RESPONDENT'S EXHIBIT 6

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: **June 29, 2015**

SUBJECT: **EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals**

PC Code: See table, Attachment A
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: See table, Attachment A
MRID No.: NA

DP Barcode: NA
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: NA

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FROM: **Greg Akerman**
Health Effects Divison
Office of Pesticide Programs
And
Amy Blankinship
Environmental Fate and Effects Division
Office of Pesticide Program

THROUGH: **Jess Rowland**
Deputy Division Director
Health Effects Division
Office of Pesticide Programs

TO: **Jolene Trujillo**
Chemical Review Manager
Pesticide Re-Evaluation Division
Office of Pesticide Programs

EPA has completed its Weight of Evidence (WoE) assessment evaluating results of the Endocrine Screening Program (EDSP) Tier 1 screening assays for the List 1 chemicals. The WoE documents for the 52 chemicals are listed in Attachment A along with the chemical and report identifiers.

Attachment A. EDSP List 1 Chemicals

Chemical Name	PC Code	TXR Number
2,4-D	030001	0057151
Abamectin	122804	0057152
Acephate	103301	0057153
Acetone	044101	0057154
Atrazine	080803	0057155
Benfluralin	084301	0057156
Bifenthrin	128825	0057157
Captan	081301	0057158
Carbaryl	056801	0057159
Carbofuran	090601	0057160
Chlorothalonil	081901	0057161
Chlorpyrifos	059101	0057162
Cyfluthrin	128831	0057163
Cypermethrin	109702	0057164
DCPA	078701	0057165
Diazinon	057801	0057166
Dichlobenil	027401	0057167
Dimethoate	035001	0057168
EPTC	041401	0057169
Esfenvalerate	109303	0057170
Ethoprop	041101	0057171
Fenbutatin-Oxide	104601	0057172
Flutolanil	128975	0057173
Folpet	081601	0057174
Glyphosate	417300	0057175
Imidacloprid	129099	0057176
Iprodione	109801	0057177
Isophorone	847401	0057178
Linuron	035506	0057179
Malathion	057701	0057180
Metalaxyl	113501	0057181
Methomyl	090301	0057182
Metolachlor	108801	0057183
Metribuzin	101101	0057184
MGK-264	057001	0057185
Myclobutanil	128857	0057186
Norflurazon	105801	0057150
o-Phenylphenol	064103	0057146
Oxamyl	103801	0057142
PCNB	056502	0057138
Permethrin	109701	0057149
Phosmet	059201	0057145
Piperonyl Butoxide	067501	0057141
Pronamide	101701	0057137
Propargite	097601	0057148
Propiconazole	122101	0057144
Pyriproxyfen	129032	0057140
Simazine	080807	0057136
Tebuconazole	128997	0057143
Tetrachlorvinphos (TCVP)	083701	0057147
Triadimefon	109901	0057139
Trifluralin	036101	0057135

EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH ESTROGEN, ANDROGEN OR THYROID PATHWAYS

CHEMICAL: CHLORTHAL DIMETHYL (DCPA)

OFFICE OF PESTICIDE PROGRAMS

OFFICE OF SCIENCE COORDINATION AND POLICY

U.S ENVIRONMENTAL PROTECTION AGENCY

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Abbreviations

Abbreviation	Terminology
A	Androgen (hormonal pathway)
ADME	Absorption, Distribution, Metabolism, Excretion
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMA	Amphibian Metamorphosis Assay
ARTA	Androgen Receptor Transcriptional Activation
AST	Aspartate Aminotransferase
ANOVA	Analysis of Variance
AOP	Adverse Outcome Pathway
AR	Androgen Receptor
B_{max}	Binding at maximum
BROD	Benzyloxyresorufin-O-dealkylase
BUN	Blood Urea Nitrogen
CAR	Constitutive Androstane Receptor
CFR	Code of Federal Regulations
CG	Cowper's Gland
ChE	Cholinesterase
ChEI	Cholinesterase inhibition
CMC	Carboxymethyl cellulose
CTA	Comparative Thyroid Assay
CV	Coefficient of Variation
CYP	Cytochrome 450
DER	Data Evaluation Record
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DP	Dorsolateral Prostrate
E	Estrogen hormonal pathway
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDRT	Endocrine Disruptor Review Team
EDSP	Endocrine Disruptor Screening Program
EE	Ethinyl Estradiol
ELISA	Enzyme Linked Immunosorbent Assay
EOGRTS	Extended One-Generation Reproductive Toxicity Study (Rat)
ER	Estrogen Receptor
EROD	Ethoxyresorufin-O-dealkylase (or deethylase)
ERTA	Estrogen Receptor Transcriptional Activation
EtOH	Ethanol
F	Female
F1	First filial generation

Abbreviation	Terminology
F2	Second filial generation
Fcd	Fecundity
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Field Observation Battery
FQPA	Food Quality Protection Act
Frt	Fertility
FSH	Follicle Stimulating Hormone
FSTRA	Fish Short-Term Reproduction Assay
FT	Flutamide
GD	Gestation Day
GGT	Gamma-glutamyl Transpeptidase
GnRH	Gonadotropin-releasing hormone
GP	Glans Penis
GSI	Gonado-Somatic Index
H	High
HLL	Hind Limb Length
HPG	Hypothalamic-Pituitary-Gonadal Axis
HPLC/MS/MS	High Pressure Liquid Chromatography/Mass Spectroscopy
HPT	Hypothalamic-Pituitary-Thyroidal Axis
I	Inadequate
IC50	Inhibitory Concentration at 50% of response
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative
K_d	Equilibrium Dissociation Constant
K_{OW}	Octanol/Water Partition Coefficient
L	Low dose
LABC	Levator Ani-Bulbocavernosus
LAGDA	Larval Amphibian Growth and Development Assay
LC50	Lethal Concentration in 50% of test organisms
LD	Lactation Day
LH	Luteinizing hormone
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Limit of Quantitation
M	Male
MDL	Minimum Detection Level
MEOGRT	Medaka Extended One Generation Reproduction Test
MH	Medium high
ML	Medium low
MoA	Mode of Action
MOE	Margin of Exposure
MRID	Master Record Identifier
MROD	Methoxyresorufin-O-dealkylase
MTC	Maximum Tolerated Concentration

Abbreviation	Terminology
MTD	Maximum Tolerated Dose
N	Negative
NE	Not examined/evaluated
NF stage	Nieuwkoop and Faber's Staging Atlas
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NS	Not Statistically Significant
NR	Not Reported
OCSPP	Office of Chemical Safety Pollution and Prevention
OECD	Organization for Economic Co-Operation and Development
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSCP	Office of Science Coordination and Policy
OSRI	Other Scientifically Relevant Information
P	Positive
P	Parental generation
PC	Positive Control
PC_{10}	Positive Control at 10% of response
PC_{50}	Positive Control at 50% of response
PND	Post-Natal Day
POD	Point of Departure
PPS	Preputial Separation
PROD	Pentaoxyresorufin-O-dealkylase (or depentylase)
PXR	Pregnane X receptor
QC	Quality Control
RBA	Relative Binding Affinity
RBC	Red Blood Cells
RfD	Reference Dose
RPC_{max}	Relative to Positive Control at maximum
SAP	Scientific Advisory Panel
SC	Solvent Control
s.c	Subcutaneous
SDH	Sorbitol dehydrogenase
SDWA	Safe Drinking Water Act
SEP	Standard Evaluation Procedure
SD	Standard Deviation or Sprague-Dawley
SVL	Snout-to-Vent Length
SV	Seminal Vesicles
T	Thyroid (hormonal pathway)
T1WoERC	EDSP Tier 1 Weight of Evidence Review Committee
T3	Triiodothyronine
T4	Thyroxine (tetraiodothyronine)

Abbreviation	Terminology
TP	Testosterone Propionate
TR	Thyroid Receptor
TSH	Thyroid Stimulating Hormone
UDPGT	Uridine Diphosphate Glucuronyltransferase (also known as UGT)
VC	Vehicle Control
VO	Vaginal Opening
VP	Ventral Prostate
VTG	Vitellogenin
WoE	Weight-of-Evidence

Executive Summary

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

In determining whether a pesticide chemical interacts with E, A, or T hormone pathways, the number and type of effects induced, the magnitude and pattern of responses observed across studies, taxa, and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

On February 15, 2014 the Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of evidence (WoE) analysis of the potential interaction of DCPA with the E, A or thyroid T hormone pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 *in vitro* assays followed by *in vivo* mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

For DCPA, there was no convincing evidence to support potential interaction with the estrogen pathway. Although DCPA increased estradiol production in the Tier 1 steroidogenesis assay and the Tier 1 ER binding assay results were equivocal, the Tier 1 ERTA and aromatase assays were negative and there were no estrogen-related effects observed in the Tier 1 uterotrophic and female pubertal assays or in the Part 158 mammalian toxicity studies. There was a non-treatment-responsive decrease in fecundity at the mid dose and a slight decrease in fertility at the high dose in the FSTRA; no other complementary responses were observed across other endpoints within those treatment groups. In the wildlife Part 158 studies, in the bobwhite quail avian reproduction study, all observed reproductive effects were seen only at an overtly toxic dose, while in the mallard duck study decreases in viable embyros/hatching were observed (no other reproductive effects observed). Therefore, there is a lack of redundancy across the battery, with no or limited effects observed without complementary responses.

There was no convincing evidence of an interaction with the androgen pathway. DCPA was found to be untestable (due to insolubility) in the Tier 1 AR binding assay and testosterone production was decreased In the Tier 1 steroidogenesis assay. DCPA was negative in the

Hershberger assay and there were no treatment-related androgen effects in the male pubertal assay. There were no androgen-related effects observed in the mammalian Part 158 studies. There was a decrease in fertility at the high dose in the FSTRA; however there was a lack of complementary responses across other endpoints. In the wildlife Part 158 studies, for the bobwhite quail reproduction study, all effects were observed in the presence of overt toxicity, while in the mallard decreases in viable embyros/hatching were observed (no other reproductive effects observed).

DCPA demonstrated a potential for interaction with the thyroid hormone pathway in the absence of overt or systemic toxicity. In the pubertal assays, serum T4 levels were dose-dependently decreased in males at 500 mg/kg/day (78%) and 1000 mg/kg/day (81%) and in females at 500 mg/kg/day (47%) and 1000 mg/kg/day (55%). In the amphibian metamorphosis assay (AMA), increased incidence of thyroid gland hypertrophy and follicular cell height increases were reported at the high concentration compared to the control. In addition, development changes related to DPCA treatment seen in the AMA include an increase in the normalized HLL on day 7 and 21.

In the mammalian Part 158 studies, DCPA-induced thyroid effects included thyroid follicular hypertrophy in a subchronic toxicity study in rats, and increased thyroid weights, follicular cell hyperplasia/hypertrophy, decreased T4 levels and an increased incidence of thyroid follicular cell neoplasms in both sexes of rats in a combined chronic toxicity/ carcinogenicity study.

In the EDSP Tier 1 male and female pubertal assays as well as in the OSRI, there was evidence for potential interaction with the thyroid pathway in studies conducted with adult animals, but no such data exists for potential thyroid toxicity in the young animals.

For DCPA, the current point of departure (POD) of 10 mg/kg/day for human health risk assessment is based on decreased level of T4 and increased incidence of histopathological lesions in the thyroid and liver at 10.0 mg/kg/day (LOAEL) in a chronic toxicity/carcinogenicity study in rats. This POD is used to derive the chronic Reference Dose (RfD) for dietary exposure risk assessments and the Margins of Exposure (MOEs) for non-dietary exposure risk assessments.

In general, since the POD and the toxicity endpoints of concern are based on data obtained from adult animals, there usually would be a concern that the POD may not be protective of potential thyroid toxicity in the young. Therefore, a special thyroid assay in pregnant animals, fetuses, postnatal animals, and adult animals is recommended. This special study should use a mechanistic approach to generate specific data on the thyroid (*i.e.*, the primary target of DCPA) to protect the developing nervous system from thyroid hormone disrupting chemicals. The specific purpose of this study is to establish a POD (*i.e.*, NOAELs and LOAELs or benchmark doses) that may be used for human health risk assessment. The POD from this special study

would address the concern for the potential ability of DCPA to disrupt thyroid function in pregnant females and in the fetus or newborn. However, it is noted that the agency has issued a Data Call-In notice for this special study under the Registration Review program.

For the wildlife, to add to the understanding of potential thyroid effects, the EDSP Tier 2 Larval Amphibian growth and Development Assay (LAGDA) is recommended since there is no chronic aquatic amphibian toxicity data available. Chronic toxicity data with fish (surrogates for aquatic-phase amphibians) are not currently available for DCPA.

