Appendix 4: Identification of Biological Endpoints for Mathematical Characterization of the Dose-response Curve

A4.0 Identification of Biological Endpoints for Preliminary Mathematical Characterization

The TG recognized that it would be difficult to attempt to characterize the PAC content – toxicity relationships for all the biological endpoints for which it had collected data (see **Section 2**, body of the report). Consequently, it was decided to identify a smaller number of biological endpoints that would undergo preliminary quantitative assessment for dose-response relationship(s) between PAC content and an effect. This subgroup of endpoints was selected based on three general considerations:

1. the endpoints were among those that were most often statistically significantly affected in the studies from which data had been extracted, (see **Section A4.1**)
2. the endpoints were among those that were most often statistically significantly affected at the LOEL (Lowest Observable Effect Level) in the studies from which data had been extracted, i.e. those effects that would be predictive of a significant biological effect (see **Section A4.2**), and
3. the TG judged effects on an endpoint could be used to define/characterize a point of departure in the dose-response curve for a study of the types in HPV.
4. Consistent with reported effects of PACs or PAC-containing petroleum substances.

A4.1 Identification of Endpoints That Were Most Often Statistically Affected in the Studies from which Data was Extracted

The TG identified those endpoints that were most often statistically significantly affected by highlighting, in the data capture spreadsheets (see **Section 3**, body of the report), ***all*** those cells that contained values that had been reported as statistically significant in the respective study reports. Statistically significant values were:

1. highlighted for all effects, across all studies and doses,
2. included in the tabulation without knowledge of the corresponding PAC content and irrespective of:
   - whether the effects were dose-related, or
   - whether the study report author considered the effects treatment-related.

An example of the highlighted cells for a single study within a small portion of one of the spreadsheets is shown in **Figure 4A-1**.

Figure 4A-1. Example of Repeat-dose Data Capture Sheets Highlighted for Significant Values

Sample No./CRU No.	Dose Levels	Body Wt, final	Brain Wt		Liver Wt		
			Abs	Rel/BW	Abs	Rel/BW	Rel/Brain
86268	0						
86268	125						
86268	500						
86268	1000						

The results of the highlighting for each study were then condensed into a single row, leaving one cell per endpoint per study. See **Figure 4A-2** for an example of this condensing as applied to the study shown in **Figure 4A-1**.

Figure 4A-2. Example of Repeat-dose Data Capture Sheet with Condensed Highlighting for Significant Values

Sample No./CRU No.	Dose Levels	Body Wt, final	Brain Wt		Liver Wt		
			Abs	Rel/BW	Abs	Rel/BW	Rel/Brain
86268							

Again, endpoints were highlighted in this condensed version, irrespective of:

- the PAC content of the test samples, or
- whether the effect(s) appeared to be dose-related.

For example, in the condensed table, "Absolute Liver Wt" is highlighted (**Figure 4A-2**), although as shown in **Figure 4A-1**, it does not appear to be dose-related.

An example of the highlighted cells across a number of studies, within a small portion of one of the spreadsheets is shown in **Figure 4A-3**. In the example, "Liver Weights" (absolute and relative to body weight) are affected more often than either brain or adrenal weights and therefore would have initially been identified as an effect for which the dose-response data should undergo preliminary mathematical characterization.

Figure 4A-3. Example of Repeat-dose Data Capture Sheets Highlighted for Significant Values

Sample No. /CRU No.	Body Wt, final	Brain Wt		Liver Wt			Adrenals Wt	
		Abs	Rel/BW	Abs	Rel/BW	Rel/ Brain	Abs	Rel/BW
F-188								
F-115								
F-73-01								
83366								
85244								
86187								
86484								
heavy								
89106								
F-233								

The tabulated frequency that each biological endpoint on the data collection worksheets was significantly affected, across all the studies reviewed by the TG, is shown in **Tables 4A-1** (repeat-dose)**,** and **4A-2** (developmental) on pages 18-22 of this appendix.

Based on the results shown in **Tables 4A-1** and **4A-2 (**pages 18-22 of this appendix)**,** the TG identified as most frequently affected the endpoints shown in **Table 4A-3**.

Table 4A-3. Biological Endpoints Most Often Statistically Significantly Affected

Repeat-dose toxicity	Developmental toxicity
Body weight, Terminal	Maternal endpoints
Liver weight (absolute & relative)	Maternal body weight and weight gain (prenatal and postnatal studies)
Thymus weight (absolute & relative)	Food consumption (prenatal and postnatal studies)
Erythrocyte count	Liver weight (relative) (prenatal studies)
Hemoglobin concentration	Thymus weight (absolute & relative) (prenatal studies)
Hematocrit	Uterine weight (absolute) (prenatal studies)
Platelet count	Prenatal studies
	Number of live fetuses
	Number of resorptions/litter
	% resorptions
	Fetal body weight
	Skeletal anomalies (primarily delayed ossification)
	Postnatal studies)
	Total pups per litter (PND 0[a])
	Live pups per litter (PND 0[a])
	Pup body weight (PND 0 and 4[a])

[a] PND= postnatal day

[b] Blood urea nitrogen (BUN) was among those endpoints most statistically affected. The TG did not consider it for modeling because it is only an indicator of renal function, and is relatively insensitive to small effects on the kidney given that BUN does not increase notably until approximately 75% of the kidney's nephrons are non-functional (Principles & Methods Tox pages 1027-1028). In the studies available to the TG, there was not a high incidence of renal pathology observed, leading the TG to think BUN was not an endpoint that would define a Point of Departure (POD) for these studies.

A4.2 Assessment of How Often Each Endpoint was Statistically Significantly Affected at the Study LOEL (Lowest Observable Effect Level)

A second assessment tabulating how often each biological endpoint was statistically significantly affected at the LOEL (Lowest Observable Effect Level) of each study further supported the TG's identification of the endpoints shown in **Table 4A-3**. This second assessment was done independently of the one described in **Section A4.1** and was conducted in the following manner.

For each repeat-dose study, all the study specific data on organ weights, body weights, and hematology and clinical chemistry measurements (see **Section 2**, body of the report & **Appendix 5**), were assimilated onto two spreadsheets, "male values" and "female values". Since developmental toxicity data were not sex specific, there was only one spreadsheet for each developmental study. An example of the cells within a small portion of one of the spreadsheets (in this case for a repeat-dose study) is shown below in **Figure 4A-4**.

Figure 4A-4. Example of Assimilated Data Spreadsheets

Study No.	Sample No./CRU No.	Dose Levels	Sex	Thymus Wt		Chloride	Calcium	Erythrocyte (RBC) Count
				Abs	Rel/BW			
		mg/kg		Group mean	Group mean	mEq/L	mg/dl	Mil/mm3
63456	86271	0	Male	0.365	0.074	100	9.7	9.58
63456	86271	30	Male	0.331	0.069	99	9.5	9.37
63456	86271	125	Male	0.297	0.063	99	9.2[a]	8.83[a]
63456	86271	500	Male	0.143[a]	0.033[a]	99	9.4	6.7[a]

[a] statistically significant

Endpoints that did not have statistically significant changes ("chloride" in **Figure 4A-4**) were then deleted from the spreadsheet, **Figure 4A-5**.

Figure 4A-5. Example of Assimilated Data Spreadsheets – Endpoints With Statistically Insignificant Effects Deleted

Study No.	Sample No./CRU No.	Dose Levels	Sex	Thymus Wt		Calcium	Erythrocyte (RBC) Count
				Abs	Rel/BW		
		mg/kg		Group mean	Group mean	mg/dl	Mil/mm3
63456	86271	0	Male	0.365	0.074	9.7	9.58
63456	86271	30	Male	0.331	0.069	9.5	9.37
63456	86271	125	Male	0.297	0.063	9.2[a]	8.83[a]
63456	86271	500	Male	0.143[a]	0.033[a]	9.4	6.7[a]

[a] statistically significant

For those endpoints that remained, an entry was made on the spreadsheet noting the lowest dose level at which the statistically significant effect was seen (shading in **Figure 4A-6**). These lowest dose level values at which the statistically significant effects were seen (shading in **Figure 4A-6**) were then plotted for each study, **Figure 4A-7**.

