Irreversible Binding and Adrenocorticoalytic Activity of the DDT Metabolite 3-Methylsulfonyl-DDE Examined in Tissue-Slice Culture

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The persistent adrenocorticoalytic DDT metabolite 3-methylsulfonyl-DDE (MeSO2-DDE) was originally identified in Baltic grey seals, a population suffering from adrenocortical hyperplasia. In mice, MeSO2-DDE induces mitochondrial degeneration and cellular necrosis in the adrenal zona fasciculata. In this study, we used precision-cut tissue slice culture to examine local CYP11B1-catalyzed irreversible binding of MeSO2-DDE in the murine adrenal cortex. We also examined effects on steroid hormone secretion, histology, and ultrastructure. As determined by microautoradiography, selective binding occurred in zona fasciculata of slices exposed to MeSO2-[14C]-DDE. Quantification of binding by phosphorautoradiography revealed a 3-fold reduction of binding in slices co-exposed to the CYP11B1 inhibitor metyrapone. As measured by HPLC, corticosterone and 11-deoxy-corticosterone secretion to the medium increased linearly for at least 24 hr. Addition of the ACTH analog tetracosactide caused an 8-fold increase in corticosterone secretion. Addition of metyrapone reduced corticosterone secretion 4-fold. Exposure of slices to MeSO2-DDE (50 μM) reduced the rate of corticosterone secretion by 90% after 24 hr of incubation. As determined by electron microscopy, vacuolated mitochondria were present in zona fasciculata of slices exposed to MeSO2-DDE (50 μM) for 24 hr. Our findings show that all effects of MeSO2-DDE previously reported in vivo could be reproduced in a simple ex vivo test system with which to examine the adrenocorticoalytic activity of xenobiotics in human and wild animal tissues. We propose adrenal slice culture as a simple ex vivo test system with which to examine the adrenocorticoalytic activity of xenobiotics in human and wild animal tissue. Key words: adrenal cortex, DDT, endocrine disrupters, irreversible binding, 3-MeSO2-DDE, tissue-slice culture, toxicity. Environ Health Perspect 109:105–110 (2001). [Online 10 January 2001]

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The chlorinated insecticide DDT is a persistent environmental pollutant that undergoes long-range atmospheric transport and is biomagnified in food chains. Although its biological degradation in the environment is slow, DDT is biotransformed to numerous lipophilic and persistent metabolites that are found in human tissues and in wild mammals, birds, and fish. These degradation products include the dechlorinated metabolites DDD and DDE and the sulfur-containing DDE metabolite 3-methylsulfonyl-DDE (MeSO2-DDE). All metabolites have a capacity to interact with the endocrine system and therefore, deleterious effects on humans and experimental and/or wild animals (1).

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Materials and Methods

Chemicals. 3-Methylsulfonyl-2,2’-bis(4-chloro-[14C]-phenyl)-1,1’-dichloroethene (MeSO2-DDE; 13.4 mCi/mmol), unlabeled MeSO2-DDE, and 2-(2-chlorophenyl)-2-(4-chloro-[14C]-phenyl)-1,1-dichloroethane (op’-[14C]-DDE; 11.2 mCi/mmol) were prepared as previously described (25, 26). DL[4,5-3H]Leucine (40 Ci/mmol) was purchased from Amersham Life Science (Amersham, England). The radiochemical purity was ≥99% for all compounds. Tetracosactide (Synacthen Depot, 1 mg/mL) was obtained from Ciba (V. Frölunda, Sweden). Bovine serum albumin (BSA), dimethylsulfoxide (DMSO), and agarose (Type VII, low melting temperature) were obtained from Sigma (St. Louis, MO, USA). 2-Hydroxyethyl-methacrylate Technovit (Type VII, low melting temperature) were obtained from Sigma (St. Louis, MO, USA). 2-Hydroxyethyl-methacrylate Technovit

