MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

July 14, 1995

TO: File for Diisononyl Phthalate (DINP) (CAS # 28553-12-0)

FROM: Dan O'Brien

SUBJECT: Initial Threshold Screening Level (ITSL) for diisononyl phthalate

The initial threshold screening level for diisononyl phthalate is 75 µg/m^3 based on an annual averaging time.

The following references or databases were searched to identify data to determine the ITSL: AQD chemical files, IRIS, HEAST, ACGIH TLV Booklet, NIOSH Pocket Guide to Chemical Hazards, RTECS, NTP Management Status Report, EPB Library, IARC Monographs, CAS On-line and NLM/Toxline (1967 - February 3, 1995), CESARS, Handbook of Environmental Data on Organic Chemicals, Patty's Industrial Hygiene and Toxicology, Merck Index and Condensed Chemical Dictionary.

The only compound-specific data available concerning the use of DINP was obtained from the Material Safety Data Sheet (MSDS) (Aristech, 1993) and from a medical case report (Brodell and Torrence, 1992). A handwritten notation on the copy of the former attached to the screening level request form indicates the compound is used as a "thinning agent," while the latter refers to DINP as a commonly used plasticizer. The phthalates as a group are widely used as plasticizers for a variety of plastics, particularly in vinyl and cellulose resins to lend flexibility and toughness. They are also used as dental and surgical plastics (Bisesi, 1994).

Searches located only one report of human toxicity from exposure to DINP, that of Brodell and Torrence (1992). These authors report on one case of a series of five in which a patient developed irritant dermatitis on the hands after exposure to the contents of a toy ("Sqwish Ball") which she had dissected. The core of the toy consisted of a sealed sphere containing a gel comprised of a number of chemicals which were a proprietary secret. On consultation with the manufacturer, the authors report that DINP was considered the likely causal agent, having caused similar clinical signs in four other individuals who had tampered with the toy and subsequently reported dermatitis. Due to the mixed exposure, however, it is impossible to tell whether DINP or another chemical was responsible.

Three mutagenicity assays of DINP (Cifone, 1986; Zeiger et al., 1985; NTP, 1983) all report a negative mutagenic response for DINP, both with and without rat and/or hamster liver S-9 metabolic activation. Two of these studies (Zeiger et al., 1985; NTP, 1983) used *Salmonella spp*. as the test organism; the other (Citone, 1986) utilized the mouse lymphoma forward mutation assay *in vitro* at the thymidine kinase (TK) locus of the L5178Y cell line.

All of the available animal toxicity data of potential use in the derivation of a screening level for DINP are in the form of unpublished studies performed for industry. All of these used the oral route of exposure. A 21-day study concentrating on hepatic effects was carried out for the

Chemical Manufacturer's Association (Ford and Gaunt, 1986). Groups of five male and five female Fischer 344 rats, approximately 6 weeks of age, were fed DINP incorporated into their diets at levels of 0, 0.6, 1.2 or 2.5% for three weeks. A positive control group was fed di(2-ethylhexyl)phthatlate (DEHP) at 1.2% for the same period. Assignment to groups was accomplished using a random number generator. Efficiency of mixing and concentration of the agent in the diets was considered adequate if within 5% of the target. Parameters measured included observations for clinical signs (daily), physical examinations (weekly), body weights (prior to exposure, at exposure initiation, and twice weekly thereafter), and feed consumption (twice weekly). Following exsanguination under ether anesthesia on day 21, necropsies were performed, and gross lesions, liver, kidney and (in males) testes were retained and weighed. Sections were formalin fixed for histopathology, fixed for electron microscopy, or homogenized for biochemical analyses (protein, cyanide-insensitive palmitoyl-CoA, and differential microsomal fractions). Blood was obtained at time of death for determination of serum cholesterol and triglycerides. Data were analyzed by ANOVA (with multiple comparison of means by the least significant difference test), or via Welch-corrected pooled students' *t* tests. Statistical significance was considered attained at a level of $p < 0.05$.

