Ward EM, Schulte P, Grajewski B, et al. Serum organochlorine levels and breast cancer: a nested case-control study of Norwegian women. *Cancer Epidemiol Biomarkers Prev* 2000; 9(12):1357-1367.

Weintraub M, Birnbaum LS. Catfish consumption as a contributor to elevated PCB levels in a non-Hispanic black subpopulation. *Environ Res* 2008; 107: 412-417.

Winter CK, Segall HJ, Haddon WF. Formation of cyclic adducts of deoxyguanosine with the aldehydes trans-4-hydroxy-2-hexenal and trans-4-hydroxy-2-nonenal in vitro. *Cancer Res* 1986; 46: 5682-5686.

Wolff MS. Occupational exposure to polychlorinated biphenyls (PCBs). *Environ Health Perspect* 1985; 60: 133-138.

Wolff MS, Fischbein A, Selikoff IJ. Changes in PCB serum concentrations among capacitor manufacturing workers. *Environ Res* 1992; 59: 202-216.

Zettner MA, Flor S, Ludewig G, Wagner J, Robertson LW, Lehmann L. Quinoid metabolites of 4-monochlorobiphenyl induce gene mutations in cultured Chinese hamster v79 cells. *Toxicol Sci* 2007; 100: 88-98.

Zhao G, Xu Y, Han G, Ling B. Biotransfer of persistent organic pollutants from a large site in China used for the disassembly of electronic and electrical waste. *Environ Geochem Health* 2006; 28: 341-351.

Zhao HX, Adamcakova-Dodd A, Hu D, Hornbuckle KC, Just CL, Robertson LW, Thorne PS, Lehmler HJ. Development of a synthetic PCB mixture resembling the average polychlorinated biphenyl profile in Chicago air. *Environ Int* 2009; in press.

Zheng GJ, Leung AO, Jiao LP, Wong MH. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans pollution in China: sources, environmental levels and potential human health impacts. *Environ Int* 2008; 34: 1050-1061.

Di(2-ethylhexyl) phthalate (DEHP)

by Tamie Nakajima PhD, Nancy B. Hopf PhD, and Paul A. Schulte PhD

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The use of di(2-ethylhexyl) phthalate (DEHP) has been modified since the last Monograph. An estimated 90% of DEHP is used as a plasticizer for PVC (Toxics Use Reduction Institute

at the University of Massachusetts, Lowell, USA (2004); www.turi.org). Consumer products such as footwear, shower curtains and toys, medical devices (IV bags, tubing), and commercial/industrial uses such as resilient flooring, wall covering, roofing, aluminum foil coating/laminating, paper coating, extrudable molds and profiles, electrical component parts, and wire and cable coating are all main users of DEHP.

Current evaluation

Conclusion from the previous Monograph:
DEHP is *not classifiable as to its carcinogenicity to humans (Group 3)* because peroxisome proliferation has not been documented in human hepatocyte cultures exposed to DEHP nor in the liver of exposed non-human primates. Therefore, the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans (IARC, 2000).

Metabolism in rodents

DEHP absorbed in the body is first metabolized by the catalytic action of lipase to produce mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) (Albro et al., 1989). Some MEHP is then conjugated with UDP-glucuronide by UDP-glucuronosyltransferase (UGT) and excreted in the urine. The remaining MEHP is excreted directly in the urine or is oxidized by cytochrome P450 4A, then further oxidized by alcohol dehydrogenase (ADH) or aldehyde dehydrogenase (ALDH) to dicarboxylic acid or ketones. 2-EH is metabolized mainly to carboxylic acid (mainly 2-ethylhexanoic acid, 2HEA) via 2-ethylhexanal by catalytic action of ADH and ALDH (Albro PW and Lavenhar SR, 1989).

Species difference in metabolism of DEHP

Lipase may be a rate-limiting step in the metabolism of DEHP and therefore species difference in the lipase activity may indicate the difference in DEHP metabolism. Recently, the i*n vitro* activities of lipase, UGT, ADH and ALDH for DEHP metabolism in several organs were measured and compared among mice, rats and marmosets (Ito et al., 2005). Marmosets were used as a reference for human metabolic activity. Clear-cut species differences were seen in the activities of the four enzymes involved in the DEHP metabolism among mice, rats, and marmosets. Of these, the difference in the lipase activity was most prominent. The constitutive levels in lipase (Vmax) and the affinity of lipase for DEHP (Km) were as follows: mice > rats >> marmosets: rodents had higher levels with greater affinity than marmosets. Thus, MEHP and perhaps 2-EHA and other di-carboxylic acid concentrations in the body were higher in mice or rats than marmosets when the same dose of DEHP was administered (Ito et al., 2007). However, there may be limitation using *in vitro* data to extrapolate to an in vivo kinetics, and also from marmosets to humans by these available data alone.