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supplemented with \(^{3}H\)-leucine (0.4 µCi/mL) for up to 96 hr. Slices and medium (1 mL) were sampled every 12 hr. After sampling, slices were washed in PBS and homogenized in distilled water (1 mL). We determined protein content using a fluorescence-based protein assay (27). Samples of homogenate (50 µL) were added in duplicate to a 96-well plate. The wells were then supplemented with PBS (85 µL, pH 7.8) and fluorescamine (140 µL) dissolved in acetonitrile 0.3 mg/mL) and kept at room temperature for 15 min. Fluorescence was then measured in a Fluostar microplate reader (SLT Labinstruments GmbH, Grödig/Salzburg, Austria) excitation at 390 nm and emission at 460 nm. We calculated protein content using a standard curve prepared with BSA (0–250 µg/mL) (27).

Incorporation of \(^{3}H\)-leucine into protein was measured in aliquots of the homogenate (0.5 mL), to which 2 M potassium hydroxide (0.5 mL) was added. After mixing, 15 M acetic acid (1 mL) was added and the samples were centrifuged (300 g, 20 min). The protein pellets were washed with 1.5 M acetic acid and centrifuged twice as above, before being dissolved in 0.5 M sodium hydroxide (0.5 mL). The solution was neutralized with 2 M hydrochloric acid (125 µL) and an aliquot (500 µL) was dissolved in scintillation liquid (Aquasea 300 Plus; 5 mL). Radioactivity was measured in a Tri-Carb 1900CA (Packard, Dowsen Grove, IL, USA) liquid scintillator counter.

**Hormone analysis.** Corticosterone, 11-deoxycorticosterone, and aldosterone concentrations in the medium were measured with HPLC using UV detection (241 nm). Methyl ether (1 mL) was removed and steroid hormones were extracted twice with chloroform:methanol (2:1, 15 mL). The combined chloroform phases were evaporated to dryness, redissolved in acetonitrile (50%), and injected into the HPLC system (Lichrosorb RP 18 column, 20 cm, 5 µm particle size; Merck). The steroid products were separated using a linear gradient of 40–80% acetonitrile (1 mL/min) and mixed with 40% methanol over 25 min. The amounts of steroids were expressed as nmol/slice. The detection level of steroid hormones was 5 pmol/mL medium.

**Autoradiography.** Micromicroautoradiography. Slices were incubated with M eSO\(_2\)\(_2\)-DDE (dissolved in DM SO) to the wells in amounts corresponding to a final concentration in the medium of 50 µM. Following incubation, slices were embedded in methacrylate and prepared for light microscopy, as above. For reference purposes, some adrenal slices were fixed directly after sectioning. For transmission electron microscopy, slices were fixed overnight in glutaraldehyde (2.5% in 0.067 M Na-cacodylate buffer). They were then treated with osmium tetroxide (1%, 2 hr at 4°C) rinsed in PBS buffer, dehydrated in ethanol as described above, treated with aceton, and embedded in TAAB 812 resin. After we selected areas to be examined, we cut ultrathin sections (50 nm) in methacrylate. Particular care was taken to orient the slices with the sliced plane parallel to the sectioning plane. In a two-step embedding procedure, the sections were fixed in the correct position with a minimal volume of methacrylate and then mounted on a plastic holder with the remaining methacrylate. The mounted slices were sectioned (2 µm) in a rotating microtome (H M 360; Mikrom Laborgeräte GmbH, Waldorf, Germany). Slides carrying the sectioned slices were dipped in NT B2 liquid emulsion (Kodak) diluted with an equal volume of distilled water. To enable localization of irreversible binding in metyrapone-treated mouse slices and rat slices, an exposure time of 60 weeks (4°C) was required to show clearly the localization. General exposure time was 6–10 weeks. Autoradiograms were developed, stained with toluidine blue, and examined in a Leica (DM RXE) light microscope. Photographs were taken with a digital camera (Leica, Wetzlar, Germany) and processed in Adobe Photoshop 5.5 (Adobe, San Jose, CA, USA). Selected autoradiograms were mounted and printed on a Fujix Pictography 3000 (Fujifilm, Japan).

