000) American Journal of Epidemiology, 152 363-70</p> <p>Case Control study</p> <p>120 cases and 126 controls</p>	<p>Mean serum levels</p> <p>DDE 3.84µg/g lipid (cases) 2.51µg/g lipids (controls)</p> <p>p,p' DDT 0.15µg/g lipid (cases) 0.23µg/g lipid (controls)</p>	<p>Adjusted Odds Ratios (n=120 for cases, n= 126 for controls)</p> <p>DDE (not stratified) Age adjusted OR = 1.59 (95% CI=95% CI 1.09 – 2.32) per loge, unit of lipid adjusted DDE in serum</p> <p>DDE (stratified) Adjusted OR=2.16 (95% CI = 0.85-5.50), p for trend=0.06</p> <p>DDT (not stratified) OR=1.03 (95% CI = 0.74-1.43) per loge, unit of lipid adjusted DDT in serum</p>	<p>Evidence that levels of DDE may be positively associated with increased breast cancer risk</p> <p>No evidence that levels of DDT are associated with increased breast cancer risk</p>
<p>Woolcott <i>et al.</i> (2001) Cancer Causes Control, 12, 395-404</p> <p>Hospital-based case-control study</p> <p>217 cases (ER & PR determined), 213 controls</p>	<p>Geometric means (breast adipose tissue)</p> <p>DDE 596µg/kg lipid (controls) 906µg/kg lipid (ER-) 638µg/kg lipid (ER+)</p> <p>β-HCH 41.5µg/kg lipid (controls) 56.2µg/kg lipid (ER-) 39.3µg/kg lipid (ER+) 54.9µg/kg lipid (large tumours) 39.8µg/kg lipids (small tumours)</p> <p>DDT 19.3µg/kg lipid (controls) 23.5µg/kg lipid (ER-) 21.3µg/kg lipid (ER+)</p>	<p>Odds Ratio (OR)</p> <p>DDE (ER-) OR=2.4 (95% CI=1.0-5.4), P for trend=0.03 (n=51 for cases, n=208 for controls)</p> <p>(ER+) OR=1.1 (95% CI=0.6-1.9), P for trend not shown (n=147 for cases, n=208 for controls)</p> <p>β-HCH (ER-) OR=1.4 (95% CI=0.6-3.2), P for trend not shown (n=51 for cases, n=208 for controls)</p> <p>(ER+) OR=0.7 (95% CI=0.4-1.3), P for trend not shown (n=147 for cases, 208 for controls)</p> <p>DDT OR not shown</p>	<p>Evidence that DDE is associated with increased breast cancer risk in estrogen negative breast tumour cases.</p> <p>No evidence of an association between DDT or β-HCH levels and increased breast cancer risk.</p>

Discussion

11. Brief tabulations of the data previously seen by the committee are given. Table 2 summarised papers seen up to 1995. Table 3 summarises papers between 1995 and 1999 (see pages 14-15 of this report).
12. The COC concluded in 1999 that the available data did not support an association between DDT (and isomers/metabolites), β-HCH, dieldrin and Lindane, and an increasing risk of breast cancer. However, the committee agreed that literature concerning this topic should be kept under review.

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13. Of the nineteen new studies identified, thirteen report no significant effect of organochlorines on breast cancer risk, twelve of these refer to p,p' DDE. Seven of the thirteen reports of negative findings also report no significant effect of p,p' DDT on breast cancer risk. Finally, four of the reports detail that levels of beta-HCH were not associated with increased breast cancer risk, and one study (Gammon *et al.*, 2002) reported that there was no association between levels of dieldrin and breast cancer risk.
14. Members will note that four out of the six studies that report positive findings, refer to data published by a single group (Hoyer *et al.*). Of these reports, three describe the results of dieldrin. Two of these studies (Hoyer *et al.*, 2000a; Hoyer *et al.*, 2001) present significant odds ratios when comparing stratified layers. The third study (Hoyer *et al.*, 2002) presented a very high odds ratio (3.53) in patients with p53 mutations, however the test for trend was not significant ($P = 0.12$). Members will recall that a study undertaken by Hoyer *et al.* (The Lancet, 352, 1816-1819, 1998) claiming a significant association between exposure to dieldrin and breast cancer was reviewed at the March 1999 meeting. Members considered that the study had been adequately conducted but considered that the results might represent a chance finding as a large number of statistical comparisons had been undertaken in the study ($n=46$). In addition there was no convincing evidence from animal studies that dieldrin had any oestrogenic activity in-vivo and members questioned the basis of the a priori hypothesis. Members noted that the authors had not obtained blood samples from subjects who had fasted and this might affect the validity of the analyses when expressed in terms of lipid content. The Chairman of the committee wrote to the editors of Lancet on this topic (appended as Annex 4). The information given in the most recent papers from this group suggest multiple comparisons have been made.
15. The remaining Hoyer *et al.* study (Hoyer *et al.*, 2000b) reports a significant positive association between the p,p' DDT and breast cancer risk. This study uses the average serum organochlorine concentration over the course of two examinations to test a possible association between organochlorine exposure and breast cancer risk. The authors use the fact that no association was seen when considering the first examination only, to suggest that repeated assessment of exposure may provide a more precise estimate than a single exposure. None of the other studies identified since 1999 report an association between DDT and breast cancer risk.
16. Of the nineteen newly reported studies, only three report levels of organochlorines in breast adipose tissue (Aronson *et al.*, 2000; Stellman *et al.*, 2000; Woolcott *et al.*, 2001). Adipose tissue samples have the advantage compared to blood or serum estimations in that breast adipose samples can reflect organochlorine levels to which the breast is exposed over time. Two out of the three studies that measure organochlorines in breast adipose tissue show no association between DDT, DDE and β -HCH

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and increased breast cancer risk (Aronson *et al.*, 2000; Stellman *et al.*, 2001). Although the third study (Woolcott *et al.*, 2001) reports a similar result for DDT and β -HCH, the authors report an association between DDE and increased breast cancer risk in oestrogen negative breast tumour cases. The remaining sixteen studies report organochlorine levels in serum or plasma. Of these, two report organochlorine levels without correcting for lipid (Mendonca *et al.*, 1999; Schrecker *et al.*, 1997). As organochlorines may be carried in the lipid fraction of the blood, expressing levels adjusted for lipid content would be considered a more desirable method for reporting. However, data from these two studies suggest no association between organochlorines and increased breast cancer risk.

17. The study conducted by Romieu *et al.* (2000) in Mexico City reported significantly higher levels of p,p' DDE in the serum of women with breast cancer (mean serum levels = 3.84 μ g/g lipid) than in women without (mean serum levels = 2.51 μ g/g lipid). In a previously published study (Lopez Carrillo *et al.*, 1997), which also used subjects from Mexico City, mean serum levels were 0.56 and 0.51 μ g/g lipid in cases and controls respectively, after converting to the same units. This latter study found no evidence of increased cancer risk associated with DDE levels. The reason for the different results from essentially the same population is not clear. In addition, it is not apparent why the magnitude of the serum DDE levels would be so different (approximately seven-fold) between populations recruited from the same area. Population characteristics were slightly different in that subjects in the Romieu *et al.* study were parous, whereas nulliparity was associated with a higher breast cancer risk in the Lopez-Carrillo *et al.* study. Alternatively, differences in the analytic methods between laboratories may have contributed to the variation. Hoyer *et al.* (2000a) also reported evidence of an association with DDE and breast cancer risk, however this was no longer seen when adjusted for tumour characterisation.
18. At the present time, no new data has been identified regarding Lindane. This may be either be due to levels of this chemical being below the limit of detection, or due to a lack of evidence to support a biologically plausible association with breast cancer.
19. This review has not examined potential mechanisms in detail. More information will be submitted at the June meeting. However, members may wish to comment on the findings reported by Payne *et al.* (2001) in Annex 4.