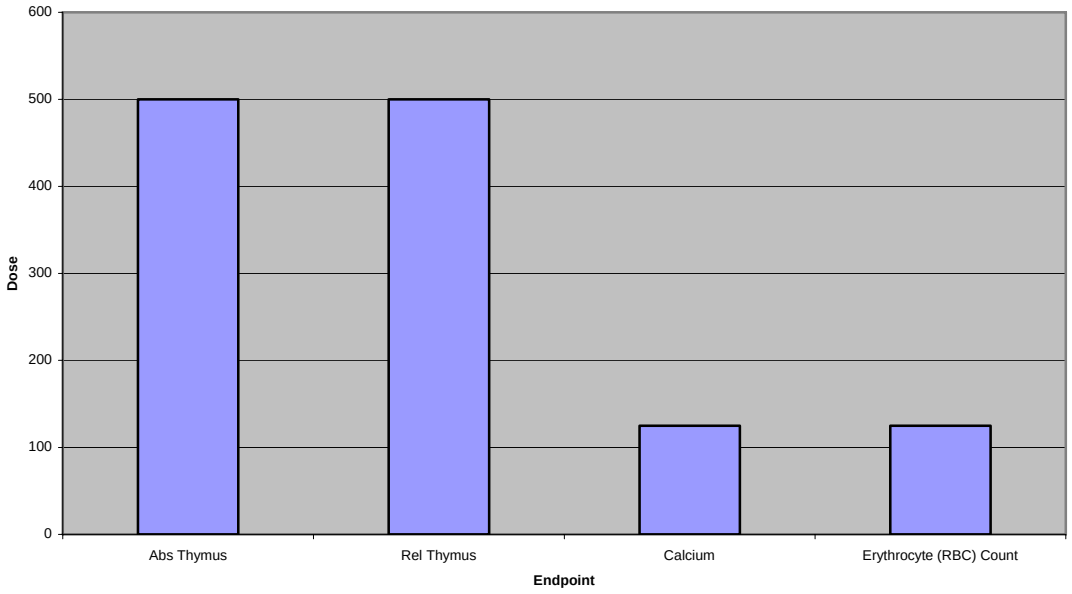
Figure 4A-6. Example of Assimilated Data Spreadsheets – Lowest Dose Level Affected Entered

Lowest dose level at which significant effect seen

Study No.	Sample No./CRU No.	Dose Levels	Sex			Calcium	Erythrocyte (RBC) Count
				Abs	Rel/BW		
		mg/kg		Group mean	Group mean	mg/dl	Mil/mm^3
				500	**500**	**125**	**125**
63456	86271	0	Male	0.365	0.074	9.7	9.58
63456	86271	30	Male	0.331	0.069	9.5	9.37
63456	86271	125	Male	0.297	0.063	9.2[a]	8.83[a]
63456	86271	500	Male	0.143[a]	0.033[a]	9.4	6.7[a]

[a] statistically significant

Figure 4A-7. Example of a Plot of the Lowest Dose Level Values at Which Statistically Significant Effects Were Seen



From the resulting plot, a study's LOEL could be determined, i.e. 125 mg/kg in **Figure 4A-7**, and the endpoints that were statistically affected at this level could be readily seen. For each endpoint, across all similar studies from which data was available (e.g. all 28-day repeat-dose studies), the TG counted the number of times the endpoint was significantly different from control at a study's LOEL. The TG conducted this assessment using all the studies from which data had been extracted, regardless of whether the study had or had not been selected for use in model development (see **Section 3**, body of the report). The TG did not count an effect if:

- the report's study director did not consider the effect compound-related, or
- the report's study director considered the effects secondary to other (e.g. dermal) effects,
- the TG did not think the effect was dose-related or

- in the case of the developmental studies, if the effect occurred in a dose group that had not been dosed on gestation days 0-19, as a minimum.

For example, in **Figure 4A-7**, "Calcium" would not have been counted as being affected at the LOEL since in the data spreadsheet (**Figure 4A-6**), the effect does not appear to be dose-related. Thus, in **Figure 4A-7**, only "Erythrocyte (RBC) Count" would have been counted as being statistically affected at the study's LOEL.

As can be seen in **Tables 4A-4** (repeat-dose) and **4A-5**/**4A-6** (developmental) on pages 23-31 of this appendix, the results of this assessment show that the endpoints previously identified as being most frequently statistically different from controls (**Table 4A-3**, page 3 of this appendix) also tended to be most often statistically significantly affected at the studies' LOELs.

43.3 Identification of Endpoints for Final Mathematical Characterization of the Dose-response

After completing the preliminary quantitative assessment of the dose-response relationship(s) for each of the endpoints shown in **Table 4** (body of the report), the number of endpoints being characterized was reduced again considering the following:

- the overall degree of the reported statistical significance from all relevant individual study dose-response assessments
- whether similar endpoints had also been characterized, thus making the analysis redundant, e.g. among hematocrit, hemoglobin, and erythrocyte count, only hemoglobin was identified for final modeling, and
- whether the effect on an endpoint would be considered an adverse effect or predictive of an adverse effect.

Preference was given to selecting endpoints that the TG considered biological significant. Biological significance was the determination that the observed effect (a biochemical change, a functional impairment, or a pathological lesion) was likely to impair the performance or reduce the ability of an individual to function or to respond to additional challenge from the agent. [A review of the reference dose and reference concentration process; EPA/630/P-02/002F Risk Assessment Forum, December, 2002; Pg 4-11]

As a result, the endpoints listed in **Table 6** (body of the report) were selected for final mathematical characterization. A brief summary of the TG's rationale for selecting each endpoint is given below.

Repeat-dose Studies

Absolute thymus weight

Absolute thymus weight was statistically significantly affected in more than half of the 90-day studies and in the one 28-day study in which thymus weight was recorded (see **Table 4A-1**, page 18 of this appendix). Furthermore, the thymus was frequently identified in the study reports as a "target" organ. Feuston et al (1994) also reported decreases in thymus weight as being related to 3-7 ring PAC content.

Thymus weight changes can be a general indication of potential adverse effects on the immune system, specifically a separate cell line (T lymphocytes). Available regulatory guidance documents indicate a significant decrease in thymus weight would be considered an adverse effect (ATSDR, 1996, 2006; EPA, 1994, 2002). The TG thinks absolute rather than relative thymus weight is a better measure of effect since thymus weight is relatively unaffected by changes in body weight.

Although the identification of absolute thymus weight for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.88 (see **Tables 4 and 6**, body of the report).

Hemoglobin concentration
Hemoglobin concentration was statistically significantly affected in more than half of the 90-day studies and in approximately 20% of the 28-day studies in which hemoglobin concentration was measured (see **Table 4A-1**, page 19 of this appendix). The related parameters, erythrocyte count and hematocrit, had similar, highly affected incidence rates.

Hemoglobin concentration is one of three parameters that can be used as an estimate of RBC mass, the other two being erythrocyte count and hematocrit. A decrease in the circulating RBC mass is an indication of anemia, and is characterized by an absolute decrease in the hematocrit, hemoglobin concentration and RBC count. All three measurements provide information concerning the oxygen-carrying capacity of the blood and the bone marrow erythropoietic activity. Given that these three measurements are indicative of RBC mass, and therefore, probably inter-related, it was decided that only one should be selected for detailed statistical modeling.

Available regulatory guidance documents suggest a significant decrease in hemoglobin concentration would in all probability be considered an adverse effect (ATSDR, 1996, 2006; EPA, 1994, 2002).

Although the identification of hemoglobin concentration for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.89 (see **Table 7**, body of the report).

Platelet count
Platelet count was statistically significantly affected in approximately 50% of the 90-day repeat-dose toxicity studies (see **Table 4A-1**, page 19 of this appendix).

In addition to prothrombin time and activated partial thromboplastin, platelet count is one of the core recommended tests for assessment of hemostasis. Along with the hemoglobin measurements and thymus weights, the TG thought platelet count gave an indication of potential effects on a third line of blood cells, megakaryocytes.

The effects seen in the studies reviewed by the TG were quite substantial, both in magnitude and frequency of occurrence. Available regulatory guidance documents suggest a significant decrease in platelet count would likely be considered an adverse effect (ATSDR, 1996, 2006; EPA, 1994, 2002).

Although the identification of platelet count for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.95 (see **Table 7**, body of the report).

Liver to Body Weight Ratio
Liver to body weight ratio was the endpoint statistically significantly affected most frequently in the repeat-dose toxicity studies. It was affected in approximately 25% and 75%, respectively of the 28- and 90-day repeat-dose studies in which liver to body weight ratios were recorded (see **Table 4A-1**, page 18 of this appendix). Furthermore, the liver was frequently identified in the study reports as a “target” organ. Feuston et al (1994) also reported increased liver weight as being related to 3-7 ring PAC content.

Although the identification of “liver to body weight ratio” for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.93 (see **Table 6**, body of the report).

