

TEDX

The Endocrine Disruption Exchange
P.O. Box 1407, Paonia, CO 81428
970-527-4082
tedx@tds.net
www.endocrinedisruption.org

SUMMARY AND COMMENTS ON THE LOW DOSE BPA SPREADSHEET

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INTRODUCTION

A 1997 study reported significant changes in the male reproductive system of offspring exposed *in utero* to low levels of bisphenol A (BPA). Prior to this research, no BPA studies had been conducted at levels below EPA's safety standard of 0.05 mg/kg/day.¹ The changes reported in the 1997 study were found at much lower doses.

The above research posed two challenges to the assumptions of BPA's safety: first, significant changes in the male reproductive system (prostate weight, daily sperm production and epididymis weight) resulted from exposure to BPA at doses well below levels deemed safe for human exposure. Second, the findings suggested BPA's activity may be much more potent and diverse than previously assumed. These assumptions initiated a series of low dose BPA studies that continue today.

We used a spreadsheet to organize the extensive number of studies that followed. We selected all papers in the published, peer-reviewed literature that include doses at or below 1 mg/kg/day (1 ppm), including *in vivo* and *in vitro* studies.² This dose range is more stringent than that suggested by the National Toxicology Program's (NTP) Low Dose Peer Review Panel (2001)³. The NTP Panel suggested using doses of 5 mg/kg/day or less as the low dose range, less than a tenth of the lowest observed adverse effect level (LOAEL). We chose to be more conservative.

The spreadsheet is designed to allow the user to break out the data in any grouping desired. The results (endpoints) are categorized as either expressing or not expressing change. Endpoints cover a wide range of parameters from the gene, to the molecular, cellular, tissue, organ, and system level. In order to deal with the vast number of endpoints, we chose to sort them by broad categories such as male reproductive system, female reproductive system, brain and behavior, organ systems, etc.

¹ <http://www.epa.gov/IRIS/subst/0356.htm>

² Many studies utilized doses above and below 1 ppm. When this occurred, only the effects from exposure at or below 1 ppm were recorded in the database.

³ National Institute of Environmental Health Sciences, NIH. 2001. National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review. P. 1-3.

Each broad category is broken into specific endpoints which then can be broken out further. For example, the broad category, male reproductive system, includes the endpoint, sperm, which is comprised of a diversity of measurements including sperm count, sperm production, sperm motility, and damage to sperm. When an organ is the listed endpoint, multiple changes in the organ, including structural, functional and genetic changes are included.

SUMMARY

Eighteen low dose BPA papers were published between 1997 and 2000. In 2000, 23 studies were published and since then the number of publications has continued to increase. As of April 2008, there are 335 studies that examined BPA effects at less than 1ppm entered in this spreadsheet. Early on, in the late 1990s, the vast majority of the studies examined the effects on the male reproductive system. As interest in BPA increased, the number of endpoints and systems under examination expanded as well. Currently there are 49 different endpoints in this spreadsheet which include the functioning of organisms from the cell to the system level.

Of the total studies (335) included in the spreadsheet, 81% reported one or more significant effects at 1 ppm or less. Of these studies, 33% were *in vitro* studies, 61% were *in vivo* studies, and 6% used both *in vivo* and *in vitro* methods. Seventy-three percent (244 studies) of all studies used mammals or mammalian tissue. The remaining studies utilized fish, amphibians, birds, invertebrates, and other wildlife species.

Twelve studies looked at 10 or more endpoints. Four of these studies found no effects. The studies often examined only traditional toxicological endpoints such as organ and body weight, litter size, sex ratio, etc. This contrasts with the majority of the other studies that examined more inconspicuous effects such as histological, biochemical, behavioral, and/or physiological alterations in endocrine function.

The following tables provide summaries of the data as of April 2008. (We continuously update the database as more studies are published, and summary tables are adjusted accordingly). These tables are a compilation of all the studies in the spreadsheet. Half of the studies included more than one endpoint and 13% contained 5 or more. Twelve studies (3%) had between 10 and 19 endpoints. As a result, the number of studies in each of the columns of the summary tables, when combined, will exceed the total number of studies in the spreadsheet. The total number of studies reporting a specific endpoint is listed, as well as the number and percentage of studies that did and did not find statistically significant changes. The purpose of this summary is to provide a general overview of the data. A list of the specific results is available upon request.