Overall Conclusions of the review

20. It would appear more information is available on dieldrin and DDE/DDT. Would members consider any revision to existing conclusions on the four organochlorines is required, in particular, to the conclusions on dieldrin?

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(Conclusions on epidemiology from COC 1999 statement)

- DDT* There is considerably more epidemiological data now than in 1995 on environmental exposure to DDT and its isomers and metabolites and a possible association with breast cancer. All of the eight studies published since 1995 investigated p, p DDE, but only two relatively small retrospective studies found evidence for an association between p, p DDE and increased risk of breast cancer. Overall, there is no convincing evidence from epidemiology studies for an elevated relative risk of breast cancer in association with DDT (as measured by p, p DDE).
- Dieldrin* There is very little epidemiological information available on dieldrin and its possible association with breast cancer. Of the two recent studies published after 1995, which considered this insecticide, one found no evidence for an association⁶¹ and the other found a positive association, which was considered likely to be a chance finding. Overall, there is no convincing evidence from epidemiological studies for an elevated relative risk of breast cancer associated with dieldrin.
- γ-HCH* There is very little epidemiological information available on γ-HCH and its possible association with breast cancer. Of the three recent studies published after 1995, which considered γ-HCH, none reported a statistically significant association with increased risk of breast cancer.
- Lindane* There is very little epidemiological information available on lindane (gamma-HCH) and its possible association with breast cancer. Of the three recent studies published after 1995, which considered lindane, none found evidence for an association with increased risk of breast cancer. The available evidence for environmental exposure to lindane suggests that body burdens of this chemical are very small, being undetectable in most individuals. It is therefore unlikely that further epidemiological investigations of breast cancer based on assessment of levels of lindane in adipose tissue, blood, or breast tissue would provide additional relevant information.

21. It is proposed that a draft statement is prepared and any additional literature on estrogenic effect of OCI's and epidemiology studies brought to the November COC meeting.

Secretariat, July 2003

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Table 2: Summary of Epidemiological studies considered by the COC in 1995

Study	Exposure	Result	Conclusion
Unger <i>et al.</i> 1984 (Cross sectional)	DDE measured in mammary adipose tissue from women either with or without breast cancer (Data set 1 (autopsy specimens), set 2 from patients undergoing surgery)	Only results from set 2 were interpretable. No evidence, after adjustment for age that DDE levels were higher in women with breast cancer	No evidence of an association between DDE and breast cancer (n=18, of 35 controls set 1. n=14, 21 controls set 2)
Mussaki-Rauhamaa <i>et al.</i>, 1990 (cross-sectional)	p,p' DDT (p,p' DDE and other metabolites), and β -HCH. Levels in mammary adipose tissue measured in 44 women undergoing surgery and from 33 controls (accidental fatalities)	Significantly higher levels of β -HCH reported in cases compared to controls. OR in person with >0.1mg/kg, after adjustment for parity and age was 10.51 (95% CI 2.00-55.26)	Evidence for association between β -HCH and breast cancer but not for DDT. Data adjusted for other breast cancer risk factors.
Flack <i>et al.</i>, 1992 (cross sectional)	p,p' DDT, p,p' DDE and γ -HCH measured in mammary adipose tissue in 20 women with breast cancer and 20 women with benign breast disease	Levels of p,p' DDT, p,p' DDE were significantly higher on a wet basis (DDE were also elevated when analysed on a lipid basis). No increase in γ -HCH levels reported.	Evidence for association between DDT (DDE) and breast cancer. Data adjusted for BMI and for age but not for other breast cancer risk factors
Wolff <i>et al.</i>, 1993 (Nested case-control from prospective study. Serum samples taken from 14920 women between 1985 -91)	58 women who developed breast cancer 1-6 months after giving a blood sample were studied. Matched control (2/post menopausal (34) and 4 per premenopausal case (24)), n=171 samples examined	Significantly higher DDE levels were reported for cases. An incremental increase in OR was claimed when DDE levels were analysed by quintile which was evident after adjustment for first degree family history, lifetime lactation, age at first full term pregnancy. OR upper quintile compared lowest was 3.68 (95% CI 1.01-13.50)	Evidence for association between DDT (DDE) and breast cancer after adjustment for a number of risk factors.
De Wailly <i>et al.</i> 1994 (Case control study of 20 women with breast cancer with assessment of estrogen receptor status)	DDE and β -HCH measured in breast adipose tissue. (Controls had benign breast disease (adenoma/fibroma, n=17)	DDE levels were significantly higher in ER positive patients (n=9) compared to controls (age adjusted). No differences were documented for DDE RE negative patients or for β -HCH analyses.	Evidence for association between DDT (DDE) and breast cancer in ER positive patients, but analyses based in very limited numbers of cases. No evidence for association between β -HCH and breast cancer.
Krieger <i>et al.</i> 1994 (Prospective study 42,629 white, 8,123 black and 2,288 asian women enrolled from 1964-1969. Follow up to 1990. Random selection of 50 cases from 1805 white, 230 black and 62 asian cases)	Serum DDE measured. Matched controls (n=150) selected from medical programme, using race, age at entering programme and year of examination	No overall difference in serum levels of DDE (unadjusted for lipid level). Serum DDE levels in black cases were higher; the difference was significant after adjustment for BMI age at menarche, age menopause (>55y).	Evidence for association between DDT (DDE) and breast cancer in black women, but overall OR (1.02 (95% CI 1.00-1.04) very small and data on breast cancer risk factors was incomplete

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Table 3: Summary of epidemiological studies considered by the COC between 1995 – 1999