**Phosphorautoradiography.** Semi-quantification of tissue-bound radioactivity was performed by apposing tissue sections to imaging plates (BAS-1P M P 2040S; Fuji, Japan) for 14 days, before subjecting them to microautoradiography. The radioactivity in the labeled areas of the adrenal sections was recorded by reading the imaging plate in a phosphorimager (BAS 1500; Fujifilm, Japan) (28,29). For semiquantification of the tissue-bound radioactivity, we used a M acintosh-based bioimaging analyzer program (MacBAS, ver. 2.2; Fujifilm, Fujifilm, Japan).

To correlate radioactivity and metabolically active regions in the incubated slices, we marked the labeled areas of the images selectively at 1 pixel resolution (1 pixel = 100 µm). Values obtained were expressed as phosphostimulated luminiscence (P S L) minus background (B G) per square millimeter of 2-µm thick tissue sections (P S L-B G)²/mm².

**Histopathology and electron microscopy.** We added MeSO\(_2\)\(_2\)-DDE (dissolved in DM SO) to the wells in amounts corresponding to a final concentration in the medium of 50 µM. Following incubation, slices were embedded in methacrylate and prepared for light microscopy, as above. For reference purposes, some adrenal slices were fixed directly after sectioning. For transmission electron microscopy, slices were fixed overnight in glutaraldehyde (2.5% in 0.067 M Na-cacodylate buffer). They were then treated with osmium tetroxide (1%, 2 hr at 4°C) rinsed in PBS buffer, dehydrated in ethanol as described above, treated with aceton, and embedded in TAAB 812 resin. After we selected areas to be examined, we cut ultrathin sections (50 nm)
with a diamond knife. Sections were placed on copper grids and counterstained with uranyl acetate (4%, 30 min) and lead citrate (0.1 M, 5 min), and then examined with a Philips EM 420 transmission electron microscope at 60 kV (Philips, Eindhoven, the Netherlands).

**Statistical evaluation of data.** All statistical analyses of hormone concentrations and bound radioactivity were made with a one-way analysis of variance (ANOVA) (using Dunnet’s posttest) or linear regression test with GraphPad Prism software version 3.0 for Windows (GraphPad Software, San Diego, CA, USA).

**Results**

**Protein synthesis.** Adrenal slices exposed to tetracosactide maintained a constant rate of $^{3}H$-leucine incorporation into protein (counts per minute (CPM) per milligram protein), which remained linear for 60 hr (Figure 1A).

**Steroid hormone secretion.** The rate of corticosterone secretion from nontreated adrenal slices remained constant for 30 hr. We could detect no secretion of 11-deoxycorticosterone from these slices.

In slices exposed to tetracosactide, accumulation of corticosterone and 11-deoxycorticosterone in the medium increased linearly for about 48 hr. Incubation for more than 48 hr reduced the rate of secretion of corticosterone and 11-deoxycorticosterone (Figure 1B). Tetracosactide exposure for 24 hr increased corticosterone content in the medium 8-fold ($p < 0.05$), compared with that in nontreated slices (Figure 2). Aldosterone content increased almost 4-fold ($p < 0.01$) under the same conditions (Figure 2).

In slices exposed to the CYP11B1 inhibitor metyrapone, the rate of corticosterone secretion was reduced about 4-fold during 30 hr of incubation, compared with nontreated slices. Exposure of slices to both metyrapone and tetracosactide for 24 hr produced no significant decrease in corticosterone secretion, compared with slices exposed to tetracosactide only. 11-Deoxy-corticosterone secretion from slices exposed to both tetracosactide and metyrapone showed a 6-fold increase ($p < 0.01$), while aldosterone secretion was more than halved ($p < 0.01$), compared with slices exposed to tetracosactide only (Figure 2).

