Chapter 4

Behavioral and Somatic Disorders in Children Exposed in Utero to Synthetic Hormones: A Testimony-Case Study in a French Family Troop

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Additional information is available at the end of the chapter

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1. Introduction

Development and maturation of the human brain especially genesis of neurones and synaptogenesis are under the genetic control of neurohormones secreted by hypothalamus during fetal and early postnatal life. So the lesser modification in the hormonal status might influence either the neurodevelopment course or the sexual differentiation. This study shows that there are serious effects on the psychological and physical health of the descendants of women treated with synthetic hormones during their pregnancy. Preliminary results by a group in Paris have recently been published [1], based on observations and diagnoses of psychiatric disorders in a small sample of children from the HHORAGES families. As synthesized by Dodds in 1938, but not patented, diethylstilbestrol (DES), a synthetic non-steroid estrogen, given among others to pregnant women, was considered at the time as "an indisputable progress in the therapeutics of ovarian deficiency", and was described in such terms as early as May 1939 in an advertisement shown in "*Le Progrès Médical*", a French journal. Despite various alerts which were published as early as 1940, and after research work was conducted on animals proving carcinogenic effects, and despite Dieckmann et al. [2], which demonstrated the ineffectiveness of DES to prevent miscarriages or premature births in a large cohort of DES-treated women versus placebo-treated women, the product has been much used worldwide, and has caused a long list of damaging effects in the past, the present and very likely in the future [3-5]. The discovery of vaginal clear cell adenocarcinomas in "DES daughters" [6] led to DES being banned for pregnant women in 1971 in the US, but it was only in 1977 that it was banned in France, where this particular use of DES was removed from the Vidal pharmaceutical handbook. However, DES was still sporadically prescribed until 1981. Meanwhile, another steroid estrogen, also synthesized in 1938 by H. Herloff Inhoffen and W.

Hohlweg, 17-alpha-ethinylestradiol, was often added to DES in cocktails, or at a later stage was used as a replacement, sometimes with synthetic delayed progestin. An example of prescription, time and doses is shown in **Table 1**.

History: Miscarriage, two years before, at 6 weeks amenorrhea.
1st pregnancy: 1966-67. At 7 weeks amenorrhea: Ethinylestradiol (EE) 100mg/d. From 10th to 20th week: Distilbene 25mg/d + EE 100mg/d. From 20th to 37th week: Distilbene 15mg/d + EE 250mg/d+ Delay Progestin 500mg/week. Full term delivery: girl, 3,870kg weight, Apgar score 10/10. No problem during pregnancy and delivery.
2nd pregnancy: 1970-71. At 6 weeks amenorrhea: EE 500mg/d. From 8th to 37th week: EE 500mg/j + Distilbène 25 mg+ Delay Progesterone 500mg/d. Full term delivery, boy, 3,900 kg weight, Apgar Score 10/10. No problem during pregnancy and delivery.
Twenty years after: 1stchild (girl): Recurrent depressions and eating disorders, 14 suicide attempts, then suicide in 1995 (28 years old). 2ndchild (boy): Borderline Schizophrenia. Suicide in 1998 (27 years old).

Table 1. An example of synthetic hormone prescription during two successive pregnancies (dose and exposure periods), confirmed by medical file and its "20 years after" consequences (MOSG personal data).

As visible in Table 1 the doses/kg/day prescribed to this pregnant woman for her first pregnancy were: ethinyl-estradiol (EE): 19µg/kg^{-1}/d; DES: 28.8µg/kg^{-1}/d; Progestin delay: 1,37 mg/kg/d. For the second pregnancy, prescribed doses were slightly higher except for synthetic progestin delay prescribed at identical dose. Similar doses were administered to other pregnant French women and in the whole world, not only to women who had miscarriages (as the prevalent idea at the time was that they suffered from a hormonal deficiency which caused the miscarriage, whilst it is well known nowadays that the deficiency is caused by the miscarriage itself), but also to other women as a pain-relieving medicine, or even as a morning-after pill or for comfort.

Although DES and 17-alpha-ethinylestradiol belong to different estrogenic categories (non-steroid and steroid) and degrade in different ways, they do bind to the same ER-beta-estrogen receptors. The (natural) 17-beta-estradiol belongs to the family of steroid estrogens, which are lipophilic compounds. So it should bind to lipids, but thanks to the metabolization enzymes Cytochromes P-450 [7] it will be disposed of in the form of hydrosoluble products such as estriol, which can be found in the urine in the sulphate form. The (synthetic) 17-alpha-ethinylestradiol undergoes different metabolization pathways relative to its acetylenic function. These pathways deactivate the metabolization enzymes Cytochromes P-450, unlike the hydrosoluble natural estradiol, estrogen, which degrades in estriol. Hence the 17-ethinylestradiol remains bound to the lipids. Diethylstilbestrol, a non-steroid oestrogen, is a very lipophilic synthetic diphenol. Its metabolization is also different from natural estradiol; it is a molecular degradation by means of a very harmful oxidation reaction, as it will release toxic "quinone"-type structures, which are highly reactive to proteins and to DNA in particular **(Table 2)**.

Up to now, many research studies have been carried out on animals (mice and rats), showing the toxicity of such synthetic oestrogens upon the descendants, and inducing in particular

behavioral disorders – along with other effects such as cancers [8,5] with multigenerational carcinogenesis effects on mice (9). Prenatal exposure to three different synthetic chemicals, DES and two pesticides (DDT and its chemical analogue, methoxychlor), was studied [10]: it affects the behavior of young mice, showing increased aggressiveness in males (increase of the number of attacks and decrease in reaction time before the attack). However, the DES doses were 1000 times smaller (0.02 and 0.2 μg/kg) than the DDT ones (and analogue) (20 and 200μg/kg), while the subsequent aggressive reactions were far more severe, showing the tremendous impact of DES, even at low doses. The treatment period of the mothers from the 11th to the 17th day of the gestation also played a key role, as it represents a crucial period in the differentiation of the reproductive system and in the development of the brain in the rodents studied [11]. Newbold [4] unambiguously demonstrated the validity of the "rodent model" transposed to humans. Injection of 17-alpha-ethinylestradiol (EE) in pregnant rats not only induces many abortions, but also anxiety and depression-type disorders in pups (strain Dark Agouti) [12, 13] (15μg/kg^{-1}/d). In terms of brain cytology, an alteration of the frontal part of the hippocampus in young rats (Long Evans strain) which were EE-treated *in utero* was also demonstrated [14] at the same doses which were calculated to be relatively comparable to the doses prescribed to pregnant women (see Table 1).