Study/population	Exposure	Results	Conclusions reached
<p>Lopez-Carillo (1997) Cancer Research, 57, 3728</p> <p>141 cases/141 hospital controls (age matched \pm 3y). Approximately 50% of women in case and control group were postmenopausal.</p>	<p>Arithmetic mean serum p,p' DDE in cases 562.48 ± 678.18 ng/g (lipid adjusted)</p> <p>Arithmetic mean serum p,p' DDE in controls = 505.46 ± 567.22 ng/g (lipid adjusted)</p>	<p>Age adjusted OR for tertile 3 compared to tertile 1 was 0.97 (95% CI 0.56-1.70)</p>	<p>No evidence of increased serum between p,p' DDE and breast cancer following analysis based on tertiles of exposure adjusting for known breast cancer risk factors (which were more prevalent in cases).</p>
<p>van't Veer (1997) BMJ, 315, 81.</p> <p>265 postmenopausal cases, 341 age matched population/hospital controls. (Subjects with > 5kg weight loss in past year excluded)</p>	<p>Needle aspirates of subcutaneous fat from buttocks.</p> <p>Mean p,p' DDE concentration in cases = $1.35 \mu\text{Lg/g}$ (95%, CI 1.15-1.58)</p> <p>Mean p,p' DDE concentration in controls = $1.51 \mu\text{g/g}$ (95%, CI 1.31-1.73)</p>	<p>Significant inverse trend between p,p' DDE in subcutaneous fat and adjusted Odds Ratio for breast cancer based on analysis using tertiles of p,p' DDE levels and adjusting for age, centre, BMI, age at first birth and alcohol consumption (OR = 0.48, 95% CI 0.25-0.95).</p>	<p>Thus no evidence for association between DDE and breast cancer.</p>
<p>Hunter (1997) NEJM, 337, 1253-1258.</p> <p>Prospective study of 121,700 nurses from 1976-1992. 32,836 provided blood samples in 1989/90. 240 developed breast cancer. Age matched controls (240) also provided a blood sample.</p>	<p>Plasma lipid p,p' DDE levels in polar solvent extract of plasma lipids</p> <p>Mean concentration 6.01 ± 4.56 ppb after adjustment for cholesterol level (cases)</p> <p>Mean concentration 6.97 ± 5.99 ppb after adjustment for cholesterol level (controls)</p>	<p>Estimate of relative risk for highest P.P' DDE decile compared to lowest was 0.38 (95% CI 0.13-1.09).</p>	<p>No evidence of an association between p,p' DDE and breast cancer following multivariate analysis.</p>
<p>Gutte et al. (1998) Arch Env Contam Tox, 35, 140-147.</p> <p>Breast tissue from 45 breast cancer cases and 20 with benign breast disease.</p>	<p>Arithmetic mean ($\mu\text{g/kg}$) in breast tissue (cases) p,p' DDT 30 p,p' DDE 805 β-HCH 79</p> <p>Lindane found in only 3 samples.</p> <p>Data not adjusted for age. (Cases on average 11y older)</p> <p>Arithmetic mean ($\mu\text{g/kg}$) in breast tissue (controls) p,p' DDT 28 p,p' DDE 496 β-HCH 93</p>	<p>After age adjustment a significant increased P.P' DDE concentration was documented in breast cancer patients A slightly lower β-HCH concentration was reported in cases</p> <p>The authors considered the results to show weakly significant differences but concluded that no definite conclusions could be drawn in the light of other published studies</p>	<p>There is no convincing evidence in this study of an association between DDT/DDE or β-HCH and breast cancer.</p>

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Study/population	Exposure	Results	Conclusions reached
<p>Hoyer <i>et al.</i> (1998) Lancet, 352, 1816-2820</p> <p>Nested case control study (240 cases compared with 477 controls) from a cohort of 7712 women enrolled in Copenhagen City Heart Study in 1976 and followed for 17 years. Two matched controls were used for each case.</p>	<p>Levels (ng/g) of organochlorines in blood (cases and controls)</p> <p>Total DDT 1325.95 p,p' DDT 141.35 p,p' DDE 1182.98 β-HCH 118.94 Dieldrin 24.42</p>	<p>Dieldrin was associated with a significantly increased dose-related risk of breast cancer (unadjusted OR 2.25, 95% CI 1.32-3.84; adjusted OR 2.05, 95% CI 1.17-3.57 highest vs lowest quartile). Linear trend observed for association</p> <p>Slight but not significant increased risk associated with β-HCH (adjusted OR 1.36, 95% CI 0.79-2.33). No linear trend was seen.</p> <p>No association with breast cancer risk with p,p' DDT (or metabolites)</p>	<p>Dieldrin was associated with a significantly increased dose-related risk of breast cancer.</p> <p>Slight but not significant increased risk associated with β-HCH.</p> <p>No association with breast cancer risk with p,p' DDT (or metabolites)</p>
<p>Olaya-Contaras <i>et al.</i> (1998) Cad. Saude Publica, 14, 125-132</p> <p>Hospital based case control 153 histologically confirmed incident cases of breast cancer 153 controls matched for age.</p>	<p>Case patients had mean level of 3.30 ± 4.12 ng/ml DDE in plasma</p> <p>Controls had mean levels of 2.50 ± 3.60 ng/ml DDE in plasma</p>	<p>Risk for breast cancer in the case group were higher than those in the controls (adjusted OR = 1.95, 95% CI 1.10-3.52, last tertile compared with lowest exposure)</p> <p>No linear trend observed for this association.</p>	<p>This study lacks any convincing evidence to support the theory that exposure to DDE is associated with an increase in risk of developing breast cancer.</p>
<p>Dorgan <i>et al.</i> (1999) Cancer causes and Control, 10:1-11</p> <p>Nested case control study from a cohort of 6426 women who donated blood in 1977-1987 as part of the Columbia Missouri Breast Cancer Serum Bank. 105 cases of histologically diagnosed breast cancer were traced during 9.5 years follow-up. Two matched controls were used for each case. 208 control subjects were studied.</p>	<p>45 organochlorine insecticides were measured in the serum</p> <p>Levels of total DDT were 4.8% and 4.3% higher than LOD for cases and controls respectively.</p> <p>Levels of p,p' DDT were 84.8% and 87.4% higher than LOD for cases and controls respectively.</p> <p>Levels of p,p' DDE were 98.1% and 99.5% higher than LOD for cases and controls respectively.</p> <p>Levels of β-HCH were 79.0% and 85.6% higher than LOD for cases and controls respectively.</p> <p>Levels of Dieldrin were 56.2% and 61.8% higher than LOD for cases and controls respectively.</p>	<p>No evidence of an increased risk of breast cancer among women with elevated serum levels of DDT, DDE, β-HCH or Dieldrin.</p>	<p>No evidence of any association between serum concentration of OC insecticide and risk of breast cancer was documented. However the power of the study was low.</p>
<p>Moysich <i>et al.</i> (1998) Cancer Epidemiology, Biomarkers and Prevention, 7, 181-188</p> <p>154 histologically diagnosed post-menopausal cancer patients and 192 post-menopausal community controls were presented.</p>	<p>Case patients had mean level of 11.47 ± 10.49 ng/g DDE in serum</p> <p>Controls had mean levels of 10.77 ± 10.64 ng/g DDE in plasma</p>	<p>There was no evidence of an adverse effect of serum levels of DDE (OR = 1.34; 95% CI 0.71-2.55)</p>	<p>No association between serum DDE levels and risk of breast cancer were reported</p>

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ANNEX 1

Summaries of Additional Epidemiological Studies

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COMMITTEE ON THE CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

UPDATE ON THE BREAST CANCER RISK FROM EXPOSURE TO ORGANOCHLORINE INSECTICIDES: CONSIDERATION OF THE EPIDEMIOLOGY DATA ON DIELDRIN, DDT AND CERTAIN HEXACHLOROCYCLOHEXANE ISOMERS

Summaries of Additional Epidemiological Studies

Negative Findings

Aronson et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9:55-63

22. A hospital-based case-control study was conducted in Ontario, Canada to evaluate the association between breast cancer risk and breast adipose tissue concentrations of various organochlorines. Organochlorines were determined in 217 women diagnosed with in situ or invasive breast cancer, and 213 age-matched controls, who had biopsies negative for malignancy. Breast adipose tissue was analysed for organochlorine content by gas chromatography coupled with electron capture detectors, after standard extraction and cleanup on Florosil columns.
23. Results suggest that levels of organochlorines measured in the breast adipose tissue were different between cases and controls for p,p' DDE (geometric mean = 693 and 596 $\mu\text{g}/\text{kg}$ lipid respectively), but not for p,p' DDT (geometric mean = 22.0 and 19.3 $\mu\text{g}/\text{kg}$ respectively) or β -HCH (geometric mean = 43.1 and 41.5 $\mu\text{g}/\text{kg}$ lipid respectively).
24. Odds ratios were calculated for the whole sample and by menopausal status. For p,p' DDE and to a lesser extent p,p' DDT, there was evidence of increased level of risk, when comparing the upper two quartiles with the lowest, in the whole sample (adjusted OR for p,p' DDE; adjusted OR for p,p' DDT = 1.18, 95% CI 0.61-2.29). However, there was no measure of significance of trend and the authors did not discuss these results further. Similarly, the odds ratio for β -HCH suggests a decreased risk (adjusted OR = 0.69, 95% CI 0.34-1.40, upper two quartiles compared to the lowest), however significance of trend was not calculated. It should be noted that odds ratios for this study were calculated using a confounder model for each specific organochlorine. Confounders were left in confounder model if their deletion caused any organochlorine odds ratio to change more than 10%. Hence, for p,p' DDT, age last breast fed was included in the confounder model, but was not used in the p,p' DDE confounder model. All confounder models included age, study site and menopausal status. Finally, the risk of breast cancer seemed to be higher

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in pre-menopausal women exposed to p,p' DDE (OR = 1.52, 95% CI 0.70-3.33) compared postmenopausal women (OR = 1.05, 95% CI 0.50-2.19) when comparing the upper two quartiles with the lowest.