Metabolism in human

DEHP is also oxidized to MEHP in human, which is secondarily oxidized to mono-(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), which reflect the short-term exposure, and mono-(2-ethyl-5-carboxypentyl)phthalate

(MECPP) and mono-(2-(carboxymethyl)hexyl]phthalate (2cx-MMHP) (Koch et al., 2004; Koch et al., 2005; Koch et al., 2006). They represent the major share of DEHP metabolites excreted in urine (about 70% for these secondary oxidized metabolites vs. about 6% for MEHP. Half-life times have been estimated to about 5 h for MEHP, 10 h for5-OH MEHP and 5oxo-MEHO, 24 h for 2ox-MEHP and 12–15 h for MECPP in humans (Koch et al., 2006). Thus, long half-times of elimination make MECPP and 2cx-MEHP excellent parameters to measure the time-weighted body burden to DEHP (Koch et al., 2006). These half-life estimates are based on oral exposures, and those after inhalation or dermal contact are not known. Oral exposures reflect the environmental exposures experienced by the general population, while inhalation and dermal exposures reflect typical occupational exposures. The ratios between secondary metabolites have been shown to differ depending on DEHP exposure levels; high exposure where MEHP is predominant (26% MEHP) and low exposures where the secondary metabolites (e.g., MECPP) are higher than MEHP (6%) (Dirven et al., 1993).

Exposure and biomonitoring

Exposure to DEHP is common in the general population and in occupational settings. Occupational exposure to DEHP may occur during its manufacture and its use mostly as a plasticizer of PVC (compounding, calendering and coating operations). In the current Monograph it is stated that urinary levels of DEHP, its metabolites and total phthalates have been shown in a few studies to be higher in DEHP-exposed workers than in non-exposed workers and in post-shift samples than in pre-shift samples. No standard method had been proposed for biological monitoring of exposure to DEHP (IARC, 2000).

Since the Monograph was published in 2000, a standard method for biological monitoring of DEHP metabolites has evolved (Adibi et al., 2003; Barr et al., 2003; Becker et al., 2004; Blount et al., 2000; Jonsson et al., 2005; Kato et al., 2003; Kato et al., 2004; Koch et al., 2003; Koch et al., 2003; Nuti et al., 2005; Preuss et al., 2005; Silva et al., 2003) and verified (Silva et al., 2008), showing reproducible and accurate results for DEHP metabolites. In addition, it was found that the phthalate metabolites in urine at −70 ◦C were stable for several years (Silva et al., 2008).

The metabolites of DEHP as biomarkers of exposures, are specific to DEHP but may stem from occupational and environmental sources because phthalates are used in consumer products as seen in Hines et al., (2009) where workers had urinary metabolite levels from phthalates not used in the workplace.

Occupational exposure

Gaudin et al. (2008) reported biological monitoring of three urinary metabolites from PVC factory workers (n=25) exposed to DEHP (33% in plasticol used) and controls (n=19) in pre- and post-shift urine samples 5 consecutive days. Median concentrations of pre- and post-shift urinary samples in the exposed workers (controls) were 16.1 and 55.9 (12.0 and 10.4) µg/l for MEHP, 37.6 and 103.7 (38.1 and 11.4) µg/l for MCEPP and 46.3 and 72.1 (31.9 and 46.0) µg/l for 2-EHA, respectively. The authors found a significant increase of post-shift excretion in the exposed workers versus unexposed controls and in post-shift versus pre-shift

concentrations only in the exposed workers. While MEHP and MCEPP are specific biomarkers of DEHP exposure (Gaudin et al., 2008), 2-EHA is *not*.- These results are lower than levels reported by Driven et al. (1993); however the sensitivity of the laboratory methods were different, and the lower level could be potentially explained by the difference in analytical methods applied.

In a cross-sectional study of 156 workers (Hines et al., 2009), DEHP exposures were assessed in eight industry sectors by comparing urinary DEHP metabolites in post-shift samples. Evidence of occupational exposure to DEHP was strongest in PVC film manufacturing, PVC compounding, and rubber boot manufacturing where geometric mean (GM) end-shift concentrations of DEHP metabolites exceeded general population levels (NHANES 2003-2005) by 8-, 6- and 3-fold, respectively. Using urinary metabolites, this study identified workplaces with likely occupational phthalate exposure. However, metabolites of some phthalates not used in the workplace were detected in urine. It is difficult to distinguish occupational from non-occupational sources in low-exposure workplaces. This conclusion is in accordance with Gaudin et al. (2008). No controls (i.e., subjects with no occupational exposures) were surveyed in this study.