Slices exposed to both Meso2-DDE (50 µM) and tetracosactide showed a reduction in the rate of corticosterone secretion (90%) at time points exceeding 6 hr of incubation, compared with slices exposed to tetracosactide where the rate of corticosterone secretion remained constant for 30 hr of incubation (Figure 3). The levels of 11-deoxycorticosterone after 30 hr of incubation were similar for both treatments.

**Autoradiography.** As determined by light microscopy, autoradiograms of adrenal slices co-exposed to Meso2-$^{14}$C]DDE and tetracosactide were characterized by a marked labeling of zona fasciculata (Figure 4 A,B). Labeling of zona glomerulosa, zona reticularis, and the adrenal medulla did not exceed that of the background. The localization of Meso2-$^{14}$C]DDE in nontreated slices did not differ from that in tetracosactide-exposed slices. The images of tissue-bound radioactivity semiquantified with phosphorautoradiography matched the images of the microautoradiograms closely. Phosphorautoradiography showed no significant increase in amount of tissue-bound Meso2-$^{14}$C]DDE in slices exposed to tetracosactide, compared with non-exposed slices. The Meso2-$^{14}$C]DDE derived labeling in zona fasciculata in slices exposed to both tetracosactide and metyrapone was reduced about 3-fold ($p < 0.01$), compared with slices exposed to tetracosactide only (Figure 4 C,D). Localization of CYP11B1 mRNA using in situ hybridization produced autoradiograms that corresponded well with that of Meso2-$^{14}$C]DDE labeling (unpublished data). Corticosterone secretion from slices exposed to Meso2-$^{14}$C]DDE (<7.5 µM) was roughly identical to that from nontreated slices after 24 hr of incubation.

**Effect of Meso2-DDE (50 µM) on corticosterone secretion.** All time points represent secretion by four slices obtained from the same four animals. Corticosterone secretion to the medium was similar in all slices during the first 6 hr of culture. After 24 hr the Meso2-DDE (50 µM)-exposed slices showed a marked reduction in corticosterone secretion. Medium (1 mL) was removed from the wells after 3, 6, 24, and 30 hr and replaced with fresh medium; values were adjusted for dilution.
Histologic changes could be observed after 24 hr of culture of tetracosactide-exposed slices, compared with noncultured slices fixed for histology immediately after slicing (Figure 5 A-B). In slices exposed to MeSO2-DDE, the nuclei in zona fasciculata cells appeared more condensed (Figure 5C).

**Electron microscopy.** Ultrastructural examination of slices fixed immediately after sectioning revealed numerous mitochondria and smooth endoplasmic reticulum (SER) in zona glomerulosa and zona fasciculata cells. In slices kept 24 hr in culture, we observed a reduced amount of SER in both zones. Notably, the mitochondrial membranes were largely intact in zona glomerulosa and zona fasciculata. Slices incubated with MeSO2-DDE (50 µM) for 24 hr showed mitochondrial vacuolation in zona fasciculata. Mitochondria in zona glomerulosa remained largely intact and similar to those in nonexposed slices incubated for 24 hr (Figure 6 A-F).

**Discussion**

In the present study, we developed an ex vivo test system based on precision-cut adrenal slice culture to examine metabolism-dependent binding, ultrastructural changes, and effects on steroid synthesis in mouse adrenal tissue exposed to MeSO2-DDE. The results showed that most metabolic and toxic events previously reported in vivo or in vitro can be reproduced ex vivo. In addition, the rate of steroid hormone synthesis in tetracosactide-exposed and nonexposed slices was readily recorded. The results suggest that adrenal tissue slice culture will become a useful test system for examination of metabolism-activated adrenal toxicants in both human and wild animal tissue.