The majority of the endpoints displayed below contain studies that found both change and no change. Most of the endpoints (67%) have more than 50% of their studies showing change and eight endpoints have more than 90% reporting changes. Some of these endpoints are in certain areas of the brain and the pancreas as well as the blood, behavior, and the breast. The least sensitive endpoints to low dose exposure appear to be the preputial gland and LH with 86% of the results showing no change. Every endpoint in the spreadsheet shows at least one positive result.

MALE REPRODUCTIVE SYSTEM ENDPOINTS

	testes		sperm		prostate		preputial gland		epididymis		penis		seminal vesicles		testosterone		Leydig		feminization	
No change	24	44%	11	33%	13	46%	6	86%	14	70%	5	56%	16	84%	13	68%	1	20%	2	22%
Change	30	56%	22	67%	15	54%	1	14%	6	30%	4	44%	3	16%	6	32%	4	80%	7	78%
Total	54		33		28		7		20		9		19		19		5		9	

FEMALE REPRODUCTIVE SYSTEM ENDPOINTS

	ovaries		vagina		uterus		breast		estrus		♀ organs		eggs/oocytes	
No change	3	13%	5	38%	11	33%	2	6%	2	25%	2	33%	6	24%
Change	20	87%	8	62%	22	67%	29	94%	6	75%	4	67%	19	76%
Total	23		13		33		31		8		6		25	

MISCELLANEOUS ENDPOINTS

	body weight		genital distance		maturation		growth		mortality		LH		enzymes		proteins & other		DNA/gene expression		binding affinity	
No change	41	65%	5	50%	9	43%	5	56%	12	63%	6	86%	1	11%	3	21%	4	13%	5	25%
Change	22	35%	5	50%	12	57%	4	44%	7	37%	1	14%	8	89%	11	79%	26	87%	15	75%
Total	63		10		21		9		19		7		9		14		30		20	

EARLY OFFSPRING ENDPOINTS

	embryos		placenta		preg rate		birth rate		sex ratio	
No change	4	24%	0	0%	9	75%	28	85%	20	83%
Change	13	76%	1	100%	3	25%	5	15%	4	17%
Total	17		1		12		33		24	

BRAIN & BEHAVIOR ENDPOINTS

	brain		behavior		dendrites		hippocampus		amygdala		hypothalamus		pituitary		dopamine	
No change	9	24%	1	3%	0	0%	2	25%	0	0%	2	33%	5	56%	0	0%
Change	28	76%	31	97%	3	100%	6	75%	1	100%	4	67%	4	44%	4	100%
Total	37		32		3		8		1		6		9		4	

ORGAN & SYSTEM ENDPOINTS

	adrenal		liver		thyroid		pancreas		kidney		spleen		bones		immune system		blood	
No change	2	67%	13	37%	4	44%	0	0%	9	75%	3	38%	2	25%	2	15%	0	0%
Change	1	33%	22	63%	5	56%	5	100%	3	25%	5	63%	6	75%	11	85%	17	100%
Total	3		35		9		5		12		8		8		13		17	

The following provides a brief overview of the data when sorted by timing of exposure, animal model, *in vivo* and *in vitro*. Summary tables are available upon request.

In vivo mammalian effects (156 studies)

Gestational and/or neonatal exposure

Forty-two percent of the mammalian studies (66 studies) examined effects from maternal exposure at any time during gestation and/or early neonatal life (to postnatal day 5). When animals are exposed at this stage of development, the effects of BPA are far ranging. Multiple changes in genes expression (mRNA, methylation, etc.) are found in the sex organs, internal organs and the brain, resulting in structural, organizational and functional changes. Adverse changes are also found in immune system functioning. The four areas that have been most frequently studied are: male and female reproductive organs, the breast, and the brain.

In the male, BPA harms the testes, penis and prostate. The changes found in the prostate are known cancer precursors. Female exposure causes changes in the structure and function of the female reproductive organs, and adversely affects the development of eggs and embryos. Multiple changes are found in developing breast tissue, many of which have been linked to the formation of breast cancer in later life. BPA also alters the organization of brain tissue and the behavior of animals exposed. Some of the changes seen are hyperactivity, changes in responses to morphine and amphetamines, and the elimination of sex specific behaviors.

The only areas of study where BPA did not show any significant results were in the kidney and sex ratio.