Demers et al., (2000) Cancer Epidemiology, Biomarkers & Prevention, 9:161-166

25. A hospital based case control study was conducted between October 1994 and March 1997 in Quebec City, Canada. 315 women with histologically confirmed infiltrating primary breast cancer and 219 controls (free of gynaecological illness, but admitted for surgery that was not breast cancer related (e.g. digestive, orthopedic, vascular etc) were recruited. A second control group included 307 women randomly selected from the general population. Cases and control were matched for age and region of residence. Hospital controls had to be free of gynaecological illnesses.
26. In order to measure levels of organochlorines, a standard extraction procedure was used, followed by cleanup on Florisil columns. Quantification was by gas chromatography equipped with electron capture detectors and was adjusted for lipids. Mean plasma levels of β -HCH were 21.1 $\mu\text{g}/\text{kg}$ lipid, 19.4 $\mu\text{g}/\text{kg}$ lipid and 17.5 $\mu\text{g}/\text{kg}$ lipid for cases, hospital controls and population controls respectively. Mean plasma levels of p,p' DDE were 508.9 $\mu\text{g}/\text{kg}$ lipid, 462.7 $\mu\text{g}/\text{kg}$ lipid and 480.4 $\mu\text{g}/\text{kg}$ lipid for cases, hospital controls and population controls respectively. Mean plasma levels of p,p' DDT were 12.7 $\mu\text{g}/\text{kg}$ lipid, 12.5 $\mu\text{g}/\text{kg}$ lipid and 11.0 $\mu\text{g}/\text{kg}$ lipid for cases, hospital controls and population controls respectively. High concentrations of β -HCH were not related to breast cancer risk, whatever the control group used (e.g. the adjusted RR was 0.8, 95% CI 0.47-1.35, highest quintile relative to the lowest quintile and comparing population controls). The results suggest that levels of DDE and DDT were associated with an increase in breast cancer risk when compared to hospital controls (RR=1.36 (95% CI = 0.71-2.63) and 1.37 (95% CI = 0.73-2.56), respectively), but significance of trend was not determined and the association was not seen when compared to population controls (RR=1.00 and 0.81, respectively).
27. The authors carried out additional statistical analysis to investigate the possible relation of the aggressiveness of breast cancer to plasma organochlorine concentrations. After adjusting for age, region of residence, BMI, breast feeding duration, number of fertile years, and time separating blood sampling from surgery, the relative risk of having a large tumour increased with plasma concentrations of most organochlorines, but were reported to be only statistically significant for β -HCH (OR = 2.27, 95% CI 1.11-4.65, comparing the highest to the lowest tertiles). Further investigation of the relationship of breast cancer aggressiveness to plasma concentration of p,p' DDE was undertaken by considering both tumour size and lymph-node involvement in the same model. Results indicated that that levels of p,p' DDE were associated with a dose related increased relative risk of exhibiting both lymph-node involvement and a large tumour.

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28. Although the data was not shown, the authors reported that women with ER-negative tumours and those with ER positive tumours had similar organochlorine plasma levels and furthermore, there was no interaction between organochlorine exposure and the hormonal status of the tumour with regard to either axillary-lymph-node involvement or tumour size.

Gammon et al. (2002) Cancer Epidemiology, Biomarkers & Prevention, 11:686-697

29. A population-based case-control study is reported, based on data collected as part of the Long Island Breast Cancer Study. Blood samples from 646 cases (415 random, 184 *in situ*, 5 African-American, 42 invasive) and 428 controls were selected between 1996 and 1997. It perhaps should be noted that the authors report that the participants were primarily white women.

30. Organochlorines were measured in plasma by a method involving solid phase extraction (no other details given). Geometric means of DDE were 671.96ng/g lipid and 645.74ng/g lipid in cases and controls respectively. Geometric means of DDT were 68.98ng/g lipid and 69.32ng/g lipid in cases and controls respectively. Geometric means of dieldrin were 20.40ng/g lipid and 21.29ng/g lipid in cases and controls respectively.

31. Slightly elevated, but not significant, multivariate-adjusted ORs were noted for the highest quintile compared with the lowest, of DDE (OR=1.20, 95% CI, 0.76-1.90), DDT (OR=1.15, 95% CI=0.74-1.79) and dieldrin (OR=1.37, 95% CI=0.69-2.72). No dose response relationship was evident for any of these organochlorines (test for trend $p > 0.05$).

32. No increase in risk in relation to organochlorines was apparent among women who had not breastfed, or who were overweight, postmenopausal, or long term residents of Long Islands, or with whether the case was diagnosed with invasive rather than *in situ* disease, or with a hormone receptor positive tumour.

Helzlsouer et al. (1999) Cancer Epidemiology, Biomarkers & Prevention, 8:525-532

33. A nested case control study was conducted to examine the association between serum concentrations of p,p' DDE and the development of breast cancer up to 20 years later. 346 cases and 346 controls were selected from cohorts of women who donated blood in 1974, 1989, or both. Cases were matched to controls based on age, race, menopausal status, and month and year of blood donation. Analyses were carried out according to cohort participation because the median DDE concentration among controls was 59% higher in 1974 than 1989. By using cohorts from time periods allowed the examination of long-term effects of exposure to high levels of organochlorine compounds near to the time the compounds were banned as well as more recent, lower levels of exposure.

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34. DDE was assayed using solid-phase extraction followed by gas chromatography with electron capture detection. Median serum levels of DDE in 1974 were 1698.9ng/g lipid and 1920.3ng/g lipid for cases and control respectively. In 1989, median serum levels were 1311.9ng/g lipid and 1586.3ng/g lipid for cases and control respectively. The authors noted that the risk of breast cancer did not increase with increasing serum concentrations of DDE. In fact, the risk of breast cancer seemed to be lowest among women with the highest concentration of DDE (1974, OR = 0.73, upper quintile compared with lowest quintile, 95% CI 0.40-1.32, p for trend = 0.13; 1989 OR = 0.58, upper tertile compared with lowest tertile, 95% CI 0.29-1.17, p for trend = 0.15). These odds ratios are unadjusted, as the authors claim that known and suspected risk factors (family history of breast cancer, BMI, age at menarche, age at first birth and duration of lactation) did not alter the point estimates of the odds ratios.
35. The authors report that there was no evidence that the putative high-risk polymorphisms of GSTM1, GSTT1, GSTP1 and COMT influenced the susceptibility to organochlorine compound effects.