Developmental toxicity studies

The most sensitive endpoints observed among the developmental toxicity studies were measurements of fetal/pup growth and survival. Only the most sensitive endpoints were considered for final statistical analysis and modeling. The TG assumed that regulatory agencies and others will focus on endpoints that define the LOAEL and NOAEL. Consequently, the TG selected endpoints that were among those most often affected at the studies' LOELs. An indication of the endpoints EPA considers LOAEL- and NOAEL-defining can be found in an article by Benedict, et al (2006). Based on a review of human and animal studies of PAHs, primarily benzo(a)pyrene, the author stated: "The available literature was reviewed to identify studies related to PAH-induced reproductive and developmental toxicity and potential mode of action. Evidence in laboratory animals indicates that exposure to PAHs may lead to impaired fertility, altered folliculogenesis, increased incidence of fetal resorptions, and decreased fetal body weight and survival. Human studies have been less definitive in providing an association between PAHs and adverse reproductive and developmental outcomes. The available human and animal evidence suggests that PAHs may affect the developing fetus, but this association has not been unequivocally defined."

"Prenatal" Studies

Defined as studies in which test material was applied during gestation and pregnant dams underwent a caesarean section on day 20 of gestation.

Fetal body weight

Fetal body weights were statistically significantly affected in approximately 70% (19/28) of the prenatal studies in which fetal body weights were recorded (see **Table 4A-2**, page 21 of this appendix). Fetal body weight was statistically significantly decreased at the LOEL in 50% (8/16) of the prenatal studies used for final model development in which fetal body weights were reported (see **Table 4A-5**, page 26 of this appendix).

Fetal body weight is considered to be a reliable and sensitive endpoint of developmental toxicity.

Although the identification of fetal body weight for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.95 (see **Table 6**, body of the report).

Percent Resorptions

Percent resorptions (resorption sites/implantation sites) were statistically significantly affected in approximately 70% (19/28) of the prenatal studies in which resorptions were recorded (see **Table 4A-2**, page 21 of this appendix). The percentage of resorptions was statistically significantly increased at the LOEL in 37% (6/16) of the prenatal studies used for final model development in which resorptions were reported (see **Table 4A-5**, page 26 of this appendix). The studies also reported the mean number of resorption sites per litter, which showed a similar incidence figure. Since these parameters are measuring essentially the same endpoint, the percentage of resorptions was used for the statistical analysis and modeling.

Resorptions are an indication of embryonal and fetal death. The percentage of resorptions is commonly measured in developmental toxicity studies.

Although the identification of percent resorptions for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.95 (see **Table 7**, body of the report).

Live fetuses per litter

The number of live fetuses per litter was statistically significantly affected in approximately 70% (19/28) of the prenatal studies in which live fetuses were counted (see **Table4A-2**, page 21 of this appendix). The mean number of live fetuses per litter was statistically significantly decreased at the LOEL in 50% (8/16) of the prenatal developmental toxicity studies in which the number of live fetuses was reported (see **Table 4A-5**, page 26 of this appendix).

Although the identification of live fetuses per litter for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.97 (see **Table 6**, body of the report).

Malformations

Among the twenty-eight dermal prenatal developmental toxicity studies initially evaluated for the current project (studies where the test material was given on at least gestation days 0-19 of pregnancy), there were a few studies in which the authors of the study concluded that there was weak or suggestive evidence of teratogenicity. However, no conclusive evidence of teratogenicity (i.e., statistically significant increase in the incidence of malformations) was demonstrated for any of the test materials applied dermally to pregnant rats among the studies where the test material was given on gestation days 0-19 as a minimum. For example, in two dermal developmental toxicity studies, there was a low incidence (typically one or two fetuses affected) of right-sided esophagus (studies 50541 and 63836). But, right-sided esophagus was not statistically significantly increased among the offspring of pregnant rats exposed to any test material when the litter was considered the statistical unit (the proper statistical unit). Similarly, cleft palate was observed in one and two fetuses of dams exposed dermally to the high- and mid-dose, respectively, of one of the test materials; however, these incidences of cleft palate were not significantly increased compared to the control group (study 50541).

In order to further evaluate the potential teratogenicity of a subset of these test materials, additional developmental toxicity studies were conducted in which the test material was given at higher oral dose levels for a single day during pregnancy to increase the survival of fetal offspring. Fueston and Mackerer (1996) reported clear evidence of teratogenicity for certain test materials in pregnant rats given a single, large gavage dose of the test materials. By administering the test material on a single day of gestation, it was possible to limit embryolethality and demonstrate the teratogenic potential of clarified slurry oil, syntower bottoms and distillate aromatic extract. When given as a single, large oral dose, the authors reported a common pattern of fetal malformations for these three test materials that included cleft palate, diaphragmatic hernia, and paw and tail defects. Other refinery streams tested by the same authors have also been reported to produce evidence of teratogenicity when administered as a single oral dose of 2000 mg/kg/day on gestation day 13 (studies 65370 and 65371).

The same investigators also conducted a small number of studies to further evaluate the potential teratogenicity of these test materials (many evaluated in the current project) given dermally at a higher dose for a shorter duration of time to limit embryolethality. These experimental conditions presented limited evidence of teratogenicity. A statistically significant increase in cleft palate was observed among fetuses and litters of pregnant rats given 1000 mg/kg/day of clarified slurry oil dermally on gestation days 9-12 (study 62492). When given orally as a single dose (2000 mg/kg/day on gestation days 11, 12, 13, or 14), this same test material produced a statistically significant increase in cleft palate, as well as paw and tail defects (study 62122).

Another test material, syntower bottoms, produced a statistically significant increase in cleft palate among fetuses (but not among litters) when pregnant rats were given 500 mg/kg/day

on gestation days 10-12 (study 62934). In comparison, single oral administration of syntower bottoms (2000 mg/kg/day on gestation days 12, 13, or 14) statistically significantly increased the incidence of cleft palate, diaphragmatic hernia, tail and paw defects among both fetuses and litters (study 63123). Of note, a statistically significant increase in the incidence of right-sided esophagus was observed among fetuses (but not among litters) of pregnant rats exposed to a single oral dose of 2000 mg/kg/day of syntower bottoms on gestation day 13 (but not 12 or 14). When the test materials were given dermally on gestation days 0-19 at a minimum, this same malformation was also observed at a low incidence (not statistically significant) in a few dermal studies (but not with syntower bottoms).

The results of these specialized studies indicate that some of the test materials exhibit teratogenic potential when given at large doses either orally or dermally for a short period of pregnancy to reduce embryolethality. Under conditions of high, short-term exposure, teratogenic potential is more clearly demonstrated with oral administration than with dermal administration. In comparison, these test materials exhibit little evidence of teratogenicity in more traditional dermal developmental toxicity studies, in which the test material is given throughout the majority of the gestation period. However, while no malformations were statistically significantly increased among litters, some of the malformations observed at a low incidence are consistent with the types of malformations significantly increased in the studies where the test material was given at a high dose for a shorter duration.

No malformations were selected for mathematical evaluation and modeling because there was no statistically significant increase in malformations (when the litter was considered the statistical unit) among any of the studies selected for evaluation (studies which included exposure on gestation days 0-19 as a minimum). As such, no malformation was identified as an endpoint most often statistically significantly affected in the studies, and no malformation was an endpoint most often affected at the study's LOEL. While some of these test materials may have limited teratogenic potential under certain conditions, it is clear that the most sensitive endpoints of developmental toxicity for these materials are endpoints of fetal survival and growth, not malformations (see earlier discussion of other endpoints of developmental toxicity in this section).

"Postnatal" Studies

Defined as studies in which test material was applied during gestation, dams were allowed to deliver and pups were monitored for 4 days of lactation.

Pup body weight (PND 0)

Pup body weights at postnatal day 0 (PND 0) were statistically significantly affected in over 50% (19/34) of the postnatal studies in which pup fetal body weights were recorded (see **Table 4A-2**, page 22 of this appendix). This is consistent with the results of the prenatal studies in which fetal body weights were statistically significantly affected in approximately 70% (19/28) of the studies in which fetal body weights were recorded (see **Table 4A-2**, page 21 of this appendix). Pup body weights (PND 0) were also statistically significantly decreased at the LOEL in approximately 65% (17/26) of the postnatal studies used for final model development in which pup body weights were reported (see **Table 4A-5**, page 28 of this appendix).

Pup body weight on PND 0 is considered to be a reliable and sensitive measure of developmental toxicity due to prenatal exposure to a chemical. Pup body weights on both PND 0 and PND 4 showed similar, highly affected incidence rates. The TG considered pup weights on both days to be measurements of the same effect, and consequently chose to use only pup body weight on PND 0.

Although the identification of pup body weight (PND 0) for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.83 (see **Table 6**, body of the report).