Concentrations of DINP and of mixing were within 5% of target. Due to an error, rats in the 1.2% DEHP dose group were fed a 0.6% DEHP diet for the first three days of the study. There were no exposure-related clinical signs. Males in the two highest DINP dose groups and females in the highest dose group weighed significantly less than controls throughout the study (2.5% males and females) or from day seven to the end of the study (1.2% males). Food consumption during the first three days of exposure was significantly depressed to half of control values in the males and to 59% of controls in the females; these remained depressed in the males for the remainder of the study, while the female consumptions rebounded to normal. The positive control females were significantly lighter than control for the last two weeks of the study. There was an initial depression in food consumption in both sexes of the DEHP rats, but this returned to normal over the course of the study. Mean calculated intakes of DINP in mg/kg/day (males:females) were: 0.6% group (639:607); 1.2% group (1192:1193); and 2.5% (2195:2289). Mean calculated intakes of the 1.2% DEHP group were 1084 mg/kg/day (males) and 1063 mg/kg/day (females). The authors estimated that had the dosing mistake not occurred in this group, mean intakes would have been 1273 and 1221 mg/kg/day, respectively. There were no significant dose-related gross lesions at necropsy. There were significant dose-related increases in both mean absolute and mean relative liver weights in all DINP exposed animals of both sexes. Mean relative kidney weights were also significantly increased in both sexes in a dose-related manner. Mean absolute kidney weights were only significantly increased in the low dose males; they were significantly decreased in the high dose males. Absolute kidney weights in the exposed females were not significantly different from controls. Mean relative testes weights were significantly increased in the high dose males compared to controls. Mean relative liver and kidney weights in the 1.2% DEHP group were significantly increased in both sexes; mean absolute liver weights were significantly increased in both sexes as well. Comparing the 1.2% DINP and 1.2% DEHP groups, increases in relative liver weights were higher in both sexes exposed to DEHP; the increases in the DEHP groups were about 10 to 30% higher (relative to controls) than were the increases in the DINP groups. Serum cholesterol was significantly decreased in all male and female rats exposed to DINP or DEHP, but the changes were not dose-related. Serum triglycerides showed significant dose-related deviations from control that decreased in males and increased in females for both DINP and DEHP. Cyanide-insensitive palmitoyl-CoA oxidation was increased in a dose-related manner in both sexes, significantly so at the two highest DINP dose levels and also in the DEHP rats. Lauric acid hydroxylase activities were significantly increased in all DINP exposed males, but only the high dose DINP females; increases were significant and dose-related in both sexes of the DEHP

dosed rats. Enzyme increases in the DEHP rats exceeded those of DINP rats dosed at a comparable level, Enzyme levels in the DEHP rats ranged from two to several times higher than DINP rats at comparable doses, with reference to control values. Total hepatic protein was slightly but significantly higher in all of the DINP dosed rats of both sexes, but a dose-response relationship was not evident with respect to microsomal protein levels. Changes in DEHP rats were similar. Electron microscopy revealed marked proliferation of peroxisomes in both sexes fed 2.5% DINP, and males fed 1.2% DEHP; proliferation was moderate to marked in the 1.2% DEHP females. Histopathological exams revealed primarily changes in the staining characteristics of the hepatocytes which were more prevalent at the higher DINP dose, but the authors considered these adaptive, rather than toxic, effects. As both liver weights and serology were significantly altered relative to controls in both sexes at even the lowest level of DINP, and liver enzymes were also significantly deviated from controls in males at the lowest dose level, a NOAEL was not identified under the conditions of this study.

A subchronic feed study in $B6C3F_1$ mice was conducted by Hazleton Labs in 1991 (Myers, 1991). DINP (reported as 99.9% active ingredient) was incorporated into a basal diet of Purina® Certified Rodent Chow® at concentrations of 0, 3,000, 6,000, 12,500 and 25,000 ppm and fed *ad libitum* to groups of 10 six-week-old mice of each sex for at least four weeks. A body weight-dependent computerized randomization procedure was used which eliminated the animals with extreme body weights and produced homogeneity of the mean body weights and their variances among groups/sex. At initiation of the study, body weights ranged from 21-26 g in the males and 16-24 g in the females. Animals were observed twice daily for clinical signs and mortality, with more careful cageside observations for toxic effects once daily. Body weights were recorded at the outset and weekly thereafter; food consumption was recorded weekly, with spilled feed collected, weighed and added to feeder weight at the end of each week. During the fifth week, blood was collected after an overnight fast for hematology and clinical chemistry. Clinical sampling was conducted over two days, with samples collected for hematology first on sampling day 1 and those for chemistry collected first on sampling day 2. This was done in an attempt to obtain adequate blood volume for both batteries of tests, despite the small size and limited blood volume of the mice. Bone marrow smears were collected at necropsy to assess myeloid/erythroid ratio. Following bleeding, all animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Gross necropsies were conducted; major organs and all gross lesions were excised and formalin fixed for histopathology. Organ weights were obtained for liver with gall bladder, kidneys, and testes with epididymides. Fixed tissues were paraffin embedded, sectioned and stained, and examined for all control and high-dose animals. Livers, spleens, kidneys, testes with epididymides (males), and all gross lesions for all animals in all dose groups were subjected to histopathologic exam as well. “Target organs” identified on examination of the high dose group (thymus, ovaries and uterus) were also examined in all dose groups. Statistical analysis consisted of analysis of variance (ANOVA) with multiple comparisons of group means to the control mean by Dunnett’s Test; a 5% two-tailed probability level was considered significant. In cases where data violated the required assumption of homogeneous variances (as noted by Levene’s test), they were transformed to achieve homogeneity. When that failed, analyses were performed on rank-transformed data.