Laden et al., (2001) Int. J. Cancer 91:568-574

36. Previously this group published an analysis of DDE and breast cancer risk in a case-control study of 238 incident cases, nested in the Nurses' Health Study (Hunter *et al.*, 1997). Members of the COC reviewed this analysis in CC/98/24 and agreed with the authors that there was no evidence of an increased risk of breast cancer among women with relatively high levels of plasma DDE. Laden *et al.* (2001) report an extended follow-up in the Nurses' Health Study in which an additional 143 cases (diagnosed between June 1992 and June 1994) of invasive postmenopausal breast cancer are considered.
37. The method used to measure organochlorines in the plasma was essentially by chromatographic cleanup of plasma lipids and subsequent analysis by gas chromatography with electron-capture detection. Median plasma levels of DDE in cases (n=372) and controls (n=372) were 0.768 and 0.817µg/g lipid, respectively. The addition of the extra 143 cases and their controls, however, did not substantially change the relationship between levels of DDE and breast cancer risk compared to that observed before (Hunter *et al.*, 1997). There was no evidence of an increased risk of breast cancer among women with relatively high levels of plasma DDE (fifth quintile compared with the first: multivariate RR = 0.82, 95% CI 0.49-1.37, adjusted for history of breast cancer in mother or a sister, history of benign breast disease, age at menarche, body mass index, number of children and age at birth of first child and duration of lactation, p for trend = 0.15). The authors reported that exclusion of 55 pairs in which the case was diagnosed within 6 months of blood sampling had little change (multivariate RR = 0.76, 95% CI = 0.44-1.32, upper quintile compared to the lowest, p for trend = 0.09) on the findings.

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Lopez-Carillo et al. (2002) European J. Cancer Prevention, 11:129-135

38. Results from a hospital-based case-control study among Mexican women are presented, which focus on the potential relationship between serum levels of β -HCH and the risk of breast cancer. 95 histologically confirmed new breast cancer patients and 95 age-matched non-cancer controls were recruited from Mexican hospitals between March 1994 and April 1996. The study was performed as an arm of a larger hospital-based case-control study.
39. β -HCH levels were measured in serum by electron capture gas-liquid chromatography using a protocol recommended by the US Environmental Protection Agency. Levels of β -HCH did not differ between cases (median = 104.16ng/g lipid) and controls (median = 92.98ng/g lipid) in the study population. When adjusted by age at menarche, number of children and age at first birth, lifetime lactation, family history of breast cancer, menopausal status and Quatelet status, the odds ratio for breast cancer by serum level of β -HCH was 1.05 (95% CI = 0.46-2.40, upper tertile compared with the lowest), which was not statistically significant ($p=0.80$).

Mendonca et al., (1999), 83:596-600

40. A case control study was carried out in a Brazilian hospital. 177 cases of invasive breast cancer and 350 controls were selected between May 1995 and July 1996. Information was collected on occupational and environmental exposure to pesticides and other factors associated with breast cancer. Serum was analysed for a number of organochlorines including DDT and its metabolites, β -HCH and Dieldrin, however, only p,p' DDE was detected in a high proportion of cases.
41. Organochlorine residues were analysed by gas chromatography equipped with electron capture and quality-control protocols were employed. Mean serum levels of DDE were 4.8 and 5.1ng/ml in control and cases, respectively. Presumably, there was no attempt to adjust for lipids. No statistically significant association was found between breast cancer risk and serum level of DDE or history of exposure to organochlorine pesticides. The age-adjusted odds ratio of breast cancer for women in the upper quintile compared with those in the lowest quintile was 0.90 (95% CI, 0.47-1.73), however this trend was not statistically significant (p for trend = 0.78). The OR's of breast cancer associated with serum concentration of p,p' DDE did not change when controlling for possible confounders (age, educational level, parity, lactation, tobacco smoking, family history of breast cancer and breast size).

Schrecker et al. (1997) Arch. Environ. Contam. Toxicology, 33:453-456

[missed in last review]

42. The authors present results of a case-control study that was conducted in Hanoi during the summer of 1994. It is important to consider that only a

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small group of patients were recruited. 21 patients, recruited as study cases, had histologically confirmed invasive adenocarcinomas of the breast. The controls were women who were diagnosed with fibrocystic disease of the breast at the same hospital and during the same calendar time. The controls were individually matched to the cases by place of residence and by age at diagnosis.

43. Organochlorine levels were measured by gas chromatography with electron capture detection, after standard solvent extraction and fractionation using column (Florisil) chromatography. The mean serum concentration of p,p' DDE was slightly higher among the controls (16.67ng/ml) than the cases (12.17ng/ml), although the difference was small and not statistically significant. It should also be noted that there was no lipid adjustment in the determination of organochlorines. There was also no evident case-control difference in serum p,p' DDT levels (2.37ng/ml and 2.33ng/ml in controls and cases, respectively). The authors reported that there was no apparent association between p,p' DDE and p,p' DDT and breast cancer among this population of northern Vietnamese residents. An unadjusted odds ratio of 1.14 (95% CI 0.23-5.98) was reported for p,p' DDE and 1.21 (95% CI 0.23-5.68) for p,p' DDT. There was no effect (data not shown) of adjustment for potential confounders (age at menarche, parity, age at first full-term pregnancy, breast-feeding history and Quetelet index).

Stellman et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9:1241-1249

44. A hospital-based case control study was conducted from October 1994 through October 1996 in New York City. 232 cases (199 invasive and 33 carcinoma *in situ*) and 323 controls (250 benign breast and 73 surgical patients) were selected. Cases were women with newly diagnosed malignant breast cancer or carcinoma *in situ*. Controls included patients diagnosed with benign breast diseases and women undergoing non-breast-related surgery in which small amounts of adipose tissue would ordinarily be removed.
45. Levels of organochlorines were determined using a method based on supercritical fluid extraction and simultaneous *in situ* removal of the bulk of fat on a partially deactivated neutral alumina sorbent and cleanup by adsorption chromatography followed by capillary gas chromatography with electron capture detection. Measured levels of p,p' DDE were above LOD for all women. Median levels of p,p' DDE were 419.2ng/g for cases and 374.1ng/g for controls. Median levels of p,p' DDT were 12.3ng/g and 12.1ng/g for cases and controls respectively. Median levels of β -HCH were 19.8ng/g and 15.8ng/g for cases and controls respectively. The authors report no significant difference between cases and controls for all pesticides except β -HCH. β -HCH exceeded LOD in a significantly higher proportion of cases than controls (98.3% vs 94.1%).

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46. There were no significant odd ratios or trends when the authors considered associations between breast cancer risk and body burden of p,p' DDE (OR = 0.74, 95% CI 0.44-1.25, upper tertile compared to the lowest, adjusted for age and BMI, hospital and race, p for trend = 0.3). In addition, there were no significant associations when the odds ratios were stratified by estrogen receptor (ER) level (ER+ or ER-). Although the data was not shown, the authors report no significant interaction terms between menopausal status and body burden of exposure to organochlorine compounds.

Ward et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9:1357-1367

47. The authors report a case control study of serum organochlorine levels in relation to breast cancer risk using stored sera collected from 1973 through 1991 from the Janus Serum Bank in Norway. Breast cancer cases were ascertained prospectively from among 25,431 female serum bank donors. A total of 272 individuals who developed breast cancer were identified in this cohort and from this group, 150 individuals who had a blood sample taken 2 or more years before diagnosis were randomly selected. 150 controls were matched to cases by birth dates and dates of sample collection.

48. Serum organochlorines were isolated using a solid phase extraction and subsequent cleanup procedure. Analytes were separated in a capillary column and quantified using selected ion monitoring mass spectrometry. Mean serum concentration of p,p' DDE was 1260ng/g lipid in controls and 1230ng/g lipid in cases. Mean serum concentration of p,p' DDT was 137.7ng/g lipid in controls and 119.5ng/g lipid in cases. Mean serum concentration of beta-HCH was 63.4ng/g lipid in controls and 60.0ng/g lipid in cases.