The viability of cultured slices was defined largely by the toxicity-related test variables examined. As determined by a series of control experiments, nonexposed slices maintained functional activity for at least 48 hr. The lack of apparent histologic change in

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**Figure 4.** Specific tissue binding in MeSO2-[14C]DDE-exposed adrenal mouse (A-D) and rat (E-F) slices cultured in medium supplemented with tetracosactide (11 nM). Abbreviations: M, adrenal medulla; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis. MeSO2-[14C]DDE binding was confined to zona fasciculata in mouse adrenal slices, demonstrated by bright-field (A) and corresponding dark-field (B) images. MeSO2-[14C]DDE binding in mouse zona fasciculata was reduced after addition of the CYP11B1 inhibitor metyrapone (50 µM) (C, D). In rat adrenal slices MeSO2-[14C]DDE binding was very weak but distinct in zona fasciculata after exposure to tetracosactide (11 nM) (E, F). Magnification x220; bar = 100 µm.

**Figure 5.** Histologic examination of a control section from an adrenal slice not placed in culture (A), a slice exposed to tetracosactide (11 nM) (B), and a slice co-exposed to MeSO2-DDE (50 µM) plus tetracosactide (11 nM) for 24 hr (C). Abbreviations: M, adrenal medulla; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis. A slight swelling of zona fasciculata cells could be observed after 24 hr, as compared with noncultured slices, in MeSO2-DDE-exposed slices, the cell nuclei in zona fasciculata appeared more condensed. Hematoxylin-eosin-stained sections; magnification x220; bar = 100 µm.
the nonexposed slices following 24 hr in culture also supports the good viability of slices. Ultrastructural examination, however, revealed reduced numbers of SER vesicles after 24 hr in culture, showing that the metabolic functions of the SER could be affected at this time point. It is noteworthy that the mitochondrial membrane structure was largely intact, compared with that of tissue fixed immediately after slicing. This observation is particularly important because MeSO2-DDE is activated by CYP11B1, an enzyme residing in the mitochondrial inner membrane of zona fasciculata cells.

Phosphorautoradiography proved a sensitive and quick tool to quantitatively detect irreversible binding in the adrenal cortex. Combined with the exact localization of binding obtained by microautoradiography, phosphorautoradiography is an efficient technique with which to measure irreversibly bound adduct levels in restricted target cell populations in the cultured slices. The digitized autoradiograms representing sites of irreversible MeSO2-[14C]DDE binding correlated well with the localization of irreversibly bound radioactivity in conventional microautoradiograms. The irreversible MeSO2-[14C]DDE binding was confined to zona fasciculata, while zona glomerulosa, zona reticularis, and the adrenal medulla were devoid of bound radioactivity (above the background levels). These results are consistent with findings reported previously in mice dosed with MeSO2-[14C]DDE in vivo. They also confirm that CYP11B1 enzyme activity was maintained in the cultured slices.

The CYP11A1-catalyzed cholesterol side-chain cleavage is the first and rate-limiting step in corticosterone synthesis. CYP11B1 catalyzes the last step in this pathway but also, as mentioned, the metabolic activation of MeSO2-DDE in the adrenal zona fasciculata (12,30,31). Since tetracosactide stimulates both CYP11A1 and CYP11B1 activity (22,24,32–35), this peptide would be expected to increase both corticosterone synthesis and irreversible MeSO2-[14C]DDE binding in the slice culture. The observed 8-fold induction of corticosterone secretion by tetracosactide was therefore expected and supports the conclusion that the cultured slices retained their functional stability throughout the experiment. Unexpectedly, however, no increased MeSO2-[14C]DDE binding in zona fasciculata was recorded by phosphorautoradiography. A possible explanation for this discrepancy could be that the increased concentration of 11-deoxycorticosterone induced by tetracosactide produced increased competition with MeSO2-[14C]DDE for the induced activating enzyme (11).