Concentration of the agent in feed was within two percent of the target in all exposure levels. There was no premature mortality. There were no clinical signs noted that were considered to be exposure-related. Both males and females in the high dose group had markedly and significantly lower mean body weights than corresponding controls from week 3 to termination; high dose males and females lost weight from week 1 and week 2, respectively, to the end of the study. By week five, the mean body weights in these groups were only 77% of controls. Mean weekly food consumption and total food consumption were significantly decreased for all

high dose females, but not for males. Mean compound consumption (in mg/kg/day; male:female) was as follows: 3,000 ppm (636:781), 6,000 ppm (1378:1672), 12,500 ppm (2690:3287) and 25,000 ppm (6518:6921). The small size of the mice prevented adequate blood being obtained for all clinical pathology analyses, and while some significant deviations from control were noted in the 12,500 ppm females and the 25,000 ppm males and females, they were considered to be an artifact of the small sample sizes rather than due to exposure to DINP. Alanine aminotransferase (ALT) and blood urea nitrogen (BUN) were significantly increased in the high dose males and females, and in the high dose males, respectively, although these results were based on four or fewer animals. Mean terminal body weights were significantly reduced in the high dose males and females compared to controls. Mean absolute and relative liver weights were significantly increased in all treated male groups and all but the low dose females as a result of exposure. Significant and dose-related decreases in mean absolute and relative kidney and testes with epididymes weights were seen in the 6,000 and 12,500 males; in the 25,000 ppm males, mean absolute weights for these organs were significantly decreased, while relative kidney weights were increased and relative testes with epididymes weights were decreased, both significantly. High dose females also experienced significant increases in mean relative kidney weights. Gross liver lesions (consisting of enlarged, pale or dark areas) were recorded in 2/10 males in the 6,000 ppm dose group, 5/10 females in the 12,500 ppm group, and in 5/10 males and 3/10 females in the 25,000 ppm group. The same lesions were also found in 2/10 females in the controls. Other gross lesions occurred only sporadically, and were not considered related to treatment. Histopathological lesions were recorded in the liver, kidneys, testes with epididymes, ovaries, uterus, spleen and thymus. Hepatocellular enlargement was present in the livers of males of all exposed groups, and in the livers of all but the low dose females, “with a clear dose response relative to incidence and severity.” Incidence was 0/10, 4/10, 10/10, 10/10 and 10/10 in males and 0/10, 0/10, 7/10, 8/10 and 10/10 in females in the 0, 3,000, 6,000, 12,500 and 25,000 ppm groups, respectively. This change was centrilobular to diffuse in distribution. There were focal areas of coagulative necrosis, mostly non-inflammatory, in many (8/10) of the high dose males, and to a somewhat lesser extent in the mid-high (4/10) and high (1/10) dose females. Separate foci of chronic inflammation were also noted in 6/10 high dose males and 1/10 mid-high dose females. The study pathologists noted these reactions were consistent with the elevations of ALT observed. Renal tubular nephrosis was seen in 1/10 males in the 12,500 ppm group and in all of the high dose animals of both sexes. This finding is also consistent with the observed elevations in BUN in the high dose males. Splenic atrophy was present in 3/10 males and 3/10 females in the 25,000 ppm group, and increased necrosis of thymic lymphocytes suggest some effect of the agent on the reticuloendothelial system as well, although the latter lesion was observed in all of the control females as well. Reproductive effects were prominent in both sexes at the higher doses, with all ten high dose males exhibiting increased cellular debris in the epididymes, and all high dose females showing ovarian and uterine atrophy. The pathologists considered the epididymal lesions to be indirect evidence of compromised spermatogenesis, while the ovarian (virtual lack of *corpora lutea*) and uterine (endometerial glandular atrophy) lesions suggested an “arrest of ovulation.” It was noted, however, that the female reproductive lesions may have also been due to the “nutritional deficiency” from decreased feed intake. Based on the results of this study, the target organ for DINP toxicity appeared to be the liver, with males more sensitive than females and a NOAEL not identified.

While the text, and so the details, of a 13-week dietary oral toxicity study of DINP in $B6C3F_1$ mice was not available for review, a rough draft of a pathology report was (Voelker and Pearson, 1992). Separate reports were made for two interim (after 3 and 31 days of exposure, respectively) and for the main study. The two interim reports compared histologic sections of liver from mice dosed at 0 and 10,000 ppm DINP to a positive control, “WY 14,463,” dosed at