Gestation to after postnatal day 5 exposure

Twenty percent of the mammalian studies (31 studies) dosed maternal animals from the beginning of gestation to lactation, and, in some studies, through weaning. Changes found in the reproductive systems of males and females are much less pronounced than in the previous exposure group with no changes found in many endpoints. The areas most frequently studied were the brain and behavior with multiple changes found in both.

All structures of the brain studied expressed morphological or functional changes. Areas of the brain which typically develop differently in males and females failed to do so, and sex specific behaviors were likewise eliminated. Responses to amphetamines and morphine were altered in both sexes, and memory and learning in males.

Late postnatal exposure (after postnatal day 5)

In eight percent of the mammalian studies, animals were exposed after postnatal day 5, but before adulthood (13 studies). Brain and behavioral changes were reported and these effects included changes in neurons and neuronal signaling, changes in sex-typed learning and reductions in male sexual response. In the remaining endpoints, positive results were sporadic.

Adult exposure

Twenty-eight percent of the mammalian studies (43 studies) investigated exposure during adulthood. Many of the studies examined the male and female reproductive systems. In the male, changes were found in all areas of sperm functioning and physiology. Changes in the

females were broader, with alterations in uterine structure and function from the gene to cell to the whole organ, changes in breast tissue, and in egg development. Exposure to BPA was correlated with polycystic ovarian syndrome, an increase in miscarriages and a decrease in successful births. In both sexes, the brain showed changes in cell structure and the activity of neurotransmitters. Compromises to the immune system were documented and changes in blood chemistry.

Multigenerational studies

Three mammalian studies (2%) examined effects of exposure on multiple generations. Most of the effects were reported in the male reproductive system and included changes in the testes and sperm function. Weights of ovaries, liver, uterus and testes showed some changes. With the exception of sperm, no measures of genetic changes or alterations in morphology or function were studied.

In vivo non-mammalian effects (78 studies)

In non-mammals (fish, amphibians, birds, etc.), exposure of the eggs/embryos (13 studies) resulted in morphological changes and abnormal development and maturation. An increase in the incidence of testes/ova was reported in males. Multiple changes were noted in the increase and decrease of enzymes and hormones, and in gene expression.

Exposure of immature animals (13 studies) revealed feminization of the males which included a reduction in the number males, reduced testosterone, changes in gonad development and increased numbers of testes/ova. Changes in sperm number were documented, and a decrease in reproductive success. Again, maturation problems were noted and changes in gene activity and behavior.

In adult exposure experiments (46 studies), feminization of the males was again found, and superfeminization of the females. The latter included a large increase in the number of eggs produced and the spawning mass, but a reduction in the successful growth of eggs and embryos. Both males and females displayed reproductive organ damage and malformation, a decline in the number and quality of embryos, and reduced sperm quality in all measured parameters. Multiple changes were found in gene expression and enzyme levels.

When the organism was exposed through multiple life stages (6 studies), male reproductive morphology and sperm quality were adversely affected. The females also displayed reproductive organ changes and an increase in egg production, but the weight and growth of the larvae were reduced.

In vitro studies (127 studies)

This group of studies included those that utilized only *in vitro* methods (109 studies), as well as those that demonstrated both *in vitro* and *in vivo* effects (18 studies). Overall, BPA exposure altered the binding and transcriptional activities of ER α and ER β , the functioning of enzymes and proteins, and gene expression in many types of cells, organs and systems. Specifically in male reproductive organs tissues, exposure resulted in cell proliferation and deformities, altered progesterone synthesis in the prostate, upregulated androgen receptors, and changed gene

expression in Leydig cells. In females, altered hormone production and hormone receptor activity was found. Cell viability decreased and cancer cell activity increased. BPA also altered endometrial cell proliferation. In breast tissue, increased cell proliferation and decreased apoptosis were found along with multiple changes in genes and gene transcription.

BPA increased conversion of fibroblasts to adipocytes and enhanced glucose uptake in adipocytes. Decreased prolactin production and growth hormone content was found in the pituitary, and in thyroid cells, gene expression and cell activity was altered. BPA also blocked T3 binding to thyroid hormone receptors. In pancreatic tissues, BPA increased insulin secretion and altered glucose metabolism. In fish, altered vitellogenin production and synthesis was found in the liver. Immune functioning was compromised with multiple changes noted in phagocyte, macrophage, lymphocyte, immunoglobulin and splenocyte proliferation and functioning. Changes in calcium and potassium content as well as osteoblast-like cell proliferation were found in bone tissue. In the brain, BPA altered intercellular Ca^{++} levels and inhibited gap junction-mediated intercellular communication and cellular signaling. It also changed dendritic growth and activation and increased neuronal damage.