49. The study revealed that negative associations were observed between levels of β -HCH (OR = 0.7, upper quartile compared to the lowest) and p,p' DDT (OR = 0.3, upper quartile compared to the lowest) and breast cancer risk. Although a positive association between levels of p,p' DDE and breast cancer risk was observed (OR = 1.2, upper quartile compared to the lowest), the significance of trend was not determined.

Wolff et al. (2000) Cancer Epidemiology, Biomarkers & Prevention, 9:271-277

50. The Committee previously considered a paper by the group, which reported elevated risk for breast cancer and DDE in 58 women with breast cancer from New York City Women's Health Study (see table 1). This new paper details the selection of 148 breast cancer cases and 295 matched controls from the same study, which originally enrolled 14,275 healthy women between 1985 and 1991. From this cohort, breast cancer cases identified through active follow-up, which were diagnosed up to October

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1994 with a lag phase of 6 months or more between blood donation and diagnosis, were eligible.

51. Geometric mean serum levels of p,p' DDE were 977ng/g lipid and 1097ng/g lipid for cases and controls respectively. The authors report no evidence of a positive association between DDE serum levels and risk of breast cancer (OR = 1.30, 95% CI 0.51-3.35, upper quartile compared to the lowest; adjusted for age at menarche, number of full-term pregnancies, age at first full-term pregnancy, family history of breast cancer, lifetime history of lactation, height, BMI, menopausal status; p for trend = 0.99). Although the data is not shown, the authors also note that women with DDE and PCB levels in the highest tertile did not have an elevated risk of breast cancer when compared with women with levels of these organochlorines in the lowest tertile. In addition, there was no evidence of effect modification by menopausal status or lactation history (data also not shown). ER status was available for 90 women. Levels of DDE were higher in ER negative cases than in their controls, but these differences were not statistically significant. Levels of DDE were very similar in the ER positive cases and their controls. In addition, there were no statistically significant differences in OC levels between ER+ and ER- cases.

Wolff et al. (2000) Environmental Research, 84:151-161

52. A hospital-based case control study of breast cancer risk related to organochlorine exposure was carried out in New York City. 175 breast cancer patients and 355 control patients were selected, with an overall distribution of 57% Caucasian, 21% Hispanic and 22% African-American. Serum was analysed for a number of organochlorines including DDT and its metabolites.

53. Geometric mean serum levels of p,p' DDE were 0.61 and 0.66µg/g lipid for cases and controls respectively. Geometric mean serum levels of p,p' DDT were 0.030 and 0.028µg/g lipid for cases and controls respectively. Higher serum levels of p,p' DDE were found in African-American women than in Hispanic or Caucasian women. When adjusted for age, menopausal status and race, there was no evidence of an association between p,p' DDE levels and breast cancer risk (adjusted OR = 0.93, upper tertile compared with lower tertile, 95% CI 0.56-1.5). Using similar adjustment there was a suggestive increase in the risk of breast cancer with DDT (adjusted OR = 1.34, upper tertile compared with lower tertile, 95% CI 0.82-2.2), however the trends were not significant (p = 0.499 and 0.241, respectively). Although the data was not shown, the authors reported that analysis of breast cancer with respect to organochlorine exposure stratified by BMI, family history, lactation history and parity revealed no significant findings.

54. In addition, the authors examined levels of organochlorines in relation to tumour markers. DDE and DDT were higher in women with ER-positive tumours than in those with ER-negative tumours, however the differences

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were not significant after adjusting for age, BMI, menopausal status and race.

Zheng et al. (2000) *Cancer Epidemiology, Biomarkers & Prevention*, 9:167-174

55. A case-control study was designed to investigate the relationship between DDE and breast cancer risk in Connecticut. Cases were incident breast cancer patients and controls were randomly selected from local residents or from patients who had newly diagnosed benign breast diseases or normal tissue. 475 cases and 502 controls had their serum samples analysed for DDE between 1995 and 1997.
56. In order to measure serum levels of DDE the authors used a standard solvent extraction, Florisil purification, and gas chromatography with electron capture detection procedure. Age and lipid adjusted geometric mean was 460.1ppb for cases and 456.2ppb for controls. After adjustment for confounding factors (BMI, age at menarche, lifetime months of lactation, age at first full-term pregnancy, number of live births, lifetime months of hormone replacement therapy, dietary fat intake, family breast cancer history, income, race and study site), an odds ratio of 0.96 (95% CI. 0.67-1.36) for DDE were observed when the third tertile was compared with the lowest (p for trend = 0.58). Further stratification by parity, lactation, and menopausal and estrogen receptor status also showed no significant association with serum levels of DDE.

Positive Findings

Høyer et al. (2000a) *Journal of Clinical Epidemiology*, 53:323-330

57. These authors have previously reported that women with high serum concentration of Dieldrin had more than a two-fold increased risk of developing breast cancer compared to those with the lowest concentration. These results have been previously considered by Committee members (see Table 3). The current paper details the results of breast cancer survival analysis presented in relation to organochlorine concentrations in banked serum samples.
58. A cohort of 7,712 women was recruited between 1976-1978 and volunteers were given follow-up questionnaires and examinations at two time points (at 1976-1978 and 1981-1983) where blood samples were taken. A total of 195 women who attended these examinations developed breast cancer and were included in the analysis. Forty of the cases were diagnosed between the first and second examination and 155 were diagnosed after the second examination.
59. Organochlorines were measured using a two-stage solid-phase extraction and gas chromatography analysis. All analytes were expressed on a lipid basis. Mean serum levels of organochlorines declined between the two

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examinations (mean p,p' DDE concentration decreased from 1379.29ng/g lipid to 1368.26ng/g lipid; mean p,p' DDT concentrations decreased from 166.19ng/g lipid to 119.84ng/g lipid; mean β -HCH concentrations decreased from 284.31ng/g lipid to 271.21ng/g lipid; mean dieldrin concentration decreased from 38.41ng/g lipid to 27.78ng/g lipid). It should perhaps be noted that DDE and β -HCH decreased only a small amount between 1976/78 and 1981/3.

60. The authors report that a high serum dieldrin concentration, measured after the first examination, was significantly associated with an increased overall mortality (RR = 2.71, 95% CI 1.54-4.77, upper quartile compared to the lowest quartile, adjusted for age). Confounders such as BMI, age at menopause, and hormone replacement therapy, did not influence this result. In addition, a significant dose response relationship was observed (p for trend = <0.01). A similar, but stronger association was seen among women diagnosed with breast cancer after the second examination, when breast cancer cases with the highest average serum concentration of dieldrin had a more than fivefold increased risk of dying compared to those with an average serum concentration in the lowest quartile (OR= 4.55, 95% CI=1.80-11.47, p for trend < 0.01).
61. No significant association was found between overall survival and any other organochlorine, although a dose response relationship was seen for p,p' DDE (RR=2.21, 95% CI = 1.07-4.58, p for trend=0.02) after the second examination. This relationship, however, was not significant when adjusted for tumour characteristics. In addition, beta-HCH was not association with breast cancer risk (RR=1.19 (95% CI=0.7-2.02, trend not significant (not shown)) after the first examination or the second (RR=1.31, 95% CI = 0.61-2.83, trend not significant (not shown)).
62. The authors therefore propose that past exposure to estrogenic organochlorines such as dieldrin may not only affect the risk of developing breast cancer but also the survival.