The reduced corticosterone secretion in MeSO2-DDE-exposed slices may result from mitochondrial toxicity, but also from inhibition of CYP11B1 enzyme activity, as previously reported in adrenal Y1 cells (31,36). As demonstrated by electron microscopy, the mitochondrial membranes of zona fasciculata cells (the site of CYP11B1 localization) were vacuolated in the MeSO2-DDE treated slices. The mitochondria in zona glomerulosa remained largely intact. This finding observed after 24 hr in culture and the delayed inhibition of corticosterone secretion suggests that toxicity was responsible (Figures 3 and 6). The mitochondrial changes observed were similar to those previously described in vivo, but the inhibition of corticosterone synthesis was more pronounced in the cultured slices. This difference may be explained by the pituitary feedback loop, which in the intact animal compensates for decreasing glucocorticoid serum levels by increasing the release of ACTH.

In vivo, the potent CYP11B1 inhibitor metyrapone blocks both synthesis of corticosterone from 11-deoxycorticosterone (37), and irreversible binding of MeSO2-[14C]DDE to adrenal homogenate and the mitochondrial fraction (11). Metyrapone treatment of slices would consequently be expected to inhibit both 11-deoxycorticosterone hydroxylation to corticosterone and metabolism of MeSO2-[14C]DDE to a reactive intermediate that becomes irreversibly bound in the zona fasciculata cells. In the present study, metyrapone did indeed reduce corticosterone synthesis about 4-fold and reduce irreversible MeSO2-[14C]DDE binding in zona fasciculata to a similar degree. A comparable inhibition of irreversible MeSO2-[14C]DDE binding was observed following

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**Figure 6.** Ultrastructural examination of sections from adrenal slices. Abbreviations: m, mitochondria; n, nucleus; s, smooth endoplasmatic reticulum. Numerous mitochondria and SER characterize cells in zona glomerulosa (A) and zona fasciculata (B) in reference slices fixed immediately after sectioning. In slices kept 24 hr in culture (C,D), a reduced amount of SER is observed. Notably, the mitochondrial membranes are largely intact in zona glomerulosa (C) and zona fasciculata (D). Slices exposed to MeSO2-DDE (50 µM) for 24 hr show mitochondrial vacuolation in zona fasciculata (F). Mitochondria in zona glomerulosa (E) remain largely intact and similar to those in slices incubated without MeSO2-DDE for 24 hr (C). Magnification x27,000; bar = 0.5 µm.
exposure of slices to both tetracosactide and metyrapone. It is noteworthy, however, that the rate of corticosterone synthesis was not significantly reduced by metyrapone in tetracosactide-exposed cultures, in contrast to cultures where slices were exposed only to metyrapone. Notably, the concentration of the CYP11B1 substrate 11-deoxycorticosterone increased 6-fold in the co-exposed cultures, supporting an inhibition of CYP11B1. The failure to inhibit corticosterone synthesis by metyrapone may consequently be due to the increased substrate concentration for the CYP11B1 substrate 11-deoxycorticosterone. These results are consistent with previous findings indicating that the increased substrate concentration for the CYP11B1 substrate 11-deoxycorticosterone halved the secretion of aldosterone.

In conclusion, we have shown that the bioactivation and mitochondrial toxicity of MeSO₂-DDE observed in vivo could be reproduced ex vivo. We have also demonstrated that steroid synthesis and secretion of both mineralocorticoids and glucocorticoids can be studied in adrenal slice cultures. We propose adrenal tissue slice culture as a simple ex vivo test system with which to examine the adrenocorticotrophic activity of environmental pollutants and drugs in human and wild animal tissues.