For birds, Part 158 avian reproduction studies with both northern bobwhite quail and mallard duck are available for DCPA. In both species, effects on reproductive parameters were observed. The type of data obtained from Part 158 avian reproduction studies (OCSPP 850.2300) are considered sufficient for evaluating potential reproductive effects to birds from DCPA exposure. Relative to EDSP, additional testing is not recommended.

I. Introduction

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

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On February 15, 2014 the Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of evidence analysis of the potential interaction of DCPA with the E, A or T hormonal pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 *in vitro* assays followed by *in vivo* mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

DCPA has a water solubility of 0.5 mg/L, a partition coefficient (log octanol/water) of 4.28, and a vapor pressure of 2.5×10^{-6} mm Hg at 20°C. DCPA is stable in water, and is stable to photolysis in water and on soil. Bioconcentration factors in bluegill sunfish are 1894, 777, and 2574 in whole fish, edible tissue, and viscera, respectively, and depuration appears to be complete after 14 days.

The available information considered in determining the potential interaction of DCPA with the E, A and/or T pathways include submitted EDSP Tier 1 assays and/or other scientifically relevant information (OSRI) such as general toxicity studies and other published articles. These data are summarized in Sections III.A through III.C. An analysis of the data submitted to the Agency, using the WoE approach outlined by the Agency (USEPA, 2011), is presented in Section IV. The EDSP Tier 2 studies recommendations are presented in Section V.

II. Sources of Scientific Data and Technical Information

A. EDSP Tier 1 Screening Assays

The Tier 1 assays and/or other scientifically relevant information (OSRI) submitted to satisfy the agency's test order are shown below in Table 1. Executive Summaries are presented in Appendix 1.

B. Other Scientifically Relevant Information (OSRI)

In response to the Agency's Test Orders, data believed to be relevant to one or more of the Tier 1 assays were submitted by the Test Order recipient. The Agency has also conducted a more recent search of available scientific literature for any additional relevant information. Summaries of the available OSRI are presented in Appendix 2. Additionally, literature/studies considered but not utilized for the WoE analysis are listed in Appendix 3.

III. Weight of Evidence (WoE) Evaluation

The principles, criteria and approach used in the WoE determination on the potential of a substance to interact with endocrine-related processes (*i.e.,* E, A, or T hormone pathways) were as described in the WoE guidance document (USEPA, 2011) and presented at the 2013 FIFRA Scientific Advisory Panel (SAP) (USEPA, 2013). The weight of evidence process identifies how the individual lines of evidence are assembled and integrated along two concepts (*i.e*., complementarity and redundancy) within the conceptual framework of an adverse outcome pathway). Broadly, there are four main steps outlined in the guidance which provide the foundation for WoE evaluations. The first step is to the individual studies for their scientific quality and relevance in evaluating potential endocrine interaction(s). The second step is to integrate the data along different levels of biological organization while examining the extent of concordance of complementarity (*i.e*., the concordance of endpoints within an assay that measures multiple endpoints) and redundancy (*i.e*., the concordance of endpoints/responses across assays) in the observed responses across these different levels of biological organization. The third step is to characterize the main lines of evidence as well any conclusions. Finally, the last step is to evaluate whether additional testing is needed based on the evidence and conclusions described above.

As mentioned, the first step is to assemble and evaluate the available scientific data. Data for the EDSP Tier 1 WoE evaluation falls into one of two categories: 1) EDSP Tier 1 data, or 2) other scientifically relevant information (OSRI). The EDSP Tier 1 data include a battery of 11 assays consisting of *in vitro* and mammalian and wildlife *in vivo* assays. The Tier 1 assays were designed specifically to evaluate a number of key biological events including potential effects on receptor binding (estrogen and androgen agonist and antagonist), steroidogenesis, and other effects on the HPG and HPT axes. OSRI may include published literature studies as well as

studies conducted under USEPA (often referred to as Part 158 data) or OECD guidelines submitted in support of pesticide registrations. Each study is evaluated for scientific quality and relevance for informing interactions with the E, A, or T pathway. Additionally, the consistency of the responses in the individual study is evaluated. For the Tier 1 *in vivo* assays, often multiple endpoints are measured in each assay.

Evaluation of the potential confounding effects of overt toxicity in the study, as well as the relative degree of diagnostic utility of a specific endpoint for discerning whether or not the chemical has interacted with the endocrine system, are considered. The collective response of the individual endpoints, as well as the conditions under which they were expressed, are considered when evaluating an overall indication of potential interaction as measured by the study.

The second step in this WoE process is to formulate hypotheses and integrate the available data along different levels of biological organization. Two keys elements in the integration of data as well as characterizing the extent to which the available data support a hypothesis that a chemical has the potential to interact E, A, or T pathways, are the concepts of complementarity and redundancy. These two concepts provide a basis for considering the plausibility, coherence, strength, and consistency of the body of evidence. The current EDSP Tier 1 screening assays are meant to evaluate whether or not a chemical can interact with E, A, and T consisting of different levels of biological organization from a molecular initiating event, such as receptor binding, through potential adverse effects in apical endpoints such as sexual development and fecundity at the whole organism level. The extent of expression of responses at higher levels of biological organization can indirectly provide information on potential compensatory capabilities of an individual organism.

After the data have been assembled and integrated, the third step is to characterize the main lines of evidence along with the conclusions; this characterization involves three components. The first component is whether the data provide relevant, robust and consistent evidence in terms of complementarity and redundancy as well as biological plausibility. Second, is at what level of biological organization were the responses observed and whether organisms exhibit compensatory responses at higher-levels of biological organization? Finally, under what conditions did the responses occur including consideration of whether the responses were observed in the presence of overt or systemic toxicity?. The presence of overt and/or systemic toxicity introduces uncertainty in the ability to distinguish effects specifically related to an endocrine-related effect from a non-endocrine toxic response.

This uncertainty in distinguishing whether the responses were endocrine-related was discussed at the FIFRA SAP meeting that evaluated scientific issues associated with the WoE evaluation of the EDSP Tier 1 screening process. In October 2013, the SAP stated that , "*In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms*

of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis" (USEPA, 2013). For these WoE analyses, overt toxicity was generally defined in accordance with EPA's current approach as used by OPP in reviewing 40 CFR Part 158 studies for both human and ecological risk assessments. Specifically, in these analyses, the effects that EPA considered to be potential evidence of overt toxicity included, but were not limited to: mortality; clinical signs such as tremors, ataxia and abnormal swimming (fish and aquatic-phase amphibians); and body weight decreases of ≥10% in mammals. Additionally, other effects including morphological (*e.g.*, organ weights/histopathology), biochemical (*e.g.,* blood chemistry), and other clinical signs (*e.g.*, lethargy) were also considered when evaluating overt toxicity, especially if the effects were extreme. In some instances, one parameter (*i.e.,* death or >10% decrease in mammalian body weight) was sufficient to consider a dose/concentration to be overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. For example, in the FSTRA, generally, body weight decreases were considered along with other responses when assessing potential overt toxicity. Additionally, effects which were considered to be signs of systemic toxicity were also captured and these effects were generally considered as less severe forms of toxicity (*e.g.*, changes in organ weights or blood chemistry). The circumstances for which a dose/concentration was considered overtly toxic for a particular study are described in Section IV.A.

In summary, EPA considers multiple lines of evidence in including the observed responses in the Tier 1 assays and OSRI in the context of a chemical's physical/chemical properties and its known modes of action in its overall characterization of a chemical's potential to interact with the E, A or T pathway. Adequately addressing the three main components described above is fundamental to the WoE process and in determining whether additional data are needed. In addition to characterizing the WoE, reviewers also considered: 1) uncertainties and their potential impact to conclusions; 2) discussion of key studies; 3) description of inconsistent or conflicting data; 4) overall strength of evidence supporting a conclusion; and, 5) what, if any, additional data are needed and why. Assessing the need for additional data is based on a case-by-case analysis which took all available toxicity data into account.

The WoE approach involved consideration of data (i.e., lines of evidence) from the EDSP Tier 1 assays and OSRI which are depicted in **Tables 2 - 4**. *These tables contain data that are considered scientifically and biologically relevant in regards to a treatment-related effect which supports a conclusion of whether a substance has the potential to interact with the E, A, or T pathway. Effects that occurred in the presence of overt toxicity are discussed in the text for each respective pathway (E, A or T) but are not reported in the table for E, A or T.*

A. EDSP Tier 1 Screening Assays

The Tier 1 assays submitted in response to the agency's test order for DCPA are shown below in **Table 1**.

Table 1: Tier 1 Screening Assays for DCPA.

Tier 1 Assays	Test Guideline	Test Order Status
Estrogen Receptor (ER) Binding Assay (Rat uterine cytosol)	OCSPP 890.1250	Requirement Satisfied (MRID No. 48615904)
ERα Transcriptional Activation Assay (Human cell line HeLa 9903)	OCSPP 890.1300; OECD 455	Requirement Satisfied (MRID No. 48675901 & 48443201)
Androgen Receptor (AR) Binding Assay (Rat prostate cytosol)	OCSPP 890.1150	Requirement Satisfied (MRID No.48670302)
Steroidogenesis Assay (Human cell line H295R)	OCSPP 890.1550; OECD 456	Requirement Satisfied (MRID No. 48615906)
Aromatase Assay (Human recombinant microsomes)	OCSPP 890.1200	Requirement Satisfied (MRID No. 48615903)
Uterotrophic Assay (Rat)	OCSPP 890.1600; OECD 440	Requirement Satisfied (MRID No. 48443203)
Hershberger Assay (Rat)	OCSPP 890.1400; OECD 441	Requirement Satisfied (MRID No. 48615902)
Pubertal Female Assay (Rat)	OCSPP 890.1450	Requirement Satisfied (MRID No. 48615905)
Pubertal Male Assay (Rat)	OCSPP 890.1500	Requirement Satisfied (MRID No. 48615905)
Fish Short-term Reproduction Assay	OCSPP 890.1350; OECD 229	Requirement Satisfied (MRID No. 48670303)
Amphibian Metamorphosis Assay (Frog)	OCSPP 890.1100; OECD 231	Requirement Satisfied (MRID No. 48670304)

B. Effects on Hypothalamic-Pituitary-Gonadal (HPG) Axis

1. Effects on Estrogen Pathway

The potential for DCPA to interact with the E pathway is summarized in **Table 2.** The various targets of the estrogen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for estrogenic, anti-estrogenic, or HPG axis effects. This table also includes HPG-relevant findings from the data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

In the Tier 1 ER binding assay, DCPA displaced >50% of the radiolabeled estradiol in one competitive binding run but less than 25% of the radiolabeled estradiol in the other two runs. Based on the results from the three runs (one interactive run and two not interactive runs), DCPA was classified as equivocal in this assay. In the Tier 1 ERTA assay, the responsiveness of the cells to the very weak positive control chemical (17α-methyltestosterone) was lower than the expected values, indicating a decreased sensitivity of the assay to very weak agonists. Although the conditions of this assay were not optimal to detect very weak activity, DCPA responses were similar to those of the negative control corticosterone and not comparable to the responses seen for the very weak positive control 17α-methyltestosterone, therefore, DCPA was considered negative for ER transcriptional activation in this test system. In the Tier 1 steriodogenesis assay, DCPA treatment induced a 1.24- to 2.31-fold increase in estradiol production compared to controls. DCPA did not inhibit aromatase activity in the Tier 1 aromatase assay. No estrogen-related effects were observed in the Tier 1 *in vivo* uterotrophic or female pubertal assays.

In the FSTRA, fecundity was significantly decreased by 44% at the mid-dose (as compared to a non-significant 10% decrease in the high dose, and 7% increase in the low dose), and fertility was significantly decreased by 1.3% at the high-dose in the absence of overt toxicity.

There were no effects on estrogen endpoints in the mammalian Part 158 studies. Effects observed in the bobwhite quail reproduction study included decreases in embryo viability, hatching and survival, but these effects co-occurred with overt toxicity (mortality, clinical signs, and reduced weight gain in the parental generation). In the mallard duck reproduction study, effects on egg viability (hatching) were observed in the absence of overt toxicity.