In the Swedish PVC-processing factory (Hagmar et al., 1990) 2,031 workers employed for at least 3 months between 1945 and 1980 and were followed until 1985. Exposure to plasticizers was stable over the entire study period, and the time-weighted average (TWA) breathing-zone level of phthalates was > 0.5 to 3, > 0.1 to 0.5, and up to 0.1 mg/m^3 for highly, moderately and low exposed workers, respectively. Significant excesses of total cancer morbidity (SIR = 1.28, 95% CI = 1.01 to 1.61; 75 observed cases) and respiratory cancer morbidity (SIR = 2.13, 95% CI = 1.27 to 3.46; 17 observed cases) were seen among the PVC-processing workers, but these excesses were not significantly associated with cumulative exposure to *plasticizers*. Only 6% of the cohort was exposed only to plasticizers. Smoking and other phthalates were not included as confounders.

A case-cohort study (Selenskas et al., 1995) of U.S. workers in a plastics manufacturing and research and development plant included 28 men case subjects who died from pancreatic cancer and had held at least one job as an hourly worker, and 140 men as control subjects (5 per case) randomly selected from the cohort and matched to cases by year of birth and survival. Smoking was not considered. Workers were classified into major production and nonproduction areas. Individuals with potential exposure to phthalates worked in either the vinyl and polyethylene processing department or the fibers and fabrics department. Potential exposure to DEHP, specifically mentioned as being used in this plant, occurred in the production of flexible plastics. Quantitative exposure measures were not available; however, duration of exposure (from employment history) and time since first exposure (latency) were known. A significantly increased risk of pancreatic cancer was observed only for workers in the vinyl and polyethylene processing department (RR = 7.15, 95% CI = 1.28 to 40.1, 5 exposed cases) exposed longer than 16 years. Limitations of this study include the exposure assessment because workers in the vinyl process might be exposed to phthalates, while workers in polyethylene processing are not (phthalates are not used with polyethylene plastics). Also, there are small number of exposed case subjects and potential confounders not considered.

Environmental exposures

Median MEHP values for the controls in (Gaudin et al., 2008) were 10.4-12.0 µg/L (n=25). This result was about the same reported in two previous studies 10.3 µg/L (n=85) (Koch et al., 2003; Koch et al., 2003), and 9.8 (n=19) (Preuss et al., 2005), but higher than environmental surveys; 0.9 – 4.5 µg/L (Barr et al., 2003; Blount et al., 2000; CDC 2003, 2005; Kato et al., 2003). There is currently no defined level for "background" DEHP exposure in the general population. Based on the metabolite levels found in environmental monitoring of DEHP and in controls of the occupational study, levels above 12 µg/L MEHP can be considered to be above background levels, and can be used to identify possible occupational exposures. However, other sources of environmental exposure such as use of DEHP consumer products cannot be ruled out and cannot be default be contributed to occupational exposures.

Donors undergoing apheresis (blood transfusion) are exposed to DEHP from disposables used in this process. In a small study, DEHP exposures in 18 donors were measured using biological monitoring (Koch et al., 2005) pre- and post-plateletpheresis. Maximum concentrations of metabolites after the continuous-flow plateletpheresis procedure was 826 µg/l for 5OH-MEHP, 774 µg/l for 5oxo-MEHP and 266 µg/l for MEHP (mean of the six volunteers); all levels are well above what is found in the general population, and were the highest in samples taken shortly after plateletpheresis.

Peroxisome proliferator-activated receptor(PPAR) and DEHP

MEHP, not DEHP, is an exogenous ligand of PPARα and PPARγ (Maloney and Waxman, 1999; Hurst and Waxman, 2003). In rats, mice, and marmosets exposed to DEHP (rodents for 2 weeks and marmoset for 15 months), Ito et al. (2007) reported constitutive expression to be 5-7 times greater in the rodents and to induce peroxisome keto-acyl-CoA thiolase mRNA and protein expression in mice and rats, but not in marmosets. The treatment, however, did not influence mitochondrial enzymes in any animals.