1,000 ppm. After 3 days, there was comparable diffuse hepatocellular enlargement in both sexes dosed with either DINP or the positive control. After 31 days, the hepatocellular enlargement was considered to be of moderate severity, and was accompanied by increased incidence of focal liver necrosis in both the DINP and positive control groups. Severity was considered greater in the positive controls, as was individual cell degeneration and necrosis. The main study noted that the mice were exposed to DINP in feed at concentrations of 0, 1,500, 4,000, 10,000 or 20,000 ppm for at least 90 days. All mice were necropsied, and a comprehensive collection of tissues examined histopathologically for the control and high dose groups, while all gross lesions, liver, kidney, spleen, testes with epididymides (males) and uterus (females) were examined for all dose groups. Exposure-related lesions were noted in the liver, kidneys, epididymides, spleen, thymus, ovaries and uterus of the 20,000 ppm mice, and in the liver of the 10,000 ppm mice. Compound-related alterations were not observed in tissue sections from mice at the low and mid-low doses. Liver changes consisted primarily of hepatocellular enlargement, diffuse and moderate to moderately severe in the high dose mice, and centrilobular to midzonal and moderate in the mid-high group. Minimal to slight cell degeneration/necrosis and pigmentation accompanied the enlargement in the high dose group, and focal necrosis was present in four (one male, three female) mice in that group as well. The pathologists note that these changes were accompanied by increased liver weights in both of these dose groups; absolute and relative liver weights were also elevated in the 4,000 ppm mice. The remaining exposure-related lesions were confined to the high dose level. These included: 1) slight to moderate renal tubular nephrosis; 2) significantly reduced testicular/ epididymal weights with minimal numbers of immature/abnormal sperm; 3) lymphoid depletion in the spleen of four mice and the thymus of 13 mice; 4) uterine hypoplasia; and 5) absence of *corpora lutea*. This last lesion was also noted in a female from the 10,000 ppm group which died during the second week of the study. The clinical portion of this pathology report notes significant elevations in ALT and aspartate aminotransferase (AST) in the high dose males, which would be consistent with the other reported liver lesions. Other alterations occurred sporadically and were not considered related to exposure.

A report was available for a 13-week subchronic feeding study of DINP in F344 rats (Myers, 1992). DINP (reported as 99.9% pure) was incorporated into a basal diet of Purina® Certified Rodent Chow® at concentrations of 0, 2,500, 5,000, 10,000 and 20,000 ppm and fed *ad libitum* to groups of 10 six-week-old rats of each sex for at least 13 weeks. Animals exhibiting clinical signs or those with ocular abnormalities based on ophthalmologic exam were excluded from the study prior to assignment. A body weight-dependent computerized randomization procedure was used which eliminated the animals with extreme body weights and produced homogeneity of the mean body weights and their variances among groups/sex. At initiation of the study, body weights ranged from 110-131 g in the males and 90-108 g in the females. Animals were observed twice daily for clinical signs and mortality, with more careful cageside observations for toxic effects once daily. A thorough physical examination was conducted once a week. Body weights were recorded at randomization, prior to exposure and weekly thereafter; food consumption was recorded weekly. Indirect ophthalmoscopic exams were performed on all animals prior to treatment and during the thirteenth week. After 13 weeks, urine samples were collected in collection racks during an overnight fast; blood was collected the next day for hematology and clinical chemistry. Femoral bone marrow smears were collected at necropsy to assess myeloid/erythroid ratio. Following bleeding, all animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Gross necropsies were conducted; major organs and all gross lesions were excised and formalin fixed for histopathology. Organ weights were obtained for liver, kidneys, brain with stem, lung, spleen, uterus and testes with epididymides. Fixed tissues were paraffin embedded, sectioned and stained, and examined for all control and high-dose animals. Livers, spleens, kidneys, testes with epididymides (males), non-glandular

stomach and all gross lesions for all animals in all dose groups were subjected to histopathologic exam as well. Statistical analysis consisted of analysis of variance (ANOVA) with multiple comparisons of group means to the control mean by Dunnett's Test; a 5% two-tailed probability level was considered significant. In cases where data violated the required assumption of homogeneous variances (as noted by Levene's test), they were transformed to achieve homogeneity. When that failed, analyses were performed on rank-transformed data.