Live pups per litter (PND 0)
The number of live pups per litter on postnatal day 0 (PND 0) was statistically significantly affected in over 50% (19/35) of the postnatal studies in which the number of live pups per litter was recorded (see **Table 4A-2**, page 22 of this appendix). Live pups per litter (PND 0) was also statistically significantly decreased at the LOEL in 65% (17/26) of the postnatal studies used for final model development in which live pups per litter were reported (see **Table 4A-5**, page 27 of this appendix). This is consistent with the results of the prenatal developmental toxicity studies in which the mean number of live fetuses per litter was statistically significantly decreased at the LOEL in 50% (8/16) of the prenatal studies (see **Table 4A-5**, page 26 of this appendix).

Although the identification of live pups per litter for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.87 (see **Table 6**, body of the report).

Total pups per litter (PND 0)
The mean number of total pups per litter on postnatal day 0 (PND 0) was statistically significantly affected in over 50% (19/35) of the postnatal studies in which the total pups per litter on PND 0 were recorded (see **Table4A-2**, page 22 of this appendix). The total pups per litter on PND 0 was statistically significantly decreased at the LOEL in approximately 60% (16/26) of the postnatal studies used for final model development in which total pups per litter on PND 0 were reported (see **Table 4A-5**, page 27 of this appendix). The TG also noted that every study with a statistically significant decrease in the mean number of total pups per litter on PND 0 also exhibited a statistically significant decrease in the mean number of live pups per litter on PND 0.

The mean number of total pups per litter on PND 0 is another important measure of embryonal/fetal survival. Both live and dead pups are counted in the number of total pups per litter.

Although the identification of total pups per litter for final modeling was based on biological considerations, final modeling indicated there is a high correlation between the PAC content and response, r = 0.85 (see **Table 6**, body of the report).

A3.4 Biological Endpoints Not Identified for Final Modeling

It should be noted that a number of endpoints were initially identified as candidates for model development, subsequently underwent preliminary statistical modeling, but were not used in the final statistical modeling. The TG's reasons for not including these endpoints in the final evaluations were:

Repeat-dose toxicity studies

Body Weight, terminal
Significant, treatment-related decreases in terminal body weight were recorded in 9 of 44 studies in males and 7 of 43 in females (see **Table 4A-1**, page 18 of this appendix). However, it was clear that body weight changes occurred only in animals in which other endpoints had also been affected.

The TG concluded that terminal body weight was not a sensitive endpoint in the context of the evaluation being undertaken.

Erythrocyte Count and Hematocrit
Erythrocyte count and hematocrit are two of three parameters that can be used as an estimate of RBC mass, the other being hemoglobin concentration. All three provide information concerning the oxygen-carrying capacity of the blood and bone marrow erythropoietic activity. Given that these three measurements are indicative of RBC mass, the TG decided only one should be undergo final statistical modeling. Preliminary modeling of hemoglobin concentration indicated it could be modeled with a higher degree of confidence than the other two endpoints (see **Table 5**, body of the report).

Dermal Effects
Although not identified as an endpoint "most often statistically significantly affected", the TG did consider and decided not to include dermal effects in the modeling exercise.

The TG identified three potential issues associated with the dermal effects.

1. A hypothesis that effects on the skin are a critical endpoint and as such need to characterized or modeled.

   With regard to the PAC TF objective, the TG does not think the data it reviewed support this hypothesis. A high proportion of the studies it reviewed reported dermal effects seen only at the site of test material application. This is a common and well-documented finding in studies utilizing dermal application as the route of administration. The TG concluded that these dermal findings were local effects that were not associated with any systemic effects. Evidence arguing against skin effects being a critical endpoint included several instances of materials that produced high skin irritation scores, but had no internal toxicity. Conversely, several materials with low skin scores produced internal toxicity.

   While the TG recognizes selected PAHs are known skin carcinogens, and dermal irritation is believed to be involved in the dermal carcinogenesis seen with selected middle distillate materials, the focus of the current effort is on non-carcinogenic endpoints. Other than local inflammatory responses at the site of application, no indication of more serious skin effects, e.g. carcinogenicity, would be expected to be observed in studies of the length that are included in this project.

   Consequently, the TG believes a more in-depth and resource intensive review of the studies is not warranted and is beyond the charter of the current project.

2. Independent of any systemic toxic effects produced by PACs, dermal irritation alone could produce the pattern of effects seen in the studies reviewed by the TG. This would lead the group to erroneously correlate irritation-produced effects with PAC concentration.

   The TG does not think the available data support this hypothesis. There did not appear to be a consistent correlation between the degree of dermal effects and statistically significant effects on any of the other endpoints. For instance, in several studies "none to minimal" irritation was reported, yet there were statistically significant effects on thymus weight and hemoglobin concentration. Conversely, there were also instances in which "severe" skin irritation was reported, yet there were no statistically significant changes in either thymus weights or hemoglobin content.

3. Irritation could have led to alterations in the barrier properties of the stratum corneum, allowing increased PAC absorption.

While this may be true, the TG believes the effects seen from this increased absorption of PACs would be captured in its current set of endpoints. Furthermore, if true, this simply means that the outcomes represent a "worst case" relative to the consequences of exposures at lower, non-irritating levels. Consequently, the TG does not believe this would alter the accuracy of the predictive models. Finally, to attempt to define the mechanism of how the PACs are producing their adverse effects is beyond the scope of the current exercise.

Developmental toxicity studies

Skeletal anomalies (Delayed Ossification)
Delayed ossification (observed at the time of caesarean section) was an endpoint "most often statistically significantly affected" (see "skeletal anomalies" **Table 4A-2**, page 21 of this appendix). However, the TG did not include this endpoint in the statistical analysis and modeling for three reasons:

1. Increased incidences of delayed ossification were associated with decreased fetal body weights in virtually every "prenatal" developmental toxicity study. Both fetal body weight and delayed ossification are indications of the same effect, i.e., an effect on growth. It is unlikely that additional information would be gained by adding delayed ossification to the list of endpoints evaluated.

2. The skeletal examination procedures varied from study to study. Therefore, it is very difficult to compare the incidences of delayed ossification across different studies and laboratories. In contrast, fetal body weight is easily compared across studies since the method for determining body weight is standardized.

3. Delayed ossification of skeletal bones is not considered a malformation but rather a minor skeletal variation since it is usually reversible and does not affect the quality of life.

Other Developmental Toxicity Endpoints
The TG evaluated a number of other developmental toxicity endpoints but decided not to include them in the final modeling exercises.

A number of the "postnatal" developmental toxicity studies exhibited decreases in (1) the number of dams delivering a litter/number of dams mated and (2) the number of dams delivering a litter/number of dams pregnant. These decreases appeared to reflect a high incidence of resorptions and fetal loss at high doses in some studies. It is clear that, at high doses, some of the test materials produced 100% fetal loss, resulting in no litters being born even though the dams appeared to be pregnant prior to the time of delivery. But, in no case were these endpoints the most sensitive parameters; there were always other effects (e.g., decreased litter size, decreased pup weight) at lower doses.

A statistically significant decrease in the percent of pups surviving from PND 0-4 was observed in 7/35 of the postnatal studies in which developmental effects were reported (see **Table 4A-2**, page 22 of this appendix). This endpoint was not affected as frequently as the endpoints included in the statistical analysis and modeling.

Interestingly, in light of the other developmental effects seen, fetal malformations were not commonly reported, and there was no consistent pattern of malformations. Based on the studies reviewed by the TG, as a class, these test materials have low potential to cause fetal malformations. Rather, they are more likely to produce other signs of embryotoxicity and fetotoxicity, such as effects on growth and survival, which were parameters covered by the modeling exercise.

Maternal Toxicity Endpoints

Several indicators of maternal toxicity constituted endpoints "most often statistically significantly affected" (see **Table 4A-2**, page 21 of this appendix). These included: maternal terminal body weight and body weight gain, food consumption, skin irritation, and various organ weights. These endpoints were not included in the statistical analysis and modeling of developmental toxicity, because they are not routinely used to define the LOAELs and NOAELs for developmental toxicity. The relationship between maternal and developmental toxicity were evaluated, as described in the next section.

Relationship between Maternal and Developmental Toxicity

Summary

Developmental toxicity was strongly associated with maternal toxicity (i.e., decreased body weight, body weight gain, food consumption) in both the prenatal and postnatal studies. For example, among the 23 prenatal studies, developmental toxicity was never observed in the absence of maternal toxicity. In addition, maternal skin irritation was observed in the vast majority of the developmental toxicity studies, although in 30% and 18% of the prenatal and postnatal studies, respectively, developmental toxicity was observed in the absence of maternal skin irritation. It is quite possible that maternal toxicity and skin irritation play a role in producing developmental toxicity. It is also possible that maternal toxicity/skin irritation and developmental toxicity are not causally related; there may be an association because the mother and the fetus are equally sensitive to the effects of the test material.