Constitutive androstane receptor (CAR) and DEHP

Eveillard et al. (2009) reported that DEHP activates not only PPARα but also CAR. Wild–type and PPARα-null adult mice were exposed to different doses of DEHP (0, 20 and 200 mg/kg for 21 days by gavages). Cyp2b10, CAR target gene in mice, transcript was markedly up-regulated by DEHP in wild-type mice (6.6-fold-change at 200 mg/kg/day), and slightly in PPARα-null mice (2.8-fold-increase). Similar result was also found in an *in vitro* experiment when recombinant JWZ-CAR cell line. In this cell line, androstenol abolished the induction of Cyp2b10 by DEHP(100µM), supporting the involvement of CAR in the regulation. Interestingly, MEHP was unable to increase Cyp2b10 mRNA in the JWZ-CAR cell line. The authors also investigated whether the activation of CAR by DEHP could be extrapolated to humans. DEHP dose-dependently increased the expression of CYP2B6, target gene of CAR in human (Maglich et al., 2003), at 50 and 100 µM of DEHP in human primary hepatocyte cultures. Thus, CAR also represents a transcriptional regulator sensitive to DEHP. These effects may provide additional pathways for induction of endpoints of DEHP toxicity.

Biomarkers of effect

DEHP exposures have been reported to significantly increase of 8-OHdG with high dose of DEHP (1000 mg/kg) in rats (Seo et al., 2004). Limitations of this biomarker of effect are that it has not been used in humans exposed to DEHP and it is not specific to DEHP. The high levels of 8-OHdG found in animal studies might be from mitochondrial DNA, which is a confounder in the methods used (Rusyn et al., 2006).

Cancer in humans

(*inadequate***,** Vol 77, 2000)

The current IARC Monograph (IARC, 2000) states that there is only limited DEHP-specific human carcinogen data are available. It included one epidemiological study; a mortality study (Thiess and Fleig 1978) of 221 workers in a DEHP production plant in Germany followed between 1940 and 1976. The Monograph Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced, and that the methods for this study were poorly described.

No epidemiologic studies specifically of DEHP exposure have been published since the last Monograph (IARC, 2000).

There are studies from the plastics industry with possible DEHP exposures focusing on different types of cancers. A Swedish case-control study (Hardell et al., 2004) from 1993 to 1997 included 791 cases of germ cell tumors and 791 controls matched by 5-year age group. A non-statistically significant increased risk was reported for exposure to soft plastics (containing plasticizer) (OR = 1.48, 95% CI = 0.94 to 2.34, 54 cases and 37 controls) but not to rigid plastics (containing little plasticizer) (OR = 1.06, 95% CI = 0.55 to 2.01, 23 cases and 26 controls).

A population-based case-control study among Danish men (Heineman et al., 1992) evaluated the relationship between multiple myeloma and exposure. There were 1,098 cases and 4,169 control subjects matched to the case by age. Exposure to phthalates was associated with nonsignificantly elevated ORs for multiple myeloma, with a higher estimated risk for probable exposure (OR = 2.0, 95% CI = 0.9 to 4.4, 11 cases and 21 controls) than possible exposure (OR = 1.3, 95% CI = 0.9 to 2.0, 34 cases and 99 controls). Stratified analysis conducted to separate the effects of exposure to phthalates from exposure to vinyl chloride, showed a non-statistically significant increased risk.

Cancer in experimental animals

(*sufficient***,** Vol 77, 2000)

The current IARC Monograph (IARC, 2000) concluded that DEHP produces liver tumors in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation; and

peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of DEHP in rats and mice.

However, recent animal studies suggest two additional cancer sites in rats of pancreatic acinar-cell adenoma (David et al., 2000) and testicular Leydig cell tumors (Voss et al., 2005). David et al.(2000) treated male and female Fisher 344 rats with 0-12,5000 ppm DEHP in the diet for up to 104 weeks and reported that incidences of bilateral aspermatogenesis in the testes increased after exposure to ≥ 500 ppm DEHP in male rats, and spongiosis hepatis in males exposed ≥ 1,250 ppm DEHP. David et al. (2000) also reported that incidences of castration cells in the pituitary gland and pancreatic acinar cell adenomas increased at 12,500 ppm DEHP in male rats; no adenomas were seen in female rats. Voss et al. (2005) reported that chronic exposure of DEHP at 300 mg/kg increased the incidences of hepatocellular tumors but that DEHP-induced testicular tumors developed earlier in lifetime than hepatocellular tumors, and their multiplicity increased with time. In addition, 300 mg/kg dose of DEHP showed a significantly increased rate of testicular atrophy. Although PPAR agonists have been hypothesized to induce Leydig cell tumors by inhibiting testosterone biosynthesis and/or by inducing aromatase, thereby increasing estradiol levels (Klaunig et al., 2003), DEHP was found to induce high-levels of gonadotropin-lutenizing hormone and to increase serum concentrations of estradiol and testosterone in Long-Evans rats exposed to 10 and 100 mg/kg per day DEHP for up to 100 days (Akingbemi et al., 2004).