Concentration of the agent in feed was within ten percent of the target in all exposure levels, with the exception of two periods: 1) weeks 1-3, where the diet for the 20,000 ppm group had a DINP concentration that was above the target by >10%, and 2) weeks 8-11, where the diet for the 2,500 ppm group was below the target concentration by >10%. All animals survived to termination. The only clinical signs noted that were considered to be exposure-related were urine stains (incidence 0/10, 0/10, 0/10, 0/10 and 1/10, males, and 0/10, 1/10, 1/10, 6/10 and 9/10, females) and thin appearance of 2/10 high dose females at the thirteenth week. Body weights were significantly lower than their respective controls in the high dose males at weeks nine and fourteen and for the study (weeks 1-13) as a whole; they were significantly lower in the high dose females at weeks 5, 9, 14 and for the study as whole. Mean food consumptions were significantly increased in high dose males at week one and decreased in high dose females at weeks four and eight. Mean compound consumption (in mg/kg/day; male:female) was as follows: 2,500 ppm (176:219), 5,000 ppm (355:438), 10,000 ppm (720:824) and 20,000 ppm (1545:1687). There were no exposure-related ophthalmic lesions at any dose level. Slight but significant decreases in erythrocyte and hemoglobin counts were noted in the 5,000, 10,000 and 20,000 ppm males and the 10,000 and 20,000 ppm females. BUN concentrations were significantly elevated in mid-high dose males and in high dose males and females; creatinine and total protein were significantly decreased in the high dose females. Albumin was elevated significantly in mid-high males and females and high dose males; globulin was decreased significantly in the two highest male and three highest female dose groups. With the exception of increases in incidence of leukocytes and amorphous material in the 10,000 and 20,000 ppm males, and in specific gravity in 20,000 ppm males, urinalyses among exposed animals were comparable to controls. Findings on gross pathology consisted only of "dark areas" in the glandular stomach of 3/10 males and 3/10 females in the high dose group, in a single female control, and enlarged and dark livers in two high-dose males. There were marked dose-related alterations in organ weights and organ to terminal body and organ to brain weight ratios in both sexes. Mean absolute liver and kidney weights, liver to terminal body weight, kidney to terminal body weight, liver to brain weight and kidney to brain weight ratios were significantly elevated in both sexes at dose levels of 5,000, 10,000 and 20,000 ppm. Mean absolute kidney and kidney to brain weight ratios in females, kidney to terminal body and liver to terminal body weight ratios in males were also significantly elevated in the 2,500 ppm groups. Mean absolute lung weights and lung to brain weight ratios were significantly depressed at the high-dose level in both sexes. With respect to reproductive organs, mean absolute uterine weights, uterus to terminal body and uterus to brain weight ratios were all significantly lower than controls in the high dose group, and testis with epididymis to terminal body weight ratios were elevated in the 10,000 and 20,000 ppm males. Histopathology revealed the following lesions where exposed rats differed from controls: 1) hepatocellular enlargement in all of the high dose rats of both sexes, and one female of the mid-high dose group (the lesion was centrilobular in females and periportal in males); 2) presence of granular casts in 6/10, 10/10 and 10/10 males in the 5,000, 10,000 and 20,000 ppm groups, respectively; and 3) "dark areas" in glandular stomach of three animals of each sex in the high-dose group; these were attributed by pathologists to acute inflammation, and were minimal to slight in severity. While regenerative/basophilic renal tubules were present with dose-related severity in the male rats (reaching "moderate" severity in the high dose group), these lesions were also present in all of the male controls as well. Even in the 20,000

ppm females, this lesion was present in only 2/10 individuals, and appears mainly associated with sex. Other lesions occurred sporadically, did not appear dose-related, and were considered unrelated to exposure. The authors of the study concluded that the target organs for DINP in rats were liver, kidney and glandular stomach, that males appeared more sensitive to anemia and renal changes and that females were more sensitive to body weight changes. They also noted that a no observed effect level (NOEL) was not identified in this study.

Two earlier 13-week feeding studies in rats exist, one in Sprague Dawley strain (Kapp, 1983*a*) and one in Fischer 344 strain (Kapp, 1983*b*). Both were performed at the same lab and are of similar design. Groups of 15 male and 15 female, four-week old Sprague-Dawley (S-D) rats were screened from a larger group to exclude sick animals and those with outlying body weights (> ± 10 of mean), and randomly assigned to dose groups of 0, 3000 or 10,000 ppm DINP incorporated into Purina® Certified Rat Chow, fed *ad libitum,* for 13 weeks. The F344 rats were eight weeks old at initiation of the study, and were similarly assigned (15 male, 15 female/grp) to dose levels of 0, 1000, 3000 (the study lists 13,000, but context gives the impression that this was a typographical error), 6000, 10,000 or 20,000 ppm. The animals weighed ~100-200 g. Animals were checked twice daily "for viability" and once weekly for signs of toxicity. Body weights were recorded predose, at initiation of dosing, and weekly thereafter, including at termination. Animals were individually housed; food consumption was recorded weekly by weighing the feeder. A battery of hematology and serology tests were performed on all animals at sacrifice; ten animals per sex per group were selected for urinalysis tests at weeks four, eight and thirteen. The same group of ten animals was sampled each time; urine was gathered in metabolic cages. After thirteen weeks of dosing, all animals were anesthetized with methoxyflurane and exsanguinated following blood collection. Gross necropsies were performed on all animals, and organ weights obtained for heart, liver, lungs, spleen, brain, kidneys (both) and testes with epididymides (both, males). Samples from these and other major organs, and all gross lesions, were fixed and sectioned for histopathology. In addition, two animals per sex per group were selected for electron microscopy evaluation. Statistical analyses consisted of evaluation of homogeneity of variance (Bartlett's test, two-tailed, at a 1% significance level), followed by ANOVA and Dunnett's test for data with homogeneous variances, or Kruskal-Wallis and Dunn's test for data with non-homogeneous variance. Data were also evaluated to assess trend in dose levels. For all of these tests, $p < 0.05$ was considered significant, and $p < 0.01$ highly so.