Høyer et al. (2000b) Cancer Causes and Control, 11:177-184

63. A nested case-control study was carried out, based on a random sample of 7,712 females who participated in the Copenhagen City Heart Study. Participants donated blood twice, in 1976-1978 and 1981-1983. 155 cases and 274 matched breast cancer free controls were selected.
64. Samples were analysed for organochlorines, at both time points, using a two-stage solid phase extraction and clean-up procedure followed by dual-column gas chromatography separation and electron detection. Serum organochlorine levels decreased between the two examinations, however results are reported independent of case-control status. Levels of β -HCH decreased from 119.0ng/g lipid to 60ng/g lipid. Levels of dieldrin decreased from 25.3ng/g lipid to below the limit of detection. Serum levels of p,p' DDT and p,p' DDE were 144.2ng/g lipid and 1196.6ng/g lipid

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(respectively) measured after the first examination and 45.7ng/g lipid and 1168ng/g lipid (respectively) measured after the second examination.

65. A significant positive association between the average serum concentration of p,p' DDT (over the two examinations) and breast cancer risk was observed (OR=3.6, 95% CI 1.1-12.2, adjusted for weight changes between the two examinations, upper quartile compared to the lowest, p for trend = 0.02). β -HCH and p,p' DDE showed no significance of trend (adjusted OR for β -HCH = 1.2, 95% CI = 0.5-3.0, upper quartile compared to the lowest, p for trend not significant (not reported); adjusted OR for p,p' DDE = 1.4, 95% CI = 0.7 - 2.8, upper quartile compared to the lowest, p for trend not significant (not reported)). Odds ratios for dieldrin were not reported. It should be noted that it is difficult to understand the differences between this study and the previous (Hoyer *et al.* 2000a) when it seems that the same subjects from the same cohort are used.

Høyer et al. (2001) BMC Cancer, 1:8

66. The authors report a cohort-nested case-control study to investigate breast cancer risk and survival according to estrogen receptor status, where the case group consisted of 268 women diagnosed with primary breast cancer. The women were selected from a cohort of 10,317 women randomly recruited between 1976-78 for the Copenhagen City Heart Study and followed-up until 1993. A random sample of 536 women were recruited as controls from the remainder of the cohort and matched to cases on the basis of age and vital statistics. It is not known how this population differs from the two previous studies.

67. Stored serum was analysed for organochlorine compounds using a two-stage solid-phase extraction and clean up followed by dual-column gas chromatographic separation and electron capture detection. 161 cases (67.1%) and their matched controls were eligible for breast cancer risk according to estrogen receptor. Median serum level of dieldrin among breast cancer cases was 28.3ppb. Median serum level of p,p' DDE among breast cancer cases was 1,129.75ppb. Serum levels of organochlorines in controls were not reported.

68. Women with the highest serum concentration of dieldrin had a more than seven-fold increased risk of developing an estrogen receptor negative breast cancer compared to women with the lowest concentration. This risk increased in a stepwise fashion with increasing dieldrin exposure level (OR=7.6, 95% CI 1.3-46.1, upper quartile compared to the lowest quartile, adjusted for age, weight, parity and hormone replacement therapy, p value for trend 0.01). No association was observed for estrogen positive tumours (adjusted OR = 1.4, 95% CI 0.8-2.5, p for trend > 0.2). For the remainder of the organochlorine compounds OR's for the highest exposure levels tended to be higher for women who developed an estrogen receptor positive breast cancer than those who developed an estrogen receptor negative breast cancer, although none of the

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relationships were statistically significant (OR for p,p' DDE (estrogen receptor positive) = 0.9, 95% CI 0.6-1.5, upper quartile compared to the lowest, p for trend >0.2; OR for p,p' DDE (estrogen receptor negative) = 0.6, 95% CI 0.2-1.7, upper quartile compared to the lowest, p for trend >0.2)

69. Results from the survival analysis indicated an approximately 2-fold increased risk of dying among women in the highest dieldrin exposure level compared to the lowest, for both estrogen receptor positive and negative tumours. However, these increases in risk were not statistically significant.

Høyer et al. (2002) Breast Cancer Research and Treatment, 71:59-65

70. The authors investigated whether mutation in the tumour suppresser gene p53 affected organochlorine exposure related breast cancer risk and survival. Between 1976 and 1978, 7712 women were recruited to the Copenhagen City Heart Study. 268 were diagnosed with breast cancer during the 17-year follow-up. From the remainder of the cohort, two women per case were selected randomly to act as controls, after matching for age and vital statistics at the time of diagnosis. It is not known how this population differs from the three previous studies

71. Organochlorine analysis involved a two-stage solid phase extraction and clean-up followed by dual-column gas chromatographic separation and electron capture detection. The results (not reported) were adjusted for total serum lipid content. Valid organochlorine analysis could be obtained from 240 cases and 477 controls. 162 cases had suitable specimens for the detection of p53 mutations by denaturing gradient gel electrophoresis of PCR amplified DNA.

72. A three-fold increase in the risk of breast cancer associated with the highest exposure levels of dieldrin among cases (n=36) with p53 mutations, however this was not significant (OR = 3.53, 95% CI 0.79-15.79, p for trend = 0.12). This was not seen in cases (n=123) with wildtype p53 (OR = 1.20, 95% CI=0.56-2.58, p for trend = 0.60). For p,p' DDT, p,p'DDE and total DDT there was no difference in breast cancer risk between cases with p53 mutation (OR=0.95 (95% CI=0.30-2.98), p=0.98; OR=0.81 (95% CI=0.23-2.84), p=0.61; OR=0.88 (95% CI=0.19-4.17), p=0.78; respectively; upper quartile compared to the lowest) or without (OR=1.32, p=0.85; OR=0.86, p=0.38; OR=0.70, p=0.98; respectively; upper quartile compared to the lowest).

73. There was no indication of a poorer prognosis among cases exhibiting mutant p53 when adjusted for other tumour characteristics. When the relative risk of dying according to organochlorine levels stratified by p53 mutation status was evaluated, dieldrin exposure appeared to be significantly associated with breast cancer cases with 'wild-type' p53 mutation status (RR = 3.31, beta-coefficient = 0.0055, p-value = 0.01).

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74. The authors propose that these preliminary results suggest that p53 mutations may have a modifying effect on at least the breast cancer risk associated with exposures to organochlorines.

Romieu et al. (2000) American Journal of Epidemiology, 15:363-70

75. The paper details an investigation into the relationship between lactation history, organochlorine serum levels and the risk of breast cancer. In particular, serum levels of p,p' DDT and p,p' DDE were measured in a sub-sample from a larger breast cancer case-control study conducted among women living in Mexico City between 1990 and 1995. 260 subjects were selected from the original study. Analysis was restricted to 120 cases and 126 controls. Subjects had all given birth to at least one child and had complete information on all key variables.

76. In order to measure serum levels of organochlorines the authors used a standard solvent extraction, Florisil purification, and gas chromatography with electron capture detection procedure. A QC procedure included running internal controls with each batch of samples and accuracy was verified by inter-laboratory comparisons. Overall the mean DDE levels were significantly higher among cases (mean = 3.84µg/g lipid) than among controls (mean = 2.51µg/g lipids). These differences were more apparent for postmenopausal women than for pre-menopausal women. In contrast, serum levels of p,p' DDT were slightly higher in controls (mean = 0.23µg/g lipid) than cases (mean = 0.15µg/g lipid).