Table 2: Estrogenic/Anti-Estrogenic Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for DCPA[1]															
Study Type / Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight / GSI	Gonadal Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assay															
ER Binding (MRID 48615904)	E														
ERTA (MRID 448675901 &48443201)		N[4]													
Aromatase (MRID 458615903)			N												
Steroidogenesis (MRID No. 48615906)			P												
Uterotrophic (MRID 48443203)					N									N	N
Female Pubertal Rat (MRID 48615905a)					N	N	N	N	N	N				↑AW[5] (H)	N
FSTRA (MRID No. 48670303)				NE		N	N				N	Fcd: ↓44% (M); Frt: ↓1.3% (H)	N		N[6]
OSRI															
Subchronic toxicity (Rats; MRID 41767901)							N							X (M, MH, H)	N
Subchronic toxicity (Mouse; MRID 41064801)							N							X (H)	N
Chronic toxicity (Dog; MRID 00083584)						N	N							N	N

Table 2: Estrogenic/Anti-Estrogenic Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for DCPA[1]															
Study Type / Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight / GSI	Gonadal Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Combined chronic toxicity / carcinogenicity (Rats; MRID 42731001)						N	N							X (ML, M, MH, H)	X (H)
Carcinogenicity (Mouse; MRID 40958701)						N	N							X (H)	N
Two-generation reproduction toxicity (MRID 41750103)							N					N		X (M,H)	X (M, H)
Developmental toxicity (Rats; MRID 00160685)						N	N							N	N
Developmental toxicity (Rats; MRID 00158010)						N	N							N	N
Developmental toxicity (Rabbits; MRID 41054820)						N	N							N	N
Developmental toxicity (Rabbits; MRID 45820101)						N	N							N	N
Avian reproduction (Bobwhite quail; MRID 47550001)							N					N		N	X (M, H)

Table 2: Estrogenic/Anti-Estrogenic Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for DCPA[1]															
Study Type / Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight / GSI	Gonadal Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Avian reproduction (Mallard duck; MRID 47550002)							N					↓18% F1 hatching (H)		N	N

1 Key to responses: L=Low treatment, M=Medium treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay. AW=adrenal weight

2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

4. In the main assays, the responsiveness of the cells to the very weak positive control 17α-methyltestosterone was lower than the expected values, indicating a decreased sensitivity of the assay to very weak agonists. Although the conditions of this assay were not optimal to detect very weak activity, DCPA responses were similar to those of the negative control corticosterone and not comparable to the responses of 17α-methyltestosterone. DCPA is considered negative in this assay.

5. Relative adrenal weights ↑14% ($p \leq 0.05$) and absolute adrenal weights ↑17% at 1000 mg/kg/day.

6. Decreases in male body weight observed; no other clinical signs of toxicity.

P Positive findings

N Negative findings (*in vivo)* / No effect (*in vivo)*

E Equivocal

2. Effects on Androgen Pathway

The potential for DCPA to interact with the A pathway is summarized in **Table 3.** The various targets of the androgen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for androgenic, anti-androgenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

DCPA was found to be untestable in the Tier 1 AR binding assay according to the guideline criteria as precipitation was observed at concentrations above 10^{-6} M. In the Tier 1 steroidogenesis assay, testosterone production was decreased to 0.91- to 0.71-fold relative to the control. No effects on androgen-sensitive tissue weights were observed in the Hershberger assay. In the male pubertal assay, non-dose dependent weight decreases were observed in the LABC (↓15% at 500 mg/kg/day, ↓14% at 1000 mg/kg/day) and seminal vesicles without coagulating fluid (↓17% at 500 mg/kg/day, ↓14% at 1000 mg/kg/day). The changes in AST weights were considered to be not treatment related since the effects seen at ½ the limit dose were comparable to those seen at the limit dose (1000 mg/kg/day) and there were no effects on serum testosterone levels or gonadal histopathology. Fertility was decreased 1.3% in the FSTRA at the high test concentration; male body weights were significantly decreased at all treatment groups, but no other clinical signs of toxicity were noted.

There were no androgen-related effects observed in the mammalian Part 158 data. As noted previously, effects observed in the bobwhite quail reproduction study included decreases in embryo viability, hatching and survival, but these effects co-occurred with overt toxicity (mortality, clinical signs, and reduced weight gain in the parental generation). In the mallard duck reproduction study, effects on egg viability (hatching) were observed in the absence of overt toxicity.

Table 3: Androgenic/Anti-Androgenic Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for DCPA[1]														
Study Type / Literature Citation	AR Binding	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/2° Sex Characteristics	Age and Weight at PPS	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assay														
AR Binding (MRID 48670302)	U[4]													
Steroidogenesis (MRID 48615906)		P												
Hershberger (MRID 486159092; 48443202)				N		N	N		N				↑LW (H)[5]	N
Male Pubertal Rat (MRID 48615905b)			N	N	N	N	N	N	N	N			N	N
FSTRA (MRID No. 48670303)			NE	N	N						Frt: ↓1.3%	N		N[6]
OSRI														
Subchronic toxicity (Rats; MRID 41767901)							N						X (M, MH, H)	N
Subchronic toxicity (Mouse; MRID 41064801)							N						X (H)	N
Chronic Toxicity (Dog; MRID 00083584)				N	N	N	N						N	N
Combined chronic toxicity / carcinogenicity (Rats; MRID 42731001)						N	N						X (ML, M, MH, H)	X

Table 3: Androgenic/Anti-Androgenic Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for DCPA[1]														
Study Type / Literature Citation	AR Binding	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/2° Sex Characteristics	Age and Weight at PPS	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Carcinogenicity (Mouse; MRID 40958701)						N	N						X (H)	N
Two-generation reproduction (MRID 41750103)					N				N				X (M,H)	N
Avian reproduction (Bobwhite quail; MRID 47550001)							N				N		N	X (M, H)
Avian reproduction (Mallard duck; MRID 47550002)											↓18% F1 hatching (H)		N	N

1. Key to responses: L=Low treatment, M=Medium treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay.
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. DCPA is classified as untestable under the conditions of this assay because precipitation was observed at concentrations $>10^{-6}$ M.
5. 20% increase in liver weight in the anti-androgenic phase of the Hershberger assay at 1000 mg/kg/day.
6. Male body weights were decreased at all treatment levels, 0.0083, 0.025, and 0.075 mg/L, in the FSTRA (17-22%, $p < 0.05$); no other clinical signs of toxicity were observed.

P Positive findings
N Negative findings (*in vivo)* / No effect (i*n vivo)*
NE Not Examined
U Untestable (*i.e.* compound is not soluble above 10^{-6} M)

C. Effects on Hypothalamic-Pituitary-Thyroidal (HPT) Axis

The current EDSP Tier 1 battery does not have a specific *in vitro* assay to detect chemicals with the potential to affect hypothalamic or pituitary regulation of thyroid hormone production, but it does include three *in vivo* assays that provide data to detect changes in the HPT axis, ***i.e.***, the female and male (rat) pubertal assays, and the AMA (frog).

The potential for DCPA to interact with thyroid regulation is summarized in **Table 4.** The various targets of the T pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for thyroid or HPT axis effects. This table also includes HPT-relevant findings from the available OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

Serum T_4 levels were dose-dependently decreased by 78-81% in the Tier 1 male pubertal assay, and by 47-55% in the Tier 1 female pubertal assay (at dose levels of 500 and 1000 mg/kg/day). No other thyroid-related effects were observed in the pubertal assays. In the AMA, thyroid gland histopathology observations included hypertrophy and follicular cell height increases at the mid and high doses compared to the negative and solvent controls. At day-7, in the mid-dose, growth effects included SVL and body weight increases of 10%, and 32%, respectively, as well as a 12% increase in the developmental endpoint (normalized HLL) compared to the negative control. Significant differences between the negative control and solvent control were observed for the day 21 growth and developmental endpoints and so the treatment groups were compared to the solvent control. The following development changes related to treatment were also noted at the mid-dose: compared to solvent control, there were effects approaching statistically significance ($p = 0.06$ or 0.07) including increases in day-21 NF stage and normalized HLL (NF Stage median = 58 vs. 57 in solvent control, normalized HLL increase = 15%). Additional growth effects compared to solvent control included 21-day SVL increase of 4% at the mid dose and 21-day wet weight decrease by 10% at the low dose. The thyroid histopathology/developmental effects observed in the AMA co-occurred with overt toxicity (12.5 % increase in mortality) only at the high dose.

In the Part 158 studies, thyroid follicular hypertrophy was observed in the subchronic toxicity study in rats. In a combined chronic toxicity/carcinogenicity study in rats, the following effects on the thyroid were also noted: treatment-related increases in thyroid weights (both sexes); increased follicular cell hyperplasia/hypertrophy (10 mg/kg/day males; ≥50 mg/kg/day both sexes); decreased T_4 levels (10 mg/kg/day males; ≥50 mg/kg/day both sexes); and a treatment-related increase in thyroid follicular cell neoplasms (both sexes). In a two generation reproduction study in rats, thyroid gland histological changes (basophilic clumped colloid and follicular cell hypertrophy) were observed at and above 250 mg/kg/day in F0 and F1 males and/or females; thyroid hormones were not tested in this study. No thyroid effects were observed in the mouse or dog studies.

Table 4: Thyroid Pathway for DCPA

Lines of Evidence Indicating Potential Interaction with the Thyroid Pathway for DCPA[1]									
Study Type/ Literature Citation	Thyroid Weight	Thyroid Histopathology	T_4 Levels	TSH Levels	Pituitary Weight	Body Weight (BW) or Snout-Vent-Length (SVL)	Developmental Stage or Asynchronous Development or Hind Limb Length	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1									
Male Pubertal (Rat) (MRID 48615905b)	N	N	↓78% L; ↓81% H	N	N	N		N	N
Female Pubertal (Rat) (MRID 48615905a)	N	N	↓47% L; ↓55% H	N	N	N		N	N
AMA (Frog) (MRID No. 48670304)		P[4] (M)				↓10% BW (L) and ↑4% SVL (M)	P[5] (M)		X (H)
OSRI									
Subchronic toxicity (Rat; MRID 41767901)	N	P (H)						X (M,MH,H)	N
Subchronic toxicity (Mouse; MRID 41064801)	N							X (H)	N
Chronic toxicity (Dog; MRID 00083584)	N	N						N	N
Combined chronic toxicity/ carcinogenicity (Rats; MRID 42731001)	P[6]	P[6]	P[6]					X (ML, M, MH, H)	X
Carcinogenicity (Mouse; MRID 40958701)	N	N						X (H)	N
Two-generation reproduction (MRID 41750103)		P (M, H)						X (M, H)	N

1. Key to responses: L=Low treatment, M=Medium treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay.
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Significant differences between the negative control and solvent control were reported, so all effects in the AMA in this table are in comparison to the solvent control. Thyroid gland hypertrophy and follicular cell height increases at the mid dose compared to the solvent control.
5. The increases in the mid-treatment group were notable but not statistically significant (NF Stage median=58 vs. 57 in solvent control, Jonckheere p=0.07; normalized HLL increase=15%, Dunnett p=0.06).
6. Effect seen at doses ≥ 10 mg/kg/day (low dose) in males and ≥50 mg/kg/day (mid-low dose) in females.

P Positive findings
N Negative findings

IV. Committee's Assessment of Weight of Evidence

This section of the document describes the weight of evidence (WoE) determination on the potential of DCPA to interact with endocrine related processes (*i.e.,* E, A or T hormonal pathways) as well as recommendations regarding Tier 2 testing.. The results of the Tier 1 assays are considered, along with other scientifically relevant information (*e.g.,* 40 CFR Part 158 test guidelines and published or publicly available peer-reviewed studies). WoE analysis in the context of the EDSP follows the Agency's guidance (USEPA 2011) and is conducted on a case-by-case basis by first assessing the different lines of evidence (*i.e.,* specific Tier 1 Assays and OSRI), then performing an integrated analysis of those lines of evidence.

The WoE evaluation includes considerations of biological plausibility of the findings from the different lines of evidence by examining the consistency, coherence, and interrelationships among the measured endpoints within and across studies. The available findings from standard toxicology studies on the substance may contribute to the WoE evaluation in helping elucidate if effects seen in the Tier 1 assay are related to perturbations of the endocrine system *per se* or alternatively sequelae of systemic effects. Endocrine modes of action may elicit a number of phenotypic consequences other than those evaluated in the Tier 1 assays.

Endocrine-related findings in the presence of overt toxicity may result in uncertainty as to whether or not the responses are related through an endocrine pathway, therefore non-endocrine toxic responses (including but not limited to mortality or body weight changes) are also considered in this WoE evaluation. The FIFRA SAP that evaluated scientific issues associated with weight of evidence evaluation of the results of the Tier 1 assays stated that *"In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis"* (USEPA, 2013).

A. Systemic/Overt Toxicity in the *in vivo* Tier 1 Assays and OSRI

Effects that were considered to be systemic or overt toxicity for the *in vivo* Tier 1 assays and OSRI studies are described below. In addition to the endocrine-related effects described above for the *in vivo* Tier 1 assays and OSRI, other effects observed which may be considered overt/systemic toxicity are also describe below. Generally, one parameter (i.e., death or >10% decrease in mammalian body weight) was sufficient for a dose/concentration to be considered overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. Effects which were considered to be signs of systemic toxicity were generally less severe forms of toxicity (e.g., changes in organ weights or blood chemistry).