The (natural) 17-beta-estradiol belongs to the family of steroid estrogens, which are lipophilic compounds. So it should bind to lipids, but thanks to the metabolization enzymes of the Cytochromes P-450 type, it will be disposed of in the form of hydrosoluble products such as Estriol, which will be found in the urine in the sulphate form.
The (synthetic) 17-alpha-ethinylestradiol undergoes different metabolization pathways relative to its acetylenic function. These pathways induce the inactivation of the Cytochromes P-450. Hence the 17-Ethinyl-Estradiol will remain bound to the lipids.
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Table 2. Difference between natural sexual hormone, the 17-beta-estradiol type, and the synthetic hormones of the 17-alpha-ethinylestradiol or diethylstilbestrol type.

In humans, research work on the effects of synthetic hormones on women is scarce, but as early as 1977, June Reinisch [15] published a paper in Nature showing that the personality of the children whose mothers had been treated with synthetic oestrogens and progestin could be affected. Behavioral disorders starting in post-adolescence have since been reported in the children exposed *in utero* to the two mentioned estrogens: depression [16, 17], anxiety [17- 19], schizophrenia [20, 21], anorexia and bulimia [22]. These observations were summarized by Pillard et al [17] and Verdoux [21]. As early as 1987, was described [20] the case of 4 male adults prenatally exposed to DES and showing psychotic disorders. It is only during late adolescence that psychotic disorders develop, which often requires a neuroleptic treatment, even in the absence of family history of same. They hypothesized then that there could be a causal relationships between disruptions in the neuro-development linked to DES, and the subsequent appearance of psychotic disorders. Pillard et al. [17] showed that the

frequency of major recurrent depressive episodes was significantly higher in DES sons than in their unexposed brothers, which was recently confirmed in 2010 [23] in DES daughters from a cohort of 74,628 women (known as "Nurses Health Cohort"), 1,612 of whom were exposed to DES. In critical literature reviews Kébir and Krebs [24, 25] analyse in particular the results of the only three epidemiological large cohort studies, as well as smaller cohorts in relation to the effects of DES on the onset of psychiatric disorders. Out of the three epidemiological studies on large cohorts, two favour the hypothesis of an existing causal link [18, 23], and one reaches a different conclusion (study based on 1,352 mothers) [26].

The mothers' exposure to synthetic hormones, and in particular to DES and 17-alpha-ethinylestradiol, during pregnancy, and the study of its impact on exposed children, represents an almost experimental model to evaluate the toxicity of these products: the French HHORAGES troop, the results of which are presented in this work, is a "real-world" experimental group, in real-life conditions.

2. Materials and methods: Gathering questionnaires and the evidence

This study is not epidemiological. Its purpose is to put together a database that will enable HHORAGES-France to advance the evidence of serious effects on the psychological and physical health of the descendants of women treated with synthetic hormones during their pregnancy. This study has been carried out based on spontaneous testimonies from families affected. Families were alerted to this initiative either by TV, by radio, or by direct confidential communication. After an initial contact with the association (by phone, post, or via e-mail), a detailed questionnaire **(Table 3)**, prepared by researchers and doctors, was sent without consideration of race only to the families affected by psychiatric disorders in one or more of their children with or without somatic problems. More often mothers answered but also daughters or sons if the mother was deceased or too old or ill or in denial of the effects. Concerning the age of the children, psychiatric diseases appearing generally at the post adolescence after 18 years or later, testimonies concern people born between 1946 to 2000. In order to avoid some deviations in the sample, other factors as contamination with pesticides or other chemicals were questioned in the paragraph "professional exposure".

An authorization request about such questionnaires and files was sent to the CNIL (French "*Commission Nationale de l'Informatique et des Libertés*") and obtained. Subsequently the data were synthesized in the form of synoptic data for further studies. In 2006 a first detailed analysis of 2002-2004 and 2005 data was carried out in the *Laboratoire d'Endocrinologie-Pédiatrique* of the Montpellier University Hospital (CHU Lapeyronie), and was conducted according to specific descriptive criteria: exposed and unexposed children, daughters and sons, ranking in the sibling order, treatment by synthetics (oestrogens, oestrogen-progestin, or progestin), and pathologies, malformations and other disorders. In May 2009, all files with prescriptions attached were validated in psychiatric terms by representatives of the CERC (*Centre d'Etude et de Recherche Clinique*, Hôpital Sainte-Anne, Paris, run by Professor M.O. Krebs), a research laboratory with which HHORAGES established a partnership from 2007 via a PICRI project (*Partenariat Institution Citoyen pour la Recherche et l'Innovation*) subsidized by the Île-de-France region, and for the benefit of said Laboratory.

Mother's Situation: Surname, First Name, Date of Birth, Address, Home phone number, Professional phone number, Mobile number, E-mail address
Family Situation, Number of children, children's first names and dates of birth. Professional Situation.
Pill taken: before first pregnancy, and between pregnancies
Hormonal treatment before pregnancy (or pregnancies). (If a treatment was prescribed, please indicate the nature of the treatment and the time elapsed between the end of treatment and the beginning of the pregnancy)
Miscarriage(s): Indicate the time when it occurred, in relation to the other full term pregnancies, if any (*before the first one, or between two full term pregnancies?*)
Professional Exposure to hormones, to chemicals (pesticides, etc...)
Psychiatric or psychopathological family history (father, mother, …)
Health problems after first child birth
Pregnancy and childbirth (For more than 2 children, T.O.P.)
1st child 2nd child
Treatment during pregnancy: *(Yes/No)* - Nature of medicines - Doses – What time during pregnancy (first and last day of treatment, expressed in weeks from the beginning of pregnancy)?
What medical reason was put forward (possible miscarriage, comfort, etc...)?
At what month did the delivery occur, from the start of the pregnancy?
General condition of the child, Sex, Weight, Health problems at birth *(mother and child)*
Existing documents (prescriptions, medical files, etc...) and testimonies
Would you be so kind as to send us the copies of the files and documents? If yes, in order to save time, please attach the copies of all relative documents in your possession.
Health problems of your children (*For more than 2 children, T.OP)*
1st child, 2nd child First name, Sex, Birth Date, Rank in the sibling order
Physical disorders: Which ones? What age? Sterility treatment? Surgery?
Psychological disorders: Nature of first symptoms? What age? Subsequent aggravation? And at what age?
Other data: Hospitalization, Violence, Suicide attempt(s), Medical Treatments, Diagnosis, AAH, Disability*?*
Relational difficulties: in married life, in professional life?
Children, grandchildren: How many? Full term? Health condition? Malformations or observed disorders?