COMMENTS

1. In this spreadsheet, exposure to BPA at 1 ppm or less resulted in changes across multiple endpoints and systems, *in vivo* and *in vitro*.
2. It has become apparent that traditional toxicological endpoints, such as litter size, sex ratio, and organ weight do not reveal the full effects of BPA. Some of the most profound effects were seen in morphological, histological, and/or functional changes in organs and the organism as a whole. Minute changes in the developing organism have consequences which are not often seen at birth, and may only become apparent as the organism matures and attempts to reproduce. These changes are often not addressed in the traditional toxicological paradigm, and as such, the subtle effects of BPA could have been overlooked or underestimated in some experiments in this spreadsheet.
3. Realizing that BPA was recognized as an estrogen in the 1930s, it is not surprising that female reproductive system endpoints were among some of the most affected. For example, 87% of studies looking at the ovaries and 94% of the studies examining the breast show change. Researchers have consistently found that BPA changes the pattern of growth and development in breast tissues - changes which have been implicated in the development of breast cancer.
4. Not as many changes have been reported in the male reproductive system. Perhaps this is because most of the male studies limited the end points to organ weight or size. For example, of the 13 studies of the prostate gland that did not find change, all of them looked at prostate weight as the endpoint. One study looked at the incidence of prostate cancer and found none. Of the 15 studies that found changes, five looked at prostate weight or size. The other studies that found changes examined morphological or histopathological changes, such as prostatic intraepithelial neoplasia (PIN) lesions, cellular growth, proliferation or androgen receptor changes.
5. The brain and changes in behavior are sensitive endpoints for BPA exposure. Seven of the eight endpoints in this area demonstrate change in more than 65% of their studies. Ninety-seven percent of the 32 behavioral studies reported change. One of the most consistent findings was the feminization of masculine behavior and the masculinization of feminine

behavior. Other consistent effects were changes in neuronal and brain structure, numbers of neurons, neuronal activity, hormone receptor activity, or neurotransmitters.

6. In recent years the spleen and immune system have become the target of BPA research. The vast majority (76%) of the 21 studies done in these systems found changes in many types of cells. These changes were found in phagocytes, macrophages, lymphocytes, splenocytes, and immunoglobulin. The results demonstrate the proliferative and/or suppressive effects of BPA and consistently show interference in the normal functioning of the immune system.
7. Body weight is the most cited endpoint in the spreadsheet. Two-thirds of the studies that examined body weight found changes, either increases or decreases; 22% of rat studies found a change, while 39% of the mouse studies showed changes. Timing of exposure was important. Across species, in 17 of the 20 studies where animals were exposed postnatally, no change in body weight was reported, while prenatal exposure caused changes about 57% of the time.
8. Recent studies have opened the door to the concept that the origin of certain disorders, such as prostate and breast cancer, and behavioral and brain disorders, may be the result of prenatal exposure to BPA.
9. While there is a pattern of positive results in mammals and mammalian tissues, some of the most striking and consistent results were found in the non-mammalian species. Experiments performed on immature and adult non-mammals consistently found changes due to BPA exposure.
10. Some of the results were discovered at doses 1,000 to 10,000 times lower than what is currently considered safe. Effects have been found at doses in parts per trillion that are within ambient exposure levels. Dose response curves are often non-linear, in the shape of a *U*, or an inverted *U*. These curves do not conform to the traditional expectations of toxicology which states that an increase in dose is matched by an increase in effect. Because the endocrine system acts like a thermostat, through self-regulating feedback loops, BPA can harm systems at very low doses while at higher doses it will shut the system down before harm can occur. Very high doses, however, can overwhelm the system and cause damage and even death. It is the body's variable responses to BPA at very low doses, operating well under traditional toxicology's no observed effect level, that results in harm.
11. The spreadsheet demonstrates that inconspicuous effects are expressed at every stage of life and in every organ and system examined. These effects range from changes in gene expression, to the operation of hormones and hormone receptors, lymphocytes, enzymes and proteins which are then expressed as changes in the functional activity of organs, systems and the animal as a whole. While the effects may vary from organ to organ, or system or system, at these ambient exposure levels there is no safe dose of BPA.

More detailed reviews of the data are available from TEDX upon request. This summary sheet should not be used for citation purposes, or copied without prior permission.