Among the S-D rats, there was a single death, a male in the high dose group, during the final week of the study. Some of the animals experienced a mistaken disconnection of their water supply during the during the twelfth study week, with resulting highly significant depressions of body weight and food consumption in effected animals. These had begun to rebound following discovery of the error (last week of the study). The authors also attributed some coincident clinical signs (increases in ocular discharge, emaciation and urine staining) to this problem. A low incidence of red nasal discharge was also observed, spread across all dose groups, and was not attributed to exposure. Erythrocyte indices (hematocrit, hemoglobin and red blood cell count) were all slightly but consistently lower than controls in all groups of exposed rats of both sexes, "suggestive of the development of mild anemia." Serology showed these clinically and statistically significant deviations of dosed means from controls: 1) alkaline phosphatase, elevated in the high dose group, both sexes; 2) triglycerides, decreased in all exposed animals, with a clear dose response relationship; and 3) chloride, decreased in all exposed animals, clearly dose-related. There was a statistically significant increase in ALT levels in the high dose males, but this was not judged to be clinically significant. Urinalysis noted clinically and statistically significant alterations in urine volume (increased in both groups of dosed males, weeks 4, 8 and 13), urine glucose (increased in both groups of dosed males, with a clear dose

relationship, weeks 4 and 13), urine protein (increased in both groups of dosed females, week 4 and decreased in low dose males, week 13), renal epithelial cell count (increased in both groups of dosed males, weeks 4 and 8, with a clear dose relationship, week 4). Mean terminal body weights were significantly lower than controls for both exposed groups of males, and for the high dose females. The 10,000 ppm males had significantly increased mean absolute liver and kidney weights, as did both dose levels of exposed females. Mean organ to terminal body weight ratios were increased for both dose levels of males for lung, kidney, brain and liver. Ratios were significantly increased also for spleen:body weight in the low dose males and for testes and epididymides:body weight in the high dose males. Among the females, mean organ to terminal body weight ratios were increased for both dose levels for kidney and liver; the high dose females also had significantly increased ratios for brain:body weight and heart:body weight. The most frequent lesions revealed on gross necropsy were discoloration, scattered tan foci and dilatation of the renal pelvis of the kidneys. Though these were noted more frequently in the high dose, there was no clear relation to dose level. There were no other exposure-related gross lesions. With respect to histopathology, there was a dose-related hepatocellular hypertrophy in all exposed animals of both sexes. Among the males, there was also an exposure-related increase in the occurrence of nephrosis (4/15 in the 3000 ppm group, 11/15 in the 10,000 ppm group) with the presence of granular tubular casts and increased tubular regeneration.

Among the Fischer strain rats, there were no pre-termination deaths. Signs of toxicity were limited to ocular discharge, and urine and “A-G” (ano-genital?) staining; urine staining was most prevalent in the high dose group. Body weight gains were normal, except for the high dose group, which showed significant depressions in weight compared to controls; these were more pronounced among the males. Food consumption was generally decreased in the 10,000 and 20,000 ppm groups; females tended to have more frequent statistically significant differences from controls than did the males. With respect to serology, the only statistically and clinically significant differences from controls noted were 1) increased alkaline phosphatase in males of the two highest doses and the highest dose in females; 2) decreased triglycerides in the 6000, 10,000 and 20,000 ppm groups of both sexes, with a clear dose-response trend; and 3) increased albumin:globulin ratios in the two highest male dose groups and the three highest female dose groups. There was a slight trend towards the mild anemia seen in the S-D rats, but although these alterations were significant statistically, they were not judged to be clinically so. Clinically and statistically significant deviations from control noted in urinalyses consisted of 1) increases in urine volume (weeks 4 and 8) and consequent decreases in osmolarity (weeks 4, 8 and 13) among the three highest male dose groups; 2) increases in urine glucose in the three highest male groups (weeks 4, 8 and 13) and in the 3000 ppm group as well (week 13); 3) increased urine protein in the three highest female dose groups (week 4), and in the 10,000 ppm males and the 20,000 ppm females (weeks 8 and 13); and 4) increases in renal epitbelial cell counts in the four highest male dose groups (weeks 4, 8, and 13 [excepting the 6000 ppm level at week 13]) with a clear dose-response relationship. Qualitative urinalysis noted occult blood in 5/10 of the high dose males at week 8, and in 1/10, 10/10, 9/10 and 2/10 males in the control, 1000, 3000 and 10,000 ppm groups, respectively at week 13. Mean absolute liver weights were significantly increased in 6,000, 10,000 and 20,000 ppm males and females, as were the lung, heart and spleen weights in the high dose males group. Mean absolute kidney weights were significantly increased in the 3000 ppm females, while mean heart and lung weights were significantly decreased in the high dose females. Mean relative liver weights were significantly increased in the top four male dose groups and the top three female dose groups; mean relative kidney weights were increased in the top two male and the top four female dose groups. The high dose group also showed increased relative brain weights in both sexes and testes/epididymides weights in the males. Increased relative spleen weights were reported in