Mechanisms of carcinogenicity:

The use of evidence of the PPAR-α activation to dismiss the human relevance of effects observed in laboratory animals has been questioned (Guyton et al., 2009; Caldwell et al., 2008; Melnick, 2001) based on the lack of experimental studies empirically challenging the mode of action hypothesis. Guyton et al. (2009) explain that PPAR-α activating compounds are pleiotropic and have been reported to exhibit a diversity of responses in addition to the hallmark effect of peroxisome proliferation, including genotoxicity, epigenetic alterations, oxidative stress, and effects on other receptors and other organelles within parenchymal cells. Importantly, DEHP reportedly affect non-parenchymal liver cells that do not express PPAR-α as well as other organ systems. Rusyn e al. (2006) also suggested that combination of molecular signals and pathways rather than a single hallmark event (such as induction of PPAR*α* and peroxisomal genes, or cell proliferation that contribute to tumors should be focused.

Two studies conducted after the IARC monograph suggest that DEHP can induce PPAR-*α* independent tumors without any loss of potency (Ito et al., 2007), and a robust hepatocyte and peroxisome proliferative response in itself is insufficient to cause tumorigenesis in transgenic model of PPAR-*α* activation in hepatocytes (Yang et al., 2007). Ito et al. (2007) reported that DEHP tumorigenesis in mice was not dependent on the PPARα pathway as both wild-type and *Pparα*-null mice fed diets containing 0, 0.01 or 0.05% DEHP for 22 months showed the incidence of liver tumors to be higher in *Pparα*-null mice exposed to 0.05% DEHP (25.8%) than in similarly exposed wild-type mice (10.0%).

Takashima et al. (2008) explored potential differences in the mechanisms of tumorigenesis between wild-type mice and *Pparα*-null mice using hepatocellular adenoma tissues of both

genotyped mice. Microarray profiles showed that the up- or down-regulated genes were quite different between hepatocellular adenoma tissues of wild-type mice and *Pparα*-null exposed to DEHP, suggesting that the mechanism of tumorigenesis might be different from each other. The authors suggested that DEHP may induce hepatocellular adenomas, partly via suppression of G2/M arrest regulated by Gadd45α and caspase 3-dependent apoptosis in *Pparα*-null mice but that these genes may not be involved in DEHP-induced tumorigenesis in wild-type mice. However, more study is needed whether DEHP promoted the spontaneous liver tumor in *Pparα*-null mice, because spontaneous hepatocellular tumors are known to occur in these mice at 24 months of age (Morimura et al., 2006)

To determine the difference in PPARα activation between mice and humans by PPAR agonists, two kinds of humanized PPARα mouse lines have been developed, hPPARα^{TetOff} mice (Cheung et al., 2004; Morimura et al., 2006) and hPPARα^{PAC} mice (Yang et al., 2008). The former line expresses the human receptor in liver in a *Pparα*-null background by placing the hPPARα cDNA under control of the Tet-Off system of doxycycline control with the liver-specific LAP1 (C/EBPβ) promoter. The hPPARα^{TetOff} mice express the human PPARα protein at levels comparable to or greater than that expressed in wild-type mice

Another transgenic mouse has the complete human PPARα gene on a P1 phage artificial chromosome (PAC) genomic clone, introduced onto the mouse *Pparα*-null background (Yang et al., 2008). This new line, designated hPPARα^{PAC}, expresses human PPARα not only in liver but also in kidney, heart, intestine and brown adipose tissue, that is, tissues with high fatty acid catabolism. hPPARα^{PAC} mice exhibited responses similar to wild-type mice when treated with fenofibrate lowering of serum triglycerides and induction of PPARα target genes encoding enzymes involved in fatty acid metabolism. Treatment of hPPARα^{PAC} mice with fenofibrate did not cause significant hepatomegaly and hepatocyte proliferation similar to hPPARα^{TetOff} mice, suggesting that the resistance to the hepatocellular proliferation found in the hPPARα^{TetOff} mice is not due to lack of expression of the receptor in tissues other than liver.

In a recent review (Guyton et al., 2009), available DEHP data were reviewed and meta-analyses performed on published data. The result raise questions about whether the hypothesized PPAR-*α* activation is either necessary or sufficient for rodent hepatocarcinogenesis. The authors question whether the proposed hepatocyte proliferation play a causal role in tumorigenesis or are merely correlated with cancer. The authors concluded the adequacy of the scientific basis for the conclusion that PPARα agonists pose no carcinogenic risk to humans requires re-examination. With regard to the hepatic, testicular, and pancreatic cancers associated with phthalates exposure, a recent National Research Council report (Committee on the Health Risks of Phthalates NEC, 2008) concluded that there is evidence that these cancer type may be mediated by mechanism independent of PPARα.