Table 3. Questionnaire sent to families.

3. Results

3.1. Data analysis

Chronological evolution of number of cases issued from the HHORAGES-France association was studied from testimonies collected from 2002 to 2007 (**Figures 1-4**). From 2002 (foundation of the HHORAGES association) to 2004 our first crude analysis was based on the testimonies of 297 mothers with a total number of 511 children, the birth years ranging from 1946 to 1994 **(Figure 1)**. 161 unexposed children had no disorder ("control"), 35 children exposed *in utero* had no disorder, 297 children exposed *in utero* showed various psychiatric pathologies, while 18 unexposed children also showed psychiatric disorders. It must be noted that in most families without any psychiatric antecedent and having several children, only the exposed child showed psychiatric disorders.

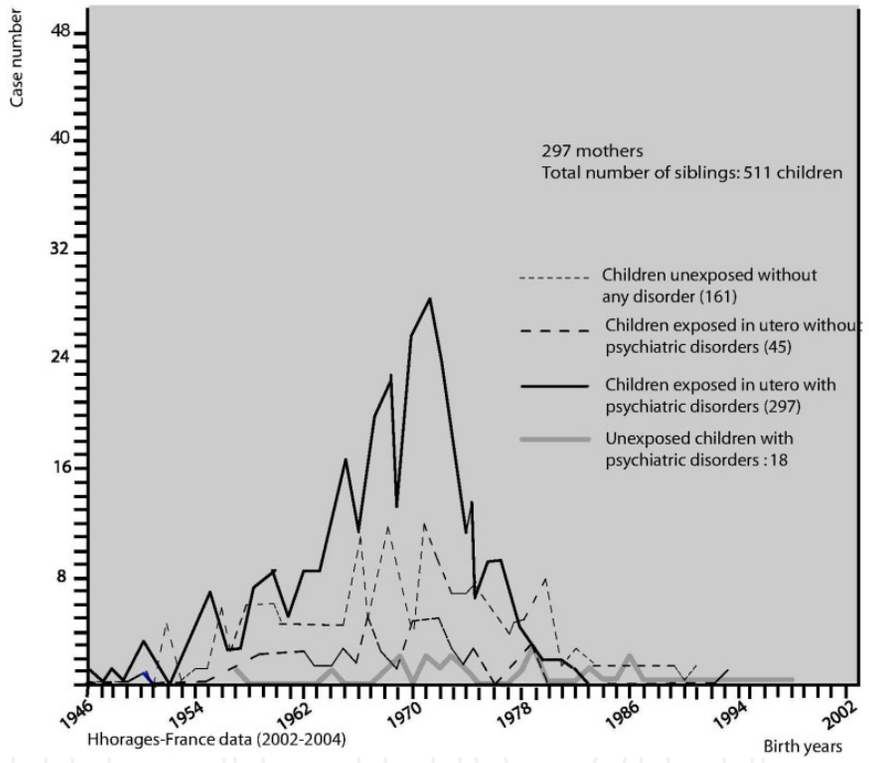


Figure 1. Representation of case numbers as a function of administered or not synthetic hormonal treatments during pregnancies (children born between 1946 and 1996), in a cohort of 511 children born from 297 mothers. The x-axis shows the birth years and the y-axis the number of cases. A prescription peak can be observed in the seventies. Black line: children exposed *in utero* with psychiatric disorders; dotted line: children unexposed without any disorders; dash line: children exposed *in utero* without psychiatric disorders; thick grey line: unexposed children with psychiatric disorders (HHORAGES-FRANCE data 2002-2004).

In 2005, a larger overall analysis based the testimony of 470 mothers **(Figure 2)** with a total number of 967 siblings: 381 of whom were not exposed, 345 exhibited no disorder, and 18 of whom showed psychiatric disorders. Interestingly, 9 of those unexposed with psychiatric disorders were born after their mother had been treated with synthetic hormones during a

previous pregnancy. 586 children were exposed, during their mother's pregnancy, to DES only or to DES in association with synthetic ethinylestradiol, and sometimes with synthetic delayed progestin, 538 children displayed psychiatric disorders and 35 were without any psychiatric disorders nor any malformation whatsoever and 13 were stillborn.