1. Tier 1 *in vivo* Assays

No adverse systemic toxicity or overt toxicity were seen when tested up to the limit dose (1000 mg/k/day) in the in the mammalian *in vivo* Tier 1 assays except for a 20% increase in liver weight in the antiandrogenic phase of the Hershberger assay at the limit dose. In fish, male body weights were decreased at all treatment levels, 0.0083, 0.025, and 0.075 mg/L, in the FSTRA (17-22%, $p<0.05$). In frogs, survival was decreased at the highest treatment level, 0.58 mg/L in the AMA (12.5%, $p<0.05$).

2. OSRI

In the longer term toxicity studies with DCPA, thyroid toxicity was characterized by decreased levels of thyroid hormone, microscopic thyroid changes and increased thyroid weight. Liver toxicity included increased liver weight, elevated liver enzyme activity, increased cholesterol and liver hypertrophy. Data showed that the liver effects are precursor events to the thyroid effects, with increased metabolism of thyroid hormone by the liver resulting in a compensatory stimulation of the thyroid.

In a subchronic mice study, absolute and relative liver weights were increased in males and females at the limit dose (1000 mg/kg/day); significantly increased incidence of minimal centrilobular hepatocyte enlargement was seen at the same dose in males and females. Thyroid hormones were not measured in this study.

In the subchronic rat study, absolute liver weights increased in males at the high dose (1000 mg/kg/day) and male relative liver weights increased at 150 and 1000 mg/kg/day; in females absolute and relative liver weights increased at doses ≥50 mg/kg/day but not in a dose-dependent manner. There were no changes in liver enzymes. Changes in absolute and relative kidney weights were also seen at ≥150 mg/kg/day. Thyroid follicular cell hypertrophy was increased in males and females at 1000 mg/kg/day; clumped colloidal and cystic follicles were increased in thyroid in males at 1000 mg/kg/day. Thyroid hormones were not measured.

In the combined chronic toxicity/carcinogenicity study with rats, there was increased mortality in both sexes at the high dose (1000 mg/kg/day) and decreases in body weight in females at the mid dose (500 mg/kg/day) and in both sexes at the high dose. Thyroid effects included decreases in T4 at various time intervals at doses ≥10 mg/kg/day and T3 levels at doses ≥50 mg/kg/day. There were compensatory increases in thyroid stimulating hormones in both sexes throughout the study, but increases did not show statistical significance. Absolute and relative thyroid weights were increased in both sexes at the high dose. Histopathological changes included increases in thyroid follicular cell hyperplasia, hypertrophy, and/or basophilic clumped colloid in males at ≥10 mg/kg/day and in females at ≥50 mg/kg/day. Thyroid tumors were seen in males (follicular cell adenomas only) and females (follicular cell adenomas and carcinomas) and liver tumors in females (adenomas, carcinomas, and cholangiocarcinomas). Possible evidence of liver injury

was shown by elevated serum GGT activity in both sexes at the mid (500 mg/kg/day) and high dose (1000 mg/kg/day) groups. Absolute and relative liver weights were variably increased in the two higher dose groups in both sexes. Histopathological liver lesions included increased incidence of eosinophilic foci and increases in centrilobular hepatocyte swelling at doses of 10 mg/kg/day and greater.

In the carcinogenicity study in mice, liver toxicity manifested as alterations in the liver enzymes (SDH, ALT) and increased liver weight and the presence of hepatic adenomas (not carcinomas) only in females. Thyroid hormones were not evaluated and there were no treatment-related change in the thyroid glands.

In the rat 2-generation reproductive study, thyroid changes in 250 mg/kg/day and 1000 mg/kg/day F0 and F1 males and/or females included basophilic clumped colloid and follicular cell hypertrophy. Centrilobular hepatocyte swelling occurred in 5000 ppm and 20000 ppm males and females. Thymic involution was increased in 20000 ppm males. Thyroid hormones were not tested. Liver (centrilobular hepatocyte swelling) and lung (interstitial pneumonitis and thickened alveolar) microscopic pulmonary changes were observed in F0 and F1 males and females at 250 and above.

In bobwhite quail, mortality (9-25%), weight loss (14-35%, $p < 0.05$), and clinical signs including emaciation, reduced reaction to external stimuli, lethargy, ruffled appearance, wing droop, and lower limb weakness were observed at 3170 and 8020 mg/kg-diet in a reproduction study. No effects were seen at 1280 mg/kg-diet.

B. Estrogen Pathway

There was no convincing evidence to support potential interaction with the estrogen pathway. Although DCPA increased estradiol production in the Tier 1 steroidogenesis assay and the Tier 1 ER binding assay results were equivocal, the Tier 1 ERTA and aromatase assays were negative and there were no estrogen-related effects observed in the Tier 1 uterotrophic and female pubertal assays or in the Part 158 mammalian toxicity studies. There was a non-treatment-responsive decrease in fecundity at the mid dose and a slight decrease in fertility at the high dose in the FSTRA; no other complementary responses were observed across other endpoints within those treatment groups. In the wildlife Part 158 studies, in the bobwhite quail avian reproduction study, all observed reproductive effects were seen only at an overtly toxic dose, while in the mallard duck study decreases in viable embyros/hatching were observed (no other reproductive effects observed). Therefore, there is a lack of redundancy across the battery, with no or limited effects observed without complementary responses.

C. Androgen Pathway

There was no convincing evidence of an interaction with the androgen pathway. DCPA was found to be untestable (due to insolubility) in the Tier 1 AR binding assay and testosterone production was decreased In the Tier 1 steroidogenesis assay. DCPA was negative in the Hershberger assay and there were no treatment-related androgen effects in the male pubertal assay. There were no androgen-related effects observed in the mammalian Part 158 studies. There was a decrease in fertility at the high dose in the FSTRA; however there was a lack of complementary responses across other endpoints. In the wildlife Part 158 studies, for the bobwhite quail reproduction study, all effects were observed in the presence of overt toxicity, while in the mallard decreases in viable embyros/hatching were observed (no other reproductive effects observed).

D. Thyroid Pathway

DCPA demonstrated a potential interaction with the thyroid hormone pathway in the absence of overt or systemic toxicity. In the pubertal assays, serum T4 levels were dose-dependently decreased in males at 500 mg/kg/day (78%) and 1000 mg/kg/day (81%) and in females at 500 mg/kg/day (47%) and 1000 mg/kg/day (55%). In the AMA, increased incident of thyroid gland hypertrophy, follicular cell height increases were reported at the high concentration compared to the control. In addition, development changes related to DPCA treatment seen in the AMA include an increase in the normalized HLL on day 7 and 21.

DCPA also induced thyroid effects in several Part 158 studies. Thyroid follicular hypertrophy was observed in a subchronic toxicity study in rats. In a combined chronic toxicity/carcinogenicity in rats, increased thyroid weights, follicular cell hyperplasia/hypertrophy, decreased T4 levels, and an increased incidence of thyroid follicular cell neoplasms were seen in both sexes.

E. Conclusions

Overall, there is no convincing evidence for interaction with the estrogen or androgen pathway. However, there is evidence for a potential interaction with the thyroid pathway in mammals and wildlife based on thyroid-related responses seen in the Tier 1 pubertal assays and AMA, as well as in the mammalian Part 158 studies.

V. EDSP Tier 2 Testing Recommendations

In the EDSP Tier 1 male and female pubertal assays as well as in the OSRI, there was evidence for potential interaction with the thyroid pathway in studies conducted with adult animals, but no such data exists for potential thyroid toxicity in the young animals.

For DCPA, the current point of departure (POD) of 1.0 mg/kg/day for human health risk assessment is based on decreased level of T4 and increased incidence of histopathological lesions in the thyroid and liver at 10.0 mg/kg/day (LOAEL) in a chronic toxicity/carcinogenicity study in rats. This POD is used to derive the chronic Reference Dose (RfD) for dietary exposure risk assessments and the Margins of Exposure (MOEs) for non-dietary exposure risk assessments.

In general, since the POD and the toxicity endpoints of concern are based on data obtained from adult animals, there usually would be a concern that the POD may not be protective of potential thyroid toxicity in the young. Therefore, a special thyroid assay in pregnant animals, fetuses, postnatal animals, and adult animals is recommended. This special study should use a mechanistic approach to generate specific data on the thyroid (*i.e.*, the primary target of DCPA) to protect the developing nervous system from thyroid hormone disrupting chemicals. The specific purpose of this study is to establish a POD (*i.e.*, NOAELs and LOAELs or benchmark doses) that may be used for human health risk assessment. The POD from this special study would address the concern for the potential ability of DCPA to disrupt thyroid function in pregnant females and in the fetus or newborn. However, it is noted that the agency has issued a Data Call-In notice for this special study under the Registration Review program.

For the wildlife, to add to the understanding of potential thyroid effects, the EDSP Tier 2 Larval Amphibian growth and Development Assay (LAGDA) is recommended since there is no chronic aquatic amphibian toxicity data available. Chronic toxicity data with fish (surrogates for aquatic-phase amphibians) are not currently available for DCPA.

For birds, Part 158 avian reproduction studies with both northern bobwhite quail and mallard duck are available for DCPA. In both species, effects on reproductive parameters were observed. The type of data obtained from Part 158 avian reproduction studies (OCSPP 850.2300) are considered sufficient for evaluating potential reproductive effects to birds from DCPA exposure. Relative to EDSP, additional testing is not recommended.

VI. References

Akhurst, L.C. (2011). Chlorthal dimethyl (DCPA) estrogen receptor transcriptional activation (Human Cell Line (HeLa-9903)). Huntingdon Life Sciences, Cambridgeshire, England. Laboratory report number BDG0009. Unpublished. (MRID 48443201).

Akhurst, L.C. (2011). Chlorthal dimethyl (DCPA) H295R steroidogenesis assay. Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England. Laboratory Study No.: BDG0013. Unpublished. (MRID 48615906)

Benz, G., Lucas, F., and Lovell, R. (1990). A two generation reproduction study in rats with technical DCPA. Ricerca, Inc.: Lab Project Number: 173088-0176-TX-003: 88-0176. Unpublished. (MRID 41750103).

Chun, J., Lucas, F., and Killeen, J. (1989). A teratology study with technical DCPA in rabbits. Ricerca, Inc.: Proj. ID 87-3204, 1116-87-0067-TX-002. Unpublished. (MRID 41054820).

Donington, S. (2011). Amended Final Report, Chlorthal Dimethyl (DCPA): Hershberger bioassay in the castrated rat (oral administration). Huntingdon Life Sciences, Cambridgeshire, England. Laboratory Project Study ID: BDG0016. Unpublished. (MRID 48615902).

Donington, S. (2011). Chlorthal Dimethyl (DCPA): Hershberger bioassay in the castrated rat (oral administration). Huntingdon Life Sciences, Cambridgeshire, England. Laboratory Project Study ID: BDG0016. Unpublished. (MRID 48443202).

Frizell, E.R. and Parker, R.M. (2011). Chlorthal dimethyl (DCPA): assessment of pubertal development and thyroid function in juvenile male and female rats. Huntingdon Life Sciences, East Millstone, NJ. Laboratory Report # 10-4359. Unpublished. (MRID 48615905 a & b).

Ford, W. (1986). A teratology study in rats with technical DCPA. SDS Biotech Corp.: Document No. 712-5TX-85-0039-003: Report SDS-893. Unpublished. (MRID 00160685).

Ford, W. (1986). A 90-day dietary study in mice with technical DCPA. SDC Biotech Corp.: Document No.: 696-5TX-84-0082-002. Unpublished. (MRID 41064801).

Frey, L.T., et al. (2003). Chlorthal-Dimethyl (DCPA): A Reproduction Study with the Northern Bobwhite. Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 246-115. Study sponsored by Amvac Chemical Corporation, City of Commerce, CA. Study initiated June 17, 2002 and submitted May 28, 2003. (MRID 47550001).

Frey, L.T., et al. (2003). Chlorthal-Dimethyl (DCPA): A Reproduction Study with the Mallard. Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 246-116. Study sponsored by Amvac Chemical Corporation, City of Commerce, CA. Study initiated June 17, 2002 and submitted June 20, 2003. (MRID 47550002).

Hazleton, L.W., and Dieterich, W.H. (1963). Final report: two-year dietary feeding — dogs. Diamond Shamrock Agricultural Chemicals: Lab Study No.: CDL:090443-T. Unpublished. (MRID 00083584).

Lucas, F., and Killeen, J. (1988). A combined chronic toxicity and tumorigenicity study in mice with technical DCPA. Ricerca, Inc.: Document No. 1098-85-0057-TX-002. Unpublished. (MRID 40958701).

Lucas, F., and Benz, G. (1991). A 90-day feeding study in rats with technical DCPA. Ricerca, Inc.: Lab Project Number: 89-0208. Unpublished. (MRID 41767901).