References and Notes

1. Brandt I, Berg C, Hallidén K, Brunström B. Developmental and reproductive toxicity of persistent environmental pollutants. Arch Toxicol Suppl 20:111-119 (1998).
2. Bergenstal DM, Hertz R, Lipszt M, Móy RH. Chemotherapy of adrenocortical cancer with o,p-DDE. Int J Cancer 22:3762 (1980).
3. Nelson AA, Woodard S, Severe adrenal cortical atrophy (cystotic) and hepatic damage produced in dogs by feeding 2,2-bis-(para-chlorophenyl)-1,1-dichlorethane (DDD or DDE). Arch Pathol 48:303 (1949).
4. Jõnsson C-J, Berg C, Mäenpää A, Brandt I. Toxicity of o,p-DDE and p,p-DDE in the adrenal cortex of mice. Toxicology 24:53-59 (1993).
5. Benecke R, Keller E, Vetter B, de Zeeuw RA. Plasma level monitoring of 3-methylbenzylol (o,p-DDE) and its metabolite (o,p-DDE) during long-term treatment of Cushings disease with low doses. Eur J Clin Pharmacol 41:259-261 (1991).
6. Jõnsson C-J, Jannson B. Methyl sulfoxone metabolites of PCB and DDE in the cultured adrenal gland. Eur J Pharmacol 80:363-366 (1981).
7. Bergman Å, Norén K, Mäenpää A, Bergman M. Methylsulfonyl metabolites of PCBs and DDE in human milk in Sweden, 1972-1979. Eur Environ Health Perspect 104:766-772 (1996).
8. Westlund C, Norén K, Methylsulfonyl metabolites of PCBs and DDE in human tissue. Eur Environ Health Perspect 105:644-649 (1997).
9. Lund BO, Bergman Å, Brandt I, Methylal activation and toxicity of a DDT metabolite, 3-methylsulfonyl-DDE, in the adrenal zona fasciculata of mice. Chem Biol Interact 65:25-40 (1988).
10. Jõnsson C-J, Rodríguez M, Aréchiga H, Lund BO, Bergman Å, Brandt I. Adrenocortical toxicity of 3-methylsulfonyl-DDE in mice. II. M Chondrosial changes following ecologically relevant doses. Fundam Appl Toxicol 16:365-374 (1991).
11. Lund BO, Lund J, Novel involvement of a mitochondrial steroid hydroxylase (3beta-hydroxy-5alpha-reductase) in the pituitary gland. J Biol Chem 270:20895-20897 (1995).
12. Krumdieck CL, Santos J, Ho K-J. A new instrument for the rapid preparation of tissue slices. Anal Biochem 164:118-123 (1987).
13. Bränden K, Mekel RL, Hruby VJ, Jõnsson DG, Gadoffli AJ, Krumdieck CL. Precision cut tissue slices in culture: a new tool in pharmacology. Proc West Pharmacol Soc 30:291-293 (1987).
14. Gadoffli AJ, Bränden K, Fisher RL, Michaud JP. Use of tissue slices in chemical mixture toxicity and interspecies investigations. Toxicology 105:285-290 (1995).
15. Parrish AR, Gadoffli AJ, Bränden K. Precision-cut tissue slices: applications in pharmacology and toxicology. Life Sci 57:1987-1991 (1995).
16. Bach PH, Vickers AEM, Fisher R, Baumann A, Brito E, Carlile DJ, Koster HJ, Lake BG, Salomon F, Sawyer TW, et al. The use of tissue slices in pharmacological and toxicological studies. ATLA-A Altern Lab Anim 24:893-923 (1996).
17. Barbara JG, Christophe PJ, McClinney RA, Kenneth T. An adrenal slice preparation for the study of cholesterol and its cholesterol-1 incorporation into adrenal steroids. Biochim Biophys Acta 104:303-314 (1965).
18. Hart MM, Straw J. Effects of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane and pyrrolmoc on adrenocorticotropic hormone-induced catabolism and on amino acid incorporation into urine of dog adrenal cortex. Biochem Pharmacol 20:257-263 (1971).
19. Ali S, Bassett R, The release of corticosterone and a corticosterone-binding protein by incubated rat adrenal slices. J Steroid Biochem Mol Biol 39:129-130 (1991).