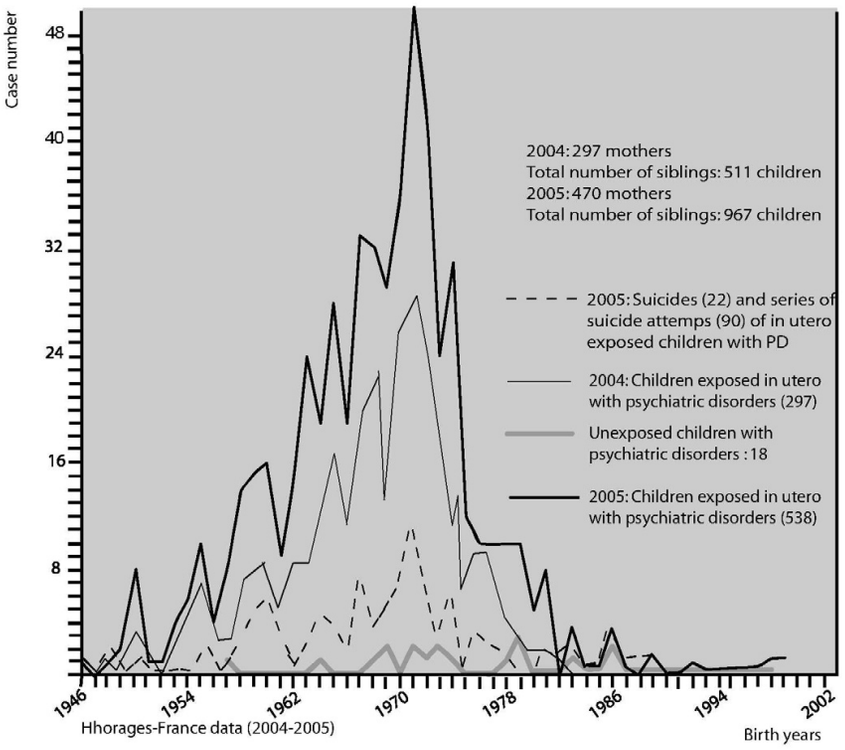


Figure 2. A detailed representation of case numbers in a group of 967 children born from 470 mothers. 363 unexposed children do not present any disorder. 586 children were exposed *in utero*, 48 of whom without disorders and 538 with psychiatric disorders (PD). 18 unexposed children showed PD, 9 of whom after their mother was treated in a previous pregnancy. Thin grey line: children exposed *in utero* with psychiatric disorders collected in 2004; black line: children exposed *in utero* with psychiatric disorders (2005). Thick grey line: unexposed children with psychiatric disorders. Dash line: Suicides and series of suicide attempts of *in utero* exposed children with psychiatric disorders. Comparison between 2004 and 2005 curves and suicide curve show the same prescription peak than in Fig.1 as observed in the seventies (1971) (HHORAGES-France data 2005).

By refining these observations, it appeared that out of the 538 children exhibiting psychiatric disorders, 200 suffered also from genital malformations. However, 74 presented genital malformations or other somatic disorders only, whereas 126 exhibited somatic and psychiatric disorders. In total, 538 children had psychiatric disorders. There were 18 non-exposed children who presented psychiatric disorders, 9 of which after their mothers had been exposed in a previous pregnancy. Among the children showing psychiatric disorders, it must be stressed that a significant number of suicides (22 S) and 90 series of suicide attempts were observed (dash line curve). The comparison between the 2004 and 2005 curves represented on Figure 2 shows a homothety, the peak of children suffering psychiatric disorders being for children born on the 1971-1972 years as well as the peak of suicide and suicide attempts.

In 2006-2007, a detailed analysis covered 529 testimonies, representing a total number of 1182 children as shown in the synthetic tree diagram of **Figures 3.**