Lucas, F., Mizens, M., and Laveglia, J. (1993). A combined chronic toxicity/oncogenicity study in rats with DCPA. Ricerca, Inc.: Lab Project Number: 3339-90-0005-TX-006. Unpublished. (MRID 42731001).

Matthews, A. (2011). Chlorthal dimethyl (DCPA): estrogen receptor binding assay using rat uterine cytosol (ER-RUC). Huntingdon Life Sciences, Huntingdon, Cambridgeshire, UK. Laboratory Study No.: BDG0008. Unpublished. (MRID 48615904).

Matthews, A. (2011). Chlorthal Dimethyl (DCPA): Aromatase (Human Recombinant) Assay. Huntingdon Life Sciences Ltd (Huntingdon Research Centre [Woolley Rd, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK]). Laboratory Study Number: BDG0007. Unpublished. (MRID 48615903).

Sahrif, I. (2011). Chlorthal dimethyl (DCPA): uterotrophic bioassay in ovariectomised rats (oral administration). Huntingdon Life Sciences, Cambridgeshire, England. Laboratory Project Study ID: BDG0014. Unpublished. (MRID 48443203).

Schneider, S.Z., K.H. Martin, T.Z. Kendall and H.O. Krueger. (2012). Chlorthal dimethyl (DCPA): Fish short-term reproduction Assay with the fathead minnow (*Pimephales promelas*). Wildlife International, Ltd., Easton, Maryland. Lab Study No.: 246A-101A. Unpublished. (MRID 48670303).

Schneider, S.Z., T.Z. Kendall, and Krueger, H.O. (2012). Chlorthal dimethyl (DCPA): amphibian metamorphosis assay. Wildlife International, Ltd., Easton, MD. Laboratory report number 246A-102. Unpublished. (MRID 48670304).

USEPA (2011). Endocrine Disruptor Screening Program — Weight of Evidence: Evaluating Results of EDSP Tier 1 Screening to Identify the Need for Tier 2 Testing. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency, Washington, DC.

USEPA (2013) Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held July 30 - August 1, 2013 on Weight-of-Evidence: Evaluating Results of EDSP Tier 1 Screening. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency, Washington, DC.

Willoughby, J.A. (2012). Chlorthal dimethyl: androgen receptor binding (rat prostate cytosol). CeeTox Inc., Kalamazoo, MI. Laboratory Study No.: 9150V-100601ARB. Unpublished. (MRID 48670302).

APPENDIX 1. EDSP Tier 1 Screening Assays

Amphibian Metamorphosis Assay (Frog); OCSPP 890.1100

The 21-day assay (MRID 48670304) of DCPA on amphibian metamorphosis of African clawed frog (*Xenopus laevis*) was conducted under flow-through conditions. Amphibian larvae at Nieuwkoop-Faber (NF) stage 51 (80 larvae per control and treatment group) were exposed to negative and solvent controls (0.020 mL/L dimethylformamide; DMF) and DCPA (98.3% purity) at nominal concentrations of 0.0083, 0.025, and 0.075 mg a.i./L. Mean-measured concentrations were <0.00400 (<LOQ; negative and solvent controls), 0.0071, 0.021, and 0.58 mg a.i./L, respectively. The test system was maintained at 21.0 to 22.4°C and a pH of 7.9 to 8.4.

Larval survival on Day 7 ranged from 90 to 100% in the controls and treatment groups. By Day 21, survival was 97.5 and 96.3% in the negative and solvent controls, respectively, and 95.0, 97.5, and 87.5% in the low, mid and high treatment groups, respectively. At the high treatment group, the observed mortality of 12.5% was a statistically significant increase from the negative control (Fisher's Exact Test $p<0.05$). These high treatment group mortalities occurred in all replicates and 8 of the 10 mortalities occurred on Days 5 and 6. Curved/crooked (bent) tails were observed in 18-30 (36 to 53%) surviving tadpoles among both controls and all treatment groups. It is unclear if the low food ration (which was half of the recommended ration during the definitive portion of the test) influenced the condition of the test organisms; however, due to similar incidence rates in both the negative and solvent controls and treatment groups, it does not appear to be a treatment-related effect.

At the mid treatment level, DCPA significantly increased ($p<0.05$) Day 7 hind-limb length (HLL; ↑23%), normalized (for snout-vent length) HLL (↑12%), wet weight (↑32%), and snout-vent length (SVL; ↑10%) compared to the negative control. By Day 21, DCPA significantly increased ($p<0.05$) HLL (↑14, 43 and 24%) and wet weight (↑8, 28 and 20%) relative to the negative control at the low, mid and high treatment levels, respectively. Additionally, Day 21 SVL was significantly increased ($p<0.05$) by 3 to 9% and Day 21 normalized HLL was significantly increased ($p<0.05$) by 11-32% relative to the negative control at all treated levels. No significant acceleration or delay of NF development stage was observed on either Day 7 or 21, and no asynchronous development was observed. Three late-stage (NF stage >60) tadpoles (two from the mid treatment and one from the high treatment group) were excluded from analyses of continuous endpoints (SVL, weight, and normalized HLL). Effects on thyroid gland histopathology, including thyroid gland hypertrophy and follicular cell height increases, were observed at the mid and high treatment groups.

Unless otherwise indicated, all effects are reported based on comparison to the negative (clean water) control. The reviewer found no significant differences between the negative control and the solvent control for any endpoints on Day 7. However, significant differences between the

negative and solvent controls were detected ($p<0.05$; 2-sided t-test) on Day 21 for wet weight, SVL, and HLL. Therefore, it is unclear to what extent the results observed may be related to the presence of the solvent. Treatment groups were compared to the solvent control for the Day 21 endpoints. There were no statistically significant differences between the solvent control and treatment groups for development stage or normalized HLL, however the increases in the mid treatment group were notable (NF Stage median=58 vs. 57 in solvent control, Jonckheere $p=0.07$; normalized HLL increase=15%, Dunnett $p=0.06$). For HLL and SVL, there were significant increases (Dunnett $p<0.05$) in the mid treatment group. For wet weight, the lowest treatment group was significantly decreased (Mann-Whitney $p=0.03$) compared to the solvent control. While effects were still observed in the mid-treatment group compared to the solvent control, the results were more pronounced when compared to the negative control.

AR Binding Assay (rat prostate cytosol); OCSPP 890.1150

In an androgen receptor (AR) binding assay (MRID 48670302), ventral prostate cytosol from Sprague Dawley rats was used as the source of AR to conduct a competitive binding experiment, which measured the binding of a single concentration of [^{3}H]-R1881 (1 nM) in the presence of increasing concentrations (10^{-12} to 10^{-3} M) of DCPA (98.3% a.i., Batch# 090614-2). Dimethyl sulfoxide (DMSO) was used as the solvent vehicle at a final assay concentration of approximately 3.2%. A total of three independent runs were performed, and each run included dexamethasone as a weak positive control, and R1881 as the ligand reference standard.

Saturation binding data were not provided in the original study report; however, summarized saturation binding data (MRID 48843501) from the performing laboratory were submitted following a request by the Agency. The dissociation constant (K_d) for [^{3}H]-R1881 was 0.613±0.041 nM and the estimated B_{max} was 0.817±0.049 fmol/100 µg protein for the single batch of prostate cytosol used for this assay. The mean and individual K_d and B_{max} values were below the expected ranges reported in the EPA validation program (0.685 to 1.57 nM and 7 to 16 fmol/100 µg protein, respectively). Confidence in these numbers is high according to the goodness of fit (R^2 = 0.957-0.984) and the small variation among runs.

In the competitive binding experiment, precipitation of DCPA was visually observed at concentrations of 10^{-5} to 10^{-3} M in Run 1 and 10^{-5} and 10^{-4} M in Run 2; therefore, the data at these concentrations were not evaluated. Although no precipitation was observed at the highest concentration tested in Run 3, the 10^{-5} M concentration was not evaluated due to the precipitation observed at this level in the first two runs. Therefore, 10^{-6} M was the highest concentration evaluated in all three runs.

In all three runs, specific binding of [^{3}H]-R1881 was 84.2-102.3% at DCPA concentrations of 10^{-12} to 10^{-6} M.

The estimated average log IC_{50}s for R1881 (−9.0 M) and the weak positive control, dexamethasone (−4.6 M), were within the expected ranges, and the mean RBA for the dexamethasone was 0.0040%. Confidence in the numbers for the reference standards is high as there was no variation in the IC_{50} values between runs. The solvent control responses indicated no drift in the study assay, and the performance criteria were generally met.

DCPA is classified as untestable under the conditions of this assay because precipitation was observed at concentrations $>10^{-6}$ M.

ER Binding Assay (Rat uterine cytosol); OCSPP 890.1250

In an estrogen receptor (ER) binding assay (MRID 48615904), uterine cytosol from Sprague-Dawley rats was used as the source of ER to conduct saturation and competitive binding experiments. A saturation binding experiment was conducted to demonstrate that the ER in the rat uterine cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radio-labeled reference estrogen prior to conducting ER competitive binding experiments. The competitive binding experiment was conducted to measure the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) in the presence of increasing concentrations of DCPA (logarithmic increase from 10^{-10} to 10^{-4} M). DMSO was used as the vehicle at a final concentration of 2%. The assay included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control, and 17-β-estradiol as the natural ligand reference material.

For the saturation binding assay, the appropriate protein concentration was determined to be 0.5 mg/mL as it bound 26% of the [^{3}H]-17β-estradiol (within the Guideline range of 25-35% binding). The K_d for [^{3}H]-17β-estradiol was 0.380 nM, and the estimated B_{max} was 0.1287 nM for the prepared rat uterine cytosol. The K_d for each run was within the expected range of 0.03 to 1.5 nM.

For the competitive binding assay, the appropriate protein concentration was determined to be 0.35 mg/mL as it bound approximately 11% (by interpolation) of the [^{3}H]-17β-estradiol (within the Guideline range of 10-15% binding). The mean IC_{50} from the three runs was 9.7×10^{-10} M for 17β-estradiol and 3.57×10^{-6} M for the weak positive control, 19-norethindrone. The mean relative binding affinity (RBA) was 0.026% for 19-norethindrone. DCPA displaced >50% of the radiolabeled estradiol in one competitive binding run with an IC_{50} of 5.16×10^{-5} M, and a corresponding RBA of 0.0014%. However, in the remaining two competitive runs, DCPA displaced <25% of the radiolabeled estradiol. Based on the results from the three runs (one

interactive run and two not interactive runs), DCPA is classified as Equivocal in the Estrogen Receptor Binding Assay.

ERα Transcriptional Activation Assay (Human cell line HeLa 9903); OCSPP 890.1300

In an estrogen receptor transcriptional activation assay (MRID 48675901, and 48443201) conducted by Huntingdon Life Sciences, hERα-HeLa-9903 cells cultured *in vitro* were exposed to DCPA (98.3% a.i., Lot # 090614-2) at concentrations increasing logarithmically from 7.5×10^{-11} to 7.5×10^{-5} or 3.75×10^{-11} to 3.75×10^{-5} in DMSO (0.1% v/v) for approximately 24 hours. The experiments were performed using 96-well plates and each DCPA concentration was tested in triplicate (3 wells/plate). Cells were exposed to DCPA for 23±1 hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor by DCPA was measured upon addition of a luciferase substrate and detection with a luminometer (sensitivity not reported). DCPA was tested up to the limit of solubility, 7×10^{-5} M. No cytotoxicity was observed at any concentration.

The mean RPC_{max} for DCPA was 6.8%, 5.7% and 2.0% for the first, second, and third runs, respectively, and the associated PC_{max} values were 7.5×10^{-5}, 3.5×10^{-5}, and 3.5×10^{-5} M. Precipitation was observed in the first run at the PC_{Max} (7.5×10^{-5} M).

In the main assays, the responsiveness of the cells to the very weak positive control 17α-methyltestosterone was lower than expected, indicating a decreased sensitivity of the assay to very weak agonists. Although the conditions of this assay were not optimal to detect very weak activity, DCPA responses were similar to those of the negative control corticosterone and not comparable to the responses of 17α-methyltestosterone, which was able to reach a maximum 41.3-75.2% PC. DCPA was only able to reach a maximum of 2.0-6.8% PC when tested up to the highest concentration possible based on cytotoxicity. Because the $RPC_{Max} < PC_{10}$ in all three assay runs, DCPA was considered negative for estrogen receptor transcriptional activation in this test system.