the 6000 and 10,000 ppm females as well. As was the case with the S-D rats, the principle gross lesion was discoloration of the kidneys, and this was clearly dose-related in the males, going from 4/15 in the 6000 ppm group, to 9/15 in the 10,000 and 14/15 in the 20,000 ppm groups. Electron micrographs of liver tissue samples revealed dose-related proliferation of peroxisomes in the three highest dose groups, which was worse in males. Histopathology found diffuse hepatocellular hypertrophy in all exposure levels of both sexes, the severity of which was clearly related to the dose. Compared to controls, there was also an exposure-related increased incidence of kidney lesions, with nephrosis of increasing severity noted in the 3000, 6000, 10,000 and 20,000 ppm males, and granular casts in the top three of those dose levels.

Considering the results of all of these studies taken together, it should be noted that consistent patterns of effects recur across species and strains. Decreases in body weights among animals dosed at high concentrations are a consistent finding, as is evidence of both liver and kidney effects at considerably lower levels of exposure (dose related increases in relative and absolute liver and kidney weights, accompanied by changes in serum biochemical/hematological tests, and evidence of histopathologic changes in these organs). This concordance of results across studies makes it appear unlikely that the effects reported are an artifact of a particular species or strain, or reflect a bias specific to a particular laboratory or team of researchers. In choosing a key study, duration becomes the first exclusion criteria. The reports of Ford and Gaunt (1986) and Myers (1991) are both of shorter duration than the remaining studies, and so are ruled out as key studies. In addition, neither report studied effects at a DINP consumption level below 600 mg/kg/day, a dose level at which effects were seen in other studies. The 13-week study in mice (Voelker and Pearson, 1992) lacks sufficient detail to critically assess the quality of the study. Of those remaining, the uncoupling of the water supply to some of the S-D rats reported in Kapp (1983*a*) complicates interpretation of results, especially since dehydration could potentially have had a marked effect on the critical endpoints (organ weights, renal lesions). This study is excluded as well. Both the Kapp (1983*b*) and Myers (1992) studies appear well conducted, and because they used different dose levels at the low end of the range (1000 and 3000 ppm, Kapp, and 2500 and 5000 ppm, Myers), they more adequately describe the threshold area of the adverse effect dose. These studies are used together as key studies (Table 1).

The lowest dose level at which an effect is reported in Myers is 2500 ppm, at which mean liver to terminal body weight and kidney to terminal body weight ratios were significantly elevated in the males, and mean absolute kidney weights and mean kidney to brain weight ratios were significantly increased in females. There was no reported evidence of histopathological or biochemical alterations in either sex at this dose level. However, at the next highest dose level (5000 ppm), both absolute and relative liver and kidney weights are significantly increased in both sexes, and are accompanied by histopathologic changes (renal tubular casts) in males, and by hematological/serological alterations (evidence of mild anemia in males and decreased globulin levels in females) that provide further evidence of an adverse renal effect in males (and possibly an adverse hepatic effect in females) at this dose level. By way of comparison with Kapp's results in the same strain, at 1000 ppm the only significant change reported was diffuse hepatocellular hypertrophy, unaccompanied by organ weight or biochemical/hematological changes. Using the guidelines for adversity of effect outlined by EPA (EPA, 1990), this change would suggest that 1000 ppm is an NOAEL. At the 3000 ppm level, increases in relative liver weights in males are accompanied by a greater degree of hepatocellular hypertrophy, and increases in both absolute and relative kidney weights in the females are accompanied by histopathologic evidence of nephrosis and increases in shed renal epithelial cells in the urine. Given the close proximity of this dose to the 2500 ppm level in Myers (1992), the latter dose level may be interpreted as a LOAEL. Since it is not clear from these two studies which sex, if either, is more sensitive to DINP exposure, the calculated mean daily DINP consumption of both

sexes combined in the 1000 ppm group of Kapp (1983*b*) [73 mg/kg/day) is used to drive the ITSL.

ITSL Derivation: Applying Rule 232(1)(e) promulgated pursuant to Article II, Chapter 1, Part 55, of Act 451:

$$ITSL = \frac{NOAEL\ (mg/kg/day)}{35 \times 100} \times \frac{W_A}{I_A} \times \frac{b}{a}$$

where:
W_A = Mean final body weight of F344 rats at the 1000 ppm dose level in Kapp (1983*b*,)
I_A = Daily inhalation rate of a sex-unspecified F344 rat (default value from MDNR, 1991)
b = Absorption efficiency by the oral route of exposure
a = Absorption efficiency by the inhalation route of exposure

The factor of 35 in this equation is a safety factor to account for using a NOAEL from a seven-day exposure period to estimate a NOAEL for a lifetime exposure, as outlined by the MATPC (1989). Since the exposure period in Myers (1992) and Kapp (1983*b*) was 13 weeks, this safety factor is reduced from 35 to 10, consistent with EPA methodology.