Research needs and recommendations

Possible cohort for future epidemiologic studies:

DEHP is commonly used in PVC and other plastics. Epidemiologic studies have been performed for a Swedish PVC-processing factory (Hagmar et al., 1990; Hardell et al., 2004), a mortality study of U.S. workers in a plastics manufacturing and research and development plant (Selenskas et al., 1995), and a population-based case-control study among Danish men (Heineman et al., 1992) exposed to PVC and phthalates.

Now that specific biomarkers of exposures for DEHP exist, these work populations identified above may serve as potential future DEHP biomarker epidemiological studies in addition to cohorts defined by Gaudin et al. (2008) and Hines et al. (2009). Also, in future biomarker epidemiological studies it would be necessary to identify what phthalates are used in the workplace and only count these metabolites as occupational exposure. DEHP metabolites should be determined in pre- and post-shift urine samples because an increase over the work-day would indicate occupational exposures, and a decrease a non-occupational exposure. In occupational and environmental settings, DEHP is often accompanied by other phthalates (i.e., to get the optimal flexibility in plastics a combination of phthalates are often used). Given that other phthalates may have similar carcinogenic properties; a cumulative phthalate exposure index should be used in future epidemiological studies.

The outcome variable could be cancer incidence, mortality, but preference would be to use an effect biomarker such as 8-OHdG with the exposure biomarkers may be useful (see section on oxidative stress).

In terms of human data, another suggested research need would be to look at studies of testicular germ cell cancer. DEHP causes reproductive effects (testicular dysgenesis, Leydig-cell dysfunction, cryptorchidism, and hypospadias) in male rodents, which are similar to risk factors for testicular germ cell cancer in humans. Furthermore, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient, and other modes, mechanisms, toxicity pathways and molecular targets may contribute to or be required for the observed adverse effects. Similarly, the epidemiologic data are inadequate to inform conclusions of human relevance of peroxisome proliferators as a class (Guyton et al., 2009).

Future toxicological studies

Previously, there have not been no reports concerning the effects of exposure of DEHP to transgenic mice with hPPARα^{TetOff} or hPPARα^{PAC} , but Ito and Nakajima (2008) reported that at a relatively high dose of DEHP (5.0 mmol/kg for 2 weeks) PPARα was activated in the liver of both genotyped mice. Although the magnitude of response was not large in hPPARα^{TetOff} mice from the standpoint of the target gene expression in the liver, the induction was beyond doubt. Hurst and Waxman (2003) reported a 5-fold lower sensitivity to the DEHP metabolite MEHP of human compared with mouse PPAR (see discussions in Guyton et al., 2009 regarding receptor activation). The results from the typical peroxisome proliferator, Wy-14643, may not always be similar to those of DEHP: Future studies are needed using hPPARα^{TetOff}, which expresses the human receptor only in liver, or hPPARα^{PAC}, which expresses the human receptor not only in liver but also in kidney, heart, intestine and

brown adipose tissues, mouse models to elucidate the role of human PPARα in DEHP carcinogenesis. Further characterization of DEHP exposures in industry is needed in order to reduce exposure misclassification in epidemiological studies. Since no epidemiologic studies have been published since the last Monograph (IARC, 2000) it is encouraged to use already established cohorts in the PVC-processing factories.

References

Adibi JJ, Perera FP, Jedrychowski W, et al. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. *Environ Health Perspect* 2003; 111: 1719-1722.

Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP. Phthalate-induced leydig cell hyperplasia is associated with multiple endocrine disturbances. *Proc*. *Natl Acad Sci USA* 2004: 101; 776-780.

Albro PW, Chapin RE, Corbett JT, Schroeder J, Phelps JL. Mono-2-ethylhexyl phthalate, a metabolite of di-(2-ethylhexyl) phthalate, causally linked to testicular atrophy in rats. *Toxicol Appl Pharmacol* 1989; 100: 193-200.

Albro PW, Lavenhar SE. Metabolism of di(2-ethylhexyl)phthalate. *Drug Metab Rev* 1989, 21: 13-34.

Barr DB, Silva MJ, Kato K, et al. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. *Environ Health Perspect* 2003; 111: 1148-1151.

Becker K, Seiwert M, Angerer J, et al. DEHP metabolites in urine of children and DEHP in house dust. *Int J Hyg Environ Health* 2004; 207: 409-417.