Fish Short-term Reproduction Assay (FSTRA); OCSPP 890.1350

The 21-day short-term reproduction assay (MRID 48670303) of DCPA (chlorthal dimethyl) with fathead minnows (*Pimephales promelas)* was conducted under flow-through conditions. Adult fish, 20 spawning groups (2 males and 4 females in each group; 6 months old), were exposed to DCPA (98.3% purity) at nominal concentrations of 0 (negative control), 0 [solvent control; dimethylformamide (DMF; 20 µL/L)], 0.0083, 0.025, and 0.075 mg a.i./L; mean-measured concentrations were <LOQ (<0.005), <LOQ (<0.005), 0.0074, 0.022, and 0.058 mg a.i./L, respectively. The test system was maintained at 24.0 to 25.9ºC and a pH of 7.9 to 8.5.

Unless otherwise noted, the DCPA treatment groups were compared to the negative control. There were no significant effects ($p>0.05$) on survival. Male body weight exhibited significant (Jonckheere-Terpstra; $p<0.05$) decreases of 17, 22 and 20% at the low, mid, and high treatment levels, respectively, relative to the negative control; female body weights and male and female body lengths in treated fish were similar to controls. No apparent treatment-related effects on clinical signs (i.e., behavioral and other sublethal effects) were reported relative to the negative control.

Spawning frequency in controls was at least every 4 days in 2 and 1 of the 4 replicates in the negative and solvent control, respectively, and fecundity was 20.8 and 16.2 eggs/female/day/replicate in the negative and solvent control, respectively. Fertilization success was 98.2% in the negative controls and 98.9% in the solvent controls. There was a significant (Dunnett's; $p<0.05$) reduction in fecundity of 46% at the mid treatment level relative to the negative control, and there was a slight but significant (Jonckheere-Terpstra; $p<0.05$) reduction in fertility of 1.3% at the high treatment level. Fecundity effects were not concentration-responsive. Compared to the negative control, fecundity was decreased by 22% in the solvent control and 10% in the high dose, and increased by 7% in the low dose (all of which were not statistically significant).

There were no significant effects ($p>0.05$) on male or female vitellogenin (VTG), male or female gonadal-somatic index (GSI), or male tubercle scores (no tubercles detected in females) when treated groups were compared to the negative control. Plasma testosterone and 17β-estradiol (sex steroids) were not measured in this study.

One male fish showed the presence of testis-ova in one gonad in the mid treatment group; additionally, another male was observed to be small and had increased spermatogonia with decreased secondary spermatocytes and spermatids. These observations were isolated to the mid treatment level and no other males in this treatment or the next higher treatment levels had remarkable findings, therefore, these observations were considered not to be related to DCPA exposure. There were no apparent treatment-related effects on secondary sex characteristics or gonadal staging. Fat pad (dorsal nape pad) scores among males in all treatment groups tended to be slightly lower than both controls, but it was not concentration-dependent nor considered treatment related.

Hershberger Assay (Rat); OCSPP 890.1400

The Hershberger Assay consists of androgenic and anti-androgenic components. To screen for potential androgenic activity, DCPA (98.3% a.i., Lot # 090614-2) in 0.5% methylcellulose was administered daily via oral gavage to ten 54- to 58-day old, castrated male Sprague Dawley rats

at dose levels of 0 (vehicle), 300 or 1000 (limit dose) mg/kg/day. An androgenic positive control group consisted of ten castrated rats exposed to 0.4 mg/kg/day testosterone propionate (TP) by subcutaneous injection.

To screen for potential anti-androgenic activity, DCPA (98.3% a.i., lot # 090614-2) in 0.5% methylcellulose was administered daily via oral gavage to ten 54- to 58-day old, castrated male Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1000 mg/kg/day in conjunction with a daily dose of reference androgen TP at 0.4 mg/kg/day by subcutaneous injection. The anti-androgenic positive control group consisted of ten castrated rats exposed to 0.4 mg/kg/day TP and 3.0 mg/kg/day flutamide (FT).

For both components of the assay, the animals were dosed for 10 consecutive days and necropsied approximately 24 hours after the final dose administration to determine weights of the five androgen-dependent tissues.

All animals survived until scheduled termination, and there were no clinical signs of toxicity at the dose levels tested.

In the androgen agonist assay, there were no effects on body weights or body weight gains in the DCPA treated groups. Animals dosed with 0.4 mg/kg/day TP had increased body weights ($p<0.05$) on Day 11 by 8%, and cumulative body weight gains were increased ($p<0.01$) for Days 1-11 (↑44%). There were no statistically significant increases in any of the accessory sex organs of animals dosed with DCPA. Animals in the positive control group (TP treated) had accessory sex organ weight increases ($p<0.05$) as follows: 530% in seminal vesicles; 522% in ventral prostate; 181% in the levator ani/bulbocavernosus (LABC) muscle complex; 285% in Cowper's gland; and 49% in glans penis. Liver weights in the 0.4 mg/kg/day TP group were increased 12% ($p<0.01$). The CV value for 1000 mg/kg/day glans penis (25%) was slightly higher than the maximum permissible value (22%), but CVs for the remaining tissues were below the maximum recommended values.

In the anti-androgen assay, there were no treatment-related effects on body weights in any dose groups, and there were no effects on body weight gains in the 100 mg/kg/day group or the positive control group. However, cumulative body weight gains were increased in the 300 mg/kg/day (↑28%, $p<0.05$%) and 1000 mg/kg/day (↑38%, $p<0.01$) dose groups. At 1000 mg/kg/day, liver weight was increased by 20% ($p<0.01$). A statistically significant decrease in organ weight was observed in only one androgen sensitive tissue in animals treated with DCPA: at 1000 mg/kg/day, the weights of Cowper's glands were decreased 25% ($p\leq0.05$). Animals in the positive control group (TP + FT) had decreased ($p\leq0.05$) accessory sex organ weights as follows: 62% in seminal vesicles; 59% in ventral prostate; 46% in LABC; 58% in Cowper's gland; and 20% in glans penis. Liver weights were unchanged in this group.

Statistically significant weight changes were not seen in two or more of the five androgen sensitive tissues. DCPA was negative for androgenicity and anti-androgenicity in the Hershberger assay.

Pubertal Female Assay (Rat); OCSPP 890.1450

In a Female Pubertal Assay (MRID 48615905), 15 Sprague-Dawley (Crl:CD® [SD] IGS BR) rats/dose group were treated daily via oral gavage with chlorthal dimethyl (DCPA; 98.3% a.i., lot # 090614-2) in 0.5% aqueous methyl cellulose at doses of 0, 500, or 1000 (limit dose) mg/kg/day from post-natal day (PND) 22 to 42. Animals were examined for vaginal opening (VO) daily beginning on PND 22, and age and weight at day of attainment was recorded. Following sacrifice on PND 42, total thyroxine (T4) and thyroid stimulating hormone (TSH) levels were analyzed using radioimmunoassays. Pituitary, adrenal glands, liver and urogenital organ weights were recorded, and microscopic examinations of the ovaries, uterus, thyroid/parathyroids, and kidneys were performed.

There were no clinical signs of toxicity related to treatment, and no effects of treatment on mortality, body weights, body weight gains, age of attainment of VO, weight at VO, food consumption, mean age at first vaginal estrus, mean cycle length, percent cycling, percent regularly cycling, clinical chemistry parameters, or gross and microscopic pathology.

Relative (to body) kidney weights were increased ($p \leq 0.05$) by 6% at 500 mg/kg/day, and absolute, adjusted, and relative (to body) kidney weights were increased ($p \leq 0.05$) by 7-9% at 1000 mg/kg/day. Relative (to body) liver weights were increased ($p \leq 0.05$) by 5-6% at 500 mg/kg/day and above. At 1000 mg/kg/day, absolute, adjusted, and relative (to body) adrenal weights were increased ($p \leq 0.05$) by 14-16%. Serum T_4 levels were decreased ($p \leq 0.001$) by 47-55% at 500 mg/kg/day and above. However, plasma TSH levels were not affected by treatment and there were no changes in thyroid histology.

The doses tested were adequate since the high dose was the Limit Dose (1000 mg/kg/day).

Pubertal Male Assay (Rat); OCSPP 890.1500

In a Male Pubertal Assay (MRID 48615905), 15 Sprague-Dawley (Crl:CD® [SD] IGS BR) rats/dose group were treated daily via oral gavage with chlorthal dimethyl (DCPA; 98.3% a.i., lot # 090614-2) in 0.5% aqueous methyl cellulose at doses of 0, 500, or 1000 (limit dose) mg/kg/day from post-natal day (PND) 23 to 53. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and weight at day of attainment was recorded. Following sacrifice on PND 53, total serum testosterone, thyroxine (T_4), and thyroid stimulating hormone

(TSH) levels were analyzed using radioimmunoassays. The weights of adrenals, liver, pituitary, thyroid, and urogenital organs were recorded, and microscopic examinations of the testes, epididymides, thyroid, and kidneys were performed.

DCPA was tested up to the limit dose (1000 mg/kg/day). At 1000 mg/kg/day, the adjusted body weight on the day of acquisition of PPS was increased ($p<0.05$) by 10%. The age of attainment of PPS at all doses was comparable to controls and within the acceptable range of the performance criteria. Treatment-related organ weight effects were limited to decreases ($p<0.05$) at 500 and 1000 mg/kg/day in the adjusted weights of the levator ani and bulbocavernosus muscle (LABC) complex (14-15%) and the seminal vesicles and coagulating glands without fluid (14-17%). The unadjusted values for all organ weights in the control group were within the acceptable range of the performance criteria. Treatment-related effects on hormone levels were limited to decreases ($p<0.001$) in T_4 levels (78-81%) at 500 and 1000 mg/kg/day. TSH and testosterone levels were unaffected by treatment. The hormone values for the control group were within the acceptable range of the performance criteria. All thyroids examined in all dose groups were considered within normal limits of control tissues. There were no treatment-related histopathological findings noted in the testes, epididymides, or kidneys at any dose.

Steroidogenesis Assay (Human cell line H295R); OCSPP 890.1550

In a steroidogenesis assay (MRID 48615906), H295R cells cultured *in vitro* in 24-well plates were incubated with DCPA, (98.3% purity, Lot # 090614-2) at log concentrations from 0.0001 to 100 µM in triplicate for 48 hours. Dimethyl sulfoxide (DMSO) was used as the vehicle, at a final concentration of 0.1%.

Testosterone and estradiol concentrations were measured using ELISA methods. Four independent experiments were performed. A Quality Control (QC) plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentration levels; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production. The highest suitable concentration of DCPA in all three runs was 20 µM due to cytotoxicity and precipitation at higher concentrations.

Minimum basal hormone production was met in all blank and solvent control (SC) wells. Basal hormone production on the QC plates met the fold-increase criteria in SC of ≥2.5-fold for estradiol above the minimum detection limit (MDL) of the assay, but two of the four runs were below the ≥5-fold recommended increase for testosterone (4.46- to 4.65-fold). Exposure to 10 µM forskolin induced testosterone production 1.51- to 2.02-fold (average 1.86-fold) over SC which is below the Guideline criteria (≥2-fold). Estradiol production was induced by ≥7.5-fold (actual 7.78- to 14.93-fold) over SC. Exposure to 1 µM prochloraz inhibited synthesis of

estradiol by ≥50% (≥65%) compared to the SC; inhibition of testosterone synthesis was reduced by ≥50% in only one run of four. The decreases ranged from 26 to 61%, with a mean decrease of only 43%. Hormone measurement sensitivity on the test plates met Guideline requirements with the exception of Run 2 for testosterone (4.78-fold increase over the MDL instead of 5-fold). Although the estradiol data met the performance criteria recommended by the Guideline, the poor inhibition responses after incubation with 1 μM prochloraz may indicate an analytical problem. Interference by DCPA with the ELISA assays was ruled out, as hormone cross-reactivity tests met performance criteria.

The % CVs for absolute testosterone and estradiol concentrations in the SC well replicates for within- and between-plate determinations on the QC and test chemical plates met the performance criteria of ≤30% as recommended by the Guideline.

The significant fold-induction of estradiol concentrations across the three runs ranged from 1.24- to 2.31-fold, with the maximum induction of 2.3-fold achieved at 20 μM. The apparent inhibition of testosterone in Run 2 was not concentration-dependent, and the results were dissimilar from Runs 1 and 3, therefore, a fourth run was conducted and analyzed for testosterone only. Testosterone production was significantly decreased 0.91- to 0.71-fold of control.

Based on the hormone responses in at least three independent runs, DCPA treatment resulted in statistically significant and reproducible decreases in testosterone production and increases in estradiol production.

Uterotrophic Assay (Rat); (OCSPP 890.1600)

In an uterotrophic assay (MRID 48443203) conducted to screen for potential estrogenic activity, DCPA (98.3% a.i., lot # 090614-2) in 0.5% methylcellulose was administered daily via oral gavage to groups of 61- to 68-day old ovariectomized female Sprague-Dawley rats at dose levels of 0 (vehicle), 100, 300, and 1000 (limit dose) mg/kg/day. A positive control group was treated with 17α-ethynyl estradiol (EE) by daily subcutaneous injection at a dose level of 0.3 mg/kg/day. All animals were terminated and necropsied approximately 24 hours after the final dose on PND 65-72 to determine wet and blotted uterine weights, and liver weights.