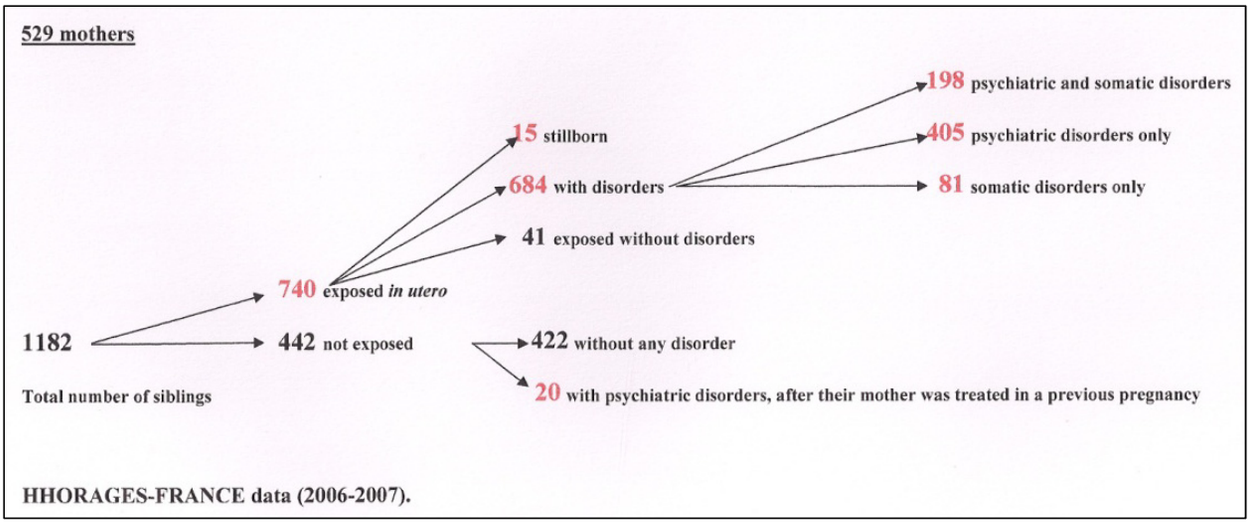


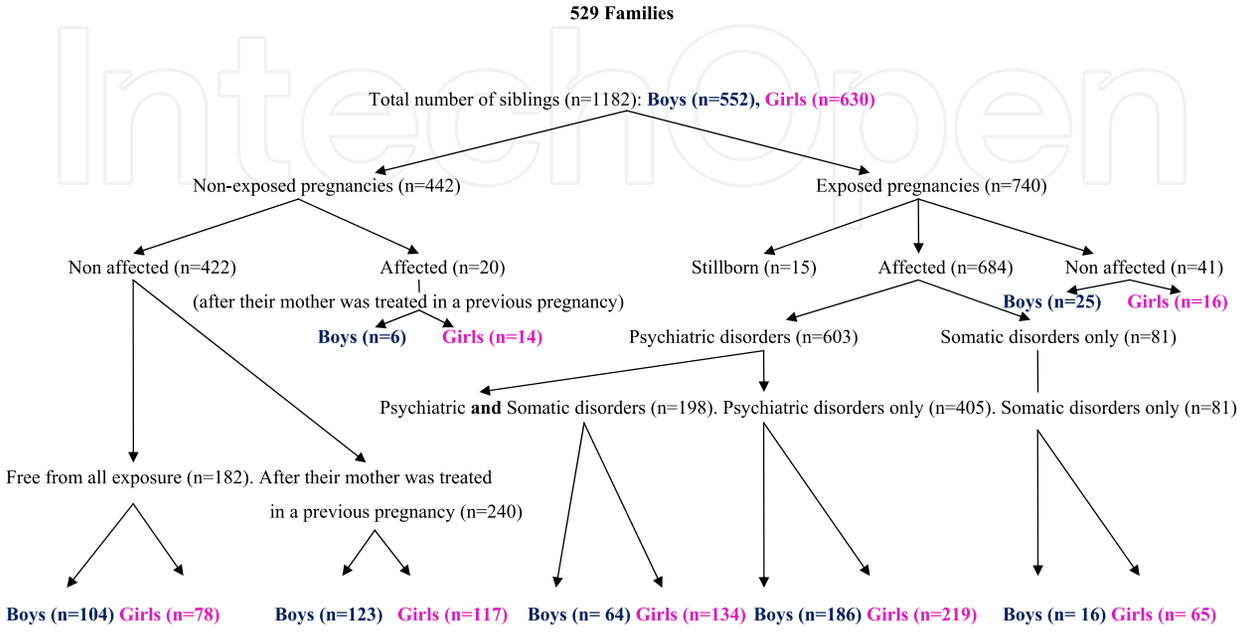
Figure 3. Tree diagram showing the distribution of a cohort of 1182 children born from 529 mothers, 740 of whom were exposed to synthetic hormones during their mothers' pregnancies. 41 exposed children did not show any disorder, 15 were stillborn and 684 were affected either by psychiatric disorders only, or by somatic disorders only, or by both (HHORAGES-France data 2006-2007).

442 children were not exposed, of which 422 did not exhibit any disorder or malformation; while 20 whose mothers had been exposed in a previous pregnancy exhibited psychiatric disorders. Among the 740 who were exposed *in utero*, 15 were stillborn (10 boys and 5 girls), 41 were not affected (25 boys and 16 girls) and 684 were affected, 405 of which with psychiatric disorders only, 198 psychiatric disorders associated with genital malformations and other somatic disorders, and 81 were affected only physically.

In **Figure 4** is shown the ratio of Boys/Girls, as well as their numbers. Thus 740 children were exposed and 684 were affected either by psychiatric disorders only, or by somatic ones, or by both. 65 girls and 16 boys were affected by somatic disorders only, while 134 girls and 64 boys were affected by psychiatric and somatic disorders. 219 girls and 186 boys are affected by psychiatric diseases only. Our group of 1182 total children is composed of 630 girls and 552 boys, so the gender ratio is 1.14. For children suffering from psychiatric diseases only, gender ratio is 1.17, psychiatric and somatic disorders 2.09, and somatic disorders only, 4.06.

Among the 442 "non-exposed" pregnancies, 422 children were not affected and 20 (mothers previously treated) were affected presenting psychiatric and/or somatic disorders. Among the 422 not affected 182 are free from all exposure (104 boys and 78 girls) and 240 were born after their mother was treated in a previous pregnancy (123 boys and 117 girls); so we observed a majority of unaffected boys. Among the 20 affected are 14 girls and 6 boys. Of these 14 girls, 7 suffered psychiatric disorders only, 3 presented psychiatric and somatic disorders, 4 somatic disorders only. Of the 6 boys, 4 presented psychiatric disorders, 2

psychiatric and somatic, and none with somatic disorder only. It is clear overall that disorders in girls are more numerous than in boys.



HHORAGES-FRANCE data (2006-2007).

Figure 4. Detailed testimony group included in the study showing the distribution of girls and boys of second generation in a cohort of 1,182 children born from 529 mothers: 740 of whom were exposed to synthetic hormones during their mothers' pregnancies collected from 2002 to 2007. 15 were stillborn and 684 were affected either by psychiatric disorders only, or by somatic malformations only, or by both (HHORAGES-FRANCE data 2006-2007). In all cases girls suffered more than boys either from psychiatric, and/or somatic disorders, while boys are more likely than girls to be unaffected even after exposure: 41 exposed children did not show any disorder (25 boys versus 16 girls) (HHORAGES-France data 2006-2007).

For boys among somatic disorders associated or not with psychiatric disorders (on a total of 80 boys), we have counted 28 cryptorchidia, 22 hypospadias, 14 sterility, azoospermia or semen abnormalities, 12 cancers or others, 4 micropenis. (**Figure 5**).

Among a total of 210 girls, somatic disorders associated or not with psychiatric disorders are: 70 womb malformations, 50 sterility, 31 difficulty to procreate (primary and secondary infertility), miscarriage, extra uterine pregnancies, 31 cancers (often of breast) or others, 21 ovarian cysts, 8 endometriosis. (**Figure 6**).

As of today (April 2012), we are receiving an ever-increasing number of testimonies, the total number of testimonies collected by HHORAGES is 1,223, which represents 2,674 children from them 409 unexposed, 1,676 children exposed to synthetic hormones after

medical prescriptions and 589 (post-DES) born after a previous exposure from which 20 presented psychiatric and/or somatic disorders. Amongst this total amount of 1,676 exposed children, 1,549 children are affected: 916 present psychiatric disorders, 418 somatic plus psychiatric disorders, 183 somatic disorders, 126 exposed are non affected. In addition, we numbered 48 suicides and 128 series of suicide attempts. Many HHORAGES families collaborated in genetic and epigenetic studies, as carried out in the Inserm U796 Laboratory in St-Anne Hospital, Paris, in order to understand the cellular and molecular mechanisms disrupted by these xenooestrogens [1] in the cadre of the PICRI (*Partenariat Institution Citoyens pour la Recherche et l'Innovation*) project. In addition, the HHORAGES families participate to a vast study on the origin of schizophrenia, this study, granted by the French National Research Agency (ANR), is larger than the PICRI one and also developed in the St Anne Hospital, Paris, by the Professor M.O. Krebs team.