20. Engelhardt D, Weber MM, Micksch T, Abendnour F, Jaspers C. The influence of ketocazone on human adrenal steroidogenesis: incubation studies with tissue slices. Clin Endocrinol 31:651-652 (1991).
21. Gröndal S, Grimelius L, Thoren M, Hamberger B, Salsby A and ACTH-stimulated cortisol and aldosterone release from adrenocortical adenomas in vitro. Eur J Surg 177:108-113 (1991).
22. Bergman Å, Wachtmeister CA. Synthesis of methylsulfonyl derivates of 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (o,p-DDE) present in seal from the Baltic. Acta Chem Scand 31:98-101 (1977).
23. Lindholm A, Klasson-Welhinder E, Wehler T, Bergman Å. Synthesis of 1C-labelled DDE isomers of high specific activity, J Label Compd Radiopharm 24:1011-1017 (1987).
24. Lorenzen A, Kennedy SW. A fluorescence-based protein assay procedure with a microplate reader. Anal Chem 214:346-348 (1993).
25. Morii K, Hamaoka T, Laseropography System (BAS). Protein, Nucleic Acid 35:136-191 (1994).
26. Motafl, Hayama S, Kuroda A, Radioimmunoassay for quantitative autoradiography of 1C. Eur J Drug Metab Pharmacokinet 20:89-105 (1989).
27. Domak J, Chaplin DD, Kirkman M, Wu RC, Liu WW, Howard TP, Seldin MF, Parker KL. Different isoforms of mouse 11 beta-hydroxylase produce mineralocorticoids and glucocorticoids. Mol Endocrinol 5:1853-1861 (1991).
28. J ohnsson M, Larsson C, Bergman Å, Lund BO. Structure-activity relationships for inhibition of CYP11B1-dependent glucocorticoid synthesis in YLS cells by aryl methyl sulfovinylpharmacol. Toxicol Sci 32:225-230 (1998).
29. Hart MM, Straw J, Effects of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane in vivo on basaline and adrenocorticotropic hormone-induced steroid production in dog adrenal slices. Biochim Pharmacol 20:1689-1691 (1991).
30. Muller J, Oertle M. Separate induction of the two isozymes of cytochrome P-45011b1 in beta rat adrenal zona glomerulosa cells. J Steroid Biochem Mol Biol 47:213-221 (1993).
31. Oertle M, Muller J. Two types of cytochrome P-45011b in rat adrenal: separate regulation of gene expression. Mol Cell Endocrinol 91:201-209 (1995).
32. Shen T, Suzuki Y, Poyard M, Best Belpomme M, Defter N, Hanoue J. Localization and differential expression of adenylyl cyclase messenger ribonucleic acids in rat adrenal gland determined by in situ hybridization. Endocrinology 138:4591-4598 (1997).
33. Jõnsson C-J, Brunström B, Lund BO, Brandt I. Toxicity and irreversible binding of two DDT-metabolites - 3-methylsulfonyl-DDE and o,p-DDE in adrenal interrenal cells in birds. Eur Environ Health Perspect 12:1303-1305 (1993).
34. Sillence M, Rodway RG. Effects of metyrapone and etomidate on adrenal function and growth rate in female rats. J Endocrinol 213:473-487 (1997).
35. Brandt I, Jõnsson C-J, Lund BO. Comparative studies on adrenocorticotropic DDT-metabolites. Amibio 21:602-606 (1992).
36. Bergman Å, Olsson M. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? Finn Game Res 44:67-62 (1985).
37. Bergman Å. Breed health condition of the Baltic grey seal (Halichoerus Grypus) during two decades. Gynaecological health improvement but increase in prevalence of colonic ulcers. Apmis 107:270-282 (1999).
38. Lund BO. In vitro adrenal bioactivation and effects on steroid metabolism of DDT, PCBs and their metabolites in the grey seal (Halichoerus Grypus). Environ Toxicol Chem 13:911-917 (1994).