All animals survived until scheduled termination. No clinical signs of toxicity were observed in animals from any dose groups. Body weights in the 100, 300, and 1000 mg/kg/day dose groups were comparable to the controls throughout the duration of the assay. Animals dosed with 0.3 mg/kg/day EE had decreased ($p<0.01$) body weight gains for Days 1-5 (↓71%). Uterine weights in the dose groups were comparable to the controls. Absolute wet and blotted uterus weights for

the 0.3 mg/kg/day EE group were increased ($p<0.001$) by 944% and 588%, respectively. These increased uterine weights were in the expected range.

No statistically significant changes were seen in uterine weights in this assay. DCPA was negative in the uterotrophic assay.

APPENDIX 2. Other Scientifically Relevant Information (OSRI)

90-Day (Subchronic) Oral Toxicity in Rodents (Rat)

In a subchronic toxicity study in rats (**MRID 41767901**), DCPA (98.0%, lot # 10148, T-170-2) was fed in the diet for 90 days. Dietary concentrations were adjusted to provide constant doses of 0, 10, 50, 100, 150, or 1000 mg/kg/day throughout the study. There were 15 CD VAF/Plus SD rats/sex/dose group. Two satellite groups of 10 rats/sex/group received 0 or 1000 mg/kg/day for 60 days and were used for evaluations of lung toxicity.

Mortality and clinical signs were unaffected by treatment. Body weights were comparable among treatment groups, but body weight gain was decreased in high-dose males (92% of controls) and females (86% of controls) and was accompanied by decreased food consumption in high-dose females. There were no treatment-related effects on hematology, clinical chemistry, or urinalysis. Thyroid hormone levels were not determined.

At necropsy, white foci were seen in lungs of high-dose males (8 vs 0 in controls) and high-dose females (6 vs 0 in controls). There was a dose-related increase in foamy macrophages in the lungs seen microscopically, principally in high-dose males and females, which correlated with the white foci seen grossly in lungs.

Absolute and relative liver weights were increased, principally in high-dose males and females. Liver weights were also increased in 50-150 mg/kg/day females, but not in a dose-related manner. There were dose-related increases in centrilobular hypertrophy of the liver seen microscopically, in the three higher dose male and female dose groups.

Absolute and relative kidney weights were increased in the two highest dose groups in males. Renal epithelial hyperplasia was increased in high-dose males; two animals in this dose group had kidneys with a granular surface at necropsy. Thyroid follicular cell hypertrophy was increased in high-dose males and females; clumped colloid and cystic follicles were increased in thyroids of high-dose males.

The NOAEL is 50 mg/kg/day and the LOAEL is 100 mg/kg/day based upon centrilobular hypertrophy of the liver. At 1000 mg/kg/day there were gross and microscopic lesions of lungs and kidneys and microscopic lesions in thyroids. Thyroid hormones were not evaluated in this study, however, the purpose of a subchronic rat study is to select doses for the rat chronic toxicity/carcinogenicity study.

90-Day (Subchronic) Oral Toxicity in Rodents (Mice)

In a 90-day toxicity study in mice (**MRID 41064801**), DCPA (96.7%, batch # 893-0501) was administered in the diet at concentrations of 0, 625, 1250, 2500, or 7500 ppm in males and 0, 1000, 2500, 5000, or 10000 ppm in females. Corresponding doses were 0, 100, 199, 406, or

1235 mg/kg/day in males and 0, 223, 517, 1049, or 2198 mg/kg/day in females. There were 15 CD-1 mice/sex/dose group.

There were no treatment related effects on mortality, clinical signs, body weight, hematology parameters, clinical chemistry, or urinalyses. There was an increase of enlarged livers in high-dose males (13/15 vs 3/15 in controls) and females (11/15 vs 1/15) noted on gross examination. Absolute and relative liver weights were increased in high-dose males and females (115-130% of control values). There was an increased incidence of centrilobular hepatocyte enlargement, classified "minimal," in 10,000 ppm males (14/15 vs 6/15 in controls) and 10,000 ppm females (11/15 vs 0/15 in controls). The NOAEL is 406 mg/kg/day in males and 1049 mg/kg/day in females. The LOAEL is 1235 mg/kg/day in males and 2198 mg/kg/day in females based upon centrilobular hepatocyte enlargement.

Chronic Oral Toxicity in Non-Rodents (Dog)

In a chronic toxicity study (**MRID 00083584**), beagle dogs were administered 0, 2.5, 25, or 250 mg/kg/day DCPA in the feed for two years. Adverse effects were not observed. Therefore, the NOEL was ≥250 mg/kg/day.

Chronic Oral Toxicity/Carcinogenicity in Rodents (Rat)

In a combined chronic/carcinogenicity study in rats (**MRID 42731001**), DCPA (97.7%, batch # 10148) was administered in the diet for 104 weeks. There were 70 SD CD VAF/Plus® rats/sex/dose group. Concentration of DCPA in the diet was adjusted to provide doses of 0, 1, 10, 50, 500, or 1000 mg/kg/day throughout the study. Test material contained 0.13% hexachlorobenzene (HCB) as a contaminant and doses of HCB were calculated to be 0, 0.0013, 0.013, 0.065, or 1.3 mg/kg/day.

There was increased mortality in males in the 1000 mg/kg/day group during the second year of the study (73% mortality vs 52% mortality in controls). Mortality rates in 1000 mg/kg/day females were decreased in comparison to controls (40% vs 52% in controls). Males in the two highest dose groups had signs of poor physical health such as anogenital staining, thin appearance, material around the mouth, increases in few feces, soft feces, dark urine, and red urine/penile discharge. Females in the two highest dose groups appeared thin.

Body weight decrements occurred during the second year of the study for females in the 500 mg/kg/day group (81% of control body weight at the end of the study), and in males and females in the 1000 mg/kg/day group (86% and 72% of control body weight, respectively).

There were no changes in hematology parameters in the first year of the study. At termination, erythrocyte counts were decreased in males and females (87% of controls for both sexes).

Thyroid toxicity was shown by decreases in the thyroid hormone, T4. At various time intervals, T4 levels were significantly decreased ($p < 0.01$) in 10 mg/kg/day males (62% of control levels at 83 weeks), in 50 mg/kg/day males and females (43% and 45% of controls, respectively, at 104 weeks), in 500 mg/kg/day males and females (16% and 19% of controls, respectively, at 104 weeks), and 1000 mg/kg/day males and females (10% and 15% of controls, respectively, at 104 weeks). The thyroid hormone, T3, was decreased in 1000 mg/kg/day males at 52 weeks (77% of control levels) and in 50 mg/kg/day and greater female groups. There were compensatory increases in thyroid stimulating hormones in both sexes throughout the study, but increases did not show statistical significance.

Absolute and relative thyroid weights were increased in 1000 mg/kg/day males after both one year and two years of treatment (approximately 124-134% of controls). In the 1000 mg/kg/day female group, relative thyroid weights were increased at one year and two years (120% and 147% of controls, respectively. Histopathological changes included increases in thyroid follicular cell hyperplasia, hypertrophy, and/or basophilic clumped colloid in the 10 mg/kg/day and greater male dose groups at interim and terminal sacrifices. Thyroid histology was altered in females at doses of 50 mg/kg/day and greater.

Possible evidence of liver injury was shown by elevated serum GGT activity in 500 mg/kg/day females (368% of control activity at 104 weeks) and in 1000 mg/kg/day males and females (435% and 616% of controls, respectively). Cholesterol was elevated in the two higher dose groups, though the toxicological significance of this finding is not clear. Absolute and relative liver weights were variably increased in the two higher dose groups in males and females. Histopathological liver lesions included increased incidence of eosinophilic foci and increases in centrilobular hepatocyte swelling at doses of 10 mg/kg/day and greater.

Evidence of kidney injury was shown by variable increases in BUN and creatinine in the two higher male dose groups. Absolute and relative kidney weights were variably increased in the two higher male and female dose groups. Severity of chronic nephropathy in males was increased in males in the three higher dose groups. Both incidence and severity of chronic nephropathy were increased in females in the three higher dose groups. The study pathologist stated that exacerbation of this common aging lesion was apparently the main cause of death or moribundity in the high-dose group.

Several dose-related microscopic changes seen in the lungs, principally in the two higher dose groups, included pneumonitis (granulomatous or interstitial), thickened alveolar walls, accumulation of foamy macrophages, and cholesterol clefts. The foamy macrophages correlated with white foci seen at necropsy.

No changes to the eyes were attributed to treatment after ophthalmological examinations. On histopathological examination, retinal atrophy was increased in eyes of females at doses of 10 mg/kg/day and greater. A subsequent chronic toxicity study in rats with DCPA found no ocular

toxicity at 20,000 ppm DCPA in the diet (approximately 1000 mg/kg/day) after treatment for two years (MRID 41750102, Tox Doc 0008373).

In males, thyroid follicular cell adenomas were increased (1, 2, 2, 8, 10, 7, in respective dose groups), but thyroid follicular cell carcinomas were not increased (1, 1, 1, 0, 1, 0). In females, incidences were as follows: thyroid follicular cell adenomas (1, 1, 2, 4, 1, 4) and thyroid follicular cell carcinomas (0, 0, 1, 0, 1, 4).

Hepatocellular adenomas were increased in females (0, 0, 1, 1, 5, 7 in respective dose groups) and hepatocellular carcinomas were also increased (0, 0, 1, 0, 3, 3 in respective dose groups). The incidence for hepatocholangiocarcinomas in females was 0, 0, 0, 0, 0, 2 in respective dose groups. There was no treatment-related increase in liver tumors in males. This study was used to determine the Q1* for DCPA of 1.49 x 10-3 based upon the three combined types of liver tumors in females (Bernice Fisher memo, 12/20/94).

The NOAEL is 1 mg/kg/day and the LOAEL is 10 mg/kg/day based upon decreased level of the thyroid hormone, T4, and increased incidences of thyroid and liver histological changes in males.

Chronic Oral Toxicity/Carcinogenicity in Rodents (Mice)

In a carcinogenicity study in mice (**MRID 40958701**), DCPA was administered in the diet to CD-1 mice for 104 weeks. Purity of test compound was 96.7% and the batch # was 5TX-85-0057 (130985, 190686/11, 170687/2), lot # JK8401. Dietary doses were 0, 100, 1000, 3500, or 7500 ppm, equivalent in males to 0, 12, 123, 435, or 930 mg/kg/day; and in females to 0, 15, 150, 510, or 1141 mg/kg/day.

There were no treatment-related effects upon survival, clinical signs, body weight, food consumption, or hematology.

Liver enzymes were slightly elevated in females, more pronounced in week 76 than in week 102. GPT enzyme activities in females during week 76 were 24, 20, 55, 57, 41 and in week 102 were 33, 28, 51, 31, 54 mU/mL in respective dose groups. SDH activities in females in week 76 were 18.5, 15.1, 35.0, 47.0, 36.5 and in week 102 were 22.1, 22.6, 39.5, 26.5, 47.1 mU/mL. Liver enzyme activities in male treatment groups were comparable to controls. There were slight increases in cholesterol in high-dose females, principally at week 76 (156% of controls), an event of uncertain toxicological significance.

Absolute liver weights were increased in high-dose males at termination (130% of control value) and in high-dose females at termination (126%). Relative liver weights were increased at termination in high-dose males (138% of controls) and high-dose females (119%). Incidence of centrilobular hepatocyte enlargement (minimal) in high-dose males was increased relative to

controls at the interim sacrifices of weeks 27, 53, and 79, but not at terminal sacrifice. Incidence of centrilobular hepatocyte enlargement was not increased in females.

There were no changes in thyroid histology in either sex. Thyroid hormones were not evaluated. Incidence of corneal opacity was 0, 1, 2, 3, 5 in males. Based upon evaluation of ocular effects in a chronic rat study (MRID 41750102), it was concluded that this was not a treatment related effect and may have been due to ocular irritation from the powdered diet (memo, 9/12/90, HED document 0008095).

Hepatic adenomas were increased in females (2, 0, 2, 3, 8 in respective dose groups), but carcinomas were not increased (0, 1, 0, 1, 1). In males, the incidences were as follows: hepatic adenomas (14, 16, 13, 9, 22) and carcinomas (6, 6, 9, 8, 11). Toxicity in this study was minimal and the mice could clearly have tolerated a larger dose. Dosing was considered adequate to evaluate the carcinogenicity of DCPA.