So,

$$ITSL = \frac{73\ mg/kg/day}{1000} \times \frac{0.204\ kg}{(0.969\ m^3/kg/day \times 0.204\ kg)} \times \frac{1}{1}$$

$$= 0.073\ mg/kg/day \times (0.969\ m^3/kg/day)^{-1} \times 1$$

$$= 0.075\ mg/m^3 \times \frac{1000\ \mu g}{1\ mg}$$

$$= \mathbf{75\ \mu g/m^3}$$

with (*b/a*) taking on the default value of 1 in the absence of data to the contrary.

Per 232(2) (c), an annual averaging time applies.

Finally, it is important to note that the MSDS for DINP (Aristech, 1993) references a 104-week feed study of di-C9-alkylphthalate ("Santicizer 900") in S-D rats which found increases in neoplastic and pre-neoplastic liver lesions in exposed animals, as well as interstitial cell testicular tumors. An attempt was made to obtain this study for review, but the file microfiche number listed in the MSDS did not correspond to the referenced study, but to a document concerning an unrelated chemical. A Chemical Abstracts Service (CAS) on-line structure search was performed in an attempt to determine whether this chemical and DINP are the same structurally, but 'di-C9-alkylphthalate' did not return any search results, and the MSDS does not list a CAS number for "di-C9-alkylphthalate" to use as a search term. The structure search for

DINP noted that one of the proprietary names for DINP is “Sansocizer DINP,” but it remains unclear whether the 104-week study referred to by the MSDS used DINP as the agent. It must be recognized that if that study becomes available for review in the future, the screening level derived in this risk assessment should be re-evaluated in light of any new information, and the screening level itself revised, if deemed appropriate.

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Table 1: Comparison of results from two principal studies of Diisononyl phthalate (DINP) [28553-12-0] ($p < 0.05$)

	Kapp, 1983*b*					Myers, 1992			
Dose level (ppm)	1000	3000/ (13,000?)	6000	10,000	20,000	2500	5000	10,000	20,000
Species/ Strain	Rat/F344					Rat/F344			
Animals/grp	15/sex/group (except for urinalysis, which tested 10/sex/group)					10/sex/group			
Age	8 weeks at initiation of dosing					6 weeks at initiation of dosing			
Duration (wks)	13+					13+			
Terminal body weight					M: ↓ F:↓				M: ↓ F:↓
Food Consumption				M: ↓ F:↓	M: ↓ F:↓				M: ↑ (wk.1) F:↓ (wks. 4.8)
Abs. Liver weight			M:↑ F:↑	M:↑ F:↑	M:↑ F:↑		M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Liver:Body Weight Ratio		M:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Liver:Brain Weight Ratio	Not Measured	Not Measured	Not Measured	Not Measured	Not Measured		M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Liver-related Clinical Pathology			M&F: ↓ triglycer. F: ↑ alb.:globul.	M&F: ↓ triglycer. ↑ alb.:globul. M: ↑ alk. phos.	M&F: ↑ alk. phos. ↓ triglycer. ↑ alb.:globul.		F: ↓ Globul.	M&F: ↑ Album. ↓ Globul.	M&F: ↓ Globul. M:↑ Album.
Liver Histopathology	M&F: hepatocell. hypertrophy	M&F: hepatocell. hypertrophy	M&F: hepatocell. hypertrophy	M&F: hepatocell. hypertrophy	M&F: hepatocell. hypertrophy			F:hepatocell. hypertrophy (1/10)	M&F: hepatocell. hypertrophy (10/10)
Abs. Kidney weight		F:↑					M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Kidney:Body Weight Ratio		F:↑	F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Kidney:Brain Weight Ratio	Not Measured	Not Measured	Not Measured	Not Measured	Not Measured		M:↑ F:↑	M:↑ F:↑	M:↑ F:↑
Kidney-related Clinical Pathology							M: ↓ Hb ↓ RBCs	M: ↑ BUN M&F: ↓ Hb ↓ RBCs	M&F: ↓ Hb ↓ RBCs ↑ BUN F: ↓ Creat.
Kidney Histopathology		M: nephrosis	M: nephrosis, granular casts	M: nephrosis, granular casts	M: nephrosis, granular casts		M: granular casts (6/10)	M: granular casts (10/10)	↓ Tot. Prot. M: granular casts (10/10)
Urinalysis		M: ↑ glucose ↑ renal epith. cells	M: ↑ volume ↓ osmolarity ↑ glucose ↑ renal epith. cells F:↑ protein	M: ↑ volume ↓ osmolarity ↑ glucose ↑ renal epith. cells M&F:↑ prot.	M: ↑ volume ↓ osmolarity ↑ glucose ↑ renal epith. cells F:↑ protein			M:↑ WBC ↑ amorph. material	M:↑ WBC ↑ amorph. material ↑ sp. grav.