For purposes of this project, it does not matter whether maternal toxicity and/or skin irritation cause developmental toxicity of the test materials. The goal of the project is to determine whether developmental toxicity can be predicted on the basis of PAC profile. The model has value if PAC profile accurately predicts developmental toxicity regardless of the mechanism of action (i.e., whether it is a direct effect or an indirect effect of maternal toxicity).

It should not be presumed that the test materials cause a direct effect on the embryo or fetus simply because mathematical analysis demonstrates a high correlation between PAC profile and endpoints of developmental toxicity. In fact, based on a review of the study NOAELs, none of the test materials are selective developmental toxicants (i.e., chemicals which cause developmental toxicity in the absence of maternal toxicity), with the exception of one questionable result in one study.

Prenatal Studies and Maternal Toxicity (Excluding Skin Irritation)

The ratio of the NOAEL for maternal toxicity (i.e. decreased body wt., wt. gain or food consumption) divided by the NOAEL for developmental toxicity was calculated for each study, and the results are summarized in **Table 4A-8**. For the calculations in **Tables 4A-8** and **4A-9**, the presence of maternal skin irritation at the site of test material application was not considered. A ratio >1 indicates that the test material is a selective developmental toxicant (i.e., developmental toxicity occurs in the absence of maternal toxicity). None of the 23 studies had a ratio >1. A ratio of 1 indicates that the NOAELs for maternal toxicity and developmental toxicity are identical. ten of 23 studies had ratios of 1. A ratio of <1 indicates that maternal toxicity occurred at a lower dose than developmental toxicity. Thirteen of 23 studies had ratios <1.

Table 4A-8. Comparison of Ratios of NOAELs for Maternal Toxicity and Developmental Toxicity in the Prenatal Studies

	Maternal Tox NOAEL/Developmental Tox NOAEL		
	Ratio <1	Ratio = 1	Ratio >1
Incidence*	13/23	10/23	0/23
* The incidence is shown as the number of studies with ratio shown/total number of studies			

Maternal toxicity was observed in 22/23 prenatal studies. No maternal or developmental toxicity was observed in one study, in which the only dose level tested was low (i.e., 0.05 mg/kg/day). Statistically significant decreases in maternal body weight (GD20) and body weight gain (GD0-20) were seen in 17/23 and 19/23 studies, respectively.

These results indicate that none of the test materials produced developmental toxicity in the absence of maternal toxicity. Doses that produced developmental toxicity were always associated with maternal toxicity. Therefore, there is a clear association between maternal and developmental toxicity among the prenatal studies.

Postnatal Studies and Maternal Toxicity (Excluding Skin Irritation)

Similarly, for the postnatal studies, the ratio of the NOAEL for maternal toxicity (i.e. decreased body wt., wt. gain or food consumption) divided by the NOAEL for developmental toxicity was evaluated for each study (Table **3A-9**). A ratio >1 indicates that the test material is a selective developmental toxicant (i.e., developmental toxicity occurs in the absence of maternal toxicity).

Only three of 34 studies had a ratio >1, indicating that developmental toxicity occurred in the absence of observed maternal toxicity (excluding skin irritation). In two of these studies, a statistically significant decrease in fetal body weight was reported at a dose that did not significantly affect maternal body weight, weight gain or food consumption. However, maternal skin irritation was reported at all doses in both of these studies, raising the possibility that maternal skin irritation may have played a role in producing developmental toxicity in these two studies. In the third study, the study authors concluded that there was no NOAEL for developmental toxicity since pup survival to PND 4 was significantly decreased at all doses. But, the incidence of pup survival to PND 4 among controls, low, middle and high dose was 99, 95, 96, and 81%, respectively. The decreased survival at the low and middle doses may have been an artifact of an unusually high survival rate in the concurrent control group; the range of control values among the other postnatal studies was 87-100%. Therefore, when the possibility of an unusual control group in one study and skin irritation in the other two studies are considered, it is not clear that developmental toxicity in the absence of maternal toxicity (including skin irritation) was observed in any study.

Table 4A-9. Comparison of Ratios of NOAELs for Maternal Toxicity and Developmental Toxicity in the Postnatal Studies

	Maternal Tox NOAEL/Developmental Tox NOAEL		
	Ratio <1	Ratio = 1	Ratio >1
Incidence*	8/34	23/34	3/34
* The incidence is shown as the number of studies with ratio shown/total number of studies			

A ratio of 1 indicates that maternal toxicity and developmental toxicity occurred at the same dose. Twenty-three of 34 studies had a ratio of 1.

A ratio of <1 indicates that maternal toxicity occurred at a lower dose than developmental toxicity. Eight of 34 studies had a ratio <1.

These results indicate that, in the vast majority of studies, doses that produced developmental toxicity were associated with maternal toxicity. Consistent with the results of the prenatal studies, the postnatal studies demonstrated an association between maternal and developmental toxicity. None of the test materials evaluated in this report are selective developmental toxicants, except for one questionable result in one study.

Prenatal Studies and Maternal Skin Irritation

Skin irritation was observed in 15/23 prenatal studies. The degree of skin irritation ranged from mild to severe.

Table 4A-10. Comparison of Ratios of NOAELs for Maternal Skin Irritation and Developmental Toxicity in the Prenatal Studies

	Maternal Skin irritation NOAEL/Developmental Tox NOAEL		
	Ratio <1	Ratio = 1	Ratio >1
Incidence*	15/23	1/23	7/23
* The incidence is shown as the number of studies with ratio shown/total number of studies			

The ratio of NOAELs for maternal skin irritation divided by the NOAEL for developmental toxicity was evaluated for each study. A ratio >1 indicates that the test material produced developmental toxicity at a dose which was not associated with skin irritation. Seven of 23 studies had a ratio >1 (**Table 4A-10**).

A ratio of 1 indicates that maternal skin irritation and developmental toxicity occurred at the same dose. Only one of 23 studies had a ratio of 1.

A ratio of <1 indicates that skin irritation occurred at a lower dose than developmental toxicity. Fifteen of 23 studies had a ratio <1.

These results indicate that seven of the test materials produced developmental toxicity in the absence of maternal skin irritation. Therefore, skin irritation was not responsible for developmental toxicity in at least 7 of the prenatal studies. Among the other 16 studies, it is possible that skin irritation played some role in producing developmental toxicity. It is also possible that maternal skin irritation and developmental toxicity, while associated in 16 studies, are not related causally.

Postnatal Studies and Maternal Skin Irritation

Skin irritation was reported in 30/34 postnatal studies. The degree of skin irritation, when it was reported, ranged from mild to severe.

Table 4A-11. Comparison of Ratios of NOAELs for Maternal Skin Irritation and Developmental Toxicity in the Postnatal Studies

	Maternal Skin irritation NOAEL/Developmental Tox NOAEL		
	Ratio <1	Ratio = 1	Ratio >1
Incidence*	15/34	13/34	6/34
* The incidence is shown as the number of studies with ratio shown/total number of studies			

The ratio of NOAELs for maternal skin irritation divided by the NOAEL for developmental toxicity was calculated for each study. A ratio >1 indicates that the test material produced developmental toxicity at a dose which was not associated with skin irritation. Six or 34 studies had a ratio >1 (Table 4A-11).

A ratio of 1 indicates that maternal skin irritation and developmental toxicity occurred at the same dose. Thirteen or 34 studies had a ratio of 1.

A ratio of <1 indicates that skin irritation occurred at a lower dose than developmental toxicity. Fifteen of 34 studies had a ratio <1.