Blount BC, Milgram KE, Silva MJ, et al. Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. *Anal Chem* 2000; 72: 4127-4134.

Caldwell JC, Keshava N, Evans MV. Difficulty of mode of action determination for trichloroethylene: An example of complex interactions of metabolites and other chemical exposures. *Environ Mol Mutagen* 2008; 49: 142-154.

CDC. Second National Report on Human Exposure to Environmental Chemicals. 2003.

CDC. Third National Report on Human Exposure to Environmental Chemicals. 2005. http://www.cdc.gov/exposurereport/pdf/thirdreport.pdf. (accessed 16 May 2008).

Cheung C, Akiyama TE, Ward JM, et al. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. *Cancer Res* 2004; 64: 3849-3854.

Committee on the Health Risks of Phthalates NRC. Phthalates and Cumulative Risk Assessment; 2008.

David RM, Moore MR, Finney DC, Guest D. Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci* 2000; 55: 433-443.

Dirven HA, van den Broek PH, Jongeneelen FJ. Determination of four metabolites of the plasticizer di(2-ethylhexyl)phthalate in human urine samples. *Int Arch Occup Environ Health* 1993; 64: 555-560.

Eveillard A, Mselli-Lakhal L, Mogha A, et al. Di-(2-ethylhexyl)-phthalate (DEHP) activates the constitutive androstane receptor (CAR): a novel signalling pathway sensitive to phthalates. *Biochem Pharmacol* 2009; 77: 1735-1746.

Gaudin R, Marsan P, Robert A, et al. Biological monitoring of occupational exposure to di(2-ethylhexyl) phthalate: survey of workers exposed to plastisols. *Int Arch Occup Environ Health* 2008; 81: 959-966.

Guyton KZ, Chiu WA, Bateson TF et al. A reexamination of the PPAR-α activation mode of action as a basis for assessing human cancer risks of environmental contaminants. *Environ Health Perspect* 2009; 117: 1668-1672.

Hagmar L, Akesson B, Nielsen J, et al. Mortality and cancer morbidity in workers exposed to low levels of vinyl chloride monomer at a polyvinyl chloride processing plant. *Am J Ind Med* 1990; 17: 553-565.

Hardell L, Malmqvist N, Ohlson CG, Westberg H, Eriksson M. Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. *Int J Cancer* 2004; 109: 425-429.

Heineman EF, Olsen JH, Pottern LM, Gomez M, Raffn E, Blair A. Occupational risk factors for multiple myeloma among Danish men. *Cancer Causes Control* 1992; 3: 555-568.

Hines CJ, Nilsen Hopf NB, Deddens JA, et al. Urinary phthalate metabolite concentrations among workers in selected industries: a pilot biomonitoring study. *Ann Occup Hyg* 2009; 53: 1-17.

Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicol Sci* 2003; 74: 297-308.

IARC Monographs. Di(2-ethylhexyl) phthalate. *IARC Monogr Eval Carcinog Risks Hum* 2000; 77: 41-148.

Ito Y, Nakajima T. PPARalpha- and DEHP-Induced Cancers. *PPAR Res* 2008; 2008: 759716.

Ito Y, Yamanoshita O, Asaeda N, et al. Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *J Occup Health* 2007; 49: 172-182.

Ito Y, Yamanoshita O, Kurata Y, Kamijima M, Aoyama T, Nakajima T. Induction of peroxisome proliferator-activated receptor alpha (PPARalpha)-related enzymes by di(2-ethylhexyl) phthalate (DEHP) treatment in mice and rats, but not marmosets. *Arch Toxicol* 2007; 81: 219-226.

Ito Y, Yokota H, Wang R, et al. Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets. *Arch Toxicol* 2005; 79: 147-154.

Jonsson BA, Richthoff J, Rylander L, Giwercman A, Hagmar L. Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* 2005; 16: 487-493.

Kato K, Silva MJ, Brock JW, et al. Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *J Anal Toxicol* 2003; 27: 284-289.

Kato K, Silva MJ, Reidy JA, et al. Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. *Environ Health Perspect* 2004; 112: 327-330.

Klaunig JE, Babich MA, Baetcke KP, et al. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol* 2003; 33: 655-780.

Koch HM, Bolt HM, Angerer J. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch Toxicol* 2004; 78: 123-130.

Koch HM, Bolt HM, Preuss R, Eckstein R, Weisbach V, Angerer J. Intravenous exposure to di(2-ethylhexyl)phthalate (DEHP): metabolites of DEHP in urine after a voluntary platelet donation. *Arch Toxicol* 2005; 79: 689-693.

Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure - an update and latest results. *Int J Andrology* 2006: 29; 155-165
.
Koch HM, Gonzalez-Reche LM, Angerer J. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; 784: 169-182.

Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure-- an update and latest results. *Int J Androl* 2006; 29: 155-165.

Koch HM, Rossbach B, Drexler H, Angerer J. Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. *Environ Res* 2003; 93: 177-185.

Maglich JM, Parks DJ, Moore PL, et al. Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. *J Biol Chem* 2003; 278; 17277-17278.

Maloney EK, Waxman DJ. trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol* 1999: 161; 209-218.

Melnick RL. Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? *Environ Health Perspect* 2001; 109: 437-442.

Morimura K, Cheung C, Ward JM, Reddy JK, Gonzalez FJ. Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis* 2006; 27: 1074-1080.

Nuti F, Hildenbrand S, Chelli M, Wodarz R, Papini AM. Synthesis of DEHP metabolites as biomarkers for GC-MS evaluation of phthalates as endocrine disrupters. *Bioorg Med Chem* 2005; 13: 3461-3465.

Preuss R, Koch HM, Angerer J. Biological monitoring of the five major metabolites of di-(2-ethylhexyl)phthalate (DEHP) in human urine using column-switching liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 816: 269-280.

Rusyn I, Peters JM, Cunningham ML. Modes of action and species-specific effects of di-(2-ethylhexyl)phthalate in the liver. *Crit Rev Toxicol* 2006; 36: 459-479.

Selenskas S, Teta MJ, Vitale JN. Pancreatic cancer among workers processing synthetic resins. *Am J Ind Med* 1995; 28: 385-398.

Seo KW, Kim KB, Kim YJ, Choi JY, Lee KT, Choi KS. Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats. *Food Chem Toxicol* 2004; 42: 107-114.

Silva MJ, Malek NA, Hodge CC, et al. Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; 789: 393-404.

Silva MJ, Preau JL, Jr., Needham LL, Calafat AM. Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; 873: 180-186.

Takashima K, Ito Y, Gonzalez FJ, Nakajima T. Different mechanisms of DEHP-induced hepatocellular adenoma tumorigenesis in wild-type and Ppar alpha-null mice. *J Occup Health* 2008; 50: 169-180.

Thiess AM, Fleig I. [Chromosome studies in workers exposed to di-2-ethylhexyl phthalate]. *Zentralbl Arbeitsmed Arbeitsschutz Prophyl* 1978; 28: 351-355.

Voss C, Zerban H, Bannasch P, Berger MR. Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology* 2005; 206: 359-371.

Yang Q, Ito S, Gonzalez FJ. Hepatocyte-restricted constitutive activation of PPAR alpha induces hepatoproliferation but not heatocarcinogenesis. *Carcinogenesis* 2007; 28: 1171-1177.

Yang Q, Nagano T, Shah Y, Cheung C, Ito S, Gonzalez FJ. The PPAR alpha-humanized mouse: a model to investigate species differences in liver toxicity mediated by PPAR alpha. *Toxicol Sci* 2008; 101: 132-139.

Atrazine

by David M. DeMarini PhD and Shelia Hoar Zahm PhD

Citation for most recent IARC review

IARC Monographs 73, 1999

Current evaluation

Conclusions from the previous Monograph:
Atrazine is not classifiable as to its carcinogenicity to humans (Group 3). There is *inadequate evidence* in humans for the carcinogenicity of atrazine. There is *sufficient evidence* in experimental animals for the carcinogenicity of atrazine. The Working Group concluded that the animal mammary tumors associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism that is not relevant to humans.

Exposure and biomonitoring

Atrazine (6-Chloro-N-ethyl-N'-(1-methylethyhl)-1,3,5-triazine-2,4-diamine) is a triazine herbicide used widely on a variety of crops, especially maize, sorghum, and sugar-cane, for the pre- and post-emergent control of broad-leaved weeds. Occupational exposure may occur through both inhalation and dermal adsorption during the manufacture of atrazine, its formulation, and its application. It is found widely, together with its dealkylated degradation products, in rivers, lakes, estuaries, groundwater, and reservoirs. In drinking-water, the levels rarely exceed 1 μg/L. Surveys of various foods and feeds have generally found no detectable atrazine residue.

A Collaboration Project between International Agency for Research on Cancer (IARC) and National Occupational Research Agenda (NORA)

IARC Technical Publication No. 42

Identification of research needs to resolve the carcinogenicity of high-priority IARC carcinogens

Views and Expert opinions of an IARC/NORA expert group meeting

Lyon, France: 30 June – 2 July 2009

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Contents