Somatic disorders :boys	Impregnation:Synthetic hormones (DES, EE..)
Hypospads	22
Cryptorchidia	28
Micropenis	4
Sterility, Azoospermia, Abnomal.spz.	14
Cancers, others	12
Total	80

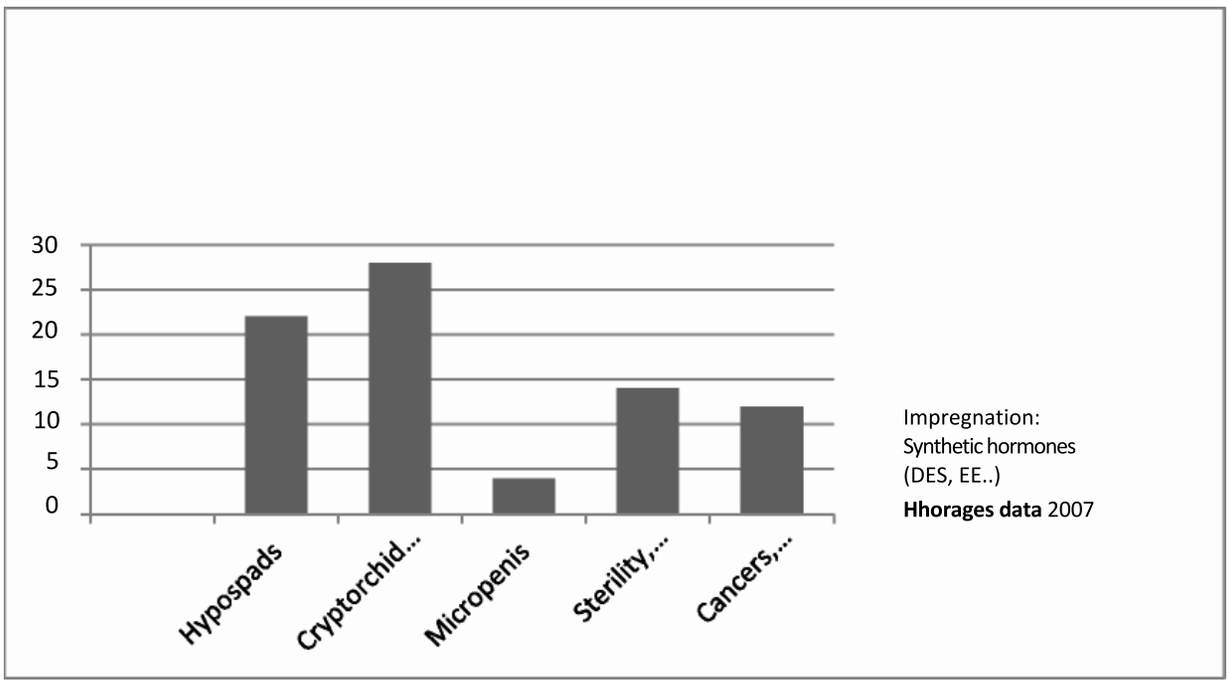


Figure 5. Somatic disorders in boys after exposition to synthetic hormones (DES, EE,.). Cryptorchidia and hypospadias are the most numerous disorders. These disorders are whether or not associated with the psychiatric ones (HHORAGES-France data 2007).

Somatic disorders : girls (associated or not to psychiatric disorders)	Impregnation: synthetic hormones (DES, EE..)
Uterine malformations	70
Sterility	50
Difficulty procreating, miscarriages, extra-uterinepregnancies	31
Ovarian cyst	21
Endometriosis	8
Cancers, others	31
Total	210

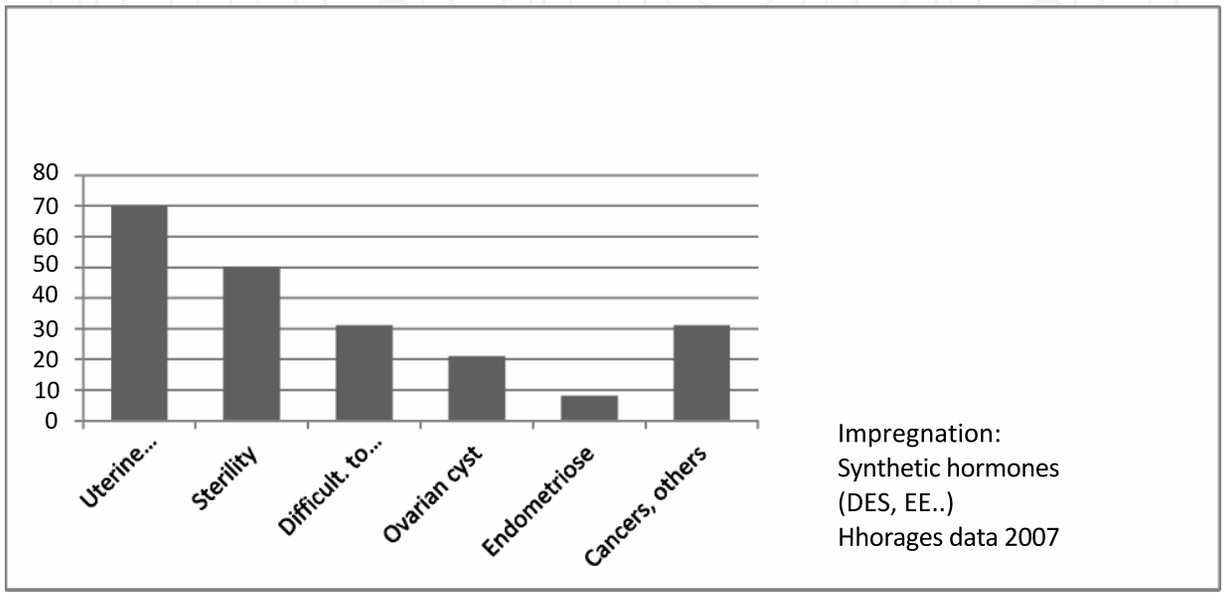


Figure 6. Somatic disorders in girls after exposure to synthetic hormones (DES, EE,). Womb malformations and sterility are the most numerous disorders. These disorders are whether or not associated with psychiatric ones (HHORAGES-France data 2007).