Table 4A-1. Frequency of Statistically Significant Effects Per *Repeat-dose* Biological Endpoint

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
Weight Data	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Weight, Terminal	3/18	2/18	1/1	0/1	0/8	0/7	5/17	5/17
Brain								
Absolute	0/18	1/18	0/1	0/1	0/7	0/6	2/13	1/13
Rel. to body weight	3/18	0/18	1/1	1/1	0/7	0/6	0/13	1/13
Liver								
Absolute	3/18	4/18	0/1	0/1	4/8	2/7	8/16	13/16
Rel. to body weight	4/18	4/18	0/1	0/1	5/8	3/7	16/17	16/17
Rel. to brain weight	3/18	4/18	0/1	0/1	0/0	0/0	1/1	1/1
Adrenals								
Absolute	0/17	0/17	0/1	0/1	1/8	0/7	0/16	3/16
Rel. to body weight	1/17	0/17	0/1	0/1	1/8	0/7	4/16	4/16
Rel. to brain weight	0/17	0/17	0/1	0/1	0/0	0/0	0/1	0/1
Heart								
Absolute	0/0	0/0	0/0	0/0	0/8	0/7	2/16	2/16
Rel. to body weight	0/0	0/0	0/0	0/0	0/8	0/7	6/16	5/16
Rel. to brain weight	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/1
Testes/Ovaries								
Absolute	0/18	1/18	0/1	0/1	0/8	0/7	2/16	1/16
Rel. to body weight	1/18	0/18	0/1	0/1	0/8	0/7	4/16	2/16
Rel. to brain weight	0/18	1/18	0/1	0/1	0/0	0/0	0/1	0/1
Kidneys								
Absolute	1/18	1/18	0/1	0/1	0/8	0/7	3/16	1/16
Rel. to body weight	1/18	0/18	0/1	0/1	1/8	0/7	4/17	5/17
Rel. to brain weight	0/18	1/18	0/1	0/1	0/0	0/0	1/1	0/1
Prostate								
Absolute	0/0	-	0/0	-	0/7	-	2/12	-
Rel. to body weight	0/0	-	0/0	-	0/7	-	2/12	-
Rel. to brain weight	0/0	-	0/0	-	0/0	-	0/0	-
Thymus								
Absolute	1/1	1/1	0/0	0/0	1/8	0/7	13/16	12/16
Rel. to body weight	1/1	1/1	0/0	0/0	1/8	0/7	13/17	11/17
Rel. to brain weight	1/1	1/1	0/0	0/0	0/0	0/0	1/1	1/1
Epididymis								
Absolute	0/0	-	0/0	-	0/7	-	2/12	-
Rel. to body weight	0/0	-	0/0	-	0/7	-	2/12	-
Rel. to brain weight	0/0	-	0/0	-	0/0	-	0/0	-

Table 4A-1 (cont.). Frequency of Statistically Significant Effects Per *Repeat-dose* Biological Endpoint

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
Weight Data	Male	Female	Male	Female	Male	Female	Male	Female
Spleen								
Absolute	0/1	1/1	0/0	0/0	1/8	0/7	2/16	2/16
Rel. to body weight	0/1	1/1	0/0	0/0	2/8	0/7	5/16	5/17
Rel. to brain weight	0/1	1/1	0/0	0/0	0/0	0/0	1/1	1/1
Uterus								
Absolute	-	0/0	-	0/0	-	0/6	-	2/13
Rel. to body weight	-	0/0	-	0/0	-	0/6	-	1/13
Rel. to brain weight	-	0/0	-	0/0	-	0/0	-	0/0
Lung								
Absolute	0/0	0/0	0/0	0/0	0/1	0/1	1/2	1/1
Rel. to body weight	0/0	0/0	0/0	0/0	0/1	0/1	1/2	1/1
Rel. to brain weight	0/0	0/0	0/0	0/0	0/0	0/0	1/1	1/1
Hematology Data								
Erythrocyte count	2/18	1/18	0/1	0/1	1/8	0/7	13/17	11/17
Total white cell count	0/18	0/18	0/1	0/1	1/8	2/7	2/17	6/17
% Neutrophils	2/18	2/18	1/1	1/1	1/7	0/6	3/12	2/12
% Lymphocytes	1/18	2/18	1/1	1/1	2/7	0/6	3/12	2/12
% Monocytes	0/18	0/18	0/1	0/1	1/6	0/6	1/12	0/12
% Eosinophils	1/18	1/18	0/1	0/1	0/6	0/6	0/12	2/12
Hemoglobin concentration	3/18	4/18	0/1	0/1	2/8	1/7	14/17	13/17
Hematocrit	3/18	4/18	0/1	0/1	2/8	1/7	14/17	11/17
Platelet count	0/17	2/17	0/1	0/1	1/7	0/6	10/15	9/15
MCV	0/9	0/10	0/1	0/1	0/7	1/6	1/11	1/11
MCHC	0/0	0/0	0/0	0/0	0/7	0/6	0/11	4/11
MCH	0/0	0/0	0/0	0/0	1/7	1/6	1/11	2/11
Clinical Chemistry Data								
Sodium	0/17	0/17	0/1	0/1	1/8	0/7	2/15	0/15
Potassium	0/17	0/17	0/1	0/1	0/8	0/7	0/15	6/15
Chloride	0/17	0/17	0/1	0/1	3/8	2/7	5/15	0/15
Calcium	0/17	0/17	0/1	0/1	3/8	1/7	4/15	3/15
Phosphorus	0/17	0/17	0/1	0/1	3/8	1/7	0/15	2/15
Blood Urea Nitrogen	2/17	1/17	0/1	0/1	4/8	1/7	10/17	12/17
Glucose	1/18	1/18	0/1	0/1	0/8	3/7	5/16	4/15

Table 4A-1 (cont.). Frequency of Statistically Significant Effects Per *Repeat-dose* Biological Endpoint

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
Clinical Chemistry Data (cont.)	Male	Female	Male	Female	Male	Female	Male	Female
Creatinine	0/16	0/16	0/1	0/1	2/8	1/7	3/12	4/12
Cholesterol	0/16	3/16	0/1	0/1	0/8	2/7	5/16	11/16
Triglycerides	0/17	0/17	0/1	0/1	0/8	0/7	1/15	7/15
Total Protein	0/17	2/17	1/1	1/1	4/8	0/7	2/16	1/17
Albumin	0/16	0/16	1/1	0/1	6/8	0/7	3/16	1/16
Globulin	0/16	1/16	1/1	1/1	1/8	0/7	0/13	1/13
A/G ratio	0/16	1/16	1/1	1/1	2/8	0/7	1/15	2/15
Alkaline phosphatase	0/17	1/17	0/1	0/1	0/8	1/7	3/16	4/16
SGOT	0/17	0/17	0/1	0/1	1/8	1/7	3/16	2/16
SGPT	1/17	1/17	0/1	0/1	2/8	1/7	4/16	3/16
Sorbitol dehydrogenase	0/0	0/1	0/0	0/0	4/6	4/6	5/13	6/13
Bilirubin	0/0	0/0	0/0	0/0	2/8	2/7	4/15	3/15
Uric acid	0/0	0/0	0/0	0/0	3/8	0/7	3/15	5/15

[a] statistically significant effect
[b] number of studies from which data was extracted
[c] the tabulations presented in this table were performed before modeling began. They are presented in this table grouped into "not used in modeling" and "used in modeling" to facilitate reference to the modeling that was subsequently performed.

Table 4A-2. Frequency of Statistically Significant Effects Per *Developmental* Biological Endpoint

Endpoint	No. Studies With Effect[a]/ No. Studies[b]	
Prenatal Studies	**Not used in final modelling[c]**	**Used in final modelling[c]**
Maternal endpoints		
Skin irritation	4/7	14/21
Body weight (Day 20)	5/7	17/21
Body weight gain (Days 0-20)	5/7	19/21
Food consumption (Days 0-20)	3/7	12/21
Liver weight		
Absolute	1/1	5/12
Rel. to body weight	1/1	10/11
Thymus weight		
Absolute	1/1	10/12
Rel. to body weight	1/1	7/9
Uterine weight		
Absolute	4/7	10/13
Rel. to body weight	0/0	0/0
Vaginal discharge	3/7	9/21
Developmental endpoints		
Females mated	0/7	0/21
% Pregnant	0/7	0/21
Implantation sites, mean	0/7	1/21
Corpora lutea	0/7	2/21
Pre implantation loss, %	1/6	0/15
Resorptions/litter, mean	4/7	14/21
Resorptions, %	4/7	15/21
Dams with resorptions (%)	3/7	11/21
Proportion male fetuses	0/7	1/21
Fetal body weight, mean		
Combined	3/7	16/21
Male	3/7	14/21
Female	3/7	16/21
Fetuses/litter, mean		
Live	5/7	14/21
Dead	0/7	0/21
External anomalies	1/7	5/21
Visceral anomalies	3/7	4/20
Skeletal anomalies	3/6	14/20
Postnatal Studies		
Maternal endpoints		
Skin irritation	4/5	26/30
Maternal deaths	0/5	0/30
Body weight		
Day 20	3/5	23/29
Lactation Day 4	2/5	13/28

Table 4A-2 (cont.). Frequency of Statistically Significant Effects Per *Developmental* Biological Endpoint