Increases in liver enzyme activities occurred only in females, were of slight magnitude, did not exhibit a good dose-response relationship, and were unaccompanied by histological signs of hepatocellular injury. Although liver enzymes showed transient elevations in the 150 and 510 mg/kg/day female groups at week 76, the 1141 mg/kg/day female group had elevated SDH and GPT activity at both weeks 76 and 102. The NOAEL is 510 mg/kg/day and the LOAEL is 1141 mg/kg/day based upon elevated liver enzymes and increased liver weight in high-dose females.

Developmental Toxicity in Rodents (Rat)

In a developmental toxicity study in rats (**MRID 00160685**), DCPA (96.7%, batch JK-8401) was administered to pregnant SD Crl:COBS® CD rats (25/sex/dose group) by gavage from gestation days 6-15. Dose groups were 0, 500, 1000, and 2000 mg/kg/day.

There were no treatment-related effects on survival, clinical signs, body weight, food consumption, or cesarean parameters in maternal rats. The maternal NOAEL is ≥2000 mg/kg/day, the highest dose tested. The maternal LOAEL is >2000 mg/kg/day.

There were no treatment-related effects on developmental parameters. The developmental NOAEL is ≥2000 mg/kg/day, the highest dose tested. The developmental LOAEL is >2000 mg/kg/day.

Developmental Toxicity in Rodents (Rat) - Metabolite

In a developmental toxicity study in rats (**MRID 00158010**), TPA (>99%, batch #12826-87-12) was administered to Crl:COBS CD (SD) BR rats by gavage from gestation days 6-15. Doses were 0, 625, 1250, or 2500 mg/kg/day. There were 25 pregnant female rats per dose group.

There was no effect upon maternal mortality. Maternal clinical signs included excess salivation and soft or liquid stools, accompanied by reddened anal region and red-colored mucus in feces. These signs may be due to irritation as the compound has two carboxylic acid functional groups. There were no treatment-related effects upon body weight, gross necropsy, or caesarean sectioning of dams. The maternal NOAEL is 1250 mg/kg/day and the maternal LOAEL is 2500 mg/kg/day based upon soft stools and excess salivation.

There was no effect upon developmental parameters and no developmental toxicity was noted. The developmental NOAEL is ≥2500 mg/kg/day, the highest dose tested.

Developmental Toxicity in Non-Rodents (Rabbit)

In a developmental toxicity study in rabbits (**MRID 41054820**), DCPA (95.5%, lot JK8401) was administered to HRA:(NZW)SPF rabbits by gavage from gestation days 7-19. Dose groups were 0, 125, 250, and 500 mg/kg/day with 20 rabbits per dose group.

There were no treatment-related effects on survival, clinical signs, body weight, food consumption, cesarean parameters, or developmental parameters in any of the dose groups. The maternal NOAEL is ≥500 mg/kg/day, the highest dose tested. The developmental NOAEL is ≥500 mg/kg/day, the highest dose tested. This study is classified acceptable/guideline. Due to the lack of toxicity, this study alone does not satisfy guideline requirements for a developmental study, but should be considered in conjunction with another developmental study in rabbits (MRID 45820101) which used higher doses.

In a developmental toxicity study in rabbits (**MRID 45820101),** DCPA (96.5%, lot JK8401) was administered to HRA: (NZW) SPF rabbits by gavage from gestation days 6-19. Dose groups were 0, 500, 1000, and 1500 mg/kg/day with 20 rabbits per dose group.

Mortality was high in all treatment groups (1, 4, 13, 12 deaths in respective dose groups). Clinical signs (ataxia, decreased motor activity, loss of righting reflex) and gastric ulcers were associated with animals that died or aborted. Weight gains during the dosing period were decreased in treatment groups (0.16, 0.10,
-0.03, 0.10 kg in respective groups). Food consumption during the dosing period was slightly decreased in the mid-dose group (39.6, 39.2, 32.5, 41.4 g/kg/day in respective dose groups). Cesarean-section and developmental parameters were not affected by treatment, although due to the high maternal mortality, definite conclusions cannot be reached in this regard.

The maternal NOAEL is <500 mg/kg/day and the maternal LOAEL is 500 mg/kg/day, the lowest dose tested, and is based upon maternal mortality.

Two-Generation Reproduction Toxicity in Rodents (Rat)

In a 2-generation reproduction study in rats (**MRID 41750103**), DCPA (96-98%, batch # JK8401 and 10148) was administered in the feed to SD CD VAF/Plus rats. The F0 parental generation produced two litters, F1a and F1b. The F1b generation was mated to produce two litters, F2a and F2b. There were 35 rats/sex/dose group in the F0 and F1 generations with a ten week growth phase for the F0 generation before the first mating and a ten week growth phase for the F1b generation before the first mating. There were 20 rats/sex/dose group in the F2b generation which were observed for a six week growth period.

Dietary concentrations were 0, 1000, 5000, or 20000 ppm (equivalent to 0, 50, 250, or 1000 mg/kg/day using a 0.05 mg/kg/day per ppm conversion factor). Doses were changed to 0, 200, 500, or 20000 ppm on day 0 of lactation for the F2b litters (equivalent to 10, 25, or 1000 mg/kg/day using a 0.05 conversion factor) in order to ensure a NOAEL for F2 pup body weight decrements.

Compound consumption was estimated by converting dietary ppm to mg/kg/day using a 0.05 conversion factor. This was done because food consumption during the reproduction phase of the study was not reported. Based on food and compound consumption, parental doses at the time of mating were approximately 45, 233, 952 mg/kg/day in males and 0, 63, 319, 1273 mg/kg/day in females for the 1000, 5000, and 20000 ppm dose groups, respectively. Doses during the growth phase of the F2b generation (calculated with food and compound consumption) were higher than parental doses at the time of mating: 17, 43, 1932 mg/kg/day in males and 18, 47, 1946 mg/kg/day in females in the 200, 500, and 20000 ppm groups.

Parental toxicity: Body weight was not affected by treatment in parental F0 and F1 males. Body weights in F0 dams in the 2000 ppm group were approximately 92-94% of controls during pregnancy. Body weights in F1 dams in the 5000 and 20000 ppm groups were 92-95% of controls during pregnancy.

Kidney toxicity occurred in 5000 ppm and 20000 ppm males and females of F0 and F1 generations. F1 5000 ppm and 20000 ppm males had red urine in the cages. Urinalyses of affected animals found red blood cells in urine in the 20000 ppm males. Kidneys in 5000 ppm males and 20000 ppm males and females appeared abnormal at gross necropsy and had microscopic changes indicating an early onset of chronic nephropathy.

Pulmonary toxicity occurred in 5000 ppm and 20000 ppm F0 and F1 males and females. Interstitial pneumonitis and thickened alveolar walls were seen microscopically. Small, white foci on the lungs correlated with foamy macrophages seen microscopically in 5000 ppm and 20000 ppm females and in 1000 ppm and higher female dose groups. Phospholipidosis/ alveolar proteinosis was diagnosed by electron microscopy.

Thyroid changes in 5000 ppm and 20000 ppm F0 and F1 males and/or females included basophilic clumped colloid and follicular cell hypertrophy. Centrilobular hepatocyte swelling occurred in 5000 ppm and 20000 ppm males and females. Thymic involution was increased in 20000 ppm males. Thyroid hormones were not tested.

The parental NOAEL is 50 mg/kg/day and the parental LOAEL is 250 mg/kg/day based upon body weight decrements, gross and microscopic changes in kidneys and lungs, and microscopic changes in liver and thyroids.

Reproductive toxicity: There were no treatment-related effects upon reproductive indices. Mating index, fertility index, pregnancy rates, and litter size were not affected by treatment.

The stillborn index was increased in the 20000 ppm F2b group, but was comparable to historical control ranges and was not attributed to treatment. The reproductive NOAEL is ≥1000 mg/kg/day, the highest dose tested.

Offspring toxicity: On day 21 of lactation there were body weight decrements in the 5000 ppm F1a and F1b groups (92 and 89% of controls, respectively) and the 20000 ppm F1a and F1b groups (81 and 84%) of controls. On lactation day 21, there were body weight decrements in 5000 ppm F2a pups (89% of controls) and in 20000 ppm F2a and F2b pups (76-77% of controls). Pup body weights in the 500 ppm F2b litters were not affected by treatment. Body weight decrements in weaned pups were accompanied by decreased food consumption in F1 animals but not in F2 animals.

There were no treatment-related effects seen at pup necropsy. The offspring NOAEL is 50 mg/kg/day and the offspring LOAEL is 250 mg/kg/day based upon pup body weight decrements.

Avian Reproduction Toxicity

In two reproductive toxicity studies in avian species, mallard duck (**MRID 47550002**) and Northern bobwhite quail (**MRID 47550001**) were administered DCPA in diet over 21 weeks at levels of 0, 1280, 3170, and 8020 mg a.i./kg-diet.

Bobwhite quail was the more sensitive species tested. Fourteen treatment-related mortalities were observed during the study, including 8 at the highest treatment level. A NOAEC of 1280 mg a.i./kg-diet and a LOAEC of 3170 mg a.i./kg-diet were determined based on mortality, survivor weight, signs of toxicity, and effects on reproduction and offspring. Treatment-related clinical signs of toxicity were also observed at the 3170 and 8020 mg a.i./kg levels and included thinness or emaciation, reduced reaction to external stimuli, a ruffled appearance, lethargy, wing droop, and lower limb weakness. Treatment related reductions in multiple reproductive parameters were detected at the top two treatment levels, including the ratios of live 3-week embryos to viable embryos, number hatched to live 3-week embryos, and hatchling survivors to

eggs set and to number hatched. Furthermore, at the highest treatment level, the reviewer's analysis detected significant reductions in eggs laid, eggs set, and viable embryos. A slight reduction in food consumption was also observed, but this was not statistically significant.

In the mallard duck study, No mortalities or overt signs of toxicity were observed in the control or in any of the treatment concentrations during the test. There were no treatment-related effects on any adult or offspring parameter at the 1280 and 3170 mg a.i./kg diet levels. At the 8020 mg a.i./kg level, there was a treatment-related reduction (not statistically significant) in the percentage of viable embryos of eggs set (75%) compared to the control (92%). A NOAEC of 3170 mg ai/kg-diet and a LOAEC of 8020 mg ai/kg-diet were determined based on embryo viability (i.e., # eggs hatched/eggs set).

APPENDIX 3: References Not Utilized in the DCPA WoE Analysis

In 2009, after public review and comment, a final list of 67 chemicals and schedule for issuing Test Orders for the EDSP Tier 1 screening battery was made available in a Federal Register Notice issued October 21, 2009 (74 FR 54422). The agency's review of the initial data submitted as "other scientifically relevant information (OSRI) was provided in the Report of the Endocrine Disruptor Review Team (USEPA, 2010).

Beginning in 2011, the agency has reviewed data cited as "OSRI which included Part 158 studies previously submitted to the agency for registration/reregistration, published literature articles and/or Tier 1 assays. The agency also conducted a more recent search (2009 to 2014) of available scientific literature for any additional relevant information for their weight of evidence (WoE) evaluations. These articles were evaluated in accordance with the agencies Evaluation Guidelines for Ecological Toxicity Data in Open Literature, May 2011 (http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/PDF_rot/esa_evaluation_open_literature.pdf) and the 2012 Guidance for considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (http://www.epa.gov/pesticides/science/lit-studies.pdf).

The following published and unpublished references were considered for use in the WoE analysis for DCPA but were not utilized due to one or more of the following reasons: 1) the article was not available in English; 2) the compound of interest was not used in the study; 3) the test material was not adequately described; 4) a formulated end-use product or mixture of chemicals was utilized as the test material; 5) only acute mortality toxicity data were provided; 6) the experimental conditions were not adequately described; 7) only an abstract of the study was available; 8) the reference is a review article or book chapter and does not contain primary study data; 9) insufficient information was available to adequately assess the validity of the study results; 10) the 40 CFR Part 158 guideline study was classified as unacceptable/inadequate; 11) the study dealt only with non-EDSP assay development; 12) no specific endocrine-related endpoints were assessed in the study; and 13) the study contained only data on invertebrates.

Davidson, C., Stanley, K., and Simonich, S.M. (2012) Contaminant residues and declines of the Cascades frog (Rana cascadae) in the California Cascades, USA. *Environ Toxicol Chem*. 31(8): 1895-1902.

Navarro, A., Weißbach, S., Faria, M., Barata, C., Piña, B., and Luckenbach, T. (2012). Abcb and Abcc transporter homologs are expressed and active in larvae and adults of zebra mussel and induced by chemical stress. *Aquat Toxicol*. 122-123:144-52.

Weldon, R.H., Barr, D.B., Trujillo, C., Bradman, A., Holland, N., and Eskenazi, B. (2011). A pilot study of pesticides and PCBs in the breast milk of women residing in urban and agricultural communities of California. *J Environ Monit*. 13(11):3136-3144.