4. Discussion

1. Research work on behavioral disorders in humans after prenatal exposure to synthetic hormones is scarce, as people affected by severe psychotic disorders and their families are not usually very inclined to answer epidemiological inquiries. Similarly, as suggested in a preliminary work [27] the number of families who spontaneously gave their testimony to HHORAGES (more than 1220 to date) is rather low in comparison to the total number of pregnancies effectively treated with these products, about 160,000 in France according to [28,29]. Nevertheless, in our results presented in Figure 2, we observe in addition to the fact that curves deducted from successive year data are homothetic and that the peak of the curve representing children suffering psychiatric disorders as well as the peak of suicide and suicide attempts correspond to the years 1971-72. This could be put in parallel to the curve published in Figure 1 in [28] and calculated from the DES plaques sold in our country (EE sold plaques not estimated).

   But some testimonies remained anonymous, or some families were not properly informed, or failed to answer the questionnaires, in these cases, certain factors should

be taken into account: -The fact that psychiatric disorders appear during post-adolescence implies on average a twenty-year gap between the time when the mothers were treated and the appearance of the disorder, hence the difficulty finding the prescriptions and the medical files.– Psychiatric disorders are often treated with denial and shame, which presents problems connecting families with HHORAGES, or in their answering any inquiry whatsoever; and the children (DES sons and daughters for instance), if and when they are informed (often very late) about their family (mother) history, they first try to find a cure, as psychotic disorders can often be very incapacitating, especially if these disorders are in addition to genital malformations, sterility, semen abnormalities or total azoospermy.

2. Somatic disorders of boys and girls as genital malformations (see Figures 5 and 6), sterility, azoospermie, endometriosis, cancers, are now completely recognized as being a consequence of DES or EE *in utero* exposure. In our troop, they are found associated with psychiatric disorders for 198 children, while 81 were only somatically affected. The total amount of 279 children represent more than 1/3 of the 684 affected children exposed to synthetic estrogens that demonstrate the DES and EE signature.

   A preliminary result concerning a small part of the total number of children linked to the HHORAGES families was published in 2009 in a congress communication [1] (**Table 4**). A series of cases were studied, consisting of 31 files about 31 mothers and 72 children born alive. Among the 72 children, the following were found: 43 children exposed and affected, 4 exposed, but not affected, 23 non-exposed and not affected, 1 non-exposed but affected. The involved hormones were DES and 17-alpha-ethinylestradiol and synthetic progestin. The psychiatric disorders found were: Alcohol addiction: 3, Learning disorders: 3, Eating disorders: 9, Behavioral disorders (aggressiveness, impulsivity): 5, Sleeping disorders: 3, Anxiety disorders: 7, Mood swings: 12, Major depressive episodes: 2, Bipolar disorders, Personality disorders: 3, Schizophrenia: 10, Acute psychotic episodes: 4, Series of suicide attempts: 9, and Suicides: 5. As concluded in this communication "The clinical pictures relative to the children studied are quite complex and involve some atypical associations, for instance mood swings associated with psychotic features." A larger study pertaining to this cohort is under way.

   It is known that psychosis as schizophrenia for example affects about 1% of the world population, a few part being of genetic familial origin. Neuro developmental and environmental causal factors of schizophrenia (or of other psychoses as bipolar diseases) are yet badly known, the epigenetic track and the conjunction gene X environment theory being the best hypothesis from several years [24, 25, 30]. So the HHORAGES family group participates also to a study largest than PICRI on the origin of schizophrenia also developed in the St Anne Hospital by Professor M.O. Krebs team.

3. In this study, we observed that girls seem more vulnerable to synthetic estrogens than boys either for somatic and psychiatric disorders (see **Figure 4**). The preferential effects of DES on female *versus* male are not fully understood. It is likely that prenatal DES exposure affected behavior through its action on estrogen receptor alpha or beta of the

hypothalamic area which concentration may be higher in female fetus as shown in [30]. A sex difference in DNA methylation and gene regulation after prenatal DES exposure cannot be excluded [31] and last, hypomethylation of COMT promotor, a major risk factor for schizophrenia and bipolar disorder, may be different between male and female fetus and infant [32]. Our observations are reinforced by these of Braun et al [33] about BPA exposure and cognitive disorders or hyperactivity observations in *in utero* exposed children. In their study authors observed this BPA exposure affected behavioral and emotional regulation domains from 3 years of age, especially among girls which present aggressiveness and impulsivity. Authors suggest that girls would be more sensitive than boys *in utero* to BPA, which acts as mimetic of natural estrogen hormones, and in surplus to them.

Among the 72 children born alive, 40 exposed children were affected, 7 exposed were not affected, 1 exposed but deceased by the age of 10 months, 23 children were not exposed and not affected, et 1 unexposed child was affected. The psychiatric disorders are as follows :
Alcohol addiction: 3; Learning disorders: 3; Eating disorders : 9
Behavioral disorders (aggressiveness, impulsivity): 5
Sleeping disorders: 3; Anxiety disorders: 7
Mood swings: 12; Major depressive episodes, 2
Bipolar disorders, Personality disorders: 3
Schizophrenia: 10; Acute psychotic episodes: 4
Series of suicide attempts: 9 and Suicides: 5.

Table 4. Analysis of the psychiatric cases of 72 children from 31 testimonies [1]. From this observation, authors suggest that the vulnerability toward psychiatric disorders could be enhanced after impregnation with diethylstilbestrol (DES), ethinyl estradiol and/or synthetic delay progestin during the mother's pregnancy.

Concerning the manifestation of psychiatric disorders in boys and girls, appearing often post adolescence, Verdoux [21] had suggested that these diseases could be due to the exposed status of the children after being informed of it by the mother: In our cohort, during our contacts with families, we carefully questioned them about this point: most of children had never been previously informed of the exposure, or were never informed at all.

Concerning the 41 children exposed and not affected (25 boys/16 girls) and the 422 children unaffected (226 boys/196 girls) of which 242 mothers had been treated in a previous pregnancy, a majority of boys is observable: we think that this fact could be linked to the specific and unequal detoxification potential of each individual, and could

concern either the treated mother or/and the fetus in correlation with Cytochromes P450 gene activity and their regulation, as well analysed in the recent work of the Seralini group [34].