Endpoint	No. Studies With Effect[a]/ No. Studies[b]	
Postnatal Studies (cont.)	**Not used in final modeling[c]**	**Used in final modeling[c]**
Maternal endpoints		
Body weight gain		
Days 0-20	2/5	25/30
Up to Day 4 lactation	0/4	7/28
Food consumption		
Days 0-20	5/5	13/28
Up to Day 4 lactation	0/3	11/23
Liver weight		
Absolute	0/1	3/3
Rel. to body weight	0/1	2/2
Thymus weight		
Absolute	0/1	0/3
Rel. to body weight	0/1	0/2
Uterine weight		
Absolute	0/0	0/0
Rel. to body weight	0/0	0/0
Vaginal discharge	0/5	16/30
Developmental endpoints		
Number of females mated	0/5	0/30
Number of females pregnant	0/5	0/30
Number of females delivered	0/5	0/30
Gestation length	0/5	7/30
Implantation sites (mean)	0/5	7/30
Pups/litter, mean (PND[d] 0)		
total	0/5	19/30
live	0/5	19/30
% dead pups (PND[d] 0)	0/5	4/30
% pup survival to PND[d] 4	0/5	7/30
Proportion of males		
at PND[d] 0	0/3	4/23
at PND[d] 4	0/3	3/23
Pup body weight		
at PND[d] 0	0/4	19/30
at PND[d] 4	0/4	17/30
Pup observations	0/5	1/30

[a] statistically significant effect
[b] number of studies from which data was extracted
[c] the tabulations presented in this table were performed before modeling began. They are presented in this table grouped into "not used in modeling" and "used in modeling" to facilitate reference to the modeling that was subsequently performed
[d] PND= postnatal day

Table 4A-4. Number of *Repeat-dose* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study Systemic LOEL

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
Weight Data	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
	Male	Female	Male	Female	Male	Female	Male	Female
Body Weight, Terminal	2/3	0/5	1/1	0/1	0/7	0/7	1/16	2/16
Brain								
Absolute	0/3	0/5	0/1	0/1	0/6	0/6	0/12	0/12
Rel. to body weight	2/3	0/5	1/1	1/1	0/6	0/6	0/12	0/12
Liver								
Absolute	1/3	2/5	0/1	0/1	2/7	1/7	1/15	5/15
Rel. to body weight	3/3	2/5	0/1	0/1	2/7	1/7	10/16	11/16
Rel. to brain weight	2/3	3/5	0/1	0/1	0/0	0/0	0/1	0/1
Adrenals								
Absolute	0/2	0/4	0/1	0/1	0/7	0/7	0/15	1/15
Rel. to body weight	0/2	0/4	0/1	0/1	1/7	0/7	1/15	1/15
Rel. to brain weight	0/2	0/4	0/1	0/1	0/0	0/0	0/1	0/1
Heart								
Absolute	0/0	0/0	0/0	0/0	0/7	0/7	0/15	0/15
Rel. to body weight	0/0	0/0	0/0	0/0	0/7	0/7	1/15	0/15
Rel. to brain weight	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1
Testes/Ovaries								
Absolute	0/3	0/5	0/1	0/1	0/7	0/7	0/15	0/15
Rel. to body weight	1/3	1/5	0/1	0/1	0/7	0/7	1/15	0/15
Rel. to brain weight	0/3	0/5	0/1	1/1	0/0	0/0	0/1	0/1
Kidneys								
Absolute	0/3	0/5	0/0	0/1	0/7	0/7	0/15	0/15
Rel. to body weight	0/3	0/5	0/0	0/1	0/7	0/7	0/16	0/16
Rel. to brain weight	0/3	0/5	1/0	0/1	0/0	0/0	0/1	0/1
Prostate								
Absolute	0/0	--	0/0	--	0/6	--	0/11	--
Rel. to body weight	0/0	--	0/0	--	0/6	--	0/11	--
Rel. to brain weight	0/0	--	0/0	--	0/0	--	0/0	--
Thymus								
Absolute	0/1	0/1	0/0	0/0	0/7	0/7	4/16	3/15
Rel. to body weight	0/1	0/1	0/0	0/0	0/7	0/7	2/17	2/16
Rel. to brain weight	0/1	0/1	0/0	0/0	0/0	0/0	0/1	0/1
Epididymis								
Absolute	0/0	--	0/0	--	0/6	--	1/12	--
Rel. to body weight	0/0	--	0/0	--	0/6	--	1/12	--
Rel. to brain weight	0/0	--	0/0	--	0/0	--	0/0	--

Table 4A-4 (cont.). Number of *Repeat-dose* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study Systemic LOEL

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
Weight Data (cont.)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Spleen								
Absolute	0/1	0/1	0/0	0/0	1/7	0/7	0/15	0/15
Rel. to body weight	0/1	0/1	0/0	0/0	1/7	0/7	1/15	1/16
Rel. to brain weight	0/1	0/1	0/0	0/0	0/0	0/0	0/1	0/1
Uterus								
Absolute	--	0/0	--	0/0	--	0/6	--	0/11
Rel. to body weight	--	0/0	--	0/0	--	0/6	--	0/11
Rel. to brain weight	--	0/0	--	0/0	--	0/0	--	0/0
Lung								
Absolute	0/0	0/0	0/0	0/0	0/1	0/1	0/2	0/2
Rel. to body weight	0/0	0/0	0/0	0/0	0/1	0/1	0/2	1/2
Rel. to brain weight	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/1
Hematology Data								
Erythrocyte count	2/3	1/5	0/1	0/1	0/7	0/7	3/16	2/16
Total white cell count	0/3	0/5	0/1	0/1	1/7	2/7	0/16	3/16
% Neutrophils	0/3	0/5	0/1	0/1	0/6	0/6	0/11	1/11
% Lymphocytes	0/3	0/5	0/1	0/1	1/6	0/6	1/11	1/11
% Monocytes	0/3	0/5	0/1	0/1	0/6	0/6	1/11	0/11
% Eosinophils	1/3	0/5	0/1	0/1	0/6	0/6	0/11	0/11
Hemoglobin concentration	3/3	1/5	0/1	0/1	0/7	1/7	5/16	5/16
Hematocrit	3/3	1/5	0/1	0/1	0/7	0/7	5/16	2/16
Platelet count	0/2	0/4	0/1	0/1	0/6	0/6	3/14	3/14
MCV	0/0	0/1	0/1	0/1	0/6	1/6	0/10	0/10
MCHC	0/0	0/0	0/0	0/0	0/6	0/6	0/10	1/10
MCH	0/0	0/0	0/0	0/0	1/6	1/6	1/10	0/10
Clinical Chemistry Data								
Sodium	0/2	0/4	0/1	0/2	0/7	0/7	0/14	1/14
Potassium	0/2	0/4	0/1	0/2	0/7	0/7	0/14	2/14
Chloride	0/2	0/4	0/1	0/2	1/7	2/7	1/14	0/14
Calcium	0/2	0/4	0/1	0/2	1/7	1/7	1/14	1/14
Phosphorus	0/2	0/4	0/1	0/2	1/7	0/7	0/14	1/14
Blood Urea Nitrogen	0/3	0/5	0/1	0/2	2/7	0/7	3/16	4/16
Glucose	0/3	0/5	0/1	0/2	0/7	2/7	2/15	1/14
Creatinine	0/2	0/4	0/1	0/2	1/7	1/7	2/11	0/11

Table 4A-4 (cont.). Number of *Repeat-dose* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study Systemic LOEL

Endpoint	28-day studies				90-day studies			
	No. Studies With Effect[a]/ No. Studies[b]				No. Studies With Effect[a]/ No. studies[b]			
	Not used in final modeling[c]		Used in final modeling[c]		Not used in final modeling[c]		Used in final modeling[c]	
Clinical Chemistry Data (cont.)	Male	Female	Male	Female	Male	Female	Male	Female
Cholesterol	0/2	0/4	0/1	0/1	0/7	1/7	1/15	5/15
Triglycerides	0/2	0/4	0/1	0/1	0/7	0/7	0/14	2/14
Total Protein	0/3	0/5	0/1	0/1	2/7	0/7	0/15	0/16
Albumin	0/2	0/4	0/1	0/1	2/7	0/7	1/15	0/15
Globulin	0/2	1/4	0/1	0/1	1/7	0/7	0/12	0/12
A/G ratio	0/2	1/4	0/1	0/1	2/7	0/7	0/14	0/14
Alkaline phosphatase	0/3	0/5	0/1	0/1	0/7	1/7	0/15	0/15
SGOT	0/3	0/5	0/1	0/1	0/7	0/7	1/15	0/15
SGPT	1/3	0/5	0/1	0/1	0/7	0/7	0/15	0/15
Sorbitol dehydrogenase	0/0	0/0	0/0	0/0	3/5	4/5	1/12	3/12
Bilirubin	0/0	0/0	0/0	0/0	0/7	1/7	2/14	1/14
Uric acid	0/0	0/0	0/0	0/0	1/7	0/7	0/14	0/14

[a] Number of studies in which a statistically significant change occurred in the endpoint at the study LOEL. Changes occurring at the LOEL but judged by the Study Director to be non-compound-related or secondary to dermal effects were not included, nor were changes occurring at the LOEL that did not appear to be dose-related.
[b] Number of studies that had a LOEL and in which data on the endpoint was available to the TG
[c] The tabulations presented in this table were performed before modeling began. They are presented in this table grouped into "not used in modeling" and "used in modeling" to facilitate reference to the modeling that was subsequently performed.