77. Serum DDE levels were significantly related to breast cancer risk (age adjusted OR = 1.59 per loge, unit of lipid adjusted DDE in serum, 95% CI 1.09 – 2.32 per loge unit of lipid adjusted DDE in serum). The authors further considered the DDE data as quartiles, using three different models according to the adjustment factors used. When adjusting for age only there was a positive trend in the risk of breast cancer with increasing levels of serum DDE (OR = 2.16, 95% CI 0.96-5.07, comparing upper quartile with the lowest, test for trend p = 0.03). This positive trend was also reflected when there was adjustment for age, age at menarche, duration of lactation, Quetelet index (measure of obesity) and menopausal status (Model 2, OR=2.16, 95% CI = 0.85-5.50, upper quartile compared to the lowest, p for trend=0.06) and also Model 3 (OR=3.81, 95% CI = 1.14-12.8, upper quartile compared to the lowest, p for trend=0.02) where the additional factor of serum DDT levels were used.

78. When the relation between DDE and menopause status was evaluated, the risk of breast cancer was reported to be stronger in postmenopausal women than that observed in the total sample (adjusted OR (model 1) = 3.46, 95% CI 0.78-15.37, when upper quartile was compared to the lowest, test for trend p = 0.02). When there was adjustment for age, age at menarche, duration of lactation, Quetelet index and menopausal status (Model 2) the positive trend was less significant (adjusted OR = 2.41, 95% CI 0.47-12.30, upper quartile compared to lowest, test for trend, p = 0.02).

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When the additional factor of serum DDT levels was added (Model 3) the positive became more significant (adjusted OR = 5.26, 95% CI 0.8-34.30). In contrast, the risk of breast cancer was reported to be less strong in pre-menopausal women in each of the models used [NB: CIs almost all span 1.0].

79. Serum DDT levels were not related to breast cancer risk (age adjusted OR = 1.03 per loge, unit of lipid adjusted DDT in serum, 95% CI 0.74 – 1.43 per loge unit of lipid adjusted DDT in serum). This data was not treated as for DDE, i.e. stratified, therefore no assessment of trend was made.

Woolcott et al. (2001) Cancer Causes Control, 12:395-404

80. The authors evaluated the association between organochlorines and breast cancer subtype defined by tumour characteristics (estrogen receptor status, progesterone receptor status, tumour size, and grade). A hospital biopsy case-control study was carried out using 217 cases (*in situ* or invasive breast cancer) and 213 controls (biopsy negative for malignancy). Controls were frequency-matched to the cases by age in 5-year groups and study site.

81. Organochlorines were measured by gas chromatography with electron capture detectors. Levels of estrogen receptor (ER) and progesterone receptor (PR) in the tumours of the cases were determined by immunohistochemistry and/or enzyme immunoassay. In general, the control group had lower breast adipose tissue concentrations of all organochlorines than cases. Furthermore, the concentration of DDE and β -HCH was higher in ER-negative cases (906 μ g/kg lipid and 56.2 μ g/kg lipid, respectively) than ER-positive cases (638 μ g/kg lipid and 39.3 μ g/kg lipid, respectively), but there was no difference between PR-negative cases and PR-positive cases (not shown). The concentration of β -HCH was significantly higher in cases with large tumours (54.9 μ g/kg lipid) than cases with smaller tumours (39.8 μ g/kg lipid) ($p < 0.05$). Similar levels of DDT were seen in controls (19.3 μ g/kg lipid), estrogen negative tumour cases (23.5 μ g/kg lipid) and estrogen positive tumour cases (21.3 μ g/kg lipid). The concentration of DDE, DDT and β -HCH was higher among cases with more poorly differentiated tumours than cases with moderately or well-differentiated tumours ($p < 0.05$). Levels of DDE, DDT and β -HCH in controls were 596 μ g/kg lipid, 19.3 μ g/kg lipid and 41.5 μ g/kg lipid respectively.

82. Breast adipose tissue concentrations of DDE were more strongly positively associated with risk of ER-negative breast cancer. The OR for risk of ER-negative breast cancer in the uppermost tertile, compared with the lowest tertile, was 2.4 (95% CI 1.0 - 5.4), and the p-value for the test of the linear trend was 0.03. In explanation of this finding, the authors suggest that tumours that are receptor-negative may have a faster rate of progression than tumours that are receptor-positive after diagnosis.

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83. Breast adipose tissue concentrations of β -HCH were more strongly negatively associated with risk of ER positive breast cancer (OR = 0.7, 95% CI 0.4-1.3, upper tertile compared with the lowest tertile, test of trend not given). Results also suggest that there was no association between DDE and progesterone receptor status. Breast adipose tissue concentrations of DDE were only weakly associated with risk of tumours less than 2cm diameter, but were not associated at all with tumours 2cm or larger. In addition, the OR for DDE was only slightly higher with risk of breast cancer of a higher grade than the risk of breast cancer of a low or medium grade.

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New information on interaction of oestrogenic chemicals

84. Members will recall paragraph 8 of the 1999 statement (Annex 3), which concluded that there was no evidence of interaction in mammals with regard to xeno-oestrogens. There are a number of new studies available concerning this subject. The secretariat intend to screen these papers and relevant literature will be submitted to the November 2003 COC meeting.
85. One published paper has been identified concerning interaction of organochlorine insecticides. It should be noted that the paper does refer to *in vitro* data and hence may be of limited value, however members initial views are required.

Payne et al. (2001) Env. Health Perspect. 109:391-397

86. The combined effects of four organochlorines (o,p' DDT, p,p' DDE, beta-HCH and p,p' DDT) on the induction of cell proliferation in MCF-7 cells were studied. Concentration-response analyses were performed with single agents to predict the effects of two mixtures of all four compounds with different mixture ratios. The predictions were calculated using models of concentration addition and independent action, and subsequently tested experimentally.
87. Results indicated that a mixture of o,p' DDT, p,p' DDE, beta-HCH and p,p' DDT could act together to produce proliferative effects in MCF-7 cells. The authors assessed the combined effects of the four organochlorines on cell proliferation in relation to the effects of the most potent mixture component and in relation to the expected responses, as predicted by the models of concentration addition and independent action. It was noted that the combined effect could be predicted on the basis of data on a single agent concentration-response relationship. Combination effects were assessed in two ways. Firstly, evaluations based on proliferative responses induced by single mixture components indicated that the combined effects might be synergistic. Secondly, comparing observed with expected results suggested that the combination of all four components might produce an additive effect. In addition, the findings suggest that there are mixture effects even when each mixture component is present at concentrations that individually produce insignificant effects.

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ANNEX 2

Additional Epidemiological Studies

Høyer et al. (2000a) Journal of Clinical Epidemiology, 53:323-330

Høyer et al. (2000b) Cancer Causes and Control, 11:177-184

Høyer et al. (2001) BMC Cancer, 1:8

Høyer et al. (2002) Breast Cancer Research and Treatment, 71:59-65

Romieu et al. (2000) American Journal of Epidemiology, 152:363-70

Woolcott et al. (2001) Cancer Causes Control, 12:395-404

Interaction of Oestrogenic Chemicals

Payne et al. (2001) Env. Health Perspect. 109:391-397

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ANNEX 3

COC statement (July 1999 – COC/99/S3)

Breast cancer risk and exposure to organochlorine insecticides: Consideration of the epidemiological data on dieldrin, DDT and certain hexachlorocyclohexane isomers

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ANNEX 4

Letters to The Lancet

Hoyer et al. (2001) "Breast cancer and dieldrin" The Lancet, 356:1852-1823

P. G. Blain "Effects of insecticides" The Lancet, 357:1442

M. Joffe "Effects of multiple exposures to insecticide" The Lancet, 358:587-588