Concerning the 20 boys and girls (14 girls and 6 boys) "post DES" (whose mothers were only exposed during a previous pregnancy) and suffering psychiatric and somatic disorders, this could be linked with the lipophily (see Table 2) of the synthetic estrogens (DES and/or EE) administrated to mothers during a previous pregnancy, due to their likely residuum (remanence) in the maternal tissues.

4. There is a large amount of convincing research on animals (rodents) showing an indisputable link between synthetic estrogen exposition during gestation of the females and various disorders in exposed pups. Newbold et al [35] did not hesitate to extrapolate from rodents to humans where anatomical disorders observed in older mice are concerned after they were prenatally exposed to DES (cancers, lesions of the male as cryptorchidism, testicular hypoplasia, semen abnormalities [36] or female genital abnomalies, also of DNA and genetic lesions) as well as a function of the doses as of exposure time. She believes that the DES makes an excellent predictive model for other environmental estrogens. Indeed, O'Reilly *et al*. [23], in their epidemiological work on the depression rates in DES daughters, suggest that studies on this pathology may be extrapolated to children exposed to BPA *in utero*. Endocrine disrupter Bis-Phenol A (BPA) was nearly chosen instead of DES, but ultimately was not. However, its action inside plastic materials is even more insidious, as it is present in a large number of materials and consumer goods and as it contaminates therefore the whole population, including fetuses and infants via their breast feeding mothers. Among the many research works carried out on animals (rats, mice, monkeys) showing that there is a detectable effect for doses lower than the ADI (Acceptable Daily Intake) of 50μg/kg, one is specially interesting: it has been demonstrated in mice, that a prenatal exposure to the low dose of 10μg/kg (from the 11th day of gestation) unambiguously triggers behavioral disorders, besides other effects [37]. As recently published [38] the effects of low dose cannot be predicted by the effects observed at high doses. And as far as some organophosphate pesticides that mimic oestrogens are concerned, it has been recently shown that residues of such chemicals in urine samples of 1139 children aged 8-15 came from the family food (based on garden fruits and vegetables), and that their presence was strongly correlated to behavioral disorders of hyperactivity type and cognitive disorders (attention deficit disorder) type [39].

5. A multi-generational effect? By what mechanism?

Multi-generational carcinogenesis studies were realized on mice after diethylstilbestrol impregnation with impressive and undisputable results [8, 9]. Our observations presented in this present work from the French HHORAGES troop raises the question of the mechanism through with synthetic hormones as DES cause either psychiatric disorders in exposed children and/or adverse effects in subsequent generations. Since Abdomaleky et al

[40,41,30] concluded that modulation of gene-environment interactions may be trough DNA methylation, in [42] and [24, 25] authors put forward hypothesis that DES-induced changes in epigenetic background and alteration of DNA methylations could be significant factors. The pregnant mother's exposure to DES at very early neurodevelopment time and/or at time of sex determination would appear to be sufficient to alter the remethylation of neuron precursors and/or of the fetus germ line. Only a few third-generation children suffering psychiatric illness are mentioned in testimonies. This is understandable because third generation exposed children are still too young (excepted in some cases) to present psychiatric disorders as schizophrenia which is not the case for hypospads that are detectable from birth in male children and grand-children [42]. Work is already under way concerning the gene X environment DES impact hypothesis by comparing DES and EE exposed children, various genetic and epigenetic factors to those of mother and unexposed children of the same family as studied by the INSERM team U796 in collaboration with the HHORAGES families.

6. Conclusions

In the present familial case control study, we have shown that there are serious effects on the psychological and physical health of the descendants of women treated with synthetic hormones during their pregnancy: psychiatric illnesses are often found associated with somatic disorders which are well known to be the DES and EE signature. Synthetic hormones, acting as endocrine disturbers, are toxic for humans, especially for pregnant women and their children, probably partly in relation with their toxic degradation status. In all cases girls suffered more than boys either of somatic and/or psychiatric disorders due to the estrogen receptor alpha or beta concentration higher in female fetus than in male. It is also clear that in all the families most of the exposed children are ill while quite the unexposed are not.

So what now? As the precautionary principle was not applied in the past, and still is not in force today, and since the lessons of recent history were never taken into account [33], it is our common duty to repair the damage by supporting the devastated families, and by pursuing research work on the observation of trans-generational effects. Such effects are already highlighted by the demonstration that cancers are observed even in the fourth generation in mice [9]. According to the Skinner's mini review [43] "the ability of an environmental compound (as DES or EE) to promote the reprogramming of the germ-line appears to be the causal factor in the epigenetic transgenerational phenotype," we observed an increase of the genital malformations in the third generation in male infants whose mothers were treated with xenoestrogens [42]. In the HHORAGES troop, DES and EE-exposed infants are already pointed out as bodily and/or psychologically impaired after their mothers were treated with clomifene citrate (an ovulation stimulator previously used for IVF-type medically assisted procreation). Another concern is the putative future effect of ethinylestradiol containing oral estrogenic contraception on future generations due to its lipophily after its metabolization and its future release in fetus through the placenta. As for

the demonstration of the causality link within the HHORAGES troop, will we have to wait for a large-scale epidemiological study, or are we allowed to think that the impressive figures that we are publishing in this work are not merely random? The only way now is to respect absolutely the precautionary principle and to delete completely or to give the less possible toxic (synthetic) hormone medication: for example Clavel Chapelon and her Endogenous Hormones and Breast Cancer Collaborative Group in Villejuif [44] informed that natural hormone as micronized (natural) progestin associated with estrogens (synthetic alas!) is more often ordered for SHT (Substitutive Hormonal Treatment) in order to avoid breast cancer. Unfortunately, she said also that in the contrary the same SHT is not recommended to avoid the endometrium cancer [45]…

As Newbold et al [35] said after they reviewed the damages caused by DES [4], "only new advances in the knowledge of genetic and epigenetic mechanisms of the disruptions of fetal development will enable us to be aware of the risks entailed by the other estrogenic disruptors which are present around us and in ourselves, even at very low doses", whilst Theo Colborn [46] insists on the fact that the foetus cannot be protected against endocrine disruptors, whatever they may be, except at zero level.

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