Table 4A-5. Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b]/ No. Studies[c]		
Prenatal Studies	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Maternal endpoints			
Skin irritation	14/20	--	--
Body weight (Day 20)	4/20	10/20	--
Body weight gain (Days 0-20)	5/20	14/20	--
Food consumption (Days 0-20)	2/20	7/20	--
Liver weight			
Absolute	0/12	0/12	--
Rel. to body weight	2/11	3/11	--
Thymus weight			
Absolute	0/12	3/12	--
Rel. to body weight	0/10	2/9	--
Uterine weight			
Absolute	2/13	4/13	5/11
Rel. to body weight	0/0	0/0	0/0
Vaginal discharge	2/20	6/20	7/16
Developmental endpoints			
Females mated	0/20	0/20	0/16
% Pregnant	0/20	0/20	0/16
Implantation sites, mean	0/20	0/20	0/16
Corpora lutea	0/20	1/20	1/16
Pre implantation loss, %	0/15	0/15	0/13
Resorptions/litter, mean	2/20	5/20	6/16
Resorptions, %	2/20	5/20	6/16
Dams with resorptions (%)	1/20	1/20	1/16
Proportion male fetuses	0/20	1/20	1/16
Fetal body weight, mean			
Combined	2/20	6/20	8/16
Male	3/20	8/20	9/16
Female	2/20	7/20	9/16
Fetuses/litter, mean			
Live	3/20	6/20	8/16
Dead	0/20	0/20	0/16
External anomalies	0/20	1/20	1/16
Visceral anomalies	0/19	2/19	2/16
Skeletal anomalies	3/19	7/19	9/16

Table 4A-5 (cont.). Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b]/ No. Studies[c]		
Postnatal Studies	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Maternal endpoints			
Skin irritation	22/30	--	--
Maternal deaths	0/30	0/29	--
Body weight			
Day 20	13/29	16/28	--
Lactation Day 4	6/28	6/27	--
Body weight gain			
Days 0-20	12/30	13/29	--
Up to Day 4 lactation	3/28	3/27	--
Food consumption			
Days 0-20	3/28	4/27	--
Up to Day 4 lactation	2/23	4/22	--
Liver weight			
Absolute	2/3	2/3	--
Rel. to body weight	1/2	1/2	--
Thymus weight			
Absolute	0/3	0/3	--
Rel. to body weight	0/2	0/2	--
Uterine weight			
Absolute	0/0	0/0	0/0
Rel. to body weight	0/0	0/0	0/0
Vaginal discharge	11/30	12/29	13/26
Developmental endpoints			
Number of females mated	0/30	0/29	0/26
Number of females pregnant	0/30	0/29	0/26
Number of females delivered	0/30	0/29	0/26
Gestation length	2/30	3/29	3/26
Implantation sites (mean)	1/30	2/29	2/26
Pups/litter, mean (PND[d] 0)			
total	11/30	14/29	16/26
live	12/30	15/29	17/26
% dead pups (PND[d] 0)	2/30	2/29	2/26
% pup survival to PND[d] 4	3/30	4/29	4/26
Proportion of males			
at PND[d] 0	2/23	2/22	2/21
at PND[d] 4	1/23	1/22	1/21

Table 4A-5 (cont.). Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b]/ No. Studies[c]		
Postnatal Studies (cont.)	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Developmental endpoints			
Pup body weight			
at PND[d] 0	10/30	14/29	17/26
at PND[d] 4	9/30	12/29	15/26
Pup observations	0/30	0/29	0/26

[a] The tabulations presented in this table were performed before modeling began. They are presented in this table grouped into "not used in modeling" and "used in modeling" to facilitate reference to the modeling that was subsequently performed.
[b] Number of studies in which a statistically significant change occurred in the endpoint at the study LOEL. Changes occurring at the LOEL but judged by the Study Director to be non-compound-related or secondary to dermal effects were not included, nor were changes occurring at the LOEL that did not appear to be dose-related.
[c] Number of studies that had a LOEL and in which data on the endpoint was available to the TG
[d] PND= postnatal day

Table 4A-6. Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Not Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b] / No. Studies[c]		
Prenatal Studies	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Maternal endpoints			
Skin irritation	4/6	--	--
Body weight (Day 20)	1/6	2/4	--
Body weight gain (Days 0-20)	1/6	2/4	--
Food consumption (Days 0-20)	1/6	0/4	--
Liver weight			
Absolute	0/0	0/0	--
Rel. to body weight	0/0	0/0	--
Thymus weight			
Absolute	0/0	0/0	--
Rel. to body weight	0/0	0/0	--
Uterine weight			
Absolute	1/6	1/4	2/3
Rel. to body weight	0/0	0/0	0/0
Vaginal discharge	1/6	1/4	1/3
Developmental endpoints			
Females mated	0/6	0/4	0/3
% Pregnant	0/6	0/4	0/3
Implantation sites, mean	0/6	0/4	0/3
Corpora lutea	0/6	0/4	0/3
Pre implantation loss, %	0/5	0/3	0/2
Resorptions/litter, mean	1/6	1/4	2/3
Resorptions, %	1/6	1/4	2/3
Dams with resorptions (%)	1/6	1/4	1/3
Proportion male fetuses	0/6	0/4	0/3
Fetal body weight, mean			
Combined	1/6	1/4	2/3
Male	1/6	1/4	2/3
Female	1/6	1/4	2/3
Fetuses/litter, mean			
Live	0/6	0/4	1/3
Dead	0/6	0/4	0/3
External anomalies	0/6	0/4	0/3
Visceral anomalies	0/6	0/4	1/3
Skeletal anomalies	2/6	2/4	3/3

Table 4A-6 (cont.). Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Not Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b]/ No. Studies[c]		
Postnatal Studies	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Maternal endpoints			
Skin irritation	4/5	--	--
Maternal deaths	0/5	0/3	--
Body weight			
Day 20	1/5	2/3	--
Lactation Day 4	0/5	0/3	--
Body weight gain			
Days 0-20	1/5	1/3	--
Up to Day 4 lactation	0/4	0/2	--
Food consumption			
Days 0-20	2/5	2/3	--
Up to Day 4 lactation	0/3	0/1	--
Liver weight			
Absolute	0/1	0/1	--
Rel. to body weight	0/1	0/1	--
Thymus weight			
Absolute	0/1	0/1	--
Rel. to body weight	0/1	0/1	--
Uterine weight			
Absolute	0/0	0/0	0/0
Rel. to body weight	0/0	0/0	0/0
Vaginal discharge	0/5	0/3	0/0
Developmental endpoints			
Number of females mated	0/5	0/3	0/0
Number of females pregnant	0/5	0/3	0/0
Number of females delivered	0/5	0/3	0/0
Gestation length	0/5	0/3	0/0
Implantation sites (mean)	0/5	0/3	0/0
Pups/litter, mean (PND[d] 0)			
total	0/5	0/3	0/0
live	0/5	0/3	0/0
% dead pups (PND[d] 0)	0/5	0/3	0/0
% pup survival to PND[d] 4	0/5	0/3	0/0
Proportion of males			
at PND[d] 0	0/3	0/1	0/0
at PND[d] 4	0/3	0/1	0/0

Table 4A-6 (cont.). Number of *Developmental* Studies in Which Each Biological Endpoint Showed Statistically Significant Findings at the Study LOEL

Studies *Not Used* in Final Modelling[a]

Endpoint	No. Studies With Effect at LOEL[b]/ No. Studies[c]		
Postnatal Studies (cont.)	**Skin Irritation & Maternal Endpoints Included**	**Skin Irritation Not Included Maternal Endpoints Included**	**Skin Irritation & Maternal Endpoints *Not* Included**
Developmental endpoints			
Pup body weight			
at PND[d] 0	0/4	0/2	0/0
at PND[d] 4	0/4	0/2	0/0
Pup observations	0/5	0/3	0/0

[a] The tabulations presented in this table were performed before modeling began. They are presented in this table grouped into "not used in modeling" and "used in modeling" to facilitate reference to the modeling that was subsequently performed.
[b] Number of studies in which a statistically significant change occurred in the endpoint at the study LOEL. Changes occurring at the LOEL but judged by the Study Director to be non-compound-related or secondary to dermal effects were not included, nor were changes occurring at the LOEL that did not appear to be dose-related.
[c] Number of studies that had a LOEL and in which data on the endpoint was available to the TG
[d] PND= postnatal day

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